

Three novel paralogs of the rodent prolactin gene family

G Dai, D Wang, B Liu, J W Kasik¹, H Müller², R A White³,
G S Hummel³ and M J Soares

Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas 66160, USA

¹Magee Women's Hospital, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15209, USA

²Department of Obstetrics and Gynecology, University of Rostock, Rostock, Germany

³Section of Medical Genetics and Molecular Medicine, Children's Mercy Hospital, Kansas City, Missouri, 64108, USA

(Requests for offprints should be addressed to M J Soares, Department of Molecular and Integrative Physiology, University of Kansas Medical Center, 39th and Rainbow Boulevard, Kansas City, Kansas 66160, USA; Email: msoares@kumc.edu)

Abstract

The prolactin (PRL) family consists of a collection of genes expressed in the uterus, placenta and anterior pituitary. These cytokines/hormones participate in the control of maternal–fetal adaptations to pregnancy. In this report, we establish the presence of three new members of the PRL family. Novel expressed sequence tags (ESTs) with homology to PRL were isolated from embryonic and placental cDNA libraries. The cDNAs were sequenced and compared with those of other members of the PRL family. The three new cDNAs were assigned to the PRL family on the basis of sequence similarities and were referred to as PRL-like protein-J (PLP-J), PRL-like protein-K (PLP-K) and PRL-like protein-M (PLP-M). Both rat and mouse PLP-J cDNAs were identified. Rat PLP-J cDNA encodes for a predicted 211 amino acid protein containing a 29 amino acid signal peptide and two putative *N*-linked glycosylation sites, whereas the mouse PLP-J cDNA encodes for a 212 amino acid protein containing a 29 amino acid signal peptide with a single *N*-linked glycosylation site. Rat and mouse PLP-J proteins share approximately 79% and 70% nucleotide and amino acid sequence identity, respectively. A full-length rat PLP-K cDNA and a partial tentative mouse PLP-K

cDNA were identified. The rat PLP-K cDNA encodes for a predicted 228 amino acid protein containing a 31 amino acid signal peptide and one putative *N*-linked glycosylation site; the mouse PLP-M cDNA encodes for a predicted 228 amino acid protein containing a 28 amino acid signal peptide and one putative *N*-linked glycosylation site. Genes for PLP-J, PLP-K and PLP-M are situated at the Prl family locus on mouse chromosome 13. PLP-J was exclusively expressed in decidual tissue from both the mouse and rat. PLP-K was expressed in trophoblast cells of the chorioallantoic placenta and showed an apparent species difference. In the mouse, virtually all trophoblast lineages expressed PLP-K, whereas in the rat, PLP-K expression was restricted to the labyrinthine trophoblast cells. Mouse PLP-M expression was restricted to the junctional zone of the chorioallantoic placenta. In summary, we have identified three new members of the rodent PRL gene family that are expressed in uterine and placental structures. Future experimentation is needed to determine the specific roles of each of these ligands in the biology of pregnancy.

Journal of Endocrinology (2000) **166**, 63–75

Introduction

Uteroplacental tissues of the rat and mouse are known to express a relatively large family of proteins structurally related to prolactin (PRL; Soares *et al.* 1998, Linzer & Fisher 1999). Discovery of many members of the PRL family proceeded along a somewhat linear path. Initially, PRL family member identification was based on the isolation of functional PRL receptor agonist activities from the placenta. During the characterization of this initial placental lactogen (PL) and its cDNA, additional members of the PRL family were identified, and as these members were characterized at the protein, cDNA and genomic

levels, other members were discovered. Two notable exceptions to this method of discovery have been evident. The first involves proliferin (PLF), which was initially identified as a relative of PRL specifically expressed in mitogen-stimulated fibroblasts (Linzer & Nathans 1984) and subsequently found to be expressed in the mouse placenta (Linzer *et al.* 1985). The second exception emanated from the mouse and rat genome projects. Expressed sequence tags (ESTs) isolated from mouse and rat uterine and extraembryonic cDNA libraries with homology to members of the PRL family have been found in the National Center for Biotechnology Information (NCBI) database (Bethesda, MD, USA). Perusal of this

repository resulted in the demonstration of previously unidentified orthologs for mouse and rat PRL family members (Lin *et al.* 1997a, Orwig *et al.* 1997b, Müller *et al.* 1998a, Sahgal *et al.* 2000) and the discovery of novel members of the mouse and rat PRL gene families (Lin *et al.* 1997b, Müller *et al.* 1998b, Ishibashi & Imai 1999).

It is assumed that the expansion of the PRL family in rodents has functional significance to the biology of gestation. Many of the PRL family members have been shown to participate in the orchestration of maternal adaptations to pregnancy (Soares *et al.* 1998, Linzer & Fisher 1999). Maternal target tissues include the ovary (Galosy & Talamantes 1995), uterus (Nelson *et al.* 1995), vasculature (Jackson *et al.* 1994), mammary glands (Thordarson *et al.* 1986, Colosi *et al.* 1988a, Dai *et al.* 1996), brain (Bridges *et al.* 1996, 1997, Voogt *et al.* 1996), pancreas (Brelje *et al.* 1993), hematopoietic cells (Lin & Linzer 1999) and immune cells (Robertson *et al.* 1982, 1994, Cohick *et al.* 1996, Dai *et al.* 1996, Müller *et al.* 1999).

In this report, we identify three novel paralogs of the rodent PRL family expressed in uterine or placental tissues. Examination of the EST database at the NCBI indicated the existence of novel cDNA clones related to PRL that had not been previously reported. The cDNAs are referred to as PRL-like protein-J (PLP-J), PRL-like protein-K (PLP-K) and PRL-like protein-M (PLP-M).

Materials and Methods

Reagents

All restriction enzymes were purchased from New England Biolabs (Beverly, MA, USA). Mouse and rat cDNAs were obtained from the University of Iowa Rat Gene Discovery Program or the IMAGE consortium through either the American Type Culture Collection (ATCC, Manassas, VA, USA) or Research Genetics (Huntsville, AL, USA). DNA extraction kits were purchased from Qiagen (Chatsworth, CA, USA). Nitrocellulose and nylon membranes were obtained from Schleicher and Schuell (Keene, NH, USA). Radiolabeled nucleotides were purchased from DuPont-NEN (Boston, MA, USA). Unless otherwise noted, all other chemicals and reagents were purchased from Sigma (St Louis, MO, USA).

Animals and tissue preparation

Holtzman rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN, USA). CD-1 mice were obtained from Charles River Inc. (Wilmington, MA, USA). The animals were housed in an environmentally controlled facility, with lights on from 0600 to 2000 h, and allowed free access to food and water. Timed pregnancies and tissue dissections were performed as previously described

(Soares 1987, Orwig *et al.* 1997a,b). Pseudopregnancy was induced by mating with vasectomized males. Deciduomal reactions were induced on day 4 of pseudopregnancy by injection of 50–75 µl sesame oil per uterine horn (Orwig *et al.* 1997a,b). Animal care and use procedures were approved by the University of Kansas Animal Care and Use Committee.

Characterization of PLP-J, PLP-K and PLP-M cDNAs

Examination of the NCBI EST database revealed the presence of several cDNA clones exhibiting sequence similarity with members of the PRL gene family. Clones were obtained from the ATCC or Research Genetics. DNA sequencing was performed using an Applied Biosystems Model 310 sequencer and Applied Biosystems Dye Terminator Cycle Sequencing kits (Foster City, CA, USA). Both strands of the cDNAs were completely sequenced. Analyses of the cDNAs and their predicted amino acid structures were performed with software programs available through ExPASy Proteomics tools (<http://www.expasy.ch/tool/>). Comparisons of PLP-J, PLP-K and PLP-M sequences with those of other members of the PRL family were performed with CLUSTAL W (version 1.7, Thompson *et al.* 1994). Phylogenetic tree construction was performed with the Molecular Evolutionary Genetics (MEGA, version 1.01) software program (Kumar *et al.* 1994). Bootstrap values were calculated as previously described (Efron *et al.* 1996).

Chromosomal assignments of mouse PLP-J, PLP-K and PLP-M genes

Chromosomal mapping of the mouse PLP-J, PLP-K and PLP-M genes was determined using the Jackson Laboratory Interspecific Backcross Panel (Rowe *et al.* 1994). Genomic DNAs from C57BL/6J, *Mus spretus* and a (*M. spretus* × C57BL/6J)F₁ × *M. spretus* (BSS type) backcross were analyzed by Southern blotting as previously described (White *et al.* 1992). Approximately 5 µg genomic DNAs from the C57BL6/J and *M. spretus* progenitors were digested with 28 different restriction enzymes to find a restriction fragment length variation (RFLV) suitable for mapping. Southern blots were probed with mouse PLP-J, PLP-K or PLP-M radiolabeled cDNAs. For each sample, approximately 2 µg DNA from the BSS type backcross panel were digested with HaeIII 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).

Analysis of PLP-J, PLP-K and PLP-M expression

The expression of PLP-J, PLP-K and PLP-M mRNAs in the rat and mouse was assessed by *in situ* hybridization. PLP-J, PLP-K and PLP-M mRNAs were detected in


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GGCACGAGGCTGATTCAGCAGACAAACTAGGGTGTGACTTCTCAGGG 47
-30 -20
ATG CTA CTG TAT TTG CCT CAA ATA TTT TCC TCA AGG GCA TCA TCG CTC CTG 98
M L L Y L P Q I F S S R A S S L L 17
-10 -1 +1
TTC CTG GTG CCC TAC TTG CTC TTT TGG GAG AAT GTA GCA TCT ATA TCC ACC 149
F L V P Y L L F W E N V A S I S T 34
+10 +20
TGT GCA GAG AGG GAT GCC ACC ATT CAG CAT TCC TTA GAG AAA CTT CTT ACA 200
[C] A E R D A T I Q H S L E K L L T 51
+30
TTG ACA ACC TTT ATG TCT CAT GTC ATG AGT ATT GAA ACT GCA AAA CTC TTC 251
L T T F M S H V M S I E T A K L F 68
+40 +50
ACT GAA TTT AAT AAT CAG TAT GCC CAG GGT AAG AGA TAC AAT GAC AGG ATC 302
T E F N N Q Y A Q G K R Y N D R I 85
+60 +70
CCT GGA ACG TGT CAC ACT GCT TTT TTT GAT ACT CCA GTA AAC AAG GAG CAA 353
P G T [C] H T A F F D T P V N K E Q 102
+80
TCT CTA GGA AGT GAT CCG AAA ACA CTA CTG AAA TTG GTA CGC AGT TTA TTG 404
S L G S D P K T L L K L V R S L L 119
+90 +100
AAT TCC TGG ACC AAT GCT CTA AAT CAT CTT GTG AAT GAA ATA TCT GCA ATG 455
N S W T N A L N H L V N E I S A M 136
+110 +120
CAA GGA GAC CCT TCT TTT CTT TTC TCC AAA GCC AGA GAG ATT CAG GCA AAA 506
Q G D P S F L F S K A R E I Q A K 153
+130
TTT GAT GAA CTC ACG ACG GGT GTT AAA ACA ATT CTC AGC ATG ATT GGA GAG 557
F D E L T T G V K T I L S M I G E 170
+140 +150
AGA GAT AAT GAC ACC CAC CTT GCT TGG TCC GGG CTG TCA TCC TTG CAG TCA 608
R D [N D T] H L A W S G L S S L Q S 187
+160 +170
AGC AAT GAA GAT GTT CGC TGC TTT TCT TTT TAT ACC CTG ATT CGC TGC CTG 659
S N E D V R [C] F S F Y T L I R [C] L 204
+180 +190
CTC CGA GAC TCT CGA AAA GTA AAT ACT TAT CTT GAG GTT ATC AAG TAC CAA 710
L R D S R K V N T Y L E V I K Y Q 221
ATA TTC AAT CAA AAC AAC TGC TAA TAGTAAAGCAATATAATCTGTCTTGAGATATCCCT 769
I F N Q N N [C] * 228

TTATAAAACATTACTGCAAAGCTCCCTTTTCGATTTCTGTTAACCTTCTTTAAAAATAAAAGCTTCTTG
GAATTGTTAAAAAAAAAAAAAAAAAAAAA

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Figure 2 Nucleotide and predicted amino acid sequences for rat PLP-K. Encoded amino acids are indicated by single letter designations beneath their respective codons. Translation is assumed to begin at the first ATG (nucleotides 48–50) and to continue until the termination codon, TAA (nucleotides 729–731). An arrow indicates the predicted signal peptide cleavage site between Ser⁻¹ and Ile⁺¹. The identity of this site as the cleavage site is based on similarities with other members of the PRL family. Putative N-linked glycosylation sites are denoted by the amino acids enclosed in black shaded boxes. Cysteines are identified by outlined boxes and the predicted polyadenylation site is underlined.

GAAGAATTCCTGACGCTGTAGTCAGCCTGCTGTGGAACCTCTCAGAG 47
-20

ATG CAG CTA TCT GTA ACT CAT CCT TGC TGC AGG ACC CTG ATT CTG CTC CTG 98
M Q L S V T H P C C R T L I L L L 17

-10 -1 +1

GTG TCT AAC CTG CTT CTG TGG GAA AGT GAG GCC GTG CTG CCC ATA TGC TCA 149
V S N L L L W E S E A ▼ V L P I C S 34

+10 +20

GTA AGG AAT GGA CGC TGC TTT GGT TCC TTT GAA GAA CTG CTT GAA AGA GCT 200
V R N G R C F G S F E E L L E R A 51

+30 +40

GTC AGT TTG TCT GAA GAG ATA AGT AAA AAA GCC TTT GAA CTC TTC ACT GCA 251
V S L S E E I S K K A F E L F T A 68

+50

TTT GAT AGT CAG TAT GCC CAG AGC CAC CAG CTC ATT GTC AAG AGC CTC AAA 302
F D S Q Y A Q S H Q L I V K S L K 85

+60 +70

AAA TGT CAC ACA TCT TCT CTC GAC CTT CCA AAA CCA GGG AGT CAA GCC ATG 353
K C H T S S L D L P K P G S Q A M 102

+80 +90

CAG ACA CAT CCT GTA ACC CTA CTG AAA TTA GCA AGC AAA TTA TTG AGA GCC 404
Q T H P V T L L K L A S K L L R A 119

+100

TGG CAA GTC CCT CTG AAC CAT CTA GTG AAC AAC CTG CCA TCC TTG AAA AAC 455
W Q V P L N H L V N N L P S L K N 136

+110 +120

GTC TCT CCT TCT ATC CTC TCT AAA GCC AAA GAG ATT GAG GAA AAG AGC AAT 506
V S P S I L S K A K E I E E K S N 153

+130 +140

GGA CTC CTG GAG GGA GTT AAG AGC ATT CTC ATC CAA ATG CAA AAT GGA GAT 557
G L L E G V K S I L I Q M Q N G D 170

+150

ACA GAA GAT GAG AAC TAC CCT GGC TGG TCT GGA CTG GCA TCC TTG AAG TCA 608
T E D E N Y P G W S G L A S L K S 187

+160 +170

GAG ACT GAA GAT ATT CGC CTC TTT GCA TAT TAT AAC ATG ATC CGC TGT GAG 659
E T E D I R L F A Y Y N M I R C E 204

+180 +190

GGC AGA GAC ACT CAG AAG GTT GAA ACT GCT CTC AAG ATG GTG AAA TGC AAA 710
G R D T Q K V E T A L K M V K C K 221

+200

ATT TCA AAT GAA AAC AAC TGC TAA GCCCTTTTCATCATATCTTCGTCTGAGCCACTGCT 769
I S N E N N C * 228

TGATGATATATTTGCTGTGAAACTTTCCTTGAATTTTTTCTCTGTAAATGCATGCCCAGTGGTGT
TATCTTCTTTTCCAAATAAAAAATTGACCCCATGTAAAAA

Figure 3 Nucleotide and predicted amino acid sequences for mouse PLP-M. Encoded amino acids are indicated by single letter designations beneath their respective codons. Translation is assumed to begin at the first ATG (nucleotides 49–51) and to continue until the termination codon, TAA (nucleotides 685–687). An arrow indicates the predicted signal peptide cleavage site between Ala⁻¹ and Val⁺¹. The identity of this site as the cleavage site is based on similarities with other members of the PRL family. Putative N-linked glycosylation sites are denoted by the amino acids enclosed in black shaded boxes. Cysteines are identified by outlined boxes and the predicted polyadenylation site is underlined.

possesses six cysteine residues in its predicted mature protein sequence that are situated in locations homologous to the six cysteine residues in mouse PRL (Linzer & Talamantes 1985).

Comparative analysis of PLP-J, PLP-K and PLP-M with members of the rat and mouse PRL family

Relationships of rat and mouse PRL family members were determined with the CLUSTAL W (version 1.7) software program (Thompson *et al.* 1994) and the Molecular Evolutionary Genetics Analysis software (MEGA, version, 1.01; Kumar *et al.* 1994). Mouse and rat PLP-J orthologs were most closely related to a subfamily of PRL members, which included placental lactogen-I (PL-I) and PL-II (Fig. 4), both effective ligands for the PRL receptor (Soares *et al.* 1998). Rat PLP-K and mouse PLP-M exhibit some distant relatedness to each other and to PLF (Fig. 4), a known regulator of angiogenesis (Linzer & Fisher 1999).

Chromosomal mapping

The gene symbols, *Prlpj*, *Prlpk* and *Prlpm*, have been assigned to the mouse PLP-J, PLP-K and PLP-M loci, respectively, in accordance with nomenclature previously approved by the International Mouse Nomenclature Committee. HaeIII RFLVs were identified for each of the PLP genes. *Prlpj* was identified by the presence of a 3.2 kb genomic DNA fragment in C57BL/6J or the presence of a 4.0 kb fragment in *M. spretus* (Fig. 5, top panel). *Prlpk* was identified by the presence of a 4.1 kb genomic DNA fragment in C57BL/6J or the presence of a 3.3 kb fragment in *M. spretus* (Fig. 5, top panel). *Prlpm* was identified by the presence of a 1.2 kb genomic DNA fragment in C57BL/6J or the presence of a 1.0 kb fragment in *M. spretus* (Fig. 5, top panel). Mapping data from this article have been deposited with the Mouse Genome Database. Haplotype analysis of these mapping data (Fig. 5, bottom panel) indicated that the *Prlpj*, *Prlpk* and *Prlpm* loci are closely linked to *Dtprp* and *Pl1* on chromosome 13 in the mouse. Allelic segregation patterns for *Prlpj*, *Prlpk*, *Prlpm*, *Dtprp* and *Pl1* are identical, indicating a distance of less than 1 centimorgan (cM) among these genes. The calculated map distances between *Prlpj*, *Prlpk* and *Prlpm* loci and adjacent loci *Gpld1* (glycosylphosphatidylinositol-specific phospholipase D) and D13 Bwg0938e (DNA segment, Chr 13, Brigham and Women's Genetics 0938 expressed), including 95% confidence limits were determined as:

Gpld1-1.1 ± 1.1 cM-*Prlpj*, *Prlpk* and *Prlpm*-2.1 ± 1.5 cM-D13 Bwg0938e

Cellular localization of PLP-J, PLP-K and PLP-M expression in the uteroplacental compartment

In order to resolve the cellular sources of PLP-J, PLP-K and PLP-M within the developing uteroplacental

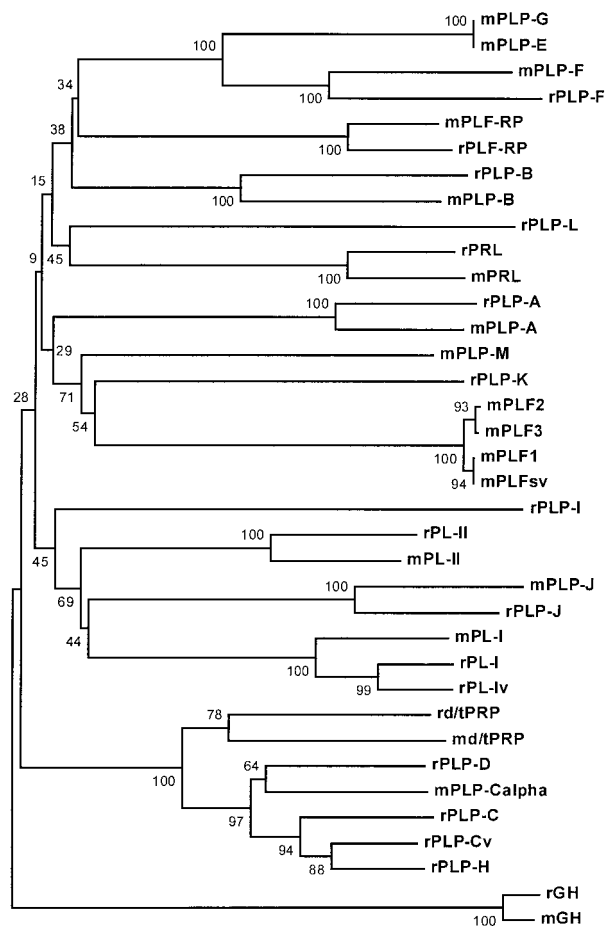


Figure 4 Mouse (m) and rat (r) orthologous and paralogous PRL family members. Comparisons of PLP-J, PLP-K and PLP-M sequences with those of other members of the PRL family were performed with the CLUSTAL W (version 1.7) software program (Thompson *et al.* 1994). Phylogenetic tree construction was performed with the Molecular Evolutionary Genetics Analysis (MEGA, version 1.01) software program (Kumar *et al.* 1994). Bootstrap values were determined as previously described (Efron *et al.* 1996). Input sequences for the analysis were derived from the present report and the following PRL family members (listed with their GenBank accession numbers): rPRL, CAA24547.1; rGH, CAA24562.1; rPL-I, BAA04662.1; rPL-IV, P34207; rPL-II, AAA41887.1; rPLP-A, AAA41890.1; rPLP-B, AAA41891.1; rPLP-C, AAA41945.1; rPLP-Cv, AAB51593.1; rd/tPRP, A46603; rPLP-D, BAA19054.1; rPLP-F, AAD52848.1; rPLF-H, BAA83728.1; rPLP-I, BAA83728.1; rPLP-L, BAA84972.1; mGH, CAA26650.1; mPRL, LCMS; mPL-I, AAA39404.1; mPL-II, P09586; mPLP-A, AAB68824.1; mPLP-B, AAB68825.1; mPLP-Calpha, AAD09012.1; mPLF1, A05086; mPLF2, P04768; mPLF3, S05648; mPLFsv, CAA53234.1; md/tPRP, AAB69861.1; mPLP-E, AAB92397.1; mPLP-F, AAB80728.1; mPLP-G, AAB80729.1; mPLF-RP, CAA26437.1. PRL, prolactin; PLP, PRL-like protein; PRP, PRL-related protein; PL, placental lactogen; PLF, proliferin; PLF-RP, proliferin-related protein; GH, growth hormone. Please note that PLP-G=PLP-E and mPLF1=mPLFsv.

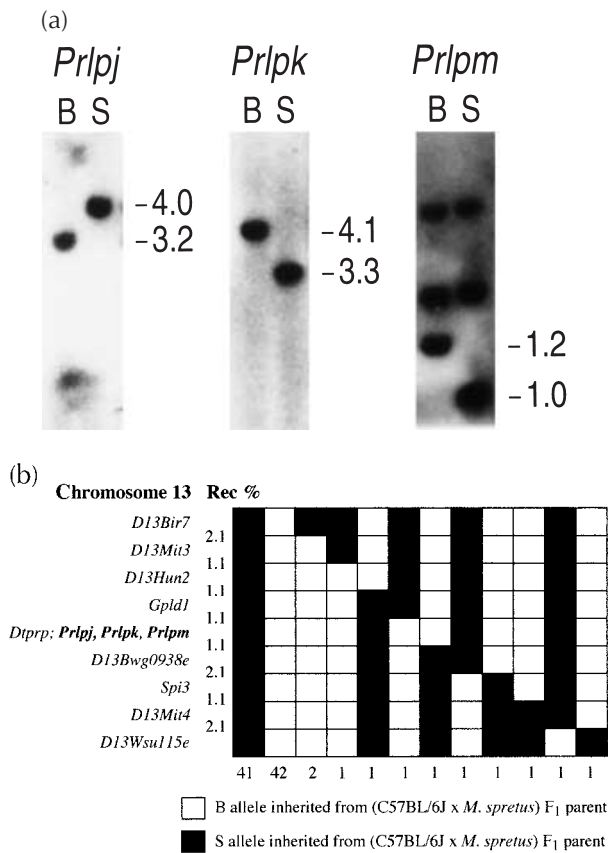


Figure 5 Chromosomal localization of the mouse PLP-J, PLP-K and PLP-M genes. (a) HaeIII restriction patterns for C57BL/6J (B) genomic DNA and *M. spretus* (S) genomic DNA probed with mouse PLP-J, PLP-K and PLP-M cDNAs. The sizes of the fragments in kb are indicated. (b) Haplotype analysis of chromosome 13 genetic markers in (C57BL/6J × *M. spretus*)F₁ × *M. spretus* (BSS type) backcross mice showing linkage and relative position of *Prlpj*, *Prlpk* and *Prlpm* genes. Closed boxes indicate inheritance of the C57BL/6J (B) allele and open boxes indicate the inheritance of the *M. spretus* (S) allele from the (C57BL/6J × *M. spretus*)F₁ parent. Gene names and references to these loci can be found in the Mouse Genome Database (MGD). These data are accessible through the MGD (<http://www.jax.org>). The first two columns indicate the number of backcross progeny with no recombinations; the following columns indicate recombinational events between adjacent loci (signified by a change from an open box to a closed box). The number of recombinants are listed below each column and the recombination frequency (Rec %) between adjacent loci is indicated.

compartment, we performed *in situ* hybridization with antisense and sense probes. Sense probes did not provide any hybridization signals in any of the tissues investigated, demonstrating the specificity of the mRNA detection.

Messenger RNA for PLP-J was specifically detected in decidual cells of pregnant and pseudopregnant rodents (Fig. 6). The mouse and rat expression patterns for PLP-J were very similar. PLP-J was abundantly expressed in the antimesometrial compartment of the decidua. The

temporal profile of PLP-J expression mirrored the lifespan of the antimesometrial decidua (data not shown). PLP-J was not detected in trophoblast lineages examined from day 6 to term in either the rat or the mouse.

The pattern of PLP-K expression differed in the rat and the mouse. In the rat, PLP-K expression was restricted to trophoblast cells within the labyrinth zone (Fig. 7B, C) whereas, in the mouse, PLP-K mRNA was detected in trophoblast lineages throughout both the junctional and labyrinth zones (Fig. 7D–I). PLP-K mRNA was first observed in trophoblast giant cells on day 10 of gestation in the mouse (data not shown) and then expanded to spongiotrophoblast and labyrinthine trophoblast cell populations (Fig. 7D–I). PLP-K was not expressed in non-trophoblast lineages in either the rat or the mouse.

PLP-M mRNA expression was restricted to trophoblast cell types present in the junctional zone (Fig. 8). Positive trophoblast giant cells expressing PLP-M were first detected on day 10 of gestation (Fig. 8A, D). As gestation progressed, both trophoblast giant cells and spongiotrophoblast cells were identified as sources of PLP-M (Fig. 8).

Discussion

Our knowledge of the PRL family of cytokines/hormones has expanded over the past few years. These most recent discoveries are chiefly related to the establishment of EST databases derived from rat and mouse uterine, embryonic and extraembryonic cDNA libraries. Perusal of ESTs from the University of Iowa Rat Gene Discovery Program and the IMAGE consortium within the NCBI dbEST database led to the identification of three novel paralogs of the rodent PRL family. The three new PRL family members were referred to as PLP-J, PLP-K and PLP-M, in accordance with the nomenclature used in recent reports (Ishibashi & Imai 1999, Toft & Linzer 1999).

The PRL gene family presumably arose as a result of gene duplication events (Wallis 1992). Members of the rodent PRL family possess structural similarities that justify their inclusion in the PRL family. The three new paralogs are no exception. PLP-J, PLP-K and PLP-M contain amino acid sequence similarities, especially positioning of cysteine residues, that are diagnostic of the PRL family (Nicoll *et al.* 1986). PRL family genes have also been mapped to similar regions within the genome. In the mouse, PRL family genes are located on chromosome 13 (Jackson-Grusby *et al.* 1988, Lin *et al.* 1997a,b, Orwig *et al.* 1997b, Dai *et al.* 1998, 1999, Toft & Linzer 1999). Consistent with this pattern, genes for PLP-J, PLP-K and PLP-M were also mapped to the *Prl* locus on mouse chromosome 13. The three new paralogs are expressed during pregnancy. This observation is also consistent with the patterns of expression of all other members of the PRL family (Soares *et al.* 1998) and implicates PLP-J, PLP-K and PLP-M in the biology of gestation.

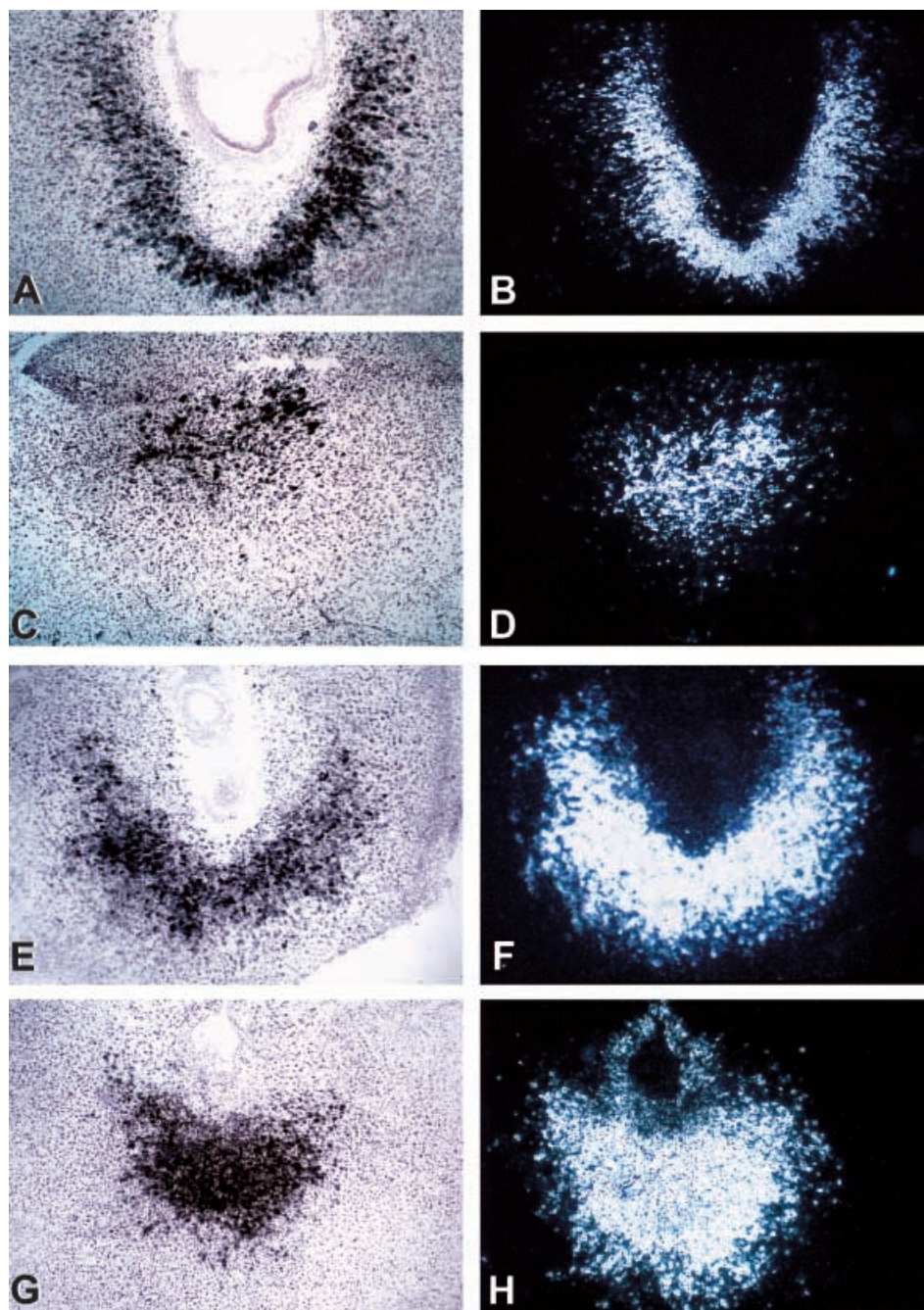


Figure 6 Localization of PLP-J mRNA in rat and mouse decidua. The *in situ* detection of mRNA expression was performed on frozen tissue sections. Full-length rat PLP-J and mouse PLP-J cDNAs were used as templates for the synthesis of [35 S]-labeled sense and antisense RNA probes. (A) Bright-field representation using a rat PLP-J antisense probe on a day 9 rat conceptus tissue section. (B) Dark-field representation using a rat PLP-J antisense probe on a day 9 rat conceptus tissue section. (C) Bright-field representation using a rat PLP-J antisense probe on a day 9 rat deciduoma tissue section. (D) Dark-field representation using a rat PLP-J antisense probe on a day 9 rat deciduoma tissue section. (E) Bright-field representation using a mouse PLP-J antisense probe on a day 8 mouse conceptus tissue section. (F) Dark-field representation using a mouse PLP-J antisense probe on a day 8 mouse conceptus tissue section. (G) Bright-field representation using a mouse PLP-J antisense probe on a day 8 mouse deciduoma tissue section. (H) Dark-field representation using a mouse PLP-J antisense probe on a day 8 mouse deciduoma tissue section. Sense probes did not provide any hybridization signal in any of the tissues investigated. Original magnifications, $\times 40$.

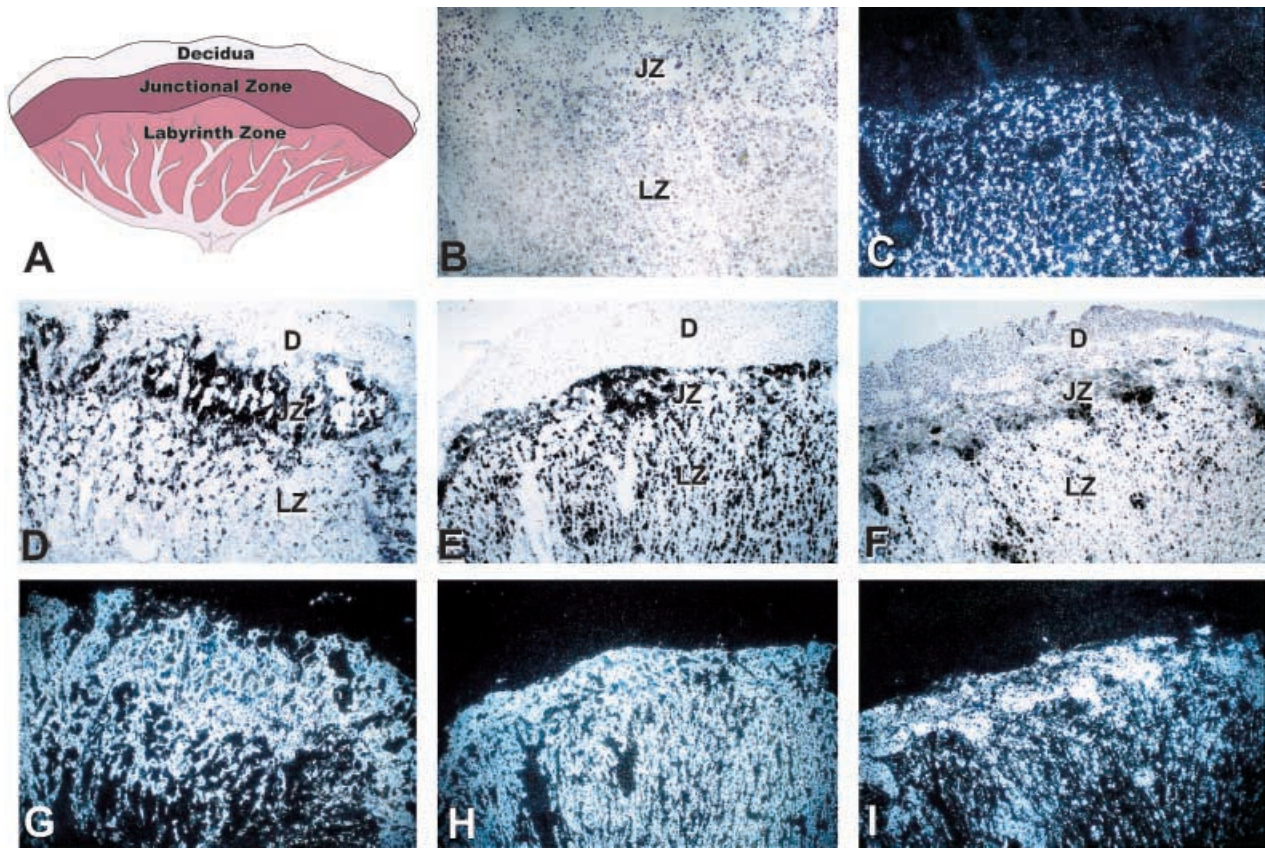


Figure 7 Cell- and tissue-specific localization of PLP-K in rat and mouse placental tissues. The *in situ* detection of mRNA expression was performed on frozen tissue sections. A full-length rat PLP-K cDNA and a 319 bp mouse PLP-K cDNA were used as templates for the synthesis of [³⁵S]-labeled sense and antisense RNA probes. (A) Schematic diagram of the mature rodent placenta. (B) Bright-field representation using a rat PLP-K antisense probe on a day 16 rat placenta section. (C) Dark-field representation using a rat PLP-K antisense probe on a day 16 placenta section. (D) Bright-field representation using a mouse PLP-K antisense probe on a day 13 mouse placenta section. (E) Bright-field representation using a mouse PLP-K antisense probe on a day 16 mouse placenta section. (F) Bright-field representation using a mouse PLP-K antisense probe on a day 19 mouse placenta section. (G) Dark-field representation using a mouse PLP-K antisense probe on a day 13 mouse placenta section. (H) Dark-field representation using a mouse PLP-K antisense probe on a day 16 mouse placenta section. (I) Dark-field representation using a mouse PLP-K antisense probe on a day 19 mouse placenta section. Sense probes did not provide any hybridization signal in any of the tissues investigated. JZ, Junctional zone; LZ, labyrinth zone. Original magnifications, × 40.

PLP-J

In this study, we identified mouse and rat orthologs for PLP-J. PLP-J has been discovered independently by three other laboratories (Hiraoka *et al.* 1999, Ishibashi & Imai 1999, Toft & Linzer 1999). Structurally, PLP-J fits within the PL subfamily, which also includes PL-I, PL-II and PL-I variant (see Fig. 4). PLs activate PRL receptor signaling pathways (Ogren & Talamantes 1988, Soares *et al.* 1998). Whether the resemblance of PLP-J to PLs reflects commonalities in receptor recognition and function remains to be determined. In both the mouse and the rat, PLP-J possesses a characteristic antimesometrial pattern of expression in the decidual compartment of the maternal uterus (Toft & Linzer 1999, present study).

Decidual expression of PLP-J is independent of either extraembryonic or embryonic factors (present study). These features are similar to the decidual patterns of expression reported for two other PRL family members, PLP-B (Croze *et al.* 1990, Cohick *et al.* 1997) and decidual/trophoblast PRL-related protein (d/tPRP; Lin *et al.* 1997b, Orwig *et al.* 1997b, Rasmussen *et al.* 1997). However, unlike PLP-J expression which appears to be restricted to decidual cells, PLP-B and d/tPRP are also expressed in trophoblast cells of the rodent chorioallantoic placenta (Cohick *et al.* 1997, Orwig *et al.* 1997b, Rasmussen *et al.* 1997). D/tPRP is not believed to have systemic targets. It avidly binds heparin-containing molecules within the decidual extracellular matrix and is a locally acting cytokine (Rasmussen *et al.* 1996).

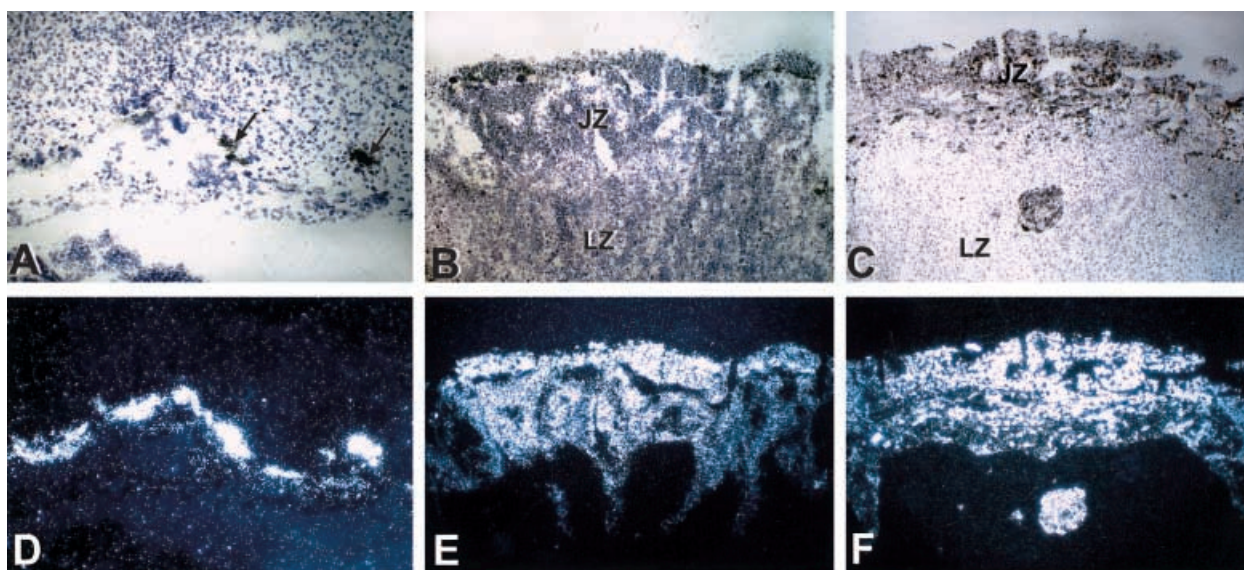


Figure 8 Cell- and tissue-specific localization of PLP-M in mouse placental tissues. The *in situ* detection of mRNA expression was performed on frozen tissue sections. A full-length mouse PLP-M cDNA was used as a template for the synthesis of [³⁵S]-labeled sense and antisense RNA probes. (A) Bright-field representation using an antisense probe on a day 10 mouse conceptus tissue section, arrows denote the location of trophoblast giant cells. (B) Bright-field representation using an antisense probe on a day 13 mouse placental tissue section. (C) Bright-field representation using an antisense probe on a day 19 mouse placental tissue section. (D) Dark-field representation using an antisense probe on a day 10 mouse conceptus tissue section. (E) Dark-field representation using an antisense probe on a day 13 mouse placental tissue section. (F) Dark-field representation using an antisense probe on a day 19 mouse placental tissue section. Sense probes did not provide any hybridization signal in any of the tissues investigated. JZ, Junctional zone; LZ, labyrinth zone. Original magnifications, A and D, × 200; B, C, E and F, × 40.

Preliminary observations using alkaline phosphatase-tagged PLP-J suggest that the targets of PLP-J may also be restricted to the uterine compartment (G Dai, D Wang, L Lu & MJ Soares, unpublished results).

PLP-K

We identified a full-length rat PLP-K cDNA and a partial mouse PLP-K cDNA. Rat PLP-K has been independently discovered by others (Ishibashi & Imai 1999). PLP-K is somewhat unique among members of the rodent PRL family, but does exhibit a distant structural relationship to PLF (see Fig. 4), a known regulator of blood vessel development and uterine growth (Jackson *et al.* 1994, Linzer 1995, Nelson *et al.* 1995). PLF regulates angiogenesis via interactions of its carbohydrate motifs with the insulin-like growth factor-II/mannose-6 phosphate receptor (Lee & Nathans 1988, Volpert *et al.* 1996, Groskopf *et al.* 1997). PLP-K possesses a putative *N*-linked glycosylation site within its amino acid sequence. The possible involvement of PLP-K in the regulation of angiogenesis or uterine growth, or the existence of carbohydrate motifs within PLP-K capable of interacting with the mannose-6 phosphate receptor represent testable hypotheses for investigating the biology of PLP-K.

Differences were observed in the expression patterns of PLP-K in the rat and mouse (present study). In the rat, PLP-K expression was restricted to trophoblast cells within the labyrinth zone whereas, in the mouse, PLP-K mRNA was detected in multiple trophoblast lineages. Expression was initiated in trophoblast giant cells at midgestation and then extended to spongiotrophoblast and labyrinthine trophoblast cell types as gestation progressed (present study). Species differences have also been observed for at least one other member of the PRL family, PLF-related protein (PLF-RP). In the rat placenta, PLF-RP originates in a non-trophoblast giant cell component of the chorioallantoic placental primordium (ectoplacental cone) and continues predominantly in labyrinthine trophoblast of the chorioallantoic placenta (present study). In contrast, PLF-RP in the mouse placenta is expressed initially in the trophoblast giant cell layer of chorioallantoic and choriovitelline placental primordia and subsequently in giant and spongiotrophoblast cell layers of the mature chorioallantoic placental junctional zone (Colosi *et al.* 1988b, Carney *et al.* 1993, Sahgal *et al.* 2000). Unlike PLP-K, PLF-RP expression is never observed in the labyrinthine zone of the chorioallantoic placenta. The spatial pattern of PLP-K expression in the rat labyrinth zone was punctate and most closely resembled the cellular distribution of PL-II and PLF-RP, which are

restricted to labyrinthine giant cells (Campbell *et al.* 1989, Deb *et al.* 1991, Sahgal *et al.* 2000, present study).

The significance of this apparent species difference in PLP-K expression is difficult to resolve fully. The different locations of PLP-K in the rat and the mouse may influence access to their potential targets. In the mouse, PLP-K may act in maternal, intraplacental and fetal compartments whereas, in the rat, it may be restricted to intraplacental and fetal targets. The presence of PLP-K protein in the maternal and/or fetal circulation and the nature of its physiological actions remain to be determined. It is also important to consider that the broader distribution of PLP-K in the mouse may have reflected expression patterns of additional close relatives of PLP-K not yet identified.

PLP-M

Mouse PLP-M possesses structural characteristics dictating its inclusion as a separate paralogous gene within the PRL family. We were not successful in identifying a rat ortholog for PLP-M from any of the existing EST databases. This is probably related to the limited availability of ESTs from placental cDNA libraries representing different phases of rat gestation. Other more direct cDNA cloning strategies may be required to isolate a rat PLP-M ortholog. Mouse and rat orthologs have been identified for almost all members of the PRL family (Soares *et al.* 1998).

Structurally, PLP-M is somewhat separated from other PRL family members. Its closest relatives are PLP-K and PLF (see Fig. 4). Above, we have discussed our current understanding of PLF biology and its potential significance to understanding the physiological role of PLP-K. These comments are also relevant to the physiology of PLP-M.

PLP-M exhibits a pattern of expression in the mouse placenta that is typical of most members of the PRL family (Soares *et al.* 1998, present study). Expression is initiated in trophoblast giant cells and then extends to spongiotrophoblast cells of the chorioallantoic placental junctional zone. Such a cellular source is ideally situated for the release of signaling molecules into the maternal environment. Thus it is assumed that, at least in the mouse, PLP-M directly coordinates maternal processes associated with the gestational state.

Overview

The PRL gene family of the rat and mouse at present consists of at least 20 paralogous genes. Our appreciation of PRL gene families in other species is currently quite modest, although we have some limited insight concerning the PRL family in the cow (Schuler & Kessler 1992). Evolutionary pressures responsible for the expansion of the rodent and bovine PRL families are unknown, but must relate to needs for ensuring viviparity. The key to furthering our understanding of the significance of the PRL

family expansion is through uncovering the biological actions of its constituents. The combination of identifying cellular targets and the generation of genetically mutant animals should significantly improve our appreciation of the biology of the PRL family, including the recently identified PLP-J, PLP-K and PLP-M.

Acknowledgements

This work was supported by the J B Reynolds Foundation and grants from the National Institute of Child Health and Human Development (HD02528, HD20676, HD 29797, HD33994). We would like to thank Donald Warn for assistance with the preparation of some of the graphic materials.

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Received 12 January 2000

Accepted 7 March 2000