Spatiotemporal expression of three gap junction gene products involved in fetomaternal communication during rat pregnancy

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Summary

The expression of three different members of the gap junction multigene family, a1 (Cx43), ft (Cx32), and p2 (Cx26), was analysed in the rat implantation chamber (a structural unit containing fetal, extraembryonic and maternal components within the pregnant uterus) during mid- and late stages of gestation as well as in the delivering, post-partum and non-pregnant uterus. A differential, spatiotemporal and cell-type-specific regulation of gap junctional coexpression was observed for Pi and p2 in the placental basal zone and trophoblast giant cells coincided with the differentiation of these cells. In addition, expression of a1 in the placental basal zone and trophoblast giant cells in the visceral epithelium (visceral yolk sac=the primary route for embryonic nourishment prior to the formation of the chorioallantoic placenta) and p2 in the chorionic villi (placental barrier=the major fetomaternal exchange route) suggests that gap junctions have an important role in fetomaternal communication.

Introduction

Gap junctions (GJ) are specialized regions of the plasma membrane containing communication channels that allow ions and small molecules to pass between cells without entering the extracellular space. A single intercellular channel comprises multiple polypeptide subunits, probably six, that span the membrane of each cell (for review, see Loewenstein, 1981, 1987). Evidence for a multigene family of related GJ proteins (connexins) that form intercellular channels between mammalian cells has been deduced by molecular characterizations of GJ cDNA clones. Thus far, four mammalian clones have been described that code for proteins of different predicted sizes in mammals: (a) a1, a 43x10^3M protein identified in rat heart (Beyer et al. 1987); (b) a2, a 46x10^3M protein in rat lens (Beyer et al. 1988); (c) a3, a 32x10^3M protein identified in rat and human liver (Paul, 1986; Kumar and Gilula, 1986); and (d) ft, a 26x10^3M protein found in rat liver (Zhang and Nicholson, 1989). Based on the amino acid sequences deduced from the cDNA clones together with topological analysis, it has been possible to deduce a generalized topological structure for junctional membrane proteins that contain four transmembrane domains (Zimmer et al. 1987; Beycr et al. 1987; Milks et al. 1988; Goodenough et al. 1988; Hertzberg et al. 1988; Zhang and Nicholson 1989; Yancey et al. 1989). However, although molecular characterizations have resulted in the identification of a GJ multigene family with diverse proteins, it is not yet known if and how this sequence diversity is related to functional differences between the different GJ gene products.

In the pregnant rat, two functional placentae exist concurrently throughout the later half of gestation serving as organs for the fetomaternal exchange route: a discoidal chorioallantoic placenta, and a villiary highly vascularized yolk sac placenta (Everett, 1935; Bridgman, 1948; Lambson, 1966; Merker and Villegas, 1970). However, prior to the formation of the chorioallantoic placenta, the visceral yolk sac (VYS) is the primary route for embryonic nourishment during the critical stages of organogenesis. The placental barrier (the structural and functional unit of the labyrinth...
separating maternal blood sinuses from fetal blood capillaries) has been described previously as a three-layered trophoblast (Jollié, 1964; Enders, 1965; Davies and Glasser, 1968), with subsequent identification of GJs in layers II and III (Forsmann et al., 1975; Metz and Forsmann, 1976).

In a previous study (Risek et al. 1990), a dynamic and tissue-specific modulation of α-γ containing GJs was found in the ovaries and uterine myometrium during rat pregnancy, while α and β, GJs were identified in the uterine luminal epithelium. However, information was not available on the anatomical distribution and potential contributions of the different GJ proteins to the fetomaternal exchange routes during mammalian development. These questions are of enormous importance, since the fetus and extraembryonic membranes must be considered integral parts of the female reproductive tract during pregnancy. For these reasons, the term 'implantation chamber' will be used to designate the structural relationship between the developing fetal (fetus and the extraembryonic membranes) and maternal components within the pregnant uterus. The present study was undertaken to determine the basic framework for the expression and modulation of three different GJ gene products in various compartments of the implantation chamber during mid- and late stages of rat pregnancy.

Materials and methods

Animals and tissue collection
Timed pregnant Wistar rats (220-250g body weight), with a gestational period of 22 days, were obtained from Simonsen (Gilroy, CA). The presence of a uterine plug was defined as day 0 (D0) of pregnancy. The animals were maintained individually on a 12 h light/dark cycle and killed by decapitation at the following stages of pregnancy: d13, d15, d17, d19, d21, d22 (parturition day) and d23. Three pregnant rats were used at each gestational stage. Implantation chambers containing intact rat concepti were removed from fat and blood vessels, embedded in OCT compound (Tissue-tek, Miles Lab., Inc., Naperville, IL), and slowly frozen in an isopentane/dry-ice bath. Implantation chambers were frozen in liquid nitrogen for subsequent transverse and longitudinal sectioning. OCT embedded samples were stored at −70°C until use. For transcript and protein analysis, pregnant uteri were cut at the wall for subsequent transverse and longitudinal sectioning. OCT embedded samples were stored at −70°C until use. For transcript and protein analysis, pregnant uteri were cut at the wall for subsequent transverse and longitudinal sectioning. OCT embedded samples were stored at −70°C until use.

Immunoblot analysis and immunohistochemistry
The preparation and characterization of the affinity-purified peptide antibodies that were used in this study (ce8, 7/8 and /3) has been described previously (Risek et al. 1990). For immunoblot analysis, liquid nitrogen frozen tissues were alkali extracted (Hertzberg, 1984) and used for analysis following protein determination (Lowry et al. 1951). SDS-PAGE and immunobLOTS were performed essentially as described (Risek et al. 1990). In addition, immunobLOTS were treated with fl or α-γ peptide antibodies to the corresponding peptides (100 μg/ml) (immunoblotting procedures to determine the antibody specificities. Indirect immunohistochemistry was performed on longitudinal and transverse sections of fresh-frozen implantation chambers containing an intact rat conceptus from different stages of pregnancy. Samples were sectioned (3-5 μm in thickness) on a cryostat (Minotome, Int. Equipment Co., Boston, MA), collected on gelatinized slides, and processed for indirect immunofluorescence as described (Risek et al. 1990).

Immunolabeling was analysed using a Zeiss Axioskop microscope with epifluorescence. All photographs were taken with Kodak T-MAX 400 black-and-white film. The evaluation of spatial and temporal differences in density and intensity of immunolabeled antigens was based on interpretations of the investigators, since the immunohistochemistry was not subjected to a quantitative analysis. However, the relative differences were sufficiently striking to be deemed as high, medium, low or undetectable.

RNA preparation, normalization of poly (A) + RNA and northern blot analysis
Total RNA was isolated by pulverization and homogenization of liquid-nitrogen-frozen tissues in guanidine isothiocyanate (Fisher) with subsequent sedimentation through a CsCl gradient by ultracentrifugation (Chingwin et al. 1979). RNA was quantified by absorbance at 260 nm and normalized for poly(A)+ RNA content as described (Risek et al. 1990) using the procedure of Harley (1987). Northern blot analysis was performed on total RNA aliquots containing equal amounts of poly(A)+ RNA. Samples were separated by electrophoresis on 1% agarose gels containing 0.6 M formaldehyde, transferred to nylon membranes (MSI), and hybridized with three different GJ cDNA probes: (a) rat α, GJ cDNA: a clone isolated from a rat granulosa cell cDNA library (Risek et al. 1990) that codes for a 43x10^3 kDa protein (Kumar and Gilula, 1986); and (c) mouse & GJ cDNA: a clone isolated from a mouse liver cDNA library (Nishi et al., 1991) that codes for a 26x10^3 kDa protein. Hybridization conditions and quantitation of autoradiograms were essentially as described (Risek et al. 1990).

Results
Structural organization of the rat implantation chamber during late stages of pregnancy
The structural components of the rat implantation chamber at d21 gestational stage are illustrated in Fig. 1. The major structural features of the implantation chamber have been determined for the d13 stage of gestation (the beginning of this GJ analysis), and they did not change substantially until delivery, except at the d16 stage (see below). The developing fetus was separated antimesometrially from the uterine components (luminal epithelium (LE), endometrial stroma (ES) and myometrium (M)) and the extraembryonic membranes (EEM; amnion, visceral and parietal yolk...
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Decidua Basalis (DB) lateral
decidua Basalis (DB) central
Mesometrial Stroma (MS)
Basal Zone (BZ)
Labyrinth (L)
Parietal Yolk Sac (PYS)
Visceral Yolk Sac (VYS)
Luminal Epithelium (LE)
Uterine/Yolk Sac Cavity (UYSC)
Extraembryonic Cavity (EEC)
Fetal Skin (EP)
Endometrial Stroma (ES)
Myometrium (M)

Fig. 1. Transverse section through implantation chamber (d21 stage) indicating fetomaternal relationship in the pregnant rat. This section passes through the center of the fetus, placenta (labyrinth, L; basal zone, BZ; decidua basalis, DB), mesometrial stroma (MS) and the lining of the uterine wall (endometrial stroma, ES; myometrium, M). The fetus, which occupies the lower right half of the chamber, is enclosed in the amnion (not apparent) and in the visceral yolk sac (VYS). The umbilical cord and allantois are not present in this section. Frozen section stained with hematoxylin and eosin. Bar, 14 mm.

The parietal yolk sac (PYS) was in contact with the trophoblastic central zone and the decidua capsularis (DC). This region was characterized by the presence of trophoblastic giant cells (GC) and maternal blood sinuses. However, by d16, the parietal yolk sac (PYS) and the decidua capsularis degenerated and retracted to the peripheral margins of the chorioallantoic placenta exposing the uterine LE to the absorptive cells of the visceral epithelium (VE). Following this exposure, the structural features of the implantation chamber were reorganized as shown in Fig. 1 for the d21 stage. The definitive chorioallantoic placenta, which was vascularized by both fetal and maternal blood vessels at d12 (except the lateral margins of the BZ) was the major route for fetomaternal exchange (known as labyrinthine placental barrier). The placental basal zone (BZ), which was composed predominantly of cytotrophoblastic elements, formed the area between the labyrinth and the decidua basalis (DB), and it was characterized by the presence of maternal blood sinuses and trophoblastic GC adjacent to the DB. The decidua basalis, which was easily identified by densely packed cells, formed the outermost placental region adjacent to the uterine LE (laterally) and the mesometrial stroma (MS centrally). The term ‘mesometrial stroma’ has been used to refer to the uterine region of the mesometrial triangle, also known as the metrial gland. As pregnancy progressed, the villus structure of the epithelial layer of the VYS became more extensive in regions proximal to the placenta (mesometrial), as opposed to a more regular surface in antimesometrial regions. In addition, the placental labyrinth increased significantly in size, whereas the BZ and DB were reduced in size. Furthermore, the epidermis (Ep) of the developing fetal skin increased in thickness (the individual layers were distinguishable at d17), while the outermost cell layer (stratum corneum) was keratinized at d19.

Immunohistochemical analysis of α, β, and β GJ antigens in the implantation chamber during mid- and late stages of pregnancy

To determine spatiotemporal modulations in ex-
pression of three different GJ gene products, an immunohistochemical analysis was performed on various regions of the rat implantation chamber from d13 to one day post-partum (d23) at 48 h intervals. Results from these selected stages (d13, d17, d21, d22-parturition, and d23) are presented chronologically, to show the striking temporal transitions that occur. Finally, the results are compared with the pattern of expression in the non-pregnant uterus.

Stage d13
At this stage, the most prominent staining of GJ protein was observed using \( \beta_1 \) peptide antibodies. \( \beta_2 \) antigen was expressed at comparable levels in the uterine LE in the antimesometrial (Fig. 2A) and mesometrial portions of the implantation chamber (Fig. 2C). However, \( \beta_3 \) antigen was not detected in the glandular epithelium (GE; Fig. 2A). Although \( \beta_2 \) antigen was localized in the DS (Fig. 2B), it was not detected in the central zone containing TB GC and in the adjoining PYS. \( \beta_3 \) was present also in lateral regions of DB (Fig. 2C) adjacent to the uterine LE, with comparable amounts localized in the central portion adjacent to the MS (data not shown). The thickness of the DB was maximal at this stage, and it decreased gradually with progressing pregnancy. Regional differences for \( \alpha \) GJ expression were observed in the VE, with less antigen detected in antimesometrial regions where the surface was more regular (data not shown) compared to a slightly higher level in more villiated structures adjacent to the placenta (VE; Fig. 2D). The most prominent staining of \( \beta_3 \) was observed in the placental labyrinth (L) which is extensively vascularized with both fetal and maternal blood vessels. \( \beta_2 \) was the only GJ antigen detected in the trophoblastic epithelia of the chorionic villi (CV), the structures that form the placental barrier (Fig. 2D). Since the trophoblastic epithelium of the CV was not entirely developed at this stage of pregnancy, the stained layer was thinner than at later stages of pregnancy (see Fig. 3B, C for d17 stage) and the villi encompassed a larger area of the labyrinth. The labyrinthine area was relatively small compared to other regions of the placenta (BZ and DB), but it increased during pregnancy reaching a maximal size just prior to term. At d13, \( \alpha \) was detected in variable amounts in the epithelial layer of the developing fetal skin (Fig. 2E).

\( \alpha \) GJ antigen was localized in the DS (Fig. 2F) and in the mesothelia of the amnion and the VYS (Fig. 2G). This antigen was abundant in the lateral (data not shown) and central portion of DB (Fig. 2H). Note that at this stage, \( \alpha \) was not expressed in the adjacent layer of GC or in the placental BZ (Fig. 2H). \( \alpha \) antigen was expressed at high levels in the MS and in the longitudinal layer of the lining myometrium delimiting both sides of the mesometrial triangle (Fig. 2J). In contrast, \( \alpha \) was not detectable in antimesometrial regions of the myometrium throughout pregnancy except at parturition day (d22). \( \alpha \) antigen was localized also in the CT of the uterine myometrium and endometrium, with a higher abundance in the mesometrial regions (Fig. 2K). \( \beta_2 \) antigen was detected in the allantois (Fig. 2L). At this stage of pregnancy, punctate \( \beta_2 \) staining was detectable in the Ep of the developing fetal skin (Fig. 2M).

The most prominent spatial differences in GJ expression within the implantation chamber were observed for \( \alpha \) in the uterus. \( \alpha \) antigen was detected in the cytoplasm (annular form) of the uterine LE in some mesometrial regions (Fig. 2O), whereas in other regions the staining was also localized to the cell surface borders (macular form) of epithelial cells (Fig. 2Q). \( \alpha \) was not detected in the GE (Fig. 2Q). Furthermore, \( \alpha \) antigen was not observed in the antimesometrial regions of the uterine LE (Fig. 2R). The macular or plaque-like form of \( \alpha \) antigen was detected in the VE with less staining in the antimesometrial compared with mesometrial regions (Fig. 2O). Thus, in addition to coexpression of \( \alpha \) and \( \beta \) proteins in decidual and epidermal cells, \( \beta_2 \) was coexpressed with \( \alpha \) in epithelial cells of the uterine luminal and visceral endoderm.

Stage d17
At this stage of pregnancy, there was an increase of \( \beta_2 \) protein in antimesometrial regions of the uterus LE and VE, compared with d13, but \( \alpha \) was not detectable in the GE (Fig. 3A). The \( \beta \) expression increased in the developing fetal Ep where the antigen was localized between cells of the stratum basalis, stratum intermedium, and periderm (Fig. 3A). The stratum cornuem was not yet developed at this gestational stage. The abundance of \( \alpha \) antigen in mesometrial regions of the
VE (Fig. 3B, C) was higher than in antimesometrial portions. Structurally, this region was characterized by increased villus formation in the VYS, accompanying the proliferation of underlying embryonic vessels. Prominent temporal differences in expression were observed in the trilaminar trophoblastic epithelia of the CV (Fig. 3B); the stain density increased substantially in these layers from d13 to d17. The demarcation line between the labyrinth and the adjoining BZ was characterized by the absence of jα antigen in the latter,
Fig. 3. Immunohistochemical localization of ft; (left), -X (middle) and ft (right) GJ antigens in cross-sections of the d17 rat implantation chamber. (A) ft antigen was detected in the fetal epidermis (Ep), visceral (VE) and uterine luminal epithelium (LE) in the antimesometrial region of the implantation chamber, but not in glandular epithelium (GE). (B,C) ft was present also in the visceral epithelium (VE) adjacent to the placenta, in the chorionic villi (CV) of the placental labyrinth (L), and in the (D) lateral and (E) central portion of the decidua basalis (DB) adjacent to the mesometrial stroma (MS). (F-I) The αx antigen was detected in the fetal epidermis (Ep), in the mesothelia of the amnion (Am) and the visceral yolk sac (VYS), in the placental basal zone (BZ), as well as in the adjacent layer of giant cells (GC) and decidua basalis (DB). (J) Phase contrast micrograph of allantois (Al) illustrating the location of αx detected in (K) the allantoic mesodermal layer. (L, M) ft was expressed in the uterine luminal epithelium (LE), in the adjacent visceral epithelium (VE), and in the (L) antimesometrial and (M) mesometrial regions of the implantation chamber. (N) ft antigen was present in the visceral epithelium (VE) adjacent to the placental labyrinth. (O) Phase-contrast micrograph of the visceral epithelium (VE) and parietal yolk sac (PYS) illustrating localization of ft in the VE (P). Bar, 50μm; bar for O and P, 20μm.
although single puncta were detected in some regions of the BZ proximal to the labyrinth (Fig. 3C). It was expressed in comparable amounts in the lateral regions of DB adjacent to the uterine LE (Fig. 3D) and in the central portion adjacent to the MS (Fig. 3E). Also, differences in ft abundance were not observed in the antimesometrial and mesometral portions of the uterine LE (compare Fig. 3A, D).

α protein was coexpressed with ft in the stratum basalis, stratum intermedium, and in the periderm of the developing fetal Ep (Fig. 3F). The ocy abundance increased markedly in these layers compared with d13, as well as in the placental BZ and the adjacent layer of GC (Fig. 3H, I). ocx antigen increased also in abundance in the allantoic mesoderm (Fig. 3K).

Spatiotemporal differences were observed for ft expression in antimesometral and mesometral regions of the uterine LE compared to d13. At d17, a macular form of ft was detected in antimesometral and mesometral portions, with a higher level in the latter (Fig. 3L, M). In addition, temporal differences for ft expression were observed in the VE. The expression was much higher than at d13 with a comparable abundance in antimesometral and mesometral regions (Fig. 3L, M). However, ft connexin was not detected in the placental labyrinth (L; Fig. 3N) or in the PYS lining Reichert's membrane (Fig. 3P). Thus, relative to d13, the coexpression of ft and ft increased in visceral endoderm and uterine lumenal epithelium at d17, and the coexpression of α and ft increased in the developing fetal epidermis. In contrast, the coexpression of αx and ft proteins in decidual cells was comparable in abundance to d13.

Stage d21

The structural organization of the implantation chamber at d21 has been presented in Fig. 1. Following d17, the major structural features did not change markedly, except for the increased diameter of the implantation chamber due to the enlarging fetus and placenta, ft expression in the uterine LE did not change during late stages of gestation, and it was not detectable in the GE (Fig. 4A). However, the ft expression pattern differed markedly in the VE, compared to earlier stages. There was a substantial loss of ft antigen in the antimesometral hemisphere, and it was barely detectable in regions proximal to the placenta (Fig. 4A, B). Relative to d17, there were no changes in ft expression in the CV of the placental labyrinth at d21 (data not shown). However, although the expression level of ft in a single CV was comparable with preceding stages of pregnancy, the total ft content increased due to the increased overall growth of the labyrinth, ft content increased also in the differentiated epidermal layers of the fetal skin (stratum spinosum and stratum granulosum), but was no longer detectable in the periderm (Fig. 4B). Striking differences in ft expression were detected in lateral and central regions of the DB. The high expression level in lateral regions (Fig. 4C) was comparable to d17, whereas a dramatic loss was observed in the central region adjacent to the MS (Fig. 4E).

An increase in αx was detected one day before delivery in the fetal Ep. αx antigen was localized at high levels at the cell borders of spinous and granular layers, and to a lesser extent in the stratum basalis (Fig. 4F). αx was not detected in the periderm. αx antigen was expressed also in the mesothelia of the amnion and the VYS in comparable amounts to d17 (Fig. 4F). However, spatiotemporal differences were observed in the circular myometrial smooth muscle layer one day prior to parturition. αx was detectable in the regions proximal to the mesometrium (Fig. 4G), but was not detected in the antimesometral portion (data not shown). In contrast, ocx was not expressed in the adjacent longitudinal muscle layer at this stage (Fig. 4H) but was abundant on parturition day (compare Fig. 4G, H with Fig. 5E, F for rapid induction of myometrial GJs related to the parturition process). αx was expressed at high levels in the DB, comparable to d17. Spatial differences were not detected, since the abundance was similar in the lateral (data not shown) and in the central portion of DB (Fig. 4I), in contrast to the ft expression pattern where there was a dramatic decrease in the central region (see Fig. 4E). αx was expressed at high levels in the MS (Fig. 4I) and in the lining myometrium (data not shown), comparable to late stages of pregnancy. The level of αx in the placental BZ did not change since d17, except in the layer of GC where an increased staining was observed (Fig. 4J). An increased <cx staining was also observed in the mesodermal layer of the allantois (Fig. 4K).

As reported for d17, ft was expressed in a spatially specific manner in the uterine LE one day prior to parturition, ft was expressed at low levels in the antimesometral regions (Fig. 4M), and at higher levels in the mesometral portion (Fig. 4O). However, in contrast to ft, different results were obtained for ft expression in the uterine LE of different animals at d21. In addition to uterine regions, where ft was detected at the borders of epithelial cells, ft was also found in the cytoplasm indicative of internalized (annular) GJs (Fig. 4Q). Regional differences were detected in the VE; ft was not detectable in the antimesometral region (Fig. 4M), but it was abundant in regions proximal to the placenta (Fig. 4O). However, as observed for ft expression at this stage, the ft expression pattern varied in the VE in different animals. The abundance fluctuated between undetectable and barely detectable levels in the antimesometral regions in some animals, but remained high in the regions proximal to the placenta. Thus, at d21 (the day before parturition) a striking cell-type-specific modulation of spatiotemporal coexpression was observed for: (a) αx and ft in the maternal decidua (high coexpression of αx and ft in lateral regions versus specific loss of ft GJs in the central portion; compare Fig. 4E and 4I); (b) ft and ft GJs in the VE proximal to the placenta (specific loss of ft, while the ft level remained constant; compare Fig. 4B and 4O); and (c) ft and ft...
GJs in the LE (annular form of \( Z \), while \( \beta_2 \) remained unaffected; compare Fig. 4A and 4Q).

Stage d.22 (parturition day)
Following parturition, regional differences were detected for \( \& \) expression in the uterine LE. This antigen was expressed at lower levels in the antimesometrial region (Fig. 5B) than in the mesometrial region (Fig. 5D). The staining density in the mesometrial region of the LE was comparable to late stages of
Fig. 4. Immunohistochemical localization of ft (left), ai (middle) and ft (right) GJ antigens in cross-sections of the d21 rat implantation chamber. (A, B) At this stage of pregnancy, ft was not detected in the visceral epithelium (VE) distal and proximal to the placenta, but it was expressed in the uterine luminal epithelium (LE) and fetal epidermis (Ep). Note the absence of ft antigen in glandular epithelium (GE). (C, E) Expression of ft was abundant in lateral regions of the decidua basalis (DB) compared to a minimal level of antigen in the central portion of the decidua basalis adjacent to the mesometrial stroma (MS). (D) Phase-contrast image of (E) which is similar also to the region shown in (I). (F) <X> antigen was detected in the fetal epidermis (Ep), and in mesothelia of the amnion (Am) and the visceral yolk sac (VYS). (G, H) Punctate staining of a-i antigen was detectable in the mesometrial region of the circular myometrium (Me), but not in the adjacent longitudinal muscle layer (MI). (I-K) Expression of ai antigen in mesometrial stroma (MS), in the adjacent central portion of the decidua basalis (DBc), in the layer of giant cells (GC), in the basal zone (BZ), and in the allantoic mesoderm (AI). (L-M) Phase-contrast micrograph and immunolocalization of ft antigen in the antimesometrial region of the uterine luminal epithelium (LE), but not in the visceral epithelium (VE). (N) Phase-contrast image and (O) localization of ft in the mesometrial region of the uterine luminal (LE) and visceral epithelium (VE). (P,Q) Phase-contrast image of uterine luminal (LE) and visceral epithelium (VE), illustrating the localization of annular ft antigen in the LE, and the macular form of ft in the VE. Bar in A (for A, L, M, P, Q), 20 μm; bar in C (for C-K, N, O), 50 μm.

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The level of ft expression was reduced in the LE one day after parturition to the lowest level observed during this study (Fig. 6B). Differences were not observed in antimesometrial and mesometrial regions. In contrast, the abundance of ft increased in the GE compared to d22 (Fig. 6B). A complete loss of ai antigen was observed in the circular and longitudinal layers of the myometrium one day following parturition (Fig. 6C, D). In these layers, ai was detected only in the CT region of the smooth muscle. In addition, ai was expressed in the MS (remnants of the metrial gland; Fig. 6E) at a reduced level compared with the prepartum stages. Finally, ft was not detectable either in the LE or GE of the post-partum uterus (Fig. 6G).

Post-partum stage

The level of ft expression was reduced in the LE one day after parturition to the lowest level observed during this study (Fig. 6B). Differences were not observed in antimesometrial and mesometrial regions. In contrast, the abundance of ft increased in the GE compared to d22 (Fig. 6B). A complete loss of ai antigen was observed in the circular and longitudinal layers of the myometrium one day following parturition (Fig. 6C, D). In these layers, ai was detected only in the CT region of the smooth muscle. In addition, ai was expressed in the MS (remnants of the metrial gland; Fig. 6E) at a reduced level compared with the prepartum stages. Finally, ft was not detectable either in the LE or GE of the post-partum uterus (Fig. 6G).

Non-pregnant uterus (proestrus)

For comparative purposes, the differential expression pattern of <X> ft and ft GJ proteins was analyzed during the proestrus cycle. The results for the complete four stages of the rat estrus cycle will be presented elsewhere. At proestrus, ft was expressed at high levels in the LE, comparable to levels observed during the late stages of pregnancy (Fig. 7B). However, in contrast to late stages of pregnancy, ft was expressed also in the GE (Fig. 7B). <X> was localized in the CT region of the endometrium, as well as in the ES region proximal to the LE (Fig. 7D), and it was not detected in the myometrium except in CT areas (data not shown). Ft was coexpressed with ft in the LE and GE (Fig. 7F), with ft at lower levels than ft. Collectively, the non-pregnant uterus at proestrus was characterized by: (a) a coexpression of ft and ft in the glandular and luminal epithelia; and (b) expression of <X> in the endometrial stroma lining the uterine epithelium.

Analysis of ai and ft GJ proteins in different components of the d19 implantation chamber

Different components of the implantation chamber at d19 were analyzed by immunoblotting to confirm the results of the immunohistochemical analysis for ft and <X> localization. In addition, since equal amounts of NaOH-insoluble material were used, a semiquantitative comparison for different samples was obtained. The placental sample (Fig. 8, ft, lane A) contained the highest level of ft protein. In addition to the ft monomer (26x10^3 M), aggregates of dimeric (38x10^3 M), trimeric (47x10^3 M) and tetrameric (60x10^3 M) forms were detected. Consistent with the immunolocalization data, monomeric, dimeric and trimeric forms of the ft GJ protein were detected also in the EEM sample consisting of VYS, amnion and allantois (Fig. 8, ft, lane B). In contrast to the placental sample, the 47x10^3 M band was the most prominent component indicating a high stability of the trimer following solubilization in the presence of SDS and ft mercaptoethanol. Monomeric and dimeric forms of the ft protein were detected in the sample of MS (Fig. 8,
Fig. 5. Immunohistochemical localization of ft (left), α (middle) and β, (right) GJ antigens in cross-sections of the parturition uterus (d22, 3 h post-partum). (A) Phase-contrast micrograph illustrating glandular (GE) and uterine luminal epithelium (LE) in the antimesometrial portion of the uterus (B) with ft antigen localized in both types of epithelia. (C,D) Phase-contrast micrograph and localization of ft in the mesometrial region of the uterine luminal epithelium (LE). (E,F) Localization of α-, antigen in circular (E) and longitudinal (F) layers of the myometrium (M). (G) Localization of αβ antigen in the mesometrial stroma (MS). (H,I) Phase-contrast micrograph and localization of αβ in connective tissue (CT) adjacent to the uterine luminal epithelium (LE). (J) Detection of annular (arrowhead) and macular (arrow) forms of ft in the central region of the luminal epithelium (LE). (K) Detection of macular ft in the mesometrial region of the luminal epithelium (LE). Bar in A (for A-G), in H (for H-I), and in J (for J,K), 50 μm.

In parallel with the ft GJ protein analysis, the ocyx protein (43 x 10^3 M_r) was detected in all samples examined (placenta, extraembryonic membranes, mesometrial stroma and fetal skin (Fig. 8, a')). In addition to the ocyx polypeptide (43 x 10^3 M_r), a band of 40 x 10^3 M_r was detected in the placental material. This...
band most likely represents either proteolytic degradation of the $43 \times 10^6$ protein or a non-phosphorylated form of this product (Musil et al. 1990). Detection of $\alpha_r$ protein in the different samples by immunoblot analysis was consistent with the immunohistochemical localization results for $\alpha^+$ antigen in those same samples.

No $\beta$ protein was detected in any of these four samples.

Fig. 6. Immunohistochemical localization of $\alpha$ (left), $\beta$ (middle) and $\gamma$ (right) GJ antigens in cross-sections of the one day post-partum uterus. (A,B) Phase-contrast micrograph and immunolocalization of $\alpha$ antigen in the antimesometrial region of luminal (LE) and glandular epithelium (GE). (C,D) Localization of $\alpha_r$ antigen in the circular (Mc) and longitudinal (MI) myometrium, and in the connective tissue (CT) of both muscle layers. (E) Expression of $\alpha_{-1}$ antigen in the mesometrial stroma (MS). (F) Phase-contrast micrograph of the antimesometrial portion of luminal (LE) and glandular epithelium (GE). (G) $\beta$ antigen was not detected in these two uterine regions. Bar, 50$\mu$m.

Fig. 7. Immunohistochemical localization of $\alpha$ (left), $\beta$ (middle) and $\gamma$ (right) GJ antigens in cross-sections of the non-pregnant uterus (proestrus stage). (A,B) Phase-contrast image and localization of $\alpha$ antigen in the luminal (LE) and glandular epithelium (GE). (C,D) Phase-contrast image and immunolocalization of $\alpha_r$ antigen in the endometrial stroma (ES) and connective tissue (CT). (E,F) Phase contrast image and immunolocalization of $\gamma$ antigen in the luminal (LE) and glandular epithelium (GE). Bar, 50$\mu$m.
The specificity of peptide antibodies was demonstrated by a polypeptide of 26x10^3M_r (small arrowhead) present in all samples examined. In addition, a product of 43x10^3M_r (large arrowhead) was detected in the placenta (A). The content of α- and ft GJ mRNA was determined by α- and ft mRNA, respectively. The highest level of α-J transcript in the fetal Ep, where the abundance was 3 times the value of the placental samples.}

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**Fig. 8.** Immunoblot analysis of NaOH-insoluble homogenates (75µg each) from d19 placenta (A), d19 extraembryonic membranes (B), d19 mesometrial stroma (C), and d21 fetal skin with α J and α S peptide antibodies. [%] peptide antibodies bound specifically to a polypeptide of 26x10^3M_r (small arrowhead) present in all samples examined. In addition, a product of 43x10^3M_r (large arrowhead) was detected in the placenta (A). The specificity of peptide antibodies was demonstrated by a 'competition' assay in the presence (+) of either the /γ or the /α- S peptide respectively.

**Fig. 9.** Northern blot analysis of α-J and ft GJ transcripts in different compartments of the rat implantation chamber at different stages of pregnancy. A transcript of 3.3kb (α-J mRNA, top) was detected in RNA from the placenta (A), extraembryonic membranes (B), mesometrial stroma (C), and fetal skin (D). Following hybridization with the /α-J probe, a 2.8 kb transcript (ft mRNA, bottom) was detected in the same samples, α-J or ft transcript abundance was compared between different tissues at various gestational stages following densitometric scanning of the signal intensities.

The specificity of peptide antibodies was demonstrated by a polypeptide of 26x10^3M_r (small arrowhead) present in all samples examined. In addition, a product of 43x10^3M_r (large arrowhead) was detected in the placenta (A). The content of α- and ft GJ mRNA was determined by α- and ft mRNA, respectively. The abundance of α-J transcript was not detected in any of the samples examined (data not shown).
Table 1. Summary of immunohistochemical analysis of GJ proteins in the rat implantation chamber

<table>
<thead>
<tr>
<th>GJ gene product</th>
<th>Tissue</th>
<th>Gestational stage</th>
<th>Parturition uterus (d22)</th>
<th>Post-partum uterus (d23)</th>
<th>Non pregnant uterus (pro-est.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aGJ M'</td>
<td>M'</td>
<td>+AM</td>
<td>(+ +)</td>
<td>++</td>
<td>ES+</td>
</tr>
<tr>
<td>aGJ MS</td>
<td>MS</td>
<td>+AM</td>
<td>(+++)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>aGJ CT</td>
<td>CT</td>
<td>+AM</td>
<td>(+++)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>aGJ DB</td>
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<td>+AM</td>
<td>(++ +)</td>
<td>(+ +)</td>
<td>-</td>
</tr>
<tr>
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<td>BZ</td>
<td>+AM</td>
<td>(+ +)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
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<td>(+ +)</td>
<td>+</td>
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</tr>
<tr>
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<td>(+ +)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
</tr>
<tr>
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<td>+AM</td>
<td>(+ +)</td>
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<td>VE</td>
<td>+AM</td>
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<td>DB</td>
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<tr>
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<td>Ep</td>
<td>+AM</td>
<td>(+ +)</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

1 See list of abbreviations.
2 Qualitative evaluation of immunofluorescence data based on interpretation of investigators. In order to compare the relative abundance of antigen at different stages, the density and intensity of the fluorescent signal was defined as high (+++), medium (++), low (+), or not detectable (-).
3 Longitudinal myometrium associated with the mesometral triangle.
4 Antimesometrial (AM) and mesometrial (M) regions of the implantation chamber.
5 Lateral (L) and central (C) regions of decidua basalis.
6 Annular (an) or internalized form of GJ.

Summary of results and expression map of GJ gene products in the rat implantation chamber during late stages of pregnancy

The results of the present study are summarized in Table 1 and illustrated in Fig. 10 (see below).

aGJ M' Myometrial (M) aGJ proteins were present throughout pregnancy only in the longitudinal myometrium next to the mesometrial triangle. At the same time, aGJ M' were not detected in other areas of the uterine myometrium until d21, when a punctate staining was observed in the circular layer. At the parturition day, however, a peak of aGJ expression was observed in the circular and longitudinal myometrium throughout the uterine wall, reaching an undetectable level in the one day post-partum uterus. These observations are consistent with the modulation of the myometrial aGJ transcripts reported previously (Risek et al. 1990). Myometrial aGJ were not observed in the non-pregnant uterus. aGJ protein was abundantly expressed in decidual cells without any detectable differences in the lateral and central regions of DB. Note that aGJ antigen was not detectable in the placental BZ and TB GC at d13. The expression started at d15 and increased at d17. The abundance remained constant in BZ at d21, but increased slightly between GC. The detection of placental aGJ antigen was confirmed by immunoblot and northern blot results. aGJ were also expressed in the mesothelia of the VYS and amnion as well as in the mesodermal layer of the allantois. With progressing pregnancy, a gradual increase of aGJ expression was observed in allantois only. These observations were in agreement with protein and transcript analysis. A gradual increase of aGJ expression was also observed during development of the fetal epidermis (Ep), where aGJ was coexpressed with β1 protein (see below).

β1, GJs /β1 GJ antigen detected in epithelial cells (LE, VY, GE) was always coexpressed with β1 protein. In effect, no cells were identified that expressed /β1 connexin only. β1 was not detectable in the antimesometrial regions of LE at d13, but was present in some mesometrial portions
between the epithelial cell borders and in others within the cytoplasm, indicative of internalized GJs. The abundance of macular ft forms increased gradually in both antimesometrial and mesometrial regions. However, at parturition day the most prominent staining was localized within the cells of LE. ft antigen was not detectable in the one day post-partum uterus, but was abundantly expressed in the LE of the non-pregnant uterus at proestrus. B, expression increased gradually in VE with advancing pregnancy, with higher abundance in the regions proximal to the placenta (mesometrial). Prominent spatial differences were observed at d21, when ft was only expressed in the mesometrial regions of the VE. Note the absence of ft in the GE of the pre- and post-partum uterus, and its expression at the proestrus of the non-pregnant animals.

ft GJs

The expression pattern of ft was expressed in the LE with undetectable regional differences (antimesometrial, mesometrial), and increased in both regions with advancing pregnancy. The abundance remained high at parturition day, and decreased in the post-partum uterus. ft was not expressed in the GE of the pre-partum uterus; the expression began at parturition day, and increased following parturition reaching a level comparable with the non-pregnant uterus. ft expression gradually increased in the VE with higher abundance in the mesometrial regions during late stages of gestation. However, at d21 a dramatic loss was observed, reaching undetectable levels in some animals. The localization of ft antigen in the VE was supported by the detection of the corresponding transcript and protein in the EEM. In analogy to the VE, similar results were obtained for ft expression in the DB. ft was expressed in high abundance in decidual cells throughout gestation, except at d21. At this time, a dramatic loss of ft was observed in the central region of DB, while the abundance remained high in the lateral portions. ft antigen was very abundant in the trilaminar trophoblastic epithelia of the CV; in all other cell types ft was coexpressed with ft. ft expression was observed at sites of fetomaternal exchange: (a) coexpression of ft and ft in the VE of the uterine lumen and VYS, with higher ft abundance in both epithelial tissues. In contrast, neither ft or ft was expressed in the GE during mid- and late stages of gestation. ft and ft proteins (green) were abundantly coexpressed in cells of the decidua basalis (DB) and the fetal epidermis (Ep). ft protein (blue) was constitutively expressed in the metrial gland (mesometrial stroma; MS) and the lining mesometrial myometrium, and to a lesser extent in the connective tissue and extraembryonic membranes (EEM=mesothelia of the amnion and VYS, and mesodermal layer of the allantois). In addition, ft was expressed in the placental basal zone (BZ; trophospongium) and in the layer of trophoblastic giant cells, ft protein (yellow) was abundant also in the trilaminar trophoblastic epithelium of the chorionic villi, constituting the placental barrier.

Discussion

In the present study, the spatiotemporal expression of three different GJ gene products has been analyzed in various compartments of the rat implantation chamber using specific peptide antibodies and cDNA probes during mid- and late stages of pregnancy. Several relevant observations have resulted from this analysis. First, spatiotemporal, differential and cell-type-specific GJ coexpression has been observed for ft and ft gene products in the epithelia of the uterine lumen and VYS, as well as for ft and ft in the cells of the maternal decidua and fetal epidermis. Moreover, differential regulation of GJ coexpression has been observed for ft and ft proteins in the central portion of d21 DB and for ft and ft in VE. Second, evidence was provided for GJ expression as a consequence of cell differentiation: (a) differentiated decidual cells coexpressed ft and ft, whereas expression was not observed in the undifferentiated endometrial stroma; (b) the absence of ft antigen in the TB GC and in the BZ at d13, and expression of ft at d15 coincided with the differentiation of GC and parenchymal TB. Third, GJ expression was observed at sites of fetomaternal exchange: (a) coexpression of ft and ft in the VE of the yolk sac placenta; and (b) high expression of ft in the trilaminar TB (placental barrier) of the chorioallantoic placenta. Fourth, the maximal number of GJ gene products coexpressed at any given time was 2. Of all three GJ gene products examined, a cell-type-specific coexpression was observed only for ft/ft and for ft/ft combinations. The coexpression of ft was not observed. Fifth, a correlation was observed between a cell-type-specific GJ expression and the germ layer origin: ft was expressed in mesodermal derivatives,
Fig. 10. Schematic representation of the fetal maternal relationship during late stages of rite pregnancy illustrating the spatial expression of GJ antigens. $\text{j}_k$ and $\text{R}_{0}$ antigens (orange) were present in the epithelium of the uterine lumen (LE) and the visceral endoderm (VE). $\text{a}_1$ and $\text{j}_1$ (green) were expressed in the deciduas basalis (DB) and the fetal epidermis (Ep). $\text{a}_x$ (blue) was present in the mesometrial siroma (MS), mesometrial myometrium (M), placental basal zone (BZ), illantoi (AI), and in the mesorhelLi of the Minion (A) and the visceral yolk sac (VYS). $\text{j}_y$ (yellow) was expressed in the chorionic vilHi of the placental labyrinth (L). Note the absence of GJ antigens in the glandular epithelium (GE) during late gestation, and the absence of n/\text{R}_{0} coexpression in the ral implantation chamber.
whereas the endodermal derivatives coexpressed $f_5$ and $f_2$ GJs. The ectodermal derivatives (surface ectoderm) were characterized by the coexpression of $a$ and $f_2$. Finally, evidence was obtained that provides the basis for an additional potential fetomaternal exchange route consisting of the fetal skin (periderm/epidermis), extraembryonic membranes and the uterus.

**Spatiotemporal modulation of GJ expression in the rat implantation chamber**

$P_i$ and $f_2$ GJs were detected at the sites of the fetomaternal exchange routes, in the placental barrier and in the epithelium of the visceral yolk sac. Based on the immunohistochemical analysis, $f_5$-containing GJs were the major contributor to intercellular channels in the placental barrier. At d13, $f_5$ was expressed to a lesser extent in the trophoblastic component of the placental barrier than at later stages of gestation; it has been reported that the placental barrier is not yet completed at this stage of development (Jollie, 1964). A maximal expression of $f_2$ was observed at d17 when all residual parenchymal TB disappears; i.e., at the time that the labyrinth development is completed. Following d17, the $f_2$ abundance did not change in the structures of the placental barrier until parturition. Thus, in contrast to d13 and d17 stages, where the metabolic activity of the placental barrier was indicated by both the modulated abundance of $f_2$ expression within the trilaminar TB of a single CV (d13) as well as by an increased number of CVs (d17), during late stages of pregnancy the intercellular coupling was regulated primarily by an increased number of CVs due to the expanding labyrinth. The detection of $f_2$ antigen in the CV of the three-layered TB is also consistent with the ultrastructural identification of GJs between syncytial layers (Forssmann et al. 1975; Metz and Forssmann, 1976). Since $f_2$ expression coincided with the development of the placental labyrinth, analysis of GJs may provide an additional approach to understand the functional role of the major fetomaternal exchange route.

Although the VYS has been studied ultrastructurally (Lambson, 1966; Padykula et al. 1966; Merker and Villegas, 1970), GJs have not been reported. The presence of $f_2$ and $f_5$ connexins in the VE is consistent with the potential role of the VYS as a functional fetomaternal exchange route for passage of ions and small molecules. Consequently, $f_5$ and $f_2$ GJs could provide pathways for nonselectively absorbed material from the uterine lumen by the VE, which is subsequently transported to the fetal blood circulation. Furthermore, the loss of GJs in the peripheral fetomaternal exchange route just prior to parturition might reflect the onset of fetal independence. GJ immunoreactive elements were not detected in the three-layered PYS (TB/Reichert's membrane/endo-derm complex) which was interposed in the yolk sac placenta exchange route from maternal to fetal blood until d16. GJs were absent between the cells of the PE since there was a physical separation between these cells, exposing the Reichert's membrane directly to the yolk sac cavity. As a consequence of this physical separation of the parietal cells from each other, transport to the absorptive VE is most likely by diffusion from Reichert's membrane directly across the yolk sac cavity as suggested by Jollie (1968).

The expression of $f_5$ at the borders of TB GC and in the BZ started at d15 and coincided with the differentiation of TB GC and trophospongium, which occurred at the same gestational stage as reported by Jollie (1965). In addition, $a$ expression by GC might be related to diverse secretory and endocrine functions of these cells approaching parturition (Deane et al. 1962; Sherman, 1983).

The transformation of endometrial fibroblast-like stromal cells into decidual cells is characterized by large numbers of GJs between the decidual cell population in the pregnant mouse uterus (Finn and Lawn, 1967), in the decidua of the pseudopregnant rat uterus (Kleinfeld et al. 1976) and in the pregnant rat uterus (Welsh and Enders, 1985; Parr et al. 1986). The high level of $a$ and $f_2$ antigens observed between decidual cells is consistent with these observations. Accordingly, the decidual GJs may contribute to synchronizing decidual cells for secretory, differentiation and degradation processes, as suggested by Ono et al. (1989). Furthermore, junctional communication between decidual cells may form functional syncytia, also known as 'communication compartments' (Lo and Gilula, 1979; Pitts and Kam, 1985). Consequently, the undifferentiated endometrial fibroblast-like stromal cells, which are not joined to the decidual cells via GJs, would remain outside the communication compartments. The loss of $f_2$ GJs in the central region of DB one day before delivery could result in the reduction of intercellular coupling activity; this might be important for facilitating the process of placental detachment from the uterus (placental attachment site) since the detachment is initiated in the central region adjacent to the MS. The unaltered $f_2$ abundance at this stage suggests that there is a differential regulation of $a$ and $f_2$ expression in decidual cells. Consequently, cultured decidual cells may be useful for studying hormonal regulation of $a$, and $f_2$ GJ coexpression.

In contrast to decidual cells (differentiated endometrial stromal cells), which coexpressed $a$ and $f_2$, cells of the MS expressed $a$ only. This difference may be notable since cells of the mesometrial stroma (metrial gland) were fibroblast-like cells, which did not differentiate into typical decidual cells but continued to proliferate (Peel, 1989). The spatiotemporally regulated $a$ antigen expression between smooth muscle cells of the uterine myometrium was consistent with the modulation of myometrial $a$. GJ transcripts as reported previously (Risek et al. 1990). Further, the detection of $a$ in the mesometrial myometrium lining the mesometrial stroma (metrial gland) may reflect regional differences in the local cell environment in two different uterine compartments, i.e., undifferentiated endometrial stroma versus proliferating mesometrial stroma.

Although dynamic spatiotemporal changes in $f_2$ and $f_5$ coexpression were observed between proliferating...
cells of the luminal and glandular epithelium, there are currently no explanations for these results. However, one possibility is that the expression patterns may be related to the secretory activities of these two uterine glands.

GJs have been described in fetal and adult mammalian skin by ultrastructural (Breathnach et al. 1972; Caputo and Peluchetti, 1976) and dye-coupling analysis (Salomon et al. 1988; Kam and Pitts, 1989). In the present study, two different members of the GJ multigene family (\( c \) and \( f \)) were identified in the fetal epidermis, where the \( a^x/f \) coexpression coincided with the keratinocyte differentiation and proliferation process.

Possible physiological and developmental implications

During the fetal development of mammals, the free surface of the epidermis is covered by periderm that is continuous with the lining of the amniotic cavity. Direct physiologic evidence demonstrating that the periderm (before epidermal keratinization) is definitely involved in maternal transport was provided by Parmley and Seeds (1970). It is reasonable to extend these observations to the less analysed rat periderm since: (a) these cells display structural properties of an absorbing epithelium (Bonneville, 1968); and (b) injection of a radioactive tracer (\(^{3}H\)thymidine) into the amniotic fluid was used to demonstrate the uptake of the label by fetal rat epidermis (Stern et al. 1971). Consequently, the identification of \( c \) and \( f \) proteins in the outermost epidermal layer may provide an additional parameter that can be used to understand the function of the periderm. The high levels of \( a^x \) and \( f \) GJs throughout the fetal body surface (including periderm) may facilitate indirect interaction of developing skin with the prenatal environment (for example, amniotic fluid) for essential functions such as absorption and secretion of metabolic products. The presence of \( a^x \) in the mesothelia of the amnion and visceral yolk sac lining the amniotic or visceral yolk sac cavity respectively, could provide an additional, bidirectional peripheral pathway for the transport of small molecules and ions between the maternal and fetal components. On the maternal side, \( f \) and \( f \) GJs in the luminal and visceral epithelium could provide a cell-cell mechanism for passage through the uterine/yolk sac cavity. Thus, molecules secreted by the uterine luminal epithelium have to cross three different cavities (diffusion) and two interposed extraembryonic membranes (absorption/secretion) to be absorbed by the periderm of the fetal epidermis (see Fig. 10). This potential peripheral communication route is consistent with the concept of an 'Organ Communication System' as discussed by Casey and MacDonald (1986).

The results of the present study indicate that the expression pattern of each GJ gene product was correlated with embryonic tissue origin. So far, mesodermal derivatives (connective tissue, myometrium, mesothelia, dermis) expressed \( a^x \), while the endoderm derived tissues (epithelia) expressed \( f \) and \( f \). The epidermis, which is a surface ectodermal derivative, expressed \( a^x \) and \( a^x/f \).

Although the present study has provided several examples for spatiotemporal coexpression of GJ gene products in different cell types during rat pregnancy, the biological implications are not understood yet. However, the spatial and temporal modulation of coexpression presumably indicates that there are mechanisms for regulating intercellular coupling activity between synchronized cells within communication compartments. Further, the differential regulation of coexpression and specific degradation of only one GJ gene product observed in several cell types indicates the existence of homo-oligomeric GJ channels, an observation that is not consistent with the concept of heterooligomeric GJ channels (oligomers containing different gene products) as discussed by Traub et al. (1989). It is interesting to note that high levels of \( a^x/f \) were found only in the placental barrier. Consequently, it is possible that \( P2 \) generates channels that are utilized for intercellular coupling activity in situations where little to no regulation is required. Finally, although results indicate a temporal switch in GJ coexpression (low \( a^x \) level in epidermis relative to \( f \) at d13; undetectable or internalized \( f \) in luminal epithelium during mid stages), as well as the termination of expression of certain GJ gene products in different cell types (\( f \) in visceral epithelium and in the central region of the decidua basalis at d21), the control mechanism(s) responsible for these cell-type-specific, spatiotemporally regulated expression patterns remain to be clarified.

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