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Differential Expression of Transcripts Encoding Retinoid Binding Proteins and Retinoic Acid Receptors During Placentation of the Mouse

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ABSTRACT **We report the distribution of transcripts from genes encoding the retinol binding protein (RBP), the cellular retinol binding proteins (CRBP I, II) and retinoic acid binding proteins (CRABP I, II), the retinaldehyde dehydrogenase type 2 (RALDH-2), the retinoic acid receptors (RARs), and the retinoid X receptors (RXRs) in mouse placental tissues from 6.5 to 19.5 days postcoitum (dpc). During early placentation, RBP and RALDH-2 gene expression are restricted to the endoderm of the visceral yolk sac and the outer uterine epithelium, respectively, whereas CRBP I transcripts are detected in the visceral yolk sac and in the presumptive chorioallantoic placenta. By 15.5 dpc, CRBP I expression is downregulated in the yolk sac where CRBP II becomes strongly expressed. Expression of CRBP II is also detected in the trophoblastic giant cells. Throughout placentation, the expression patterns of the CRABP I and II genes partly overlap in the decidual tissue and the vacuolar zones of the decidua, suggesting a role for these binding proteins in sequestering free retinoic acid from maternal blood, thus regulating its availability to the embryo. RARα is ubiquitously expressed in all placental tissues, except in trophoblastic giant cells, at all stages studied. During early placentation, RARβ and RARγ are co-expressed in the decidua but differentially expressed in the chorionic region (RARβ, 10.5 to 12.5 dpc) and the presumptive labyrinth (RARγ, 7.5 to 12.5 dpc). During the same stages, RXRα is strongly expressed in the presumptive placenta. RARγ remains weakly expressed in the labyrinth until 15.5 dpc, whereas RXRα exhibits a strong expression in this zone until birth, suggesting a role for these receptors in the development and function of the definitive placenta.** ***Dev. Dyn. 208:199–210, 1997.*** **© 1997 Wiley-Liss, Inc.**

Key words: cellular retinoic acid binding proteins; cellular retinol binding proteins; retinoic acid receptors; retinoid X receptors; retinol binding protein; mouse; placenta; development; in situ hybridisation

INTRODUCTION

The developmental abnormalities resulting from vitamin A (retinol) deficiency (Wilson et al., 1953) and excess (e.g. Cohlan, 1953; Shenefelt, 1972; Morriss, 1973; Geelen, 1979; Kistler, 1981) are well known. These effects involve conversion of retinol (Rol) to its active metabolite retinoic acid (RA), which can alter gene regulation after binding to nuclear retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (reviewed in Kastner et al., 1995; Chambon, 1996). The mechanisms underlying delivery of Rol from the mother to the embryo are less well understood. Provitamin A is the dietary source of maternal Rol; carotenoids in vegetables and preformed retinyl esters (long chain fatty acid esters of retinol) in animal diet. Rol is mainly stored in the Kupffer cells of the liver as retinyl esters in lipid droplets (reviewed in De Luca and Creek, 1986). In maternal serum, Rol is bound to a specific retinol binding protein (RBP) (Kanai et al., 1968), which is itself complexed with transthyretin. At the cellular level, Rol uptake may involve a cell-surface receptor for RBP (Bavik et al., 1991, 1992; Ward et al., 1996). It has been suggested that cellular binding proteins with high affinity for Rol (cellular retinol binding proteins [CRBP I and II]; Ong and Chytil, 1978a; Sundelin et al., 1985; Li et al., 1986) and RA (cellular retinoic acid binding proteins [CRABP I and II]; Ong and Chytil, 1978b; Sundelin et al., 1985; Bailey and Siu, 1988) are involved in the intracellular control of retinoid levels (Napoli, 1993; Napoli et al., 1995). The observation that all these retinoid binding proteins are expressed in a tissue- and stage-specific manner during mouse development (Perez-Castro et al., 1989; Dollé et al., 1990; Ruberte et al., 1991, 1992, 1993) has supported this assumption, which was challenged by the apparent dispensability of CRABP I and CRABP II in mice harbouring targeted disruptions of the corresponding genes (Lampron et al., 1995).

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In any event, for maternally derived Rol to reach the developing embryo, it first must pass through the maternal-fetal barrier, the functional placenta. At early stages of development (until approximately 15 days postcoitum [dpc] in the mouse), the early placenta and hence the site of nutrient uptake is the yolk sac placenta (Freeman et al., 1981; Jollie, 1986). The visceral endoderm of the yolk sac of early postimplantation rodent embryos is also the major site of RBP synthesis (Soprano, 1986; Makover et al., 1989) and Rol uptake (Ward et al., 1996). At later stages of development, the function of the yolk sac is superseded by the haemochorial placenta. Because Rol is known to be crucial for normal embryonic and fetal development, it is likely that regulated mechanism(s) exist to enable its placental uptake and transport to the conceptus throughout development. As a first approach toward the elucidation of these mechanisms, we have analysed the mRNA expression patterns for the proteins believed to control retinoid levels and for nuclear retinoic acid receptors within the tissues forming the functional placenta throughout gestation.

RESULTS

The expression patterns of transcripts from genes encoding RBP; CRBP I and II; CRABP I and II; RARα, β, and γ; and RXRα, β, and γ were analysed by in situ hybridisation on cryosections of mouse conceptuses from 6.5 to 19.5 dpc, sectioned in utero or explanted from the uterus (15.5 to 19.5 dpc), and sectioned together with the placenta and extraembryonic membranes. We have also studied the expression of the retinaldehyde dehydrogenase type 2 (RALDH-2) gene because it encodes an aldehyde dehydrogenase that appears to be required for the oxidation of retinaldehyde into RA (Zhao et al., 1996). For the sake of clarity, the expression patterns, which are summarised in Table 1 and Figure 6, are described successively during early (yolk sac) and mid–late (haemochorial) stages of placentation. Only extra-embryonic expression domains are described here, because the expression patterns in the embryo proper have been previously reported (Perez-Castro et al., 1989; Dollé et al., 1989, 1990, 1994; Ruberte et al., 1990, 1991, 1992, 1993; Zhao et al., 1996).

Early Placentation (6.5 to 12.5 dpc)

Before the appearance of a functional chorioallantoic placenta, the visceral yolk sac is the primary site of placentation. Throughout early placentation the RBP gene was selectively and strongly expressed in the endoderm of the visceral wall of the yolk sac (Fig. 1A,H,O). In contrast, the extraembryonic mesoderm contributing to the allantois and the extraembryonic ectoderm forming the chorion were not labelled (Fig. 1A,H).

At 6.5 to 7.5 dpc, the CRBP I gene was widely expressed in the entire decidua and ectoplacental cone (data not shown). At 8.5 dpc, specific expression was detected in the outer layer of the decidua (decidua basalis) and in the myometrium (Fig. 1B). In addition, there was specific labelling in the endoderm of the visceral yolk sac and the inner surface of the ectoplacental cone (presumptive chorion), where apposition and fusion of the allantoic bud with the chorion will form the chorioallantoic placenta (Fig. 1B). The decidual labelling progressively disappeared by 9.5 to 10.5 dpc, so that only the chorioallantoic placenta and yolk sac membranes remained labelled (Fig. 1I). Labelling intensity decreased in the yolk sac by 12.5 dpc, except in the regions close to the definitive placenta (Fig. 1P). The CRBP I gene remained strongly expressed in the chorioallantoic placenta including the decidual, vacuolar, labyrinthine, and spongiotrophoblastic regions and the allantois (Fig. 1P and data not shown). CRBP II transcripts were first detected at 8.5 dpc in the antimesometrial portion of the inner decidua (Fig. 1C). Whereas no specific expression of CRBP II was seen at 9.5 dpc, labelling was detected in the secondary giant cells of the chorion at 10.5 dpc (Figs. 1J, 5A,B). On some sections, labelling was also detectable in the visceral yolk sac (Fig. 5A,B). Both the giant cells and the yolk sac were clearly labelled at 12.5 dpc (Fig. 1Q).

The CRABP I and II genes were expressed in distinct regions of the decidua. CRABP I transcripts were first detected at 6.5 dpc, and CRABP II at 7.5 dpc (data not shown). CRABP I was expressed in some inner decidual cells, especially toward the mesometrial pole at 8.5 to 9.5 dpc (Fig. 1D and data not shown). Between 10.5 and 12.5 dpc, CRABP I transcripts were highly restricted to the vacuolar zones of the decidua (Figs. 1R, 5C,D). At 8.5 dpc, the CRABP II gene was expressed at the level of the antimesometrial pole of the decidua and in the ectoplacental cone (Fig. 1E). CRABP II transcripts were distributed throughout the decidua at 9.5 dpc (data not shown) and remained expressed only at the mesometrial pole of the inner decidua by 10.5 dpc (Fig. 1L). At 12.5 dpc, labelling was restricted to the decidual region adjacent to the placenta, including the vacuolar zones (Fig. 1S).

Throughout these developmental stages, the RALDH-2 gene expression was restricted to the myometrium—thus surrounding the entire implantation site—and in the outer decidual cells at the mesometrial pole (Fig. 1F,M,T).

As seen in the embryo proper (Ruberte et al., 1991), RARα gene transcripts appeared to be expressed in a diffuse ("ubiquitous") manner within cells of the uterus, yolk sac, and developing chorioallantoic placenta from 6.5 to 12.5 dpc (data not shown). However, there was no apparent labelling in the primary and secondary giant cells (data not shown). The RARβ gene was specifically expressed in the outer mesometrial region of the decidua from 7.5 dpc (Fig. 2B). In addition, RARβ transcripts were transiently expressed by 10.5 and 11.5 dpc

TABLE 1. Retinoid Binding Protein and Retinoic Acid Receptor Transcript Distributions during Mouse Placentation

	RBP	CRBP I	CRBP II	CRABP I	CRABP II	RALDH-2	RARα	RARβ	RARγ	RXRα	RXRβ	RXRγ
Early placentation (6.5–12.5 dpc)												
Uterus	−	+	−	−	−	+	+	−	+	−	−	−
Decidua	−	+ MZ 6.5–9.5 dpc	+ AMZ 8.5–9.5 dpc	+ MZ	+ AMZ/MZ 7.5–12.5 dpc	−	+	+ MZ 7.5–10.5 dpc	+ 7.5–11.5 dpc	+ AMZ 7.5–9.5 dpc	−	−
Ectoplacental cone/chorion	−	+	−	−	+	−	+	+ CZ 10.5–12.5 dpc	+	+	−	−
Visceral yolk sac	+	+	+ 11.5–12.5 dpc	−	−	−	+	−	−	−	−	−
Giant cells	−	−	+ 10.5–12.5 dpc	−	−	−	−	−	−	+	−	−
Definitive placentation (13.5–19.5 dpc)												
Uterus	+	−	−	+ vacuolar zone	+	+	+	−	+	−	−	−
Decidua	−	−	−	+	+	−	+	−	+	−	−	−
Choriallantoic placenta	−	+	−	−	−	−	+	−	+ 13.5–15.5 dpc	+	−	−
Yolk sac membranes	+	+ 13.5–15.5 dpc	+	−	−	−	+	−	−	−	−	−

AMZ, antimesometrial zone; MZ, mesometrial zone; CZ, chorionic zone.

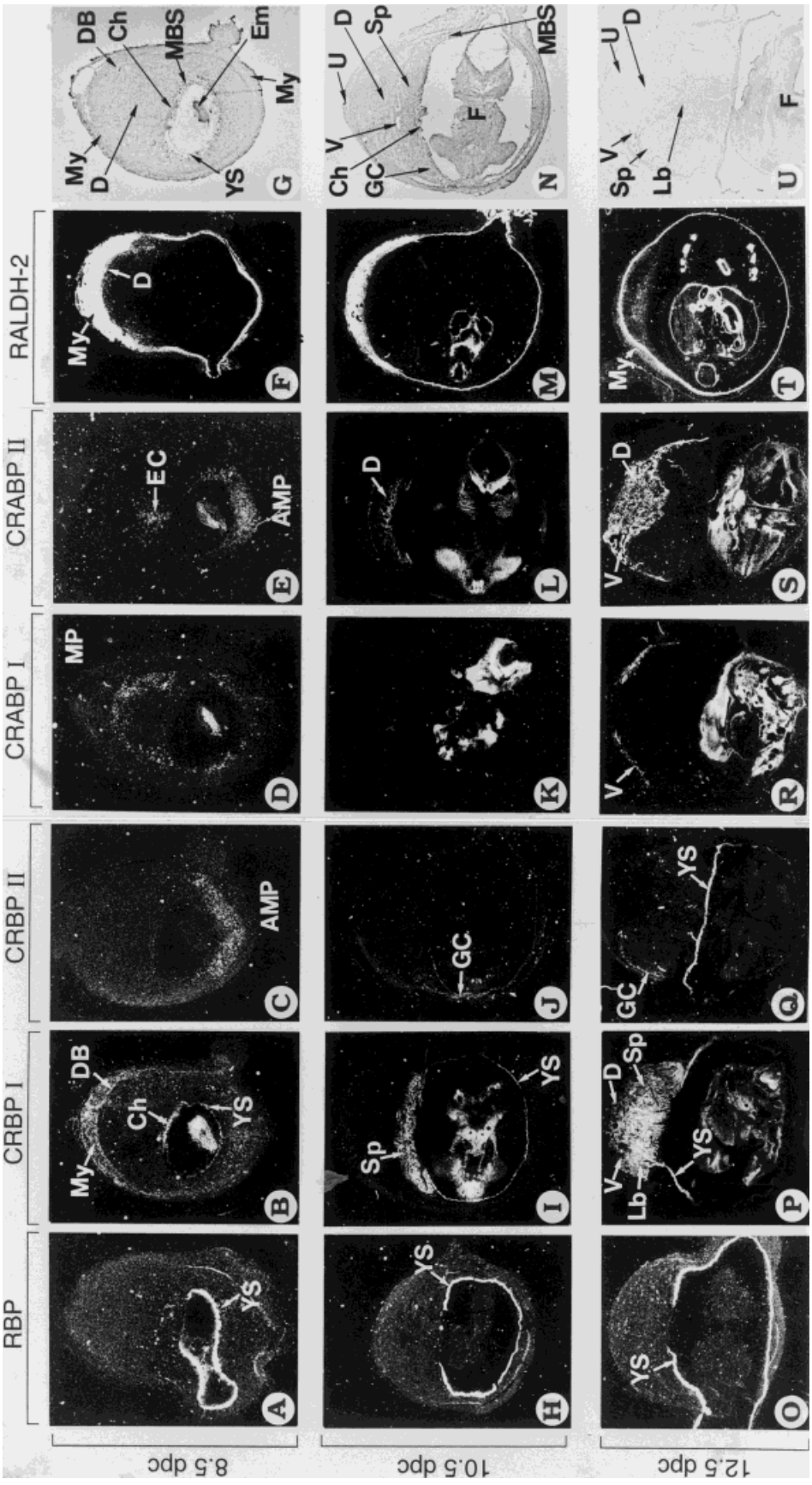

Fig. 1. Expression of retinoid binding protein and RALDH-2 genes during early placentation. Histologic sections of mouse conceptuses were hybridised to ^{35}S-labelled antisense riboprobes for RBP, CRBP I, CRBP II, CRABP I, CRABP II, and RALDH-2 transcripts. The developmental stages shown are 8.5 dpc (**A–G**), 10.5 dpc (**H–N**), and 12.5 dpc (**O–U**). The hybridised sections were viewed under darkfield illumination to reveal the signal grain in white. G,N,U: Brightfield views of selected sections to provide histologic landmarks. Note that in this and the following figures, only part of the hybridised sections are comparable to the brightfield panels, because the probes were too numerous to be hybridised serially to single embryos. AMP, antimesometrial pole; Ch, chorion; D, decidua; DB, decidua basalis; EC, ectoplacental cone; Em, embryo; F, foetus; GC, giant cells; Lb, labyrinth; MBS, maternal blood sinus; MP, mesometrial pole; My, myometrium; Sp, spongiotrophoblast; U, uterus; V, vacuole; YS, yolk sac.

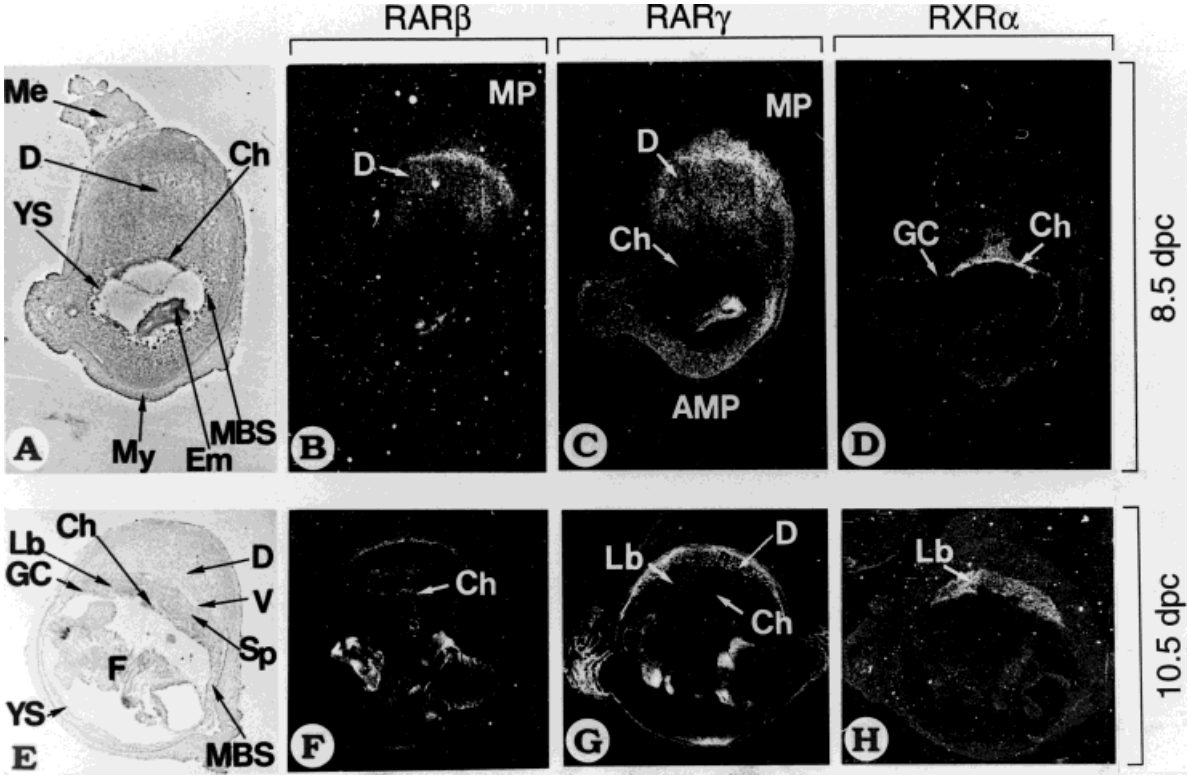

Fig. 2. Expression of RAR and RXR genes during early placentation. Histologic sections of mouse conceptuses hybridised to RARβ, RARγ, and RXRα riboprobes are shown, together with selected brightfield views at 8.5 dpc (**A–D**) and 10.5 dpc (**E–H**). AMP, antimesometrial pole; Ch, chorion; D, decidua; Em, embryo; F, foetus; GC, giant cells; Lb, labyrinth; MBS, maternal blood sinus; Me, mesometrium; MP, mesometrial pole; My, myometrium; Sp, spongiotrophoblast; V, vacuole; YS, yolk sac.

in the chorionic region, where fusion with the allantois has taken place (Fig. 2F). No specific signal for RARβ was detected in the 12.5 dpc placenta (data not shown). RARγ transcripts were first detected at 7.5 to 8.5 dpc in the entire uterus, decidua, and chorion (Fig. 2C). From 9.5 to 11.5 dpc, labelling persisted in the uterus and chorion, whereas the yolk sac and giant cells were unlabelled (Fig. 2G and data not shown). Weak labelling also appeared in the labyrinthine portion of the placenta by 10.5 dpc (Fig. 2G).

At 6.5 to 7.5 dpc, RXRα transcripts were preferentially expressed in the antimesometrial region of the decidua, whereas the yolk sac was not significantly labelled (data not shown). This preferential expression disappeared by 8.5 to 9.5 dpc, whereas strong expression became apparent in the chorion and giant cells (Fig. 2D). From 10.5 to 12.5 dpc, only the labyrinthine portion of the chorioallantoic placenta showed specific labelling (Fig. 2H and data not shown). Throughout these stages, RXRβ and RXRγ showed diffuse, nonspecific labelling patterns in placental tissues (data not shown).

Mid–Late Placentation (13.5-19.5 dpc)

Most of the expression domains that were established by 12.5 dpc could still be detected in corresponding regions at later stages. For instance, RBP gene expression persisted in the endothelium of the visceral yolk sac from 13.5 to 19.5 dpc. (Fig. 3A,H). In addition, RBP gene expression was specific to the decidua basalis at 13.5 dpc and later stages (Fig. 3A,H). CRBP I gene expression was detected in the visceral yolk sac until 15.5 dpc (Fig. 3B,I); labelling became restricted to the region of the yolk sac adjacent to the chorioallantoic placenta by 17.5 dpc and was later undetectable in this region (data not shown). The CRBP I gene was also strongly and evenly expressed throughout the chorioallantoic placenta until 15.5 dpc (Fig. 3B,I). At 17.5 and 19.5 dpc, however, transcript levels became much higher in the spongiotrophoblast zone than in the labyrinth (Fig. 5E and data not shown). CRBP II expression was detected in the visceral yolk sac, appearing at 12.5 dpc and gradually increasing to a steady level by 15.5 dpc, whereas there was no noticeable labelling pattern in the chorioallantoic placenta from 13.5 to 19.5 dpc (Fig. 3C,J and data not shown). CRABP I transcripts remained restricted to the vacuolar zones of the decidua at 13.5 to 15.5 dpc and were further restricted to the outermost cells of these zones at 17.5 to 19.5 dpc (Fig. 3D,K and data not shown). CRABP II transcripts were present within the decidual tissue and on the mesometrial side of the decidua basalis throughout these stages

(Fig. 3E,L). The RALDH-2 gene was specifically expressed in the inner layer of the myometrium of the uterus until 19.5 dpc (Fig. 3F,M).

RARα showed strong but diffuse expression in the definitive placenta, but there was no detectable signal for RARβ from 13.5 to 19.5 dpc (data not shown). RARγ transcripts could be detected in the labyrinthine portion of the placenta until 15.5 dpc (Fig. 4A,D); strong RARγ expression persisted in the decidua (including the vacuolar zone) until 19.5 dpc (Fig. 4A,D,G). From 13.5 to 19.5 dpc, RXRα was specifically expressed in the labyrinthine region of the placenta (Fig. 5G), its transcript levels appearing consistently higher than those of RARγ (compare Fig. 4A,D,G with C,F,I). There was only diffuse, nonspecific RXRβ and RXRγ labelling throughout the later stages of placentation (data not shown).

DISCUSSION

In this study, we describe the expression patterns of the transcripts corresponding to the major known proteins involved in the retinoid signalling pathway in the placental tissues of the mouse from the early postimplantation to preterm stages of pregnancy. The functional yolk sac placenta is the primary site of placentation in rodents. The parietal yolk sac forms at the time of implantation, when there is considerable nonvascularised decidual tissue separating the embryo from maternal blood vessels (Welsh and Enders, 1983, 1987; Parr and Parr, 1986, 1989). It is a discontinuous epithelium with a thick basement membrane (Reichert's membrane), continuous with the embryonic hypoblast (primitive endoderm), and comes to line the trophoblastic cell layer. With formation of the egg cylinder and the chorion, a bilaminar membrane composed of an outer layer of extra-embryonic endoderm and an inner layer of mesoderm is formed (the visceral yolk sac, see Fig. 6A). The mesodermal layer undergoes angiogenesis as the yolk sac swells and comes to surround the embryo during early stages of morphogenesis (Duval, 1891; Clark et al., 1975; Minor et al., 1976; Morriss-Kay, 1993).

The expression patterns of the various retinoid binding proteins during the phase in which the yolk sac is the functional placenta are summarised in Figure 6A and Table 1. In keeping with previous reports (Soprano et al., 1986; Makover et al., 1989), RBP transcripts were found to be strongly expressed within the endoderm of the yolk sac visceral wall. This expression persists throughout all stages of early and definitive placentation. Furthermore, there is co-expression of RBP and CRBP I within the visceral yolk sac endoderm during the stages of early placentation, although CRBP I expression also extends into the ectoplacental cone. CRBP I expression is limited to the inner surface of the ectoplacental cone, the point at which the allantoic bud will fuse to initiate formation of the chorioallantoic placenta, and is excluded from the avascular portion of the parietal yolk sac that finally lines this area. The primary function of the yolk sac membranes is to take up nutrients and transfer these to the embryo. The mesoderm of the visceral yolk sac is the first haematopoietic organ of the embryo; it is vascularised by the coalescence of blood islands to form vitelline vessels that form connections with the embryonic circulation on 8 dpc in the mouse (Batten and Harr, 1979). Synthesis and secretion of many serum proteins has been shown in the yolk sac (Nahon et al., 1987), as has that of other blood-borne products such as steroid hormones (King, 1971). Interestingly, synthesis of a protein similar to the vitamin D-dependent intestinal calcium binding protein has been demonstrated in the yolk sac membranes (Bruns et al., 1986).

The presence of RBP in the visceral yolk sac endoderm suggests that this layer is a major site for Rol uptake. Maternally derived Rol passes from maternal blood circulating in the trophoblastic blood sinuses, through the Reichert's membrane, and into the yolk sac cavity; there it comes into direct contact with the endodermal surface of the visceral yolk sac, where uptake occurs (Ward et al., 1996). The formation of vitelline blood vessels within the mesoderm of the visceral yolk sac are impaired in conditions of vitamin A deficiency or when RBP synthesis in the yolk sac is experimentally blocked (Bavik et al., 1996). Taken together, these observations suggest that a possible role for RBP within the visceral yolk sac endoderm is to facilitate transport of Rol to the embryo. The role of CRBP I, however, is less clear. CRBP I binds Rol with high affinity (Ong and Chytil, 1978b) and has been shown to facilitate Rol metabolism in vitro (Napoli, 1993). At present, there is no evidence for such a role in vivo, although embryonic tissues expressing high levels of CRBP I are also those that are most vulnerable to vitamin A deficiency (Dollé et al., 1990). Co-expression with RBP in the yolk sac membrane suggests a role for CRBP I in mediating uptake or transfer of Rol to the embryo, as previously proposed by Ruberte et al. (1991).

During early placentation, the RARα gene is almost ubiquitously expressed, but RARβ and RARγ have more restricted expression patterns. RARβ transcripts are detected in the area of the chorion where the allantois will eventually fuse, suggesting a possible role for the receptor in this process. Failure of the allantois to fuse with the chorion is a common defect associated with early embryonic exposure to RA excess (Tesh, 1988 and references therein). RARγ is expressed in the entire uterus and thus may play a role in its conditioning for maintenance of pregnancy. RXRβ and RXRγ show no preferential expression patterns, whereas RXRα is specifically expressed in the chorion and trophoblast giant cells, which may account for labelling of the labyrinth at later stages.

The function of the yolk sac placenta is eventually superseded by the definitive (haemochorial) placenta, which forms from the chorioallantoic junction. At approximately 9 dpc in the mouse, a network of intertrophoblastic maternal blood sinuses develops in the ecto-

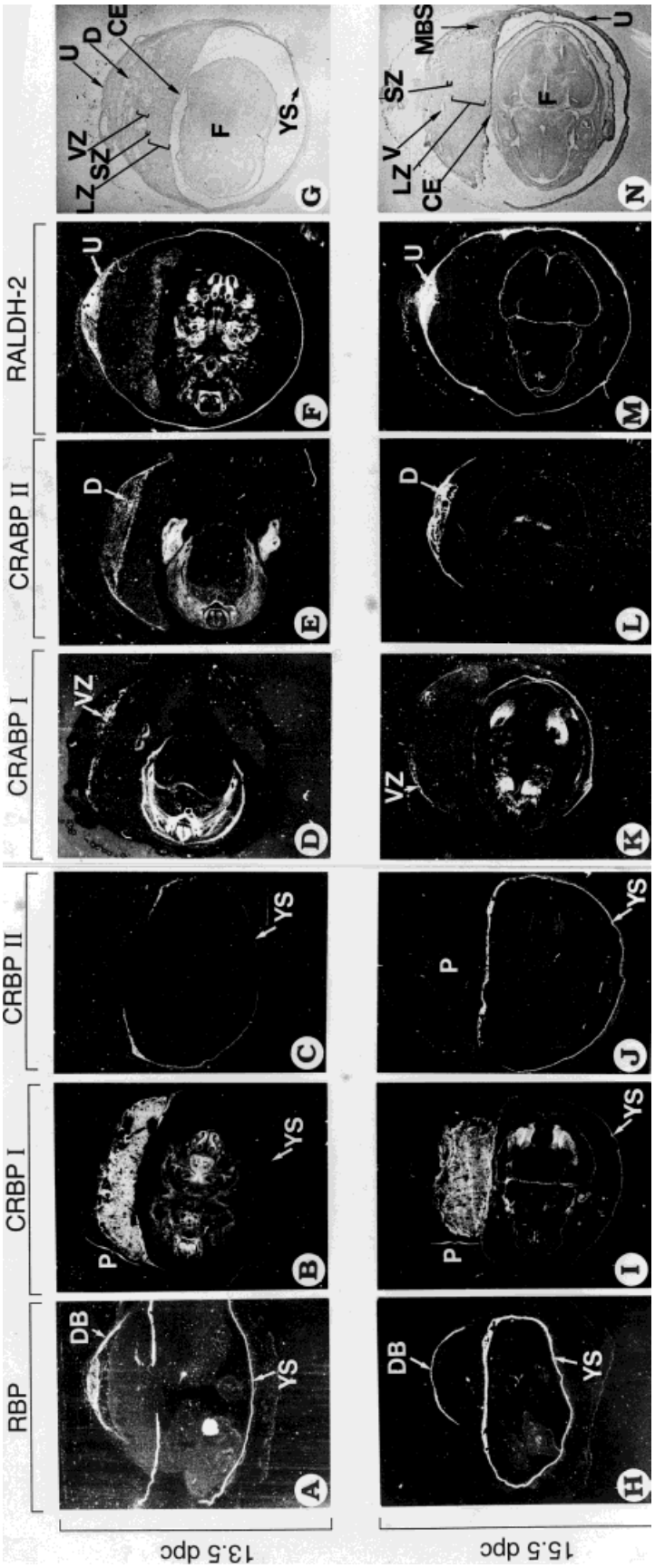

Fig. 3. Expression of retinoid binding protein and RALDH-2 genes during definitive placentation. Histologic sections of mouse conceptuses hybridised to RBP, CRBP I, CRBP II, CRABP I, CRABP II, and RALDH-2 riboprobes are shown, together with selected brightfield views at 13.5 dpc (**A–G**) and 15.5 dpc (**H–N**). CE, chorionic ectoderm; D, decidua; DB, decidua basalis; F, foetus; LZ, labyrinthic zone; MBS, maternal blood sinus; P, placenta; SZ, spongiotrophoblastic zone; U, uterus; V, vacuole; VZ, vacuolar zone; YS, yolk sac.

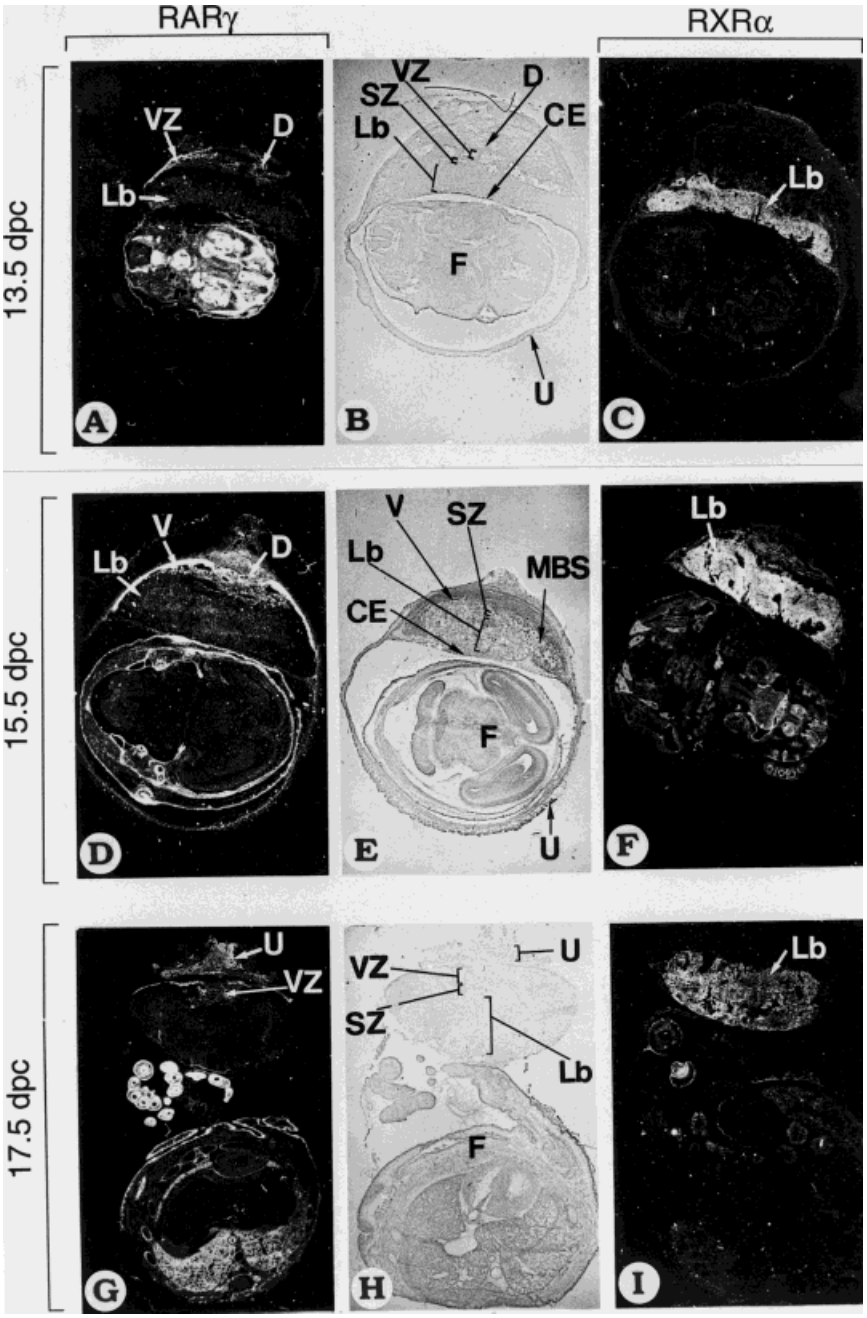

Fig. 4. Expression of RAR and RXR genes during definitive placentation. Histologic sections of mouse conceptuses hybridised to RARγ and RXRα riboprobes are shown, together with selected brightfield views at 13.5 dpc (**A–C**), 15.5 dpc (**D–F**), and 17.5 dpc (**G–I**). CE, chorionic ectoderm; D, decidua; F, foetus; Lb, labyrinth; MBS, maternal blood sinus; SZ, spongiotrophoblastic zone; U, uterus; V, vacuole; VZ, vacuolar zone.

placental cone. Once the allantois has fused with the chorion, adding its own capillary network, these structures form a functional placenta. Following these changes, at approximately 11 dpc, the parietal yolk sac begins to degenerate. The allantois becomes vascularised and forms the umbilical cord, connecting to the vascularised fetal surface of the chorioallantoic placenta. Figure 6B and Table 1 summarise the expression patterns of the various retinoid binding proteins during definitive placentation. The expression of RBP at these later stages remains localised to the visceral endoderm of the yolk sac membranes. However, RBP expression is also detected (as early as 13.5 dpc) at the junction of the uterine wall and placenta (decidua basalis). CRBP I is strongly expressed in the chorioallantoic placenta and becomes progressively restricted to the basal region.

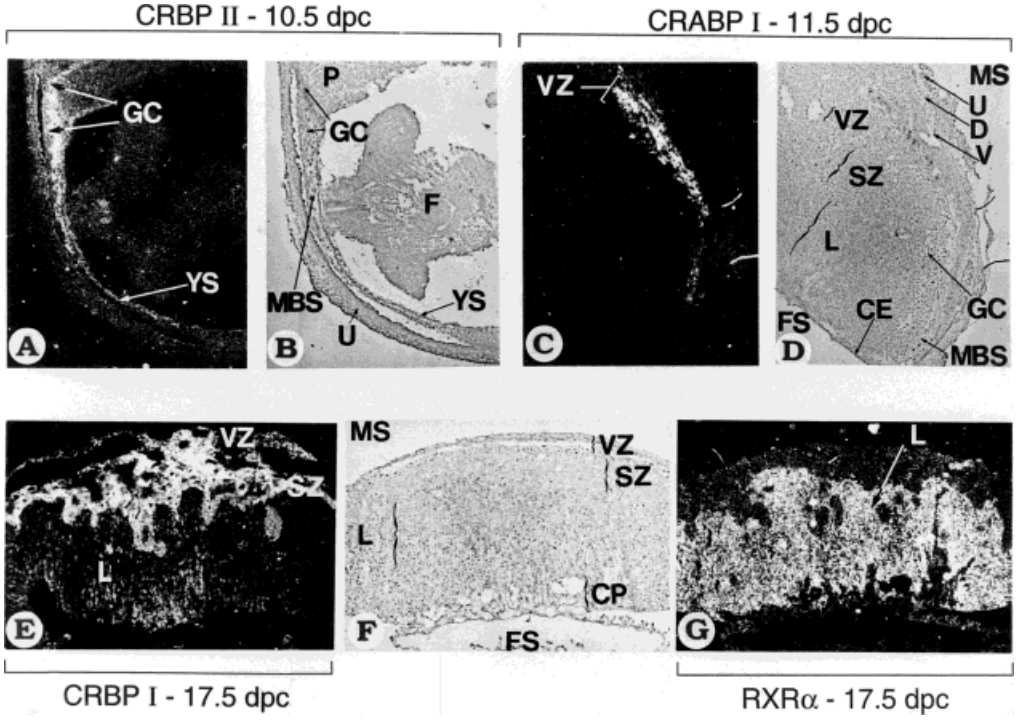

Fig. 5. Details of CRBP I, CRBP II, CRABP I, and RXRα expression patterns. **A,B:** Section through a 10.5 dpc conceptus showing CRBP II expression in the visceral yolk sac endoderm and trophoblast giant cells. **C,D:** Section through an 11.5 dpc conceptus showing restricted expression of CRABP I transcripts in the vacuolar zone of the decidua. **E–G:** Sections through a 17.5 dpc placenta showing differential expression of the CRBP I and RXRα genes in the maternal side and labyrinthic zone of the placenta, respectively. CE, chorionic ectoderm; CP, chorionic plate, D, decidua; F, foetus; FS, fetal side; GC, giant cells; L, labyrinth; MBS, maternal blood sinus; MS, maternal side; P, placenta; SZ, spongiotrophoblastic zone; U, uterus; V, vacuole; VZ, vacuolar zone; YS, yolk sac.

These data indicate that a second site for Rol uptake may correspond to the area that eventually forms the definitive placenta. However, active uptake by the yolk sac membranes may continue after the formation of the definitive placenta, as indicated by the presence of expression of RBP and CRBPs in these tissues. Strikingly, CRBP I expression decreases in the yolk sac membranes after 15.5 dpc while CRBP II transcript levels increase. CRBP II, which is the major binding protein present in the fetal and adult gut, has been implicated in the process of uptake of dietary Rol (Goda and Takase, 1989). The significance of this reversal of CRBP types in the fetal yolk sac membrane is unclear.

Only two of the nuclear receptor genes exhibit specific expression patterns in the chorioallantoic placenta. RARγ is weakly expressed in the labyrinth until about 15.5 dpc. In contrast, RXRα expression is strong in this layer and persists until birth. Whereas RARγ gene knockout does not cause fetal lethality (Lohnes et al., 1993), RXRα homozygous mutants die at various stages of fetal development (12.5 to 16.5 dpc). These animals suffer a severe deficiency in the development of the myocardium, which has been postulated to be the cause of fetal death (Kastner et al., 1994; Dyson et al., 1995). However, investigation of the chorioallantoic placenta in these mutants revealed histologic abnormalities, suggesting that functional defects of the placenta could contribute to the lethality of homozygous RXRα mutants (Sapin V., et al., unpublished data).

Expression of both CRABP I and II is limited to the decidual tissues at all stages. During early placentation, CRABP I expression is generally more prevalent toward the mesometrial pole, and CRABP II expression is greater toward the antimesometrial pole. At later stages, CRABP II expression is uniform throughout the whole decidua, whereas CRABP I expression is restricted to the vacuolar zones. The biochemical function(s) of CRABPs has not been elucidated. CRABP I has been implicated in the quantitative control of RA access to the nucleus: catabolism of RA to inactive compounds is more rapid when bound to CRABP I and, accordingly, overexpression of CRABP I decreases the level of RA-induced transcription of reporter genes (Means and Gudas, 1995 and references therein). It is feasible that the role of CRABPs in the decidua would be to sequester any free RA diffusing from the maternal blood coursing through the decidual sinuses. The importance of these proteins is challenged by the finding they are not essential for normal embryonic development or postnatal life, at least under laboratory conditions. Mutant mice harbouring homozygous disruptions of both CRABP I and CRABP II genes display no detect-

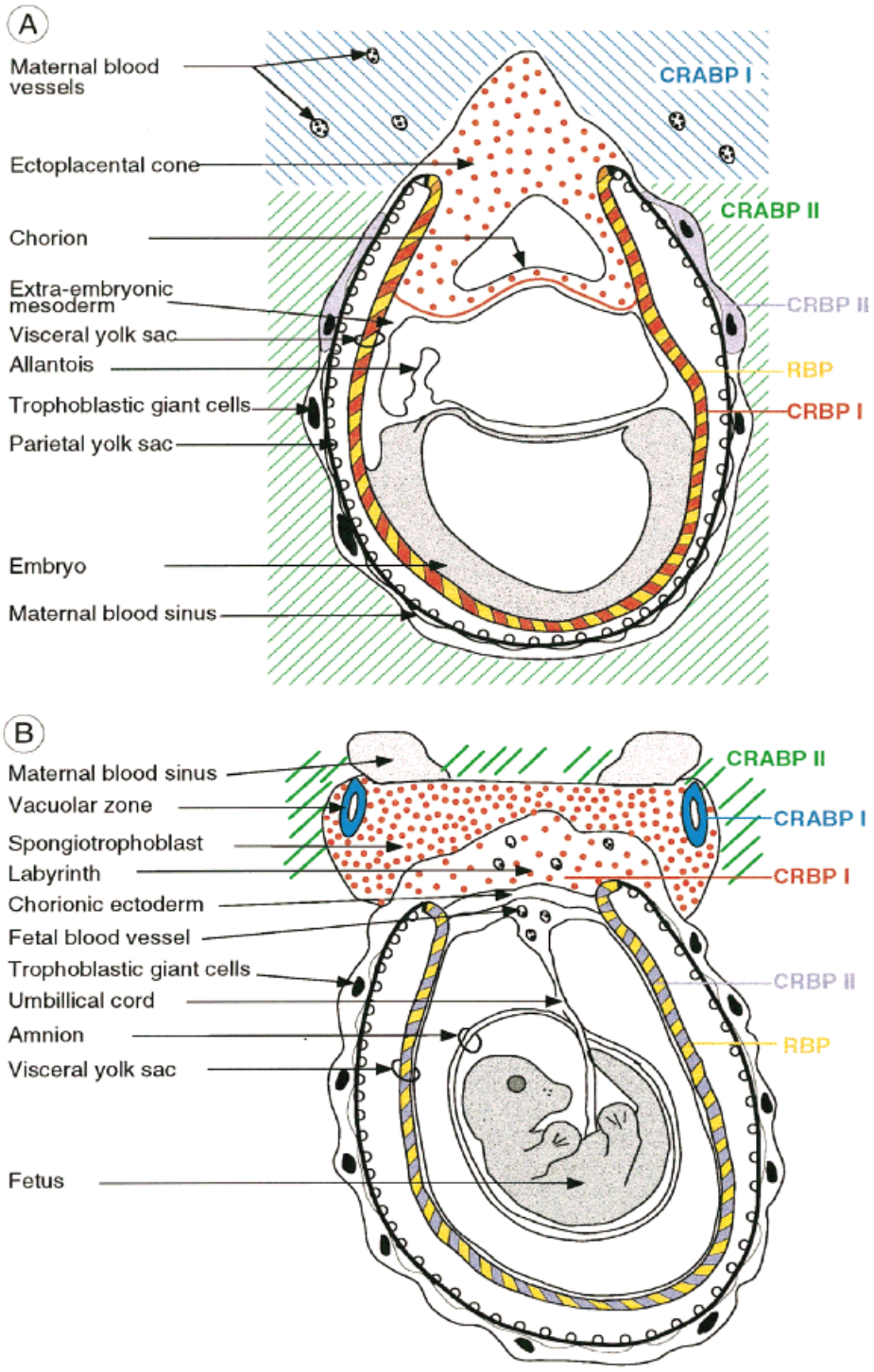

Fig. 6. Summary schemes of the distributions of RPB, CRBP I and II, and CRABP I and II gene transcripts during early (**A**, approximately 7.5 dpc) and late (**B**, approximately 15.5 dpc) placentation in the mouse.

able abnormalities, apart from an occasional supernumerary digit rudiment in the forelimbs (Lampron et al., 1995). However, the possibility remains that outside the relative protection of normal laboratory conditions, these proteins serve as moderators of fluctuations in RA levels.

Throughout all stages studied here, the RALDH-2 gene was expressed only in the uterine wall. RALDH-2 is an enzyme known to specifically oxidize retinal dehyde to RA (Zhao et al., 1996). Its specific expression during embryogenesis was found to correlate with areas where active retinoids are produced or that depend on RA for their normal development (Zhao et al., 1996; our unpublished data). Restricted expression of RALDH-2 to the uterus may reflect the requirement for RA for the support and maintenance of pregnancy.

In conclusion, the expression patterns presented here provide the basis for functional models involving members of the retinoid signalling pathway during placentation. They also provide the framework for future studies of changes in expression patterns of these genes in mutant strains or experimental conditions in which the function of the yolk sac or the chorioallantoic placenta is challenged.

EXPERIMENTAL PROCEDURES

Preparation of Mouse Placentas

Natural matings of CD1 mice were performed overnight. Pregnant females (morning of vaginal plug was considered as 0.5 dpc) were killed by cervical dislocation, and mouse conceptuses were collected in phosphate-buffered saline (PBS). Mouse conceptuses were sectioned in utero (from 6.5 to 15.5 dpc) or explanted from the uterus (from 15.5 to 19.5 dpc) and sectioned together with the placenta and extraembryonic membranes. The specimens were directly placed in moulds containing OCT medium (Miles Lab., Elkhard, IN) and frozen on the surface of dry ice, as described in Décimo et al. (1995).

In Situ Hybridisation

The plasmids used to generate ^{35}S-labelled riboprobes from cDNAs have been described previously in Dollé et al., 1989 (RARα, β, and γ; CRBP I and CRABP I), Dollé et al., 1994 (RXRα, β, and γ), Ruberte et al., 1992 (CRABP II), and Zhao et al., 1996 (RALDH-2). The CRBP II and RBP cDNA plasmids were kind gifts from H. Nakshatri and P. Bouillet, respectively. To compare the temporal and spatial distributions of the corresponding transcripts, series of four adjacent cryosections, 10-mm thick, were hybridised to these probes in a repeated manner so that the entire placentas were analysed. The procedures for probe synthesis and in situ hybridisation were as described previously (Décimo et al., 1995).

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