

Fatty Acid Transport Regulatory Proteins in the Developing Rat Placenta and in Trophoblast Cell Culture Models

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The placenta forms a selective barrier that is able to transport nutrients that are of critical use to the fetus. Delivery of essential fatty acids to the fetus is dependent upon transplacental transport and provides the backbone for the biosynthesis of biological membranes, myelin and various signalling molecules. The primary objective of this research was to elucidate the expression patterns of genes that regulate fatty acid transport across the placenta. Several fatty acid transport regulatory genes have been identified in the rat including; cytoplasmic heart fatty acid binding protein (hFABP), plasma membrane fatty acid binding protein (FABPpm), fatty acid translocase (FAT) and fatty acid transport protein (FATP). In this study, we have elucidated temporal and spatial expression patterns for these genes in the rat placenta and in cell culture models of the rat placenta by Northern blot, RT-PCR, Western blot and/or by in situ hybridization analyses. Expression of hFABP was specific to the labyrinth zone, the main barrier and site of transplacental transport in the rat placenta. In addition, the levels of hFABP expression increased with gestational age, suggesting a growing requirement for fatty acid transport with advancing stages of pregnancy. FABPpm, FAT and FATP are expressed in both the junctional and labyrinth zones of the rat placenta. FAT was predominantly localized to the labyrinth zone by in situ hybridization analysis. The placental cell expression patterns of the genes involved in fatty acid transport were supported by our observations of HRP-1 (labyrinth zone) and Rcho-1 (junctional zone) trophoblast cell culture models. Given their cell surface location, we predict that FABPpm, FAT and FATP potentially participate in placental fatty acid uptake. The predominant expression of hFABP and FAT in the labyrinth zone of the chorioallantoic placenta implicates hFABP and FAT in the transplacental movement of fatty acids from maternal to fetal compartments. © 2000 Harcourt Publishers Ltd

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INTRODUCTION

The placenta is comprised of highly specialized trophoblast cells that form a barrier between the maternal uterus and fetus. Trophoblast cells directionally regulate the transport of nutrients from the maternal blood supply to the fetal blood supply (Knipp, Audus and Soares, 1999). The active transport of nutrients, including fatty acids, across the placenta is critical for the development of a healthy fetus. For example, unsaturated fatty acids of the n-3 and n-6 classification are considered to be essential because mammals are unable to synthesize them and must acquire them through their diet (Nettleton, 1993;

Jumpsen and Clandinin, 1995). The fetus must rely on maternal circulation and transfer across the placenta as its source of these essential fatty acids (Nettleton, 1993; Jumpsen and Clandinin, 1995). Essential fatty acids of both the n-3 and n-6 series (e.g., linoleic and linolenic acid) are involved in the synthesis of several compounds involved in cell–cell signalling including: prostaglandins, prostacyclins, leukotrienes, thromboxanes and lipoxins. Essential fatty acids are also involved in the synthesis of phospholipids, a primary component of biological membranes and myelin, a principle constituent of the nervous system (Jumpsen and Clandinin, 1995; Nettleton, 1993).

The importance of fatty acid transport to the fetus is further demonstrated by the fact that transfer is highly directional from the mother to the fetus (Crawford et al., 1993; Nettleton, 1993; Jumpsen and Clandinin, 1995; Viscardi, 1995; Robillard and Christon, 1997). The ability of fatty acids to cross the placenta is critical for proper fetal growth (Hornstra et al., 1995), including the development of the fetal lungs (Viscardi,

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1995) and brain (Crawford et al., 1993; Nettleton, 1993; Jumpsen and Clandinin, 1995). Excessive fatty acid delivery to the fetus is also a potential problem and has been theorized to predispose infants to the development of cardiovascular disorders at later stages of life (Barker et al., 1989; Barker et al., 1990; Barker et al., 1993; Hornstra et al., 1995). Clearly, a better understanding of how fatty acids permeate the placental barrier is required in order to better diagnose and prevent these potential disorders.

Fatty acid transport has been shown to be regulated by several proteins; including the cytoplasmic fatty acid binding protein family (FABP), plasma membrane fatty acid binding protein (FABPpm), fatty acid translocase (FAT) and fatty acid transport protein (FATP). Of these proteins, it is believed that the FABPs are the most abundant in any cell type, comprising as much as 4 per cent of total cytosolic protein (Nettleton, 1993). There are several different FABPs (MW range from 12 000–16 000) which are identified by the tissue in which they were originally discovered (Banaszak et al., 1994). FABP is responsible for the intracellular distribution of fatty acids (Bass, 1988; Glatz et al., 1993) and is believed to play an integral role in metabolism, transport and membrane incorporation of fatty acids (Glatz et al., 1993). FABPpm, FAT and FATP are localized in the plasma membrane and have been identified as transporters of fatty acids. FABPpm is a 40 kDa membrane protein that has been identified in various organs (Schwieterman et al., 1988; Diede et al., 1992; Campbell et al., 1994; Campbell, Gordon and Dutta-Roy, 1994; Isola et al., 1995; Calles-Escandon et al., 1996) and has been shown to regulate the uptake of long chain fatty acids (Schwieterman et al., 1988). The amino acid sequence of FABPpm has also been shown to be identical to mitochondrial aspartate aminotransferase (Isola et al., 1995). FAT, an 88 kDa membrane protein, was identified and characterized in rat adipocytes, and was shown to be homologous to human CD36 glycoprotein (Abumrad et al., 1993). FAT/CD36 has been shown to facilitate the uptake of fatty acids in a variety of cell types (Harmon et al., 1991; Endemann et al., 1993; Ibrahim et al., 1996; Nozaki et al., 1995; Otnad et al., 1995; Yoshida et al., 1998). FATP is a 71 kDa protein that was originally identified in adipocytes and has been shown to be involved in facilitating lipid transport (Schaeffer and Lodish, 1994; Man et al., 1996).

Information on the presence of these fatty acid transport regulatory proteins in the rat placenta is limited. Two of these proteins, hFABP and FABPpm, have been reported to be present in both the human and rat placental tissues. The rat FABP heart isoform (hFABP) has been identified in the rat placenta by Northern blot (Heuckeroth et al., 1987) and immunocytochemical analysis (Watanabe, Ono and Kondo, 1991). FABPpm has been functionally identified in human (Campbell et al., 1994; Campbell and Dutta-Roy, 1995; Campbell, Gordon and Dutta-Roy, 1996; Campbell et al., 1997; Campbell et al., 1998a; Campbell et al., 1998b) and sheep (Campbell, Gordon and Dutta-Roy, 1994) placentae, by investigating the binding of [14 C]-oleate and [14 C]-linolenate (Campbell et al., 1994; Campbell, Gordon and Dutta-Roy,

1996; Campbell et al., 1998b). Spatial and temporal placental expression patterns for hFABP and FABPpm have not been reported in the rat placenta, nor has it been determined whether FAT or FATP are expressed in rat placental tissues. However, hFABP, the liver isoform of FABP, a placental isoform of FABPpm, FAT and FATP have been recently demonstrated to be present in the human placenta (Campbell et al., 1998a).

In order to establish a framework for investigating trans-placental fatty acid transport in the rat, we have examined the spatial and temporal expression patterns of FABP, FABPpm, FAT and FATP in the junctional (invasive/endocrine function) and labyrinth (transport barrier function) zones of the developing rat chorioallantoic placenta. In addition, the expression of these fatty acid transport regulatory genes were examined in cell culture models of the rat placenta, HRP-1 [labyrinth zone-like model (Hunt et al., 1989; Soares et al., 1987)] and Rcho-1 [junctional zone-like model (Faria and Soares, 1991; Peters, Chapman and Soares, 1999)] trophoblast cell models. We have established that FABP, FABPpm, FAT and FATP are expressed in the developing rat placenta.

MATERIALS AND METHODS

Reagents

Reagents for polyacrylamide gel electrophoresis were purchased from Bio-Rad (Hercules, CA, USA). All restriction enzymes, polymerases and DNA ligase were purchased from New England Biolabs (Beverly, MA, USA). Rat cDNAs for hFABP (Heuckeroth et al., 1987), FABPpm (Mattingly et al., 1987), FAT (Abumrad et al., 1993) and FATP (Schaap et al., 1997) were obtained from Dr Jeffrey Gordon, Washington University, St Louis, Missouri, USA; Dr Nada A. Abumrad, State University of New York, Stony Brook, New York, USA; and Dr Jan Glatz, University of Limburg-Maastricht, The Netherlands; respectively. cDNA and antibodies to rat FABPpm were generously provided by Dr Joseph Mattingly, University of Missouri, Kansas City, Missouri, USA. Transformation competent *Sure* bacterial cells and Prime-It[®] II labelling kits were obtained from Stratagene (La Jolla, CA, USA). DNA extraction kits were purchased from Qiagen (Chatsworth, CA, USA). Nitrocellulose and nylon membranes were obtained from Schleicher and Schuell (Keene, NH, USA). Radiolabelled nucleotides were purchased from DuPont-NEN (Boston, MA, USA). TRIzol Reagent for RNA extraction and reverse transcriptase-polymerase chain reaction kits were obtained from Life Technologies (Gaithersburg, MD, USA). Enhanced Chemiluminescence detection reagents kit were obtained from Amersham Life Sciences (Arlington Heights, IL, USA). Unless otherwise noted, all other chemicals and reagents were purchased from Sigma (St Louis, MO, USA).

Animals and tissue collection

Holtzman rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN, USA). The animals were housed in an

environmentally controlled facility, with lights on from 0600–2000 hours, and allowed free access to food and water. Timed pregnancies and tissue dissections were performed as previously described (Soares, 1987). The presence of a copulatory plug or sperm in the vaginal smear was designated day 0 of pregnancy. Upon isolation, the tissues were quickly frozen in liquid nitrogen and then stored at -80°C until analysed. For each of the studies, a minimum of three placentae were collected from different rats to provide a comprehensive survey of the placental expression of the mRNAs and proteins of interest. Protocols for the care and use of animals were approved by the University of Kansas Animal Care and Use Committee.

Cell culture

Two trophoblast cell lines were examined for their ability to express fatty acid transport regulatory genes. HRP-1 trophoblast cells represent a cell population derived from the labyrinth zone of the rat chorioallantoic placenta (Soares et al., 1987; Hunt et al., 1989). HRP-1 trophoblast cells were routinely maintained in RPMI-1640 culture medium containing 10 per cent FBS and the above supplements. The Rcho-1 trophoblast cell line was derived from a rat choriocarcinoma and is capable of differentiating along the trophoblast giant cell lineage characteristic of the junctional zone of the rat chorioallantoic placenta (Faria and Soares, 1991; Peters, Chapman and Soares, 1999). Rcho-1 trophoblast cells were routinely maintained in subconfluent conditions with NCTC-135 culture medium supplemented with 20 per cent FBS, $50\ \mu\text{M}$ 2-mercaptoethanol, 1 mM sodium pyruvate, 100 units/ml of penicillin and $100\ \mu\text{g}/\text{ml}$ of streptomycin (Faria and Soares, 1991; Peters, Chapman and Soares, 1999). Differentiation was induced by growing the cells to confluence in FBS supplemented culture medium and then replacing the serum supplementation with 10 per cent HS (Faria and Soares, 1991; Peters, Chapman and Soares, 1999). All cells used in these studies were between passages 7 and 22.

Northern analysis

Northern blots were performed as previously described in our laboratory (Faria et al., 1990; Orwig et al., 1997). Total RNA was extracted from tissues and cells essentially as described by Chomczynski and Sacchi (1987), using TRIzol. Blots were probed with [^{32}P]-labelled rat heart FABP (Heuckeroth et al., 1987) and rat FABPpm (Mattingly et al., 1987). Control blots were probed with ribosomal protein L7 [rpL7 (Meyuhas and Klein, 1990)] and/or rat placental lactogen-I [PL-I (Dai et al., 1996)] cDNAs.

RT-PCR and Southern analysis

Total RNA was isolated from tissues with the TRIzol reagent as described above. RT-PCR was performed with primers

specific for FAT (upstream primer: 5'ATGGGCTGCGA TCGGAAGTGT3'; downstream primer: 5'ACAGACAGT GAAGGCTCAAAGAT3') and FATP (upstream primer: 5'TGCTTTGGCTTCTGGACTT3'; downstream primer: 5'CCATAAATGAGGGCCTTG3'). The FAT primers amplified a 383 bp product (nucleotides 74–457), whereas the FATP primers amplified a 499 bp product (nucleotides 120–619). Control reactions were performed with primers to rat-actin (upstream primer: 5'ATCGTGGGCCGCCGCTAGGCA3'; downstream primer: 5'TGGCCTTAGGGTT CAGAGGGG3') which amplify a 244 bp product. Five micrograms of total RNA and $0.5\ \mu\text{g}$ of oligo(dT) were utilized for the reverse transcriptase reaction. The PCR reaction was performed for 30 cycles (denature: 94°C for 1 min; annealing: 60°C for 2 min; extension: 72°C for 2 min) using a Perkin-Elmer Thermocycler (model 480, Norwalk, CT, USA). Reaction products were electrophoretically separated in 1.5 per cent agarose gels, transferred to nylon membranes and probed with [^{32}P]-labelled FAT (Abumrad et al., 1993) or FATP cDNAs (Schaap et al., 1997).

Western analysis

Western blot analyses were performed as previously described (Faria et al., 1990). Placental cytosolic and membrane fractions were separated in 15 per cent and 10 per cent SDS polyacrylamide gels under reducing conditions, respectively, and electrophoretically transferred to nitrocellulose filters. Filters were blocked with 5 per cent non-fat milk. Placental cytosolic and membrane protein fractions were isolated by homogenizing the tissue in a hyperosmotic, Nonidet P-40 buffer, and then centrifuging at 12 000 rev/min for 20 min for subcellular fractionation. Cytosolic protein preparations were probed with antibodies for rat hFABP (Watanabe, Ono and Kondo, 1991) and placental membrane preparations were probed with antibodies to rat FABPpm (Mattingly et al., 1987). Rat heart tissue was used as a control. Immunoreactive bands were visualized using a chemiluminescence detection system.

In situ hybridization

The in situ detection of mRNA expression was performed on frozen tissue sections as previously described (Faria et al., 1990; Rasmussen et al., 1997). A full-length rat hFABP cDNA (Heuckeroth et al., 1987) and an 800 bp fragment of the rat FAT cDNA (Abumrad et al., 1993) were subcloned into pBluescript sk vector, linearized, and used as templates for the synthesis of [^{35}S]-labelled sense and antisense RNA probes. Hybridizations with sense and antisense RNA probes for placental lactogen-II [PL-II (Dai et al., 1996)] and prolactin-like protein-C [PLP-C (Dai et al., 1996a; Deb et al., 1991a)] were used as additional controls for identifying placental cell subpopulations.

Statistical Analysis

All experiments were performed a minimum of three times. Data generated from Northern and Western blot analysis were quantitated by densitometry. Data was analysed by analysis of variance. The source of variation from significant *F*-ratios was determined by Neuman–Keuls multiple comparison test (Keppel, 1973).

RESULTS

Fatty acid transport regulatory gene expression in the developing rat chorioallantoic placenta

In order to establish the rat as a model for studying placental fatty acid transport, we investigated spatial and temporal expression patterns for fatty acid transport regulatory genes during the development of the rat chorioallantoic placenta.

hFABP. The temporal and spatial expression patterns for rat hFABP mRNA and protein in the rat placenta elucidated by Northern and Western blot analyses are shown in Figure 1. In situ hybridization data for hFABP mRNA expression is presented in Figure 2. Transcript and protein sizes corresponded to previous published reports for rat hFABP (Heuckeroth et al., 1987; Watanabe, Ono and Kondo, 1991). Collectively, the results indicate that hFABP is predominantly expressed in the labyrinth zone of the mid to late gestation rat placenta. hFABP mRNA levels increased significantly from day 13 to day 21 of gestation. The in situ hybridization analysis verified the labyrinthine localization of hFABP mRNA which was quite different from the junctional zone pattern of expression noted for PLP-C (Figure 2). Within the labyrinth zone, hFABP mRNA distribution is distinct from the distribution of PL-II mRNA which is punctate and restricted to labyrinthine trophoblast giant cells. hFABP expression in the labyrinth zone is consistent with a syncytial trophoblast localization. PL-II is also expressed in trophoblast giant cells located in the junctional zone. Localization of PLP-C and PL-II mRNAs were similar to previous reports (Deb et al., 1991; Deb et al., 1991a).

FABPpm. FABPpm mRNA and protein were expressed in both junctional and labyrinth zone placental compartments (Figure 1). Transcript and protein sizes corresponded to previous published reports for rat FABPpm (Mattingly et al., 1987). Labyrinthine levels of FABPpm mRNA and protein appeared to be slightly higher than junctional zone levels.

FAT and FATP. Messenger RNA levels for both FAT and FATP were relatively less abundant than were hFABP or FABPpm levels, as determined by Northern analysis. Consequently, we utilized RT-PCR and Southern blotting to determine whether these two fatty acid transport regulatory proteins were detectable in the developing rat placenta. Both transcripts were within the limits of detection in samples from both junctional and labyrinth tissue specimens (Figure 3). Please

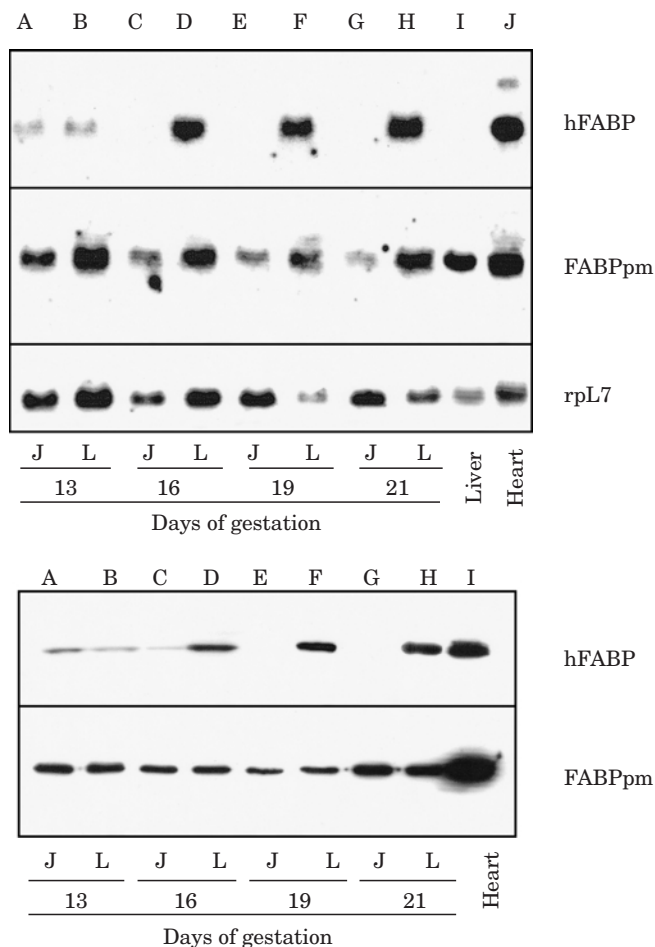


Figure 1. Expression of FABP and FABPpm in the rat placenta during the second half of gestation. (Top Panel) Northern blots showing the temporal and spatial patterns of expression of hFABP and FABPpm mRNAs in the placenta. Placental tissues were isolated, dissected into junctional and labyrinth zones, and extracted for total RNA. Total RNA (20 µg/lane) was fractionated on 1.4 per cent agarose gels transferred to nylon membranes and probed with [³²P]dATP labelled cDNAs. Heart and liver RNAs were used as positive controls. Ribosomal protein L7 (rpL7) was used to control for RNA integrity and equal loading. Lanes A–H: placental RNA samples from days 13, 16, 19 and 21 of gestation; lane I: rat liver RNA; lane J: rat heart RNA. (Bottom Panel) Western blots showing the temporal and spatial patterns of expression of hFABP and FABPpm in the placenta. Rat heart extracts were used as a positive control for hFABP and FABPpm expression. Cytosolic and membrane fractions were separated in 15 per cent and 10 per cent SDS polyacrylamide gels under reducing conditions, respectively, and electrophoretically transferred to nitrocellulose filters. Cytosolic protein preparations were probed with antibodies to rat hFABP and placental membrane preparations were probed with antibodies to rat FABPpm. Immunoreactive bands were visualized using a chemiluminescence detection system. Lanes A–H: fractionated placental protein samples from days 13, 16, 19 and 21 of gestation; lane I: rat heart RNA. Please note the predominance of hFABP in the labyrinth zone and the increase in its expression as gestation progresses. J: junctional zone; L: labyrinth zone.

note the RT-PCR analysis was not quantitative. FAT and FATP mRNA expression were further analysed by in situ hybridization. FAT mRNA was shown to have a distribution of expression within the labyrinth zone similar to FABP (Figure 2), indicating a syncytial localization. The temporal profile of FAT mRNA expression in the labyrinth zone increased as gestation advanced (data not shown), in a way

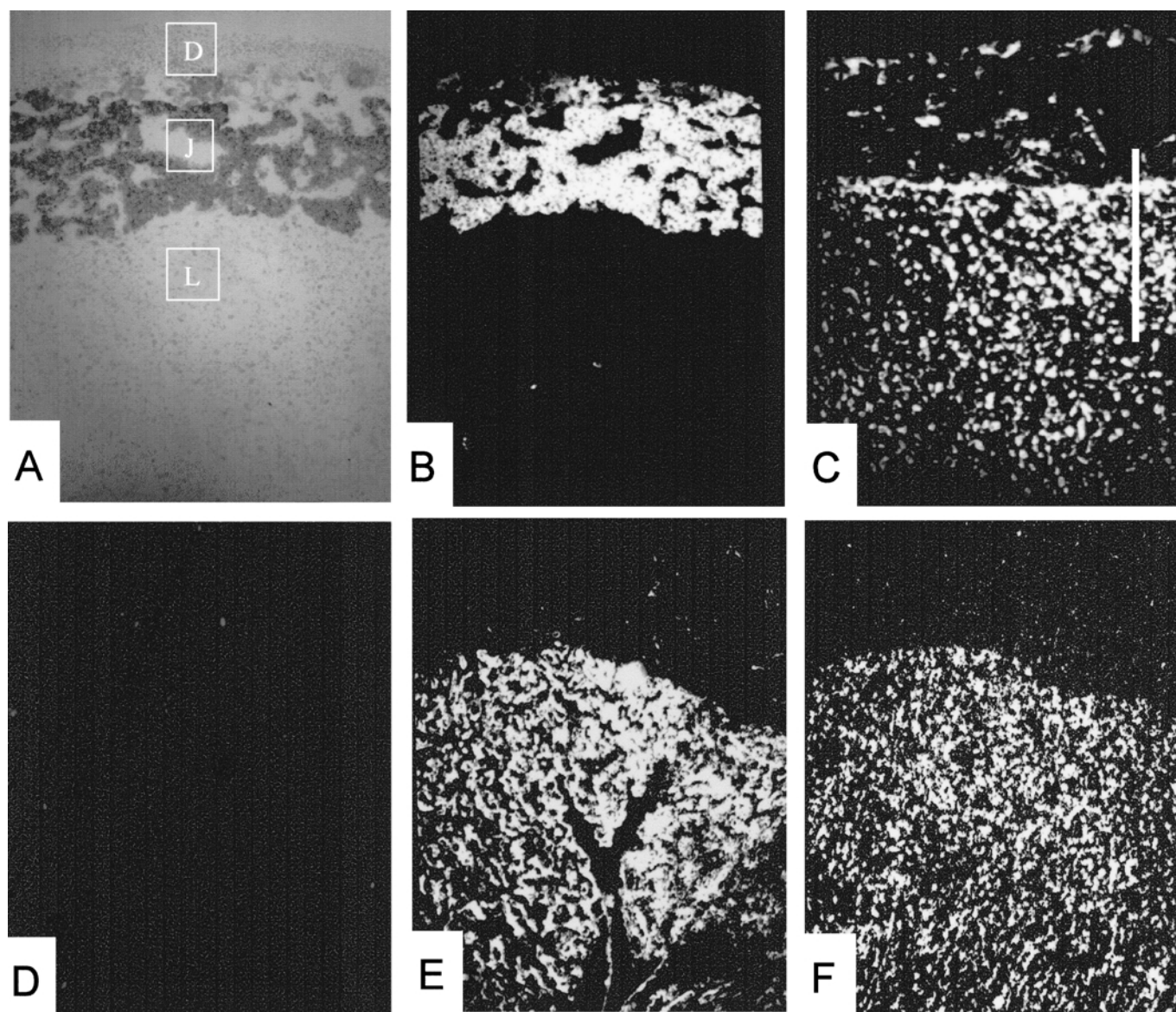


Figure 2. In situ localization of hFABP and FAT mRNAs in the rat chorioallantoic placenta from day 19 of gestation. The in situ detection of mRNA expression was performed on frozen tissue sections. A full-length rat hFABP cDNA (Faria and Soares, 1991) and an 800 bp fragment of the rat FAT cDNA were subcloned into pBluescript sk vector, linearized and used as templates for the synthesis of [³⁵S]-labelled sense and antisense RNA probes. Hybridizations with sense and antisense RNA probes for placental lactogen-II (PL-II, 19) and prolactin-like protein-C (PLP-C) were used as additional controls for identifying placental cell subpopulations. Panel A: bright field representation using a PLP-C antisense probe; Panel B: dark field representation using a PLP-C antisense probe; Panel C: dark field representation using a PL-II antisense probe; Panel D: dark field representation probed using a hFABP sense probe (line represents 1 mm in length); Panel E: dark field representation using a hFABP antisense probe; Panel F: dark field representation using a FAT antisense probe. Magnifications, × 40. D: decidua; J: junctional zone; L: labyrinth zone.

similar to the FABP pattern of expression. Discrete FATP mRNA localization within the rat placenta was not successful and may have been beyond the sensitivity of the assay procedures.

Fatty acid transport regulatory gene expression in rat trophoblast cell culture models

Two trophoblast cell culture models were evaluated for their expression of fatty acid transport regulatory genes: HRP-1 and

Rcho-1 trophoblast cell lines. HRP-1 trophoblast cells were derived from labyrinthine placental primordia (Soares et al., 1987; Hunt et al., 1989) and possess the capacity to directionally transport fatty acids (Shi et al., 1997). Rcho-1 trophoblast cells represent a stem cell population that can be induced to differentiate into trophoblast giant cells possessing a junctional zone phenotype (Faria and Soares, 1991; Peters, Chapman and Soares, 1999).

hFABP. hFABP mRNA and protein were readily detected in HRP-1 and Rcho-1 trophoblast cells (Figure 4). Expression of

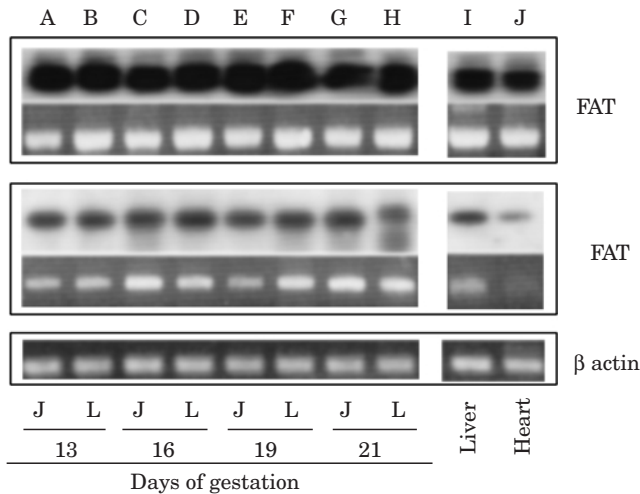


Figure 3. Expression of FAT and FATP mRNA in the rat placenta during the second half of gestation. RT-PCR analysis of FAT and FATP mRNAs in the rat placenta. Placental tissues were isolated, dissected into junctional and labyrinth zones and extracted for total RNA. RT-PCR was performed with primers specific for FAT, FATP and β -actin which amplified products of 383, 499 and 244 bp, respectively. Reaction products were electrophoretically separated in 1.5 per cent agarose gels, stained with ethidium bromide, transferred to nylon membranes and probed with [32 P]-labelled FAT or FATP cDNAs. (Top panels) FAT RT-PCR analysis: Southern analysis (upper panel) and ethidium bromide stained products (lower panel); (middle panels) FATP RT-PCR analysis: Southern analysis (upper panel) and ethidium bromide stained products (lower panel); (bottom panel) ethidium bromide stained products. Lanes A–H: placental RNA samples from days 13, 16, 19 and 21 of gestation; lane I: rat liver RNA; lane J: rat heart RNA.

hFABP mRNA and protein in Rcho-1 trophoblast cells was differentiation dependent. As Rcho-1 trophoblast cells are induced to differentiate they exhibit a pronounced change in morphology, forming trophoblast giant cells, and initiate production of the hormone/cytokine, PL-I, as well as other hormones and cytokines (Faria and Soares, 1991; Peters, Chapman and Soares, 1999; Figure 4). Interestingly, as Rcho-1 trophoblast cells differentiated they shut off synthesis of hFABP (Figure 4).

FABPpm, FAT and FATP. FABPpm mRNA and protein were readily detectable in both HRP-1 and Rcho-1 trophoblast cells by Northern and Western analyses, respectively (Figure 4). FABPpm levels did not appear to significantly change accompanying trophoblast cell differentiation. Expression of FAT and FATP transcripts was demonstrated by RT-PCR in both HRP-1 and Rcho-1 trophoblast cells (Figure 5). There was some evidence for a greater expression of FAT in HRP-1 trophoblast cells and for a modest increase in FAT and FATP expression accompanying trophoblast giant cell formation (Figure 5).

DISCUSSION

The placenta is vital for the delivery of nutrients, including fatty acids, to the fetus. In this study, we have examined the

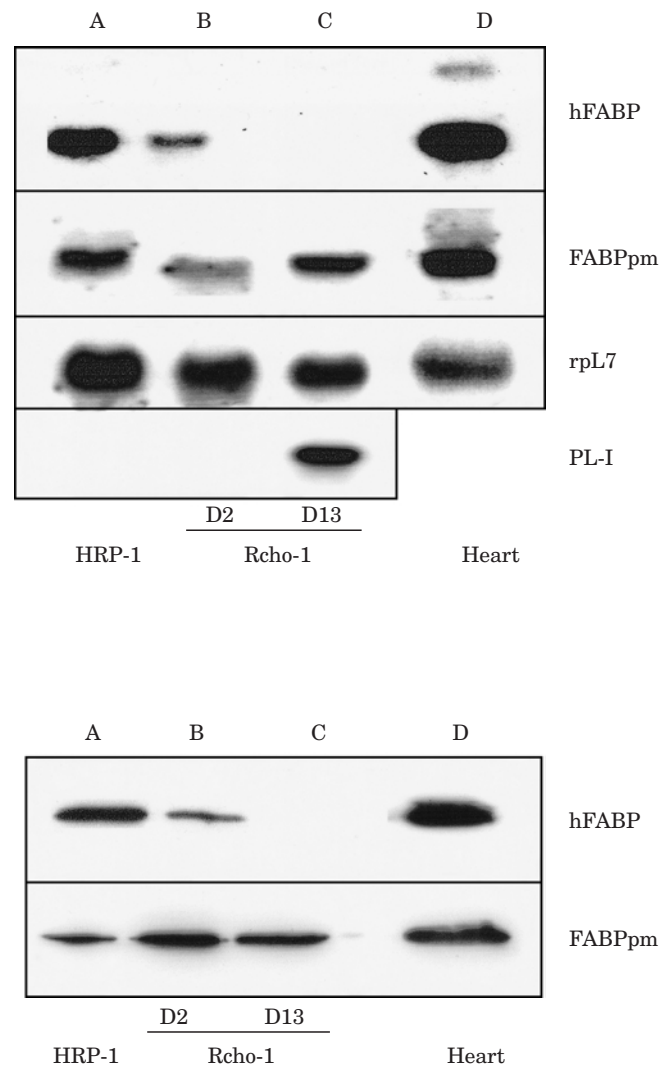


Figure 4. Expression of FABP and FABPpm in rat trophoblast cells. (Top Panel) Northern blots showing expression of hFABP, FABPpm, PL-I and rpL7 mRNAs in HRP-1 and Rcho-1 trophoblast cells. Cells were harvested and extracted for total RNA. Total RNA (20 μ g/lane) was fractionated on 1.4 per cent agarose gels transferred to nylon membranes and probed with [32 P]dATP labelled cDNAs. Heart RNA was used as a positive control. Ribosomal protein L7 (rpL7) was used to control for RNA integrity and equal loading. Lane A: HRP-1 trophoblast cells; lane B: undifferentiated Rcho-1 trophoblast cells, D2; lane C: differentiated Rcho-1 trophoblast cells, D13; lane D: rat heart. Please note that in Rcho-1 trophoblast cells, hFABP showed a differentiation-dependent decrease in expression, whereas, PL-I mRNA levels increased in differentiated trophoblast cells. (Bottom Panel) Western blots showing expression of hFABP and FABPpm proteins in HRP-1 and Rcho-1 trophoblast cells. Rat heart extracts were used as a positive control for hFABP and FABPpm expression. Cytosolic and membrane fractions were separated in 15 per cent and 10 per cent SDS polyacrylamide gels under reducing conditions, respectively, and electrophoretically transferred to nitrocellulose filters. Cytosolic protein preparations were probed with antibodies for rat hFABP and membrane preparations were probed with antibodies to rat FABPpm. Immunoreactive bands were visualized using a chemiluminescence detection system. Lane A: HRP-1 trophoblast cells; lane B: undifferentiated Rcho-1 trophoblast cells, D2; lane C: differentiated Rcho-1 trophoblast cells, D13; lane D: rat heart.

expression patterns of four key genes previously implicated in the regulation of fatty acid transport (hFABP, FABPpm, FAT and FATP). We have focused our analysis on the developing

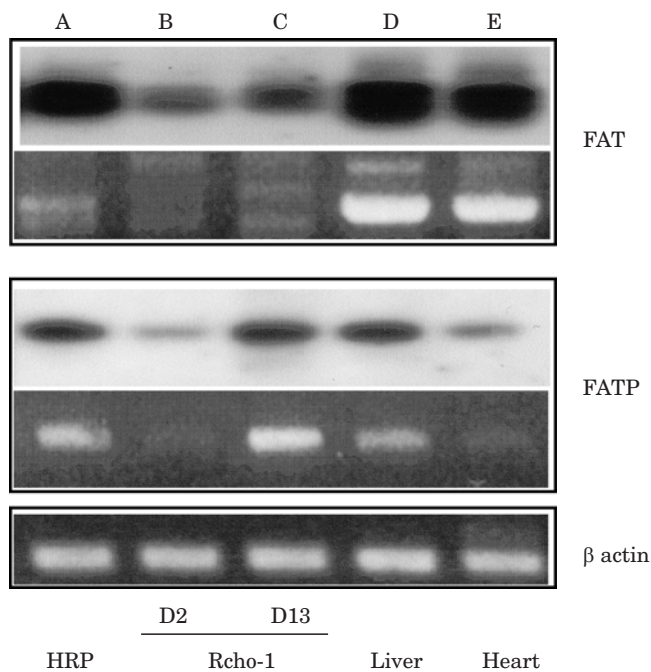


Figure 5. Expression of FAT and FATP mRNA in cell culture models of the rat placenta. RT-PCR analysis of FAT and FATP mRNAs in HRP-1 and Rcho-1 trophoblast cells. Cells were harvested and extracted for total RNA. RT-PCR was performed with primers specific for FAT, FATP and β -actin which amplified products of 383, 499 and 244 bp, respectively. Reaction products were electrophoretically separated in 1.5 per cent agarose gels stained with ethidium bromide, transferred to nylon membranes, and probed with [32 P]-labelled FAT or FATP cDNAs. (Top panels) FAT RT-PCR analysis: Southern analysis (upper panel) and ethidium bromide stained products (lower panel); (middle panels) FATP RT-PCR analysis: Southern analysis (upper panel) and ethidium bromide stained products (lower panel); (bottom panel) ethidium bromide stained products. Lane A: HRP-1 trophoblast cells; lane B: undifferentiated Rcho-1 trophoblast cells, D2; lane C: differentiated Rcho-1 trophoblast cells, D13; lane D: rat liver; lane E: rat heart.

rat placenta. The rat has been extensively used to study fatty acid uptake and transport in various cell types. Thus, homologous molecular probes for genes implicated in fatty acid uptake and transport are available and functional attributes of the regulatory proteins have been previously reported. In addition, the placenta of the rat offers two key experimental advantages: (1) functionally distinct regions (junctional zone: invasive/endocrine versus labyrinth zone: bidirectional transport) can be readily dissected (Baier, Bogardus and Sacchettini, 1996) (2) trophoblast cell culture models mimicking the behaviour of the functionally distinct regions are available (HRP-1 and Rcho-1 cells). We report that each of these four genes implicated in regulating fatty acid transport is expressed in the rat placenta and more specifically in rat trophoblast cells.

hFABP is a member of the cytoplasmic fatty acid binding protein family. Members of this family are known to be responsible for the intracellular trafficking of fatty acids (Glatz et al., 1993; Veerkamp and Maatman, 1995; Luxon, 1996; Murphy et al., 1996). In addition to regulating intracellular fatty acid trafficking, FABP family members have also been shown to contribute to the transcellular transport of fatty acids (Baier, Bogardus and Sacchettini, 1996). Functional character-

istics of FABP family members are overlapping but in some situations they are unique, cell type-specific and dependent upon cell differentiation state (Glatz et al., 1993; Veerkamp and Maatman, 1995). hFABP expression was very abundant in the rat chorioallantoic placenta and largely restricted to the labyrinth zone (present study). More specifically, hFABP mRNA was localized to the labyrinthine syncytial trophoblast layers, a finding consistent with previous immunocytochemical localization of the hFABP protein (Watanabe, Ono and Kondo, 1991), and ideally situated for participation in the transplacental movement of fatty acids to the fetus. hFABP was also abundantly expressed in HRP-1 trophoblast cells and undifferentiated Rcho-1 trophoblast cells. HRP-1 trophoblast cells were originally derived from labyrinthine primordia (Soares et al., 1987; Hunt et al., 1989) and have been previously demonstrated to transcellularly transport fatty acids from apical to basolateral compartments (Shi et al., 1997). The HRP-1 trophoblast cell model should be useful for dissecting the role of hFABP in transplacental fatty acid transport. Interestingly, hFABP expression dramatically decreased as Rcho-1 trophoblast cells differentiated into trophoblast giant cells. Trophoblast giant cells are principally involved in hormone biosynthesis, including the synthesis of steroid hormones (Soares et al., 1996). Thus, intracellular delivery of lipid precursors in steroid synthesizing trophoblast cells probably does not involve hFABP. In fact, downregulation of hFABP expression in trophoblast giant cells may be required in order to appropriately target lipid precursors to sites of steroid synthesis. Such results further emphasize the unique features of members of the FABP family. The kinetics of hFABP association and dissociation with fatty acids are consistent with its functioning as a transcellular transporter (Glatz et al., 1993). It is possible that other FABP isoforms are expressed in differentiating trophoblast giant cells which promote intracellular utilization of lipid precursors. One such possibility might be the liver FABP isoform, which has recently been demonstrated in the human placenta (Campbell et al., 1998a).

FABPpm, FAT and FATP are cell surface proteins involved in fatty acid transport (Hui and Bernlohr, 1997). This includes the uptake of fatty acids into cells and their potential for transcellular transport. Of the three fatty acid regulatory genes FABPpm was most abundant and distributed throughout the rat chorioallantoic placenta (present study), and was also highly expressed in the two rat trophoblast cell models. FABPpm has also been demonstrated to contribute to fatty acid uptake by human trophoblast cells (Campbell et al., 1994; Campbell and Dutta-Roy, 1995; Campbell et al., 1997; Campbell et al., 1998a). In general, FABPpm has a broad tissue distribution and is probably involved in providing fatty acid precursors essential for normal cellular function (Hui and Bernlohr, 1997). FAT and FATP were expressed in the rat chorioallantoic placenta and in the rat trophoblast cell lines; however, at a lower level than observed for FABPpm (present study). The RT-PCR approach for detecting FAT and FATP expression verified their presence in placental tissues and trophoblast cells but did not provide quantitative information.

In situ hybridization demonstrated a clear preference for expression of FAT in the labyrinth region of the placenta. The labyrinthine expression of FAT increased as gestation advanced and paralleled the expression of hFABP. Such a localization is consistent with participation in the transplacental movement of fatty acids from maternal to fetal compartments. Interestingly, FAT appears to be coordinately regulated with hFABP during rat heart development where both proteins act to increase fatty acid metabolism (Van Nieuwenhov et al., 1995). Consistent with our observations in the placenta, FAT expression is tissue-specific and under metabolic control (Abumrad et al., 1993) and a potential partner for hFABP in the transplacental fatty acid transport. FATP is also tissue specific in its expression pattern and, as recently demonstrated, part of a larger family of at least five homologues (Schaeffer and Lodish, 1994; Hirsch, Stahl and Lodish, 1998). In our analysis, we examined the expression of the rat homologue for what is now referred to as mouse FATP1. The patterns of FATP1 mRNA expression in the rat placenta and trophoblast cells did not add to our understanding of its potential involvement in placental fatty acid transport. It will be necessary to

examine the placental expression and function of other FATP family members which may have potentially have a greater role in placental fatty acid transfer.

In conclusion, we have determined the placental expression pattern of four key genes participating in fatty acid transport. FABPpm was abundantly expressed throughout the rat chorioallantoic placenta and probably contributes to a generalized housekeeping function. The cell surface fatty acid transporter, FAT, and the cytoplasmic fatty acid binding protein, hFABP, had a very specific distribution restricted to the labyrinthine compartment of the rat chorioallantoic placenta, the site of transcellular delivery of fatty acids to the fetal compartment. We hypothesize that FAT and hFABP may participate in the placental transfer of fatty acids to the fetus. Furthermore, we have established two in vitro models, HRP-1 and Rcho-1 trophoblast cell lines, for dissecting roles of these fatty acid regulatory proteins in the placenta. In the future it will be essential to decipher the specific intracellular functions of FAT and hFABP within trophoblast cells and determine their contribution to the placental transfer of fatty acids to the fetus.

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