Three novel paralogs of the rodent prolactin gene family

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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 prolifemin (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


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 C57BL/6J 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
frozen tissue sections as previously described (Faria et al. 1990, Rasmussen et al. 1997, Sahgal et al. 2000). Full-length mouse and rat cDNAs were linearized and used as templates for the synthesis of [35S]-labeled sense and antisense RNA probes.

**Results**

The uteroplacental PRL gene family represents a collection of cytokines that participate in mechanisms underlying the control of viviparity. The putative new members of the rat and mouse PRL family were acquired and characterized. We adapted our terminology for the new PRL family members to conform to that in recent reports (Ishibashi & Imai 1999, Toft & Linzer 1999).

**PLP-J cDNA characterization**

A rat cDNA exhibiting sequence similarity to PRL was obtained from the University of Iowa Rat Gene Discovery Program (dbEST identification No. Id: 1677325) and termed PLP-J. The rat PLP-J cDNA (GenBank Accession No. AF234638) encodes for a predicted 211 amino acid protein containing a predicted 29 amino acid signal peptide and two putative N-linked glycosylation sites. On the basis of nucleotide and amino acid similarities, a mouse cDNA orthologous to rat PLP-J was identified (dbEST Id: 1030399) and obtained from the IMAGE consortium. The mouse partial clone was 319 bp in length and possessed approximately 79% sequence identity with the corresponding rat PLP-J nucleotide sequence (nucleotides 555 to 874) and is tentatively considered a mouse ortholog for PLP-J. Asterisks below the sequences denote identity.

**PLP-K cDNA characterization**

A second novel rat cDNA was identified and obtained from the University of Iowa Rat Gene Discovery Program (dbEST Id: 1799330) and termed PLP-K. The rat PLP-K cDNA (GenBank Accession No. AF234635) encodes for a predicted 228 amino acid protein containing a predicted 31 amino acid signal peptide and one putative N-linked glycosylation site. Rat PLP-K possesses six cysteine residues (Fig. 2); three of these (Cys+58, Cys+180 and Cys+228) are positioned in locations homologous with cysteines in rat PRL (Cooke & Baxter 1982). A partial mouse cDNA clone bearing considerable sequence similarity to rat PLP-K was identified (dbEST Id: 628707) and obtained from the IMAGE consortium. The mouse partial clone was 319 bp in length and possessed approximately 79% sequence identity with the corresponding rat PLP-K nucleotide sequence (nucleotides 555 to 874) and is tentatively considered a mouse ortholog for PLP-K.

**PLP-M cDNA characterization**

A cDNA for mouse PLP-M was identified. The mouse PLP-M cDNA (GenBank Accession No. AF234636) encodes for a predicted 228 amino acid protein containing a predicted 28 amino acid signal peptide and one putative N-linked glycosylation site. Rat and mouse PLP-J orthologs shared approximately 79% and 70% nucleotide and amino acid sequence identity, respectively, and the location of four cysteine residues (Fig. 1). Most interestingly, the carboxy terminal pair of cysteine residues shared by all previously described PRL family members (see Soares et al. 1998) were not present in rat PLP-J, but were present in mouse PLP-J (Fig. 1).
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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<sup>1</sup> and Ile<sup>1</sup>. 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.

*Journal of Endocrinology (2000) 166, 63–75*
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 Ala₁ and Val₁. 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, Prlj, 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 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 Prlj, Prlpk and Prlpm loci are closely linked to Dtpp and Pl1 on chromosome 13 in the mouse. Allelic segregation patterns for Prlj, Prlpk, Prlpm, Dtpp and Pl1 are identical, indicating a distance of less than 1 centimorgan (cM) among these genes. The calculated map distances between Prlj, 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–Prlj, 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

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*Journal of Endocrinology (2000) 166, 63–75*
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 spongioptrophoblast 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 giant and spongioptrophoblast 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 expressed in 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 probably 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 Plh 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 decidual tissues. 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 [35S]-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 deciduomal tissue section. (D) Dark-field representation using a rat PLP-J antisense probe on a day 9 rat deciduomal 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 deciduomal tissue section. (H) Dark-field representation using a mouse PLP-J antisense probe on a day 8 mouse deciduomal tissue section. Sense probes did not provide any hybridization signal in any of the tissues investigated. Original magnifications, × 40.
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 antisymesmetrical 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).
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 cell types as gestation progressed (present study). 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.

**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.
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 spongiosotrophoblast 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.

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**References**


Ishibashi K & Imaz M 1999 Identification of four new members of the rat prolactin/growth hormone gene family. Biochemical and Biophysical Research Communications 262 575–578.


Lin J, Poole J & Linzer DIH 1997a Three new members of the mouse prolactin/growth hormone family are homologous to proteins expressed in the rat. Endocrinology 138 5541–5549.

Lin J, Poole J & Linzer DIH 1997b Two novel members of the prolactin/growth hormone family are expressed in the mouse placenta. Endocrinology 138 5535–5540.


Nicolis CS, Mayer GL & Russell SM 1986 Structural features of prolactins and growth hormones that can be related to their biological properties. Endocrine Reviews 7 169–203.


Volpert O, Jackson D, Bouck N & Linzer DIH 1996 The insulin-like growth factor II/mannose 6-phosphate receptor is...


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