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Structure of the Murine *lck* Gene and Its Rearrangement in a Murine Lymphoma Cell Line

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The *lck* gene encodes a lymphocyte-specific protein-tyrosine kinase that is implicated in neoplastic transformation. We have determined the germ line organization of the murine *lck* gene and have isolated and characterized a rearranged *lck* allele in the murine lymphoma cell line LSTRA. The overall exon-intron organization of the normal *lck* gene is almost identical to that of avian c-*src*. In LSTRA DNA, an internally rearranged Moloney murine leukemia virus genome is interposed between two distinct promoters that normally generate *lck* transcripts differing only in 5′ untranslated regions. The rearrangement appears to have been selected to permit splicing of transcripts that initiate from the Moloney virus promoter to an acceptor site located within the first exon 3′ to the downstream promoter, thus generating an *lck* mRNA with a novel 5′ untranslated region that may be more efficiently translated.

Protein-tyrosine kinases are implicated in the control of cell growth and differentiation. Although originally identified as products of retroviral oncogenes (4, 33), this class of molecules now includes a large family of transmembrane growth factor receptors (7, 9, 26, 32) and a more enigmatic set of membrane-associated enzymes which are regulated by unknown stimuli. Within this latter group, a family of seven protein-tyrosine kinases has been defined on the basis of their relationship to the product ($p60^{c\text{-}src}$) of the c-*src* proto-oncogene. The *src*-like protein-tyrosine kinases range between 505 and 543 amino acids in length, and each contains a closely conserved carboxy-terminal kinase domain of about 300 amino acids joined to an extremely variable amino-terminal region that is about 70 amino acids long (38; J. A. Cooper, *in* B. Kemp and P. F. Alewood, ed., *Peptides and Protein Phosphorylation*, in press). Since much of the heterogeneity within the *src* family is confined to this region, it is believed that features of this amino-terminal portion confer unique physiological properties upon each of these kinases (18, 31, 38).

Additional evidence supporting the functional specialization of *src*-like protein-tyrosine kinases has emerged with the observation that many are expressed in a cell type-restricted fashion. For example, in mouse and human cells, the product of the *lck* gene ($p56^{lck}$) normally accumulates only in cells of the lymphoid lineage (18; R. M. Perlmutter, J. D. Marth, D. B. Lewis, R. Peet, S. F. Ziegler, and C. B. Wilson, J. Cell. Biochem., in press). The *lck* gene is predominantly expressed in thymocytes and in resting T cells and at lower levels in some B-cell lines (18). Furthermore, the *lck* gene is specifically implicated in the pathogenesis of lymphoid malignancy by virtue of its rearrangement and overexpression in two independently obtained murine lymphoma cell lines, LSTRA and Thy-19 (6, 18, 37), and by its localization at a site of frequent chromosomal abnormalities (lp32-35) in human non-Hodgkin lymphomas (15). The gene is also overexpressed in some human colon and lung tumors (34).

To better understand the regulation of *lck* gene expression, we have pursued a detailed analysis of the structure of the normal murine *lck* gene and of its upstream regulatory sequences. We report here that the murine *lck* gene shares identical exon structure with the avian c-*src* gene throughout all but the most 5′ portion. Interestingly, two forms of *lck* transcripts, differing only in their 5′ untranslated regions, are normally synthesized in lymphoid cells through the action of two distinct promoter structures. We have defined the 3′ promoter for the murine *lck* gene and have identified sequences spanning 500 base pairs (bp) in the region of this promoter that are closely conserved in the human *lck* gene. We also demonstrate that in LSTRA cells, Moloney murine leukemia virus (MoMuLV) sequences have intercalated between the two promoter elements, permitting the generation of MoMuLV long terminal repeat-*lck* fusion transcripts by using a normal splice acceptor site. These observations help to provide a molecular explanation for the overexpression of $p56^{lck}$ in LSTRA cells.

MATERIALS AND METHODS

Genomic libraries and library screening. A BALB/c mouse genomic library constructed by partial digestion of sperm DNA with *Alu*I and *Hae*III has been previously described (22). A library of LSTRA DNA was constructed by *Mbo*I partial digestion followed by fractionation of 15- to 20-kilobase (kb) fragments on sucrose density gradients (14), ligation into EMBL3 bacteriophage arms, and packaging by standard techniques (11).

The LSTRA library was screened with a full-length murine *lck* cDNA probe, NT18 (18). The BALB/c library was screened with a partial-length cDNA (T9) that contained 900 bp of *lck* sequence, including most of the kinase domain (18). Plating of the phage and subsequent screening by hybridization, isolation, and large-scale culture were performed as previously described (3, 14).

Human genomic clones were isolated from a cosmid library constructed with human peripheral blood leukocyte DNA from a single female donor. The DNA was partially digested with *Mbo*I, and 40- to 50-kb fragments were purified by sucrose gradient fractionation. These were then ligated into the *Bgl*II site of the vector pTL-5, and the resulting cosmids were propagated and screened by hybridization with the NT18 probe by established procedures (29).

Cell lines and nucleic acid isolation. The cell lines Jurkat,

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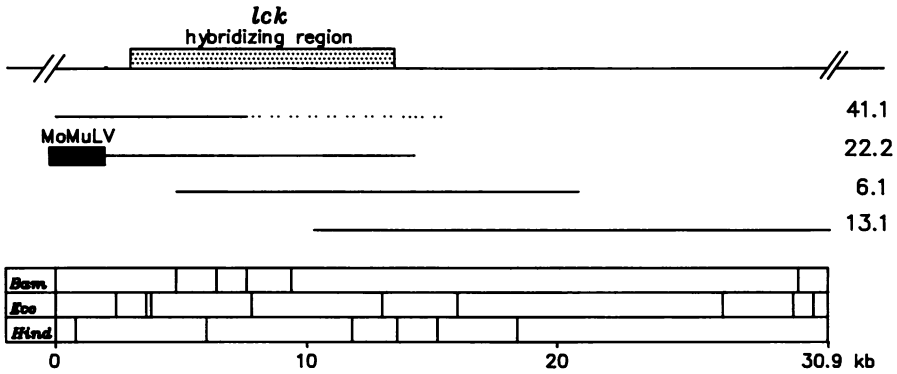

FIG. 1. Restriction map of the murine *lck* locus. Clone designations are given on the right. Clone 41.1 contains a tandem ligation event such that the 3′ end contains unrelated sequence (dotted line). Clone 22.2 contains the MoMuLV rearrangement present in LSTRA cells. Restriction enzymes are indicated as follows: *Bam*, *Bam*HI; *Eco*, *Eco*RI; *Hind*, *Hin*dIII.

LSTRA, SL-3, and YAC-1 were maintained in RPMI 1640 as previously described (18). Cells were harvested at a density of 1.5×10^6 cells per ml. Total RNA, isolated by the guanidine thiocyanate method, was passed over oligo(dT)-cellulose to obtain poly(A)$^+$ RNA (1). DNA from LSTRA cells was isolated by standard methods (14).

DNA sequencing. Appropriate DNA fragments isolated from phage and cosmid clones were subcloned into either pUC18, pUC19, or M13mp8 vectors (Pharmacia, Inc., Piscataway, N.J.) and sequenced by the dideoxy-chain termination method with specific oligomer primers (24). Oligonucleotides were synthesized with a DNA synthesizer (Applied Biosystems, Inc., Foster City, Calif.).

S1 mapping and primer extension. The transcription start site of *lck* was mapped by using a 1.1-kb *Eco*RI-*Eco*RI genomic subclone in pUC18 that included the 5′ untranslated region of the *lck* transcript. The plasmid was linearized at an internal *Stu*I site, treated with calf intestinal phosphatase, and digested with *Exo*III. A 200-ng portion of this probe was labeled to a specific activity of 10^7 cpm/pmol with T4 polynucleotide kinase (14). Poly(A)$^+$ RNA (4 μg) was incubated with 3×10^4 cpm of probe in a 60-μl reaction mix containing 50% formamide, 0.3 M NaCl, 10 mM Tris (pH 7.5), and 2 mM EDTA. After hybridization for 12 h at 42°C, the nucleic acids were ethanol precipitated and treated with S1 nuclease as previously described (25). Samples were boiled and subjected to electrophoresis on a 5% polyacrylamide gel, which was then dried prior to autoradiography.

Primer extension was performed by using oligonucleotide primers complementary to either the 5′ untranslated region or the coding region of the *lck* mRNA. Labeled primer (10^5 cpm) was annealed with 4 μg of poly(A)$^+$ RNA and extended with reverse transcriptase (Life Sciences, Inc., St. Petersburg, Fla.) as previously described (23). The primer extension products were separated by electrophoresis on a fully denaturing 8% polyacrylamide sequencing gel. A set of dideoxy sequencing reactions was run alongside to permit single-base resolution of the extension reaction.

RNA blot analysis. RNA blots were performed as previously described (18, 28) with 10 μg of total RNA isolated from Jurkat cells and from human thymus tissue (a kind gift of Christopher B. Wilson) obtained from a 5-year-old child during thoracic surgery.

RESULTS

Structure of the *lck* gene. To characterize the organization of the murine *lck* gene, genomic libraries generated with either BALB/c sperm DNA or LSTRA cell DNA were screened using the *lck* cDNA as a probe. Figure 1 presents a restriction map of the murine *lck* locus deduced from the four overlapping genomic clones that were isolated. These clones spanned 32 kb of germ line DNA and included the entire sequence of the *lck* transcript previously defined from the NT18 cDNA (18). Details of the map were confirmed by comparison with genomic blots (18) (not shown). Clone 41.1, derived from the BALB/c library, contained a tandem ligation artifact caused by the joining of two *Hae*III-*Alu*I fragments; hence, only the 5′ portion of the *lck* locus was present in this insert. As discussed below, clone 22.2, derived from the LSTRA library, was rearranged at its 5′ end compared with clone 41.1.

Appropriate fragments of the *lck* gene were subcloned and sequenced to define the exon organization. A complete map of the *lck* gene, indicating exon size and position, is presented in Fig. 2. In all, the transcribed sequences defined with the NT18 cDNA spanned 10.5 kb and were encoded in 12 exons.

***lck* is a member of the *src* gene family.** DNA sequences across 14 of the exon-intron junctions of *lck* were compared with the analogous regions from the avian c-*src* gene (30). Exons 3 through 12 were nearly identical between *lck* and *src* in both length and positions of the splice sites within codons, with only minor variations. Exons 3 and 7 were 9 and 3 bases longer, respectively, in *src* than in *lck*. Also, exon 12 in *src* had 6 more bases of coding sequence than exon 12 in *lck*. The sizes of the 3′ untranslated regions were different. We conclude that *lck* almost certainly arose by gene duplication from an ancestral *src*-like gene.

Despite the profound similarity between the *src* and *lck* genes throughout most of their lengths, exons 1 and 2 differed quite dramatically. Exon 1 of *lck* contained the entire 5′ untranslated region and 105 bases of coding sequence, whereas the first exon of *src* contained only 5′

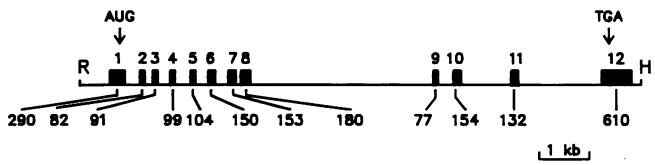

FIG. 2. Structure of the *lck* gene. Exons (■) are numbered with respect to the 3′ promoter (see text for description). The translation initiation site in exon 1 and the termination site in exon 12 are indicated. The exon sizes in base pairs are shown beneath the map.

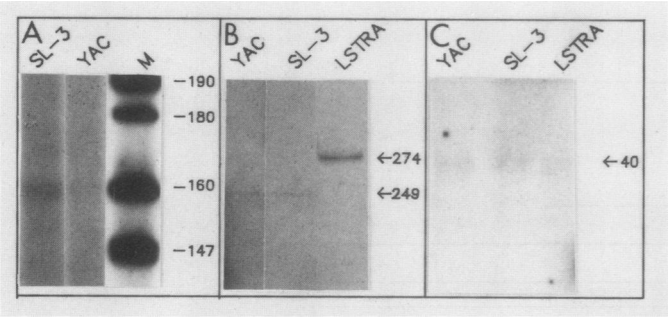

FIG. 3. S1 mapping and primer extension of the murine *lck* transcript. (A) RNA from T cell lines SL-3 and YAC-1 protected a 162-bp fragment labeled at a *Stu*I site 25 bp 5′ to the translation initiation codon. Lane M, Markers. (B) Primer extension products 249 bp in length were produced by using RNA from YAC-1 and SL-3; 274-bp primer extension products were produced by using RNA from LSTRA. The primer is complementary to codons 16 to 21 of the *lck* message. (C) Primer extension with an oligomer complementary to base pairs −166 through −146 from the initiation codon produced 40-bp products by using YAC-1, SL-3 and LSTRA RNAs. Numbers to the right of panels A, B, and C indicate numbers of base pairs.

untranslated sequence. Exon 2 of *lck* had 82 bases of coding sequence, while exon 2 of *src* had 9 bases of untranslated sequence and 241 bases of coding sequence. These observations suggest that the 5′ exons of *src* and *lck* do not share a common ancestor (but see below).

Transcription start site of *lck*. S1 mapping and primer extension were used to define the transcription start site of the *lck* gene (Fig. 3). The S1 probe was labeled at a *Stu*I site 25 bp 5′ to the translation initiation codon for p56^{lck}. RNA from both the SL-3 and YAC-1 cell lines protected a 162-bp fragment when this probe was used. (Fig. 3A). Oligonucleotides for primer extension were made complementary to base pairs −166 to −146 (relative to the translation start site) or to codons 16 to 21 of the *lck* mRNA. The longest primer extension product obtained by using the oligomer to codons 16 through 21 was 249 bp with YAC-1 and SL-3 RNA and the oligomer to codons 16 to 21 (Fig. 3B). These RNAs yielded 40-bp products when the 5′ untranslated region oligomer was used to prime reverse transcriptase. All three of these experiments agree in positioning the transcription start site in the *lck* gene at a guanine residue 186 bp 5′ to the translation initiation codon (see Fig. 5). As expected, primer extension of LSTRA mRNA confirmed the existence of a longer transcript in this cell line, presumably the previously described MoMuLV LTR-*lck* fusion transcript (37). On the basis of the published sequence (37), the size of the primer extension product from the LSTRA LTR-*lck* fusion transcript with the codon 16 to 21 *lck* oligomer was predicted to be 274 bp. When LSTRA RNA was used, a band of this size (25 bp larger than the primer extension products from YAC and SL-3 mRNA with the same oligonucleotide primer) was seen (Fig. 3B).

MoMuLV sequence upstream of *lck* in LSTRA cells. By screening a LSTRA genomic library with the *lck* probe, we also isolated part of the rearranged *lck* allele previously demonstrated by genomic blotting in LSTRA DNA (18). Clones 22.2 (LSTRA) and 41.1 (BALB/c) differed in the region immediately 5′ to the *lck* gene (Fig. 1). A 2.7-kb *Bam*HI-*Eco*RI fragment from the 5′ end of clone 22.2 was found to hybridize with a MoMuLV probe (data not shown). Detailed characterization of this region of clone 22.2 by DNA sequencing revealed that MoMuLV had been inserted

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                              -969  lck→
                                ↓
Normal Allele:          ACAGGGTTCCTCTGTGTAGC
                              :   ::::::::::
Rearranged Allele:      TGTAAGTAGGTCTGTGTAGC
                        1097 ← 1088
                           gag
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FIG. 4. *lck* rearrangement in LSTRA cells. Sequences from normal and rearranged *lck* genes are compared. The rearranged *lck* gene present in LSTRA cells contains *gag* sequences of MoMuLV (bases 1088 to 1097 are shown underlined) at position −969 from the *lck* transcription start site.

at a position 969 bp 5′ to the transcription start site of the *lck* gene. The MoMuLV sequence closest to *lck* began at position 1088 of the *gag* gene (27), and its transcriptional orientation was opposite to that of *lck* (Fig. 4). The *Bam*HI site at the end of clone 22.2 was derived from the *Bam*HI site at position 3234 in the *pol* gene of MoMuLV.

Potential regulatory sequences of *lck* conserved in mouse and humans. To assist in defining promoter structures that might direct the lymphocyte-specific expression of *lck* transcripts, we searched for regions of similarity in the human and mouse *lck* genes. A human genomic *lck* clone was isolated from a cosmid library, and subclones corresponding to exon 1 of the mouse gene were identified. We sequenced 956 bp of human *lck* sequence 5′ to the initiation codon and 778 bp of DNA 5′ to the transcription start site of murine *lck*. Figure 5 shows an alignment of the two sequences. Significant regions of sequence similarity are seen throughout the region between base pairs −500 and +1. These conserved regions may represent *cis*-linked regulatory elements for *lck* expression.

Alternative 5′ untranslated sequences in human *lck* transcripts. We and others have previously reported the structure of nearly full-length cDNA clones encoding human p56^{lck} (12; Perlmutter et al., in press). Figure 6 presents alignments of the 5′ untranslated region of the human genomic sequence to that of the human cDNA and of the murine genomic sequence to the fusion transcript found in LSTRA cells. Clearly, the human cDNA sequence diverges from its genomic counterpart at a position 5 bp 5′ to the translation initiation codon. This is precisely the position at which MoMuLV LTR sequences are spliced to *lck* sequences in LSTRA cells. Although it might be argued that the 5′ portion of the human cDNA represents a cloning artifact, we independently obtained a 5′ sequence in our human *lck* cDNA clones (Perlmutter et al., in press) identical to that reported by Koga et al. (12). Hence, it appears that the cryptic splice site employed in the LSTRA LTR-*lck* fusion transcript is a bona fide splice site in the human *lck* gene.

At the same time, the close sequence similarity between the human and murine *lck* genes across a 500-bp region 5′ to the translation initiation codon suggests that a transcription start site for the human gene should be positioned analogously to that in the mouse. Figure 7 demonstrates that this is, in fact, the case. By using a probe complementary to sequences in the 5′ untranslated region of the human *lck* cDNA YT16 (12), transcripts were easily identified in both normal thymus RNA and in RNA derived from the human T cell line Jurkat (Fig. 7A). In addition, a probe derived from the presumed 5′ untranslated region in the human *lck* genomic clone (a *Pst*I-*Bam*HI fragment from −584 to +62; [Fig. 5]) identified a slightly larger transcript on the same blot (Fig. 7B). Both transcripts appeared as a single broad band

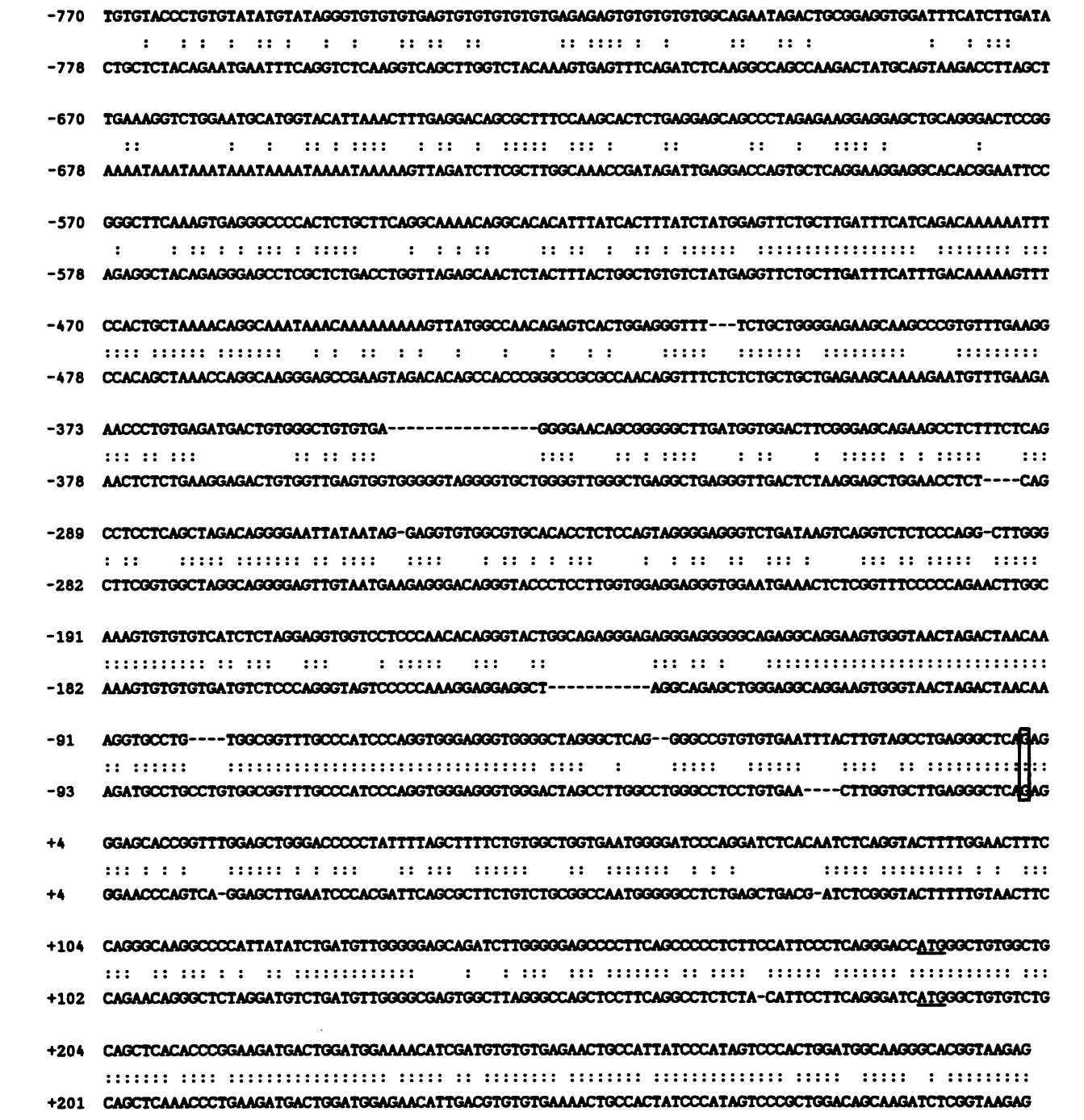

FIG. 5. Comparison of human (top lines) and murine (bottom lines) *lck* sequences flanking the transcription start site, designated +1 (boxed). The initiation codons are underlined for both sequences.

when a coding region probe was used (data not shown). Thus, the splice acceptor site 5 bp 5′ to the translation start site for $p56^{lck}$ is variably used. The 5′ untranslated region sequence in the human cDNA could not be identified on our genomic clone and is positioned at least 3 kb 5′ to the *lck* coding region (data not shown).

DISCUSSION

There are seven well-characterized members of the *src* gene family (*fgr*, *fyn*, *hck*, *lck*, *lyn*, *src*, and *yes*), all of which encode quite similar membrane-associated protein-tyrosine kinases. The avian *src* and human *fgr* genes not only encode similar products but also share a common exon structure, further emphasizing that these genes are derived from a common evolutionary precursor (20). We have now demonstrated that the murine *lck* gene is similarly configured. Differences between the *lck* gene and either *src* or *fgr* reside principally in the first two exons, which encode most of the amino-terminal portion of $p56^{lck}$. This region shares no homology with any other *src*-like protein-tyrosine kinase. It

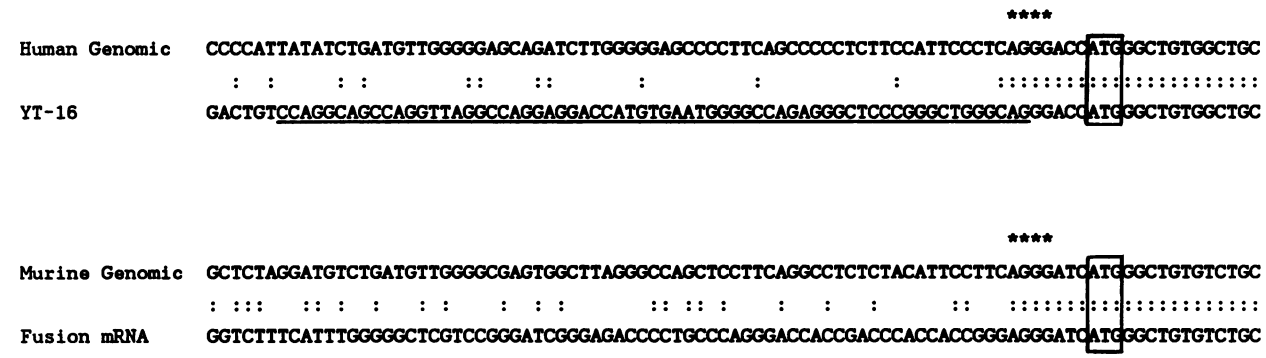

FIG. 6. Comparison of *lck* genomic and cDNA sequences. The human *lck* cDNA sequence YT16 (12) is aligned with the human genomic sequence, while the murine genomic sequence is aligned with that reported for the LTR-*lck* fusion transcript in LSTRA cells (36). The asterisks identify the splice acceptor in each genomic sequence. The underlined region of YT16 indicates the sequence used as a probe for the RNA blot analysis shown in Fig. 7. The initiation codons are boxed.

is thus tempting to speculate that the first two exons of the *lck* gene, perhaps with their associated lymphocyte-specific promoter element, arose independently from the remainder of the gene and were fortuitously juxtaposed at some time during vertebrate evolution.

At the same time, some *lck* transcripts utilize the splice site just upstream of the translated region and contain sequences derived from an alternative 5′ exon (Fig. 6). Under these circumstances, the first exon contains most of the 5′ untranslated region, and the translation initiation codon is located 5 bases 3′ to the start of exon 2, a position analogous to that used in *src* and *fgr*. It is thus remotely possible that all of the transcribed regions of *lck*, like those of *fgr* (20), diverged from a primordial *src*-like element and that a second promoter element arose subsequently in the first intron.

Structure of an *lck* promoter region. By defining a transcription start site for the murine *lck* gene, we have identified a region that probably contains a complete *lck* gene promoter. Human and mouse *lck* genes appear to be regulated in a similar manner (16, 18, 34) and would be expected to have common *cis*-acting regulatory sequences. Thus, by comparing genomic sequences in this region, we can identify elements that presumably assist in the lymphocyte-specific transcription of *lck*. This strategy has been used to identify *cis*-linked regulatory elements in a variety of genes, e.g., those for immunoglobulins and metallothioneins (21, 25). A 500-bp region 5′ to the transcriptional start site in mouse is 85% identical to the analogous sequence in humans (Fig. 5). Although neither the murine nor the human *lck* sequence contains typical promoter motifs (5), a conserved TGTGAA at position −25 of the murine gene bears some similarity to the TATAA box often found in an analogous position in other polymerase II transcriptional units, and the sequence CCAT at position −69 in the murine *lck* gene (and conserved in the human gene) bears some resemblance to the CAAT sequences found in many genes near position −70. A computer-assisted analysis revealed no significant relationship between the *lck* 5′ sequences and the lymphocyte-specific promoter elements believed to function in the interleukin-2 and gamma interferon genes (10). In addition, there are no consensus binding sites for the transcription factors SP1 (8), AP1 (13), or AP2 (19). Additional experiments will be required to define the functionally important aspects of this *lck* promoter.

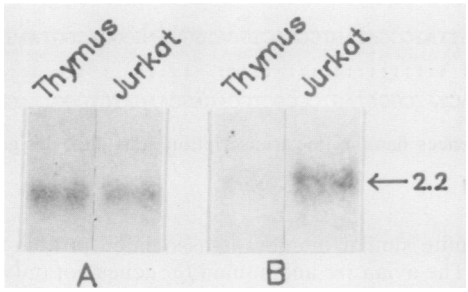

FIG. 7. Two *lck* transcripts differing in 5′ untranslated regions are expressed in human thymus and Jurkat cells. A 65-bp sequence from the 5′ untranslated region of YT16 (shown in Fig. 6) and a *Pst*I-*Bam*HI fragment from the human genomic clone that spans the region from −582 to +62 (Fig. 5) were used as probes for RNA blots in panels A and B, respectively. Each lane contains 10 μg of total RNA from the indicated cell type. The transcript in panel B is approximately 2.2 kb in size.

The murine genomic sequence presented in Fig. 5 agrees completely with that presented by Voronova et al. (35) from −6 to +219. We agree with their conclusion that the 5′ end (base-pairs 9 to 26 of NT18) of the *lck* cDNA (18) is the reverse complement of +10 to +27 of the genomic sequence and is attributible to a reverse transcriptase artifact.

Structure of the *lck* gene rearrangement in LSTRA cells. We have previously demonstrated that the *lck* gene is rearranged and overexpressed in LSTRA cells (18). Characterization of a MoMuLV LTR-*lck* fusion transcript in LSTRA mRNA indicated that MoMuLV had integrated 5′ to the *lck* gene (37). Clone 22.2 provided a molecular description of this insertion event. Surprisingly, the MoMuLV *gag* and *pol* gene sequences on clone 22.2 are positioned in the opposite transcriptional orientation relative to the *lck* gene. Genomic blot analyses confirm that clone 22.2 faithfully represents the genomic structure of the rearranged *lck* allele (data not shown; 18). Unfortunately, clone 22.2 does not contain the 5′ MoMuLV LTR. Nevertheless, the existence of the LTR-*lck* fusion transcript in LSTRA cells means that the 5′ LTR of MoMuLV must be oriented identically to *lck*. The fusion transcript cannot initiate from the 3′ LTR because it contains *gag* sequences that must be transcribed from the 5′ LTR (37). Therefore, the MoMuLV sequences in LSTRA cells must be internally rearranged. Although it is impossible at this point to determine the configuration of the original MoMuLV integrant, we suspect that the viral se-

quence initially inserted in the same transcriptional orientation as the *lck* gene and that a subsequent inversion destroyed the viral transcription unit. Regardless of the precise mechanism, this rearrangement allows a primary transcript originating from the 5′ LTR to splice to an acceptor site in *lck*, since the acceptor site in MoMuLV is no longer intact. Interestingly, the Thy-19 tumor also contains a MoMuLV insertion 5′ to the *lck* gene that generates identical fusion transcripts (35, 37). Disruption of the normal 5′ untranslated region may therefore be an important feature of *lck* activation in lymphomagenesis (see below).

Alternative 5′ exons in the *lck* gene. Comparison of the human *lck* cDNA and the human *lck* genomic sequence yielded an apparent paradox: these sequences diverge completely at exactly the point where long terminal repeat sequences appear in the long terminal repeat-*lck* fusion transcript in LSTRA cells (Fig. 6). Our studies clearly demonstrate that two alternative 5′ exons and two different promoters can be used to generate *lck* transcripts. Voronova et al. have recently reported an RNase protection analysis of murine *lck* transcripts which supports this conclusion (35). Neither the S1 probe (Fig. 3A) nor the 5′ untranslated region primer (Fig. 3C) could have detected transcripts initiating at the 5′ promoter, since these probes did not extend 3′ to the splice acceptor site at position 181 (Fig. 5). Examination of the complete set of extension products obtained by using the coding region primer (Fig. 3B) indicated that transcripts from the murine 5′ promoter included an 84-bp 5′ untranslated region (data not shown). These transcripts would therefore be 102 bp shorter than those initiating at the 3′ start site, which is consistent with the pattern observed in RNA blot analysis (Fig. 7).

Alternative use of multiple 5′ exons has been reported in another protein-tyrosine kinase gene, c-*abl* (2). In this case, the representation of each of four different transcripts appears to vary somewhat among different cell types. Interestingly, the ratio of transcripts initiating at the 5′ rather than the 3′ *lck* start site is different in thymus mRNA and mRNA from the Jurkat cell line (Fig. 7). It is thus possible that the two *lck* promoters are differentially regulated under some circumstances. However, unlike the proteins encoded by multiple forms of c-*abl*, both *lck* transcripts encode identical $p56^{lck}$ products. Why, then, are there two alternative *lck* transcripts?

We have observed that the murine *lck* transcript derived from the downstream promoter is translated very inefficiently, most likely as a result of three out-of-frame AUG codons positioned 5′ to the translational start site for $p56^{lck}$ (17). The MoMuLV-*lck* fusion transcript found in LSTRA cells lacks all but 5 bases of the *lck* 5′ untranslated sequence, including the upstream AUG codons. Interestingly, LSTRA cells have a 7-fold increase in *lck* message but a 50-fold increase in the protein product, reflecting the more efficient translation of *lck* transcripts that are not subject to translational masking. It is therefore tempting to speculate that transcripts derived from the 5′ promoter will direct the synthesis of $p56^{lck}$ more efficiently. Thus, levels of $p56^{lck}$ in normal T cells might potentially be regulated by variable activation of the 5′ promoter element. This interpretation is complicated by the fact that human *lck* transcripts generated by using the 5′ promoter, like those derived from the 3′ promoter, contain upstream AUG triplets. Direct evaluation of the translational efficiencies of these two mRNAs will be required to assess the potential importance of the *lck* 5′ untranslated regions in regulating $p56^{lck}$ expression.

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