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Calcium-Binding Proteins

Distribution and Implication in Mammalian Placenta

Louiza Belkacemi, Lucie Simoneau, and Julie Lafond

Laboratoire de Physiologie Materno-Foetale, Département des Sciences Biologiques, Université du Québec à Montréal, Montréal, Canada

During gestation, transport by the placenta is solely responsible for nutrient supply to the developing fetus. In this context, calcium (Ca^{2+}) transport machinery of the placenta thus represents the primary tissue site for regulating fetal Ca^{2+} homeostasis. In humans, the transplacental movements of Ca^{2+} increase dramatically during the last trimester of gestation, when fetal skeletal mineralization is at its highest. However, little is known about the exact mechanism of transport. Evidence suggests that some developmentally expressed cytosolic Ca^{2+}-binding proteins (CaBPs) have an important role in regulating or shuttling cytosolic Ca^{2+} since they are endowed with a high affinity for Ca^{2+} (~10^6 M^{-1}). CaBPs belong to a large family of eukaryotic proteins containing a specific helix-loop-helix structure, referred to as the EF-hand motif, which counts more than 200 members. Several of these CaBPs were identified in the placenta: CaBP9k, CaBP28k, CaBP57k, oncomodulin, S-100P, S-100α, and S-100β. This review discusses the current views in this field to guide future investigations into the localization and functions of CaBPs during Ca^{2+} intracellular homeostasis in the placenta.

Key Words: Calcium-binding protein; cytotrophoblast cells; syncytiotrophoblasts; parathyroid hormone–related protein; estrogen; vitamin D.

Introduction

Ionic calcium (Ca^{2+}) is vital for all cellular homeostasis in mammals. Increasing amounts of Ca^{2+} must be transported to the fetus across the human placental trophoblast epithelium to support fetal growth and development of bone formation throughout gestation. By the end of a normal pregnancy, the fetus acquires approx 30 g of Ca^{2+}, acting as a regulator for a variety of intracellular functions including homeostasis. In the present review, calbindins (Ca^{2+}-binding proteins [CaBPs]) are referred to as calbindin-28k (CaBP28k), calbindin-9k (CaBP9k), and calbindin-57k (CaBP57k), eliminating the letter *D* since the CaBPs in the uterus of birds and mammals are actually not vitamin D dependent albeit estrogen induced *(1,2)*, while brain CaBPs are also not vitamin D dependent but considered to be constitutively expressed *(3,4)*. The CaBPs are members of the troponin-C superfamily and were observed for the first time by Wasserman's group *(5)*. These proteins bind in EF-hand structures and are "Ca^{2+} sensitive" in that they undergo a conformational change on binding Ca^{2+} *(6)*. They occur in a number of Ca^{2+}-transporting tissues such as brain, kidney, bone, intestine, uterus, and placenta. We focus here on the localization and functions of placental CaBPs, i.e., CaBP9k *(7)*, CaBP28k *(8)*, CaBP57k *(9)*, oncomodulin *(10)*, S-100P *(11,12)*, S-100α, and S-100β *(12)*.

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Author to whom all correspondence and reprint requests should be addressed: Julie Lafond, Département des Sciences Biologiques, Laboratoire de Physiologie Materno-Foetale, Université du Québec à Montréal, C.P. 8888, Succursale "Centre-Ville", Montréal, Québec, Canada, H3C 3P8. E-mail: lafond.julie@uqam.ca

Tissue Distribution

CaBPs are present in all classes of mammalian tissues. CaBP-9k is a cytosolic CBP detected by biochemical and immunologic methods in many species (Table 1). CaBP9k is unevenly distributed in human fetal tissues *(13)*. Although the exact role of CaBP9k is not known, studies suggest that it facilitates placental Ca^{2+} transcellular transport *(14)*. In the placenta of rodents, concentrations varying between 1 and 7 μg/mg of protein have been reported *(15–21)*, whereas much higher levels of CaBP9k were found in the yolk sac membranes compared to the main placenta choriollantoic labyrinth *(22)*. In the rat uterus, Krisinger et al. *(23)* showed that CaBP9k mRNA was highest at proestrus, dropped one-fold at estrus, and was not detectable at diestrus. In bovine, caprine, and ovine families, CaBP9k expression level was about 8–10 times higher in placentomal trophoblast compared with the placentomal. In the bovine uterus, CaBP9k was localized to the maternal caruncular epithelium, and the expression level increased from the second trimester to term pregnancy *(24)*. CaBP9k was also detectable in the uninucleate trophoblast cells in the sheep and goat. In that case, CaBP9k was homogeneously distributed through cytosol and nucleoplasm *(25)*.

CaBP28k, originally characterized in avian tissue *(5)*, was reported in mouse trophoblast giant cells, which separate the deciduas from Reicherts membrane on d 10 of gestation *(8)*.

Table 1
Characterization of Placental CaBPs from Mammals

Protein	Additional name(s)	Species	Gene and protein	No. of EF regions	Role(s)
CaBP9k	Calbindin-D9k	Human, rat, mouse, rabbit, sheep, horse, bird, pig, bovine	Gene size of 5.5 kbp, localized on X-chromosome, consists of 3 exons interrupted by 2 introns; protein of 79 amino acids, molecular mass of 8.787 kDa	2	Buffering/shuttling
CaBP28k	Calbindin-D28k, originally described as a 27-kDa protein	Human, baboon rat, mouse, avians	Found with 8q21.3-q22.1 region on chromosome 8; protein 261 amino acids; molecular mass of 28 kDa	6 in which 2 have presumably lost their Ca^{2+}-binding capabilities *(47)*	May be intracellular facilitator of Ca^{2+} diffusion or intracellular buffer of Ca^{2+}
CaBP57k	Calbindin-D57k	Human, mouse	Mouse protein of 417 amino acids, molecular mass of 57 kDa		Ca^{2+} shuttling and trophoblast cell differentiation
Oncomodulin	β-Paravalbumin *(123)*	Human, cow, sheep, goat, pig, hamster, rat, mouse, avian, reptile, amphibian	Human gene on chromosome 7; small acidic protein, molecular mass of 10 kDa	3 *(123)*	Physiologic role not clear, may increase invasiveness of cytotrophoblast cells
S-100P	Placental binding protein *(10)* or S-100P*(11)*	Human	Localized to 1q21 region of chromosome 1 *(37)*; small acidic protein of 90 amino acids; molecular mass of 10–12 kDa	2 *(37)*	Cell growth and motility, cell-cycle regulation, transcription, and differentiation
S-100β	—	Human, rat, mouse	Localized on 21q22.3 region of chromosome 1 *(124)*, contains 3 exons *(37)* encoded by exons 2 and 3 *(125)*	2 *(37)*	Ca^{2+}-modulated protein *(38)*; activator of nuclear protein kinase Ndr, which regulates cell division and morphology *(38)*
S-100α	—	Human	Localized on chromosome 1, contains 3 exons	2 *(37)*	

The staining intensity of these cells, which are believed to be responsible for invading the uterine wall during blastocyst implantation *(26)*, decreased during gestation, so that by d 18 only a few cells were weakly stained. At this stage, the columnar epithelial cells of the intraplacental yolk sac were intensely stained *(8)*.

CaBP57k has been found in both human and rodent placenta and is localized exclusively in trophoblastic cells *(27, 28)*. Using a cDNA clone to the mouse placental CaBP, mRNA transcripts of CaBP57k have been localized in the mouse placenta by *in situ* cDNA-RNA hybridization *(28)*. Tuan et al. *(28)* demonstrated that CaBP57k mRNA was localized specifically to the fetal trophoblast of the mouse placenta and was expressed in a development-dependent manner. CaBP57k cDNA also hybridized strongly *in situ* to sections of human term placenta giving rise to signals localized specifically to the syncytiotrophoblastic layer of the chorionic villi *(28)*. The presence of CaBP57 on the surface of cytotrophoblast cells was also demonstrated by indirect immunofluorecence, fluorescent cytometry, and affinity chromatography *(29)*. In an Rcho-1 cell line derived from a rat choriocarcinoma, the equivalent of human trophoblastic cells, CaBP57k expression was upregulated during and/or following the differentiation of these cells into trophoblastic giant cells, supporting the importance of CaBP57 in trophoblast maturation *(30)*.

Oncomodulin is a tumor-specific CaBP, described for the first time by MacManus *(31)* in 1979. It is a develop-

mental protein, because cytotrophoblast cells of the fetal placenta are the only nontumor-associated cells synthesizing oncomodulin *(32)*. It is found in human and fetal rat placenta and preimplanted embryo tissues, as well as in several tissues of other species *(32–34)*. Using the dot-blot technique, Gillen et al. *(35)* demonstrated that oncomodulin mRNA levels in the placenta of rat were variable, as were the protein levels from day to day *(33)*, with the greatest increase occurring from d 14 to 18. Quantitative measurements by densitometry of dot blots revealed a direct relationship between oncomodulin and oncomodulin mRNA in the developing placenta, indicating transcriptional regulation of oncomodulin production *(36)*. It is conceivable that oncomodulin acts by activating enzymes or as a factor required during cell cycle in tumor cells. However, because of its restricted tissue distribution and varying expression in tumors, oncomodulin does not seem to be a general regulatory factor *(36)*.

S-100 proteins are CaBPs that are regulators of intracellular processes such as cell growth and motility, cell-cycle regulation, transcription, and differentiation *(37)*. They are not ubiquitously distributed in tissues and may, therefore, have specific cellular functions *(38)*. Twenty members have been identified so far, among which S-100P *(11)*, S-100α, and S-100β were detected in the placenta *(39)*. Regarding the trimester-dependent distribution of S-100β, the following results were found. In human first-trimester placenta, intense immunostaining for S-100β was localized at the embryomaternal interface in trophoblast cells *(38)*. In some human placentas, at a greater gestational age (10–12 wk of gestation), low levels of S-100β were also found in cytotrophoblast cells of chorionic villi. In the third trimester, cytotrophoblast and mesenchymal cells of chorionic villi stained for S-100β, as well as intermediate trophoblast cells. In late gestation, the intensity of S-100β staining varied from individual to individual. Most of the placenta at early third trimester contained low-level immunoreactive S-100β in the cytotrophoblast cells and in a few syncytiotrophoblasts of chorionic villi, whereas intermediate trophoblast cells were always positive. With advancing gestation, a more intense positive reaction was detectable in syncytiotrophoblasts of chorionic villi, which appeared to be positive in all term placenta and in the few cytotrophoblast cells still present. It is clear that the amount of S-100β in the placenta in total was greater in the second and third than in the first trimester. S-100β was localized to the amnion trophoblast cells, to the chorion decidual cells, and to the endothelial cells of cord vessels throughout gestation *(38)*.

Molecular Structure

The genes of the CaBP's EF-hand family have evolved from a common ancestor by gene duplication, transposition, and splicing *(40–42)* resulting in up to eight Ca^{2+}-binding sites. In most cases, functional Ca^{2+}-binding sites are not grouped in individual exons, indicating that the introns found presently were introduced after gene duplications and transpositions took place *(43)*.

The molecular structure of the CaBP9k gene is 5.5 kbp long and contains 3 exons interrupted by 2 introns while the first exon encodes almost the entire 5' noncoding region. The second exon codes for CaBP site I, and the third exon codes for CaBP site II and the 3' untranslated region. Gene transcription is initiated at a single site *(44)* and translated into a 9-kDa polypeptide protein.

Wilson et al. *(45)* and Fullmer & Wasserman *(46)* originally reported the complete amino acid sequence of CaBP28k for chicken intestine, and subsequently it was determined for mammalian tissues *(47,48)*. The protein consists of 261 amino acids forming a single polypeptide chain and has a computed molecular mass of 30.042 kDa *(46)*, which is comparable with the 28 kDa determined by sodium dodecyl sulfate gel electrophoresis. Mammalian interspecies homology for CaBP28k is 98% *(14)* and about 80% for chicken CaBP28k *(48)*. The chicken gene is 18.5 kbp long and is split into 11 coding exons separated by 10 introns *(49)*.

To our knowledge, only mouse CaBP57k has been mapped *(50,51)*. By combining the 5' Rapid Amplication of cDNA 5' Ends (5' RACE) technique and cDNA clone library screening, a 1.9-kbp full-length CaBP57k cDNA from mice was mapped. It encodes a protein of 417 amino acids with an acidic C-terminal domain. This CaBP57k sequence shows sequence homology to calreticulin, in particular the highly conserved P-domain of calreticulin *(52,53)*. Structural homology is also seen with other CaBPs such as calnexin *(54)* and extensin *(55)*, which share homology with calreticulin as well.

Although much work has been devoted to the elucidation of its structure and function, the biologic relevance of oncomodulin is still unclear *(33)*. Oncomodulin has three EF-hand motifs called AB, CD, and EF; only two of these are functional *(36)*. In the rat *(56,57)* and mouse *(58)*, the oncomodulin gene contains five identical exons except for the first exon, which is completely different from the others. The genes for the oncomodulin family of CaBPs are characterized by splice sites occurring within domains encoding Ca^{2+}-binding sites. By contrast, Ca^{2+} sites are separated by introns in genes for CaBP9k and the S-100 protein *(36)*. In humans, the oncomodulin gene has been mapped to the p1-p13 region of chromosome 7 *(59)*. This region corresponds to mouse chromosome 11 *(60)*, indicating that the oncomodulin locus is not contained within a syntenic group conserved in human and mouse.

The S-100 protein family is highly conserved even between species. S-100P from human placenta for instance, is not identical to any other reported member of the S-100 family *(11,61)*. However, an important aspect of the S-100 family is that most of the genes are located in a gene cluster 1q21

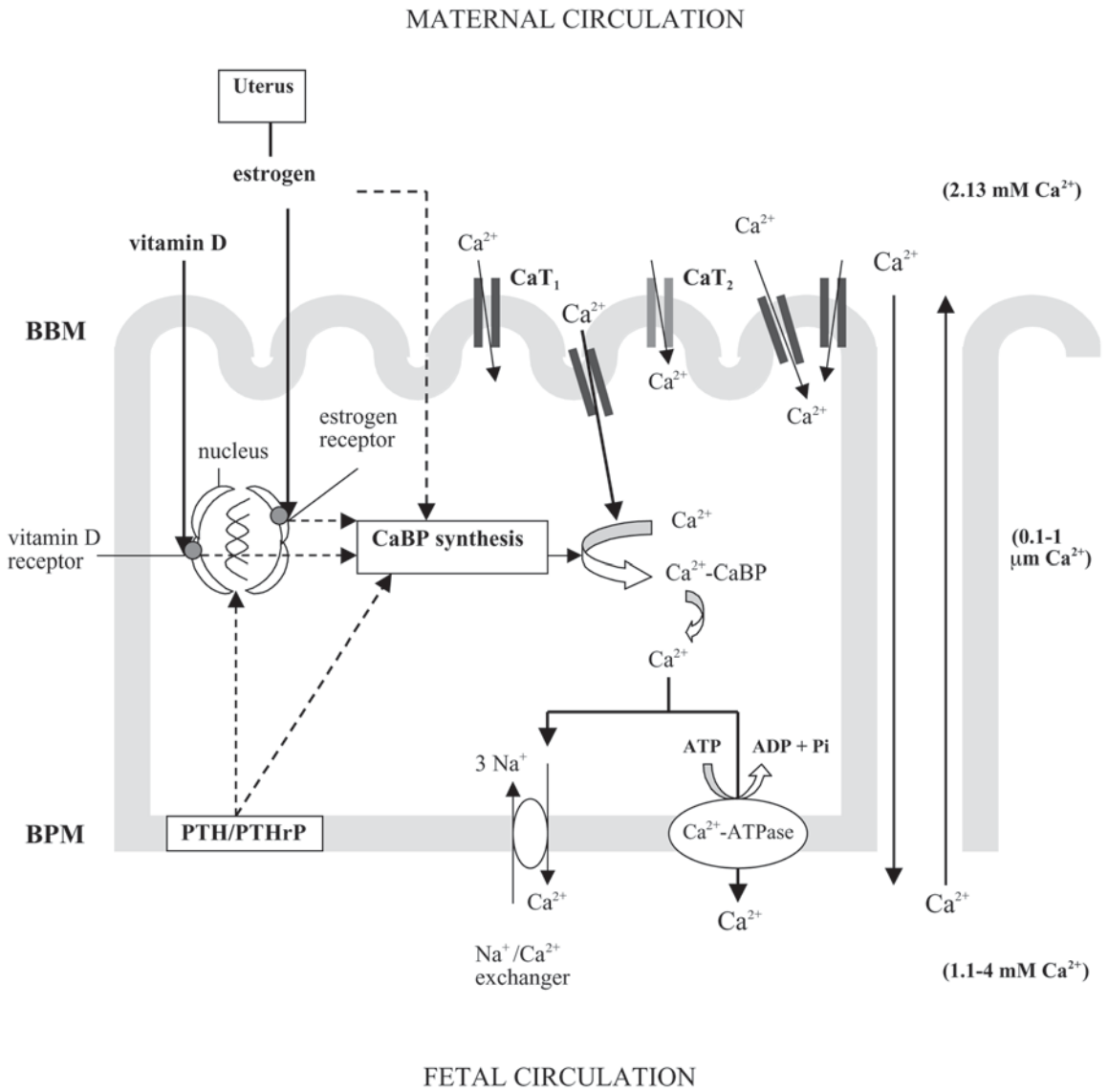

Fig. 1. Schematic representation of trophoblastic Ca^{2+} movement in correlation with CaBPs. Solid arrows represent known pathway; and dashed arrows represent hypothetical pathway.

region of the human genome structurally conserved during evolution *(62,63)*. S-100P represents a small acidic protein with 3 EF-hand motifs and a molecular mass of 10 kDa. It appears that S-100P shares 50% sequence homology with S-100β and S-100α *(11,12)*.

Ca^{2+} and CaBPs

Placental trophoblast cells are responsible for Ca^{2+} transport from the maternal to the fetal circulation. To date, no single specific factor has been conclusively shown to be involved in the exact mechanism of Ca^{2+} trophoblast transport although several factors have been postulated to play a role, including the CaBPs. Placental CaBPs were identified in the syncytiotrophoblasts *(51)*,which represent a physical and selective barrier for various nutrients crossing from the mother to the fetus. These cells are bipolar with two distinct membranes, a brush border membrane (BBM) facing the maternal circulation and a basal plasma membrane (BPM) facing the fetal circulation. The polarity of the syncytiotrophoblast structure involves certain particularities in relation to the transfer of Ca^{2+} through the placenta. BBM microvillosities provide a wider surface to facilitate absorption of nutrients. Because the concentration of Ca^{2+} in the maternal circulation is higher (2.13 m*M*) *(64)* compared with the cytosol of syncytiotrophoblasts (0.1–1 μ*M*) *(65)*, Ca^{2+} in the form of free Ca^{2+} and bound to CaBP diffuses across the BBM mostly by passive mechanisms in humans *(66)*. Following this, the ions exit into the fetal circulation by means of an active Ca^{2+}-adenosine triphosphatase (ATPase)-dependent mechanism located in the syncytiotrophoblasts of the chorionic villi *(67,68)* as well as Na^+/Ca^{2+} exchangers *(69)*

(Fig. 1). Therefore, CaBPs seem to have two proposed roles: (1) buffering and/or shuttling cytosolic Ca^{2+} from BBM to BPM, and (2) directly or indirectly regulating the Ca^{2+}-ATPase activity (BPM). As has been suggested, CaBPs may enhance transcellular Ca^{2+} transport by facilitating the action of Ca^{2+} as a second messenger *(70,71)*. The range of biochemical CaBP functions is correlated with differences in the way in which they respond to the binding of Ca^{2+}. According to Ingersoll and Wasserman *(72)* and Fullmer and Wasserman *(73)*, the order of cation binding affinity of the alkaline elements in transport machinery of the placenta relative to that of Ca^{2+} are as follows: Ca > Sr > Ba >> Mg.

In many mammalian tissues, such as intestine, pancreas, and placenta, epithelial calcium channels (CaT1 and CaT2), which represent a unique family of Ca^{2+}-selective channels *(74–76)*, are coexpressed with CaBPs *(77,78)*. In the human placenta *(78,79)*, these two channels are especially located in syncytiotrophoblasts *(80)*. Interestingly, in many tissues involved in transcellular calcium transport—namely, intestine, kidney and placenta—CaT1 is expressed more abundantly than CaT2 *(81)*. These channels seem to participate actively in transplacental calcium transport, since their expression is correlated with Ca^{2+} transport of syncytiotrophoblasts *(80)* and CaBP9k expression *(78)*.

In humans, movement of Ca^{2+} through the trophoblast cytosol would disrupt cellular processes as a consequence of large changes in Ca^{2+} unless there was sufficient buffering by CaBPs *(82)* or perhaps sequestration within intracellular organelles such as the endoplasmic reticulum *(83)*. A role for CaBP9k in maternal-fetal Ca^{2+} transport has been proposed since the mRNA *(84)* and placental levels of CaBP9k reach a maximun concentration in late gestation *(85)*. This conclusion is in accordance with studies sheep showing that the levels of CaBP9k mRNA had a pregnancy stage–related increase that correlated with fetal Ca^{2+} demand in maternal endometrial gland and fetal placentomal trophoblast epithelia *(86)*. In the case of rat placenta, Glazier et al. *(87)* demonstrated by Northern analysis that CaBP9k mRNA was temporally associated with a sharp gestational increase in Ca^{2+} transport between d 15 and 22 of gestation while rat placental Ca^{2+}-ATPase remained insignificant over the same gestation period. These trends suggest that the expression of placental CaBP9k but not Ca^{2+}-ATPase may be rate limiting to placental Ca^{2+} transport in the rat *(87)*. Conversely, in chicken shell gland, an analogous organ to the uterus, an active Ca^{2+}-ATPase has been localized to the gland cell apex *(88)*, where it seems to mediate Ca^{2+} transport out of the gland cell to known sites of mineral deposition during egg shell formation *(88)*. The gland cells show CaBP9k immunostaining in their cytosol and nucleoplasm *(89)*.

So far, there are no studies regarding the physiologic role(s) of placental CaBP28k, and very few data are available on CaBP57k. Hershberger and Tuan *(51)* have shown evidence for a functional implication of CaBP57k in placental Ca^{2+} transport and trophoblast cell differentiation. This functional role of CaBP57k in cellular Ca^{2+} handling was investigated using an Rcho-1 trophoblastic cell line, the equivalent of cytotrophoblast cells in human placenta. It was demonstrated that on differentiation, Rcho-1 cells exhibited an enhanced Ca^{2+} uptake compared with undifferentiated Rcho-1 stem cells together with an upregulation of CaBP57k. Conversely, downregulation of CaBP57k expression using antisens technology had a negative effect on Ca^{2+} uptake, Ca^{2+} transport, and trophoblast cell differentiation in Rcho-1 cells *(30)*. It is possible, therefore, that CaBP57k may play a cardinal role in placental Ca^{2+} transport by acting as an intracellular Ca^{2+} buffer/shuttle and promoting trophoblast differentiation *(30)*.

Oncomodulin is another placental CaBP, but its physiologic role(s) in correlation to Ca^{2+} transport has not been studied. However, based on the expression pattern of mammalian oncomodulin, it seems likely that this protein is involved in cytotrophoblast cell Ca^{2+}-dependent motility, which is important for invasive cytotrophoblast cells in the placenta *(43)*.

Unlike oncomodulin, S-100 proteins can be found in different cell types, where they are localized in specific cellular compartments from which some of them are able to relocate on Ca^{2+} activation *(90–93)*. Individual members of this family seem to actually utilize distinct pathways (endoplasmic reticulum–Golgi route, tubulin or actin associated) for their protein secretion before entering extracellular space *(90,94)*. In the case of S-100β, e.g., immunostaining is localized in cytoplasm, where it acts as a Ca^{2+}-modulated protein interacting with several target proteins such as adenylate cyclase, phospholipase C, and protein kinase C. These target proteins are involved in many cellular processes *(95)* including the regulation of the proliferation and/or differentiation of fetal cells.

Functions of CaBPs

Although the exact mechanisms of action of CaBPs are not known, several hormones such as vitamin D (1,25-$[OH]_2D_3$), estrogens, and parathyroid hormone–related proteins (PTHrP) seem to affect the action of CaBPs on Ca^{2+} transport. The following sections summarize experimental data and immunocytochemical localization studies.

Vitamin D and Estrogens

The maternal concentration of active vitamin D increases throughout gestation but particularly during the third trimester, during which it can be linked to an increased Ca^{2+} requirement of the fetus. In addition, the rate of Ca^{2+} uptake can be increased rapidly by an external supply of vitamin D in maternal circulation. Tanaka et al. *(96)* and Whitsett et al. *(97)* demonstrated the synthesis of vitamin D in the placenta, but its fetal levels were relatively low *(98)*. Human vitamin D nuclear receptor has been purified from nuclear fractions of the placenta *(99)*. To examine the effect of

vitamin D on Ca^{2+} transport, the concentration of vitamin D was quantified in nephrectomized sheep fetus and rat dams. In nephrectomized sheep, vitamin D concentration was reduced, and the fetal sheep became hypocalcemic after a reduction in the Ca^{2+} uptake into the maternal-fetal circulation. In addition to these characteristics—vitamin D deficiency and hypocalcemia—the rat dam and fetal skeletons were totally mineralized *(100)*.

Besides increasing Ca^{2+} transport, in vitro studies by Bruns et al. *(19)* have indicated that vitamin D can increase the synthesis of a 10-kDa CaBP in a cultured mouse yolk sac. However, in vivo experiments showed that mouse placental CaBP does not increase when vitamin D is administered to the mother *(101)*, possibly because there is only minimal transport of vitamin D from the mother to the fetus *(102)*. Jeung et al. *(103)* showed that the CaBP9k gene has a vitamin D response element (VDRE) detected about 1.1 kbp downstream from the promoter. Placental production of vitamin D together with the presence of VDRE for CaBP9k may indicate a fetal production of vitamin D that could provide an alternative source of hormone, at least for placental CaBP tissues harboring vitamin D receptors *(104)*. As for the regulation of CaBP9k synthesis, it is usually regulated by the active form of vitamin D, but in the uterus it is under the control of estrogen, as demonstrated in rat *(105)*. Furthermore, this expression is dependent on critical levels of estradiol 17β *(106,107)* and the presence of estrogen receptor *(108,109)*. Results from the levels of rat CaBP9k mRNA at different stages of gestation confirmed these observations. They showed that the CaBP9k mRNA level was highest at proestrus and not detectable at diestrous stage, implying a positive regulation of this protein by estrogen. While the exact CaBP9k estrogen response element (ERE) in humans has not been determined, Jeung et al. *(103)* reported a sequence positioned 50 nucleotides downstream from the human CaBP9k promoter showing extensive homology for the ERE at the same location within the rat CaBP9k gene. This ERE functions as an enhancer and a mediator of uterine and possibly placental CaBP9k expression in rat and probably in most other mammals.

PTHrP and PTH

PTHrP is produced throughout the embryo and during fetal development by the syncytiotrophoblasts of the placenta *(110,111)*, amnion *(112)*, chorion *(112,113)*, umbilical cord *(113)*, and fetal parathyroid glands *(110)*. The protein consists of 141 amino acids that may undergo processing to a 1-36/1-37 N-terminal fragment that resembles the PTH with 8 of the first 13 residues being identical to those of PTH *(114)*. PTH, acting through the PTH/PTHrP receptor (PTH1R), is the principal regulator of blood Ca^{2+} in human adults, while in the fetus, PTH and PTHrP share Ca^{2+} metabolism regulation through PTH1R and/or other receptors. Kovacs et al. *(115)* demonstrated that in homozygous fetal mice in which PTHrP gene or PTH1R has been ablated, the ionized Ca^{2+} was significantly lower compared with fetal heterozygous or wild-type littermates. Moreover, they showed that transplacental Ca^{2+} transport is reduced and that the maternal-fetal Ca^{2+} gradient is reversed in homozygous fetal mice. dePapp and Stewart *(116)*, while acknowledging the vital role of PTHrP in feto-placental Ca^{2+} transport, designated a region in the PTHrP sequence not shared with PTH for this function. Furthermore, Ramirez et al. *(117)*, Kovacs et al. *(115)*, and Wu et al. *(118)* found that PTHrP midregional and/or C-terminal fragments are the major fragment of PTHrP involved in Ca^{2+} transfer from mother to fetus. PTHrP is thought to function locally in an autocrine/paracrine manner *(119)*, and in an intracrine manner *(120)*, to exert many actions including translocation to the nucleus *(121)*, in order to produce growth factor effects.

Despite the importance of PTHrP and CaBPs in Ca^{2+} homeostasis in the placenta, very few investigations have been carried out to study the effect of PTHrP on the expression of CaBPs in the placenta. In 2002, Kovacs et al. *(122)* showed that PTHrP, PTH/PTHrP receptor, and CaBP9k were all highly expressed in the intraplacental yolk sac, compared to trophoblast cells in murine. They also indicated that in the PTHrP gene-deleted or PTHrP-null placenta in which placental Ca^{2+} transfer is decreased, CaBP9k expression was downregulated in the intraplacental yolk sac but not in the murine trophoblast cells *(122)*, which is not in line with the high receptor expression. The hormonal regulation of CaBP expression was analyzed in cultured placental tissues and in undifferentiated and differentiated Rcho-1 trophoblastic cell line. These cells were treated in vitro for 48 h with different agents implicated in Ca^{2+} homeostasis including PTH, PTHrP *(1–34)*, and PTHrP *(67–84)*, and the CaBP57k expression was evaluated. The results showed that in cultured placental tissues as well as in undifferentiated and differentiated Rcho-1 cells, these hormones had no effect on CaBP57k mRNA, while protein levels were significantly higher compared to the control cells. Consequently, the action of PTHrP on Ca^{2+} transport by placental trophoblast is likely to involve the regulation of CaBP57k expression, perhaps with the view to handle the increasing Ca^{2+} requirement of the developing fetus *(51)*. Further studies on the interaction between these parameters in vitro and in vivo may help to increase our understanding of the hormonal regulation of CaBP expression.

Conclusion

Various reports have appeared on the role and tissue/species distribution of CaBPs, and only a few parts of this active area of research have been touched on in this review. The actual knowledge of CaBPs in relation to Ca^{2+} in the placenta is summarized in Fig. 1 and Table 1. Despite all the progress made, it is still unclear how CaBPs operate in

the placenta—whether they serve as a diffusional facilitator or assume a totally different role—as well as how PTHrP, estrogen, and vitamin D can affect the production and functions of CaBPs. These and related questions represent challenging problems for current and future research.

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