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Homologues for Prolactin-Like Proteins A and B Are Present in the Mouse[1]

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ABSTRACT

The prolactin (PRL) family consists of a collection of proteins expressed in the uterus, placenta, and anterior pituitary. These cytokines/hormones are hypothesized to control maternal-fetal adaptations to pregnancy. Establishment of mouse models for members of the PRL family expands the experimental repertoire available for investigations on their biological activities. In this report, we establish the presence of mouse homologues for two rat members, PRL-like protein-A (PLP-A) and PLP-B. We present data on their cDNAs and describe aspects of their expression in uteroplacental tissues. A mouse genomic DNA fragment was found to hybridize with a rat PLP-A cDNA. Perusal of the National Center for Biotechnology Information dbEST database resulted in the identification of several putative mouse PLP-A cDNAs and a single putative mouse PLP-B cDNA. The cDNAs were obtained from the IMAGE consortium and Research Genetics and sequenced, and their corresponding mRNAs and proteins were characterized. Overall, mouse PLP-A and PLP-B showed considerable similarities with rat PLP-A and PLP-B in both structure and expression. PLP-A was expressed in both trophoblast giant cells and spongiotrophoblast cells, whereas PLP-B was expressed in decidual and spongiotrophoblast cells. However, some notable exceptions were evident. Mouse PLP-A contained a single putative *N*-linked glycosylation site and consisted of a single 29-kDa protein species, whereas rat PLP-A contained two putative *N*-linked glycosylation sites and consisted of two protein species, of 29 and 33 kDa. Subtle differences in the expression patterns in the mouse and rat are also apparent. In summary, we have established the presence of PLP-A and PLP-B in the mouse. The findings expand our knowledge of these two cytokines/hormones and provide additional strategies for studying their function.

INTRODUCTION

Uteroplacental tissues of the mouse and rat are known to express a family of proteins structurally related to prolactin (PRL) [1–3]. These proteins are proposed to participate in the orchestration of maternal adaptations to pregnancy [1–3]. The basic structure and expression patterns of many members of the family have been well characterized [1–3]. Biological actions for some PRL family members are also well appreciated. A subgroup referred to as placental lactogens (PLs) activate PRL receptor signaling pathways and possess biological actions similar to those attributed to pituitary PRL [4–11]. Another subgroup, including proliferin (PLF) and proliferin-related protein (PLF-RP), modulate blood vessel development [12, 13]. The physiology of the remaining PRL family members is largely unknown. PRL-like protein-A (PLP-A) and PLP-B represent two members of this latter group [14–17]. They were originally identified during the cloning of PL-II [14, 15]. Aspects of their structure and patterns of expression in rat uteroplacental tissues have been reported [18–22]; however, their physiological roles during pregnancy are yet to be resolved. Approaches for investigating the physiology of PLP-A and PLP-B during pregnancy have been limited because of the absence of mouse models and of the application of gene manipulation strategies.

In this report, we establish the presence of PLP-A and PLP-B in the mouse, characterize their cDNAs, and describe aspects of their expression in uteroplacental tissues. Our identification of mouse PLP-A and PLP-B grew out of an initial genomic DNA analysis of PLP-A and the subsequent discovery of both PLP-A and PLP-B expressed sequence tags (ESTs) in the National Center for Biotechnology Information (NCBI, Bethesda, MD) dbEST database.

MATERIALS AND METHODS

Reagents

Fetal bovine serum (FBS) and donor horse serum were purchased from JRH Bioscience (Lenexa, KS). Reagents for PAGE were purchased from Bio-Rad Laboratories (Hercules, CA). All restriction enzymes were purchased from New England Biolabs (Beverly, MA). Mouse PLP-A and PLP-B cDNAs were obtained from Research Genetics (Huntsville, AL). The pSV2-Neo plasmid and the 293 cell line of human fetal kidney origin were obtained from American Type Culture Collection (Rockville, MD). DNA extraction kits were purchased from Qiagen (Chatsworth, CA). Nitrocellulose and nylon membranes were obtained from Schleicher and Schuell (Keene, NH). T7 DNA sequencing kits were acquired from United States Biochemical (Cleveland, OH). Radiolabeled nucleotides were purchased from DuPont-NEN (Boston, MA). TRIzol reagent for RNA extraction and the pCMV-SPORT expression vector were obtained from Life Technologies (Gaithersburg, MD). Avidin-biotin immunoperoxidase kits were purchased from Zymed Laboratories (South San Francisco, CA). Reagents for the detection of immune complexes by enhanced chemiluminescence were acquired from Amersham Corporation (Arlington Heights, IL). Unless otherwise noted, all other chemicals and reagents were purchased from Sigma Chemical Company (St. Louis, MO).

Animals and Tissue Preparation

CD-1 mice were obtained from Charles River Inc. (Wilmington, MA). Holtzman rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN). The animals were housed in an environmentally controlled facility, with lights-on from 0600–2000 h, and allowed free access to food and water. Timed pregnancies and tissue dissections were performed as previously described [23]. The presence of a copulatory plug was designated Day 1 of pregnancy. Pseudopregnancy was induced by mating with a vasectom-

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ized male. Deciduomal responses were induced on Day 4 of pseudopregnancy by injection of 50–75 μl of sesame oil per uterine horn. Tissue dissections, conditioned medium collection, and cytosol preparations were performed as previously described [16, 18]. Protocols for the care and use of animals were approved by the University of Kansas Animal Care and Use Committee.

Southern Blot Analysis

The presence of PLP-A or PLP-A-related DNA fragments in rat and mouse genomic DNA preparations was determined according to the procedure of Southern [24]. Genomic DNA was isolated from rat and mouse livers and digested with *Bam*HI. Digested samples were then electrophoretically separated in agarose gels, transferred to nylon membranes, and probed with a ^{32}P-labeled rat PLP-A cDNA [14].

Characterization of PLP-A and PLP-B cDNAs

DNA sequencing was performed by the dideoxy chain termination method [25] using T7 and SP6 primers and oligonucleotide primers corresponding to internal regions of the mouse PLP-A and PLP-B sequences. Sequencing reactions utilized [^{35}S]dATP and were performed with T7 sequencing kits. Both strands of the cDNAs were completely sequenced. Reaction products were resolved in 6% polyacrylamide urea gels, dried, and exposed to Kodak XAR film (Eastman Kodak, Rochester, NY).

Heterologous Production of Mouse Recombinant PLP-A and PLP-B

The human fetal kidney cell line, referred to as 293, was used as a host for the expression of recombinant mouse PLP-A and PLP-B. The 293 cells were routinely maintained in minimum essential medium (MEM) supplemented with 20 mM HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FBS in an atmosphere of 5% CO_2:95% air at 37°C in a humidified incubator. pCMV-SPORT expression vectors containing either mouse PLP-A or mouse PLP-B cDNAs were transfected along with pSV2-Neo into 293 cells via electroporation [26]. After a 2-wk selection with 500 μg/ml G418, the cells were grown to confluence and transferred to serum-free culture medium. Conditioned medium was collected after 72 h, clarified by centrifugation, and stored at −20°C until used.

Analysis of PLP-A and PLP-B Expression

The expression of PLP-A and PLP-B in the mouse was assessed by biochemical and cytochemical procedures.

Northern blot analysis of PLP-A and PLP-B mRNA expression was performed as previously described in our laboratory [27, 28]. Total RNA was extracted from various mouse tissues essentially as described by Chomczynski and Sacchi [29], using TRIzol. Blots were probed with ^{32}P-labeled mouse PLP-A, PLP-B, or ribosomal protein L7 cDNAs [28, 30].

Western blot analyses for PLP-A and PLP-B were performed as previously described [16–18]. Samples were separated by 12% PAGE under reducing conditions and transferred to nitrocellulose membranes. Immunoreactive bands were visualized using a chemiluminescent detection system (Arlington Heights, IL). Native and recombinant preparations of rat PLP-A [17] and rat PLP-B [18] were used as controls in the immunoblotting experiments.

Tissue and cellular localization of PLP-A and PLP-B proteins was determined by immunocytochemistry using a streptavidin-biotin immunoperoxidase kit for rabbit IgG [17, 18, 31]. The immunostained sections were counterstained with hematoxylin. Specificity of the immunoreactions was demonstrated using preimmune serum and/or preadsorbed antibodies.

RESULTS

Genomic Analysis of PLP-A in Mouse and Rat

The discovery of PLP-A in the rat occurred well over a decade ago [14]. Although considerable progress has been made in studying some aspects of PLP-A biochemistry and biology, a specific function has not been assigned to PLP-A. The availability of additional genetic manipulation strategies in the mouse prompted us to determine whether a PLP-A homologue existed in the mouse. Genomic DNA was isolated from both the rat and mouse, and Southern blot analysis was performed with a ^{32}P-labeled rat PLP-A cDNA. Two prominent DNA fragments of 1.1 and 1.6 kilobases (kb) were detected in the rat, whereas a prominent fragment of 1.1 kb and a more weakly hybridizing DNA species of 8.5 kb were identified in the mouse (Fig. 1). The results of this experiment suggested that a gene related to PLP-A might exist in the mouse genome. We followed the Southern analysis with a comprehensive search of existing databases for sequences related to PLP-A. Several ESTs with significant homology to rat PLP-A were identified within the NCBI dbEST database. Additionally, a single EST was found with homology to rat PLP-B. These mouse ESTs had been isolated from cDNA libraries generated from embryonic and extraembryonic tissues as part of the IMAGE consortium [32].

PLP-A cDNA Characterization

A mouse cDNA exhibiting sequence homology to rat PLP-A was obtained from the IMAGE consortium and Research Genetics (clone No. 553090). Both strands of the full-length putative mouse PLP-A cDNA were sequenced (Fig. 2). The cDNA exhibited 88% nucleotide identity with the previously described rat PLP-A cDNA [14] and was found to encode for a 227-amino acid protein with 81% amino acid identity with the rat PLP-A protein (Fig. 3). On the basis of homology with rat PLP-A, it was further determined that mouse PLP-A contained a 31-amino acid signal peptide. Both mouse and rat PLP-A also contain five homologously positioned cysteine residues. A prominent difference between the mouse and rat structures was the presence of a single putative *N*-linked glycosylation site in the mouse and two putative *N*-linked glycosylation sites in the rat. The amino-terminally located putative *N*-linked glycosylation site of rat PLP-A was absent in the mouse (Fig. 3).

PLP-B cDNA Characterization

A mouse cDNA exhibiting sequence homology to rat PLP-B was obtained from the IMAGE consortium and Research Genetics (clone No. 557206). The clone had erroneously been identified as being related to mouse PLF-RP. Both strands of the full-length putative PLP-B cDNA were sequenced (Fig. 4). The cDNA exhibited 80% nucleotide identity with the previously described rat PLP-B cDNA [15] and was found to encode for a 234-amino acid protein with 64% amino acid identity with the rat PLP-B protein

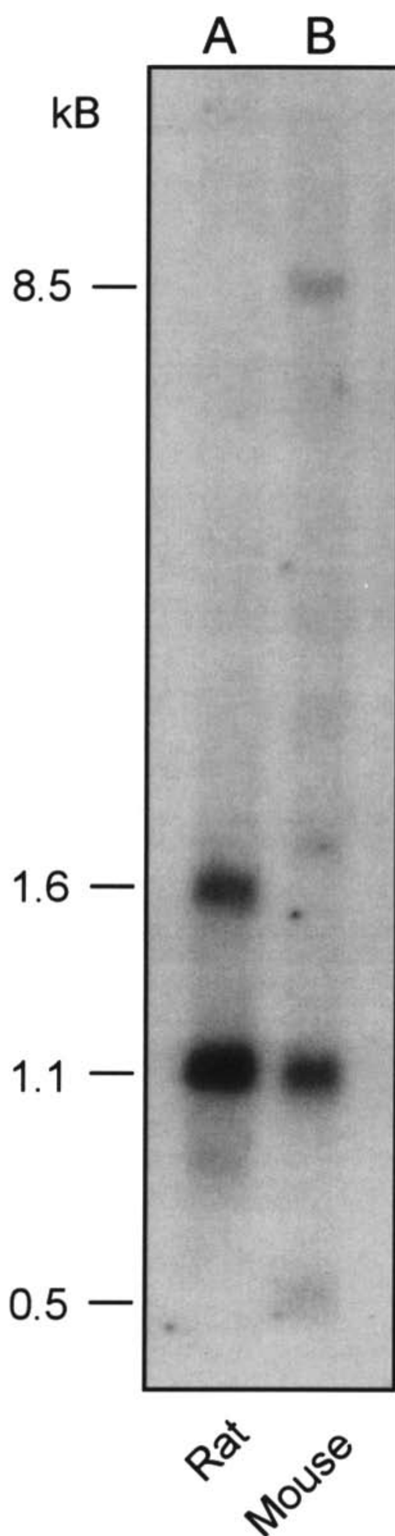

FIG. 1. Southern blot analysis of PLP-A from rat and mouse genomic DNA. Genomic DNA was isolated from rat (lane A) and mouse (lane B) livers and digested with *Bam*HI. Digested samples were then electrophoretically separated in agarose gels, transferred to nylon membranes, and probed with a ^{32}P-labeled rat PLP-A cDNA. The rat PLP-A probe hybridized with both rat (lane A) and mouse (lane B) DNA fragments.

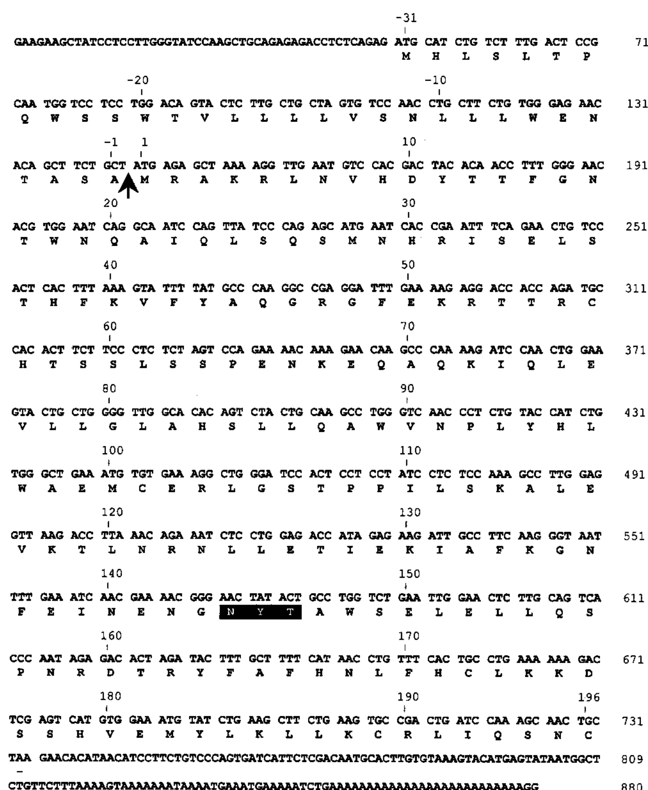

FIG. 2. Nucleotide and predicted amino acid sequences for mouse PLP-A. Encoded amino acids are indicated by single letter designations beneath their respective codons. Translation is assumed to begin at the first ATG (nucleotides 51–53) and continue until the termination codon, TAA (nucleotides 732–734). An arrow indicates the predicted signal peptide cleavage site between Ala^{-1} and Met^{+1}. The identity of this site as the cleavage site is based on homology with rat PLP-A. A putative *N*-linked glycosylation site is denoted by the amino acids enclosed in a black box.

(Fig. 5). On the basis of homology with rat PLP-B, it was further determined that mouse PLP-B contains a 29-amino acid signal peptide. This signal peptide is four amino acids shorter than the rat PLP-B signal peptide. Both mouse and rat PLP-B contain four homologously positioned cysteine residues and a single homologous putative *N*-linked glycosylation site. It should be noted that the overall homology of mouse and rat PLP-B were somewhat less than that found for mouse and rat PLP-A. The significance of this observation is not yet known.

PLP-A and PLP-B Expression Patterns

Northern blot analysis from several mouse tissues indicated that PLP-A mRNA expression was restricted to placental tissues, whereas PLP-B mRNA was dually expressed in both placental and decidual tissues (Fig. 6). Mouse PLP-A and PLP-B mRNAs migrated at approximately 1 kb. Similar findings have been observed for rat PLP-A [14]; however, in contrast, two mRNA species are typically detected for rat PLP-B, migrating at 0.9 and 1.2 kb [15]. The two rat PLP-B transcript sizes differ only in the amount of 5′-untranslated sequence associated with each transcript. Expression of mouse PLP-A and PLP-B mRNAs appeared to be somewhat higher at midgestation than during late gestation. Tissue specificity for PLP-A and PLP-B mRNA expression was similar to that previously reported [14, 15, 19, 21]. The integrity of the RNA was verified by hybridization of the samples with ribosomal protein L-7 cDNA (Fig. 6).

Polyclonal antibodies to recombinant rat PLP-A and rat

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Mouse  MHLSLTPQWSSWTVLLLLVSNLLLWENTASAMRAKRLNVHDYTTFGNTWNQAIQLSQSMN   60
Rat    MHLSLSHQWSSWTVLLLLVSNLLLWENTASAMRAKLLNVHNYTSYGDTWNQAIKISQDMN

Mouse  HRISELSTHFKVFYAQGRGFEKRTTRCHTSSLSSPENKEQAQKIQLEVLLGLAHSLLQAW  120
Rat    QYISDLSTHVKIFYAQGRGFERRTTRCHTSSLSSPENKEQAQQFQLEVLLGLSHSLLQAW

Mouse  VNPLYHLWAEMCERLGSTPPILSKALEVKTLNRNLLETIEKIAFKGNFEINENGNYTAWS  180
Rat    LNPLHHLWAEMCDRLGSTPPTLYKALMLKESNIKLLDAIKNIAKKGNFEINEKANYTAWS

Mouse  ELELLQSPNRDTRYFAFHNLFHCLKKDSSHVEMYLKLLKCRLIQSNC              227
Rat    ELGFLQSPNRDTRYFAFYNLFHCLKKDSNNVEMYLKLLKCRLIRSKC
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FIG. 3. Amino acid sequence comparison of mouse (present study) and rat PLP-A [14]. Both sequences contain five cysteine residues positioned in homologous locations; however, they differ in the presence of putative *N*-linked glycosylation sites. Mouse PLP-A contains a single site, whereas rat PLP-A contains two sites. Asterisks below the sequences denote identity, and dots below the sequences denote similarity.

```
Mouse     MLSLSQPCFSGTLLMLLASNFLLWKNVAPVPMYASLDEYGEMSIYDLLDHVTILSH   60
Rat    MVKSWLRMSKKMEAGTLLMLLMSNILLWENVASVPRHASGAGRGEMSLHGLLDHAIILAH

Mouse  NVSELTAEMHRIFMEDVRYKPGRWFSDRYLTACHTSTLTISVSKEGARQMPGVFLVKEMI  120
Rat    NVTELIAEMNSVFLEDVLYKPGRWFPERDLTACHRSPFTIAVSKEGTQQRLGVFLVKEMI

Mouse  SMLTAWRYPLYHIITELSYMEQAPDEIISRARNIEEKIIVLIEALRGILSKIQPGPPENE  180
Rat    GMLETWTFSLYHIANEMSHMEEPPDEIISRAKNIEEKIKELMDVLKGILNKIQPGSPQNE

Mouse  RYPVWNELASLQSPDEDLRHLTLFNLFQCLVKDSRKIDSSIRLLKCKLLYNRDC       234
Rat    RFPMWNELAYLRSPDEERRHFAFTNLFQCLLQDSRKFDSKVRLLKCRLIYNRDC
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FIG. 5. Amino acid sequence comparison of mouse (present study) and rat PLP-B [15]. Both sequences contain four cysteine residues and a single putative *N*-linked glycosylation site positioned in homologous locations. Asterisks below the sequences denote identity, and dots below the sequences denote similarity.

PLP-B were used to monitor the expression of mouse PLP-A and PLP-B proteins, respectively. Recombinant mouse PLP-A and PLP-B proteins were effectively expressed from the pCMV-SPORT expression vectors in 293 cells (Fig. 7). Mouse PLP-A protein species co-migrated with the 29-kDa species of rat PLP-A. A second PLP-A species migrating at 33 kDa was found only in the rat (Fig. 7). This mouse/rat difference may relate to the existence of a single putative *N*-linked glycosylation site in the mouse and two sites in the rat [14, 16]. The mouse PLP-B protein migrated as a 31-kDa species, similar to rat PLP-B (Fig. 7).

Given that antibodies to rat PLP-A and rat PLP-B recognized the mouse homologues, we attempted to determine the cellular localization of PLP-A and PLP-B within the developing mouse placenta by immunocytochemical procedures. PLP-A was localized to both trophoblast giant cells and spongiotrophoblast cells of the mouse placenta (Fig. 8). PLP-B was prominently expressed in only spongiotrophoblast cells of the mouse placenta (Fig. 8). Expression of PLP-B in mouse decidua was detectable but very weak (data not shown). The cellular expression patterns for PLP-A and PLP-B in the mouse and rat are similar [19, 20].

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                                         -29                                   -20
GCTGCATTCCGTGGCCCACAGACGTGGTCATTTCCCAG  ATG CTG TCT TTG AGT CAA CCT TGC TTC TCA    68
                                         M   L   S   L   S   Q   P   C   F   S
                                     -10                                 -1  1
GGG ACT CTC CTG ATG CTG TTG GCA TCA AAC TTC CTT CTG TGG AAG AAT GTG GCC CCT GTG   128
 G   T   L   L   M   L   L   A   S   N   F   L   L   W   K   N   V   A   P ↑ V
                                 10                                      20
CCC ATG TAT GCC AGT TTG GAT GAA TAT GGT GAG ATG TCC ATA TAT GAC CTG CTT GAT CAC   188
 P   M   Y   A   S   L   D   E   Y   G   E   M   S   I   Y   D   L   L   D   H
                                 30                                      40
GTC ACC ATA CTA TCT CAT AAT GTC AGT GAG CTC ACT GCA GAA ATG CAC AGG ATA TTT ATG   248
 V   T   I   L   S   H   N   V   S   E   L   T   A   E   M   H   R   I   F   M
                                 50                                      60
GAG GAT GTG CGA TAT AAA CCA GGC AGG TGG TTC TCT GAC AGA TAC CTT ACT GCC TGC CAC   308
 E   D   V   R   Y   K   P   G   R   W   F   S   D   R   Y   L   T   A   C   H
                                 70                                      80
ACA TCT ACA TTG ACT ATT TCA GTG TCT AAG GAG GGA GCC CGA CAG ATG CCG GGT GTA TTC   368
 T   S   T   L   T   I   S   V   S   K   E   G   A   R   Q   M   P   G   V   F
                                 90                                     100
CTT GTG AAA GAG ATG ATC AGT ATG TTG ACA GCT TGG AGA TAT CCT CTG TAT CAC ATA ATA   428
 L   V   K   E   M   I   S   M   L   T   A   W   R   Y   P   L   Y   H   I   I
                                110                                     120
ACT GAA CTG AGT TAT ATG GAG CAA GCT CCA GAT GAG ATC ATA TCA AGA GCC AGA AAT ATT   488
 T   E   L   S   Y   M   E   Q   A   P   D   E   I   I   S   R   A   R   N   I
                                130                                     140
GAA GAA AAA ATC ATA GTA CTT ATA GAA GCT CTT AGA GGG ATA CTC AGC AAG ATT CAG CCT   548
 E   E   K   I   I   V   L   I   E   A   L   R   G   I   L   S   K   I   Q   P
                                150                                     160
GGA CCC CCA GAA AAT GAG AGA TAT CCC GTG TGG AAT GAA CTG GCA TCC TTG CAG TCA CCT   608
 G   P   P   E   N   E   R   Y   P   V   W   N   E   L   A   S   L   Q   S   P
                                170                                     180
GAT GAA GAC CTT CGC CAT CTT ACA TTA TTT AAC TTA TTC CAG TGC TTG GTC AAG GAT TCA   668
 D   E   D   L   R   H   L   T   L   F   N   L   F   Q   C   L   V   K   D   S
                                190                                     200 201
CGT AAG ATT GAC TCT AGT ATC AGG CTT TTG AAG TGC AAA CTT CTC TAT AAC AGA GAT TGT   728
 R   K   I   D   S   S   I   R   L   L   K   C   K   L   L   Y   N   R   D   C
TAA TCTCACAACCATCCAATTTACTTTTAGAGTGATCTTTTTAATCTATCTTATTTTAACCAATTTATAATTTGTAAC   806
TTTGAATGAATGTTATTGTGTGAAGAATCCTTCATTAGAAATAAAAACAGACTCTTTAAACAAAAAAAAAAAAAAAAA    885
AAAAAAAAGG                                                                         895
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FIG. 4. Nucleotide and predicted amino acid sequences for mouse PLP-B. Translation is assumed to begin at the first ATG (nucleotides 39–41) and continue until the termination codon, TAA (nucleotides 726–728). An arrow indicates the predicted signal peptide cleavage site between Pro^{-1} and Val^{+1}. The identity of this site as the cleavage site is based on homology with rat PLP-A. A putative *N*-linked glycosylation site is denoted by the amino acids enclosed in a black box.

DISCUSSION

PLP-A and PLP-B represent two members of the PRL family of cytokines/hormones originally identified in the rat. Results from this study demonstrate the existence of mouse homologues for PLP-A and PLP-B. Mouse genomic DNA fragments capable of hybridizing with rat PLP-A were identified. This observation prompted a search of the NCBI dbEST database for mouse homologues of PLP-A and led to the subsequent identification and characterization of mouse PLP-A, PLP-B, and other members of the mouse PRL family (present study; unpublished findings).

Mouse PLP-A and PLP-B exhibit marked similarities with rat PLP-A and PLP-B. The two newly identified c-DNAs were identified as mouse homologues of PLP-A and PLP-B on the basis of sequence comparisons. These included the conservation of five cysteine residues and a putative *N*-linked glycosylation site in mouse and rat PLP-A and four cysteine residues and a putative *N*-linked glycosylation site in mouse and rat PLP-B. PLP-A and PLP-B also displayed cross-species homology in cell-type expression patterns. In both mouse and rat, PLP-A is expressed in trophoblast giant cells and spongiotrophoblast cells, whereas PLP-B is expressed in decidual and spongiotrophoblast cells [14, 15, 19, 21]. Thus, the basic structure and expression patterns of mouse and rat PLP-A and PLP-B are conserved.

Nonetheless, there are a few notable exceptions between the mouse and rat regarding PLP-A and PLP-B. First of all, mouse PLP-A contains a single putative *N*-linked glycosylation site and consists of a single 29-kDa protein species, whereas rat PLP-A contains two putative *N*-linked glycosylation sites and consists of two glycoprotein species (29 and 33 kDa). The correlation of the number of putative *N*-linked glycosylation sites and protein species is consistent with our previous hypothesis that the two rat PLP-A species (29 and 33 kDa) could be attributed to the addition of carbohydrate to one (29-kDa) or two (33 kDa) of the putative *N*-linked glycosylation sites [16]. Rat PLP-A contains oligosaccharide structures characterized by the presence of β1,4-linked GalNAc (*N*-acetyl galctosamine) and sialic acid

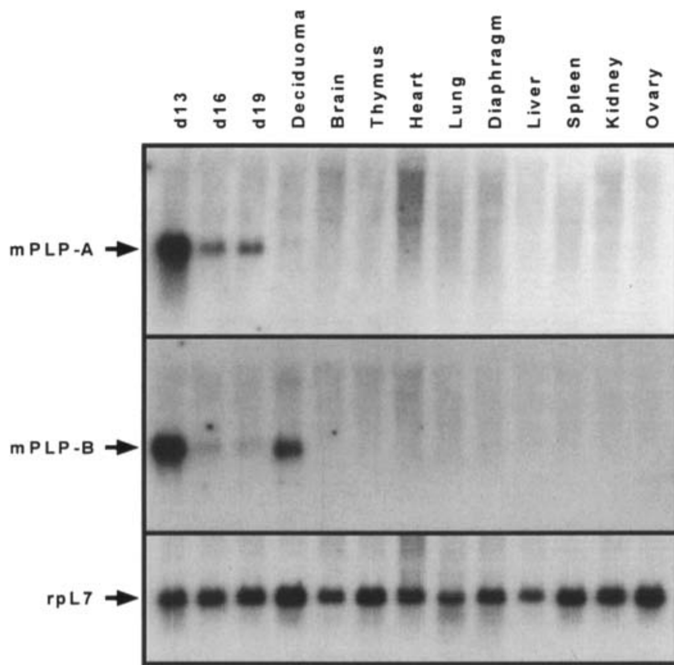

FIG. 6. Tissue-specific expression of PLP-A and PLP-B mRNAs in mouse tissues. Total RNA was collected from placental tissues isolated on Days 13, 16, and 19 of gestation; from deciduoma isolated from Day 8 of pseudopregnancy; and from brain, thymus, heart, lung, diaphragm, liver, spleen, kidney, and ovarian tissues isolated from Day 13 of pregnancy. Samples (15 μg/lane) were fractionated by electrophoresis in 1% agarose gels and transferred to nylon. Hybridization was carried out with ^{32}P-labeled PLP-A, PLP-B, and rpL7 cDNAs. Please note the restricted expression of PLP-A in placental samples and PLP-B in both placental and decidual samples.

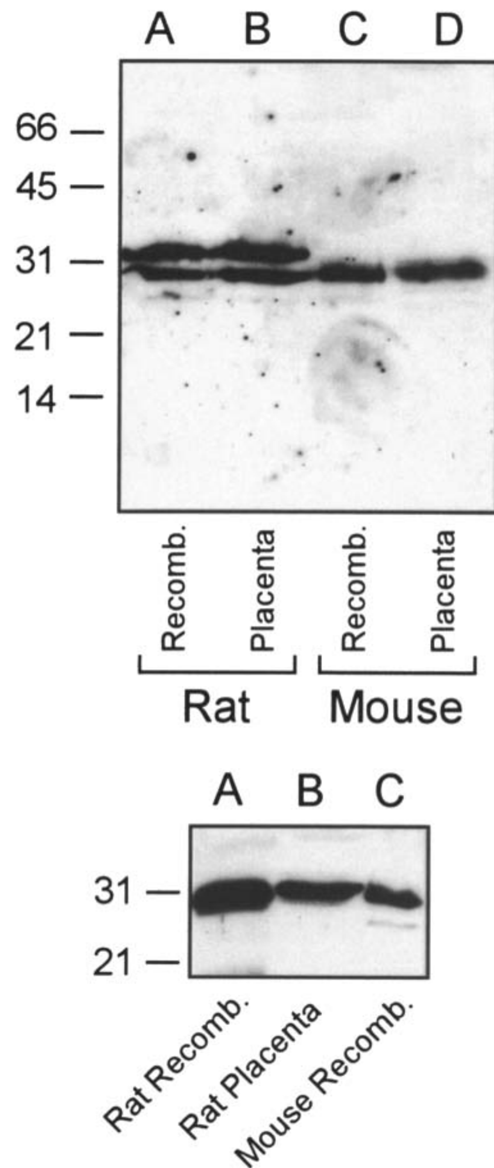

FIG. 7. Western blot analysis for mouse PLP-A and PLP-B. Samples were separated by SDS electrophoresis in 12% gels, electrophoretically transferred to nitrocellulose, and probed with antibodies to recombinant rat PLP-A or recombinant rat PLP-B at final dilutions of 1:1000. Molecular weight standards (× 10^{-3}) are shown. **Top**) Analysis of PLP-A protein species. Lane A, recombinant rat PLP-A; lane B, rat placental PLP-A; lane C, recombinant mouse PLP-A; lane D, mouse placental PLP-A. The mouse PLP-A migrates as a single species with a molecular mass approximating 29 kDA, whereas rat PLP-A migrates as two species with molecular mass approximating 29 and 33 kDa. **Bottom**) Analysis of PLP-B protein species. Lane A, recombinant rat PLP-B; lane B, rat placental PLP-B; lane C, recombinant mouse PLP-B. Please note that the mouse and rat PLP-B protein species co-migrate.

[22]. Glycosyl transferases required for this modification are restricted to the spongiotrophoblast cells of the utero-placental compartment [22]. Specific receptors for this carbohydrate structure have also been described and may participate in regulating the half-life of PLP-A, its delivery to maternal and fetal compartments, and/or its activation of cellular signal transduction pathways [22]. It remains to be determined whether mouse PLP-A contains similar oligosaccharide structures. A second difference between the mouse and rat relates to the relative contribution of PLP-A expression by various trophoblast cell types. It appears that trophoblast giant cells may be a more significant contributor to PLP-A production at midgestation in the mouse than they are in the rat (present study, [19]). This species difference may have implications on potential PLP-A post-translational modifications and access to various target cells in the two species. Finally, it appears that placental expression of PLP-A and PLP-B subsides during gestation more dramatically in the mouse than it does in the rat (present study, [18–22]). The significance of this observation will become more apparent once we have a better appreciation of the biological activities of PLP-A and PLP-B.

The identification PLP-A and PLP-B in the mouse is significant. The presence of PLP-A and PLP-B in the mouse indicates that their appearance in the rat is not a novelty. Species conservation suggests a greater overall importance for these two cytokines/hormones than if there were not species conservation. A more extensive cross-species survey of PLP-A and PLP-B homologues would provide even more insight into these members of the PRL family. Additionally, the existence of PLP-A and PLP-B in the mouse expands our experimental repertoire for studying function. The observations permit the implementation of mouse genetics and gene manipulation to investigate the actions of PLP-A and PLP-B.

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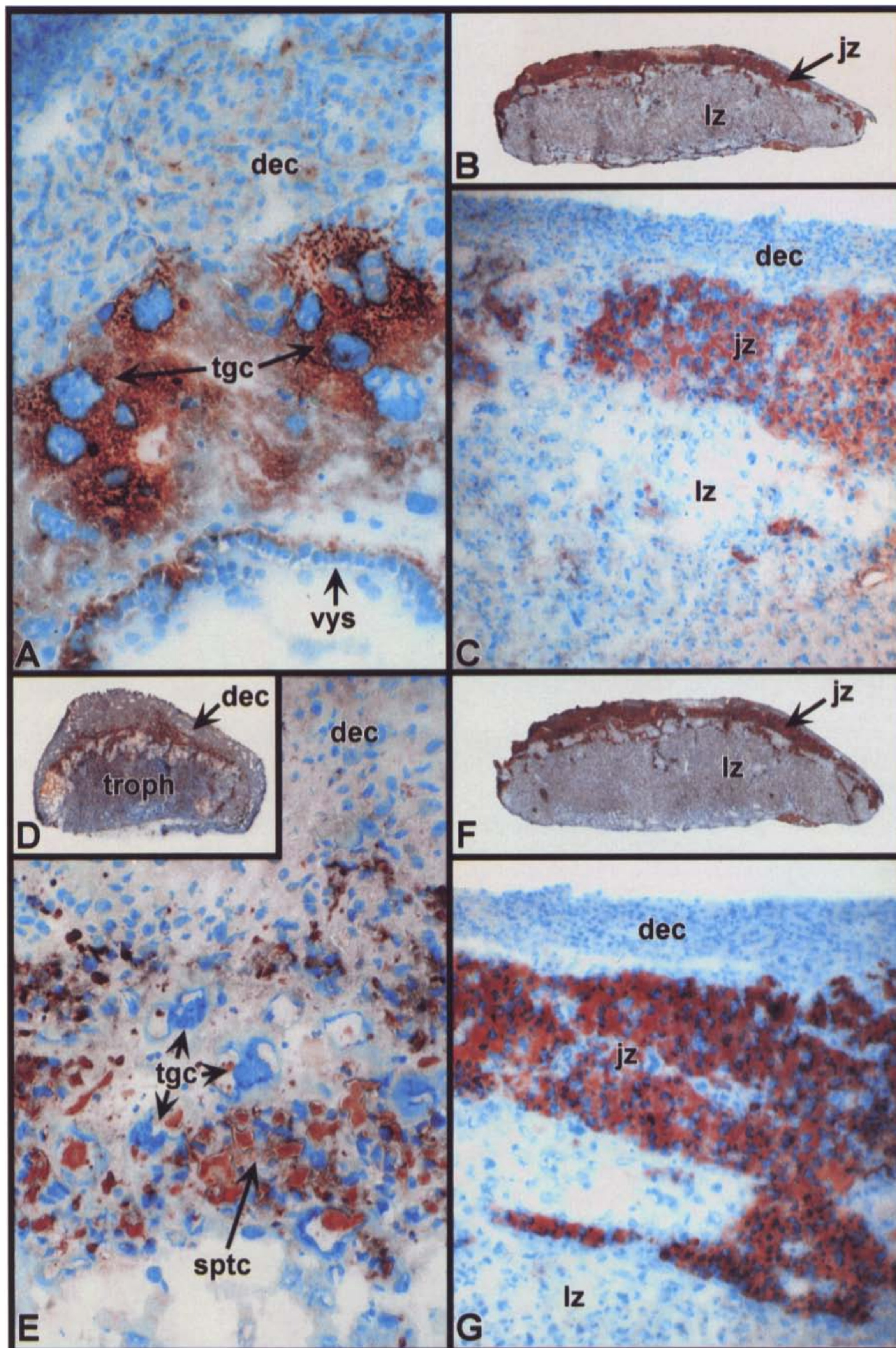

FIG. 8. Cell- and tissue-specific localization of PLP-A and PLP-B in the mouse placenta. Mouse placental sections were stained for the presence of PLP-A (**A–C**) or for PLP-B (**D–G**) using polyclonal antibodies to recombinant rat PLP-A and recombinant rat PLP-B, and a streptavidin-biotin immunoperoxidase kit. Antibodies were used at a final dilution of 1:250. **A**) Day 10 conceptus, ×500; **B**) Day 16 placenta, ×10; **C**) Day 16 placenta, ×250;

D) Day 13 placenta, ×10; **E**) Day 13 placenta, ×500; **F**) Day 16 placenta, ×10; **G**) Day 16 placenta, ×250. (Reproduced at 90%.) PLP-A is localized to both trophoblast giant cells and spongiotrophoblast cells (**A** and **C**), whereas PLP-B expression is restricted to spongiotrophoblast cells (**E** and **G**). dec, Decidua; tgc, trophoblast giant cell; vys, visceral yolk sac; jz, junctional zone; lz, labyrinth zone; sptc, spongiotrophoblast cell; troph, developing trophoblast.

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