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

Figure 1 Amino acid sequence comparison of rat and mouse PLP-J. Note similarities of positioning of cysteine residues (shown as outlined boxes) and differences in the presence of putative N-linked glycosylation sites (black shaded boxes). Rat PLP-J possesses two putative N-linked glycosylation sites, whereas mouse PLP-J possesses one such site. Asterisks below the sequences denote identity.

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. 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 PLP-J cDNA (GenBank Accession No. AF234637) encodes for a predicted 212 amino acid protein containing a predicted 29 amino acid signal peptide and a single 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).

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 (Fig. 2). 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 (Fig. 3). Mouse PLP-M

GGCACGAGGCTGATTTCAGCAGACAAACTAGGGTGTGACTTCTCAGGG 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								+40									
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
+50								+60									
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
+70								+80									
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
+90								+100									
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
+110								+120									
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
+130								+140									
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
+150								+160									
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
+170								+180									
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
+190								+200									
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
+210								+220									
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
+230								+240									
ATA	TTC	AAT	CAA	AAC	AAC	TGC	TAA	TAGTAAAGCAATATAATCTGTCTTGAGATATCCCT	769								
I	F	N	Q	N	N	C	*	228									
TTATAAAACATTACTGCAAAGCTCCCTTTTCGATTTCTGTTAAC TTCTTTAAAAATAAAAGCTTCTTG																	
GAATTGTTAAAAAAAAAAAAAAAAAAAAAAAAA																	

TTATAAAACATTACTGCAAAGCTCCCTTTTCGATTTCTGTCTTAAGCTTTTAAAAAATAAAAGCTTCTTG

GAATTGTTAAAAA

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	GCCCTTTT	CATCATATCTTCGTCTGAGCCACTGCT	769																								
I	S	N	E	N	N	C	*																											

TGATGATATATTGCTGTGAAACTTCCTTGAATTTTTTCTCTGTAAATGCATGCCAGTGGTGT
TATCTTCTTTTACAATAAAATTGACCCCATGTAAAAAAAAAAAAAAAAAAAA

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, *Prtpj*, *Prtpk* and *Prtpm*, 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. *Prtpj* 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). *Prtpk* 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). *Prtpm* 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 *Prtpj*, *Prtpk* and *Prtpm* loci are closely linked to *Dtprp* and *Pl1* on chromosome 13 in the mouse. Allelic segregation patterns for *Prtpj*, *Prtpk*, *Prtpm*, *Dtprp* and *Pl1* are identical, indicating a distance of less than 1 centimorgan (cM) among these genes. The calculated map distances between *Prtpj*, *Prtpk* and *Prtpm* 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-*Prtpj*, *Prtpk* and *Prtpm*-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 *Prllpj*, *Prllpk* and *Prllpm* 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 recombination 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 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 [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 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, $\times 40$.

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 [35 S]-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 13 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, $\times 40$.

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 [35 S]-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, $\times 200$; B, C, E and F, $\times 40$.

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.

References

- Brelje TC, Scharp DW, Lacy PE, Ogren L, Talamantes F, Robertson M, Friesen HG & Sorenson RL 1993 Effect of homologous placental lactogens, prolactins, and growth hormones on islet B-cell division and insulin secretion in rat, mouse, and human islets: implication for placental lactogen regulation of islets function during pregnancy. *Endocrinology* **132** 879–887.
- Bridges RS, Robertson MC, Shiu RPC, Friesen HG, Stuer AM & Mann PE 1996 Endocrine communication between conceptus and mother: placental lactogen stimulation of maternal behavior. *Neuroendocrinology* **64** 57–64.
- Bridges RS, Robertson MC, Shiu RPC, Sturgis JD, Henriques BM & Mann PE 1997 Central lactogenic regulation of maternal behavior in rats: steroid dependence, hormone specificity, and behavioral potencies of rat prolactin and rat placental lactogen I. *Endocrinology* **138** 756–763.
- Campbell WJ, Deb S, Kwok SCM, Joslin JA & Soares MJ 1989 Differential expression of placental lactogen-II and prolactin-like protein-A in the rat chorioallantoic placenta. *Endocrinology* **125** 1565–1574.
- Carney EW, Prideaux V, Lye SJ & Rossant J 1993 Progressive expression of trophoblast specific genes during formation of mouse trophoblast giant cells *in vitro*. *Molecular Reproduction and Development* **34** 357–368.
- Cohick CB, Dai G, Xu L, Deb S, Kamei T, Levan G, Szpirer C, Kwok SCM & Soares MJ 1996 Placental lactogen-I variant utilizes the prolactin receptor signaling pathway. *Molecular and Cellular Endocrinology* **116** 49–58.
- Cohick CB, Xu L & Soares MJ 1997 Prolactin-like protein-B: heterologous expression and characterization of placental and decidual species. *Journal of Endocrinology* **152** 291–302.
- Colosi P, Ogren L, Southard JN, Thordarson G, Linzer DIH & Talamantes F 1988a Biological, immunological, and binding properties of recombinant mouse placental lactogen-I. *Endocrinology* **123** 2662–2667.
- Colosi P, Swiergiel JJ, Wilder EL, Oviedo A & Linzer DIH 1988b Characterization of proliferin-related protein. *Molecular Endocrinology* **2** 579–586.
- Cooke NE & Baxter JD 1982 Structural analysis of the prolactin gene suggests a separate origin for its 5' end. *Nature* **297** 603–606.
- Croze F, Kennedy TG, Schroeder IC & Friesen HG 1990 Expression of rat prolactin-like protein B in decidualoma of pseudopregnant rat and in decidua during early pregnancy. *Endocrinology* **127** 2665–2672.
- Dai G, Imagawa W, Liu B, Levan G, Szpirer C, Kwok SCM & Soares MJ 1996 Rcho-1 trophoblast cell placental lactogens: complementary DNAs, heterologous expression, and biological activities. *Endocrinology* **137** 5020–5027.

- Dai G, Chapman BM, Liu B, Wang D, Orwig KE, White RA, Preuett B & Soares MJ 1998 A new mouse member of the prolactin-like protein-C subfamily: structure and expression. *Endocrinology* **139** 5157–5163.
- Dai G, Chapman BM, Wang D, White RA, Preuett B & Soares MJ 1999 Prolactin-like protein-A gene structure and chromosomal mapping. *Mammalian Genome* **10** 78–80.
- Deb S, Faria TN, Roby KF, Larsen D, Kwok SCM, Talamantes F & Soares MJ 1991 Identification and characterization of a new member of the prolactin family, placental lactogen-I variant. *Journal of Biological Chemistry* **266** 1605–1610.
- Efron B, Halloran E & Holmes S 1996 Bootstrap confidence levels for phylogenetic trees. *PNAS* **93** 13429–13434.
- Faria TN, Deb S, Kwok SCM, Talamantes F & Soares MJ 1990 Ontogeny of placental lactogen-I and placental lactogen-II expression in the developing rat placenta. *Developmental Biology* **141** 279–291.
- Galosy SS & Talamantes F 1995 Luteotropic actions of placental lactogens at midpregnancy in the mouse. *Endocrinology* **136** 3993–4003.
- Groskopf JC, Syu L-J, Saltiel AR & Linzer DIH 1997 Proliferin induces endothelial cell chemotaxis through a G protein-coupled, mitogen-activated protein kinase-dependent pathway. *Endocrinology* **138** 2835–2840.
- Hiraoka Y, Ogawa M, Sakai Y, Takeuchi Y, Komatsu N, Shiozawa M, Tanabe K & Aiso S 1999 PLP-I: a novel prolactin-like gene in rodents. *Biochimica et Biophysica Acta* **1447** 291–297.
- Ishibashi K & Imai M 1999 Identification of four new members of the rat prolactin/growth hormone gene family. *Biochemical and Biophysical Research Communications* **262** 575–578.
- Jackson D, Volpert OV, Bouck N & Linzer DIH 1994 Stimulation and inhibition of angiogenesis by placental proliferin and proliferin-related protein. *Science* **266** 1581–1584.
- Jackson-Grusby LL, Pravtcheva D, Ruddle FH & Linzer DIH 1988 Chromosomal mapping of the prolactin/growth hormone gene family in the mouse. *Endocrinology* **122** 2462–2466.
- Kumar S, Tamura K & Nei M 1994 MEGA: molecular evolutionary analysis software for microcomputers. *Computational Applied Biosciences* **10** 189–191.
- Lee S-J & Nathans D 1988 Proliferin secreted by cultured cells binds to mannose 6-phosphate receptors. *Journal of Biological Chemistry* **263** 3521–3527.
- Lin J & Linzer DIH 1999 Induction of megakaryocyte differentiation by a novel pregnancy-specific hormone. *Journal of Biological Chemistry* **274** 21485–21489.
- 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.
- Linzer DIH 1995 Placental angiogenic and anti-angiogenic factors. *Journal of National Institutes of Health Research* **7** 57–58.
- Linzer DIH & Nathans D 1984 Nucleotide sequence of a growth-related mRNA encoding a member of the prolactin-growth hormone family. *PNAS* **81** 4255–4259.
- Linzer DIH & Talamantes F 1985 Nucleotide sequence of mouse prolactin and growth hormone mRNAs and expression of these mRNAs during pregnancy. *Journal of Biological Chemistry* **260** 9574–9579.
- Linzer DIH & Fisher SJ 1999 The placenta and the prolactin family of hormones: regulation of the physiology of pregnancy. *Molecular Endocrinology* **13** 837–840.
- Linzer DIH, Lee S-J, Ogren L, Talamantes F & Nathans D 1985 Identification of proliferin mRNA and protein in mouse placenta. *PNAS* **82** 4356–4359.
- Müller H, Ishimura R, Orwig KE, Liu B & Soares MJ 1998a Homologues for prolactin-like proteins A and B are present in the mouse. *Biology of Reproduction* **58** 45–51.
- Müller H, Orwig KE & Soares MJ 1998b Identification of two new members of the mouse prolactin family. *Biochimica et Biophysica Acta* **1396** 251–258.
- Müller H, Liu B, Croy BA, Head JR, Hunt JS, Dai G & Soares MJ 1999 Uterine natural killer cells are targets for a trophoblast cell-specific cytokine, prolactin-like protein-A. *Endocrinology* **140** 2711–2720.
- Nelson JT, Rosenzweig N & Nilsen-Hamilton M 1995 Characterization of the mitogen-regulated protein (proliferin) receptor. *Endocrinology* **136** 283–288.
- Nicoll 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.
- Ogren L & Talamantes F 1988 Prolactins of pregnancy and their cellular source. *International Reviews in Cytology* **112** 1–65.
- Orwig KE, Dai G, Rasmussen CA & Soares MJ 1997a Decidual/trophoblast prolactin related protein: characterization of gene structure and cell-specific expression. *Endocrinology* **138** 2491–2500.
- Orwig KE, Ishimura R, Müller H, Liu B & Soares MJ 1997b Identification and characterization of a mouse homolog for decidual/trophoblast prolactin-related protein. *Endocrinology* **138** 5511–5517.
- Rasmussen CA, Hashizume K, Orwig KE, Vellucci S, Xu L & Soares MJ 1996 Decidual prolactin-related protein: heterologous expression and characterization. *Endocrinology* **137** 5558–5566.
- Rasmussen CA, Orwig KE, Vellucci S & Soares MJ 1997 Dual expression of prolactin-related protein in decidua and trophoblast tissues during pregnancy. *Biology of Reproduction* **55** 647–654.
- Robertson MC, Gillepsie B & Friesen HG 1982 Characterization of the two forms of rat placental lactogen (rPL): rPL-I and rPL-II. *Endocrinology* **111** 1862–1866.
- Robertson MC, Cosby H, Fresnoza A, Cattini PA, Shiu RPC & Friesen HG 1994 Expression, purification, and characterization of recombinant rat placental lactogen-I: a comparison with the native hormone. *Endocrinology* **134** 393–400.
- Rowe LB, Nadeau JH, Turner R, Frankel WN, Letts VA, Eppig JT, Ko MSH, Thurston SJ & Birkenmeier EH 1994 Maps from two interspecific backcross DNA panels available as a community genetic mapping resource. *Mammalian Genome* **5** 253–274.
- Sahgal N, Knipp GT, Liu B, Chapman BM, Dai G & Soares MJ 2000 Identification of two new nonclassical members of the rat prolactin family. *Journal of Molecular Endocrinology* **24** 95–108.
- Schuler LA & Kessler MA 1992 Bovine placental prolactin-related hormones. *Trends in Endocrinology and Metabolism* **3** 334–338.
- Soares MJ 1987 Developmental changes in the intraplacental distribution of placental lactogen and alkaline phosphatase in the rat. *Journal of Reproduction and Fertility* **79** 93–98.
- Soares MJ, Müller H, Orwig KE, Peters TJ & Dai G 1998 The uteroplacental prolactin family and pregnancy. *Biology of Reproduction* **58** 273–284.
- Thompson JD, Higgins DG & Gibson TJ 1994 CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22** 4673–4680.
- Thordarson G, Villalobos R, Colosi P, Southard JN, Ogren L & Talamantes F 1986 Lactogenic response of cultured mouse mammary epithelial cells to mouse placental lactogen. *Journal of Endocrinology* **109** 263–274.
- Toft DJ & Linzer DIH 1999 Prolactin (PRL)-like protein J, a novel member of the PRL/growth hormone family, is exclusively expressed in maternal decidua. *Endocrinology* **140** 5095–5101.
- Volpert O, Jackson D, Bouck N & Linzer DIH 1996 The insulin-like growth factor II/mannose 6-phosphate receptor is

- required for proliferin-induced angiogenesis. *Endocrinology* **137** 3871–3876.
- Voogt JL, Soares MJ, Robertson MC & Arbogast LA 1996 Rat placental lactogen-I abolishes nocturnal prolactin surges in the pregnant rat. *Endocrine* **4** 233–238.
- Wallis M 1992 The expanding growth hormone/prolactin family. *Journal of Endocrinology* **9** 185–188.

White RA, Peters LL, Adkison LR, Korsgren C, Cohen CM & Lux SE 1992 The murine pallid mutation is a platelet storage pool disease associated with the protein 4·2 (pallidin) gene. *Nature Genetics* **2** 80–83.

Received 12 January 2000

Accepted 7 March 2000