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The Mouse Gene *Ptprf* Encoding the Leukocyte Common Antigen-Related Molecule LAR: Cloning, Characterization, and Chromosomal Localization

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The human receptor-like protein tyrosine phosphatase leukocyte common antigen-related molecule (LAR; gene symbol PTPRF) closely resembles cell adhesion molecules, which suggests that it may be involved in the regulation of phosphotyrosine levels through cell–cell or cell–matrix interactions. To obtain a better understanding of LAR function, we have characterized the mouse *Ptprf* gene as a first step toward site-directed mutagenesis studies *in vitro* and *in vivo*. The cytoplasmic region of the mouse LAR (mLAR) protein is encoded by 11 exons that span only 4.5 kb of genomic DNA. Compared to the known exon–intron structures of other mammalian receptor-like protein tyrosine phosphatase genes, such as *Ptpra* (encoding LRP) and *Ptprc* (coding for Ly-5), the *Ptprf* gene part encoding the cytoplasmic region of mLAR contains not only smaller, but also fewer introns. Sequence analysis of both phosphatase domains of mLAR and its homologs MPTPδ and mRPTPσ revealed a higher evolutionary conservation of the second, C-terminal domain in comparison to the first domain. Fluorescence *in situ* hybridization was used to map the *Ptprf* gene to region C6–D1 on mouse chromosome 4.

INTRODUCTION

Protein tyrosine phosphorylation is recognized as an important reversible reaction controlling many aspects of cellular function, including growth and differentiation, cell cycle control, and cytoskeletal integrity (Tonks *et al.*, 1992; Mourey and Dixon, 1994). The role of protein tyrosine kinases (PTKs) in these processes and the induction of their activity have been extensively studied, but the recent identification and characterization of many protein tyrosine phosphatases (PTPases) suggest that dephosphorylation of signal transduction proteins is also a crucial event in signal transduction (Fischer *et al.*, 1991; Brady-Kalnay and Tonks, 1994). To date more than 30 PTPases have been identified (Mourey and Dixon, 1994). Sequence comparisons have revealed that there are two major classes of PTPases (for review see Tonks *et al.*, 1992; Mourey and Dixon, 1994), namely (i) those found in the cytoplasm or nucleus that contain only one tyrosine phosphatase domain and (ii) membrane-bound, receptor-like PTPases (RPTPases) that have two tandemly repeated catalytic domains. There also exist intermediate forms such as HPTPβ (Krueger *et al.*, 1990) and PTP-SL (Hendriks *et al.*, 1995) that are transmembrane proteins with only one catalytic domain.

The RPTPases can be subdivided into five types based on the structure of their extracellular segments (Mourey and Dixon, 1994). Type I represents the CD45 family, which comprises multiple isoforms arising from differential splicing of the RNA of a single gene. Type II members (e.g., LAR, HPTPδ, RPTPσ, RPTPμ, and RPTPκ) are characterized by the presence of multiple immunoglobulin (Ig) and fibronectin (FN) type III-like domains. Type III members carry multiple FN-III domains (e.g., HPTPβ), while type IV members (e.g., HPTPα and HPTPε) possess small glycosylated segments. Finally, type V members (e.g., HPTPζ and HPTPγ) have an amino-terminal motif with homology to carbonic anhydrase.

The extracellular region of the type II RPTPases resembles that of cell adhesion molecules (CAMs) such as N-CAM (Edelman and Crossin, 1991). Recently, homophilic adhesive properties have been attributed to RPTPμ and RPTPκ (Gebbink *et al.*, 1993; Brady-Kalnay *et al.*, 1993; Sap *et al.*, 1994). In combining cell adhesion motifs at the cell surface with intracellular

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession Nos.: mLAR (Z37988), rLAR (M60103), hLAR (Y00815), mPTPδ (D13905), hPTPδ (X54133), mRPTPσ (D28530), rPTPσ (A49104), and hRPTPσ (S78080)

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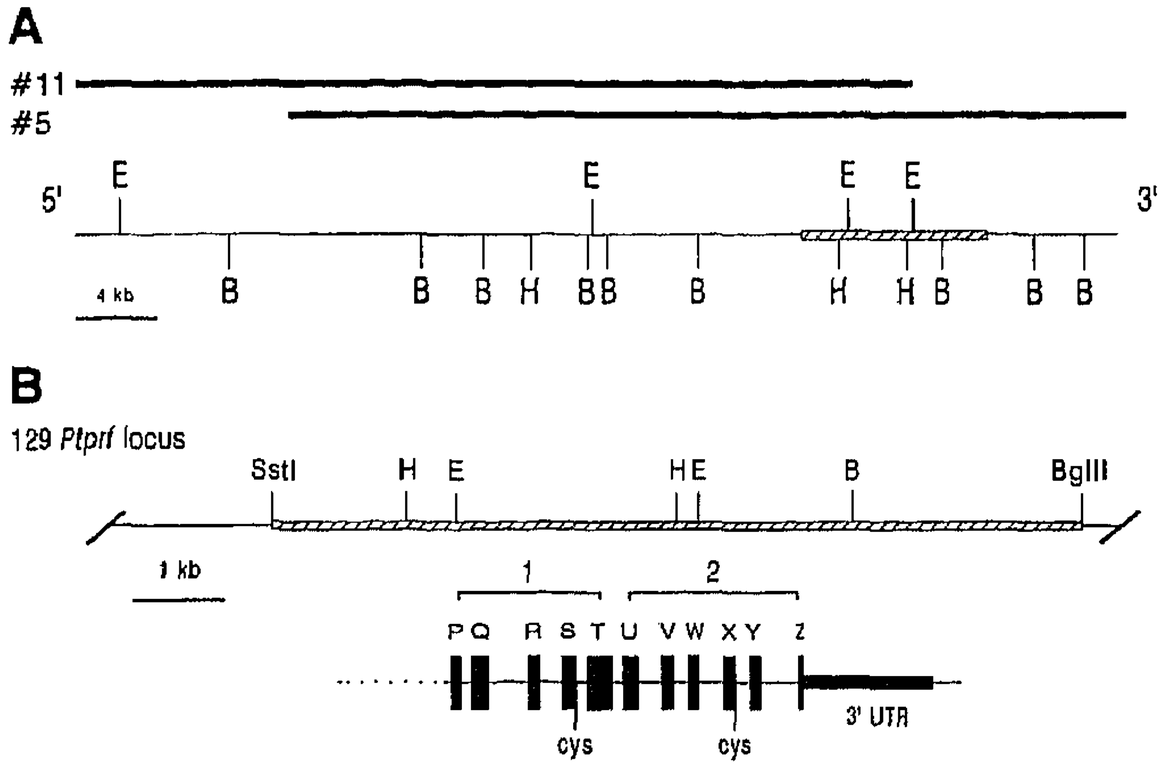

FIG. 1. Schematic representation of the *Ptprf* locus (**A**) and exon–intron structure of the region of the gene encoding the phosphatase domains of the mLAR protein (**B**). (**A**) Thick solid bars indicate the positions of the cosmid clones relative to the *Ptprf* locus. A hatched bar marks the *Sst*I–*Bgl*II fragment that is shown in more detail in **B.** B, *Bam*HI;E, *Eco*RI;H, *Hin*dIII. (**B**) Introns (thin lines) and exons (filled boxes) are drawn to scale. Exons are numbered P thru Z. The regions encoding the two phosphatase domains are indicated by brackets and numbered 1 and 2, respectively. Cys indicates the position of the codon for the conserved cysteine residue within the signature sequence of each phosphatase domain. 3′ UTR, 3′ untranslated region.

phosphatase activity, these RPTPases may be involved in the control of cell growth, motility, and differentiation in a direct response to cell–cell interaction.

The cytoplasmic region of most type II RPTPases contains two tandem phosphatase domains of approximately 260 amino acid residues each, but it is not clear at present whether both domains are enzymatically active *in vivo* or exhibit different substrate specificities. Structure–function relationships have been established only for phosphatase domains expressed in bacteria and therefore outside their natural context. Although site-directed mutagenesis studies have shown the significance of the "signature sequence" (I/V)HCXAGXXR(S/T)G for tyrosine phosphatase activity of the first, transmembrane proximal phosphatase domain (Mourey and Dixon, 1994), it remains to be determined whether the distal phosphatase domain also exhibits catalytic activity (Wang and Pallen, 1991; Tan *et al.*, 1993) or plays only a regulatory role in modulating the activity of the first domain (Streuli *et al.*, 1990; Pot *et al.*, 1991; Krueger and Saito, 1992). However, *in vitro* studies are inadequate to address such questions because specific inhibitors of PTPases are not yet at hand and substrates and ligands are unknown.

To initiate studies of the role of LAR domains in an organismal context, we set out to isolate structural and genomic mouse LAR (mLAR) DNAs. Here we describe the organization of the *Ptprf* gene region encoding the cytoplasmic part of mLAR. Compared with known exon–intron structures of other mouse PTPase genes, such as *Ptpra* (Wong *et al.*, 1993) and *Ptprc* (Saga *et al.*, 1988), the *Ptprf* gene exhibits a more compact structure.

Comparison of both phosphatase domains of mLAR with closely homologous sequences of MPTPδ (Mizuno *et al.*, 1993) and mRPTPσ (Ogata *et al.*, 1994) revealed a higher evolutionary conservation of the second, C-terminal domain compared to the proximal "catalytically active" domain.

Finally, we report the chromosomal localization of the *Ptprf* gene to region C6–D1 of mouse chromosome 4.

MATERIALS AND METHODS

Isolation of mouse LAR genomic and cDNA clones. Cosmid clones were isolated from a mouse 129/SvEv genomic DNA cosmid library (kindly provided by Dr. M. Hofker, University of Leiden, Leiden) using a random-primed (Feinberg and Vogelstein, 1983) 1.5-kb *Eco*RI–*Sal*I human LAR (hLAR) cDNA fragment (positions 4444–5933, Streuli *et al.*, 1988) as a probe. Hybridizations were carried out according to Church and Gilbert (1984), and filters were washed for 5, 10, and 30 min at 65°C in 0.25 *M* sodium phosphate/1% SDS/1 m*M* EDTA. Positive clones were purified by subsequent rounds of low-density screening.

The same hLAR cDNA probe was used to screen an oligo(dT)-primed mouse brain cDNA library in Lambda ZAPII (Stratagene). Filters were washed three times for 30 min at 65°C in 2× SSC/0.2% SDS. Positive phages were plaque-purified, and inserts were rescued as pBluescript SK plasmids according to the manufacturer's protocol.

Sequence analysis. Nested deletion mutants of cDNA clone mLAR#9 were generated using Exonuclease III (Promega), and nucleotide sequences were determined using the double-stranded DNA dideoxy sequencing method (Hattori and Sakaki, 1986). A 6.5-kb *Sst*I–*Bam*HI *Ptprf* genomic fragment was used for exon–intron determination according to the shotgun sequencing strategy of Deininger (1983). Briefly, fragments of around 500 bp were generated by sonication, blunt-ended using Klenow (Boehringer), and subcloned into the *Sma*I site of vector pGEM4 (Promega). Clones, grown in microtiter plates, were replica plated onto nitrocellulose filters and screened with the mLAR cDNA#9 insert. Thirty positive clones were used for sequence determination (Hattori and Sakaki, 1986). DNA sequence gel readings were recorded, compared, edited, and assembled using the IGSUITE 5.35 package (Intelligenetics Inc., Moutain View, CA). Sequences were aligned using the GCG Wisconsin programs PILEUP and LINEUP (Devereux *et al.*, 1984). Evolutionary reconstruction was performed using the program FITCH supplied in the phylogeny inference package PHYLIP (distributed by Dr. J. Felsenstein, University of Washington, Seattle). FITCH uses distance matrices to construct trees without allowing negative branch length (Fitch and Margoliash, 1967).

Fluorescence in situ hybridization. Fluorescence *in situ* hybridization was performed on mouse strain 129-derived E14 embryonic stem cell (Handyside *et al.*, 1989) metaphase spreads for the regional localization of the *Ptprf* gene, using mouse cosmid 5 as a probe. Biotinylated cosmid DNA was dissolved in 10 μl hybridization solution containing 2× SSC, 10% dextran sulfate, 1% Tween-20, and 50% formamide. The probe mixture was heat-denatured at 80°C for 10 min, followed by incubation at 37°C to allow reannealing of highly repetitive sequences. Hybridization of this probe to heat-denatured chromosome spreads, under a cover slip, was carried out overnight at 37°C. Hybridizing probe was detected immunohistochemically using fluorescein isothiocyanate conjugated to avidin-D (Suijkerbuijk *et al.*, 1991). Images of metaphase preparations were captured by a cooled high-performance CCD camera (Photometrics) coupled to a Macintosh II computer. Separate images of both mLAR hybridizing signals and DAPI-counterstained chromosomes were transformed into pseudocolored images using image analysis software.

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                                                                ... ATG CGA GAC CAC CCG CCC ATC        21
                                                                    M   R   D   H   P   P   I        (1306)

CCC ATC ACT GAC CTG GCA GAC AAT ATT GAG CGC CTC AAA GCC AAC GAT GGG CTC AAG TTC TCC CAG GAG TAT GAG    96
 P   I   T   D   L   A   D   N   I   E   R   L   K   A   N   D   G   L   K   F   S   Q   E   Y   E  (1331)
↓Exon P
TCC ATT GAC CCT GGA CAG CAG TTC ACA TGG GAG AAT TCC AAC TCG GAG GTG AAC AAG CCC AAG AAC CGC TAT GCA   171
 S   I   D   P   G   Q   Q   F   T   W   E   N   S   N   S   E   V   N   K   P   K   N   R   Y   A  (1356)
                                                                ↓Exon Q
AAT GTC ATT GCC TAT GAC CAT TCT CGA GTC CTC CTC ACC TCC ATT GAT GGT GTT CCT GGG AGT GAC TAC ATC AAT   246
 N   V   I   A   Y   D   H   S   R   V   L   L   T   S   I   D   G   V   P   G   S   D   Y   I   N  (1381)

GCC AAC TAC ATT GAT GGC TAC CGA AAG CAG AAT GCC TAC ATC GCC ACA CAA GGT CCG CTG CCC GAG ACC ATG GGC   321
 A   N   Y   I   D   G   Y   R   K   Q   N   A   Y   I   A   T   Q   G   P   L   P   E   T   M   G  (1406)

GAT TTC TGG AGG ATG GTG TGG GAA CAG CGC ACA CCC ACA GTG GTC ATG ATG ACC AGG CTA GAG CAG AAA TCC CGG   396
 D   F   W   R   M   V   W   E   Q   R   T   A   T   V   V   M   M   T   R   L   E   E   K   S   R  (1431)
↓Exon R
GTG AAG TGT GAT CAG TAT TGG CCA GTC CGT GGC ACT GAG ACC TAT GGC CTC ATT CAG GTG ACC CTG GTG GAC ACT   471
 V   K   C   D   Q   Y   W   P   V   R   G   T   E   T   Y   G   L   I   Q   V   T   L   V   D   T  (1456)
                                                                ↓Exon S
GTC GAG TTG GCC ACA TAC ACC ATG CGC ACC TTT GCC CTC CAT AAG AGT GGC TCC AGT GAG AAG CGT GAG CTG CGT   546
 V   E   L   A   T   Y   Y   T   M   R   T   F   A   L   H   K   S   G   S   S   E   K   R   E   L   R  (1481)

CAG TTC CAG TTC ATG GCC TGG CCA GAC CAC GGC GTT CCT GAG TAC CCC ACT CCC ATC TTG GCC TTC CTG AGA CGG   621
 Q   F   Q   F   M   A   W   P   D   H   G   V   P   E   Y   P   T   P   I   L   A   F   L   R   R  (1506)
                                                                ↓Exon T
GTC AAG GCC TGT AAC CCA CTA GAT GCG GGG CCC ATG GTG GTG CAT TGC AGT GCG GGT GTG GGG CGC ACA GGC TGC   696
 V   K   A   C   N   P   L   D   A   G   P   M   V   V   H   C   S   A   G   V   G   R   T   G   C  (1531)

TTC ATC GTC ATC GAC GCA ATG CTG GAG CGT ATG AAG CAC GAG AAG ACG GTT GAC ATC TAT GGC CAC GTG ACG TGC   771
 F   I   V   I   D   A   M   L   E   R   M   K   H   E   K   T   V   D   I   Y   G   H   V   T   C  (1556)

ATG CGC TCA CAA AGG AAC TAC ATG GTG CAG ACC GAG GAC CAG TAT GTG TTC ATC CAC GAG GCC CTG CTA GAG GCT   846
 M   R   S   Q   R   N   Y   M   V   Q   T   E   D   Q   Y   V   F   I   H   E   A   L   L   E   A  (1581)

GCC ATG TGC GGA CAC ACC GAG GTG CTC GCT CGG AAC CTC TAT GCC CAC ATC CAG AAG CTA GGC CAA GTG CCT CCC   921
 A   M   C   G   H   T   E   V   L   A   R   N   L   Y   A   H   I   Q   K   L   G   Q   V   P   P  (1606)
                                                                ↓Exon U
GGG GAG AGC GTC ACG GCC ATG GAA CTT GAG TTC AAG TTG CTG GCC AAC TCC AAG GCC CAC ACG TCG CGC TTT GTC   996
 G   E   S   V   T   A   M   E   L   E   F   K   L   L   A   N   S   K   A   H   T   S   R   F   V  (1631)

AGT GCC AAC CTG CCC TGC AAC AAG TTC AAG AAC CGC CTA GTG AAC ATC ATG CCC TAT GAG CTG ACC CGA GTG TGC  1071
 S   A   N   L   P   C   N   K   F   K   N   R   L   V   N   I   M   P   Y   E   L   T   R   V   C  (1656)
                                                                ↓Exon V
TTG CAA CCC ATC CGT GGT GTG GAG GGC TCA GAC TAC ATC AAT GCC AGC TTT CTA GAT GGC TAC AGA CAG CAG AAG  1146
 L   Q   P   I   R   G   V   E   G   S   D   Y   I   N   A   S   F   L   D   G   Y   R   Q   Q   K  (1681)

GCC TAC ATA GCT ACA CAG GGG CCT CTG GCA GAG AGC ACC GAG GAC TTC TGG CGC ATG TTA TGG GAG CAC AAT TCC  1221
 A   Y   I   A   T   Q   G   P   L   A   E   S   T   E   D   F   W   R   M   L   W   E   H   N   S  (1706)
                                                                ↓Exon W
ACC ATC ATC GTC ATG CTG ACC AAG CTT CGG CAG ATG GGC AGG GAG AAA TGT CAC CAG TAC TGG CCA GCA GAG CGC  1296
 T   I   I   V   M   L   T   K   L   R   E   M   G   R   E   K   C   H   Q   Y   W   P   A   E   R  (1731)

TCC GCT CGC TAT CAG TAC TTC GTT GTT GAC CCG ATG GCT GAG TAC AAC ATG CCC CAG TAT ATT CTG CGT GAA TTG  1371
 S   A   R   Y   Q   Y   F   V   V   D   P   M   A   E   Y   N   M   P   Q   Y   I   L   R   E   F  (1756)
                                ↓Exon X
AAA GTC ACA GAC GCC CGG GAT GGG CAG TCA AGG ACA ATC CGA CAG TTC CAG TTT ACA GAC TGG CCA GAG CAA GGA  1446
 K   V   T   D   A   R   D   G   Q   S   R   T   I   R   Q   F   Q   F   T   D   W   P   E   Q   G  (1781)

GTA CCC AAA ACA GGT GAA GGC TTC ATC GAC TTC ATC GGG CAG GTG CAC AAG ACA AAG GAG CAG TTT GGC CAG GAT  1521
 V   P   K   T   G   E   G   F   I   D   F   I   G   Q   V   H   K   T   K   E   Q   F   G   Q   D  (1806)
                                ↓Exon Y
GGG CCC ATC ACG GTG CAC TGC AGT GCT GGT GTG GGC CGC ACC GGT GTG TTC ATC ACC CTG AGC ATT GTC CTG GAG  1596
 G   P   I   T   V   H   C   S   A   G   V   G   R   T   G   V   F   I   T   L   S   I   V   L   E  (1831)

CGC ATG CGC TAT GAG GGT GTG GTT GAC ATG TTC CAG ACC GTG AAG ACC CTC CGC ACA CAG CGC CCT GCA ATG GTG  1671
 R   M   R   Y   E   G   V   V   D   M   F   Q   T   V   K   T   L   R   T   Q   R   P   A   M   V  (1856)
                ↓Exon Z
CAG ACA GAG GAC CAA TAC CAG CTG TGC TAC CGT GCG GCC CTG GAA TAC CTC GGC AGC TTT GAT CAC TAT GCA ACG  1746
 Q   T   E   D   Q   Y   Q   L   C   Y   R   A   A   L   E   Y   L   G   S   F   D   H   Y   A   T  (1881)

TAA CTACTGCTCCCCTCTCCTCCGACGCTCCCCCGCGGCTCCGGAGGGACCCAGCTCCTCTGAGCCATACCAACCATCGTCCAGCCCTCCTGCACGGA  1844
 *

TGCTGTTGCCGGCAGAGCACAGCCCACTGGGATCACAGCATTTCGGGGAACATTGCCACACCAGTCAGAGAGCCCAGAACACCTGGGCAAGTAGGCGGA  1943
CTGGCAGCCTGGCTCTGGGCCCTCGTCCACCGGGCCAAGTGGAGCCCCGCTTCAAGCTCTCTGTTCAGCTCCGCGTTCTCATGCTTCTCATGGGGTGGG  2042

AAAAGGGGGCAAAGCCCCCACTTTTTATACACTAGGCGGGGTAGACTGCGGGGTCCTAGCCTCTTCCTCCGACTTTGCTTTTGCAGGTCTTTCACTGCA  2141
GATGGGGCTGCTGTGGGAGTTGGGACTTGTTTGTTTTCCTTTTTGAGTTCACGTTGGATCC...                                      2202
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FIG. 2. Definition of the exon–intron boundaries within the partial mLAR cDNA sequence. Exon–intron boundaries are indicated by arrows at the first nucleotide of each exon. The 3′ UTR sequence is given up to the *Bam*HI site as indicated in Fig. 1. Numbers to the right indicate nucleotides as present in clone mLAR#9 and, in parentheses, amino acids according to the numbering system of Streuli *et al.* (1988). Regions that exhibit 100% identity with previously published PCR-derived mLAR fragments (Hendriks *et al.*, 1995) are shown on a gray background. Underlined sequences indicate the "active site" signature motif.

RESULTS AND DISCUSSION

Isolation and Characterization of the mLAR-Encoding Ptprf Gene

We are currently investigating the biological role of LAR and the functions of the tyrosine phosphatase domains in the LAR signal transduction pathway using reverse genetic approaches. As a first step we isolated and characterized mouse LAR genomic and cDNA clones, with special emphasis on the region encoding the phosphatase domains.

A 1.5-kb *Eco*RI–*Sal*I hLAR cDNA probe encoding

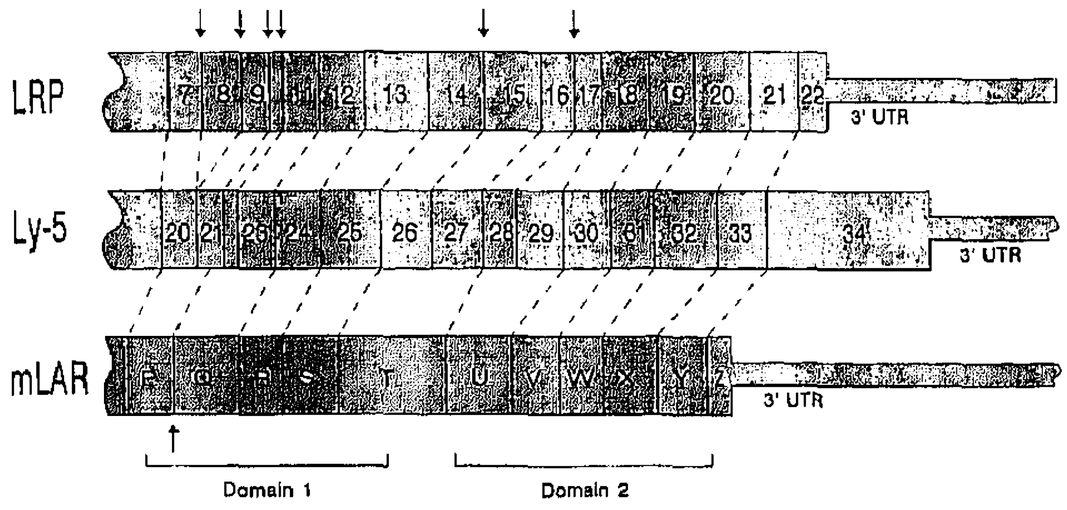

FIG. 3. *Comparison of exon segmentation as observed in the Ptpra, Ptprc,* and *Ptprf* genes within the region encoding the phosphatase domains of RPTPases LRP (Wong *et al.,* 1993), Ly-5 (Saga *et al.,* 1988), and mLAR, respectively. Dashed lines indicate identical boundaries. Downward arrows indicate introns present in *Ptpra* and *Ptprc* but missing in the *Ptprf* gene. The upward arrow points to an intron present in *Ptprf* but absent in *Ptpra* and *Ptprc.* Exon numbers for LRP and Ly-5 are according to the literature.

both phosphatase domains (Streuli *et al.,* 1988) was used to isolate cosmid clones, two of which, 5 and 11, repeatedly showed stronger hybridization signals under more stringent washing conditions. These clones were characterized further. Southern blot and restriction enzyme analyses revealed that these two clones overlap and span some 50 kb of the mouse *Ptprf* locus, as shown in Fig. 1. We assume that the other, more weakly hybridizing cosmid clones contained either *Ptprf* pseudogenes or genes with homology to *Ptprf,* such as *Ptprd* (encoding MPTPδ, Mizuno *et al.,* 1993) or *Ptprs* (coding for mRPTPσ, Ogata *et al.,* 1994).

We compared genomic and cDNA sequences to position exon–intron boundaries within the *Ptprf* gene. To this end, 10 cDNA clones were isolated from a Lambda ZAPII mouse brain cDNA library using the hLAR cDNA probe mentioned above. End-in sequencing and comparison with the cognate positions in hLAR (Streuli *et al.,* 1988), rat LAR (rLAR, Pot *et al.,* 1991), and fragments of mLAR (Hendriks *et al.,* 1995) revealed that three clones contained genuine mLAR cDNAs. The other cDNA clones contained mLAR homologous sequences. In Fig. 2, the sequence of the largest mLAR cDNA clone, mLAR#9, is depicted alongside the deduced amino acid sequence.

All three mLAR cDNAs ended in an A-rich stretch in the 3′ untranslated region (3′ UTR) also found in the hLAR mRNA message (position 6900, Streuli *et al.,* 1988). Screening of the mouse cDNA library with a more 3′ UTR hLAR probe (*Sal*I–*Eco*RI, positions 5933–7700, Streuli *et al.,* 1988) yielded additional clones that terminated at the genuine polyadenylation

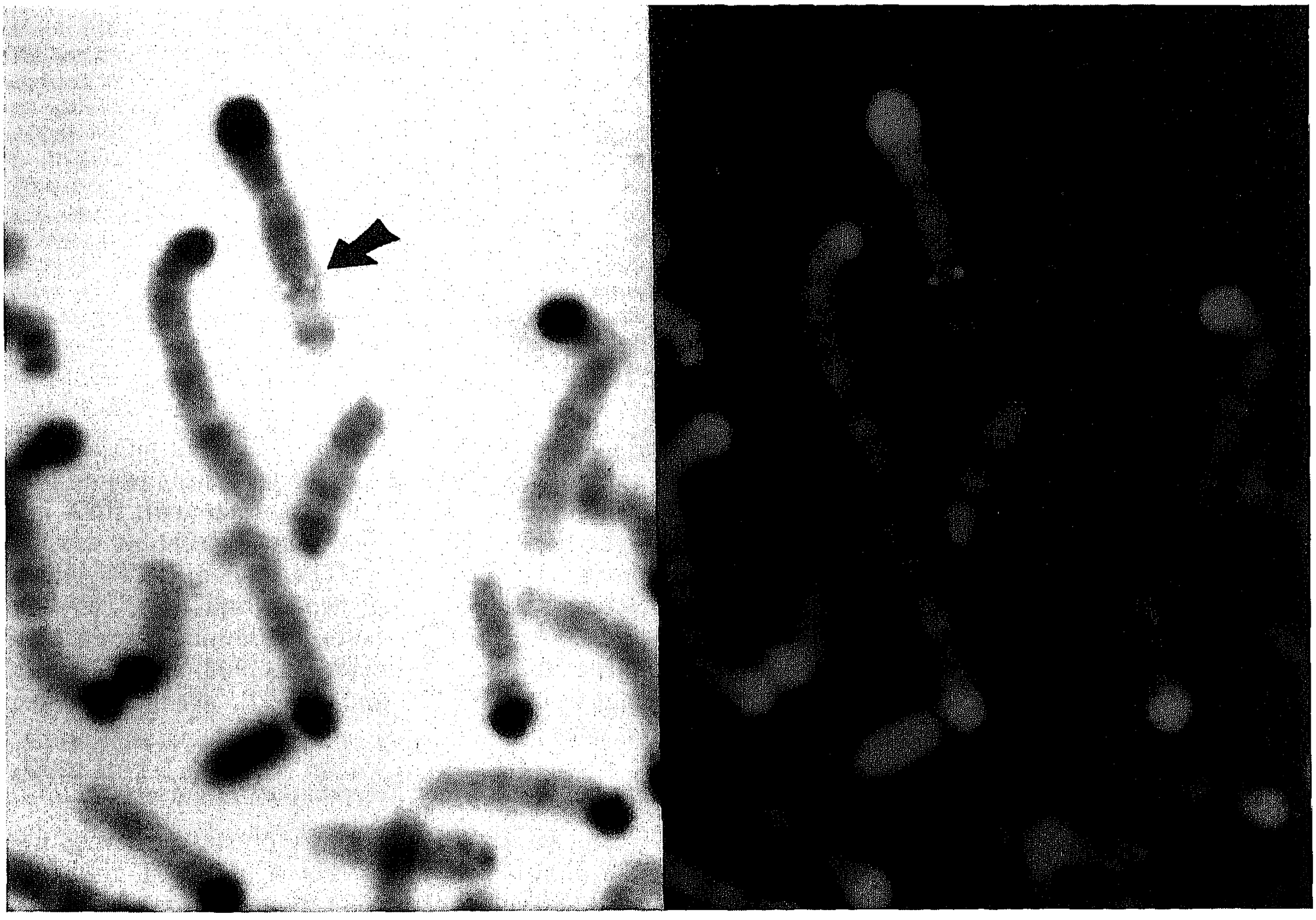

FIG. 4. Chromosomal localization of the *Ptprf* gene. Unique hybridizing signals of cosmid mLAR#5 were recorded in more than 30 metaphase spreads (**right**). Positively imaged chromosomes were counterstained with DAPI for the identification of the individual chromosomal subbands (**left**).

site as predicted from hLAR sequence data (not shown). We therefore conclude that clone mLAR#9 represents a mLAR cDNA in which the reverse transcription reaction started at the A-rich stretch within the 3′ UTR.

Southern blot analysis using cDNA clone mLAR#9 as a probe revealed that the sequence encoding the mLAR cytoplasmic region resided in a 6.5-kb *Sst*I–*Bam*HI genomic fragment. Sequence analysis showed both phosphatase domains to be encoded by 11 exons contained within a 4.5-kb *Eco*RI–*Bam*HI fragment (Fig. 1). The most 3′ exon contains the last 22 codons for the protein and the entire 3′ UTR of the mRNA. The intron lengths in the gene segment shown in Fig. 1 vary from only 80 bp to 0.5 kb. Comparison of the deduced organization of the *Ptprf* gene with that of the *Ptpra* and *Ptprc* RPTPase genes (encoding for LRP and Ly-5, respectively, Wong *et al.*, 1993; Saga *et al.*, 1988) revealed conspicuous differences (Fig. 3). Whereas the genomic organization of the first phosphatase domain is similar but not identical to the second domain in all three PTPase family members, both mLAR phosphatase domains together are encoded by only 11 exons, in contrast to the 17 (spanning ~34 kb) and 16 (spanning ~27 kb) exons for LRP and Ly-5, respectively. This finding is at variance with the conclusions of Wong *et al.* (1993), who postulated that all RPTPases exhibit a similar genomic organization of the region encoding the phosphatase domains. Our results show clear differences in the gene structure of RPTPases. We propose that an ancestral gene originally contained a single tyrosine phosphatase domain encoded by at least nine exons. This may have been duplicated later to produce RPTPases with two phosphatase domains. Subsequently, several intron losses may have occurred for both of the phosphatase domains as well as for the various types of RPTPases. One intron (between exons P and Q) still present in the *Ptprf* gene has been removed from the *Ptpra*/*Ptprc* ancestor. In the *Ptprf* gene, five introns that are still present in the other two RPTPase genes have been removed (Fig. 3). The reason why the gene structure of the *Ptprf* gene is so much more compact than other RPTPase genes remains, however, obscure for the moment.

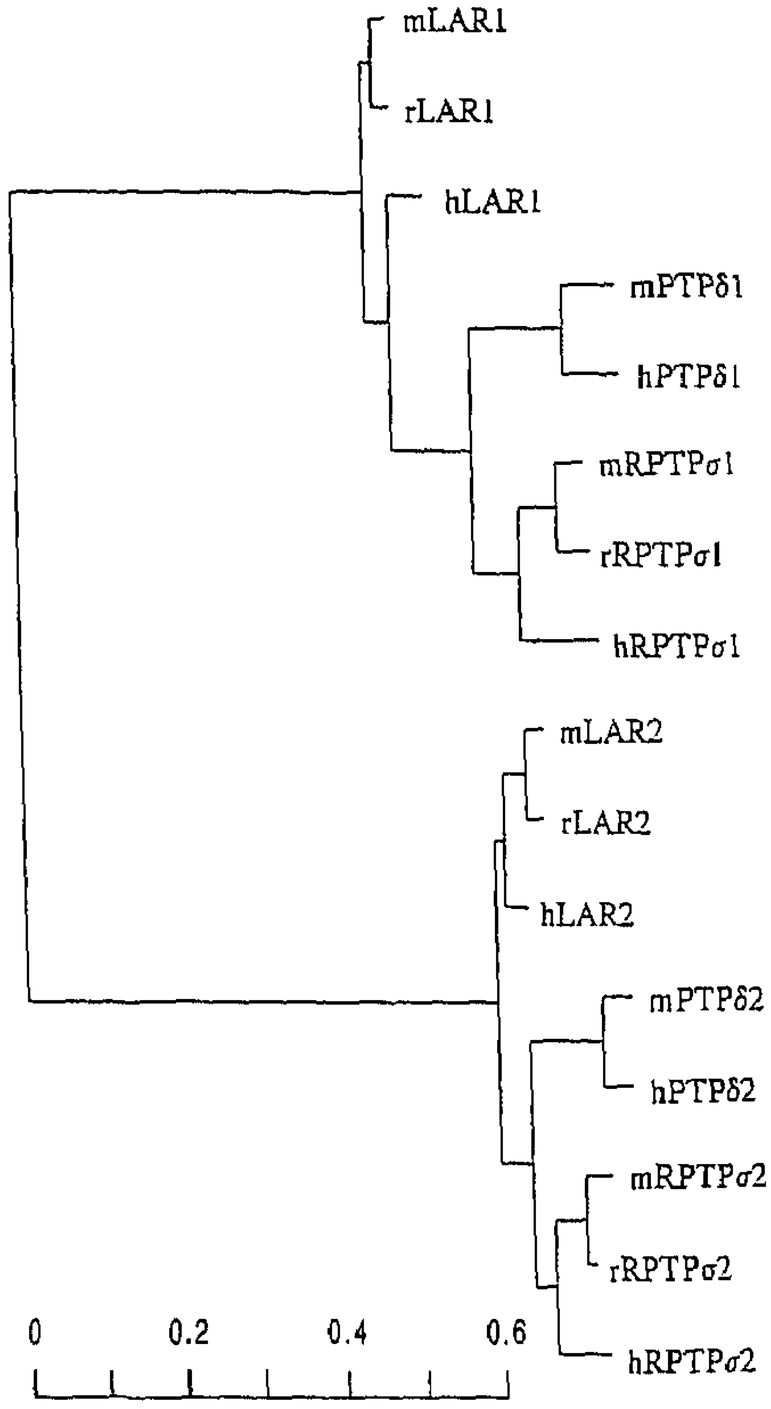

FIG. 5. A proposed phylogenetic tree of phosphatase domains 1 and 2 of the human, rat, and mouse PTPases LAR, PTPδ, and RPTPσ. Nucleotide sequences encoding the region that starts immediately after the sequence DFWRM(I/V)W(E/D) and ends before the stretch HCSAG(V/I)GR were used for both domains of mLAR (Accession No. Z37988), rLAR (M60103), hLAR (Y00815), mPTPδ (D13905), hPTPδ (X54133), mRPTPσ (D28530), rPTPσ (A49104), and hRPTPσ (S78080). This unrooted tree was constructed on the basis of a distance matrix created according to the Kimura 2-parameter model (Kimura, 1980) with a transition/transversion ratio of 2, using the program FITCH and performing global rearrangements to verify the obtained topology. Changes in sequence input order did not alter the tree topology. The scale for branch lengths (bottom) represents the expected underlying number of changes per site.

Chromosomal Localization of the Ptprf Gene

Mouse cosmid clone mLAR#5 was used as a probe for fluorescence *in situ* hybridization to determine the chromosomal localization of the *Ptprf* locus. Alignment to G-banded chromosome images showed that the *Ptprf* gene localizes within the region C6–D1 of mouse chromosome 4 (Fig. 4). Based on data on evolutionary conservation of chromosome segments in human and mouse (Lyon and Kirby, 1993), this is in agreement with the localization of the PTPRF gene within the p32–p33 region of human chromosome 1 (Streuli *et al.*, 1992). This localization is distinct from those of the *Ptprd* and *Ptprs* loci, which have been assigned to the region around the *b* locus on mouse chromosome 4 (Mizuno *et al.*, 1993) and distal mouse chromosome 17 (Yan *et al.*, 1993), respectively. Together with RNA *in situ* hybridization data, which reveal different expression patterns for mLAR, MPTPδ, and mRPTPσ (R. Q. J. Schaapveld, J. T. G. Schepens, and W. J. A. J. Hendriks, unpublished results), these mapping data are in agreement with Mizuno *et al.* (1993) and demonstrate that mLAR, MPTPδ, and mRPTPσ are indeed separate entities.

Homology to Other Receptor-like PTPases

The mLAR cDNA sequence presented here is nearly identical to that of rat (Pot *et al.*, 1991) and human LAR (Streuli *et al.*, 1988); the overall homology at the amino acid level is 99.6 and 98.2%, respectively. The homology (up to 90% at the amino acid level) to other type II RPTPases, MPTPδ and mRPTPσ (Mizuno *et*

al., 1993; Ogata *et al.*, 1994), which also contain two catalytic domains, a single transmembrane domain, and, extracellularly, eight FN-III and three Ig-like domains, is also striking. Indeed, we also isolated clones representing MPTPδ and mRPTPσ by screening both the genomic and the cDNA libraries at low stringency (not shown). Nucleotide sequence alignment of the phosphatase domains was used to gain insight into their evolutionary history. A phylogenetic tree, as depicted in Fig. 5, suggests that a common ancestor for PTPδ and RPTPσ diverged from the LAR lineage most probably before the time of mammalian radiation. These results suggest that the mutation rate for LAR is lower than those for both PTPδ and RPTPσ, reflecting the maintenance of selective pressure on the LAR lineage and the adaptation to new roles in growth and development for PTPδ and RPTPσ.

In addition, the homology among these three proteins is more conserved in phosphatase domain 2 than in domain 1. This is not seen for the other, more distant members of the type II RPTPases (i.e., RPTPμ and RPTPκ) or among type IV (e.g., HPTPα and HPTPϵ) and type V (e.g., HPTPζ and HPTPγ) RPTPases (Jiang *et al.*, 1993; Krueger *et al.*, 1990; Krueger and Saito, 1992; Kaplan *et al.*, 1990). Further studies of the biological significance of each individual phosphatase domain in RPTPases are now in progress.

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Note added in proof. An identical genomic structure has recently been described for the human LAR-encoding PTPRF gene (O'Grady *et al.*, 1994). O'Grady, P., Krueger, N. X., Streuli, M., and Saito, H. (1994). Genomic organization of the human LAR protein–tyrosine phosphatase gene and alternative splicing in the extracellular fibronectin type-III domains. *J. Biol. Chem.* **269:** 25193–25199.

REFERENCES

Brady-Kalnay, S. M., Flint, A. J., and Tonks, N. K. (1993). Homophilic binding of PTPμ, a receptor-type protein tyrosine phosphatase, can mediate cell–cell aggregation. *J. Cell Biol.* **122:** 961–972.

Brady-Kalnay, S. M., and Tonks, N. K. (1994). Protein tyrosine phosphatases: From structure to function. *Trends Cell Biol.* **4:** 73–76.

Church, G. M., and Gilbert, W. (1984). Genomic sequencing. *Proc. Natl. Acad. Sci. USA* **81:** 1991–1995.

Deininger, P. L. (1983). Random sequencing of sonicated DNA: Application to shotgun DNA sequence analysis. *Anal. Biochem.* **129:** 216–223.

Devereux, J., Haeberli, P., and Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* **12:** 387–395.

Edelman, G. M., and Crossin, K. L. (1991). Cell adhesion molecules: Implications for a molecular histology. *Annu. Rev. Biochem.* **60:** 155–190.

Feinberg, A. P., and Vogelstein, B. (1983). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132:** 6–13.

Fischer, E. H., Charbonneau, H., and Tonks, N. K. (1991). Protein tyrosine phosphatases: A diverse family of intracellular and transmembrane enzymes. *Science* **253:** 401–406.

Fitch, W. M., and Margoliash, E. (1967). Construction of phylogenetic trees. *Science* **155:** 279–284.

Gebbink, M. F. B. G., Zondag, G. C. M., Wubbolts, R. W., Beijersbergen, R. L., van Etten, I., and Moolenaar, W. H. (1993). Cell–cell adhesion mediated by a receptor-like protein tyrosine phosphatase. *J. Biol. Chem.* **268:** 16101–16104.

Handyside, A. H., O'Neill, G. T., Jones, M., and Hooper, M. L. (1989). Use of BRL-conditioned medium in combination with feeder layers to isolate a diploid embryonal stem cell line. *Roux Arch. Dev. Biol.* **198:** 48–56.

Hattori, M., and Sakaki, Y. (1986). Dideoxy sequencing method using denatured plasmid templates. *Anal. Biochem.* **152:** 232–238.

Hendriks, W., Schepens, J., Brugman, C., Zeeuwen, P., and Wieringa, B. (1995). A novel receptor-type protein-tyrosine phosphatase with a single catalytic domain is specifically expressed in mouse brain. *Biochem. J.* **305:** 499–504.

Jiang, Y.-P., Wang, H., D'Eustachio, P., Musacchio, J. M., Schlessinger, J., and Sap, J. (1993). Cloning and characterization of R-PTPκ, a new member of the receptor protein tyrosine phosphatase family with a proteolytically cleaved cellular adhesion molecule-like extracellular region. *Mol. Cell. Biol.* **13:** 2942–2951.

Kaplan, R., Morse, B., Huebner, K., Croce, C., Howk, R., Ravera, M., Ricca, G., Jaye, M., and Schlessinger, J. (1990). Cloning of three human tyrosine phosphatases reveals a multigene family of receptor-linked protein-tyrosine phosphatases expressed in brain. *Proc. Natl. Acad. Sci. USA* **87:** 7000–7004.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16:** 111–120.

Krueger, N. X., and Saito, H. (1992). A human transmembrane protein-tyrosine phosphatase, PTPζ, is expressed in brain and has an N-terminal receptor domain homologous to carbonic anhydrases. *Proc. Natl. Acad. Sci. USA* **89:** 7417–7421.

Krueger, N. X., Streuli, M., and Saito, H. (1990). Structural diversity and evolution of human receptor-like protein tyrosine phosphatases. *EMBO J.* **9:** 3241–3252.

Lyon, M. F., and Kirby, M. C. (1993). Mouse chromosome atlas. *Mouse Genome* **91:** 40–80.

Mizuno, K., Hasegawa, K., Katagiri, T., Ogimoto, M., Ichikawa, T., and Yakura, H. (1993). MPTPδ, a putative homolog of HPTPδ, is expressed in specialized regions of the brain and in the B-cell lineage. *Mol. Cell. Biol.* **13:** 5513–5523.

Mourey, R. J., and Dixon, J. E. (1994). Protein tyrosine phosphatases: Characterization of extracellular and intracellular domains. *Curr. Opin. Genet. Dev.* **4:** 31–39.

Ogata, M., Sawada, M., Kosugi, A., and Hamaoka, T. (1994). Developmentally regulated expression of a murine receptor-type protein tyrosine phosphatase in the thymus. *J. Immunol.* **153:** 4478–4487.

Pot, D. A., Woodford, T. A., Remboutsika, E., Haun, R. S., and Dixon, J. E. (1991). Cloning, bacterial expression, purification, and characterization of the cytoplasmic domain of rat LAR, a receptor-like protein tyrosine phosphatase. *J. Biol. Chem.* **266:** 19688–19696.

Saga, Y., Tung, J.-S., Shen, F.-W., Pancoast, T. C., and Boyse, E. A. (1988). Organization of the *Ly-5* gene. *Mol. Cell. Biol.* **8:** 4889–4895.

Sap, J., Jiang, Y.-P., Friedlander, D., Grumet, M., and Schlessinger, J. (1994). Receptor tyrosine phosphatase R-PTPκ mediates homophilic binding. *Mol. Cell. Biol.* **14:** 1–9.

Streuli, M., Krueger, N. X., Hall, L. R., Schlossman, S. F., and Saito, H. (1988). A new member of the immunoglobulin superfamily that has a cytoplasmic region homologous to the leukocyte common antigen. *J. Exp. Med.* **168:** 1553–1562.

Streuli, M., Krueger, N. X., Thai, T., Tang, M., and Saito, H. (1990). Distinct functional roles of the two intracellular phosphatase like domains of the receptor-linked protein tyrosine phosphatases LCA and LAR. *EMBO J.* **9:** 2399–2407.

Streuli, M., Krueger, N. X., Ariniello, P. D., Tang, M., Munro, J. M., Blattler, W. A., Adler, D. A., Disteche, C. M., and Saito, H. (1992). Expression of the receptor-linked protein tyrosine phosphatase LAR: Proteolytic cleavage and shedding of the CAM-like extracellular structure. *EMBO J.* **11:** 897–907.

Suijkerbuijk, R. F., van de Veen, A. Y., van Echten, J., Buys, C. H. C. M., de Jong, B., Oosterhuis, J. W., Warburton, D. A., Cassiman, J. J., Schonk, D., and Geurts van Kessel, A. (1991). Demonstration of the genuine iso(12p) character of the standard marker chromosome of testicular germ cell tumors and identification of further chromosome 12 aberrations by competitive in situ hybridization. *Am. J. Hum. Genet.* **48:** 269–273.

Tan, X., Stover, D. R., and Walsh, K. A. (1993). Demonstration of protein tyrosine phosphatase activity in the second of two homologous domains of CD45. *J. Biol. Chem.* **268:** 6835–6838.

Tonks, N. K., Yang, Q., Flint, A. J., Gebbink, M. F. B. G., Franza, B. R., Jr., Hill, D. E., Sun, H., and Brady-Kalnay, S. (1992). Protein tyrosine phosphatases: The problems of a growing family. *Cold Spring Harbor Symp. Quant. Biol.* **57:** 87–94.

Wang, Y., and Pallen, C. J. (1991). The receptor-linked protein tyrosine phosphatase HPTPα has two catalytic domains with distinct substrate specificities. *EMBO J.* **10:** 3231–3237.

Wong, E. C. C., Mullersman, J. E., and Thomas, M. L. (1993). Leukocyte common antigen-related phosphatase (LRP) gene structure: Conservation of the genomic organization of transmembrane protein tyrosine phosphatases. *Genomics* **17:** 33–38.

Yan, H., Grossman, A., Wang, H., D'Eustachio, P., Mossie, K., Musacchio, J. M., Silvennoinen, O., and Schlessinger, J. (1993). A novel receptor tyrosine phosphatase-σ that is highly expressed in the nervous system. *J. Biol. Chem.* **268:** 24880–24886.