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The prolactin family: effectors of pregnancy-dependent adaptations

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Prolactin (PRL) is a hormone involved in many biological functions. In some species, there is a family of PRL-related genes; such is the case in the mouse and rat. The actions of members of the PRL family can be distinguished based on the involvement of the PRL receptor signaling pathway (classical versus nonclassical). Recent insights into the biology of the PRL family have been derived from mouse mutagenesis studies. There is compelling evidence suggesting that the PRL family contributes to the regulation of pregnancy-dependent adaptations to physiological stressors.

Introduction

Prolactin (PRL) is a hormone and cytokine with a long history, ranging from its discovery over 75 years ago [1–3] to its noted involvement in a diversity of vertebrate biological functions [4]. Numerous excellent reviews have been written on the structure of PRL, its receptor and its involvement in the biology of various organisms [5–12]. A less well appreciated role for PRL or, more specifically, the ancestral Prl gene, is as an evolutionary template for the generation of new genes, encoding proteins with a breadth of actions. The birth of these new genes is linked to viviparity but is not a common feature of all viviparous species. Collectively, Prl and the Prl-related genes are referred to as members of the PRL family. Knowledge about the PRL family grows as the genomes of different species are sequenced and characterized. Thus far, it is evident that the mouse and rat represent species where the PRL gene family has undergone a particularly robust expansion [13–15]. Insights into the significance of the PRL family expansion have recently been derived from mutational analysis of the mouse [16,17].

Here, we discuss the biology of PRL family gene expansion and provide evidence that members of the PRL family are effectors of pregnancy-dependent adaptations to physiological stressors. Our focus is on the PRL family of the mouse and rat.

Mouse and rat expanded PRL families

Discovery of the expanded PRL family started early in the previous century, during the formative stages of the discipline of endocrinology (Box 1). Awareness of protein families related to PRL within the mouse and rat grew as technical breakthroughs in protein purification, cDNA cloning and genome analyses were having an impact on biomedical investigation. For the majority of expanded PRL family members, gene discovery was not driven by prior biological characterization of the ligand. Currently, several members of the PRL family remain orphan ligands with unknown biological functions.

Expanded PRL gene families have been identified in the mouse, rat and cow but not in species such as the human and dog [15] (Figure 1). Derivation of these expanded gene families displays elements of species specificity. Although the mouse and rat expanded PRL gene families are largely orthologous, the expanded bovine PRL family genes are not orthologous with rodent expanded PRL family genes [18,19]. Furthermore, the human exhibits a striking variation. Even though the human PRL locus has not expanded, the human genome has undergone amplification at a related locus, encoding growth hormone (GH). This is consistent with the evolution of PRL and GH from a common ancestral gene [10,20]. The human GH locus contains a family of five related genes [21]. In contrast to rodent and ruminant GHS, primate GHS have the dual capacity to activate both PRL and GH receptors, which probably provided plasticity in the choice of templates for the derivation of pregnancy-associated ligands [22]. Expansion of the GH family has not occurred in the mouse, rat or dog.

In an evolutionary context, the expanded PRL family arose through gene duplication and natural selection [23,24]. Gene amplification is a common cellular adaptation to environmental insults, yielding an increased capacity for the production of proteins, and thus improving survival [24]. Therefore, it is reasonable to propose that, during the origin of some species, gene duplications within the ancestral PRL locus represented adaptive responses to environmental pressures. At some point in time, as the environmental challenges were relaxed, the requirement for elevated levels of ancestral PRL was removed. These events enabled surplus copies of the ancestral Prl gene to mutate and acquire new functions or further specializations, providing the species with improved abilities to adapt to its environment.

The topography of the PRL family locus in the mouse and rat suggests that its constituent genes arose by a series of individual gene duplication events rather than block duplications [13–15]. PRL family loci seem to be dynamic, with evidence for the origin and death of genes. In the mouse, the PRL locus consists of 23 genes, spanning 1.0 megabase [13,14], whereas in the rat, the PRL locus consists of 24 genes spanning 1.7 megabases [15].
Box 1. The historical journey to the expanded mouse and rat PRL families
The path to discovery of the mouse and rat PRL families had its origins in the efforts of legendary figures of endocrinology, including individuals such as Joseph Long, Hans Selye, Edwin Astwood, Roy Greep and William Lyons. These scientists and their colleagues demonstrated that lactogenic and luteotropic hormones existed in extrapituitary sites, including the placenta. Through the leadership of Henry Friesen at the University of Manitoba, Canada, and Frank Talamanes at the University of California–Santa Cruz, USA, these lactogenic and luteotropic activities became ascribed to specific placental proteins in the late 1970s and early 1980s. During this time, Daniel Linzer, a postdoctoral fellow working with Daniel Nathans at Johns Hopkins University, USA, identified an mRNA that was dramatically upregulated during the initiation of cell proliferation. The mRNA had significant sequence similarity to PRL and was termed PLF. Marit Nilsen-Hamilton of Iowa State University, USA, independently discovered the PLF protein, which she termed mitogen-regulated protein (MRP). In the mid-1980s, Mary Lynn Duckworth, working with Henry Friesen’s laboratory and Daniel Linzer’s laboratory at Northwestern University, USA, began cloning and characterizing cDNAs for the PLs. Purification of the placental proteins and their cDNAs led several laboratories, including our own, to the identification of related proteins and cDNAs, further expanding the PRL family. Elucidation of the remaining known members of the mouse and rat PRL families occurred through mining expressed sequence tag databases and the sequenced mouse and rat genomes.

Box 2. Nomenclature of the PRL families
The nomenclature for the mouse and rat PRL families is awkward and is the result of the efforts of several independently functioning laboratories, including our own, without any oversight. At the beginning, no one had any appreciation of the magnitude of the gene family expansion. Names were originally assigned based on biological activities [PL, chorionic somatomammotropin (CS) and PLF] and on structural similarities to PRL or PLF [PRP-related protein (PRP), PLP and PLF-RP]. As the Mouse and Rat Genome Databases were established, some improvements were made in the nomenclature but it remains cumbersome. Additionally, the nomenclature can be confusing when comparisons are made across species. Terms such as PL and PRP have been used to describe ligands from other species. However, it is important to appreciate that human PLs and bovine PLs and PRPs are not orthologous with PLs and PRPs of the mouse and rat. A revised nomenclature is needed that provides a set of logical rules for organizing the current members of the PRL family, probably based on structural relatedness, and that enables the addition of new members as they are discovered. We are currently working with the Mouse and Rat Genome Databases to develop a revised nomenclature for the mouse and rat PRL families.

Table 1. The mouse and rat prolactin families

<table>
<thead>
<tr>
<th>PRL family membera</th>
<th>Symbols</th>
<th>Mouse GenBank accession no.</th>
<th>Rat GenBank accession no.</th>
<th>Major tissue source(s)</th>
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aMembers of the mouse and rat prolactin families are listed in the order of their positioning within the prolactin family locus [13–15].

bND indicates that an ortholog has not been identified.

(Table 1, Box 2). Both loci contain pseudogenes. Mouse and rat PRL family loci exhibit similar organizational features [13–15]. The Prl gene is situated at one end of each locus, and structurally related genes are located in clusters. Genes positioned at the ends of each locus exhibit a similar 5’ to 3’ orientation and 5-exon structure,
whereas genes located in the middle of each locus are aligned in the opposite orientation and have a six-exon structure. Six-exon genes are structurally similar to five-exon genes, except for an extra short exon situated between exons II and III of the five-exon genes. Exceptions to these generalizations exist, as do differences in the composition of genes within the mouse and rat PRL family loci.

Figure 1. Phylogenetic analysis of the PRL family in the mouse (Mus musculus), rat (Rattus norvegicus), cow (Bos taurus), human (Homo sapiens) and dog (Canis familiaris). Multiple amino acid sequence alignments and phylogenetic tree construction were performed as described by Alam et al. [15]. Bootstrap values are included within the phylogenetic tree. PRL orthologs from the mouse, rat, cow, human and dog are shaded in purple. The mouse, rat and cow each have expanded PRL families. The PRL families of the mouse and rat are largely orthologous (shaded in cyan), in contrast to the bovine family (shaded in orange), which seems to have evolved independently. See Table 1 for abbreviations of the rat and mouse PRL families. Other abbreviations: bPRP, bovine PRL-related protein.
PRL family genes encode proteins possessing a signal peptide and conserved alignments of cysteine residues [22]. Most PRL family ligands circulate in the blood; however, some, because of their affinity for heparan sulfate, are largely excluded from distribution through the vasculature. Post-translational modifications, including glycosylation, are present in the majority, but not all, of PRL family ligands. These modifications influence both dissemination and biological activities [22].

**PRL family gene expression and viviparity**
The PRL family is linked to pregnancy. PRL family gene expression involves the coordination of maternal, extraembryonic and fetal tissues, and spans the entire duration of pregnancy [25] (Box 3).

The anterior pituitary, uterine decidua and placenta are sources of PRL family ligands (Table 1). At the initiation of pregnancy, mating activates a neuroendocrine response, resulting in twice-daily surges of PRL from the anterior pregnancy, mating activates a neuroendocrine response, sources of PRL family ligands (Table 1). At the initiation of extraembryonic and fetal tissues, and spans the entire gene expression involves the coordination of maternal, extraembryonic and fetal tissues, and spans the entire duration of pregnancy [25] (Box 3).

The anterior pituitary, uterine decidua and placenta are sources of PRL family ligands (Table 1). At the initiation of pregnancy, mating activates a neuroendocrine response, resulting in twice-daily surges of PRL from the anterior pituitary [26]. These surges of PRL continue for the first half of pregnancy and sustain corpus luteum function [27]. Luteal steroid hormones, along with the embryo, provide essential signals for embryo implantation, including the decidualization of the uterine stroma [28,29]. Uterine decidual cells produce a subset of PRL family ligands [22,30]. The decidual PRL family cytokines can be viewed as downstream mediators of intrauterine progesterone action. The trophoblast lineage represents the major source of the remainder of PRL family ligands [22,31]. Trophoblast cells differentiate into several specialized cell types capable of producing members of the PRL family, including trophoblast giant cells, spongiosotrophoblast cells and invasive trophoblast cells [22,31,32]. Trophoblast cell lineages can be defined by the complement of PRL family ligands they express. During the final stages of pregnancy, PRL expression is initiated in the fetal anterior pituitary [22].

**Box 3. Organization of the uteroplacental compartment**
An appreciation of uterine and placental morphology provides insights into the sources and actions of members of the PRL family. The uteroplacental compartments of the rat and mouse are similar, and they share the same basic organizational plan of other species with hemochorial placentation. Structural features of the uteroplacental compartment are dynamic, changing throughout gestation. The site within the uterus where blood vessels enter is referred to as the mesometrial compartment. Embryo implantation stimulates the differentiation of uterine stromal cells into decidual cells. Following implantation, NK cells accumulate and infiltrate the mesometrial compartment. Trophoblast lineages are derived from the outer layer of the early embryo (trophectoderm). Fusion of trophoblast and allantoic tissues leads to the formation of the chorallantoic placenta, which consists of two functionally distinct regions: (i) the junctional zone: an endocrine and invasive tissue comprised of trophoblast giant cells, spongiosotrophoblast cells, glycogen cells and the source of invasive trophoblast cells; and (ii) the labyrinth zone: the site of maternal–fetal exchange and the location of cytotrophoblast, syncytiotrophoblast and labyrinthine trophoblast giant cell lineages and the allantoic vasculature. As gestation progresses, NK cells degenerate, leading to the invasion of trophoblast cells into the mesometrial compartment. Trophoblast cell invasion is more restrictive in the mouse (limited to the mesometrial decidua), whereas in the rat, trophoblast cells penetrate through the mesometrial decidua and infiltrate the entire mesometrial compartment.

**Biology of the PRL family**
Biological activities of PRL family ligands can be categorized as classical and nonclassical [22]. Classical actions are mediated by ligand interactions with the PRL receptor, and nonclassical modes of action use other signaling pathways.

**Classical actions**
PRL and the placental lactogens (PLs) are PRL receptor agonists [22]. During the early phases of pregnancy, PRL, produced by the anterior pituitary, is the only ligand available for the PRL receptor. As gestation progresses, trophoblast giant cells of the placenta first produce PL-I, which is encoded by transcripts derived from multiple PL-I genes, and subsequently PL-II. PL-II production persists until term. The PRL receptor agonists stimulate mammary epithelial cell growth and differentiation, and help to maintain corpus luteum integrity and progesterone and relaxin biosynthesis [22]. The biological relevance of the evolutionarily conserved PRL and the PRL receptor has been determined through mouse mutagenesis studies [33,34]. PRL and the PRL receptor are both crucial for the establishment of pregnancy and mammary gland development [33,34]. The importance of the PLs is less well understood. In vivo assessment of loss-of-function mutations for the PL genes has not been reported. Presumably, the existence of multiple ligands for the PRL receptor provides a selective reproductive advantage.

**Nonclassical actions**
Modest progress has been made in uncovering the biological actions of PRL family ligands not utilizing the PRL receptor signaling pathway. Although limited, the existing knowledge is intriguing. Nonclassical PRL family ligands target cell populations that are crucially involved in mediating pregnancy-dependent adjustments in maternal tissues. These targets include cells of the vasculature, hematopoietic progenitor cells and immune cells, and components of the extracellular matrix.

**Vascular remodeling** Proliferin (PLF) and PLF-related protein (PLF-RP) are products of trophoblast cells. They are concurrently expressed and function as regulators of angiogenesis [35–38]. PLF was originally discovered in serum-starved mouse fibroblasts as a growth factor-regulated gene [39], and PLF-RP was identified based on its structural relationships with PLF [40]. The actions of PLF and PLF-RP on the vasculature have been demonstrated through a series of in vitro and in vivo experiments [35–38]. These two ligands have opposing actions, with PLF promoting blood vessel development and PLF-RP inhibiting the process. PLF seems to use an endothelial cell signal transduction pathway involving the mannose 6–phosphate receptor, also known as the insulin-like growth factor II receptor [36]. The mechanism of cellular action for PLF-RP is unknown. Additional evidence indicates that PLF might also participate in wound healing [41] and the regulation of cell proliferation for a variety of cell types [42,43].

PRL has also been implicated in regulating the vasculature. Proteolytic fragments of PRL (vasoinhibins)
modulate the activities of endothelial cells by inhibiting vasodilation and angiogenesis, and promoting endothelial cell death [44]. Human GH and human PL also serve as precursors for vasoanibbins [44]. The actions of vasoanibbins are not mediated by the classical PRL receptor signaling pathway [44]. It remains to be determined whether vasoanibbins can be derived from members of the expanded rodent PRL family.

**Hematopoiesis**  PRL-like protein (PLP) E (also known as Prlpe) and PLP-F (also known as Prlpf) are expressed sequentially during gestation by mouse trophoblast cells and function to stimulate hematopoiesis, especially of megakaryocyte and erythroid cell lineages [45–49]. PLP-E stimulates hematopoiesis through signal transduction cascades involving the gp130 coreceptor and Janus kinase (Jak)–signal transducer and activator of transcription (Stat) pathways [45,46]. PLF also stimulates the expansion of bone marrow-derived hematopoietic stem cells [42].

During pregnancy, hematopoiesis is activated in extraembryonic tissues. The midgestation placenta is a site of hematopoietic stem cell development [50–52], and the visceral yolk sac is a site of erythropoiesis [53–55]. Peaks in placental hematopoietic stem cell production and visceral yolk sac erythropoiesis overlap with the expression profiles of PLP-E, PLP-F and PLF. There is evidence for the transport of PLF to the yolk sac and fetus [56]. Similar observations are not available for PLP-E or PLP-F. Whether any of these PRL family ligands are physiological modulators of hematopoietic stem cell expansion and/or differentiation in extraembryonic, embryonic or adult targets remains to be determined.

**Natural killer cells**  PLP-A (also known as Prlpa) is produced by trophoblast cells and specifically interacts with natural killer (NK) cells [57,58]. During pregnancy, NK cells accumulate around the mesometrial uterine vasculature [59,60] and stimulate vascular remodeling, facilitating nutrient flow to the placenta and fetus [61]. These vascular remodeling effects of NK cells are mediated by interferon γ (IFN-γ) [62]. PLP-A is an intermediary in trophoblast cell modulation of NK cells, including their production of IFN-γ [16,58]. Additional insights into the biology of PLP-A have been shown through phenotypic characterization of PLP-A-deficient mice (see later) [16].

**Interactions with extracellular matrix components**  Decidual PRL-related protein (dPRP, also known as Dtprp) is produced in uterine decidua and resides primarily in the decidual extracellular matrix, where it binds with high affinity to heparin-containing molecules [63–66]. Human decidual PRL also binds avidly to heparin [67]. Heparin and heparin-related structures are widely distributed and are involved in an array of physiological processes. These molecules participate in cell adhesion, migration, growth regulation, basement membrane properties and differentiation [68]. During early pregnancy, the uterus is a site of extensive remodeling that undoubtedly involves heparin and heparin-related structures. The high affinity of dPRP for heparin places it in a key position to modulate heparin-dependent events during the establishment of pregnancy, which prompted the creation and characterization of a Dtprp-null mouse (see later) [17].

**Other PRL family ligands**  Information about the biological actions of other members of the expanded PRL family is either fragmentary or not available. In some cases, there have been insights into functions that PRL family members do not possess. For example, PLP-B, PLP-C, PLP-D and PLP-H do not signal through the PRL receptor [69–71]. The physiological targets or activities of this subset of PRL family members have not been determined. Restricted expression profiles suggest that the likely involvement of other PRL family members in specific biological events (e.g., PLP-J with decidual cells; PLP-L, PLP-N and PLP-P with invasive trophoblast cells; PLP-K with labyrinthine trophoblast giant cells). From what has been learned while investigating PLF, PLF-RP, PLP-E, PLP-A and dPRP, it is likely that the remainder of the orphan PRL family ligands will contribute to the regulation of interesting biological processes.

**Roles for the PRL family in adaptations to physiological stressors**  Mouse mutagenesis has been used as a tool to discover the involvement of the PRL family in adaptations to physiological stressors. Four lines of mutant mice have been generated: one with a null mutation in the *Prl* gene [33], the second with a gain of function mutation resulting in the constitutive expression of PLP-E [72] and the third and fourth lines with null mutations in the *Prlpa* gene [16] and *Dtprp* gene [17], respectively.

**Immunological adaptations**  PRL is a multifunctional ligand with a wide range of actions, including modulation of the immune system [73]. Mice with mutations in *Prl* and the PRL receptor have been generated and characterized [33,34]. Although PRL signaling is not essential for the development of the immune system [33,74], PRL is crucial for the regulation of immune responses to stressors [75,76]. Expansion of bone marrow-derived myeloid lineages and splenic T lymphocytes following thermal injury is less effective in PRL-null mice than in wild-type mice [75]. Secretion of PRL from the anterior pituitary is also stimulated by an assortment of different physiological stressors [7,73]. Collectively, the data implicate PRL in homeostatic mechanisms controlling immunological responses to physiological stressors.

**Hematopoietic adaptations**  PLP-E is a trophoblast-derived hormone with stimulatory effects on megakaryocyteopoiesis and erythropoiesis [45–49]. A mouse line possessing a transgene consisting of the *Prlpe* cDNA under the control of the mouse metallothioine I promoter has been generated and characterized [72]. Blood PLP-E levels are constitutively elevated in the transgenic mice. These PLP-E-expressing mice recover faster from chemically induced thrombocytopenia and neutropenia than do control mice [72]. Surprisingly, recovery from phenylhydrazine-induced anemia is not accelerated
As the phenotype of Prlpa was found that two members of the PRL family (PLP-A, dPRP) (Box 4). Using mouse gene targeting strategies, it was especially by the use of hypoxia as an experimental tool [78,79] to investigate the interactions with maternal uterine structures, the development of specific extraembryonic lineages and the establishment of the hemochorial placenta requires physiological stressors through disrupted expansions in platelets and erythrocytes.

Placentation-associated adaptations
The establishment of the hemochorial placenta requires the development of specific extraembryonic lineages and their interactions with maternal uterine structures, especially the vasculature. Placentation is sensitive to environmental stimuli. This has been demonstrated effectively by the use of hypoxia as an experimental tool [78,79] (Box 4). Using mouse gene targeting strategies, it was found that two members of the PRL family (PLP-A, dPRP) facilitate placenta-associated adaptations to hypoxia [16,17].

PLP-A Prlpa is a five-exon gene expressed in trophoblast cells. Prlpa-null mice were made by replacing exons II to V of the Prlpa gene with a neomycin-resistance cassette [16]. As the phenotype of Prlpa-null mice was characterized, it became evident that the Prlpa gene was dispensable when the null mice were maintained under standard animal husbandry conditions. The only insight regarding the biology of PLP-A was that it targeted NK cells [57,58]. Because NK cells have been implicated in modulating the uterine vasculature [61], it was hypothesized that PLP-A might contribute to adaptations when the uterine vasculature is challenged. Maternal hypobaric hypoxia represents an effective method for challenging the uterine vasculature, hematopoietic precursors and the maintenance of pregnancy in wild-type mice and rats [79]. When pregnant Prlpa-null mice are challenged by exposure to hypoxia, they are not able to adapt, and their pregnancies terminate [16]. Pregnancies failed because of inadequate placentation. Under the hypoxic conditions, Prlpa-null trophoblast cells do not satisfactorily invade into and remodel the uterine vasculature. The net result is a failure of adequate nutrient flow to support extraembryonic and embryonic development.

**Box 4. Hypoxia and pregnancy-dependent adaptations**

Hypoxia can be used as an experimental strategy to investigate pregnancy-dependent adaptations. The specific effects on pregnancy are dependent upon the magnitude, duration and gestational timing of the hypoxia. Less severe exposure to maternal hypoxia results in successful adaptations, whereas more severe exposure leads to intrauterine fetal growth restriction, and sometimes pregnancy failure. Placentation can be affected by hypoxia, including the development of trophoblast cells and their interactions with the uterine vasculature. Thus, placentation exhibits plasticity. The final placental design is matched to meet environmental challenges. Disease results when pregnancy-dependent adaptations are not appropriate. Experimental maternal hypoxia exaggerates pregnancy-dependent adaptations and is an effective tool to investigate the biology of the PRL family.

Conclusion
Expansion of the mouse and rat PRL families did not occur in a controlled laboratory setting, in which nutrition and temperature are carefully regulated and exposure to pathogens limited. Instead, the expanded PRL gene family probably provided the mouse and rat with selective advantages for surviving in more hostile conditions. A ‘proof of principle’ has been established that members of the expanded PRL family are biologically relevant. Gene-targeting strategies need to be extended to the remainder of the PRL family locus. Characterizations of mice with mutations involving individual genes and clusters of genes will be informative. We expect that the impact of the mutations will be most significant when the animals are exposed to physiological stressors. In the initial experimentation, hypoxia was used as a tool to challenge pregnant mice. Future experimentation will be required to investigate the involvement of the expanded PRL family in the adaptation to other stressors (e.g. nutrient, pathogen, temperature). Given the linkage of PRL family gene expression to pregnancy and the actions of the PRL family on the vasculature, hematopoietic precursors and immune cells, it is logical to assume that the PRL family expanded in the mouse and rat to ensure pregnancy-dependent adaptations to an assortment of physiological stressors (Figure 2). This represents an effective strategy for ensuring the survival of a species in varying and unstable habitats. Among mammals, few species have achieved the reproductive success of mice and rats.

The PRL family expansion in the cow presumably ensures similar pregnancy-dependent adaptations. At least one member of the expanded bovine PRL family, PL, has been implicated as a potential regulator of maternal (corpus luteum function, uterine gland development, intermediary metabolism and mammary gland development) and fetal physiological processes [80,81].
Why study the expanded rodent PRL family?

The lack of conservation of the expanded PRL family in primates does not have a negative impact on the importance of the mouse and rat as model systems for studying pregnancy. Pregnancy in all species is characterized by adaptive responses to physiological stressors. Appropriate adaptive responses result in successful pregnancy and healthy offspring, whereas ineffective adaptations compromise pregnancy and have a negative impact on the health of the fetus, and increase its susceptibility to disease in adulthood. Although the expanded PRL family ligands are not conserved in the human, the cells they target (e.g. endothelial cells, hematopoietic and immune cells) are conserved and undergo fundamental pregnancy-dependent adaptations. Exploration of the biology of the expanded rodent PRL family provides a strategy for identifying crucial cellular and molecular mechanisms controlling pregnancy-dependent adaptations. At this juncture, there are limited insights as to how any member of the expanded PRL family acts on its cellular target(s). We need to identify the receptors for the ligands and the components of their intracellular signal transduction networks. With these insights in hand, we will have the tools to identify essential connections to conserved regulatory pathways controlling viviparity in mammals, including the human.

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