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# Genomic Structure and Chromosomal Localization of the Mouse LIM Domain-Binding Protein 1 Gene, *Ldb1*

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**The LIM domain is a structural motif that is well conserved throughout evolution in a variety of factors known to play important roles in development and cell regulation. *Ldb* genes encode LIM domain-binding (Ldb) factors. Here we report on the structural organization and chromosomal localization of the mouse *Ldb1* gene. It contains at least 10 exons and spans approximately 4 kb of genomic DNA. The transcription initiation site is located 462 bp upstream of the translation initiation codon ATG as determined by 5′-RACE. Sequencing analysis of the 5′-flanking region shows TATA and CCAAT motifs as well as potential binding sites for GATA, CF-1, PEA3, HRE, APRRE, RARE, Myc, and c-Jun. *Ldb1* maps to the distal region of mouse chromosome 19 that is syntenic with human chromosome 10q.**

## INTRODUCTION

The recently discovered LIM domain-binding (Ldb) factors are characterized by their ability to bind to LIM domains (Agulnick *et al.,* 1996; Jurata *et al.,* 1996; Bach *et al.,* 1997). This family currently consists of two members, Ldb1/NLI/CLIM-2 and Ldb2/CLIM-1. LIM domains are specialized zinc finger motifs that are found in a wide variety of regulatory proteins and are thought to mediate protein–protein interactions (for review, see Sanchez Garcia and Rabbitts, 1994; Dawid *et al.,* 1995; Gill, 1995). The observation of Ldb/LIM interactions is so far restricted to nuclear LIM proteins (Agulnick *et al.,* 1996; Jurata *et al.,* 1996; Bach *et al.,* 1997). The nuclear LIM proteins consist of LIM homeodomain factors, which contain two tandem LIM domains followed by a homeodomain, and the LMO proteins, which contain two LIM domains alone. LIM homeodomain proteins are regulators of cell fate in many species and are required for processes such as head formation (Shawlot and Behringer, 1995), forebrain and blood cell development (Porter *et al.,* 1997), mechanosensory neurogenesis (Way and Chalfie, 1988), pituitary development (Sheng *et al.,* 1996), and motor neuron formation (Pfaff *et al.,* 1996). Misexpression of LMO proteins is involved in T-cell acute lymphoblastic leukemia (Boehm *et al.,* 1991), and LMO2 is essential for erythropoiesis (Warren *et al.,* 1994). In addition, LIM and Ldb proteins can form complexes with additional regulatory factors, and these complexes can regulate gene expression (Wadman *et al.,* 1997; Bach *et al.,* 1997). Ldb1 (alias NLI and CLIM-2) is well conserved in evolution (Agulnick *et al.,* 1996) and widely expressed during development and in the adult organism (Agulnick *et al.,* 1996; Jurata *et al.,* 1996; Bach *et al.,* 1997). These findings suggests that Ldb1 is involved in transcriptional regulation of a wide array of genes both during development and in the adult. This study addresses the genomic structure and the chromosomal localization of the mouse Ldb1 gene.

## MATERIALS AND METHODS

*Genomic library screening.* A mouse 129/sv genomic library, obtained by partial digestion of chromosomal DNA with *Sau*3A and ligation into the EMBL 3A phage vector (gift from Dr. S. Tonegawa, Massachusetts Institute of Technology, Cambridge), was screened with a 1.4-kb *Ldb1* cDNA probe containing the entire *Ldb1* coding region, 237 bp of 5′ UTR, and 27 bp of 3′ UTR (Agulnick *et al.,* 1996). Three positive clones were identified, and their inserts were subcloned into the *Sal*I site of pBluescript SK (Stratagene). One of three isolated clones contained the entire coding region of *Ldb1,* which was confirmed by Southern hybridization performed with a 5′-specific (0.3-kb fragment) and a 3′-specific probe (0.25-kb fragment) derived from the 1.4-kb cDNA clone. Sequencing was performed using the dideoxynucleotide method (Sequenase Kit, U.S. Biochemical).

*Determination of the exon–intron boundaries.* The intron–exon boundaries were determined by direct comparison of the nucleotide

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sequence of the *Ldb1* cDNA and genomic DNA. The size of introns was determined by direct reading of the nucleotide sequence of the genomic clone.

*Determination of the transcription initiation site by 5′ and 3′-RACE.* 5′-RACE and 3′-RACE were performed using the 5′/3′ RACE Kit (Boehringer Mannheim) as described by the manufacturer with minor modifications. Total RNA was isolated from adult CD1 mouse brain with RNA zol (Tel-Test Inc.) and used as a template to generate first-strand cDNA. Ten micrograms of total RNA predigested with 20 units of DNase I (Boehringer Mannheim) at 37°C for 20 min was transcribed into first-strand cDNA for 5′-RACE using gene-specific primer A, situated in Exon 2. The tailing reaction was performed as described, and seminested PCR was then carried out with dA16-anchor primer B and nested gene-specific primer C, which spans Exon 1 and Exon 2 and thus prevents amplification of genomic DNA, using 28 cycles of 94°C for 1 min, 49°C for 1 min, and 72°C for 1 min. Second-round PCR was performed with anchor primer D and several nested gene-specific primers (AS-1, 2, 3, and 4) that are located in the 5′-flanking region of the genomic DNA, using 35 cycles under the same conditions as described for first-round PCR. Primer sequences are shown in Fig. 2. The 5′-RACE products were cloned into the pGEM-T Easy vector (Promega) and sequenced as described above.

For 3′-RACE, first-strand cDNA was generated by using an oligo$(dT)_{16}$-anchor primer E, followed by nested PCR with gene-specific primer F (for first PCR, sense primer) situated in Exon 8, primer G (for second PCR, sense primer) in Exon 10, and primer D (for both reactions, antisense anchor primer).

*Interspecific mouse backcross mapping.* Interspecific backcross progeny were generated by mating (C57BL/6J × *Mus spretus*)$F_1$ females and C57BL/6J males as described (Copeland and Jenkins, 1991). A total of 205 N2 mice were used to map the *Ldb1* locus (see text for details). DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization were performed essentially as described (Jenkins *et al.,* 1982). All blots were prepared with Hybond-$N^+$ nylon membrane (Amersham). The probe, an ~1.4-kb *Eco*RI fragment of mouse cDNA, was labeled with [$\alpha$-$^{32}$P]dCTP using a nick-translation labeling kit (Boehringer Mannheim); washing was performed to a final stringency of 0.8× SSCP, 0.1% SDS at 65°C. Fragments of 4.6 and 1.5 kb were detected in *Taq*I-digested C57BL/6J DNA, and fragments of 5.4 and 1.5 kb were detected in *Taq*I-digested *M. spretus* DNA. The presence or absence of the 5.4-kb *Taq*I *M. spretus*-specific fragment was followed in backcross mice.

A description of one of the probes and RFLPs for the loci linked to *Ldb1 Col 17a1* (formerly *Bpag2*) has been reported previously (Copeland *et al.,* 1993). One locus has not been reported previously for this interspecific backcross. The probe for *Pax2* was a 1.7-kb *Bam*HI/*Eco*RI fragment of mouse cDNA that detected 4.6-, 0.6-, and 0.4-kb *Taq*I fragments in C57BL/6J DNA and 5.4-, 4.6-, 0.6-, and 0.4-kb *Taq*I fragments in *M. spretus* DNA. Recombination distances were calculated using Map Manager, version 2.6.5. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

## RESULTS

### *Genomic Organization of the Ldb1 Gene*

Three *Ldb1* genomic clones obtained by screening a mouse 129/sv library were analyzed in detail. Each clone contained approximately 15 kb of genomic DNA. A composite restriction map of the *Ldb1* locus, including 12 kb of upstream sequence and 4 kb of downstream sequence, is shown in Fig. 1 (top). Approximately 5.2 kb of genomic DNA that contained the entire *Ldb1* coding region was sequenced fully. The exon–intron structure was then determined by comparison of this genomic sequence to that of the *Ldb1* cDNA sequence (1.4 kb) described in Agulnick *et al.* (1996) (Fig. 1, bottom). Exon 1 contains the 462-base 5′-untranslated region (UTR) and the translation initiation codon ATG, plus an additional six codons. The transcription initiation site was determined by 5′-RACE (see below). Exon 2 encodes 15 amino acids and extends from nucleotide position 594 to 638, Exon 3 encodes 25 amino acids from position 742 to 817, Exon 4 encodes 34 amino acids from position 904 to 1006, and Exon 5 encodes 58 amino acids from position 1145 to 1317. Following the relatively large Intron 5, Exon 6 encodes 41 amino acids and extends from position 1783 to 1905, Exon 7 encodes 28 amino acids from position 2035 to 2118, and Exon 8 encodes 41 amino acids from position 2218 to 2341. Following the largest intron, Intron 9, Exon 9 extends from position 2551 to 2699 and encodes 50 amino acids including a potential nuclear localization signal (amino acids KRRKRK and KKK; Agulnick *et al.,* 1996). Exon 10, encompassing nucleotides 3383 to 4121, encodes 76 amino acids and the stop codon TGA, plus 508 bases of 3′-UTR sequence. Both our cDNA and that reported by Jurata *et al.* (1996) terminate at or near nucleotide 4121. There is likely to be additional 3′-UTR sequence that has not yet been mapped and sequenced (see 3′-RACE results and Discussion). The *Ldb1* gene harbors short introns, which vary between 86 and 683 bases in length. The exon–intron boundaries are assigned as shown in Table 1. All exon–intron junctions conform to the 5′ donor and 3′ acceptor consensus rules (Breathnach and Chambon, 1981).

### *Transcription Initiation Site*

To determine the transcription initiation site, 5′-RACE was performed. PCR products were sequenced, revealing two potential transcription initiation sites, separated by 5 bp (Fig. 2, positions +1 and +5). The longer product mapped 462 bp upstream of the ATG translation initiation codon. The shorter product may represent an alternative initiation site or a premature termination of the RACE product. To verify the mapping of initiation sites, 5′-RACE was performed in which several different antisense primers (AS-1, 2, 3, 4; Fig. 2) that were located in slightly different positions in the 5′-UTR were used for second-round PCR to compare the size of each PCR product. In addition, RT-PCR using a sense primer that is situated 80 bp upstream of the transcription initiation site did not reveal any PCR product, whereas that using a sense primer spanning positions −2 to +23 gave a product of the expected size (data not shown).

### *The 5′-Flanking Sequence*

The sequence of the 5′ upstream region is shown in Fig. 2. A consensus TATAAA element was not found near the −30-bp position; however, this region has two A-T-rich motifs. At −118 bp, a modified CCAAT motif was found. Conserved binding sites (Faisst and Meyer,

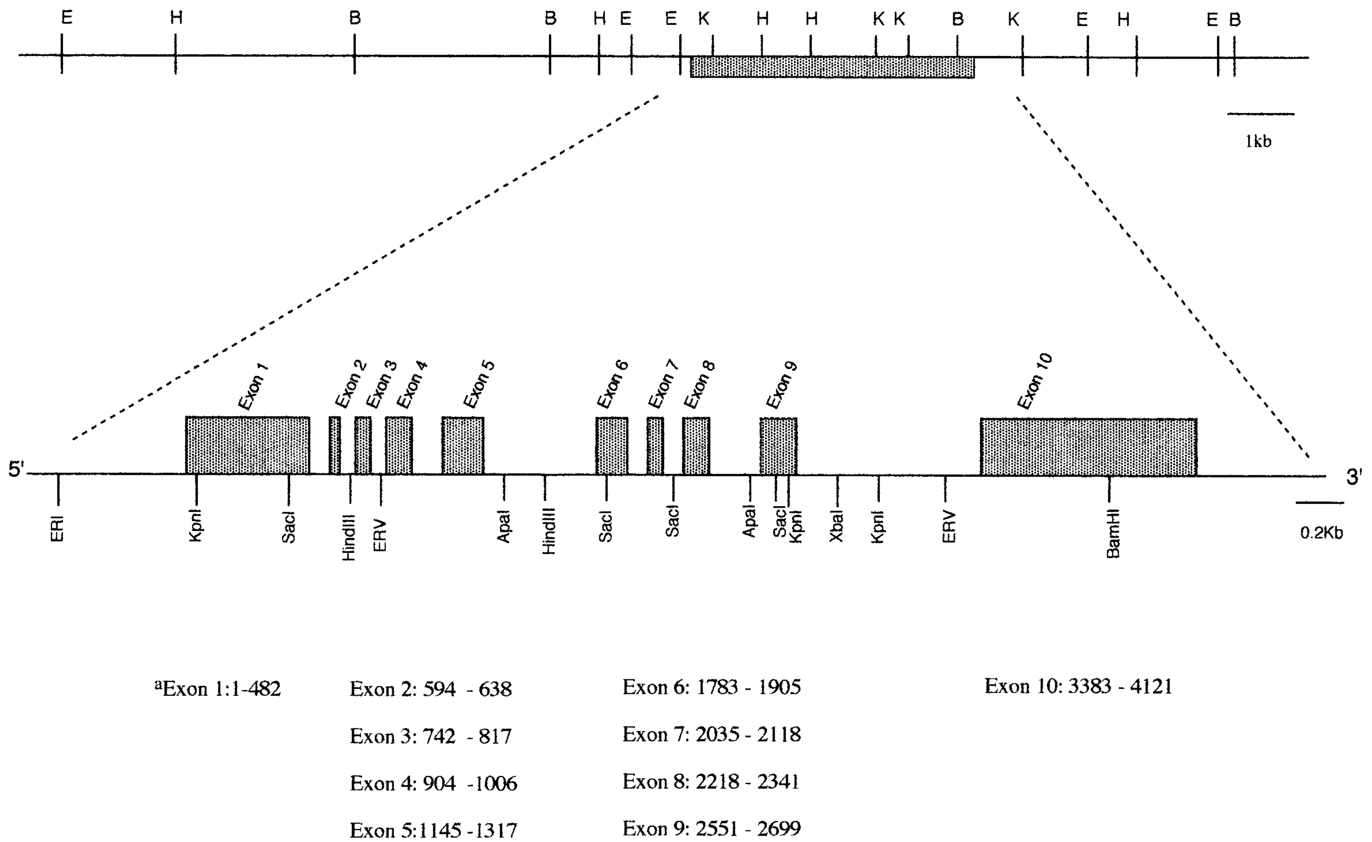



**FIG. 1.** Genomic structure of the mouse *Ldb1* gene. [a]The length of Exon 1 is based on the major transcription start site, which has been generated and determined by 5′-RACE. E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; K, *Kpn*I.

1992) for various other transcription factors such as GATA, CF-1, PEA3, Myc, and c-Jun as well as a steroid hormone responsive element, an acute phase reactant regulatory element, and a retinoic acid response element are also seen in the 5′ upstream region.

### *The 3′-Untranslated Region*

By Northern blot analysis, two major Ldb1 mRNA species of approximately 2.4 and 3.7 kb have been detected (Agulnick *et al.,* 1996). 3′-RACE, using gene-specific primers F and G located in the 3′-end of the Ldb1 ORF, detected two products of approximately 0.65 and 1.8 kb (data not shown). The length of these products, combined with the lengths of the 5′-untranslated region (462 bp) and the ORF upstream of 3′-RACE primer G (1005 bp), is consistent with the lengths of the 2.4- and 3.7-kb mRNAs.

### *Chromosome Localization*

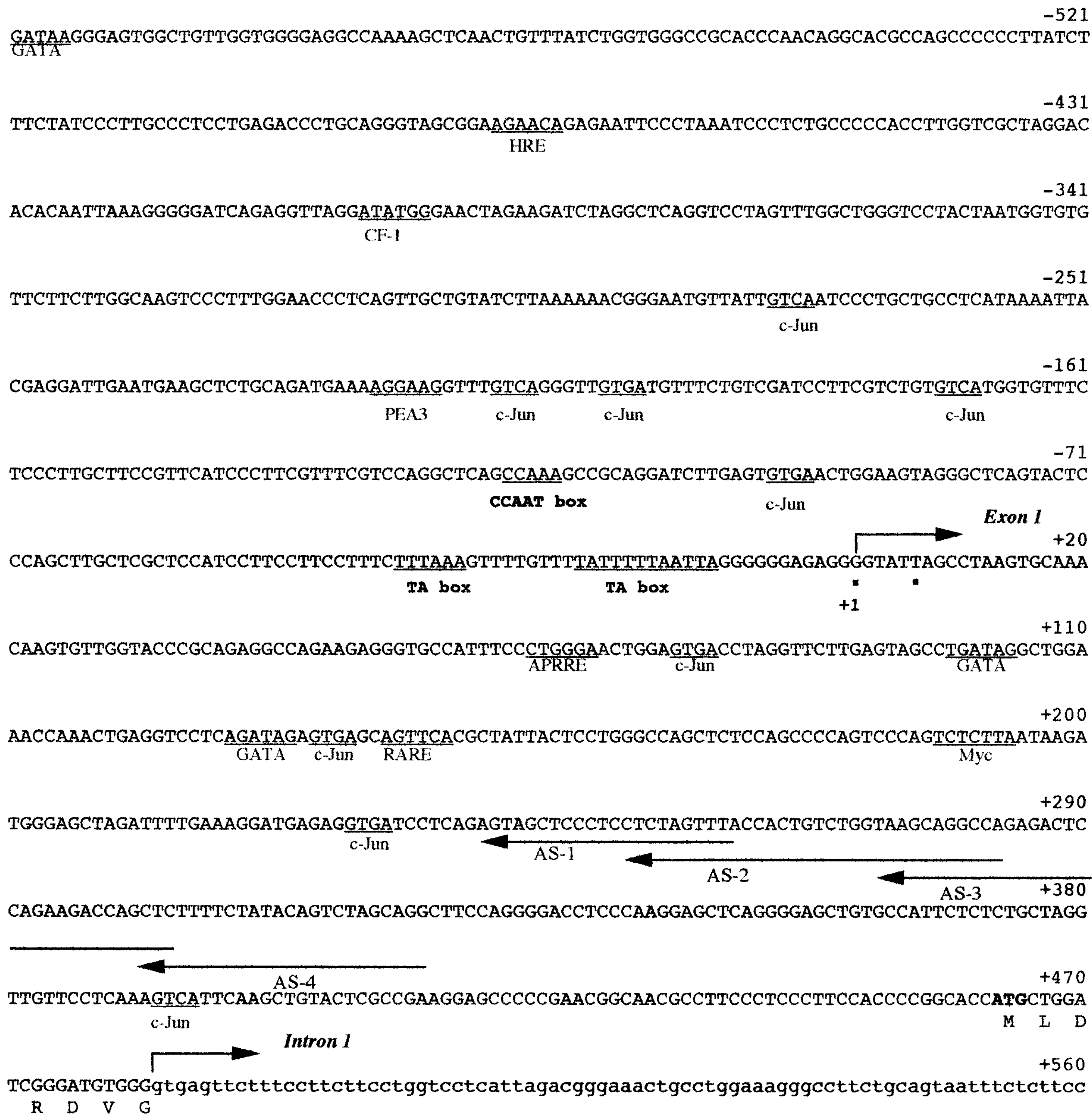
The mouse chromosomal location of *Ldb1* was determined by interspecific backcross analysis using progeny derived from matings of [(C57BL/6J × *M. spret-*

**TABLE 1**

**Exon–Intron Junctions of the Mouse Ldb-1 Gene**

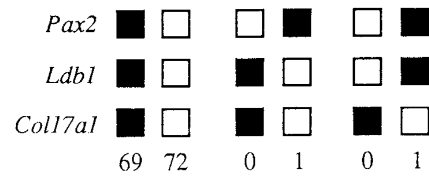
| | Splice donor site | | Splice acceptor site | |
|---|---|---|---|---|
| Exon 1. . . . . | .GTGGG**gt**gag. . . . . | .Intron 1. . . . . | .cac**ag**CCCAA. . . . . | .Exon 2. . . |
| . . .Exon 2. . . . . | .ATCGG**gt**aag. . . . . | .Intron 2. . . . . | .ccc**ag**GAGGC. . . . . | .Exon 3. . . |
| . . .Exon 3. . . . . | .CAGAG**gt**atg. . . . . | .Intron 3. . . . . | .ttc**ag**GAGTG. . . . . | .Exon 4. . . |
| . . .Exon 4. . . . . | .ATATA**gt**aag. . . . . | .Intron 4. . . . . | .tat**ag**CCATT. . . . . | .Exon 5. . . |
| . . .Exon 5. . . . . | .CCCAG**gt**gag. . . . . | .Intron 5. . . . . | .tct**ag**GTGTG. . . . . | .Exon 6. . . |
| . . .Exon 6. . . . . | .TGCAC**gt**gag. . . . . | .Intron 6. . . . . | .tct**ag**GCCCA. . . . . | .Exon 7. . . |
| . . .Exon 7. . . . . | .TCCGA**gt**aag. . . . . | .Intron 7. . . . . | .ccc**ag**CTCTG. . . . . | .Exon 8. . . |
| . . .Exon 8. . . . . | .TCCCG**gt**gag. . . . . | .Intron 8. . . . . | .cac**ag**CGGAG. . . . . | .Exon 9. . . |
| . . .Exon 9. . . . . | .TACCT**gt**aag. . . . . | .Intron 9. . . . . | .ctc**ag**GATGT. . . . . | .Exon 10 |

*Note.* The exon sequence is shown in uppercase letters, and the intron sequence has been depicted in lowercase letters. The invariant 5′ ag . . . gt 3′ sequences are shown in boldface.




Primer A : GATCCCAGGCTCCAGGTA
Primer B : GACCACGCGTATCGATGTCGACAAAAAAAAAAAAAAAAV
primer C : TACATGGGAGTTGGGCCCACAT
primer D : GACCACGCGTATCGATGTCGAC
Primer E : GACCACGCGTATCGATGTCGACTTTTTTTTTTTTTTTTV
primer F : GAGCCCATGCAGGAACTTATGTC
primer G : CATTGACGACGAGGACAG
primer AS-1 : TAAACTAGAGGAGGGAGCTACT
primer AS-2 : TGGCCTGCTTACCAGACAGTGGTAAACTAGAGG
primer AS-3 : GAGCTGGTCTTCTGGAGTCTCTGGCCTGCTTA
primer AS-4 : GCCTGCTAGACTGTATAGAAAGA

*us*)$F_1$ × C57BL/6J] mice. This interspecific backcross mapping panel has been typed for over 2400 loci that are well distributed among all the autosomes as well as the X chromosome (Copeland and Jenkins, 1991). C57BL/6J and *M. spretus* DNAs were digested with several enzymes and analyzed by Southern blot hybridization for informative restriction fragment length polymorphisms (RFLPs) using a mouse cDNA *Ldb1* probe. The 5.4-kb *Taq*I *M. spretus* RFLP (see Materials and Methods) was used to follow the segregation of the *Ldb1* locus in backcross mice. The mapping results indicated that *Ldb1* is located in the distal region of mouse chromosome 19 linked to *Pax2* and *Col 17al.* Although 143 mice were analyzed for every marker and are shown in the segregation analysis (Fig. 3), up to 179 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for recombination frequencies using the additional data. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are as follows: centromere–Pax2–3/179–*Ldb1*–2/154 *Col 17al.* The recombination frequencies (expressed as genetic distances in centimorgans ± the standard error) are *Pax2*–1.7 ± 1.0–*Ldb1*–1.3 ± 0.9–Col 17al.



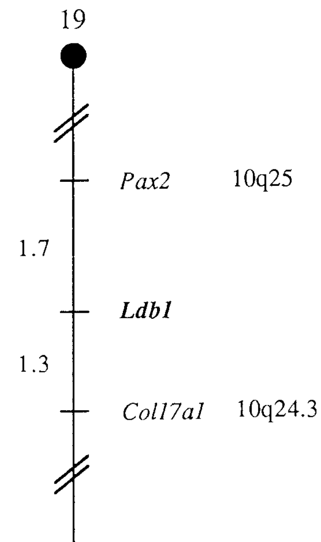




**FIG. 3.** *Ldb1* maps in the distal region of mouse chromosome 19. *Ldb1* was placed on mouse chromosome 19 by interspecific backcross analysis. The segregation patterns of *Ldb1* and flanking genes in 143 backcross animals that were typed for all loci are shown at the top. For individual pairs of loci, more than 143 animals were typed (see text). Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/6J × *M. spretus*)$F_1$ parent. The shaded boxes represent the presence of a C57BL/6J allele, and white boxes represent the presence of a *M. spretus* allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. A partial chromosome 19 linkage map showing the location of *Ldb1* in relation to linked genes is shown at the bottom of the figure. Recombination distances between loci in centimorgans are shown to the left of the chromosome, and the positions of loci in human chromosomes, where known, are shown to the right. References for the human map positions of loci cited in this study can be obtained from GDB (Genome Data Base), a computerized database of human linkage information maintained by The William H. Welch Medical Library of The Johns Hopkins University (Baltimore, MD).

## DISCUSSION

The mouse *Ldb1* gene encodes a protein that binds to the LIM domains of LMO and LIM homeodomain proteins, well known as important transcriptional regulators of mouse embryogenesis, including hematopoiesis (Xu *et al.,* 1993; Porter *et al.,* 1997), neurogenesis (Fujii *et al.,* 1994; Barnes *et al.,* 1994; Shawlot and Behringer, 1995; Pfaff *et al.,* 1996; Porter *et al.,* 1997; Sheng *et al.,* 1997), and organogenesis of the pituitary gland (Sheng *et al.,* 1996). Binding of Ldb proteins to LIM domains results in transcriptional synergy with other classes of transcription factors (Agulnick *et al.,* 1996; Wadman *et al.,* 1997; Bach *et al.,* 1997). In this study, we established the genomic organization of the mouse *Ldb1* gene.

The *Ldb1* gene contains at least 10 exons and spans approximately 4 kb of genomic DNA. The major RNA transcripts are approximately 2.4 and 3.7 kb in length (Agulnick *et al.,* 1996). Since 5′-RACE revealed two transcription initiation sites located only 5 bases apart, we investigated whether the two RNA species differ in the length of the 3′-UTR. Two 3′-RACE products of 0.65 and 1.8 kb were revealed, suggesting that two different 3′ processing sites are utilized, one that gives rise to the 2.4-kb transcript and the other that gives rise to the 3.7-kb transcript.

Interestingly, the 5′-UTR sequence upstream of position −86 bp relative to the translation start codon differs from that seen by Jurata *et al.* (1996). We did not observe this alternate 5′-UTR sequence in several 5′-

**FIG. 2.** DNA sequence of the mouse *Ldb1* Exon 1 and its immediate flanking region. Potential transcription initiation sites determined by 5′-RACE are marked with dots. The positions of antisense primers (AS-1, 2, 3, and 4) used for 5′-RACE are indicated by opposite directed arrows. The primer sequences for 5′-RACE are also shown at the bottom. The major transcription start site is indicated by an arrow and numbered as +1. The ATG translation initiation site is shown in boldface. Potential TATA and CCAAT promoter sites are indicated in boldface and are underlined. Consensus sequences for GATA, HRE, CF-1, PEA3, APRRE, RARE, Myc, and c-Jun are also underlined. The sequence of primers used in this study is shown at the bottom.

RACE clones using antisense primer AS-4, which is located downstream of the sequence divergence. This alternate sequence is not present in the approximately 700 bp of genomic sequence upstream of our transcription start sites that we have analyzed. Also, our 5′-RACE sequence is continuous with the genomic sequence of this region. However, it is possible that an alternative splicing event can occur at the −86-bp position, which implies that another promoter and noncoding exon exist upstream of the genomic region we have sequenced here.

We have compared our interspecific map of chromosome 19 with a composite mouse linkage map that reports the map location of many uncloned mouse mutations (provided from Mouse Genome Database, a computerized database maintained at The Jackson Laboratory, Bar Harbor, ME). *Ldb1* mapped in a region of the composite map that lacks mouse mutations with a phenotype that might be expected for an alteration in this locus (data not shown). The distal region of mouse chromosome 19 shares a region of homology with human chromosome 10q (summarized in Fig. 3). The placement of *Ldb1* in this interval in mouse suggests that the human homolog of *Ldb1* will map to 10q as well.

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