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Alternatively Spliced Murine *lyn* mRNAs Encode Distinct Proteins

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Two *lyn* proteins of 56 and 53 kDa have been observed in immunoprecipitates from a variety of murine and human cell lines and tissues. We report the cloning and nucleotide sequence of two distinct murine *lyn* cDNAs isolated from an FDC-P1 cDNA library. One of the cDNAs, designated *lyn*11, encodes a protein of 56 kDa which shares 96% similarity with human *lyn*. The other cDNA, designated *lyn*12, encodes a protein of 53 kDa. The proteins differ in the presence or absence of a 21-amino-acid sequence located 24 amino acids C terminal of the translational initiation codon. Using RNase protection analysis, we have identified mRNAs corresponding to both cDNAs in murine cell lines and tissues. Sequence analysis of murine genomic clones suggests that the distinct mRNAs are alternatively spliced transcripts derived from a single gene. Expression of both cDNAs in COS cells leads to the production of *lyn* proteins with the same molecular weight as the two forms of *lyn* proteins immunoprecipitated from extracts of FDC-P1 cells and mouse spleen. Subcellular fractionation studies and Western immunoblotting analysis suggest that both isoforms of *lyn* are membrane associated. The association of both *lyn* isoforms with the membrane fraction supports the notion that *lyn*, like other *src*-related kinases, may interact with the intracellular domain of cell surface receptors.

The *lyn* gene encodes a member of the *src* family of protein tyrosine kinases (53). Eight distinct genes encoding the *src*-related kinases *lyn* (53), *src* (24, 44), *lck* (23, 50), *hck* (13, 31, 56), *blk* (6), *yes* (42), *fgr* (45, 52), and *fyn* (15, 39) have been identified in the genomes of mammals. Four of these kinases, *lyn*, *hck*, *lck*, and *blk*, are expressed either predominantly or uniquely in cells of the hemopoietic system (for a review, see reference 29). *lyn*, which is expressed predominantly in B-lymphoid and myeloid cells as well as some human T-cell lymphotrophic virus type I-transformed T cells (55), shares 71% amino acid similarity with *hck* (which is also expressed in B cells and myeloid cells [13, 31, 56]), 65% amino acid similarity with *lck*, which is predominantly found in T cells (23, 50), and 63% amino acid similarity with *blk*, which is a product of cells of the B-lymphoid lineage (6). It seems probable that these *src* family members perform specialized but related functions within the cells in which they are expressed.

There are three *src*-related kinases which appear to form part of the cytoplasmic domain of different cell surface receptor complexes. Recent findings indicate that *fyn* may form part of the T-cell receptor complex and may be responsible for the phosphorylation of components of this complex following stimulation of T cells with specific antibodies (36). Observations by Yamanashi and coworkers (54) suggest that *lyn*, like *fyn*, is intimately involved in the regulation of the immune response. Their studies show that proteins which are immunoreactive with *lyn*-specific antisera are associated with the membrane-bound immunoglobulin M (IgM) on the surface of B cells (54). However, the most compelling evidence that *src*-related kinases are directly involved in signal transduction is provided by the demonstration that p56^{lck} physically associates with the CD4/CD8 receptors of T cells (34, 47). This association has been shown to depend on residues located within the N-terminal 32 amino acids of *lck* and sequences contained within the cytoplasmic domain of the CD4/CD8 receptors (40, 41, 46). In light of the postulated role of CD4 and CD8 in T-cell function, it seems probable, therefore, that *lck* is involved in signal transduction processes that ultimately lead to the functional activation of these cells (48; for reviews, see references 1 and 28).

If association with cell surface molecules proves to be a property shared by other *src* family members, the N-terminal region of individual *src* molecules is the domain most likely to contribute the required specificity in this interaction. We report here the isolation of two cDNAs which encode *lyn* proteins that differ in the presence or absence of a 21-amino-acid sequence located 24 amino acids C terminal of the translational initiation codon. Intriguingly, unlike the situation with other *src* family members for which alternative splicing has been documented, the mRNAs encoding both *lyn* isoforms appear to be present in all cells that express the *lyn* gene. While we cannot rule out the possibility that each isoform of *lyn* forms a component of separate signal transduction pathways, their coordinate expression may indicate that their functions are in some way coupled.

MATERIALS AND METHODS

Isolation and characterization of clones. A λgt10 FDC-P1 cell (5) cDNA library (a gift from A. Wilks) was screened at low stringency (35) with a radiolabeled *Eco*RI-*Bam*HI DNA fragment corresponding to the tyrosine kinase domain of murine *hck* (13). A number of hybridizing clones were plaque purified and analyzed with restriction enzymes. DNA fragments derived from each clone were inserted into M13 vectors (27) and subjected to DNA sequence analysis (37). These analyses identified a number of clones encoding proteins with a high degree of similarity to human *LYN* (53).

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The nucleotide sequences of three of these clones, designated *lyn*6, -11, and -12, were determined.

The genomic sequences spanning the splice junction points were isolated following polymerase chain reaction (PCR) analysis of murine genomic DNA with the oligonucleotides 5'-GACAATCTCAATGACGATGAAGTA-3' and 5'-CTCTGTCCTGGTAAAAGATG-3', which correspond to positions 262 to 286 and 399 to 379, respectively (Fig. 1a). The reactions were carried out in the presence of 2.5 mM $MgCl_2$, using Biotec *Taq* polymerase and the buffer supplied by the manufacturer. The temperatures and cycle times used were 95°C (60 s), 42°C (60 s), and 72°C (240 s) for 35 cycles. Following PCR, the 3.7-kb product was gel purified and treated with Klenow polymerase and polynucleotide kinase prior to being ligated into the vector pGEM3Z. The recombinants were sequenced by the method of Sanger et al. (37).

RNase protection. DNA fragments from the 5' end of the *lyn*11 cDNA to the *Xba*I and *Hin*cII sites were introduced into the vector pGEM4 to generate pGEM11X and pGEM11H, respectively. Similarly, the sequences from the 5' end of *lyn*12 cDNA to the *Xba*I site were introduced into pGEM4 to give pGEM12X. In vitro transcription of pGEM12X (which had been linearized with *Eco*RI) from the T7 promoter produced a 499-nucleotide *lyn*12 antisense RNA. In vitro transcription of pGEM11X and pGEM11H (also linearized with *Eco*RI) from the T7 promoter gave rise to antisense RNA transcripts of 414 and 498 nucleotides, respectively. The plasmids described above were transcribed in the presence of [α-^{32}P]UTP to produce radiolabeled antisense transcripts which were subsequently used for RNase protection analysis (21, 26). Protected fragments were analyzed on a 6% denaturing acrylamide gel, and a set of sequencing reactions (M13mp18 universal primer) was included as a size standard (21). Following electrophoresis, the gel was dried and subsequently subjected to autoradiography (16 h).

RNA preparation and Northern (RNA) analysis. Total RNA was prepared from murine tissues by the CsCl cushion procedure (8, 35), and poly(A)$^+$ RNA was subsequently isolated by oligo(dT)-cellulose chromatography (7). Poly (A)$^+$ RNA was prepared from tissue culture cells as described by Gonda et al. (10). Following transfer to nitrocellulose, filter hybridization was carried out in 2× SSC (SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate (SDS) at 68°C, using an antisense radiolabeled RNA probe generated by in vitro transcription of sequences corresponding to nucleotides 236 (initiation codon) to 516 (*Xba*I) in Fig. 1a. Filters were washed at 68°C in 0.1× SSC–0.5% SDS prior to autoradiography (16 h).

COS cell transfections and immunoprecipitations. *lyn* cDNAs which contained the entire coding region were constructed by fusing the 3' sequences contained within the *lyn*6 cDNA with the 5' sequences of *lyn*11 or *lyn*12. *lyn*6, *lyn*11, and *lyn*12 cDNAs were joined via a shared *Eco*RI site. This junction preserves the reading frame deduced from the human *LYN* sequence (53). Full-length cDNA clones were inserted into pJL4 (12), and the resultant vectors were introduced into COS cells (9, 25) by DEAE-dextran-mediated transfection (35). At 48 h posttransfection, cells were harvested and lysed, and the *lyn* proteins were immunoprecipitated with a *lyn*-specific antiserum, L40 (1a). This antiserum was raised by immunizing rabbits with a glutothionein-*S*-transferase/*lyn* fusion protein which contained amino acids 7 to 430 of murine p56^{lyn}. The specificity of the antiserum is demonstrated by its ability to exclusively precipitate two proteins of 56 and 53 kDa from murine spleen lysates which comigrate with those precipitated from COS cells transfected with vectors which express the two murine cDNAs. *lyn* proteins were also immunoprecipitated from lysates of FDC-P1 and W265 cells. Cells were lysed at 4°C for 30 min in 0.5 ml of modified Lau buffer (17) (100 mM NaCl, 10 mM Tris-Cl [pH 7.5], 2 mM EDTA, 0.5% sodium deoxycholate, 1% Nonidet P-40, 10 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride, 1 mM Trasylol, 0.1 mM $NaVO_4$), and the insoluble material was removed following the addition of PanSorbin (1%) and centrifugation (350,000 × *g*, 10 min, 4°C). Antiserum was added to the supernatant and incubated for 3 h (4°C) prior to the addition of proteinA-Sepharose (5% final concentration). The immune complexes were washed three times in modified Lau buffer, resuspended in kinase buffer (10 mM $MgCl_2$, 10 mM $MnCl_2$, 20 mM *N*-2-hydroxyethylpiperaze-*N*-2'-ethanesulfonic acid [HEPES; pH 7.0], 1 mM dithiothreitol, 0.1 mM $NaVO_4$, 0.5% Nonidet P-40), and incubated for 10 min (25°C) in the presence of 10 μCi of [γ-^{32}P]ATP. The kinase reactions were terminated by the addition of 1 volume of SDS sample buffer (2% SDS, 10% glycerol, 50 mM Tris-Cl [pH 7.0], 0.1% bromophenol blue), and the samples were fractionated by SDS-polyacrylamide gel electrophoresis (PAGE) (3). The gel was treated with 1 M KOH for 2 h at 55°C prior to autoradiography (4).

Subcellular fractionation and Western immunoblotting. Murine RAW8 (32) and W265 (51) cells were washed in phosphate-buffered saline (PBS), resuspended at a concentration of 10^7/ml in hypotonic lysis buffer (2.5 mM Tris-Cl [pH 7.5], 1 mM dithiothreitol, 2.5 mM KCl, 0.5 mM $MgCl_2$, 1 mM phenylmethysulfonyl fluoride, 1 mM ε-amino caproic acid), and incubated at 4°C for 15 min. Cells were lysed in a Dounce homogenizer, and the cellular debris was removed by centrifugation at 1,000 *g* for 1 min. The supernatant was then subjected to a second centrifugation step at 100,000 × *g* at 4°C for 10 min. The resultant pellet, representing total cellular membranes, was solubilized in modified Lau buffer, and the supernatant, representing the cytosolic fraction, was adjusted to 1× modified Lau buffer. Samples representing both the cytosolic and nuclear fractions were subjected to Western blot analysis using the polyclonal antiserum L40, which recognizes both isoforms of *lyn* (1a). To assess the purity and integrity of the cell fractions, a replicate filter was probed with a monoclonal antibody (ACA88) directed against the cytosolic protein HSP90 (33, 38). Briefly, proteins were transferred to Immobilon (Millipore) by using a Sartorious semidry blotting apparatus. Filters were blocked with 5% nonfat skim milk in PBS–0.1% Tween 20 (NFSM buffer) for 30 min (42°C). Antibodies were used at 1/1,000 dilution in 5% NFSM buffer for 6 h and washed three times with Tris-buffered saline (25 mM Tris-Cl [pH 8.0], 150 mM NaCl, 0.1% Tween 20). Primary antibodies were detected by using the Amersham chemiluminescence kit and horseradish peroxidase-conjugated anti-rat or anti-mouse IgG (Bio-Rad) antibodies according to the manufacturer's instructions.

Nucleotide sequence accession number. The sequence information in Fig. 1 has been given The GenBank accession number M64608.

RESULTS

DNA sequence analysis of murine *lyn* cDNA and genomic clones. We have isolated a number of cDNA clones from an FDC-P1 cDNA library which possess a high degree of sequence similarity to a cDNA encoding human *LYN* reported by Yamanashi and coworkers (53). One of these

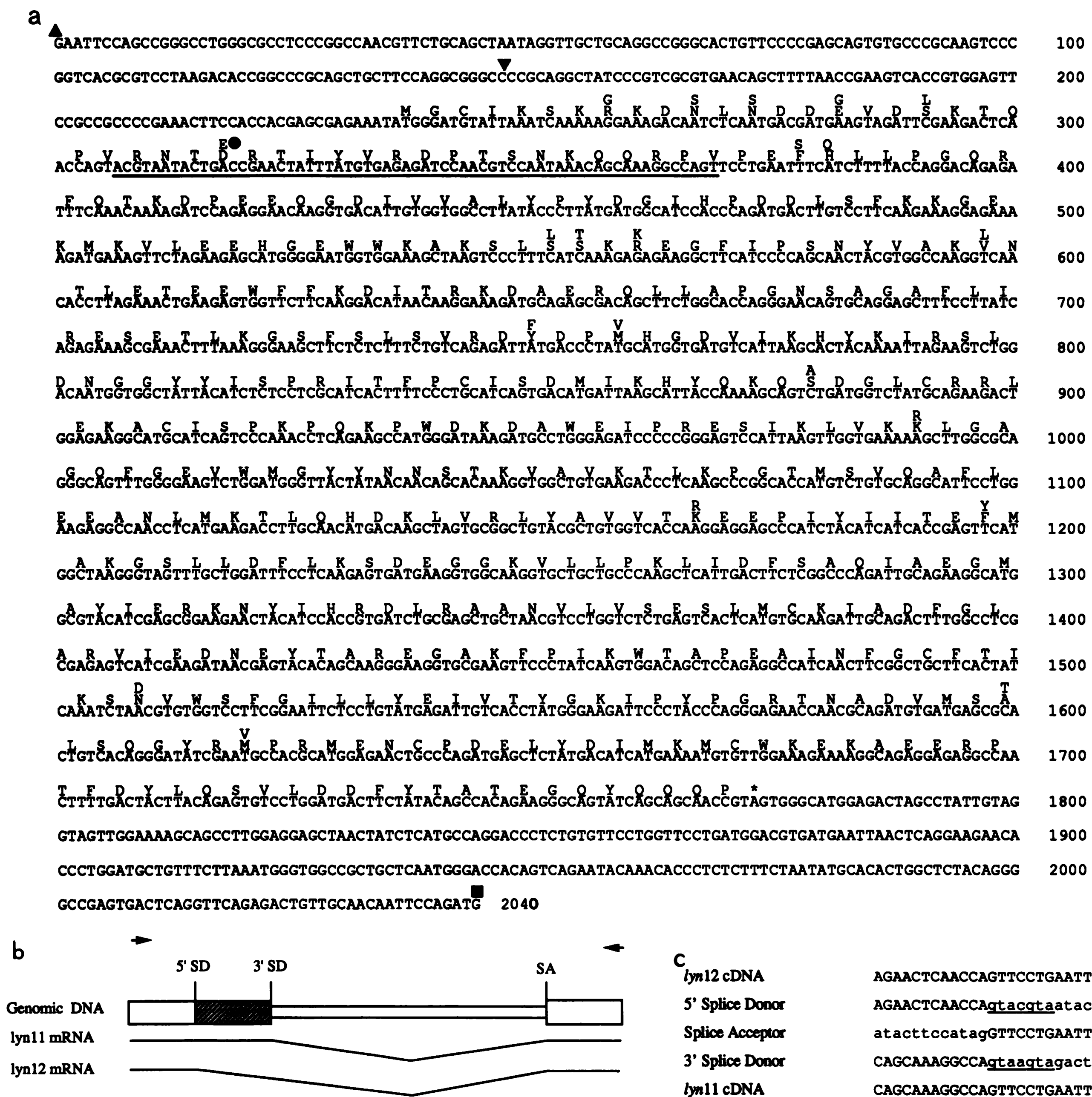

FIG. 1. (a) DNA sequence and conceptual translation of murine *lyn* cDNA clones isolated from an FDC-P1 cDNA library. The DNA sequence absent from the *lyn*12 cDNA is underlined. The 5′ endpoints of the *lyn*11 (▼) and *lyn*12 (▲) cDNAs are indicated. The 3′ endpoint of each clone is the internal *Eco*RI site at position 1530. The 5′ (●) and 3′ (■) ends of the *lyn*6 cDNA are also indicated. The sequence information 3′ of the internal *Eco*RI site (nucleotide 1525) was derived from the permuted *lyn*6 cDNA. Where the human and murine *lyn*11 cDNA-encoded amino acids differ, the human amino acid is shown above that of the murine. The stop codon (*) is indicated. (b) Schematic representation of the genomic region spanning the differences between the *lyn*11 and *lyn*12 cDNAs. Arrows indicate the positions of primers used to amplify the genomic fragment by PCR. The exon sequences are depicted by large boxes; the hatched region represents the region absent from the *lyn*12 cDNA. The two mRNAs generated by the alternative splicing event are also depicted. SD, splice donor; SA, splice acceptor. (c) Sequences spanning the splice donors and acceptors used in the generation of the mRNAs represented by the *lyn*11 and *lyn*12 cDNAs. The exon sequences are shown in uppercase, and the intron sequences are shown in lowercase. The nucleotide sequences corresponding to the splice donor sites are underlined.

cDNAs, designated *lyn*6, was permuted at a natural *Eco*RI site such that the kinase domain was inverted with respect to the remainder of the *lyn* sequences. This clone was the only representative which retained sequences encoding the C-terminal half of the catalytic domain. Two other cDNAs, designated *lyn*11 and *lyn*12, possessed sequences spanning the translational initiation codon and extended 3′ to the internal *Eco*RI site. The nucleotide sequence presented in Fig. 1a, summarizes sequencing data obtained from *lyn*6, *lyn*11, and *lyn*12 cDNAs. The protein encoded by the *lyn*11 cDNA has 96% sequence similarity to the human *LYN* protein (53). Many of the differences between the murine and

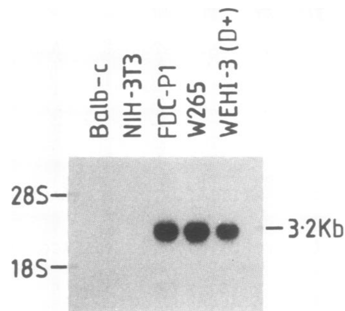

FIG. 2. Northern blot analysis of poly(A)$^+$ RNA isolated from murine cell lines. Represented are the fibroblastic cell lines BALB/c 3T3 and NIH 3T3 and the hemopoietic cell lines FDC-P1, W265, and WEHI-3 (D+). The blot was hybridized with a radiolabeled antisense riboprobe corresponding to nucleotides 236 to 516 in Fig. 1a. The hybridizing mRNA is approximately 3.2 kb as measured relative to the 28S and 18S rRNAs.

human *lyn* proteins represent conservative amino acid substitutions. The proteins encoded by the *lyn*11 and *lyn*12 cDNAs differ in the presence or absence of a 21-amino-acid sequence located 24 amino acids C terminal of the translational initiation codon.

The proposal that both cDNAs represent mRNAs derived from a single locus is supported by sequence analysis of genomic clones derived by PCR using oligonucleotides which span sequences corresponding to the region that differs between the two cDNAs. A summary of the sequence information is presented in a schematic form in Fig. 1b. As indicated, the generation of the two mRNAs corresponding to the *lyn*11 and *lyn*12 cDNAs most likely occurs as a consequence of differential usage of alternative splice donor sites. The 5′ splice donor site is thus present in the *lyn*11 cDNA and occurs at position 305 in Fig. 1a. The details of the splice junction points are presented in Fig. 1c.

Northern analysis of *lyn* mRNAs. We analyzed *lyn* expression by screening Northern blots containing various mRNAs with a radiolabeled probe corresponding to nucleotides 238 (translational initiation codon) to 524 (*Xba*I site) in Fig. 1a. Figure 2 shows that unlike cell lines of hemopoietic origin (FDC-P1, W265, and WEHI-3B) which contain high levels of *lyn* mRNA, two fibroblastic cell lines, BALB/c 3T3 and NIH 3T3, do not appear to express the *lyn* gene. However, longer exposures of the autoradiograph shown in Fig. 2 reveal the presence of low levels of hybridizing transcripts of the same size as those present in the hemopoietic cell lines (data not shown). The significance of the low level of *lyn* expression in fibroblasts, which has also been observed by Lindberg and coworkers (20), is unclear. The size of both murine *lyn* mRNAs is 3.2 kb, identical to that reported for the human *LYN* mRNA by Yamanashi et al. (53).

RNase protection analysis of FDC-P1 cell mRNA. RNase protection analysis was used to verify that both *lyn*11 and *lyn*12 cDNAs were representative of mRNA species present in vivo (Fig. 3a). RNA isolated from FDC-P1 cells was used to protect the three distinct radiolabeled RNA probes depicted schematically in Fig. 3b. Probes X11 and H11 represent sequences from the 5′ end of the *lyn*11 cDNA to the *Xba*I and *Hinc*II sites, respectively. X12 represents sequences from the 5′ end of the *lyn*12 cDNA to the *Xba*I site. The protected fragments obtained following digestion of hybrids between *lyn* mRNA and the H11 probe are shown in lane 1. The largest protected fragment of 454 nucleotides

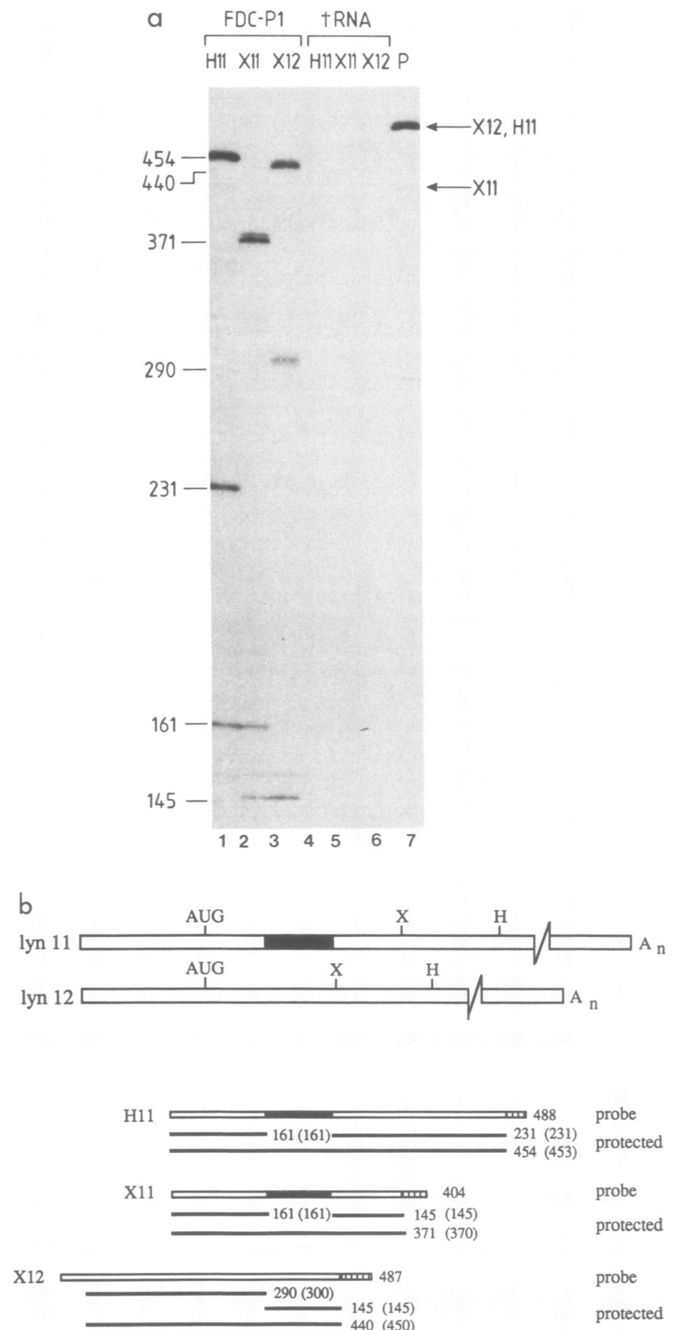

FIG. 3. (a) RNase protection analysis of poly(A)$^+$ RNA isolated from FDC-P1 cells. The fragments resulting from protection of RNA hybrids between FDC-P1 RNA or *Escherichia coli* tRNA and the H11, X11, and X12 probes are shown. The lengths (in nucleotides) of the probes prior to digestion (lane P) are indicated on the right. (b) Schematic representation of the probes, the protected fragments, and their relationship to the *lyn*11 and *lyn*12 mRNAs. Numbers immediately following the probes correspond to the sizes (in nucleotides) of the protected fragments. Numbers in parentheses are the expected sizes deduced from the nucleotide sequence. The shaded area represents the sequences absent from the *lyn*12 mRNA; the striped area represents polylinker sequences from the plasmid. AUG shows the approximate position of the translational initiation codon. X and H show the relative positions of the *Xba*I and *Hinc*II sites, respectively.

represents hybrids formed between the probe and mRNA corresponding to the *lyn*11 cDNA. Other protected fragments of 231 and 161 probably correspond to hybrids between the probe and the mRNA species represented by the *lyn*12 cDNA. Such hybrids would possess an RNase-sensitive region of single-stranded RNA between nucleotides 307 and 370 (Fig. 3a).

Protected fragments obtained with the X11 probe are shown in lane 2 of Fig. 3a. The fragment representing hybrids between the X11 probe and mRNAs corresponding to the *lyn*11 cDNA is 371 nucleotides in length. This species is 83 nucleotides shorter than that obtained with the H probe, the distance between the *Hin*cII and *Xba*I sites. The fragment of 161 nucleotides, common to both the X11 and H11 probes, represents the distance from the common end of the probes to the point at which the two cDNAs diverge (Fig. 3b). The remaining fragment, of 145 bp, represents sequences 3′ of the divergence point to the *Xba*I site and, as predicted, is 80 nucleotides shorter than the analogous fragment protected with the H11 probe.

When the X12 probe was used, three protected fragments of 145, 290, and 440 nucleotides were generated (Fig. 3a, lane 3). The 440-nucleotide fragment, which corresponds to hybrids between the X12 probe and *lyn*12 mRNA, is approximately 10 nucleotides shorter than predicted. This discrepancy may indicate that most *lyn* transcripts initiate 3′ of the point corresponding to the 5′ end of the *lyn*12 cDNA. This would imply that there is heterogeneity in the points of transcriptional initiation within the *lyn* gene, a phenomenon observed in other *src*-related genes (21, 43). The 290-bp protected fragment reflects the distance from the 5′ end of the *lyn*12 cDNA to the point of divergence between *lyn*11 and *lyn*12 cDNAs. This fragment is also 10 nucleotides shorter than predicted, suggesting that *lyn*11 and *lyn*12 mRNAs may have common sites of transcriptional initiation. The smallest fragment of 145 nucleotides presumably represents sequences 3′ of the point where the two cDNAs converge to the *Xba*I site.

RNase protection analysis of murine tissues. To investigate possible differences in the pattern of expression of the mRNAs encoding the two *lyn* isoforms, we have performed RNase protection analysis using RNA isolated from a number of murine tissues in conjunction with the X11 probe. As shown in Fig. 4, of the tissues surveyed, *lyn* transcripts are highest in spleen and lung, an observation consistent with patterns of *lyn* expression in murine and human tissues (53). Moreover, the pattern of protected fragments obtained with RNA isolated from spleen and lung is identical to that observed with RNA derived from FDC-P1 (5) and W265 (51) cells. The additional protected fragments, which are 5 nucleotides longer than the 371- and 145-nucleotide fragments predicted and observed with the X11 probe, probably correspond to incomplete digestion products, since the corresponding products are not observed when the H11 probe is used. The low levels of *lyn* RNAs detected in other tissues after prolonged autoradiographic exposure may reflect a low level of *lyn* expression within the tissues themselves or may reflect the presence of resident hemopoietic cells. Importantly, our analysis shows that in tissues or cell lines in which the *lyn* gene is expressed, both forms of *lyn* mRNA are present.

Expression of *lyn* cDNAs in COS cells. To verify that the two *lyn* cDNAs encode proteins which had been previously identified in immunoprecipitates from hemopoietic cells, we have expressed the cDNAs in a simian virus 40-based mammalian expression system. Full length *lyn*11 and -12 cDNAs were incorporated into the eukaryotic expression vector pJL (11) and transfected into COS cells (9). After 48 h of incubation, cells were harvested and *lyn* immunoprecipitates were prepared by using the anti-*lyn* serum L40 (1a). As

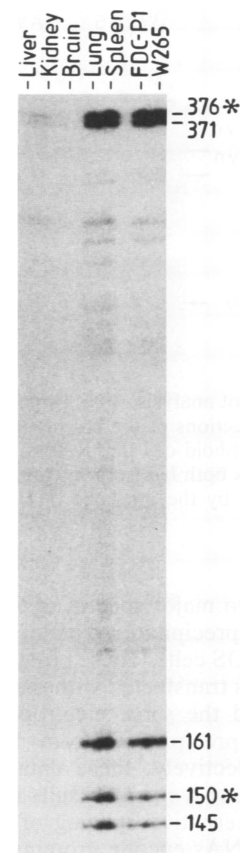

FIG. 4. RNase protection analysis of 1 μg of poly(A)$^+$ RNA isolated from murine tissues and cell lines by using the X11 probe. The fragments of 371, 161, and 145 nucleotides are as described for Fig. 3. The presence of fragments of 376 and 150 nucleotides (*) is somewhat variable, and since no equivalent products are observed with the H11 probe (see Fig. 3a), we believe that these fragments probably represent incomplete digestion products.

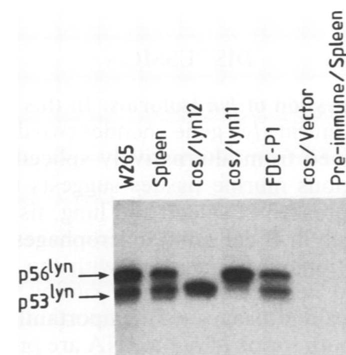

FIG. 5. Comparison of *lyn* proteins immunoprecipitated from COS cells transfected with a vector expressing the *lyn*11 and *lyn*12 cDNAs with *lyn* proteins from FDC-P1, W265, and murine spleen cells. Following immunoprecipitation with a *lyn* antiserum, the *lyn* proteins were incubated in the presence of [γ-^{32}P]ATP prior to fractionation by SDS-PAGE.

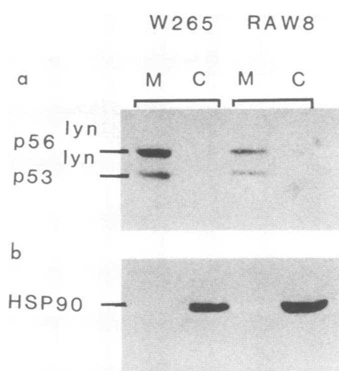

FIG. 6. Western blot analysis of *lyn* proteins from the cytoplasmic and membrane fractions of the murine myeloid cell line W265 and the murine B-lymphoid cell line RAW8, using the L40 antiserum, which recognizes both *lyn* isoforms (a). The purity of the cell fractions is supported by the presence of HSP90 in the cytosolic fraction (b).

shown in Fig. 5, two major species of 56 and 53 kDa were present in immunoprecipitates from pJL/*lyn*11- and pJL/*lyn*12-transfected COS cells. These proteins, which were not present in COS cells transfected with the pJL vector lacking an insert, displayed the same electrophoretic mobility as molecules immunoprecipitated from FDC-P1 cells and mouse spleen. Collectively, these data suggest that transcription of the mouse *lyn* gene results in the generation of two mRNAs by alternative splicing of a single precursor RNA. The two mRNAs encode proteins of 53 and 56 kDa, reflecting the presence or absence of an alternative 64-nucleotide exon.

The two *lyn* isoforms are membrane associated. To determine the subcellular localization of the two *lyn* isoforms, we prepared membrane and cytosolic fractions from two murine cell lines, W265 and RAW8, which express *lyn*. As shown in Fig. 6, both forms of murine *lyn* are associated with the membrane fraction, a characteristic shared with other *src*-related kinases. The purity and integrity of the cell fractions is supported by immunoblot analysis indicating that HSP90, a cytosolic protein (16), is present within the cytosolic fractions. The association of *lyn* with the membrane fraction is consistent with the idea that *lyn*, like other, *src*-related kinases, may be involved in the transduction of signals from the cell surface.

DISCUSSION

Coupled expression of *lyn* isoforms. In this study we have shown that the murine *lyn* gene encodes two distinct proteins which are derived from alternatively spliced mRNAs. Our analysis of various murine tissues suggests that *lyn* is predominantly expressed in spleen and lung, tissues which are known to be rich in B cells and macrophages, respectively. These observations are consistent with the conclusions of Yamanashi and colleagues in their survey of human and murine cell lines and tissues (55). Importantly, our analysis indicates that both forms of *lyn* mRNA are present in all cell lines and tissues where the *lyn* gene is expressed.

The coexpression of mRNAs encoding the two *lyn* isoforms is in distinct contrast with other examples of alternative splicing within the *src* family. The murine c-*src* gene appears to encode three proteins, two of which are found in tissues of neural origin, while the other is expressed ubiquitously (19, 24, 30, 44). Similarly, the *fyn* gene encodes alternatively spliced RNAs which are expressed mutually exclusively in either hemopoietic or nonhemopoietic tissues (2). The linked expression of the two *lyn* isoforms raises the possibility that their functions are in some way coupled, perhaps forming part of the same macromolecular complex. Consistent with this notion, Yamanashi et al. (54) have shown that both forms of *lyn* are present in membrane complexes involving IgM molecules on the surface of B cells. If the two *lyn* isoforms are closely associated in other cell types, it should be possible to coprecipitate both of them with antisera directed against the unique regions of $p56^{lyn}$.

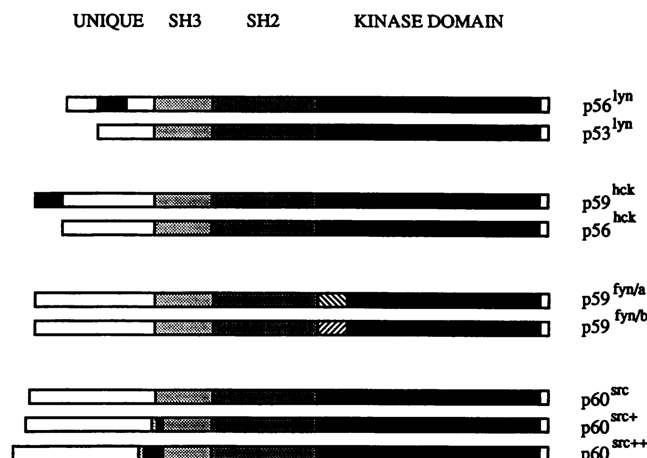

FIG. 7. Schematic representation of the alternative isoforms of *src*-related tyrosine kinases. The two *lyn* isoforms are compared with those encoded by the *hck* (21a), *fyn* (2), and c-*src* (19, 24, 30) genes. The approximate positions of supplementary amino acids (black boxes) or, in the case of *fyn*, alternative amino acids (cross-hatched boxes) are indicated. The c-*src* proteins containing 6- and 18-amino-acid insert are designated $p60^{c\text{-}src+}$ and $p60^{c\text{-}src++}$ respectively.

Regulation of the two *lyn* isoforms. The protein encoded by the *lyn*11 cDNA, $p56^{lyn}$, possesses a number of serine and threonine residues which are absent from the protein encoded by the *lyn*12 cDNA, $p53^{lyn}$. Serine and threonine residues in the N-terminal regions of $p56^{lck}$ and $pp60^{c\text{-}src}$ are known to become phosphorylated following treatment of cells with tetradecanoyl phorbol acetate (12, 22, 49). In both cases, this phosphorylation is believed to be carried out by protein kinase C. The functional consequences of phosphorylation of N-terminal serine and threonine residues is unclear, although tetradecanoyl phorbol acetate is known to promote the dissociation of $p56^{lck}$ from CD4 but not CD8 (14). In view of this, it seems plausible that phosphorylation of N-terminal residues may influence the ability of *src*-related kinases to associate with and transduce signals from cell surface molecules. It is therefore possible that the ability of $p53^{lyn}$ and $p56^{lyn}$ to complex with and transduce signals from membrane-associated molecules could be independently regulated by phosphorylation-dephosphorylation events.

Alternative isoforms of *src* family members. It is now apparent that some members of the *src* family exist in different isoforms (Fig. 7), although in most cases the functional significance of such variation remains to be established. Alternative splicing of the *fyn* and *src* mRNAs results in structural or functional alterations to the catalytic domains of the respective proteins. In the case of *fyn*, alterna-

tive splicing results in the substitution of one form of exon 7 for another, a substitution which affects the amino acid composition of the nucleotide binding site of the kinase (2). Neuronal cell-specific alternative splicing of c-*src* mRNAs results in the production of proteins which possess a 6- or 17-amino-acid insertion in their SH3 domains (24, 30) (Fig. 7). Interestingly, the protein with the 6-amino-acid insertion, src^+, has slightly elevated tyrosine kinase activity and is a more potent transforming agent than the prototypic form of c-*src* (18). In this context, it will be of interest to establish whether the two *lyn* isoforms differ in tyrosine kinase activity or transforming ability.

Recent experiments in our laboratory indicate that *hck*, which, like *lyn*, is expressed predominantly in cells of the B-lymphoid and myeloid lineages, exists as two isoforms that differ in the presence or absence of a 21-amino-acid N-terminal extension (21a; Fig. 7). The region of *lyn* affected by alternative splicing also lies in the N-terminal domain, which corresponds to the unique region of *src*-related proteins. This region is thought to mediate the interaction of the kinases with other cellular molecules and to provide the specificity whereby individual members of the *src* family of proteins can participate in distinct cellular processes. This hypothesis has gathered support following the demonstration that the physical association between CD4/CD8 and $p56^{lck}$ is mediated by the N-terminal 32 amino acids of $p56^{lck}$ and the cytoplasmic domain of the CD4 and CD8 (40). The formation of this complex has been shown to depend on two cysteine residues contained within the cytoplasmic tail of CD4/CD8 and a corresponding pair of cysteines in the N-terminal domain of $p56^{lck}$ (41, 46). If the N-terminal domain of *lyn* is involved in coupling this kinase with cell surface molecules, then the absence of corresponding cysteine residues in either *lyn* isoform would suggest that their engagement with cell surface receptors is likely to involve a mechanism distinct from that which underlies the association of *lck* with CD4/CD8.

Since the two *lyn* isoforms have structurally distinct N-terminal domains, it remains possible that each isoform is capable of associating with distinct cell surface receptors. However, the recent observations of Yamanashi et al. (54) suggest that in B cells at least, a small fraction of both *lyn* isoforms are associated with membrane-bound IgM. Whether $p56^{lyn}$ and $p53^{lyn}$ are associated with complexes involving a single receptor in other cell types which express high levels of *lyn*, but presumably lack immunoglobulin molecules, remains to be determined.

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