

## Prolactin-like protein-A gene structure and chromosomal mapping

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Prolactin-like protein-A (PLP-A) is a member of the prolactin (PRL) gene family and is expressed by trophoblast cells of the developing rat and mouse chorioallantoic placenta (Lin et al. 1997; Müller et al. 1998). PLP-A has been proposed to participate in the control of maternal immunologic responses prerequisite for the establishment of pregnancy through its specific interactions with uterine natural killer cells (H. Müller and M.J. Soares, unpublished). In this report, we present data on the structure of the mouse PLP-A gene and describe its chromosomal localization.

A genomic DNA library generated from a 129/SvEv strain mouse liver and packaged in the Lambda FIX II vector was a generous gift of Lexicon Genetics, Inc. (Houston, Tex.). Approximately  $1 \times 10^6$  pfu were screened with a mouse PLP-A cDNA (Müller et al. 1998). Positive plaques were amplified and used to inoculate LE392 *E. coli*. A series of forward and reverse oligonucleotide primer sets based on the mouse PLP-A cDNA were designed and utilized to sequence exons and exon-intron boundaries. These primers were also used to estimate 5' and 3' flanking DNA and intron sizes by PCR analysis and agarose electrophoresis. DNA sequencing was performed with an Applied Biosystems Model 310 sequencer and Applied Biosystems Dye Terminator Cycle Sequencing kits (Foster City, Calif.).

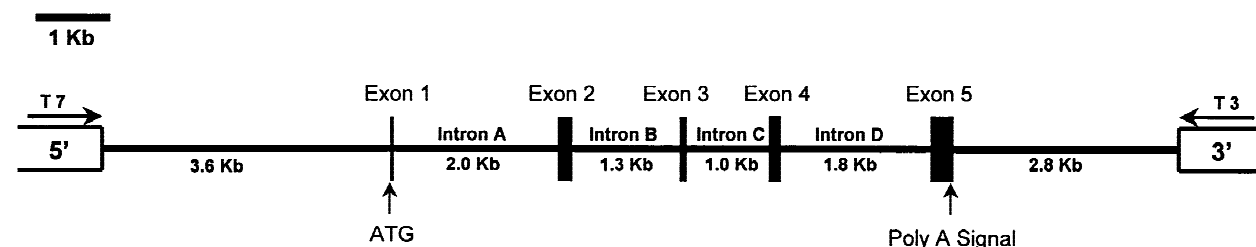
Genomic library screening resulted in the identification of two positive phage clones. A 13.3-kb clone contained the entire mouse PLP-A gene and additional 5' and 3' flanking DNA (Fig. 1). All exons and exon/intron boundaries were sequenced (Figs. 1 and 2). Exonic regions were identical to the sequence of the previously

published mouse PLP-A cDNA (Müller et al. 1998). Consensus GT and AG splicing junctions were evident in each intron. The mouse PLP-A gene is characterized by a 5 exon-4 intron gene structure (Figs. 1 and 2). An additional 3.6 kb of 5' flanking DNA and 2.8 kb of 3' flanking DNA were also present within the clone.

Chromosomal mapping of the mouse PLP-A gene was determined by use of The Jackson Laboratory Interspecific Backcross Panel (Rowe et al. 1994). Genomic DNAs from C57BL/6J, *Mus spretus*, and a (*M. spretus* × C57BL/6J) $F_1$  × *M. spretus* (BSS type) backcross were analyzed by Southern blotting as previously described (White et al. 1992). Approximately 5 µg of genomic DNAs from the C57BL/6J and *M. spretus* progenitors were digested with 28 different restriction enzymes to find a suitable restriction fragment length variation (RFLV) for mapping. Southern blots were probed with the mouse PLP-A cDNA (Müller et al. 1998). Approximately 2 µg of DNA from the BSS type backcross panel was digested for each sample with *BclI* overnight. Segregation of alleles was compared with other loci from a database at The Jackson Laboratory Backcross DNA map Panel Service (Rowe et al. 1994).

The gene symbol, *Prlpa*, has been assigned to the mouse PLP-A locus and has been approved by the International Mouse Nomenclature Committee. A *BclI* RFLV for *Prlpa* was identified by the presence of a 2.6-kb genomic DNA fragment in C57BL/6J or the presence of a 3.3-kb fragment in *M. spretus* (Fig. 3A). Mapping data from this article have been deposited with the Mouse Genome Database under Accession No. J:44997. Haplotype analysis of these mapping data (Fig. 3B) indicated that the *Prlpa* locus is closely linked to *Dtprp* and *Pli* on Chromosome (Chr) 13 in the mouse. Allelic segregation patterns for *Prlpa*,

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### mPLP-A Genomic Clone (13.3 Kb)

**Fig. 1.** Schematic representation of the 13.3-kb mouse PLP-A genomic clone. Sequence analysis of exon (E)/intron (I) boundaries revealed that the mouse PLP-A gene is comprised of five exons and four introns. The beginning of exon 1 is defined as the putative translation start site (ATG),

and the end of exon 5 is defined as the polyadenylation site (AATAAA). Shaded boxes correspond with actual exon sizes. The sizes of the 5' and 3' flanking regions were determined by PCR and agarose gel electrophoresis.



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