

# The Uteroplacental Prolactin Family and Pregnancy<sup>1</sup>

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## INTRODUCTION

The establishment and maintenance of pregnancy requires significant modifications of the maternal environment. During pregnancy, changes arise in virtually every major organ system within the mother, permitting growth and development of the embryo/fetus within the protected confines of the maternal reproductive tract. These gestational-dependent adaptations are believed to be controlled, at least in part, through the elaboration of chemical signals by the uterus and placenta referred to as hormones/cytokines. Some uteroplacental signals have been coupled to the regulation of specific maternal responses, while a specific function is not known for other putative signaling molecules that have been identified.

The subject of this review is the uteroplacental prolactin (PRL) family. PRL is a multifunctional hormone/cytokine that has been used as an evolutionary template for the generation of regulatory molecules involved in the establishment and maintenance of pregnancy. Several reviews on the PRL family have appeared over the past several years and are recommended [1–7].

## HISTORICAL BACKGROUND

The first evidence for the existence of PRL-like hormones in uteroplacental tissues arose from studies of the hypophysectomized mouse and rat by such pioneers as Long, Selye, Astwood, Greep, Deansley, Allen, and Lyons [8–13]. Removal of the anterior pituitary after midgestation was compatible with the continued function of the corpus luteum and the development of the mammary glands, two well-established actions attributed to the anterior pituitary hormone PRL [14]. Placental extracts were shown to possess both luteotropic and lactogenic activities. These early findings spurred investigations, led by Henry G. Friesen, Frank Talamantes, and Geula Gibori among others, culminating in the discovery of uteroplacental proteins possessing lactogenic/luteotropic activities from several species. A number of compelling insights resulted from this significant body of work, including 1) the existence of protein families; 2) PRL or growth hormone (GH) as an evolutionary prototype, 3) the existence of uteroplacental proteins related to PRL lacking classical PRL biological actions; and 4) the absence of placental lactogenic activities in selected species.

### 1. Existence of Protein Families

Early on it became evident that there was not a single placental lactogen (PL). As lactogens were isolated from

the placenta and characterized at biochemical and molecular levels, related proteins and cDNAs were discovered. Families of proteins expressed in the uterus and/or placenta have now been described in primates, ruminants, and rodents (Fig. 1; [3–7]). Although the significance of the gene families are not readily apparent, they most likely arose by gene duplication [15]. It has become evident that we are not dealing with a simple concept of a single hormone/cytokine corresponding with a single biological function. Groups of related proteins with overlapping and distinct expression and distribution patterns and functions have been described. Specialization would appear to be the key to appreciating the derivation of these uteroplacental protein families.

### 2. PRL or GH as the Evolutionary Prototype

Two different templates have been used for the generation of placental proteins with lactogenic/luteotropic activities. Primates have evolved a family of proteins expressed in the placenta related to GH, whereas for some ruminants and rodents, PRL is the evolutionary prototype (Fig. 1). GH and PRL genes are situated on different chromosomes in each species. In the human, the related genes expressed in the placenta are contiguous with the GH gene on chromosome 17 [16, 17], whereas in the cow, they are located on chromosome 22 along with the PRL gene [18], and in rodents they co-segregate with the PRL gene on mouse chromosome 13 [19, 20] or rat chromosome 17 [5, 21–26]. The human placenta does not appear to express PRL or any genes closely related to PRL [7]. Nonetheless, in addition to members of the placental GH family, the uteroplacental milieu contains another activator of the PRL receptor. This activator is PRL, which in addition to being expressed in the anterior pituitary is also expressed in uterine decidual cells through the use of alternative promoters [7, 27].

The use of GH or PRL prototypes for the derivation of the uteroplacental protein families has a functional corollary. Primate GHs can dually activate GH and PRL receptors, whereas GHs from other species are restricted to GH receptor activation [28]. PRLs from primates, ruminants, and rodents effectively activate the PRL receptor signaling pathway. The apparent common feature is that each evolutionary template can generate proteins capable of activating the PRL receptor signaling pathway. The significance of this observation remains to be determined.

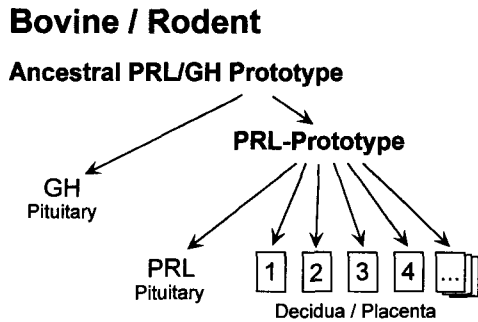
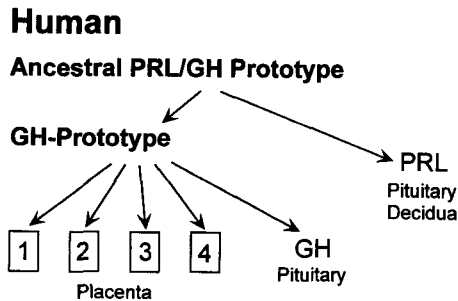
### 3. Nonclassical Members of the PRL Family

Initial discoveries and functional characterizations of members of the uteroplacental PRL family were based on biological activities previously attributed to pituitary PRL (classical actions). It is now evident that many members of the uteroplacental PRL family are not PRL receptor agonists. Investigations on the biological activities of these members of the family are actively being pursued.

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Species	Gene	Chromosomal Location
Human	PRL	6
	GH family	17
Cow	PRL family	22
	GH	19
Rat	PRL family	17
	GH	10
Mouse	PRL family	13
	GH	11

FIG. 1. Evolutionary relationships and derivation of PRL/GH gene families. Two evolutionary patterns have been described for the derivation of uteroplacental-specific hormones/cytokines. **Top)** Human model shows the derivation of GH and members of the placental family from a GH prototype. The sole PRL derivative is expressed in both the anterior pituitary and decidua. **Middle)** Bovine/rodent model shows the derivation of PRL and members of the placental and decidual family from a PRL prototype. The sole GH derivative is expressed in the anterior pituitary. **Bottom)** Shows the chromosomal location of members of the PRL and GH families in the human, cow, mouse, and rat.

**4. Absence of Placental Lactogenic Activities in Selected Species**

During the early cross-species surveys, it became apparent that some mammals such as the pig, horse, rabbit, and dog did not possess a classic PL [29]. There are many examples of divergence in evolution yielding distinct solutions for regulating physiological processes, and this may be yet another. However, complicating the issue is that the absence of PL in several species is based on limited experimentation. Some of the apparent nonexpressing species may actually possess a classic PL with a restricted expression pattern that was not adequately tested. Alternatively, nonclassical members of the PRL family may be expressed by uteroplacental tissues in these species. Possibly it is not the need for an activator of the PRL receptor that has driven the evolution of the uteroplacental PRL families but some other function(s). Future cross-species examination of structural and functional homologues for nonclassical members of the PRL family will help resolve this uncertainty.

**THE RODENT UTEROPLACENTAL PRL FAMILY**

We will concentrate the remaining discussion on the rodent uteroplacental PRL family. The rat and mouse have

been the principal models for investigation and will represent our primary resource. A number of PRL family members have been characterized in each of these species. Many PRL family members have been identified as being homologous in the mouse and rat. Although mouse or rat homologues have not been determined for other family members, this does not necessarily exclude their existence. In the remaining sections, we will initially provide a brief overview on the cells responsible for the production of members of the uteroplacental PRL family and then present our current understanding of the structure, expression, distribution, and biological activities of the uteroplacental PRL family members.

*Cellular Sources*

There are three differentiated cell types involved in the biosynthesis of members of the uteroplacental PRL family in the rat and mouse. Two of the cell types are of trophoblast origin, and the third is derived from maternal uterine stroma (Fig. 2).

*Trophoblast contribution.* Trophoblast cells are the parenchymal cells of the placenta, which can be organized into two distinct structures: choriovitelline and chorioallantoic placentas. These structures are responsible for controlling fetal and maternal environments during pregnancy. The choriovitelline placenta is a relatively simple structure consisting of trophoblast cells adhering to parietal endoderm. It forms shortly after implantation and degenerates shortly after mid-gestation. In contrast, the chorioallantoic placenta is a more complex structure of the latter half of pregnancy that is organized into an invasive/endocrine component—located at the maternal interface and referred to as the junctional zone—and a region responsible for maternal-fetal bidirectional transport and limited endocrine activity—located at the fetal interface and referred to as the labyrinth zone. Two trophoblast cell types express members of the PRL family: 1) trophoblast giant cells and 2) spongiotrophoblast cells. Additional information on the development and organization of rodent trophoblast cells is available [1, 2, 30].

1) Trophoblast giant cells. Trophoblast giant cells represent the major endocrine cell type of the choriovitelline and chorioallantoic placentas. Within the chorioallantoic placenta, trophoblast giant cells are situated at the maternal-placental interface of both the junctional and labyrinthine compartments. Trophoblast giant cells possess an amplified genome that arises by a process referred to as endoreduplication. The control of trophoblast giant cell formation is poorly understood. The Rcho-1 trophoblast cell line represents an in vitro model that can be manipulated to recapitulate and investigate many aspects of trophoblast giant cell differentiation [31].

2) Spongiotrophoblast cells. Spongiotrophoblast cells are a major constituent of the junctional zone of the chorioallantoic placenta. They are critical for pregnancy, and their development appears to be dependent upon the actions of a helix-loop-helix (HLH) transcription factor, Mash-2 [32, 33], and possibly the epidermal growth factor (EGF) receptor [34, 35]. Primary spongiotrophoblast cell cultures have been used with some success in studying their development and function [36].

*Maternal uterine stroma contribution.* The remaining cell-type involved in the biosynthesis of uteroplacental PRL family members is derived from maternal uterine stroma and is referred to as a decidual cell. Differentiation of rodent decidual cells requires ovarian steroids, progesterone

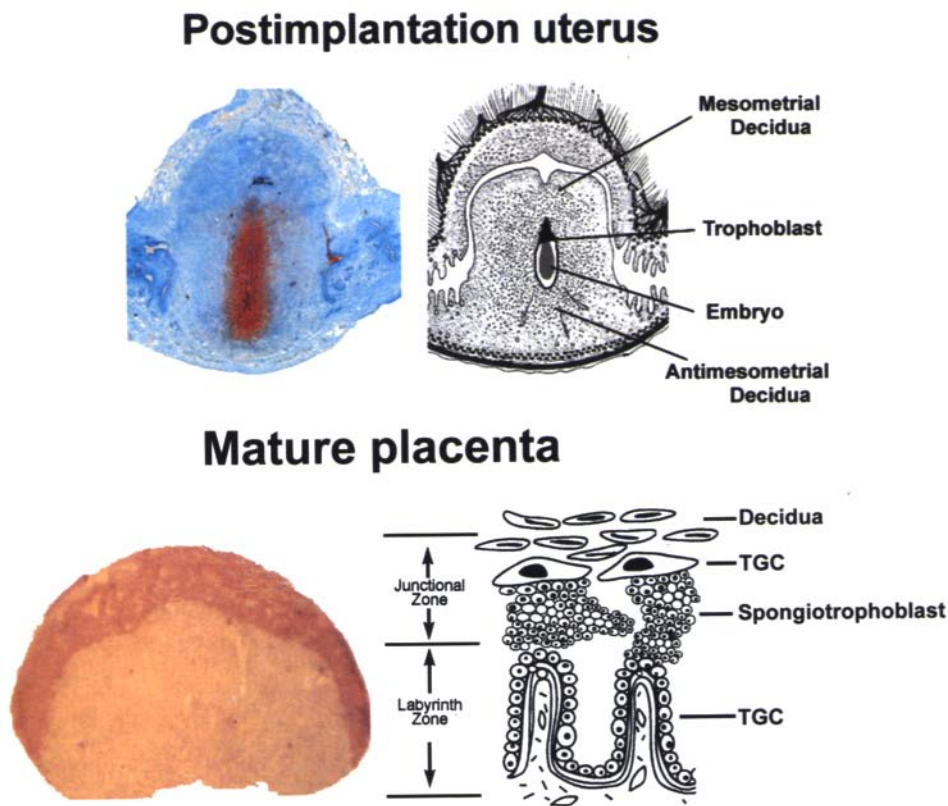


FIG. 2. Organization of the rodent utero-placental compartment. **Top**) Cross section and schematic representation of a postimplantation uterus (Day 7/8 of gestation). **Bottom**) Cross section and schematic representation of a mature rat chorioallantoic placenta. The upper dark brown staining region and the lower lighter-appearing region represent the junctional and labyrinth zones, respectively. TGC, trophoblast giant cell.

and estrogen, and the implanting blastocyst [37]. Decidual cells comprise a transient structure that first forms at the time of implantation and surrounds the developing blastocyst, providing a barrier between the blastocyst and the remainder of the uterus [37–39]. Mesometrial (closest to the incoming blood supply) and antimesometrial (furthest from incoming vasculature) decidua are morphologically and functionally distinct [38, 40]. Antimesometrial decidua degenerates in conjunction with the demise of the choriovitelline placenta [41].

#### Discovery

The successful isolation of PLs from rodent placentas resulted from technical advances in the detection of PRL-like bioactivities [42, 43]. Discovery of many members of the PRL family proceeded along a somewhat linear path. The saga begins with the identification of a functional PRL receptor agonist from late-gestation rat and mouse placental tissue. During the characterization of this initial 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 [44, 45] and subsequently found to be expressed in the mouse placenta [46]. The second exception emanates from the mouse genome project. Expressed sequence tags (ESTs) isolated from mouse uterine and extraembryonic cDNA libraries with homology to members of the PRL family have been found in the National Center for Biotechnology Information dbEST database (Bethesda, MD). Perusal of this repository resulted in the demonstration of previously unidentified mouse homologues for rat PRL family members [20] and

the discovery of novel mouse placental PRL family members (unpublished results). The current listing of rodent PRL family members and their commonly used abbreviations are provided in Table 1. We have further categorized members of the PRL family into subfamilies based on structural (e.g., PL-I and PRL-like protein-C [PLP-C] subfamilies) and functional (classical vs. nonclassical) considerations (Table 1).

#### Structural Features

In this section, we will provide an account of our present understanding of structural features of the PRL gene family.

TABLE 1. Mouse/rat PRL family.

Hormone	Species
<b>Classical members</b>	
Prolactin	Mouse, rat
Placental lactogen-I subfamily	
Placental lactogen-I (PL-I)	Mouse, rat
Placental lactogen-I variant (PL-Iv)	Rat
Placental lactogen-II (PL-II)	Mouse, rat
<b>Nonclassical members:</b>	
Prolactin-like protein-A (PLP-A)	Mouse, rat
Prolactin-like protein-B (PLP-B)	Mouse, rat
Prolactin-like protein-C subfamily	
Prolactin-like protein-C (PLP-C)	Rat
Prolactin-like protein-C variant (PLP-Cv)	Mouse, rat
Prolactin-like protein-D (PLP-D)	Rat
Prolactin-like protein-E (PLP-E) <sup>a</sup>	Rat
Prolactin-related protein (d/tPRP)	Mouse, rat
Proliferin (PLF)	Mouse
Proliferin-related protein (PLF-RP)	Mouse
Prolactin-like protein-F (PLP-F) <sup>b</sup>	Mouse
Prolactin-like protein-G (PLP-G) <sup>b</sup>	Mouse

<sup>a</sup> K. Iwatsuki and K. Shiota, personal communication.

<sup>b</sup> H. Müller and M.J. Soares, unpublished results.

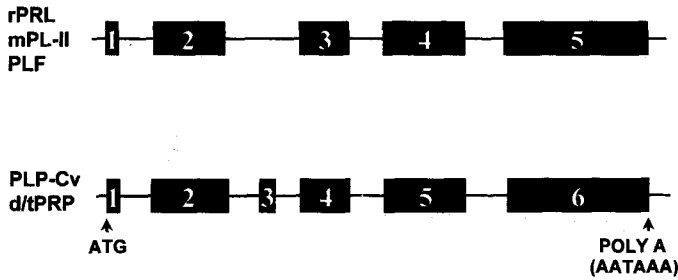


FIG. 3. Exon-intron organization of the rodent PRL gene family. The upper representation depicts the organization of the rat PRL (rPRL), mouse placental lactogen-II (mPL-II), and mouse PLF (PLF) genes; the lower representation depicts the organization of the rat PLP-Cv (PLP-Cv) and rat d/tPRP genes. Exons are depicted by shaded boxes. Please note that the PLP-Cv and d/tPRP genes contain an extra exon situated between the second and third exons of the prototypical PRL exonic organization seen in PRL, PL-II, and PLF.

The analysis includes comments at the gene, amino acid, and posttranslational levels.

**Gene structure.** As indicated above, PRL family members are arranged on chromosome 13 in the mouse and chromosome 17 in the rat. Although the precise arrangement of each gene along its respective chromosome has not been reported, it is apparent, at least in the mouse, that the PLF gene is physically separated from the PL-I and decidual/trophoblast PRL-related protein (d/tPRP) genes [20]. Information on gene structure is limited to only a few representative members (Fig. 3). A couple of exon/intron organization patterns have been described: 1) the prototypical 5-exon/4-intron arrangement found in PRL, PL-II, and PLF [47–51] and 2) a 6-exon/5-intron structure found in two members of the PLP-C subfamily, PLP-Cv and d/tPRP [24, 52]. The latter exon/intron arrangement represents the addition of a small exon between exons 2 and 3 of the prototypical PRL exon/intron organization. Based on amino acid homologies, this unique exon would probably be present in all members of the PLP-C subfamily and in PLF-related protein (PLF-RP, [2, 53]). The significance of the unique exon is unknown.

**Primary structure.** There is an assortment of structural characteristics that define a protein as a member of the PRL

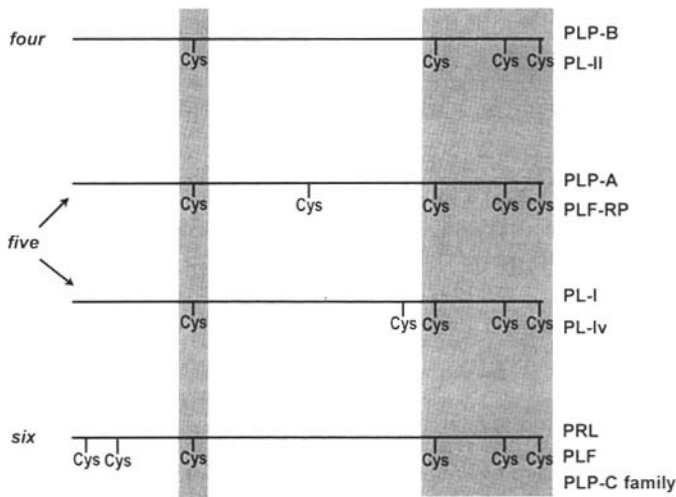


FIG. 4. Schematic comparisons of protein structures for members of the uteroplacental PRL family. Cysteine residues are shown below each line. Locations of homologous cysteine residues are shaded. Please note that there are four basic organization patterns containing four, five, or six cysteine residues.

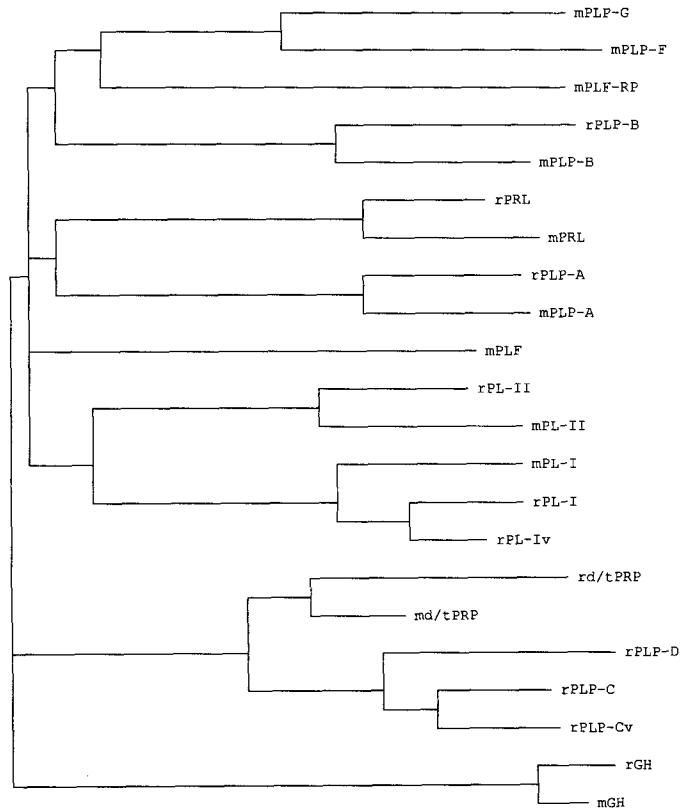


FIG. 5. Phylogenetic analysis for members of the mouse (m) and rat (r) PRL gene family.

family. These include positioning of cysteine residues (Fig. 4) and the situation of other key amino acids [28, 54]. All members of the PRL family have a backbone of four highly conserved cysteine residues. A subset, which includes PL-I, PL-I variant (PL-Iv), PLP-A, and PLF-RP possess a fifth cysteine [22, 26, 53, 55–59]. Another subgroup, consisting of PRL, PLF, and members of the PLP-C subfamily, has six homologously located cysteines [20, 23–25, 45, 47–49, 60]. Positioning cysteines within the protein structure probably influences conformational attributes and possibly influences function. Although this structure-function association dictated by the presence of cysteine residues is logical, at this juncture, it does not appear to provide a useful formula for determining functional relationships within the PRL family. Classical members of the PRL family exist with four (PL-II), five (PL-I), or six (PRL) cysteine residues.

Additional information has been obtained by comparing amino acid sequences of PRL family members (Fig. 5). The characterization of homologues in both mouse and rat has led to the identification of subfamily-, ligand-, and species-specific domains within the PRL family. This is exemplified by exon 3 from members of the PLP-C subfamily, which encodes, in part, for a region contributing to an amino acid segment rich in aromatic amino acids called the “aromatic domain.” The functional significance of the aromatic domain in the PLP-C subfamily is yet to be determined. Pivotal amino acids within PRL necessary for interactions with PRL receptors have been predicted [28]. Their absence in nonclassical members of the PRL family is consistent with the inability of nonclassical members to activate PRL receptor signaling pathways.

**Posttranslational modifications.** All uteroplacental PRL family members appear to receive some type of carbohy-

drate modification except PL-II and possibly PLP-Cv [25, 61–66]. Glycosylation patterns are cell type- and protein-dependent [67]. Each of the cell types responsible for expressing a uteroplacental PRL family member is capable of adding carbohydrate moieties to the protein backbone. PRL family members expressed by spongiotrophoblast cells receive distinct glycosylation patterns, which include Asn-linked oligosaccharides containing both GalNAc and sialic acid [67]. This type of carbohydrate structure appears to be characteristic of proteins secreted during pregnancy in a number of species, including chorionic gonadotropin (CG) in primates. Of the rodent uteroplacental PRL family members, PLP-A is most efficiently modified in this manner and is as good a substrate as is CG [67]. The addition of specific oligosaccharide structures, resembling mannose-6-phosphate, may also be important in generating biologically active PLF ([68–70]; see below). There also appear to be some mouse/rat differences regarding glycosylation state. For example, rat PLP-A has two putative *N*-linked glycosylation sites that are associated with the generation of two characteristic glycoprotein species of 29 and 33 kDa, whereas the mouse has only a single putative *N*-linked glycosylation site that is associated with a single 29-kDa protein species [59, 63]. Thus far, the species differences appear to be more quantitative than qualitative, and their physiological significance is not apparent.

Specific receptors for these carbohydrate structures have been described and may participate in regulating the half-life of the molecules, their delivery to maternal and fetal compartments, and activation of cellular signal transduction pathways (see below).

### Expression

Members of the uteroplacental PRL family are expressed in precise cell and temporal patterns. These patterns probably have relevance to the roles of each hormone in the establishment and maintenance of pregnancy. The expression patterns also provide information regarding the developmental state of the cells responsible for their expression. In the following paragraphs, we will describe expression patterns and discuss possible mechanisms controlling expression of the uteroplacental PRL family.

*Patterns of expression.* For the purpose of this discussion, we will classify expression according to cell type (Table 2). Individual PRL family members can be expressed by 1) a single cell type (monopartite), 2) two cell types (bipartite), or 3) three cell types (tripartite). Expression of the same PRL family member in more than one cell type has important implications. Cellular source directly affects posttranslational processing and thus potentially the distribution and activity of the ligand. Additionally, the location of cells involved in ligand biosynthesis affects the ligand's access to target cells.

1) Monopartite expression (trophoblast giant cell). Trophoblast giant cells are the most versatile of the cellular sources, possessing the capacity to express all but one member of the uteroplacental PRL family, and are the exclusive sources of PL-I, PL-II, and PLF [71–81]. The behavior of trophoblast giant cells differs depending upon their intraplacental location and developmental state. Expression patterns involve trophoblast giant cells residing in the choriovitelline placenta and in both the junctional and labyrinth regions of the chorioallantoic placenta. PL-I and PLF first appear immediately postimplantation [72, 74, 75]. PL-I terminates at midgestation [72–74, 77], whereas PLF

TABLE 2. Cellular sources of members of the uteroplacental PRL family.

Mode	Cellular source	Hormone
Monopartite	Trophoblast giant cell	PL-I, PL-II, PLF
Bipartite	Trophoblast giant cell and spongiotrophoblast cell	PLPs (except PLP-B) PL-IV, PLF-RP
	Decidual cell and spongiotrophoblast cell	PLP-B
Tripartite	Decidual cell, trophoblast giant cell, and spongiotrophoblast cell	d/tPRP

continues through the second half of gestation at reduced levels of production [46, 74, 81]. PL-II expression begins at midgestation and continues until the termination of pregnancy [72, 76, 78]. Although PL-I, PLF, and PL-II share cell-specific requirements for expression, they appear to be optimally activated in response to distinct factors in the trophoblast giant cell environment.

2) Bipartite expression (trophoblast giant cell, spongiotrophoblast; decidual cell, spongiotrophoblast). Two examples of bipartite expression are evident. One involves the two trophoblast cell types and the other, trophoblast and decidual cells.

Clearly, the most common pattern of gene regulation involves expression by spongiotrophoblast and trophoblast giant cells. PLP-A, PLP-C, PLP-Cv, PLP-D, PLP-E, PLF-RP, and PL-IV are dually expressed by both cell types during the second half of gestation ([23, 59, 61, 65, 76, 78, 82]; K. Iwatsuki, K. Shiota, personal communication). Biosynthesis of these hormones is restricted to the two cell populations within the junctional zone and does not involve trophoblast giant cells of the labyrinthine placenta. There is some evidence for the separation of DNA regulatory elements controlling gene activation of spongiotrophoblast cells versus trophoblast giant cells [83].

PLP-B possesses a unique bipartite expression pattern involving maternal and extraembryonic cells. Antimesometrial decidual cells are the primary source of PLP-B during early pregnancy, and spongiotrophoblast cells are the principal cellular sources during the latter half of gestation [59, 76, 84–86]. At present, PLP-B appears to be the only member of the uteroplacental PRL family not expressed by trophoblast giant cells.

3) Tripartite expression (decidual cell, spongiotrophoblast, trophoblast giant cell). The final expression pattern, exemplified by d/tPRP, involves three cell types—decidual, spongiotrophoblast, and trophoblast giant cells [20, 25, 52, 87, 88]—and represents a combination of the two bipartite patterns described above. D/tPRP is predominantly expressed by antimesometrial decidual cells during early gestation and then by spongiotrophoblast and trophoblast giant cells during the second half of pregnancy [20, 88].

*Regulation of expression.* Cell and temporal similarities in the patterns of expression of the uteroplacental PRL family members suggest potential commonalities in regulation. Both in vivo and in vitro models have been employed to investigate control of the uteroplacental PRL family.

In vivo experimentation directed toward studying placental function is inherently problematic and has been limited to a few investigations on placental PRL family members (see ref. [2] for a review). In some instances, levels of hormones in circulation or within the placenta have been impacted by a specific manipulation (removal of the anterior pituitary, ovary, fetus, etc.). However, except for a role

TABLE 3. Regulation of uteroplacental PRL family members.

Regulator <sup>a</sup>	Model system <sup>b</sup>	Response	Refs
Extracellular signaling molecules			
FGF-2	Rcho-1	↑ PL-I	[98]
EGF	Rcho-1, Pc <sub>mix</sub>	↑ PL-I, ↓ PL-II	[98–101]
LIF, OM, IL-11	Pc <sub>mix</sub>	↑ PL-I, ↓ PL-II	[102]
Progesterone	Pc <sub>mix</sub>	↓ PL-I, ↓ PL-II	[91, 103]
TGF-α	Pc <sub>mix</sub>	↓ PL-I	[101]
Insulin	Pc <sub>mix</sub>	↑/↓ PL-II	[90, 104]
Calcyclin, inhibin, GHRH	Pc <sub>mix</sub>	↑ PL-II	[104–106]
IL-6, IL-1, TNF-α, TGF-β <sub>1</sub> , activin	Pc <sub>mix</sub>	↓ PL-II	[106–112]
Retinoic acid	Pc <sub>spong</sub>	↓ PL-Iv, ↓ PLP-C	[36]
Intracellular signaling molecules			
Cyclic AMP	Pc <sub>mix</sub>	↑/↓ PL-I, ↓ PL-II, ↑ PLF, ↑ PLF-RP	[113, 114] [115]
MEK	Rcho-1	↑ PL-I	[98]
Hxt	Rcho-1	↑ PL-I	[116]
GATA-2/3	Rcho-1	↑ PL-I, ↑ PLF	[117, 118]
Id-1	Rcho-1	↓ PL-I	[116]

<sup>a</sup> LIF, leukemic inhibitory factor; OM, oncostatin M; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor; MEK, mitogen-activated protein kinase kinase.

<sup>b</sup> Rcho-1, Rcho-1 trophoblast cell line; Pc<sub>mix</sub>, mixed primary cultures; Pc<sub>spong</sub>, primary spongiotrophoblast cell cultures.

of an anterior pituitary factor, possibly GH, in regulating PL-I and PL-II clearance [89], little is known of the specific regulatory processes affected by any of the *in vivo* manipulations.

*In vitro* experimentation has been more extensive. Two approaches have been used: 1) primary cultures and 2) cell lines.

1) Primary cultures. Isolation and culture procedures have been described for obtaining primary mouse and rat trophoblast and decidual cell populations capable of synthesizing members of the PRL family [36, 52, 90, 91]. Unfortunately, primary cultures are difficult to isolate to homogeneity; thus their impact on studying the regulation of uteroplacental PRL family gene expression has been mixed.

2) Cell lines. Some progress in establishing trophoblast and decidual cells lines expressing members of the uteroplacental PRL family has been made.

The *in vitro* model best characterized is the Rcho-1 trophoblast cell line, which was derived from a rat choriocarcinoma [31, 92]. *In vitro*, Rcho-1 trophoblast cells can be manipulated to recapitulate trophoblast giant cell development, including endoreduplication and the expression of trophoblast giant cell-specific members of the placental PRL family [31, 93, 94]. Rcho-1 trophoblast cells have been used for the identification of intracellular signaling pathways controlling trophoblast giant cell development and trophoblast giant cell-specific gene expression (see below). Another cell line (RCHO), derived from the same rat choriocarcinoma and possessing similar characteristics, has also been described [95, 96].

A rat antimesometrial decidual cell line, GG-AD, has recently been characterized [97]. The cell line was established by viral transformation of antimesometrial rat decidual cells with a temperature-sensitive Simian virus. GG-AD cells can be induced to differentiate into cells resembling antimesometrial decidual cells and exhibit some capacity for PLP-B expression; however, they do not appear to be capable of d/tPRP expression. Additional investigation with the GG-AD cell line will help explain the nature of the decidual phenotype it represents. Two other uterine stromal cell lines (UI and CUS-V2) may have some potential in the study of d/tPRP gene regulation [52].

*In vitro* models are beneficial for understanding the regulatory pathways controlling differentiation of the cell lineages responsible for uteroplacental PRL family expression. The Rcho-1/RCHO and GG-AD cell lines have proven beneficial for studying trophoblast giant cell and antimesometrial decidual cell development, respectively. It is important to appreciate that *in vitro* models represent caricatures of normal development. No single *in vitro* system mimics a developmental process in its entirety. Thus, the establishment of additional cell culture models for studying the uteroplacental PRL family, especially spongiotrophoblast models, is of considerable importance.

*Regulatory factors.* We possess a modicum of knowledge about the regulation of the uteroplacental PRL family. Some insights concerning signaling pathways responsible for the activation of a few members of the PRL family are available, especially for PL-I. Unfortunately, the overall limited information regarding the regulation of uteroplacental PRL family members makes it difficult to generalize about patterns of control. A summary of extracellular factors and intracellular mediators regulating members of the uteroplacental PRL family is presented in Table 3. Additional discussion on the transcriptional control of PRL family genes can be found in a recent review [119].

#### Transport and Tissue Distribution

Most members of the uteroplacental PRL family have been shown to be present in maternal circulation, and some have been shown to exist in the fetus [2]. Access and availability to target cells are key parameters affecting the biological activities of a hormone. These parameters of hormone action can be influenced by posttranslational modifications and interactions with various transport and binding proteins. Carbohydrate appears to be the most common posttranslational modification for members of the uteroplacental PRL family. As indicated above, the nature of protein glycosylation appears to be cell type- and protein-specific [67]. The addition of carbohydrate to PL-I by trophoblast giant cells is apparently responsible, at least in part, for its lengthened half-life in circulation relative to the short half-life of the unglycosylated PL-II [120]. Specific asso-

ciations of members of the uteroplacental PRL family with transport or binding proteins has also been observed. Mouse PL-II, in part, circulates bound to  $\alpha_2$ -macroglobulin [121]. Transport proteins such as  $\alpha_2$ -macroglobulin can specifically direct ligands to their target cells or effectively protect the maternal environment from the actions of the ligand. In the rat, PLP-A achieves very high concentrations in blood and is known to circulate as a high-molecular weight complex bound to another protein(s) [64, 66]. The nature of the circulating PLP-A binding protein has not been reported. Some members of the uteroplacental PRL family appear to be restricted in their distribution because of specific interactions with components of the extracellular matrix. D/tPRP binds to heparin-containing molecules and is present in the decidual extracellular matrix [122]. Such a location is ideal for gaining access to cells that traverse the decidual compartment (trophoblast, immune, endothelial, decidual) and in limiting exposure to extrauterine sites. A few members of the uteroplacental PRL family have been reported to gain access to the fetal circulation, whereas others are selectively excluded from the fetus [63, 123–125]. Mechanism(s) responsible for a ligand's gaining access to or being excluded from the fetal compartment have not yet been determined but may involve posttranslational modifications or interactions with carrier molecules.

### Biological Functions

Our understanding of the physiological roles and modes of action of members of the uteroplacental PRL family is somewhat limited but is rapidly expanding. The recent growth in our knowledge base is directly related to the increasing availability of a wide range of experimental tools for studying the biology of the uteroplacental PRL family.

*Roles in the physiology of pregnancy.* Over the past several years, data have accumulated on the involvement of specific uteroplacental PRL family members in the regulation of maternal adaptations to pregnancy. Known target tissues for a subset of members of the uteroplacental PRL family are summarized in Table 4.

1) PRL/PL regulatory network: mammatrophic, luteotrophic, etc. The field of research described in this review grew out of classic observations implicating the rodent placenta as a source of factors influencing mammary gland development and corpus luteum function. Rodent placental hormones possessing both mammatrophic and luteotrophic actions have been isolated and characterized. PL-I and PL-II stimulate various parameters of mammary epithelial cell growth and differentiation [126–128] and promote the biosynthesis of progesterone by the corpus luteum [129, 130]. PL-I and PL-II are part of a regulatory network that also includes PRL and PL-IV. The expression of these maternal (PRL), trophoblastic (PL-I, PL-IV, and PL-II), and fetal (PRL) hormones are orchestrated in a precise temporal pattern that ensures the presence of activators of the PRL receptor signaling system throughout gestation [131–135]. PL-IV is a weak PRL receptor agonist when compared to PRL, PL-I, or PL-II [22]. In addition to the mammary gland and corpus luteum, there are several other targets for these ligands in both maternal and fetal compartments. Evidence has arisen for their involvement in the control of maternal behavior [136, 137], the regulation of PRL [138] and insulin secretion [139], and the modulation of immune cells [22, 26, 55, 131, 135]. The significance of the activation of the PRL receptor signaling pathway by multiple ligands is that such activation probably provides for complementarity and presumably some form of specialization [1].

TABLE 4. Known target tissues for members of the uteroplacental PRL family.<sup>a</sup>

Hormone	Target tissue
Placental lactogens I and II	Mammary gland Ovary Liver Pancreas Brain T-cells
Placental lactogen-I variant	Ovary Liver T-cells
Proliferin	Endothelial cells Uterine cells Muscle cells
Proliferin-related protein	Endothelial cells

<sup>a</sup> See text for additional information.

Whether the specialization lies in the biological activities of the ligands, their cellular source, or their control remains to be elucidated.

2) PLF/PLF-RP regulatory network: angiogenesis, cell growth, and differentiation. Establishment of a vascular connection between the mother and the fetus is imperative for a successful pregnancy and represents an essential role for trophoblast cells. Recently, two nonclassical members of the mouse placental PRL family (PLF and PLF-RP) have been shown to modulate blood vessel development via an effective antagonistic relationship [140, 141]. Through a series of in vitro and in vivo assays, Linzer and his colleagues have convincingly demonstrated that PLF is angiogenic and PLF-RP is anti-angiogenic [140]. These two hormones influence blood vessel formation principally via actions on endothelial cell motility [69, 140, 142]. Collectively, PLF and PLF-RP represent the major placental factors regulating angiogenesis in the mouse [141]. PLF has also been shown to stimulate uterine cell proliferation [143] and inhibit muscle cell differentiation [144, 145]. Possible effects of PLF-RP on these latter actions of PLF have not been reported.

3) Other nonclassical regulators. We have made modest progress beyond determining that the "nonclassical" members of the uteroplacental PRL family do not possess classical PRL biological activities and do not utilize the PRL receptor signaling system ([66, 84, 122, 146]; unpublished results). Some recent insight regarding possible biological actions of d/tPRP has been obtained with an in vivo transplantation model. Expression of d/tPRP by CHO cells appears to influence the ability of CHO cells to form tumors in athymic mice [122]. The data support a mode of action directed toward immune targets such as macrophages or natural killer cells. Parallels between the involvement of the immune system in tumor formation and the establishment of pregnancy are evident.

*Modes of action.* We have made the distinction between members of the PRL family that utilize the PRL receptor signaling pathway (classical) and those using other modes of action (nonclassical).

1) PRL receptor signaling pathway. Considerable information has accrued regarding PRL stimulation of the PRL receptor signaling pathway. In contrast, knowledge of the nature of the intracellular pathways activated by classical members of the placental PRL family is meager. PL-I, PL-II, and PL-IV are all capable of binding to various PRL receptor preparations [22, 124, 127, 132, 135, 147–154]. The only apparent disparity in binding characteristics is a

lower affinity of PL-IV for the PRL receptor [22, 132]. This lower affinity correlates with a reduced biopotency of PL-IV in assays for PRL-like bioactivities [22, 132]. It is assumed that PL-I, PL-II, and PL-IV activate signaling pathways downstream of the PRL receptor similar to those activated by PRL. In brief, PRL binds to one of two types of PRL receptors (short and long forms) and initiates receptor dimerization [155, 156]. The PRL receptor does not possess intrinsic enzyme activity and appears to transmit its signal via association with other regulatory molecules (see refs. [155, 156]). The long form of the PRL receptor activates a nonreceptor tyrosine kinase, Jak2, and signals to the nucleus via activation of members of the STAT family [155, 156]. There is evidence suggesting that the short form of the PRL receptor transduces signals via the mitogen-activated protein (MAP) kinase pathway [157] or, alternatively, acts as an endogenous dominant negative (see ref. [156]). Other intracellular signaling molecules have also been identified that appear to participate in PRL signal transduction pathways (see ref. [156]). Beyond the ability of PL-IV to stimulate Jak2 activity in Nb2 lymphoma cells [22], we lack information on intracellular signaling pathways triggered by classical members of the placental PRL family. It would appear likely that at least some aspects of PRL signaling are mimicked by PL-I, PL-II, and PL-IV; however, whether these three placental ligands reproduce the full spectrum of PRL action within its target cells is unknown.

2) PLF/insulin-like growth factor (IGF)-II/mannose-6-phosphate receptor pathway. Many of the actions of PLF appear to be mediated through PLF interactions with the IGF-II/mannose-6-phosphate receptor [68–70, 142]. Historically, the IGF-II/mannose-6-phosphate receptor has been viewed as a competitor for actions through the IGF-I receptor and not as a receptor possessing a fundamental signaling role [142]. This relationship of the IGF-II/mannose-6-phosphate and IGF-I receptors is similar to the proposed relationship of the short and long forms of the PRL receptor. However, in endothelial cells, PLF binds to the IGF-II/mannose-6-phosphate receptor, resulting in signaling via a pertussis toxin-sensitive G protein and MAP kinase, which culminates in specific effects on cell motility [69, 142]. Whether the IGF-II/mannose-6-phosphate receptor individually participates in the PLF signaling pathway or is part of a receptor complex has not been fully resolved. PLF has also been shown to stimulate uterine cell proliferation through interactions with a distinct receptor system [143].

3) Other modes of action. Two additional pieces of information provide some possible insight concerning the actions of members of the uteroplacental PRL family. At least one member of the family, d/tPRP, specifically associates with heparin-containing molecules, which may provide clues to its mode of action [122]. The association of heparin or heparan sulfate proteoglycans with members of the fibroblast growth factor (FGF) family directly facilitates the ability of FGFs to activate FGF receptors [158]. A similar mechanism of action may be used by d/tPRP on its target cells. Suggestions of a second strategy for target cell activation arise from the identification of the oligosaccharide structure (NeuAc $\alpha$ 2,6GalNAc $\beta$ 1,4GlcNAc $\beta$ ) on members of the PRL family synthesized by spongiotrophoblast cells [67]. This carbohydrate structure potentially binds to receptors related to CD22 or selectins and may influence immune system function [67, 159]. In summary, it is not clear whether specific receptor signaling systems have co-evolved with each of the PRL family members, whether PRL family members utilize signaling pathways for other

known ligands, or whether PRL family members act through receptor-independent mechanisms (e.g., transport proteins, binding proteins, etc.).

## CONCLUSIONS

The uteroplacental PRL family of the mouse and rat represents an intriguing biological paradigm. At least fifteen different ligands bearing some similarity to PRL have been identified. Their expression is highly coordinated between populations of maternal and trophoblastic cells, and their known actions are vital for the establishment and maintenance of pregnancy. Given these important facts, it is evident that we are dealing with central mechanisms underlying maternal and fetal adaptations to the gestational state.

The future of this research field is in establishing the biological roles of the nonclassical members of the uteroplacental PRL family. It is likely that complementarity, specialization, and/or antagonism may characterize the relationships of these ligands and their yet-to-be discovered functions. Two recent developments should greatly expand our experimental armamentarium: 1) the use of alkaline phosphatase-PRL family fusion proteins for the detection of target cells [147], and 2) the identification of additional mouse homologues and the implementation of gene manipulation strategies ([20, 59]; unpublished results). As roles for members of the uteroplacental PRL family are elucidated, it will be of interest to evaluate their involvement in autocrine/paracrine regulatory networks located outside of the uterus [160].

Understanding of the physiology of the uteroplacental PRL family in the mouse and rat has provided and will continue to provide access to important regulatory processes in other species. Observations in the hypophysectomized pregnant mouse and rat led to cross-species discoveries of PLs and cross-species characterizations of corpus luteum and mammary gland function. In some instances, similarities prevailed, while in other cases it was the differences that were most compelling. Nonetheless, our appreciation for the biology of pregnancy increased. We propose that members of the uteroplacental PRL family evolved to subserve important biological roles during pregnancy. Functional homologies among species probably exist and may include the ligands, their receptors, or components of their signaling pathways. Our pursuit of ligand function should be open-minded. Many of our past efforts to understand the biology of the uteroplacental PRL family were biased by the PRL receptor signaling pathway. Luteotrophic and mammatrophic activities of the rodent uteroplacental PRL family were the focus of our entry into this field, but they may not represent the only physiological pressures for the evolution of this protein family. The driving force for the derivation of the uteroplacental PRL family may actually stem from other more elemental functions required for viviparity, including the establishment of vascular connectivity and immunological adaptations to pregnancy.

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