MOLECULAR AND CELLULAR BIOLOGY, May 1991, p. 2391–2398
0270-7306/91/052391-08$02.00/0

Vol. 11, No. 5

Hematopoietic Cells Express Two Forms of *lyn* Kinase Differing by 21 Amino Acids in the Amino Terminus

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Received 29 October 1990/Accepted 28 January 1991

cDNAs for the murine *lyn* protein tyrosine kinase gene were cloned from mouse bone marrow-derived monocytic cells. Comparison of the human and murine genes demonstrated a 94% homology in peptide sequence. Comparable to the human gene, murine *lyn* was found to be expressed in myeloid and B-lymphoid lineage cells. During the cloning, two types of cDNAs were obtained that differed by the presence (*lynA*) or absence (*lynB*) of 63 bp within the amino-terminal coding region of the gene. The genomic structure of the murine *lyn* gene demonstrates that the two types of *lyn* transcripts are derived from alternative splicing utilizing an internal splice donor site. Transcripts for both forms were found to be expressed in myeloid cells. *lyn*-specific antisera detected comparable levels of proteins of 56 and 53 kDa in hematopoietic cells. These 56- and 53-kDa proteins comigrated with proteins produced by in vitro translation or in vivo expression of the *lynA* and *lynB* cDNAs, respectively. The two forms had comparable in vitro kinase activities in immunoprecipitates and showed similar peptide patterns, with partial V8 digestion of the in vitro-phosphorylated proteins. The potential significance of the two *lyn* proteins is discussed.

Three families of protein tyrosine kinases have been identified (12). The transmembrane protein tyrosine kinases are receptors for a variety of known growth factors or are hypothesized to be receptors for unknown ligands. A second family consists of cytoplasmic protein tyrosine kinases that are similar in structure to the *abl* proto-oncogene. Lastly, there are a number of protein tyrosine kinases that share structural homology to the *src* proto-oncogene product, including the *src*, *lck*, *fgr*, *blk*, *fyn*, *hck*, *lyn*, and *yes* protein tyrosine kinases.

The *src* gene family kinases share extensive homologies in the catalytic domains (*src* homology domain 1 [SH1]) and in two additional domains (SH2 and SH3) that are immediately 5′ of catalytic domains (23). In contrast, the amino-terminal 40 to 80 residues show little homology among the gene family members and may contribute to the unique biological functions of each member. The *src* family kinases do not have transmembrane regions, although they associate with the cytoplasmic side of the plasma membrane through the amino terminus and this association is facilitated by myristylation of the amino terminus.

The role of various members of the *src* gene family in growth regulation or differentiation is largely unknown. A potential function for the *lck* gene in signal transduction in lymphocytes was initially postulated on the basis of its physical association with the membrane glycoproteins CD4 and CD8 (8, 27, 34). Consistent with this hypothesis, cross-linking of CD4 with antibodies activates the protein kinase activity of *lck* (35). The association of *lck* with CD4 or CD8 occurs through the interaction of the unique amino-terminal domain of *lck* with the cytoplasmic tail of CD4 or CD8 (29, 32).

The human *lyn* gene (*lck*/*yes*-related novel tyrosine kinase) was initially cloned from a placental cDNA library by using a v-*yes* probe (38). Among the members of the *src* gene family, the *lyn* gene is most similar to the *lck* gene. Unlike *lck*, which is expressed in T lymphocytes and natural killer cells and to a lesser degree in some B cells (20), the human *lyn* gene is expressed at the highest levels in macrophages, platelets, and B lymphocytes and at much lower levels in a variety of other cell lineages, including granulocytes, erythrocytes, and T lymphocytes (8, 39). The structure and pattern of expression of the murine *lyn* gene have not been reported. While isolating novel protein kinases expressed in murine hematopoietic cells, we obtained cDNA clones that were highly homologous to the human *lyn* gene and were likely to be the murine homolog. The results described here report the structure and pattern of expression of the murine *lyn* gene. We also demonstrate the existence of two forms of the protein that differ in the presence or absence of 21 amino acids in the amino-terminal domain, a region which has been shown to be required for the interaction of *lck* with CD4 or CD8.

MATERIALS AND METHODS

Cells and cell culture. Mouse monocytic cells were derived from 7-day-old cultures of mouse bone marrow cells in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS) and colony-stimulating factor 1 (kindly provided by M. Roussel, St. Jude Children's Research Hospital) at 20 U/ml as previously described (40). DA-3, NFS-60, and 32Dcl cells were grown in RPMI 1640 medium supplemented with murine interleukin-3 (IL-3) at 20 U/ml (14) and 10% FCS. DA-2 cells were grown in 10% FCS–RPMI 1640 medium (13). Human M-07 cells (2) were cultured in DMEM containing 20% FCS and recombinant human IL-3 at 20 U/ml (kindly provided by S. Clark, Genetics Institute). Cos-1 cells (11) and NIH 3T3 cells were grown in 10% FCS–DMEM. Other murine IL-3-dependent cells (13) were maintained routinely in RPMI 1640 medium containing 10% FCS and murine IL-3 at 20 U/ml.

PCR amplification of *lyn* cDNA and genomic sequences. The

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polymerase chain reaction (PCR) procedure described by Wilks (36) was used to obtain cDNA fragments for the conserved regions of the catalytic domain of kinases from mouse monocytic cells. With sequencing, one of the cDNA fragments was found to be highly homologous to the human *lyn* kinase gene. Oligonucleotides complementary to the murine *lyn* cDNA sequences were synthesized and used as primers to derive 5′ and 3′ cDNA sequences by an anchored PCR method (9). The cDNA fragments were cloned into M13 vector and sequenced by dideoxy-chain termination procedures (28). Four sets of oligonucleotides were used as primers to analyze the coding region of the *lyn* transcript: primer set A (CCTCGAGCGAGAAATATG, CACCATGC ATAGGGTCATAA), primer set B (GGAATTCCTTATCA GAGAAAGCG, GGATCCTCCTTGGTGACCAC), primer set C (CCCAAACCTCAGAAGCCATG, TGTCGACTAC GGCTGCTGCT), and primer set D (CCTCGAGCGAGAAA TATG, AGAATTCTCGAGTCTCCATGCCCAC). Double-stranded cDNA was derived from RNAs of different cells and tissues by using a cDNA synthesis kit (Amersham). The cDNA was then mixed with different primers separately and amplified by PCR under the following condition: 94°C, 2 min; 65°C, 2 min; and 75°C, 3 min for 30 cycles. PCR products were analyzed by electrophoresis in 2.5% agarose gel and detected by ethidium bromide staining. In addition, cDNAs of mouse monocytes were amplified by using primer set D, digested with restriction enzyme *Xho*I, cloned into Bluescript vector, and sequenced.

Three sets of primers were used for amplification of *lyn* genomic DNA: set 1 (CCTCGAGCGAGAAATATG, GGAG CTCTCACATAAATAGTTC), set 2 (AGAGCTCCAACG TCCAATAAAC, GAAGCTTTTGTTTGAAATCTCT), and set 3 (CCTCGAGCGAGAAATATAG, GAAGCTTTTGTT TGAAATCTCT). *lyn* genomic DNA was amplified from NIH 3T3 fibroblast DNA at 92°C, 1 min; 60°C, 1 min; and 72°C, 1 min for 25 cycles. The amplified DNA was cloned and sequenced as described above.

Expression of *lyn* transcript in hematopoietic cells. Poly(A)$^+$ RNA and whole cell RNA were isolated from various cells by previously published procedures (40). Poly(A)$^+$ RNAs of different mouse tissues were from Clontech Laboratory, Inc. For each sample, 20 μg of whole cell RNA or 2 μg of poly(A)$^+$ RNA was separated in a 1% agarose-formaldehyde gel and blotted to nitrocellulose (40). Northern (RNA) hybridization was performed by using a nick translation-labeled cDNA fragment (1.1 kb) from the 3′ noncoding region of the *lyn* cDNA clone as a probe.

Characterization of *lyn* protein tyrosine kinases. *lynA* and *lynB* cDNAs were derived from PCR amplification with primer set D from RNA of mouse bone marrow-derived monocytic cells and were cloned into the Bluescript vector. *lynA* and *lynB* transcripts were generated by using a transcription kit (Stratagene) according to the manufacturer's procedure. The transcripts were then translated in rabbit reticulocyte lysate (Stratagene) in the present of 10 μCi of [^{35}S]methionine (DuPont). Translated proteins were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (17). *lynA* and *lynB* cDNAs were also inserted into pBC12 vector (7). The pBC12/*lynA* and pBC12/*lynB* plasmids were then transfected into Cos-1 cells separately (4). The transfected cells were harvested at 72 h after the transfection and used for immunoprecipitation and the in vitro kinase assay.

For ^{35}S labeling of cellular proteins, mouse bone marrow-derived monocytic cells, NFS-60 cells, DA-2 cells, and M-07 cells (10^7 cells) were washed in methionine-free DMEM and then incubated separately in 1 ml of methionine-free medium containing 10% FCS and 200 μCi of [^{35}S]methionine for 6 h at 37°C.

For immunoprecipitation, cells were washed in cold phosphate-buffered saline (PBS) once and lysed in extraction buffer (10 mM Tris, 5 mM EDTA, 50 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride) (10^7 cells per ml) on ice for 20 min. Cell lysates were incubated with normal rabbit serum or rabbit polyclonal serum directed against a synthetic peptide corresponding to residues 44 to 63 of human p56^{lyn} (38), and the immunocomplexes were collected with protein A-agarose (Bethesda Research Laboratories) and analyzed by SDS-PAGE. Immunoprecipitates were also treated with calf intestinal alkaline phosphatase (Boehringer) and potato acid phosphatase (Boehringer) as described previously (22). In the in vitro kinase assay (27), PBS-washed cells were lysed in 2% Nonidet P-40 and TEN (50 mM Tris-HCl [pH 7.4], 1 mM EDTA, 0.15 M NaCl). Immunoprecipitated proteins were suspended in 10 μl of kinase buffer containing 50 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES; pH 7.0), 1 mM MnCl, 1 mM Na_3VO_4, and 10 μCi of [γ-^{32}P]ATP) and incubated at room temperature for 20 min. The kinase reactions were analyzed by SDS-PAGE.

For partial V8 protease digestion (5), proteins were labeled with ^{32}P in the in vitro kinase assay and then were separated by SDS-PAGE. The proteins were subsequently eluted from the gel in elution buffer (0.125 M Tris [pH 6.8], 0.15% SDS, 1 mM EDTA). Following incubation in the presence of V8 protease (Boehringer) at 30°C for 30 min, the resulting peptides were separated on a 15% SDS-polyacrylamide gel. ^{35}S-labeled proteins and ^{32}P-labeled proteins in SDS-polyacrylamide gels were detected by fluorography and autoradiography, respectively.

Nucleotide sequence accession number. The murine *lynA* and *lynB* cDNA sequences are in the GenBank data base under accession numbers M57696 and M57697, respectively.

RESULTS

Cloning and sequence analysis of murine *lyn* cDNAs. To identify novel protein tyrosine kinases in murine hematopoietic cells, mixed primers complementary to the highly conserved regions of the catalytic domain of kinases were used in PCR reactions to obtain cDNA clones (36). One clone (TY18) encoded a peptide sequence that was highly homologous to the human *lyn* protein tyrosine kinase (38). By using an anchored PCR procedure (9), overlapping cDNA fragments for the 3′ and 5′ regions were cloned and sequenced. Additional cDNA clones were obtained by screening of cDNA libraries with PCR-derived cDNA fragments.

The sequence of 2.7 kb of the murine *lyn* cDNA, deduced from a compilation of the cDNA clones, is shown in Fig. 1. The sequence contains a single long open reading frame (ORF) of 1,536 bp. The first ATG of the ORF has flanking sequences that conform to the Kozak consensus for a translation initiation site (16). The ORF encoded a protein 512 amino acid residues that contained all of the conserved domains found in the *src* gene family kinases (12), including (i) a tyrosine kinase catalytic domain with an ATP-binding motif of G-X-G-X-X-G and an autophosphorylation site (Y-397); (ii) SH2 and SH3 domains in the amino-terminal region linked to the catalytic domain by a short hinge region; (iii) a carboxyl-terminal tyrosine phosphorylation site (Q-Y-Q-Q-Q) involved in regulation of kinase activity; and (iv) an amino-terminal site for myristylation (G-2–X–X–X–X–K-7).

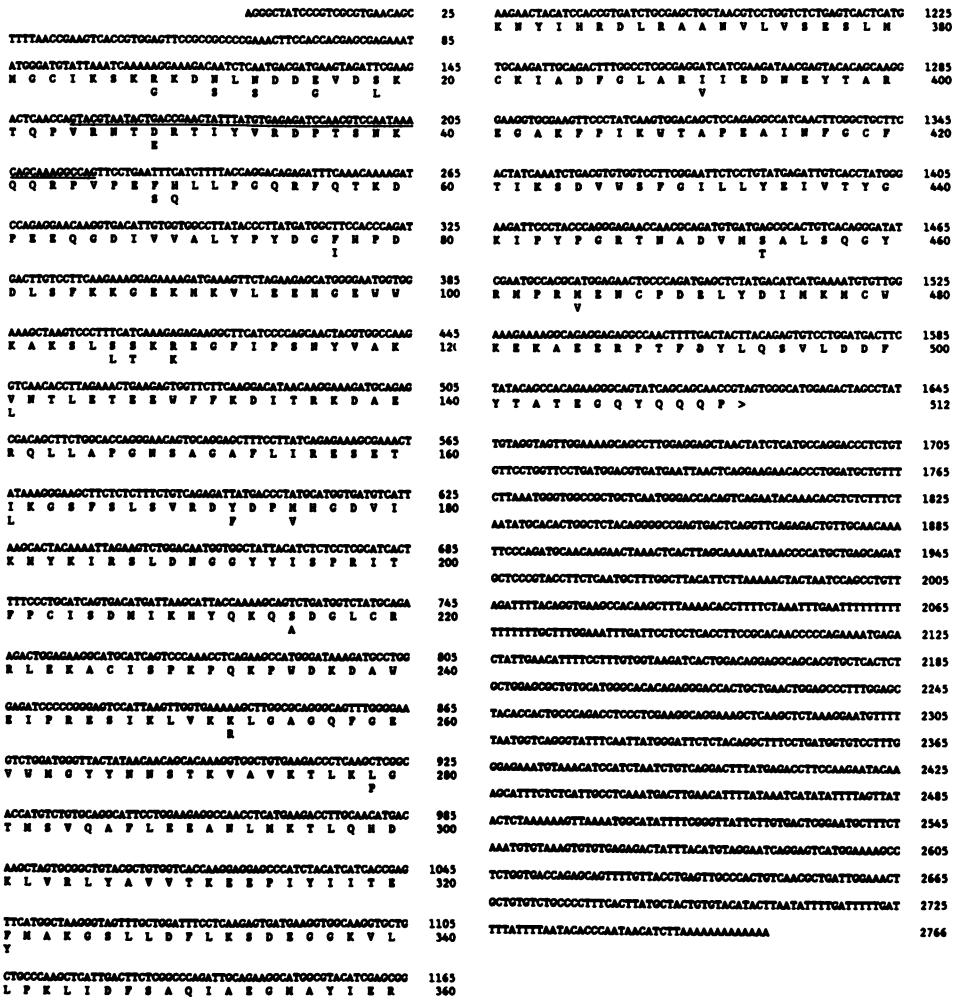

FIG. 1. Nucleotide sequence and predicted amino acid sequence of the 2.7-kb cDNA clone of the murine *lyn* protein tyrosine kinase gene. The predicted translation initiation codon is located at position 86, and the ORF region is stopped at position 1624. A 63-nucleotide region in the 5′ sequence is underlined; this region is absent in a second type of *lyn* cDNA (see below). The amino acid substitutions found in the human gene are shown below the murine sequence.

A comparison of the sequence with those available in data bases demonstrated 95.5% amino acid and 89% nucleotide homology with the human *lyn* gene (38). The amino-terminal region (amino acids 1 to 80) was highly conserved (89% homology) between the murine and human *lyn* genes.

***lyn* expression in murine hematopoietic cells.** Using the murine *lyn* cDNA as a probe for Northern hybridization, we examined *lyn* expression in a variety of cells and tissues (Fig. 2). Among 22 hematopoietic cells lines, a transcript of approximately 3.0 kb was detected in all myeloid and B-lymphoid cells (DA-8, NFS-70, and NFS-112) at comparable levels. A 3.0-kb transcript was also detected in murine macrophages (BMX) at levels that were approximately seven times higher than that observed in other hematopoietic cells. No or very low levels of transcripts were detected in a T-lymphoid cell (DA-2) or in NIH 3T3 fibroblasts. This pattern of expression is similar to that reported for the human *lyn* gene (8, 39). Northern hybridization of various murine tissues is shown in Fig. 2C. *lyn* transcripts were readily detected in RNAs from a variety of tissues, including mouse brain, heart, ovary, testes, muscle, and spleen, but were not detected in lung and liver.

lyn transcripts were detected in the myeloid cell line 32Dcl (Fig. 2B). These cells require IL-3 for growth and will terminally differentiate to neutrophilic granulocytes in the presence of granulocyte colony-stimulating factor (26). There were no changes in the levels of *lyn* expression following the removal or readdition of IL-3 or during differentiation (data not shown).

Two types of *lyn* transcript differ in their amino-terminal coding sequences. While cloning the 5′ region of murine *lyn* transcript, we obtained evidence for the existence of two size classes of transcripts. To characterize these, four sets of *lyn*-specific primers, complementary to different regions of the *lyn* cDNA sequence, were used for PCR amplification of cDNA (Fig. 3A). PCR amplification of *lyn* sequences from macrophage cDNAs by using primer sets B and C resulted in the expected production of single amplified fragments of approximately 480 and 840 bp, respectively. (Fig. 3B). However, with primer set A, a fragment of 480 bp was consistently detected in addition to the expected fragment of approximately 540 bp (Fig. 3B), indicating the presence of a second transcript that lacked approximately 60 bp in coding sequence.

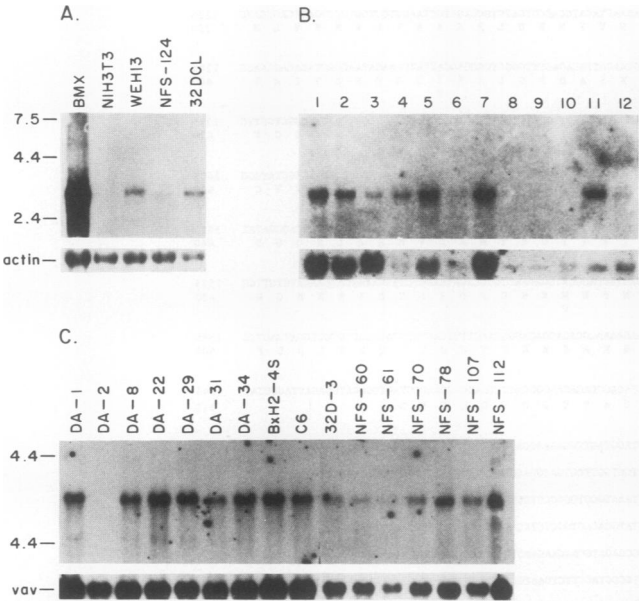

FIG. 2. Detection of *lyn* expression in murine hematopoietic cells and various tissues by Northern hybridization. (A and C) Total cellular RNA (10 μg of each sample) was analyzed. (B) Poly(A)$^+$ RNA (2 μg of each sample) was analyzed. Samples in lanes 1 to 12, respectively, were oviduct, ovary, testis, skeletal muscle, intestine, stomach, kidney, spleen, lung, liver, heart, and brain. The blots were hybridized to a ^{32}P-labeled 1.1-kb cDNA fragment of the 3′ noncoding region of the murine *lyn* cDNA. The blot were rehybridized to an actin gene probe (A), a *vav* gene probe (B) (15), or a human elongation factor probe (C).

The presence of two classes of transcripts was also indicated when 1.5-kb cDNA clones, obtained by amplification with primer set D, were examined. Digestion of these cDNA clones with *Hin*dIII yielded a fragment of 440 bp in addition to the expected three fragments of approximately 500, 270, and 800 bp; *Hin*dIII digestion of cDNA clones amplified with primer set A resulted in the same two fragments of 440 and 500 bp (Fig. 3C).

cDNA clones for both classes of transcripts were cloned from the amplified products by using primer set D and were sequenced. The sequences of the larger cDNA clones (*lynA*) were identical to the sequence in Fig. 1. The smaller cDNA clones (*lynB*) were found to differ only in the absence of a 63-bp sequence that encodes 21 amino acids (amino acids 24 to 44) (Fig. 1 and 4). This region contains no known protein motifs and no homology to other protein sequences.

To establish the origin of the two transcripts, oligonucleotides flanking and within the 63-bp sequence were used in PCR with genomic DNA. When oligonucleotides from the 5′ region and within the 63-bp sequence were used, a product of approximately 100 bp was obtained, as expected if these sequences are contiguous in genomic DNA (Fig. 5). In contrast, when oligonucleotides from either the 5′ region or within the 63-bp sequence were used in combination with 3′ sequences, a PCR product of approximately 4.5 kb was observed. These results suggested that a 4.5-kb intron separated the 5′ and 63-bp primers from the 3′ primer. To further establish this, we cloned and sequenced portions of the PCR products. The sequences (Fig. 5C) demonstrated that in genomic DNA, the 63-bp sequence was contiguous with the 5′ cDNA sequences and diverged from 3′ cDNA sequences. Importantly, splice donor sites bounded both the 5′ and 3′ junctions of the 63-bp sequence, and a splice acceptor site was located approximately 4.5 kb downstream and adjacent to the cDNA sequences 3′ of the 63-bp region. Taken together, the results demonstrate that the two *lyn* transcripts are derived from alternative splicing utilizing an internal splice donor site.

To determine whether both transcripts are commonly expressed, PCR approaches were used to characterize the transcripts present in the myeloid cell lines DA-3, NFS-60, and 32Dcl. Both forms were found in all myeloid cell lines examined but were not expressed in a T-lymphoid cell line (DA-2) (Fig. 3D). The two transcripts were also readily detected in RNAs of brain, heart, and spleen but not in RNAs of lung and liver.

Two *lyn* proteins of 56 and 53 kDa are expressed in hematopoietic cells. The sequences of the cDNA clones indicated the potential presence of two *lyn* proteins of 56 and 53 kDa. We therefore used *lyn*-specific antisera in immunoprecipitation experiments to characterize the *lyn* protein that is expressed in hematopoietic cells. Two *lyn*-specific proteins of approximately 56 and 53 kDa were detected from mouse bone marrow-derived monocytes and were present at approximately equal intensities (Fig. 6A). These proteins comigrated in SDS-PAGE with in vitro-translated proteins derived from RNAs made from cDNA clones for *lynA* and *lynB* (Fig. 6C), suggesting that they were derived from the respective transcripts. Phosphatase treatment did not affect the mobilities of these proteins (Fig. 6B). Comparable proteins were immunoprecipitated from NFS-60 and myeloid cells but not from a T-cell line (DA-2) (Fig. 6D). Two *lyn* proteins were also immunoprecipitated with *lyn*-specific antisera from a human hematopoietic cell line (M-07), suggesting that the human gene may also give rise to two transcripts.

To characterize tyrosine kinase activity of the two *lyn* proteins, the *lynA* and *lynB* cDNA clones were expressed in Cos-1 cells. The proteins were immunoprecipitated and were compared in in vitro kinase assays with the proteins immunoprecipitated from monocytes. Both the 56- and 53-kDa proteins from the Cos-1 cells had kinase activity and readily autophosphorylated the respective proteins (Fig. 7A). In addition, both the 56- and 53-kDa proteins from monocytes were phosphorylated in immunoprecipitates. Examination of the phosphoamino acid contents of these phosphoproteins showed that only tyrosine residues were phosphorylated (data not shown). Importantly, the 56- and 53-kDa phosphoproteins from monocytes comigrated with the in vivo-expressed *lynA* and *lynB* proteins, respectively.

To determine whether phosphorylation occurred on comparable residues in the 56- and 53-kDa proteins, partial V8 protease digestions were done with in vitro-phosphorylated proteins from immunoprecipitates of monocytes and Cos-1 cells expressing *lynA* or *lynB*. The 56-kDa phosphoprotein from immunoprecipitates of monocytes had a digestion pattern that was identical to that of the 56-kDa phosphoprotein from Cos-1 cells expressing *lynA* (Fig. 7). Similarly, the digestion patterns were identical for the 53-kDa phosphoproteins from monocytes or Cos-1 cells expressing *lynB*. Moreover, the V8 digestions of the 56- and 53-kDa phosphoproteins were very similar except for the slowest-migrating band, which represents undigested protein, and an 8-kDa fragment that is seen only in digestions of the 56-kDa phosphoprotein. The possible significance of this minor difference is discussed below. Nevertheless, these data demonstrate that the two proteins are comparably phosphor-

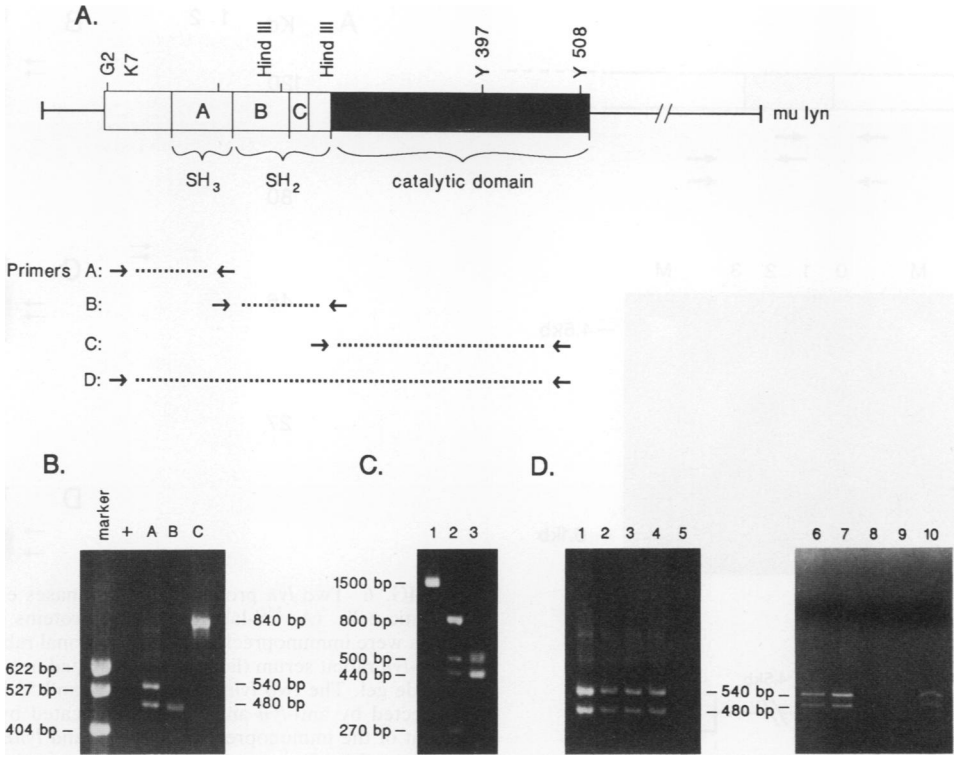

FIG. 3. PCR amplification of two types of *lyn* transcripts in mouse monocytic cells. (A) Four sets of oligonucleotides complementary to the different regions of the murine *lyn* cDNA sequence were used in PCR amplification. (B) PCR products of monocyte cDNA amplified with primer set A (lane A), primer set B (lane B), and primer set C (lane C) were electrophoresed in a 2.5% agarose gel and detected by ethidium bromide staining. Lane +, PCR product of monocyte cDNA alone without primers. (C) The PCR products of monocyte cDNA amplified with primer set D were electrophoresed prior to (lane 1) and after (lane 2) digestion with restriction enzyme *Hin*dIII. Lane 3, Primer set A amplified cDNA digested with *Hin*dIII. (D) Primer set A amplified cDNAs from mouse monocytes (lane 1), DA-3 cells (lane 2), 32Dcl cells (lane 3), NFS-60 cells (lane 4), DA-2 cells (lane 5), mouse brain (lane 6), heart (lane 7), liver (lane 8), lung (lane 9), and spleen (lane 10).

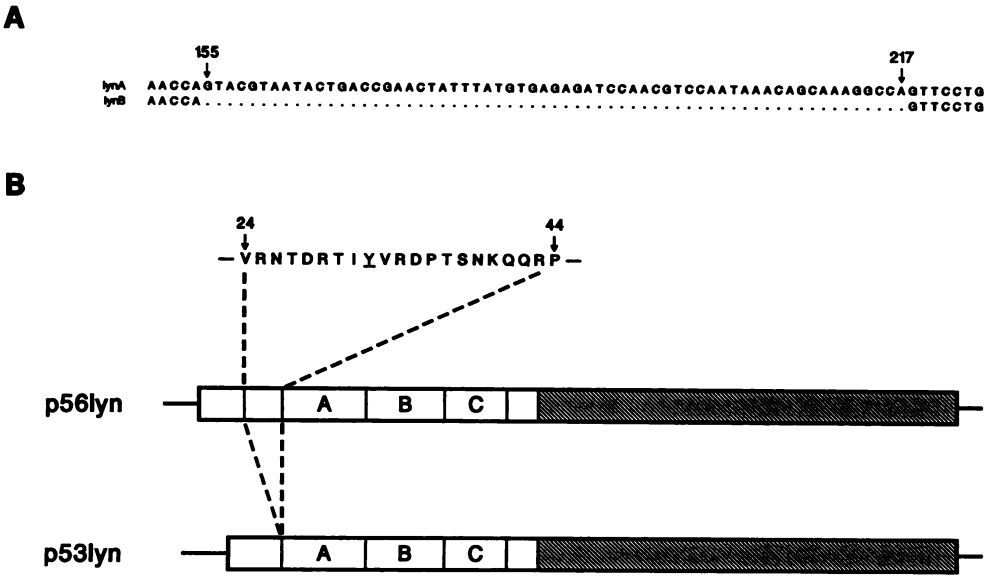

FIG. 4. Sequences of cDNA clones for *lynA* and *lynB* transcripts. Two types of cDNA clones were detected from the PCR products of monocytes obtained by using primer set D. The nucleotide sequences of the *lynA* and *lynB* cDNAs were aligned, and the regions different between them are shown (A). The 63-nucleotide sequence unique for *lynA* encodes a region of 21 amino acid residues located in the amino terminus of the *lyn* protein tyrosine kinase (B).

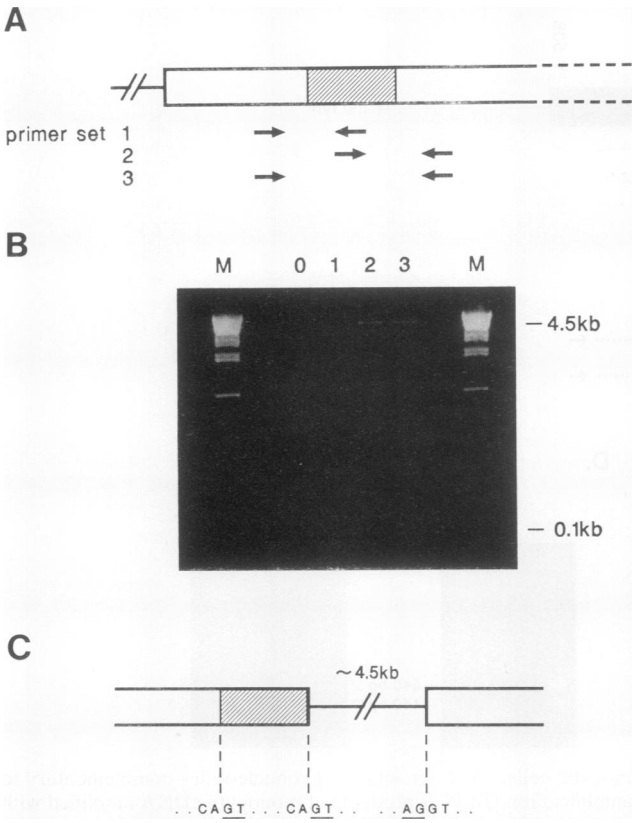

FIG. 5. Genomic structure of the murine *lyn* gene around the 63-bp sequence. Three sets of oligonucleotides (A) complementary to the *lynA* cDNA sequence flanking and within the 63-bp insertion were used in PCR to derive the corresponding genomic sequence of the murine *lyn* gene. The PCR products were separated in 1.5% agarose gels and detected by ethidium bromide staining (B). Lanes: M, DNA size markers; 0, PCR without primers; 1, 2, and 3, PCR with primer sets 1, 2, and 3, respectively. The PCR products were subsequently cloned, and regions were sequenced by using the primers shown in panel A. (C) Organization of the genomic structure and sequences at the relevant boundaries.

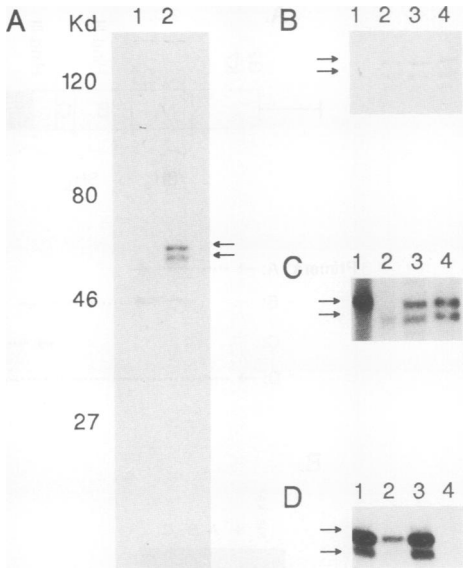

FIG. 6. Two *lyn* protein tyrosine kinases expressed in hematopoietic cells. (A) ^{35}S-labeled cellular proteins of mouse monocytic cells were immunoprecipitated with normal rabbit serum (lane 1) or anti-*lyn* rabbit serum (lane 2) and analyzed in a 10% SDS-polyacrylamide gel. The two *lyn* proteins of 56 and 53 kDa (*lynA* and *lynB*) detected by anti-*lyn* antibody are indicated by arrows. (B) Treatment of the immunoprecipitated *lynA* and *lynB* with alkaline phosphatase (lane 3) or potato acid phosphatase (lane 4) did not change the relative mobilities of the two proteins in comparison with the untreated *lyn* proteins (lane 2). Lane 1 is normal rabbit serum immunoprecipitation. (C) In vitro transcription and translation of *lynA* (lane 1) and *lynB* (lane 2) cDNA clones resulted 56- and 53-kDa proteins, respectively. The *lynA* and *lynB* proteins comigrated with the 56- and 53-kDa *lyn* proteins immunoprecipitated from mouse monocytes (lane 3) and the two *lyn* proteins labeled by ^{32}P in an in vitro kinase assay (lane 4). (D) Anti-*lyn* antibody immunoprecipitated proteins from mouse monocytes (lane 1), NFS-60 cells (lane 2), M-07 cells (lane 3), and DA-2 cells (lane 4).

ylated and further demonstrate that the 56- and 53-kDa proteins in monocytes are derived from *lynA* and *lynB*.

DISCUSSION

The sequence of murine *lyn* kinase has the typical features of *src*-related protein tyrosine kinases and is highly homologous to the human *lyn* gene protein, including the amino-terminal sequences. The murine *lyn* gene is expressed in myeloid cells and B cells, consistent with the pattern of expression of the human gene (39). The murine *lyn* gene is also expressed in a variety of other tissues. The expression pattern of the *lyn* protein in hematopoietic cells differs from that of other *src*-related protein tyrosine kinases that are expressed in hematopoietic cells (24). In particular, *lck* is expressed in T-lymphoid cells and natural killer cells and to a lesser degree in some B cells (8), *blk* is expressed in B-lymphoid cells (20), *fgr* is expressed in normal monocytic cells and granulocytes (37), and *hck* is expressed in monocytes and granulocytes and at low levels in some B cells (41). The expression pattern of *lyn* protein tyrosine kinase gene in hematopoietic cells suggests that *lyn* kinase may participate in a signal transduction pathway common to myeloid cells and B-lymphoid cells. In this regard, it is interesting to note that several membrane molecules are expressed in a similar pattern on hematopoietic cells, including the receptors for complement components and the Fc portion of immunoglobulin molecules (31, 33). Cross-linking of these cell surface molecules induces rapid tyrosine phosphorylation of several cellular proteins (31), and preliminary data indicate that cross-linking of immunoglobulin G Fc receptor leads to an increase of *lyn* kinase activity (39a).

Our data demonstrate that there exist two forms of the *lyn* kinase of 56 and 53 kDa. In particular, we have isolated murine cDNA clones containing a 63-bp deletion and have shown that transcripts for both forms exist in the cells examined. Sequencing of genomic DNA demonstrates that the two transcripts are derived from alternative splicing utilizing an internal splice donor site. Since the human *lyn* cDNA is highly homologous to the murine *lyn* sequence and is identical at the 5′ and 3′ sites of the deletion, it is likely that the human gene also undergoes alternative splicing to produce a smaller transcript. These transcripts may give rise to the 56- and 53-kDa *lyn* proteins detected in our studies in M-07 cells and other human cells as previously described (39).

A variety of alternative splicing patterns have been dem-

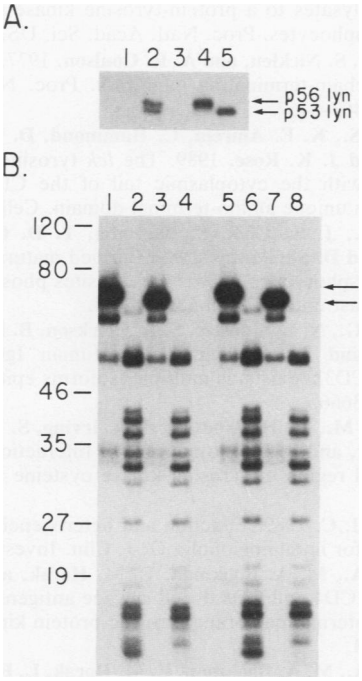

FIG. 7. In vitro kinase activities and *Staphylococcus aureus* V8 protease analysis of *lyn* protein tyrosine kinases. (A) Kinase activities of *lyn* proteins immunoprecipitated with anti-*lyn* antibody from mouse monocytes (lane 2) and Cos-1 cells transfected with pBC12/*lynA* (lane 4) or pBC12/*lynB* (lane 5) were examined in an in vitro kinase assay. Lanes 1 and 3 contain proteins precipitated with normal rabbit serum from mouse monocytes and pBC12/*lynA*-transfected Cos-1 cells, respectively. (B) V8 peptide analysis of the *lyn* proteins ^{32}P labeled in an in vitro kinase assay. The proteins were digested with V8 protease at 2.5 μg/ml (lane 1, 3, 5, and 7) or 1 mg/ml (lane 2, 4, 6, and 8). Lanes: 1 and 2, 56-kDa *lyn* protein; 3 and 4, 53-kDa *lyn* protein; 5 and 6, *lynA* protein; 7 and 8, *lynB* protein.

onstrated in genes which result in altered transcripts and protein isoforms (3). Among the *src* gene family members, various alternatively spliced transcripts have been described. In c-*src*, neuronal cell-specific exons (NI and NII) have been identified between exons 3 and 4. Incorporation of NI introduces 6 amino acids, while incorporation of both additional exons introduces 17 amino acids (18, 21, 25). In hematopoietic cells, c-*fyn* contains an alternative exon 7 which encodes an altered ATP-binding site (6). Lastly, two unique promoters give rise to c-*lck* transcripts that contain alternative 5′ noncoding exons (1, 10).

The expression of *lynA* and *lynB* in Cos-1 cells resulted in proteins of 56 and 53 kDa that were specifically immunoprecipitated with antisera against *lyn* and comigrated with the proteins that were immunoprecipitated from monocytes. Both proteins were phosphorylated on tyrosine in kinase assays with immunoprecipitates from either monocytes or Cos-1 cells expressing *lynA* or *lynB*. Lastly, partial V8 protease digestion patterns were very similar for all of the proteins, demonstrating their identity and indicating that phosphorylation occurred on comparable sites in immunoprecipitates. The one exception was a small fragment that was seen only in digests of the 56-kDa *lynA* protein. This could arise from a peptide that comes from the region that is present in *lynA* and absent in *lynB* since it contains a tyrosine residue. In vivo tyrosine phosphorylation of two residues in the amino-terminal half the *lck* protein tyrosine kinase has been shown (19). Experiments are currently in progress to map the autophosphorylation sites in the *lyn* kinases.

In the cells examined, approximately equal amounts of the 56- and 53-kDa *lyn* proteins were detected, suggesting a physiological significance. In this regard it is important to note that the deleted region corresponds to regions of the *src* kinase that may be involved in regulation or function. For example, in *src* kinase this region contains sites of phosphorylation by *cdc2* kinase during the cell cycle (30). Most importantly, the deletion removes 21 amino acids from a region that has been implicated in the interaction of *lck* with CD4 or CD8 (29, 32). Thus, it is possible that the two forms of *lyn* associate with different signal-transducing complexes in hematopoietic cells.

ACKNOWLEDGMENTS

This work was supported in part by National Cancer Institute Cancer Center Support (CORE) grant P30 CA21765, grant R01 DK42932 from the National Institute of Diabetes and Digestive and Kidney Diseases, and the American Lebanese Syrian Associated Charities. T. Yi is a Journey Fellow of St. Jude Children's Research Hospital.

We thank M. Roussel and J. Downing (Department of Tumor Cell Biology, St. Jude Children's Research Hospital) for providing colony-stimulating factor 1 and for helping in analysis of phosphoproteins. We thank S. Clark (Genetics Institute) for providing recombinant human IL-3 and J. Summer (Department of Cell Biology, University of New Mexico) for the pBC12 vector. We also thank J. Cleveland, D. Askew, and W. Lumm (Department of Biochemistry, St. Jude Children's Research Hospital) for help in cell culture and Northern hybridization experiments.

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