

Dual Expression of Prolactin-Related Protein in Decidua and Trophoblast Tissues during Pregnancy in Rats¹

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ABSTRACT

Decidual prolactin-related protein (dPRP) is a member of the prolactin gene family and is abundantly expressed in the rat deciduum. Previously, dPRP was shown to associate with heparin-containing molecules and was found to reside, at least in part, within the decidual extracellular matrix, where it was postulated to influence decidual cells and other cell types. The purpose of this investigation was to identify the cellular origin and the temporal and regional characteristics of dPRP expression in the rat uterus during pregnancy. Protein expression was evaluated by Western blot analysis, immunoprecipitation, and immunocytochemistry; dPRP mRNA expression was assessed by reverse transcriptase polymerase chain reaction and in situ hybridization. Decidual PRP was first detected at Day 6 of pregnancy or pseudopregnancy. Expression increased with the growth of the deciduum and then declined coincident with regression of decidual tissue. Throughout the first half of pregnancy or pseudopregnancy, dPRP and mRNA were predominantly localized to the antimesometrial deciduum of the developing conceptus. During the second half of gestation, expression also appeared in the chorioallantoic placenta. Trophoblast giant cells and spongiotrophoblast cells within the junctional zone of the chorioallantoic placenta expressed dPRP, as did the Rcho-1 trophoblast cell line. In conclusion, dPRP production is elevated from implantation until parturition through the participation of decidual (early pregnancy) and trophoblastic (late pregnancy) tissues.

INTRODUCTION

Significant morphological and functional adjustments take place in the uterus and placenta during the course of pregnancy in the rat. Uterine stromal cells undergo a differentiation process referred to as decidualization, which is induced by hormonal events of early pregnancy and signals from the early embryo [1–3]. Decidualization begins in the antimesometrial endometrium and eventually surrounds the developing blastocyst, providing a physical barrier between the blastocyst and the remainder of the uterus and serving as a source of hormones [1–4]. Cells of the antimesometrial and mesometrial deciduum undergo different maturational processes resulting in distinct morphological, biochemical, and physiological phenotypes [2, 5]. Regression of the deciduum begins around midgestation [6, 7]. Before the initiation of decidual regression, morphogenetic events are occurring in the chorioallantoic placenta, resulting in the formation of two prominent zones: the junctional and labyrinth zones [8, 9]. Trophoblast giant cells, spongiotrophoblast

cells, and glycogen cells arise within the junctional zone, which is located at the uteroplacental interface and is the principal site of hormone production. Labyrinthine trophoblast cells (syncytial trophoblast cells) are involved in bidirectional transport between maternal and fetal compartments and in some limited endocrine activities (trophoblast cells).

Rat uterine and placental tissues are characterized by their production of a family of hormones structurally related to pituitary prolactin (PRL; [9, 10]). The PRL family includes placental lactogen-I (PL-I), PL-I variant (PL-Iv), PL-II, PRL-like protein (PLP)-A, PLP-B, PLP-C, and decidual PRL-related protein (dPRP; [9, 10]). Each member of the family is expressed in cell- and temporal-specific patterns during pregnancy [9, 10]. PL-I and PL-II are the exclusive products of trophoblast giant cells [11–13], whereas PL-Iv, PLP-A, and PLP-C are dually expressed by trophoblast giant cells and spongiotrophoblast cells [13–17]. Decidual cells and spongiotrophoblast cells contribute to the production of PLP-B [13, 18]. Decidual PRP is expressed in the deciduum and possibly the placenta [19, 20].

Members of the PRL family can be distinguished on the basis of their biological actions (classical vs. nonclassical). Classical members (PL-I, PL-II, PL-Iv) are functional PRL homologues, whereas nonclassical members (PLP-A, PLP-B, PLP-C, dPRP) do not appear to utilize the PRL receptor signaling pathway [21]. Decidual PRP binds to heparin, resides at least in part in the decidual extracellular matrix, and alters host-tumor cell relationships, thereby facilitating tumor formation [22]. Parallel maternal-embryo relationships may be modulated by dPRP during the establishment of pregnancy.

In this study, experiments were designed to determine the cell and temporal patterns of dPRP mRNA and protein expression. Western blot, immunocytochemical, immunoprecipitation, reverse transcriptase polymerase chain reaction (RT-PCR), and in situ hybridization analyses were used to evaluate dPRP expression during gestation.

MATERIALS AND METHODS

Reagents

Fetal bovine serum (FBS) and horse serum (HS) were purchased from JRH Bioscience (Lenexa, KS). Dispase II was acquired from Boehringer Mannheim Biochemicals (Indianapolis, IN). Pansorbin *Staphylococcus aureus* cells were purchased from Calbiochem (San Diego, CA). *Trans*-³⁵S-label was purchased from ICN (Irvine, CA). Restriction enzymes and polymerases were purchased from New England Biolabs (Beverly, MA). Reagents for the synthesis of cRNA probes and random primer-labeled DNA probes were obtained from Stratagene (La Jolla, CA). *Taq* polymerase was acquired from Promega (Madison, WI). Radiolabeled nucleotides were obtained from DuPont-NEN (Boston, MA). Reagents for first-strand synthesis and PCR were purchased from Gibco BRL/Life Technologies (Gaithersburg,

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MD). Reagents for PAGE were acquired from Bio-Rad (Hercules, CA). Nitrocellulose for Western blots and nylon membranes for Southern blots were obtained from Schleicher and Schuell (Keene, NH). Avidin-biotin immunoperoxidase kits were purchased from Vector Laboratories (Burlingame, CA). Unless otherwise noted, all other chemicals were purchased from Sigma Chemical Company (St. Louis, MO).

Animals and Tissue Preparation

Holtzman rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Animals were housed in an environmentally controlled facility, with lights-on from 0600 to 2000 h, and were allowed free access to food and water. Timed pregnancies and pseudopregnancies and tissue dissections were performed as previously described [19, 23]. The day on which a copulatory plug or sperm in the vaginal smear was present was designated Day 0 of pregnancy. Pseudopregnancy was induced by vagino-cervical stimulation on the evening of proestrus with a mechanical vibrator [24, 25]. The first day of pseudopregnancy was defined as the first day of leukocytic vaginal smears after vagino-cervical stimulation. Deciduomal responses were induced in pseudopregnant rats on Day 4 of pseudopregnancy by injection of 50–100 μ l sesame oil per uterine horn [26]. Decidual cytosols were prepared as previously described [27]. Protein concentrations of cytosol preparations were estimated by the method of Bradford [28]. Tissues to be used for immunocytochemistry were immersion fixed in Bouin's fluid and embedded in paraffin. Tissues for in situ hybridization and RT-PCR were flash frozen in cold isopentane and stored at -80°C until processed. Protocols for the care and use of animals were approved by the University of Kansas Animal Care and Use Committee.

Rcho-1 Trophoblast Cell Culture

The Rcho-1 trophoblast cell line was maintained under subconfluent conditions in NCTC-135 culture medium supplemented with 20% FBS [29, 30]. Differentiation was induced by growing the cells to confluence in FBS-supplemented culture medium and replacing the FBS with 10% HS [30].

Immunoprecipitation of In Vitro Decidual Cell-Synthesized dPRP

Decidual tissue was harvested on Day 7 of pseudopregnancy by scraping, cut into 1- to 2-mm³ pieces, and washed several times with Hanks' Balanced Salt Solution. Tissue segments were incubated with Dispase II (2.4 U/ml) containing DNase I (50 U/ml) and shaken vigorously for 1 h at 37°C in an atmosphere of 95% air:5% CO_2 . Nondispersed cells were reincubated with the enzyme solution for an additional hour. Dispersed cells were pooled, centrifuged, and resuspended in Dulbecco Minimum Eagle medium (DMEM) containing 100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, and 10% FBS and filtered through a 74- μm nylon mesh. The equivalent of one uterine horn of decidual tissue was plated per 35-mm dish. Cells were allowed to attach for 2–3 h; medium and all nonattached cells were discarded. Attached cells were incubated in DMEM containing antibiotics and 1% FBS and maintained at 37°C in an atmosphere of 95% air:5% CO_2 . On Day 3 of culture, the cells were incubated with methionine-free culture medium for 30 min; this was followed by a 16-h incubation

in methionine-free culture medium containing 100 $\mu\text{Ci}/\text{ml}$ *trans* ^{35}S -label (70% [^{35}S]methionine, 1200 Ci/mmol). At the termination of the incubation, conditioned medium was precleared by incubation with Pansorbin cells. Aliquots (100 μl) of the precleared conditioned medium were incubated overnight at 4°C with preimmune serum (10 μl) or with antiserum to dPRP (10 μl) in immunoprecipitation buffer (200 μl ; 10 mM sodium phosphate buffer, pH 7.5, containing 150 mM NaCl, 10 mM L-methionine, 1% deoxycholic acid, and 2% Triton X-100). After incubation, antigen-antibody complexes were recovered by incubation with Pansorbin cells, centrifuged, and washed five times with wash buffer (50 mM Tris-HCl, pH 7.4, containing 150 mM NaCl, 5 mM EDTA, 0.02% azide, and 0.05% Nonidet P-40) according to the procedure of Kessler [31]. Immunoprecipitated proteins were eluted from Pansorbin cells by resuspension in SDS-electrophoresis sample buffer and heated at 90°C for 4 min. Samples were then centrifuged, and supernatants were recovered and electrophoretically separated in 12.5% polyacrylamide gels. The gels were sequentially incubated in water for 30 min and 1 M sodium salicylate for 30 min. Gels were dried and exposed to Kodak X-Omat AR x-ray film (Eastman Kodak, Rochester, NY) at -80°C [32].

Western Blot Analysis

Western blot analyses were performed as previously described [27, 33]. Samples were separated by SDS-PAGE in 12.5% gels under reducing conditions. Proteins from the gels were electrophoretically transferred to nitrocellulose and subjected to immunoblot analysis. Nitrocellulose filters were then developed by exposure to an alkaline phosphatase-labeled anti-rabbit IgG and subsequent histochemical staining. Antibodies generated to recombinant dPRP were used as probes [22]. In some experiments, preimmune serum or antibodies saturated with the respective antigens were used as controls.

Immunocytochemistry

Tissues were sectioned at 6 μm and mounted on poly-L-lysine-coated slides. Localization of dPRP was determined using a streptavidin-biotin immunoperoxidase kit for rabbit IgG. Diaminobenzidine was used as the chromogen. The immunostained sections were counterstained with hematoxylin. Specificity of the immunoreactions was determined by comparing the reactivity of the antibodies with that of antibodies adsorbed with excess antigen.

In Situ Hybridization

Decidual PRP mRNA was detected in frozen tissue sections as previously described [11]. A full-length dPRP cDNA cloned into a Bluescript plasmid [19] was linearized and used as a template for the synthesis of ^{35}S -labeled sense and antisense RNA probes.

RT-PCR

Total RNA was extracted from tissues by the guanidinium isothiocyanate isolation method [34]. Reverse transcription was performed according to the manufacturer's instructions (Superscript Preamplification System; Gibco BRL/Life Technologies); 5 μg of total RNA and 0.5 μg of oligo(dT) primer (12–18 mer) were utilized for the reverse transcription reaction. PCR was performed subsequent to first-strand synthesis for 35 cycles with a denaturing tem-

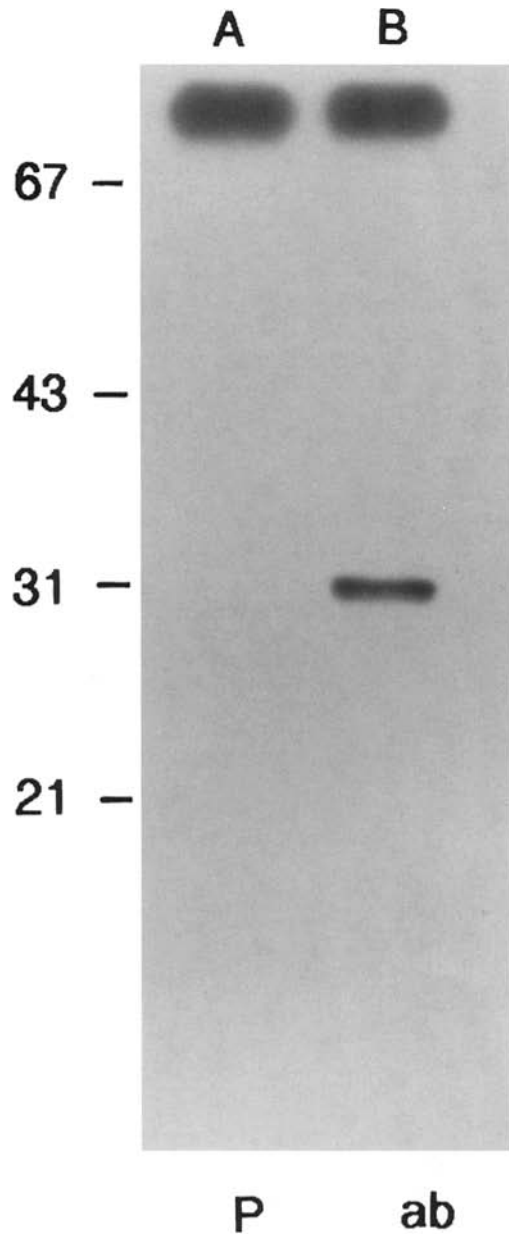


FIG. 1. In vitro incorporation of radiolabeled methionine into dPRP by decidual cells. Conditioned medium from [35 S]methionine-labeled decidual cell cultures was immunoprecipitated with an antiserum to dPRP. The precipitates were separated by SDS-12.5% PAGE and subjected to fluorography. Lane A: immunoprecipitated with preimmune serum; lane B: immunoprecipitated with antiserum to recombinant purified dPRP. Molecular weight standards ($\times 10^{-3}$) are shown.

perature of 94°C (1 min), an annealing temperature of 60°C (2 min), and an extension temperature of 72°C (2 min) using a Perkin Elmer Thermocycler Model 480 (Norwalk, CT). The dPRP-specific primers consisted of an upstream primer, 5'-CATGGACCTGAACATGAAAACATCAA-3' (sense, 325-354; located on exon 4), and a downstream primer, 5'-GTAGAATATATCAACACGTAGGCAGTG-3' (antisense, 637-666; located on exon 6). Reaction products were fractionated in agarose gels and transferred to nitrocellulose. The expected amplified product was 342 base pair. Southern blots were performed with a [32 P] random primer-labeled dPRP cDNA [19]. After hybridization, filters were autoradiographed with Kodak X-Omat AR x-ray film.

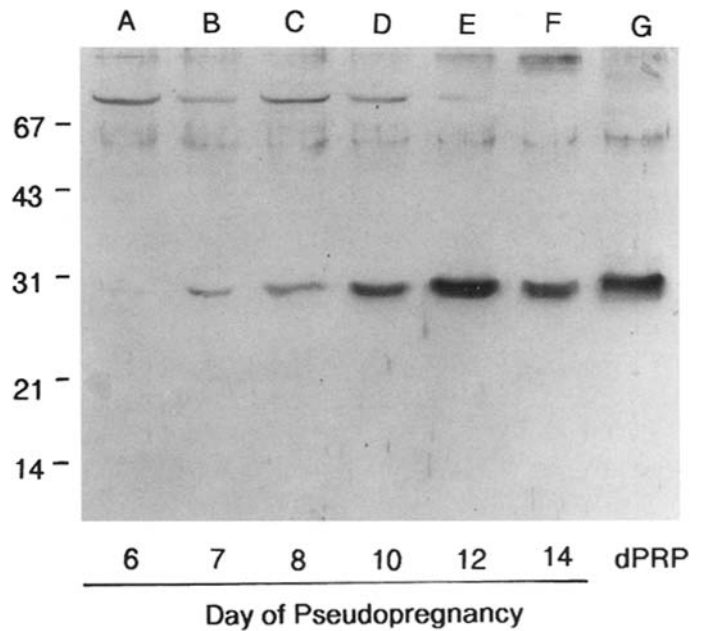


FIG. 2. Temporal pattern of expression of dPRP in decidual tissue. Cytosol preparations from decidual tissues isolated on Days 6-14 of pseudopregnancy (lanes A-F) were electrophoretically separated using SDS-12.5% polyacrylamide gels, transferred to nitrocellulose, and subjected to immunoblot analysis with an antiserum to dPRP (1:2000 final dilution). Purified recombinant dPRP was used as a control (lane G). Representative blot of experiments performed in triplicate. Molecular weight standards ($\times 10^{-3}$) are shown.

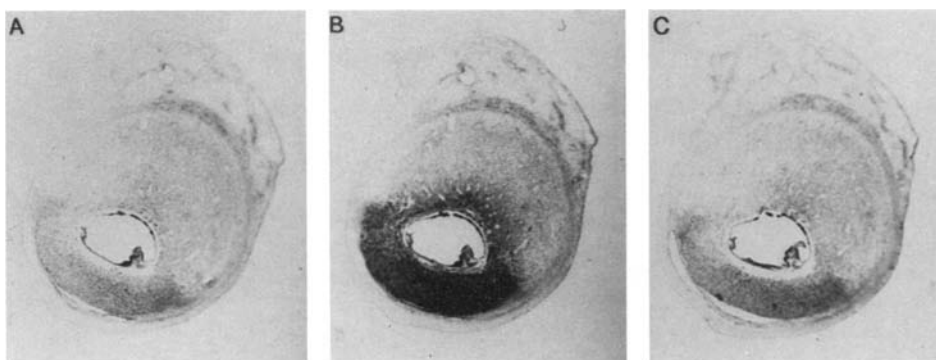
RESULTS

Characterization of Decidual dPRP Expression

De novo synthesis of dPRP was evaluated by incubating decidual cells with 35 S-labeled methionine. The radiolabeled amino acid was specifically incorporated into a 29-kDa protein recognized by antibodies directed to dPRP (Fig. 1). The temporal pattern of expression of dPRP in whole decidual tissue was assessed by Western blot analysis using specific antibodies to dPRP (Fig. 2). Decidual PRP expression in pseudopregnancy was initiated at Day 6, reached a peak by Day 12, and began to decline by Day 14. On the basis of these results, immunocytochemistry was performed to localize the dPRP within the pregnant uterus. The specificity of the immunocytochemical localization of dPRP was demonstrated by the restricted localization of dPRP to the antimesometrial deciduum and by the absence of staining with preimmune antiserum or with dPRP antibodies saturated with excess dPRP (Fig. 3). Expression of dPRP was initiated as early as Day 6 (Fig. 4, A and B). Primitive endoderm also stained positive for the presence of dPRP. On Day 10 of gestation, the expression of dPRP was restricted to the antimesometrial compartment of the deciduum (Figs. 3 and 4C). Immunoreactive dPRP was localized to decidual cells, whereas undecidualized stroma and myometrium presented only weak reactive immunostaining. By Day 13 of gestation, some limited dPRP immunoreactivity was detected in mesometrial decidual cells located proximal to trophoblast giant cells of the junctional zone (Fig. 4D).

The pattern of dPRP mRNA expression in decidua mimicked that of dPRP immunoreactivity (i.e., was restricted primarily to antimesometrial decidual cells). Expression of dPRP mRNA was intense in the antimesometrial decidua but absent in nondecidualized stroma and myometrium

FIG. 3. Immunocytochemical localization of dPRP in the uterus. Transverse sections of rat uteri at conceptus sites were stained for the presence of dPRP using polyclonal antibodies to dPRP and a streptavidin-biotin immunoperoxidase kit. The antiserum was used at a final dilution of 1:1000. **A)** Day 10 of pregnancy, incubated with preimmune serum; **B)** Day 10 of pregnancy; specific immunostaining was localized predominantly to the antimesometrial deciduum; **C)** Day 10 of pregnancy; adsorption of the antiserum with dPRP resulted in the loss of specific immunostaining. Magnifications, $\times 8$.



(Fig. 4, E and F). Mesometrial decidual cells, developing placenta, and embryonic and extraembryonic tissues and membranes did not show positive hybridization with the antisense dPRP probe (data not shown). Thus, dPRP reactivity seen in primitive endoderm and myometrium likely represents a site of dPRP uptake/binding and not a site of synthesis. The dPRP sense probe did not specifically hybridize to tissue sections (data not shown).

Characterization of Placental Expression of dPRP

Other members of the rat placental PRL family are abundantly expressed in the chorioallantoic placenta [10], and at least one member, PLP-B, is dually expressed, appearing in both decidua and placenta [13, 18]. To investigate the presence of dPRP in the placenta, immunocytochemistry and *in situ* hybridization were employed on placentas from Day 19 of gestation. Decidual PRP mRNA and protein were both specifically localized to the junctional zone of the chorioallantoic placenta (Fig. 5). Within the junctional zone, both trophoblast giant cells and spongiotrophoblast cells stained intensely for dPRP, while glycogen cells and cellular components of the labyrinth zone did not express dPRP.

Decidual PRP shares approximately 70% structural homology with another PRL family member, PLP-C [17, 19]. Furthermore, a PLP-C cDNA was originally used to identify dPRP cDNAs from a rat decidual cDNA library [19]. Consequently, in order to verify the expression of dPRP in the placenta, an RT-PCR assay for dPRP was developed. The assay was utilized to survey placental tissues and the Rcho-1 trophoblast cell line. The Rcho-1 trophoblast cell line can be manipulated to proliferate or differentiate into trophoblast giant cells capable of expressing members of the placental PRL family [29, 30]. The primers used in the RT-PCR procedure were designed to be specific for dPRP transcripts and to distinguish between complementary and genomic dPRP DNA [35].

When total RNA was subjected to RT-PCR using primers specific for dPRP, a single band was generated, yielding a product of the predicted size of 342 base pair (Fig. 6). A time-course evaluation of dPRP expression in the junctional zone of the chorioallantoic placenta shows that dPRP is detectable at Day 13 and continues to be expressed until parturition at Day 21 (Fig. 6). Southern blot analysis revealed that dPRP mRNA is expressed in differentiated Rcho-1 trophoblast cells, providing additional evidence that the signal for dPRP is expressed in the trophoblast giant cell lineage (Fig. 6).

DISCUSSION

Decidual PRP is a member of a family of proteins possessing structural and sometimes functional relatedness to pituitary PRL. A number of PRL family members are expressed during pregnancy in a cell- and temporal-specific manner [10]. In humans and some other primates, a protein identical in primary structure to pituitary PRL, and encoded by the same gene, is expressed in decidual tissue of the normal reproductive cycle and throughout pregnancy [36, 37]. The rat does not express the pituitary PRL gene in decidual tissue but instead synthesizes other PRL family members, including dPRP and PLP-B ([18, 19]; present study). In this report, we have mapped fundamental characteristics of dPRP expression and described the coordinated biosynthesis of dPRP in maternal to extraembryonic tissues as gestation progresses. This coordinated effort by uterine and placental structures ensures the continued presence of dPRP from implantation until parturition. Given the nature of the expression pattern, we now refer to the protein as decidual/trophoblast prolactin-related protein (d/tPRP).

Cell- and temporal-specific patterns of d/tPRP expression have been determined with cytochemical and biochemical procedures ([19, 20]; present study). Decidual/tPRP expression is first detected at Day 6 of pregnancy, increases with growth of the deciduum, and declines with decidual regression. Throughout the first half of pregnancy, d/tPRP mRNA and protein are predominantly localized to the antimesometrial deciduum of the implantation site. This regionally specific localization of d/tPRP expression in the deciduum underscores known structural and functional differences existing in mesometrial and antimesometrial decidual cells [2, 5]. Whether regional differences in decidual cell behavior represent the differentiation of distinct cell lineages or are the consequences of exposure of a common cell lineage to differing environmental regulators remains to be determined. During the second half of gestation, d/tPRP expression shifts to the chorioallantoic placenta. Both trophoblast giant cells and spongiotrophoblast cells within the junctional zone of the chorioallantoic placenta express d/tPRP. Additionally, a trophoblast cell line (Rcho-1) capable of differentiating along the trophoblast giant cell lineage expresses d/tPRP in the differentiated state. Thus, d/tPRP expression during pregnancy represents a complex coordination between decidual and trophoblast cells.

The coordinated pattern of decidua and trophoblast d/tPRP expression resembles the gestational expression pattern for another member of the PRL gene family, PLP-B [13, 38–40]. PLP-B was originally identified from a rat pla-

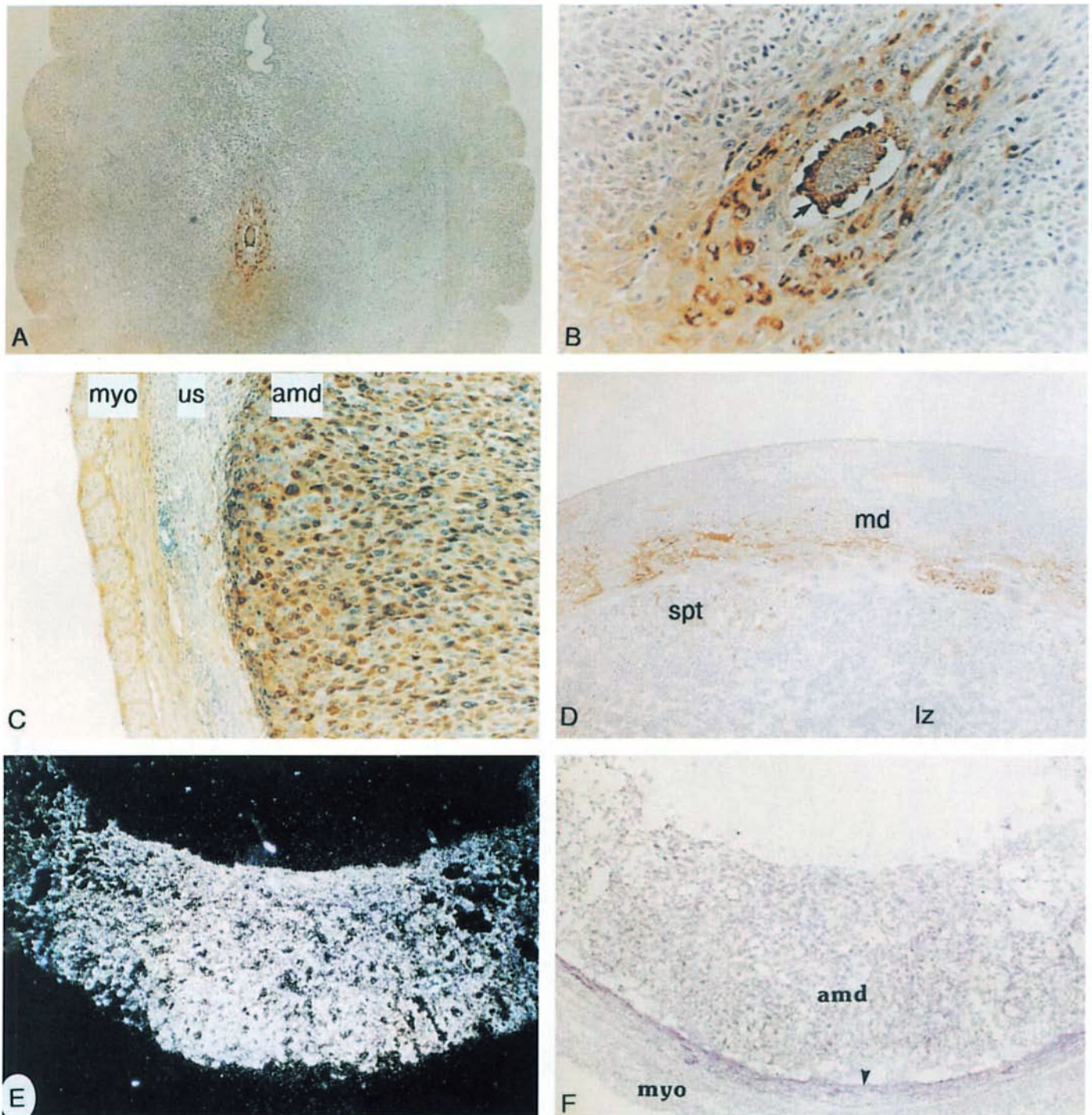


FIG. 4. Distribution of dPRP mRNA and protein in the uterus during the first half of pregnancy. Transverse sections of rat uteri at conceptus sites were stained for the presence of dPRP using polyclonal antibodies to dPRP and a streptavidin-biotin immunoperoxidase kit (A–D) or were processed for the in situ localization of dPRP mRNA (E and F). **A**) Day 6 of pregnancy; dPRP is present in decidual cells surrounding the blastocyst and is beginning to be expressed in antimesometrial decidual cells; $\times 10$. **B**) Higher magnification of Day 6 conceptus (arrow shows the site of primitive endodermal immunoreactivity); $\times 40$. **C**) Day 10 of pregnancy; decidualized stromal cells of the antimesometrial deciduum (amd) showed strong positive immunostaining; $\times 100$. **D**) Day 13 of pregnancy; note limited dPRP immunoreactivity in mesometrial decidual cells adjacent to the trophoblast giant cells of the junctional zone and some initial reactivity in spongiotrophoblast cells (spt); $\times 40$. **E**) Darkfield image from a portion of a Day 10 deciduum hybridized with radiolabeled dPRP antisense probe; $\times 100$; **F**) brightfield image of the Day 10 conceptus shown in **E**; $\times 100$. Arrowhead denotes undecidualized stroma. Note that the positive antisense signal is restricted to decidualized stroma. The dPRP sense probe did not hybridize to tissues (not shown). Abbreviations: amd, antimesometrial deciduum; md, mesometrial deciduum; myo, myometrium; us, nondecidualized uterine stroma; spt, spongiotrophoblast cells.

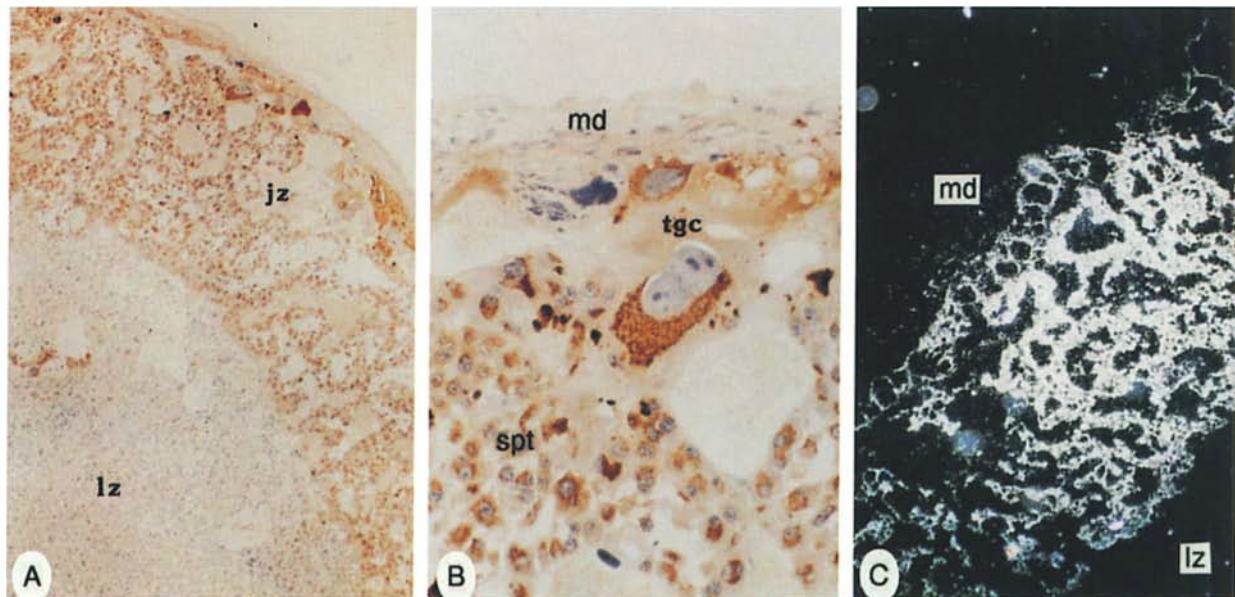


FIG. 5. Placental distribution of dPRP mRNA and protein at Day 19 of pregnancy. Immunocytochemical localization of dPRP in sections from the chorioallantoic placenta (A and B). Antiserum to dPRP was used at a dilution of 1:1000 in conjunction with an avidin-biotin immunoperoxidase kit. Note in A the junctional zone (jz) of the placenta exhibits positive staining for dPRP while the labyrinth zone (lz) does not; $\times 40$. B is at a higher magnification showing cellular distribution of dPRP in spongiotrophoblast cells (spt) and trophoblast giant cells (tgc); $\times 200$. In situ hybridization of dPRP mRNA in the chorioallantoic placenta. Sections were hybridized with [35 S]-labeled dPRP sense or antisense riboprobe. C is a darkfield photomicrograph depicting dPRP mRNA expression in the junctional zone of the placenta; $\times 100$. No specific hybridization was detected with the sense probe (not shown). Abbreviations: jz, junctional zone; lz, labyrinth zone; tgc, trophoblast giant cell; md, mesometrial deciduum; spt, spongiotrophoblast.

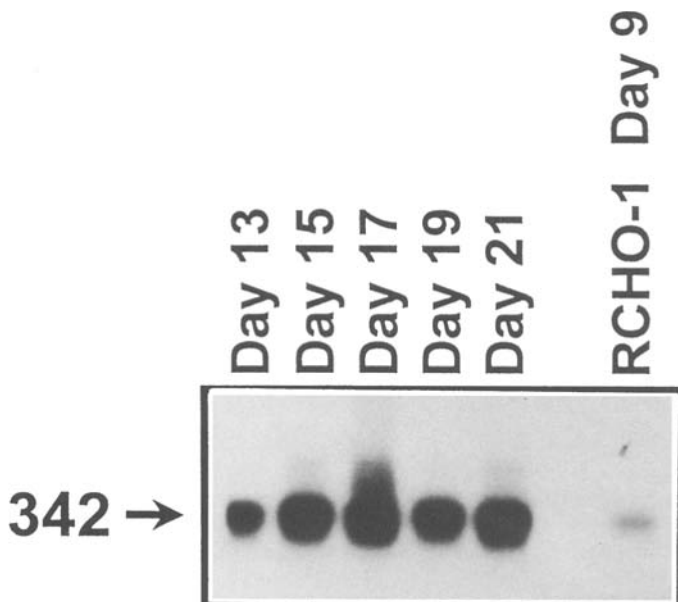


FIG. 6. Temporal expression pattern of dPRP in the junctional zone compartment of the chorioallantoic placenta and Rcho-1 trophoblast cells. Total RNA was extracted from tissues by the guanidinium isothiocyanate isolation method. Reverse transcription was performed using oligo(dT) primers. Subsequent PCR was performed using dPRP-specific primers. The reaction products were separated in agarose gels and transferred to nitrocellulose. Southern blots were performed with a [32 P]-labeled dPRP cDNA. Note that dPRP expression is evident in the junctional zone of the chorioallantoic placenta throughout the latter part of gestation and in differentiated Rcho-1 trophoblast cells (Day 9).

cental cDNA library during a search for a placental lactogen cDNA [38]. Like that of d/tPRP, expression of PLP-B coincides with the development of antimesometrial decidual tissue during the early part of pregnancy and subsequently spongiotrophoblast cells of the chorioallantoic placenta during the second half of gestation [13, 18]. There are a few features of d/tPRP and PLP-B expression that differ. First, the level of decidual PLP-B expression is very low relative to decidual d/tPRP expression [18–20]. Second, unlike d/tPRP, PLP-B is not expressed within trophoblast giant cells of the chorioallantoic placenta [13]. Consistent with this observation, PLP-B is also not expressed in the trophoblast giant cell lineage-restricted Rcho-1 trophoblast cell line [29, 41]. Third, placental PLP-B expression wanes during the latter part of gestation, whereas d/tPRP expression continues unabated ([38, 39]; present study). The overall pattern of d/tPRP expression in the chorioallantoic placenta more closely resembles the cell- and temporal-specific expression patterns of three other members of the PRL gene family, PLP-A, PLP-C, and PL-IV [13–17]. Thus, regulatory mechanisms controlling decidual and spongiotrophoblast cell d/tPRP expression may be shared with PLP-B, whereas trophoblast giant cell d/tPRP expression may be controlled by mechanisms also regulating PLP-A, PLP-C, and PL-IV.

Some information is available regarding the control of decidual, spongiotrophoblast, and trophoblast giant cell-specific gene expression that may provide insight into the control of d/tPRP expression. The human PRL gene, which is expressed in multiple tissues, including decidua, accomplishes this expression pattern, at least in part, through the use of an alternative promoter [42]. Alternative promoters may similarly account for d/tPRP expression in decidual and trophoblast cells. Progesterone, relaxin, and activators of the cAMP/protein kinase A pathway (possibly including prostaglandins and/or relaxin) have been linked to the ac-

tivation of human decidual PRL gene expression [43–45] and are logical candidate regulators of d/tPRP expression in the deciduum. An array of transcription factors and DNA regulatory regions dictating decidual, spongiotrophoblast, or trophoblast giant cell-specific expression have been identified [42, 46–50] and may similarly participate in directing cell-specific dPRP expression. Isolation and characterization of the d/tPRP gene and flanking DNA should help resolve some of these issues. Decidual/tPRP can also be viewed as a powerful tool for the elucidation of regulatory mechanisms controlling uterine stromal cell differentiation. Identification of *cis*-elements and *trans*-acting factors responsible for decidual cell-specific expression of d/tPRP represents a strategy that may lead to understanding of the mechanisms controlling uterine stromal cell differentiation. Control of uterine stromal cell differentiation is likely to be highly conserved across species, similar to molecular mechanisms underlying the differentiation of other cell lineages [51].

The coordinated pattern of d/tPRP expression in the uterus and placenta may be associated with several gestational-dependent changes in d/tPRP that are essential for the progression of pregnancy. These may include a shift in 1) the control of d/tPRP expression; 2) the posttranslational processing of d/tPRP, potentially affecting its availability, distribution, and/or biological activity; and/or 3) the site of d/tPRP production, influencing its accessibility to maternal, extraembryonic, and embryonic targets. Our current interpretation of the biology of d/tPRP actions relates to its influence on maternal-embryo relationships through mechanisms that do not involve the PRL receptor signaling pathway [21, 22]. Additional information on the biological actions of d/tPRP will undoubtedly contribute to understanding of the significance of the coordinated activities of uterine and placental structures responsible for d/tPRP production throughout pregnancy.

In conclusion, the present studies show that the expression of dPRP during gestation is coordinated through the activities of maternal uterine tissue and trophoblast tissues. Initially d/tPRP is produced by antimesometrial decidual cells. Later in pregnancy, d/tPRP production switches to the chorioallantoic placenta. The intensity and duration of expression imply an important function for d/tPRP not only during the establishment of pregnancy but throughout gestation.

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