

Prolactin-Like Protein-F Subfamily of Placental Hormones/Cytokines: Responsiveness to Maternal Hypoxia

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The prolactin (PRL) family of hormones/cytokines is involved in the maintenance of pregnancy and adaptations to physiological stressors. In this report, we identify and characterize a new member of the rat PRL family, examine the impact of maternal hypoxia on placental PRL family gene expression, and investigate maternal adaptive responses to hypoxia. Perusal of the PRL gene family locus in the rat genome resulted in the identification of a putative new member of the rat PRL family. The new member is closely related to the previously reported PRL-like protein-F (PLP-F) and has been named PLP-F α and the originally characterized PLP-F, now termed PLP-F β . The two proteins exhibit structural similarities but possess distinct cell- and temporal-specific expression profiles. *In vivo* hypoxia stimulates placental PLP-F α and PLP-F mRNA expression in the rat and mouse, respectively. Recho-1 trophoblast cells can differentiate into trophoblast giant cells,

express PLP-F α , and exhibit enhanced PLP-F α mRNA levels when cultured under low oxygen tension (2%). Exposure to hypobaric hypoxia during latter part of pregnancy did not significantly impact the expression of PLP-F β mRNA. Finally, exposure to hypobaric hypoxia during midpregnancy led to increased maternal red blood cells, hemoglobin concentrations, hematocrit, and increased concentrations of maternal splenic mRNAs for key proteins involved in hemoglobin synthesis, erythroid Krüppel-like factor, erythroid 5-aminolevulinic synthase-2, and β -major globin. In summary, adaptive responses to maternal hypoxia include activation of placental PLP-F α /E gene expression, which may then participate in maternal hematological adjustments required for maintaining maternal and fetal oxygen delivery. (*Endocrinology* 148: 559–565, 2007)

PLACENTATION IS ASSOCIATED with modifications of the maternal environment. This is accomplished, in part, through the elaboration of hormones and cytokines. In the rat and mouse, endocrine cells of the placenta, along with lactotrophs of the anterior pituitary, and decidual cells of the uterus produce a family of hormones/cytokines, related to prolactin (PRL) (1, 2). They are termed placental lactogens (PLs), PRL-like proteins (PLPs), PRL-related proteins, proliferin, and proliferin-related protein. In the mouse, 23 genes related to PRL have been identified and characterized, whereas 24 PRL-related genes are known in the rat (3–5). Most of the PRL-related genes in the two species are orthologous. The PRL family genes are located on chromosome 13 in the mouse and chromosome 17 in the rat (3, 5). Each member of the family has temporal and tissue-specific expression patterns associated with pregnancy (2). PRL family members target the reproductive tract, liver, hematopoietic and immune cells, vasculature, and brain and participate in adaptations to physiological stressors (2, 6).

Maternal hypoxia is an effective tool to investigate the in-

volvement of the PRL family in adaptations to physiological stressors (7). At least one member of the PRL family, PLP-A, has been shown to contribute to pregnancy-dependent adaptations to maternal hypoxia (8). Other members of the PRL family are involved in regulating key biological processes that are typically associated with adaptations to hypoxia. The latter PRL family members include the PLP-F subfamily, which has been shown to regulate hematopoiesis, including red blood cell development (9–15). Three PLP-F subfamily members have been reported; two from the mouse, PLP-E and PLP-F (16, 17), and one from the rat, PLP-F (18). PLP-E is expressed in trophoblast giant cells (16) and is an effective stimulator of erythropoiesis (10, 15) and could be part of a pregnancy-dependent adaptive mechanism ensuring adequate red blood cell oxygen delivery. Mouse PLP-F is expressed predominantly by spongiotrophoblast cells (16) and has been shown to similarly stimulate hematopoiesis (13). Although rat PLP-F is slightly more similar in structure to mouse PLP-F, its expression pattern more closely resembles mouse PLP-E (18).

In this report, we identify and characterize a new member of the rat PLP-F subfamily and demonstrate that the expression of some PLP-F subfamily members is regulated by maternal hypoxia.

Materials and Methods

Animals and tissue preparation

Holtzman rats were obtained from Harlan Sprague Dawley Inc. (Indianapolis, IN). CD-1 mice were obtained from Charles River Labora-

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Abbreviations: ALAS-2, 5-Aminolevulinic synthase-2; EKLF, erythroid Krüppel-like factor; EST, expressed sequence tag; FBS, fetal bovine serum; PL, placental lactogen; PLP, PRL-like protein; PRL, prolactin.

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tories (Wilmington, MA). 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 mating of animals was conducted by placing females with fertile males. The presence of sperm in a vaginal smear of a female rat was designated as d 0.5 of pregnancy. The presence of a seminal plug in the vagina of female mice was designated as d 0.5 of pregnancy. Placentation sites, including uterus, metrial gland, and placental tissues, were dissected from pregnant animals. Placental tissues collected from gestation d 12.5 in the rat were dissected into chorioallantoic and choriovitelline components. On d 18.5 of gestation, the chorioallantoic placenta was dissected into junctional and labyrinth zones. Tissues were snap frozen in liquid nitrogen for RNA analyses. For *in situ* hybridization, tissues were frozen in dry ice-cooled heptane. All tissue samples were stored at -80°C until used. Protocols for these procedures have been described (19). The University of Kansas Medical Center Animal Care and Use Committee approved all procedures for handling and experimentation with rodents.

In vivo hypoxia

On the designated day of pregnancy, female rats or mice were placed in hypobaric chambers. Two conditions were used. Rats were exposed to conditions, in which air is circulated at a barometric pressure of approximately 380 Torr, which results in an inspired PO_2 of approximately 70 Torr, equivalent to breathing 10% O_2 at sea level, whereas mice were exposed to conditions, in which air is circulated at a barometric pressure of approximately 420 Torr, which results in an inspired PO_2 of approximately 78 Torr, equivalent to breathing 11% O_2 at sea level (7). The chambers were opened daily to replenish food and water (15–20 min). Pair-fed and *ad libitum*-fed control pregnant rats were exposed to ambient conditions (barometric pressure of ~ 760 Torr and inspired PO_2 of ~ 149 Torr).

Rcho-1 trophoblast cell culture model

The Rcho-1 trophoblast cell line represents an *in vitro* model for studying rat trophoblast cells in undifferentiated and differentiated states (20, 21). Rcho-1 trophoblast cells can be maintained in a proliferative state by maintaining the cells or induced to differentiate, primarily along the trophoblast giant cell lineage (20, 21). Rcho-1 trophoblast cells were maintained in a proliferative state by culturing under subconfluent conditions with RPMI 1640 culture medium supplemented with 20% fetal bovine serum (FBS), 50 μM 2-mercaptoethanol, 1 mM sodium pyruvate, 100 U/ml of penicillin, and 100 $\mu\text{g}/\text{ml}$ of streptomycin (21). Differentiation was induced by growing cells to near confluence in FBS-supplemented culture medium and then replacing the culture medium with NCTC 135 culture medium supplemented with 1% horse serum, 50 μM 2-mercaptoethanol, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin (21). Trophoblast giant cell differentiation is facilitated by high cell density and the removal of factors capable of stimulating proliferation (removal of FBS) (21). During differentiation, cells were maintained under ambient (21% O_2) or low oxygen (2% O_2) levels in a BioSpherix OxyCycler incubator/glove box (BioSpherix Ltd., Redfield, NY). These *in vitro* conditions of low oxygen tension do not necessarily equate with the *in vivo* maternal hypoxia described above.

Identification and characterization of a new member of the PLP-F subfamily

A new member of the PLP-F subfamily was identified by BLAST analysis (22) using the rat PLP-F nucleotide sequence (18) against the public rat genome assembly (Rat Genome Sequencing Project Consortium, 2004; <http://rat.ensembl.org>, <http://genome.brc.mcw.edu/cgi-bin/hgGateway?org=Rat&db=rn3&hgsid=375405>, and <http://www.ncbi.nlm.nih.gov/genome/seq/MmBlast.html>) and found on a BAC clone localized to chromosome 17 (CH230–190E4; Children's Hospital-BACPAC Resources, Oakland, CA; Baylor Rat BACTig Assembly: <http://www.hgsc.bcm.tmc.edu/projects/rat/>). The genomic sequence corresponding to the newly identified PLP-F subfamily member was then used to search the expressed sequence tag (EST) database (National Center for Biotechnology Information, Bethesda, MD) (22). Several ESTs with nucleotide sequences matching the genomic sequence were identified

and obtained from Invitrogen (Carlsbad, CA). cDNAs were sequenced by the Northwestern Sequencing Facility of Northwestern University (Chicago, IL). The original PLP-F was renamed PLP-F α and the newly identified PLP-F family member was termed PLP-F β . Multiple amino acid sequence alignments were generated with CLUSTAL X (23). Locations of signal peptides were determined with the SignalP software program (version 2.0.b2) (24) and based on homology with other members of the PRL family.

Analysis of mRNA expression

PRL family miniarray assay. The PRL family miniarray is a hybridization-based tool for monitoring expression of each PRL family member (25). It has been used to monitor phenotypes of anterior pituitary, uterine, and placental tissues. The PRL family miniarray was performed as previously described (25). Total RNA was extracted using TRIzol reagent [Invitrogen, (26)]. [^{32}P] dCTP-labeled cDNA probes were produced by reverse transcription using 5 μg total RNA. Hybridization was conducted overnight with labeled probes at 42 C. Membranes were washed, wrapped in plastic wrap, and exposed to Bio-Max film (Kodak, Rochester, NY) for 1–4 h and later exposed for 3–5 h on PhosphorImager cassettes (Amersham Pharmacia Biotech, Piscataway, NJ).

Northern blot analysis. Northern blots were performed as previously described (27, 28). Total RNA was extracted using TRIzol, separated on 1% formaldehyde-agarose gels, and transferred to nylon membranes. Blots were probed with [^{32}P] dCTP-labeled cDNAs for rat PLP-F α (18), the newly identified rat PLP-F β , mouse PLP-E (17), erythroid Kruppel-like factor (EKLF; GenBank accession no. AA926284), β -major globin (GenBank accession no. X15009), or erythroid 5-aminolevulinic synthase-2 (ALAS-2; GenBank accession no. NM_013197). A 28S rRNA cDNA probe was used to assess RNA integrity and as a loading control.

RT-PCR. PLP-F α and PLP-F β transcripts were assessed by RT-PCR. Total RNA was extracted from Rcho-1 trophoblast cells using TRIzol. Five micrograms of total RNA and 0.5 μg of oligo dT were used for reverse transcription reactions with Superscript II RT (Invitrogen). PCR was performed using Platinum *Taq* DNA High Fidelity polymerase (Invitrogen) with PLP-F α (forward: 5'-CTA CTA TTA CAC CGA GGC CT-3'; reverse: 5'-AGA ATC TTC ACT ATT GAT GGA TAA) and PLP-F β (forward: 5'-CTG TTA AAT AAT GCC ACC AGA GT-3'; reverse: 5'-TCC AAG AGC TCC AAA TAT AAT TCC-3') specific primers. PCR was conducted for 30 cycles under the following conditions: preheat 94 C, denature 94 C for 1 min, anneal 60 C for 1 min, and extension 72 C for 1 min. PCR products were separated on 1% agarose gels and stained with ethidium bromide.

In situ hybridization. *In situ* hybridization was performed as previously described (29, 30). Ten-micrometer cryosections of tissues were prepared and stored at -80°C until used. Plasmids containing cDNAs for rat PLP-F α (18) and PLP-F β were used as templates to synthesize sense and antisense digoxigenin-labeled riboprobes according to the manufacturer's instructions (Roche Molecular Biochemicals, Indianapolis, IN). Tissue sections were air dried and fixed in ice cold 4% paraformaldehyde in PBS. Prehybridizations, hybridizations, and detection of alkaline phosphatase-conjugated antidigoxigenin were performed as previously reported (29, 30).

Red blood cell analysis

Blood samples (1–3 ml) were collected by cardiac puncture of anesthetized rats. Blood was collected into Vacutainer tubes and stored at 4 C until analyzed. Hematological measurements were performed using an Act10 hematological analysis system (Beckman Coulter, Miami, FL) at the Hematology Core Laboratory of the University of Kansas Medical Center (Kansas City, KS). Analyses focused on red blood cell numbers, hemoglobin concentrations, and hematocrit.

Statistical analysis

Data generated were analyzed with ANOVA. The source of variation from significant F ratios will be determined with Newman-Keuls multiple comparison test (31).

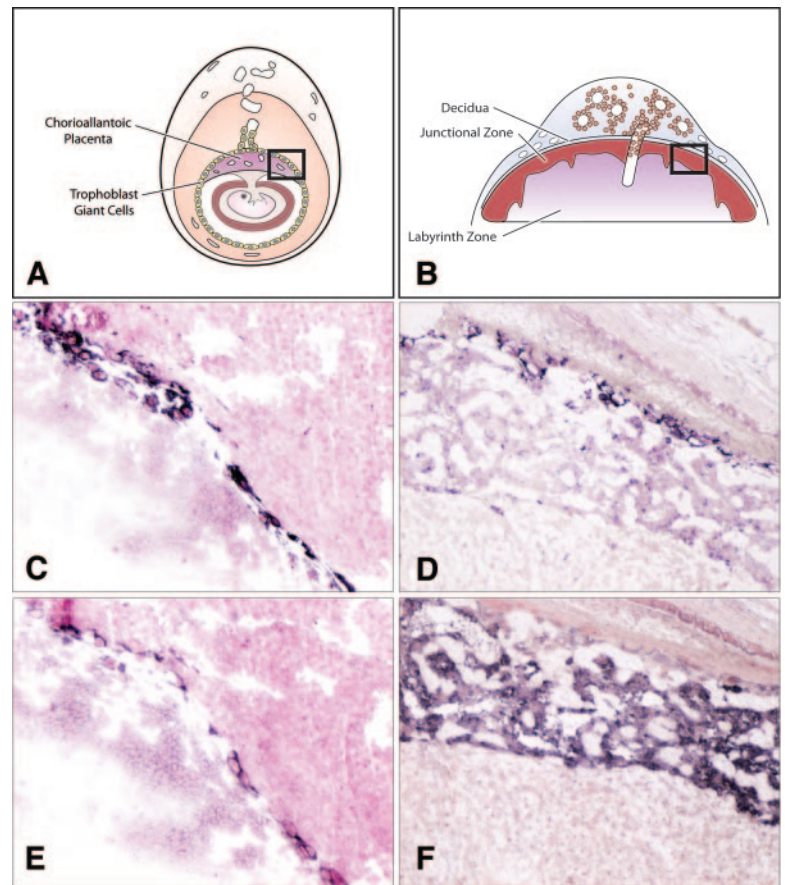


Fig. 2. Localization of PLP-F α and PLP-F β mRNAs within rat placental tissues. A, Schematic diagram of a mid-gestation uteroplacental compartment. The black-outlined box is the region of the placenta that is enlarged in C and E. B, Schematic diagram of late gestation uteroplacental compartment. The black-outlined box is the region of the placenta that is enlarged in D and F. *In situ* hybridization was used to identify the spatial distributions of PLP-Fs in the rat uteroplacental compartment. Cryosections were prepared from gestation d 11.5 (C and E) and d 18.5 (D and F) and hybridized to digoxigenin-labeled PLP-F α (C and D) and PLP-F β (E and F) antisense and sense (data not shown) probes.

cental PLP-F α mRNA responses were more robust and consistent than chorioallantoic placental PLP-F α responses (data not shown). The effects of maternal hypoxia in stimulating choriovitelline placental PLP-F α mRNA were verified by Northern blot analysis (Fig. 3C).

As indicated above, the mouse possesses two members of the PLP-F subfamily, PLP-E and PLP-F. Mouse PLP-E and rat PLP-F α share commonalities in their temporal and spatial expression patterns. Pregnant female mice were exposed to the equivalent of 11% oxygen from d 5.5 to 11.5 of gestation (Fig. 3D). Northern blot analysis revealed increased expression of PLP-E mRNA in animals exposed to hypoxia compared with pair-fed normoxic controls (Fig. 3E).

Finally, we examined the affect of maternal hypoxia on the regulation of PLP-F α and PLP-F β gene expression during the last week of gestation. Pregnant female rats were exposed to the equivalent of 10% oxygen from d 13.5 to 18.5 of gestation (Fig. 4A). Northern blot analyses of gestation d 18.5 junctional zone tissues indicated that PLP-F α and PLP-F β were not responsive to hypoxia (Fig. 4B).

In summary, mid-gestation trophoblast rat PLP-F α and mouse PLP-E expression respond positively to exposure to *in vivo* maternal hypoxia.

Impact of in vitro oxygen tension on trophoblast cell PLP-F α gene expression

Rcho-1 trophoblast cells represent an excellent *in vitro* model for investigating aspects of trophoblast giant cell dif-

ferentiation and function (21). In the following experiments, Rcho-1 trophoblast cells were induced to differentiate while exposed to ambient or 2% oxygen for 10 d (Fig. 5A). Northern blot analyses revealed that PLP-F α mRNA levels were significantly elevated in the 2% oxygen-exposed cell cultures (Fig. 5B). Thus, both *in vivo* and *in vitro* models indicate that trophoblast cell PLP-F α expression is sensitive to oxygen tension.

Erythroid responses to maternal hypoxia

To better understand the maternal response to hypobaric hypoxia, blood and spleen tissues were collected on gestation d 12.5 after 7 d of hypobaric hypoxia or in pair-fed pregnant animals exposed to ambient conditions (Fig. 6A) and analyzed for various hematological parameters. Exposure to maternal hypoxia significantly increased red blood cell numbers, hemoglobin concentrations, and hematocrit values (Fig. 6B). Additionally, key genes associated with hemoglobin synthesis (EKLF, ALAS-2, and β -major globin) were significantly elevated in splenic tissues from pregnant females exposed to hypobaric hypoxia (Fig. 6C). Collectively, the data suggest that the pregnant female rat responds to hypoxia, at least in part, through stimulation of the erythroid cell lineage.

Discussion

The PRL family has undergone species-specific expansions (5, 6). In the mouse and rat, the PRL family is exceptionally

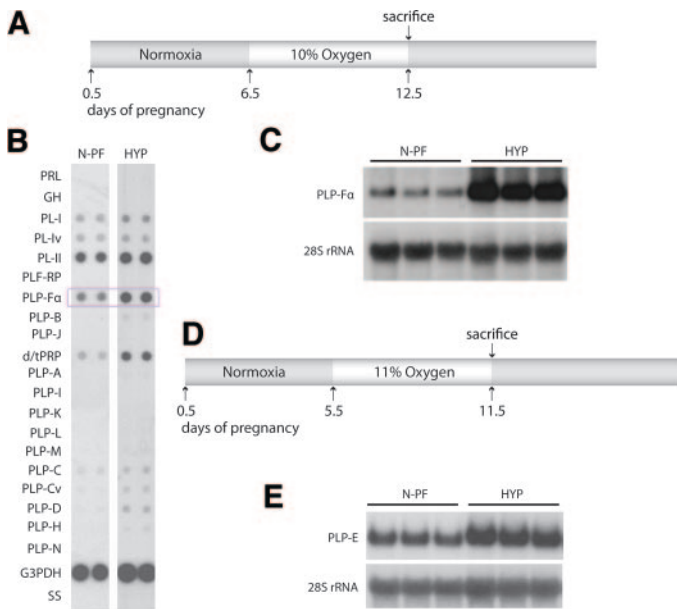


FIG. 3. Effects of hypoxia on placental PRL gene family expression. **A**, Schematic representation of *in vivo* rat experiments designed to evaluate placental responses to maternal hypoxia used to generate data presented in **B** and **C**. Pregnant Holtzman female rats were placed in hypobaric chambers in which air was circulated at a barometric pressure of approximately 380 Torr, which resulted in an inspired PO_2 of approximately 70 Torr, equivalent to breathing 10% O_2 at sea level. Hypoxia exposure was initiated on d 6.5 of gestation and animals were killed at d 12.5 of gestation (HYP). Gestationally matched pair-fed pregnant females exposed to ambient conditions were used as controls (N-PF). **B**, Examination of the effects of maternal hypoxia on choriovitelline placental PRL gene family expression using the PRL family miniarray. Total RNA was isolated from choriovitelline placental tissues on d 12.5 of gestation, radiolabeled by reverse transcription, and used as hybridization probes for rat PRL family miniarrays. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was used as a control. PLF-RP, Proliferin-related protein; d/tPRP, decidual/trophoblast PRL-related protein. **C**, Northern blot analysis of PLP-F α mRNA in choriovitelline placental tissues from female rats exposed to hypoxia and ambient conditions. 28S rRNA was used as a control to demonstrate loading accuracy and the integrity of the RNA. **D**, Schematic representation of *in vivo* mouse experiments used to generate data presented in **E**. Pregnant female CD-1 mice were placed in hypobaric chambers in which air was circulated at a barometric pressure of approximately 420 Torr, which results in an inspired PO_2 of approximately 78 Torr, equivalent to breathing 11% O_2 at sea level. Hypoxia exposure was initiated on d 5.5 of gestation and animals were killed at d 11.5 of gestation (HYP). Gestationally matched pair-fed pregnant females exposed to ambient conditions were used as controls (N-PF). **E**, Northern blot analysis of PLP-E mRNA in placental tissues from female mice exposed to hypoxia and ambient conditions. 28S rRNA was used as a control to demonstrate loading accuracy and the integrity of the RNA.

large (~24 genes in each species) and can be classified into a number of subfamilies (3, 5). These subfamilies are based strictly on structural relatedness. They exhibit elements of conservation between the mouse and rat and also species specificity. The PLP-F subfamily is of considerable interest because of recent advances in understanding its biological roles during pregnancy (9–15). This subfamily consists of two members in the mouse (PLP-E and PLP-F) and two members in the rat (PLP-F α and PLP-F β).

In this report, we investigate the rat PLP-F subfamily. PLP-F α and PLP-F β exhibit significant sequence similarities

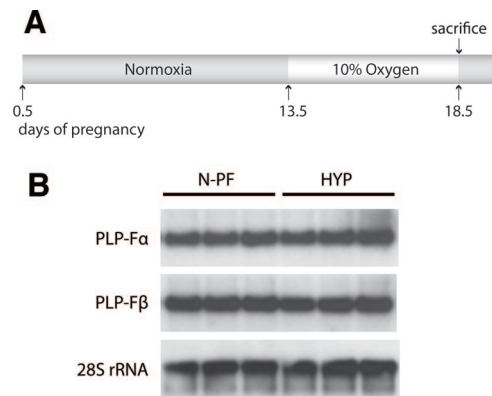


FIG. 4. Effects of maternal hypoxia during the last week of gestation on PLP-F α and PLP-F β mRNA expression. **A**, Schematic representation of *in vivo* rat experiments used to generate data presented in **B**. Pregnant Holtzman female rats were placed in hypobaric chambers in which air was circulated at a barometric pressure of approximately 380 Torr, which resulted in an inspired PO_2 of approximately 70 Torr, equivalent to breathing 10% O_2 at sea level. Hypoxia exposure was initiated on d 13.5 of gestation and animals were killed at d 18.5 of gestation (HYP). Gestationally matched pair-fed pregnant females exposed to ambient conditions were used as controls (N-PF). **B**, Expression of PLP-F α and PLP-F β mRNAs in the rat junctional zone during late gestation. Northern blot analyses were performed for PLP-F α and PLP-F β mRNAs in junctional zone placental tissues from animals exposed to hypobaric hypoxia and pair-fed normoxia controls. 28S rRNA was used as a control to demonstrate loading accuracy and the integrity of the RNA.

and structurally are both more closely related to mouse PLP-F than to mouse PLP-E. However, based on expression profiles, PLP-F α shares similarities with mouse PLP-E, whereas PLP-F β parallels mouse PLP-F. Furthermore, we demonstrated that exposure of pregnant rats to hypobaric hypoxia is an effective technique for investigating pregnancy-dependent adaptations. Maternal hypoxia activates placental signals, including members of the PLP-F subfamily,

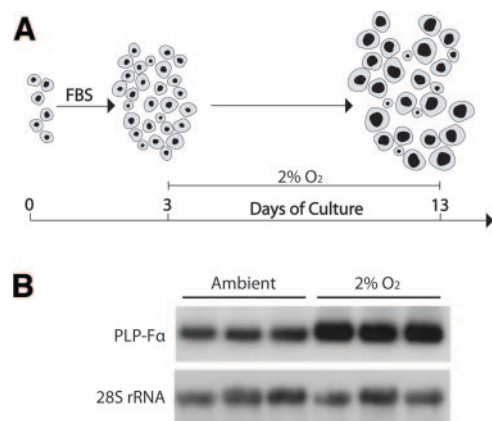


FIG. 5. Effect of low oxygen on PLP-F α mRNA expression in Rcho-1 trophoblast cells. **A**, Schematic representation of *in vitro* cell culture experiments used to generate data presented in **B**. Proliferative Rcho-1 trophoblast cells were cultured for 3 d under proliferative conditions and then under differentiating conditions in 2% or ambient O_2 for 10 d. **B**, Northern blot analysis of PLP-F α mRNA in differentiating Rcho-1 trophoblast cells exposed to ambient conditions or low oxygen. 28S rRNA was used as a control to demonstrate loading accuracy and the integrity of the RNA.

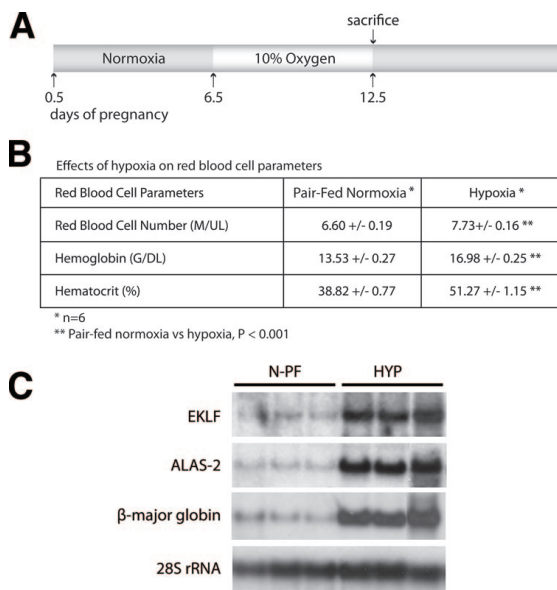


FIG. 6. Effects of maternal hypoxia on red blood cell parameters and expression of hemoglobin synthesis genes. **A**, Schematic representation of *in vivo* rat experiments used to generate data presented in **B** and **C**. Pregnant Holtzman female rats were placed in hypobaric chambers in which air was circulated at a barometric pressure of approximately 380 Torr, which resulted in an inspired PO_2 of approximately 70 Torr, equivalent to breathing 10% O_2 at sea level. Hypoxia exposure was initiated on d 6.5 of gestation and animals were killed at d 12.5 of gestation (HYP). Gestationally matched pair-fed pregnant females exposed to ambient conditions were used as controls (N-PF). **B**, Effects of maternal hypoxia on red blood cell parameters. Hematological variables were measured using an Act10 hematological analysis system. **C**, Expression of genes encoding key proteins involved in the regulation of hemoglobin synthesis from the maternal rat spleen. Northern blot analyses were performed for EKLf, ALAS-2, and β -major globin mRNAs in splenic tissue from animals exposed to hypobaric hypoxia and pair-fed normoxia controls.

and maternal homeostatic responses ensuring oxygen availability and delivery to the fetus.

PLP-F subfamily genes are expressed by different trophoblast cell lineages and are differentially activated by hypoxia. Rat PLP-F α and PLP-F β genes each possess a six-exon structure and are situated next to each other within the PRL family locus on chromosome 17 (5). Their close proximity does not correlate with their cell- and temporal-specific expression patterns. PLP-F α is expressed predominantly in trophoblast giant cells during midgestation, whereas PLP-F β is expressed primarily by spongiotrophoblast cells during the latter part of gestation (Ref. 18 and present study). Not surprisingly, the derivation of the trophoblast giant cell and spongiotrophoblast cell lineages involve distinct regulatory pathways (34) and are differentially affected by hypoxia (35–38). Midgestation rat PLP-F α and mouse PLP-E expression are stimulated by exposure to maternal hypoxia. Although the increase of PLP-F α and PLP-E expression by hypoxia is dependent on the activation of midgestation trophoblast giant cells, other trophoblast giant cell-specific genes are not similarly up-regulated by maternal hypoxia. For example, PL-I, PL-II, and PLP-M transcript levels do not change significantly in placental tissues from pregnant females exposed to hypoxia (present study). As gestation progresses, the reg-

ulation of PLP-F subfamily gene expression becomes constitutive and nonresponsive to oxygen tension. Gestational-dependent regulation of PLP-F subfamily gene expression is likely connected to the physiological demands of pregnancy and the biological activities of the PLP-F subfamily.

The PLP-F subfamily of cytokines/hormones has been linked to the regulation of hematopoiesis. PLP-E and PLP-F stimulate blood cell formation, including megakaryocyte and erythrocyte development (9–15). Hypoxia is known to differentially affect megakaryocytopoiesis and erythropoiesis. Megakaryocyte formation is inhibited by hypoxia, whereas erythrocyte development is stimulated (39, 40). Erythrocytes are key regulators of oxygen homeostasis. They synthesize hemoglobin, bind oxygen, and are fundamental for oxygen delivery to tissues (41, 42). Pregnancy represents a significant challenge to oxygen homeostasis and fetal oxygen delivery. Red blood cell numbers increase during pregnancy concomitant with expansions in blood volume and the growth demands of the fetus (43). During early postimplantation to midgestation, trophoblast giant cells are effective sensors of oxygen status. If oxygen tension is below a physiological set point, then trophoblast giant cells are activated to produce PLP-F subfamily members, especially PLP-F α and PLP-E. This midgestation regulatory loop can be further activated by exposure of pregnant rats to hypobaric hypoxia. During late pregnancy, maternal and fetal demands for oxygen are increased and PLP-F subfamily members (PLP-F β and mouse PLP-F) are constitutively produced by spongiotrophoblast cells. Collectively, these hormones enter into maternal circulation (9) and are targeted to maternal sites of hematopoiesis (bone marrow, spleen) in which they can mimic the actions of erythropoietin and stimulate red blood cell formation (10, 15). At least one member of the PLP-F subfamily can also be activated during pathological conditions (12). The cellular mechanism of action of PLP-F subfamily cytokines/hormones is not well understood but may involve gp130 and Janus kinase/signal transducer and activator of transcription signaling pathways (9, 10).

During pregnancy, hematopoiesis is also initiated in extraembryonic and embryonic tissues. The midgestation placenta is a site of hematopoietic stem cell development (44–46), and the visceral yolk sac is an active site of erythropoiesis (41, 42). The peak in placental hematopoietic stem cell production and visceral yolk sac erythropoiesis correspond to the expression profile of PLP-F subfamily members; however, whether they have access to these extraembryonic targets and are capable of modulating their functions remains to be determined.

In summary, the placenta orchestrates adaptive responses to hypoxia, at least in part, via the elaboration of hormones and cytokines. PLP-F α and PLP-E, in the rat and mouse, respectively, represent hormones/cytokines that are responsive to maternal hypoxia and possess biological actions contributing to pregnancy-dependent regulation of oxygen homeostasis.

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