Int. J. Dev. Biol. 54: 475-482 (2010)
doi: 10.1387/ijdb.082766am

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Trisomy 21- affected placentas highlight prerequisite factors for human trophoblast fusion and differentiation

ANDRÉ MALASSINÉ[1,2,3], JEAN-LOUIS FRENDO[1,2,3] and DANIÈLE EVAIN-BRION*[,1,2,3]

[1]INSERM, U767, [2]Université Paris Descartes and [3]PremUP, Fondation, Paris, France

ABSTRACT Trophoblastic cell fusion is one essential step of the human trophoblast differentiation pathway and is a multifactorial and dynamic process finely regulated and still poorly known. Disturbances of syncytiotrophoblast formation are observed in numerous pathological clinical conditions such as preeclampsia, intrauterine growth retardation and trisomy 21. In this review, we summarize current knowledge of the different membrane proteins directly involved in trophoblastic cell fusion, which we identified by using the physiological model of primary culture of villous trophoblastic cells. Connexin 43 and gap junctional intercellular communication point to the role of molecular exchanges through connexin channels preceding membrane fusion. Zona occludens-1, which can interact with connexin 43, is also directly involved in trophoblast fusion. The recently identified fusogenic membrane retroviral envelop glycoproteins syncytin 1 (encoded by the *HERV-W* gene) and syncytin 2 (encoded by the *FRD* gene) and their receptors are major factors involved in human placental development . We describe the increasing number of factors promoting or inhibiting trophoblast fusion and differentiation and emphasize the role of human chorionic gonadotropin (hCG) and its receptor. Indeed, in trisomy 21 the dynamic process leading to membrane fusion is impaired due to an abnormal hCG signaling. This abnormal trophoblast fusion and differentiation in trisomy 21-affected placenta is reversible *in vitro.* Trisomy 21 trophoblastic cell culture may therefore be useful to identify the possible large number of prerequisite factors involved in trophoblast fusion, the limiting step of trophoblast differentiation.

KEY WORDS: *connexin 43, ZO-1, syncytin, hCG, human placenta*

In the human placenta, the trophoblast differentiates along two major pathways both critical for normal placental functions (Benirschke and Kaufmann, 2000). In the extravillous trophoblast invasive pathway, the cytotrophoblastic cells of the anchoring villi in contact with the uterus wall proliferate, detach from the basement membrane and aggregate into multilayered columns of non-polarized cells that invade the uterus wall (Fig.1). These cells, which compose the extravillous cytotrophoblast (ECT), invade the endometrium, the first third of the myometrium and the associated spiral arterioles. In the villous trophoblast pathway, the cytotrophoblastic cells (CT) of the floating villi proliferate, differentiate and fuse to form a syncytiotrophoblast (ST) that covers the entire surface of the villi (Fig.1). The syncytiotrophoblast layer plays a major role throughout pregnancy, since it is the site of numerous placental functions, including ion and nutrient exchange and the synthesis of steroid and peptide hormones required for fetal growth and development (Eaton and Contractor, 1993; Ogren and Talamentes, 1994). Among them, progesterone and hCG (human Chorionic Gonadotropin) are absolutely required for human gestation. Therefore, disturbances of syncytiotrophoblast formation or functions are observed in pathological clinical conditions such as preeclampsia (Langbein *et al.*, 2008) and trisomy 21 (Frendo *et al.*, 2004; Frendo *et al.*, 2000b; Massin *et al.*, 2001; Pidoux *et al.*, 2007a; Pidoux *et al.*, 2004b) and may be implicated in abnormal fetal growth and development.

The multinucleated syncytiotrophoblast is regenerated along pregnancy by a continuous turnover process including proliferation of mononuclear CT followed by the induction of early stages of apoptosis and fusion of these CT into ST. Apoptotic progres-

Abbreviations used in this paper: CT, cytotrophoblastic cell; ECT, extravillous cytotrophoblast; GJIC, gap junction intercellular communication; hCG, human chorionic gonadotropin; ST, syncytiotrophoblast; T21, trisomy 21; ZO, zona occludens.

***Address correspondence to:** Danièle Evain-Brion. INSERM U767, Faculté de pharmacie, 4 Avenue de l'Observatoire, 75006 Paris, France. Fax: +33-1-44-07-3992. e-mail: daniele.evain-brion@univ-paris5.fr

Final author-corrected PDF published online: 21 September 2009.

ISSN: Online 1696-3547, Print 0214-6282

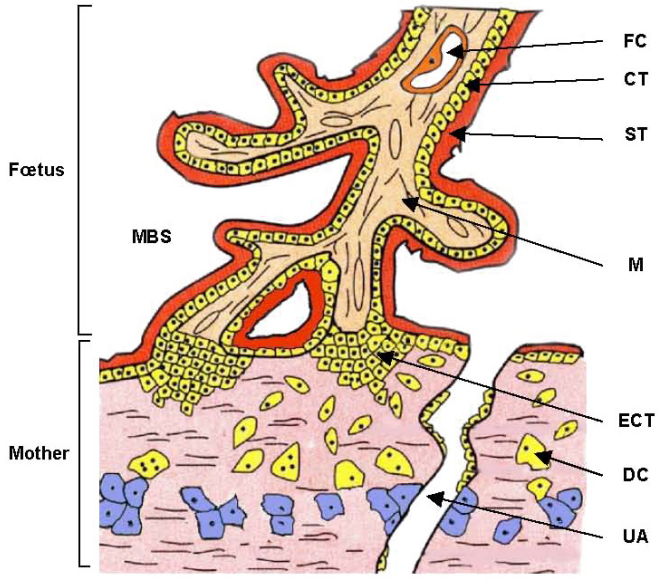


Fig. 1. Schema of human chorionic villi. *In humans, at 10-12 weeks of pregnancy, the chorionic floating villi are in contact with the maternal blood in the maternal blood space (MBS). In these villi, cytotrophoblastic cells (CT) differentiate by fusion to generate the syncytiotrophoblast (ST). In the anchoring villi the cytotrophoblastic cells proliferate and invade the decidua (DC). The extravillous cytotrophoblastic cells (ECT) invade the uterine stroma and differentiate into multinucleated giant cells and invade also the lumen of uterine arteries (UA). FC, fetal capillary; M, mesenchyme.*

sion in the ST leads to the accumulation of condensed nuclei into syncytial knots and the shedding of these aggregates into the intervillous spaces. Syncytiotrophoblast formation can be reproduced *in vitro* using different models. Choriocarcinoma cells, *ie* BeWo cells, are able to fuse in presence of cAMP to form a multinucleated syncytium. However, the last step of differentiation, the gathering of nulei into a central mount, is missing. In addition, these cells are transformed and some trophoblastic functions are not present (King *et al.*, 2000). Denude villous explants from early placenta allow to follow the *in vitro* ST repairing. In this model, the cell-cell interactions are present, but cell-cell communication studies and quantification of trophoblastic hormones are difficult to assess. Purified villous CT, cultured on plastic dishes, aggregate and fuse forming the multinucleated ST (Fig. 2A) with pregnancy specific hormonal production (*ie* hCG, Progesterone) (Kliman *et al.*, 1986). This model allows accurate biochemical studies.

Due to the key role of trophoblastic cell fusion in human pregnancy, we tried to identify the membrane proteins directly implicated in the ST formation (in normal and pathological conditions) using the physiological model of villous trophoblastic cells primary culture. The Trisomy 21 (T21) affected trophoblast model of abnormal fusion and differentiation allowed us to unravel the major factors involved in these processes.

Membrane proteins involved in trophoblastic cell fusion

Before two cells can fuse, several steps are needed. Firstly, the cells must leave the proliferative stage and express genes and proteins involved in the fusion process. Secondly, they must recognize and interact to their fusion partner. Thirdly, the cells must communicate together, allowing signals exchange. Lastly, they can fuse. Therefore, this dynamic process may be finely regulated and coordinated.

The identification of a protein directly involved in trophoblastic cell fusion, requires not only the localization of this protein at the membrane level during one step of the process, but also to demonstrate that the knock-down of it synthesis (siRNA or antisense strategy) induces an inhibition or a decrease of trophoblastic cell fusion.

During last years, we used the physiological model of villous CT primary culture to analyze the expression and the role of different membrane proteins in trophoblast fusion. Cells were isolated from first, second trimester and term placentas. Protein expression, mRNA and cellular localization of connexin 43, zona occludens-1 (ZO-1), syncytin 1 (HERV-W envelop glycoprotein) and syncytin 2 (HERV-FRD envelop glycoprotein) were analyzed during the different steps of villous trophoblastic cells differentiation: isolated cells, aggregated cells, fused cells.

Connexin 43 and Gap Junctional Intercellular Communication (GJIC)

Gap junctions are clusters of trans-membrane channels composed of connexin (Cx) hexamers. Gap junctions provide pathway for the diffusion of ions and small molecules such as cAMP, cGMP, inositol triphosphate and Ca^{2+}. Connexins represent a family of closely related membrane proteins, which are encoded by a multigene family that contains at least 20 members in humans. These connexins have different biophysical properties, functional and regulatory characteristics (Willecke *et al.*, 2002). The permeability of junctional channels is finely regulated. This regulation involves the cyclic phosphorylation and dephosphorylation of connexins and changes in intracellular Ca^{2+}, H^+ and cAMP. The exchange of molecules through gap junctions is thought to be involved in the control of cell proliferation, in the control of cell and tissue differentiation, in metabolic cooperation and in spatial compartimentalization during embryonic development (Bani-Yaghoub *et al.*, 1999; Lecanda *et al.*, 1998; Loewenstein, 1981; Saez *et al.*, 1993). Furthermore other effects of connexins expression have been attributed to the interaction of Cx to the intracellular signal cascades via the carboxy-terminus (Herve *et al.*, 2004).

We have previously demonstrated, both *in situ* and *in vitro* the expression of Cx43 mRNA in villous trophoblast and the localization of Cx43 protein between CTs and between CTs and ST whereas Cx26, Cx32, Cx33 and Cx40 were not detected. Furthermore, using fluorescence recovery after photobleaching method (gap-FRAP) we demonstrated the presence and the requirement of a functional inter trophoblastic communication via gap junctions (Cronier *et al.*, 1994; Malassine and Cronier, 2005). Futhermore, cultured CT treated with Cx43 antisense aggregate and fuse poorly. This treatment dramatically reduces the percentage of coupled cells as demonstrated by the gap-FRAP method (Frendo *et al.*, 2003a). This demonstrates that the molecular exchanges through connexins channels, preceding membrane fusion, are one essential step for trophoblastic cell fusion.

Zona occludens-1 (ZO-1)

Secondary proteins interacting with connexins have been iden-

tified: chaperones, scaffolding proteins, kinases, phosphatases, cells signaling molecules (for review see Herve *et al.*, 2004). Among these partner proteins, zona occludens-1 (ZO-1) has been demonstrated to interact with multiple connexins. ZO-1 is a 220 kDa peripheral membrane protein, which has been originally identified in association with tight junction (Anderson *et al.*, 1988; Stevenson *et al.*, 1986) and later at adherens junction. ZO-1 contains multiple protein interaction domains including three PSD95/Dlg/ZO-1 (PDZ) domain and a Src homology 3 (SH3) domain (Anderson *et al.*, 1988; Beatch *et al.*, 1996; Itoh *et al.*, 1993; Willott *et al.*, 1993). A recent study using nuclear magnetic resonance method, demonstrated that the interaction of Cx43 with ZO-1 occurs through the last 20 amino acids in the extreme carboxyl terminus of Cx43 and the second PDZ domain of ZO-1 (Giepmans and Moolenaar, 1998; Sorgen *et al.*, 2004). Divergent roles have been proposed for the interaction of ZO-1 and Cx43. Changes in Cx43-ZO-1 interaction have been noted during remodeling of gap junctions in different cell types (Barker *et al.*, 2002; Defamie *et al.*, 2001; Segretain *et al.*, 2004). Modulation of Cx43 and ZO-1 interactions may also be involved in gap junction formation, localization and activity (Duffy *et al.*, 2004; Giepmans *et al.*, 2001; Hunter *et al.*, 2005; Sorgen *et al.*, 2004; Toyofuku *et al.*, 2001). A role in internalization and remodeling of Cx43 in response to intracellular changes (Barker *et al.*, 2002) and in targeting for endocytosis (Segretain *et al.*, 2004) was also demonstrated.

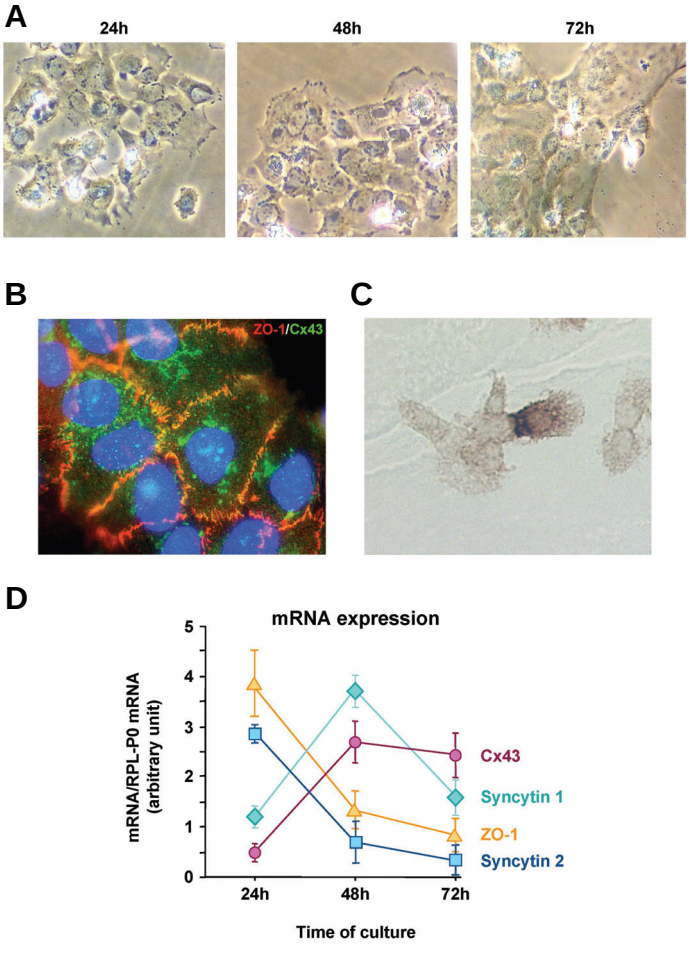


Fig. 2. Evolution of membrane protein expression during *in vitro* differentiation of trophoblastic cells isolated from normal placenta. **(A)** *Morphological differentiation of isolated cytotrophoblast cultured on plastic dishes. After one day, pseudopodia of CT are making contact with neighboring CT. After two days, CT are mainly aggregated. After three days, large syncytiotrophoblast (ST) are observed with central nuclear mounts.* **(B)** *Immuno-colocalization of Cx43 (green fluorescence) and ZO-1 (red fluorescence) on aggregated CT at 48 hours of culture.* **(C)** *Immunostaining of syncytin 2 on aggregated CT at 48 hours of culture. Staining is observed in some CT with a stronger staining at intercellular sites. Scale bars: 10 µm.* **(D)** *Real-time RT-PCR analysis of* syncytin 1, syncytin 2, ZO-1 *and* Cx43 *mRNA during* in vitro *differentiation of trophoblastic cells. Datas are expressed as the level of each mRNA normalized to that of* RPL-P0 *mRNA.*

Therefore, we have investigated the role of ZO-1 in the trophoblastic cell fusion process. *In situ*, ZO-1 and Cx43 immunocolocalized at some intercellular boundaries, between CTs and between CTs and ST. During *in vitro* trophoblast differentiation ZO-1 was localized only at the intercellular boundaries of aggregated cells and its expression decreased. Cx43 expression had the same localization but its expression increased during differentiation. Cx43 and ZO-1 co-localized only at some intercellular boundaries of aggregated cells (Fig. 2B left panel). Moreover, by using co-immunoprecipitation experiments, a physical interaction between ZO-1 and Cx43 was demonstrated. To determine a functional role for ZO-1 during trophoblast differentiation a siRNA strategy was used to knock-down ZO-1 expression. CTs treated with ZO-1 siRNA aggregated but fused poorly and less hCG secretion was detected. Furthermore, Cx43 expression was decreased in ZO-1 siRNA treated CTs. Moreover, this treatment did not alter the functionality of trophoblastic cell-cell communication assessed by Gap-Frap method. These results demonstrate that ZO-1 expression is required for trophoblastic cell fusion.

Fusogenic membrane retroviral envelop glycoproteins: syncytin 1 and 2

Human endogenous retroviruses (HERV) comprise approximately 8% of the human genome (de Parseval and Heidmann, 2005; Lander *et al.*, 2001). Most of the identified elements are defective due to mutations and/or deletions within their genes, but some elements have conserved intact open reading frames. A systematic search for non-defective endogenous retrovirus envelope genes has led to the identification of 16 genes (de Parseval *et al.*, 2003). Among them two can induce cell-cell fusion when expressed in different cells and are highly and specifically expressed in the human placenta (Blaise *et al.*, 2003; Blond *et al.*, 2000; Mi *et al.*, 2000).

The products of these two genes are glycoproteins named HERV-W Env glycoprotein (syncytin 1) and HERV-FRD Env glycoprotein (syncytin 2). Syncytin 1 and syncytin 2 originate from distinct retroviral elements, and disclose several differences. Syncytin 2 entered the primate genomes earlier than syncytin 1, namely before the split between New World and Old World Monkeys (*i.e* > 40 Myrs ago), whereas syncytin 1 entered about 25 Myrs ago, being not found in Old World Monkeys. Yet, both genes remained functional in all the corresponding primate branches, thus strongly suggesting selection for a physiological role. Syncytin 1 and syncytin 2 also differ by their receptor (still not identified for syncytin 2), as demonstrated by *ex vivo* cell-cell

fusion assays using different cell types. In addition it has been shown recently that syncytin 2 is immunosuppressive and syncytin 1 is not (Mangeney *et al.*, 2007).

We first demonstrated using monoclonal and polyclonal antibodies that *in situ* syncytin1 is immunolocalized in all cell type of human trophoblast: villous and extravillous trophoblast. Syncytin 1 is fusogenic by interacting with the D type mammalian retrovirus receptor (RDR) also known to be the neutral amino acid transporter ATB0/ASCT2/SLC1A5. We showed that the D type mammalian retrovirus receptor is also localized in the various trophoblastic phenotypes (Malassine *et al.*, 2005).

We analysed the involvement of syncytin 1 in the differentiation of CT in culture. First, the syncytin 1 mRNA and glycoprotein expressions were found to increase with cell aggregation and fusion and then to slightly decrease (Fig. 2C). Second, *in vitro* stimulation of trophoblast cell fusion and differentiation by cAMP is associated with a concomitant increase in syncytin 1 and hCG mRNAs and protein expression as well. Finally, we demonstrated by using specific antisense oligonucleotides that inhibition of syncytin1 expression lead to a decrease of trophoblast fusion and differentiation (Frendo *et al.*, 2003b), hCG secretion in the culture medium of antisense treated cells being decreased by 5 fold. Furthermore the inhibition of trophoblast fusion by overexpression of superoxide dismutatse 1 (SOD-1) is associated with an absence of increase in HERV-W env mRNA (Frendo *et al.*, 2001; Frendo *et al.*, 2000a).

All together, these results strongly support a direct role of syncytin1 in human trophoblastic cell fusion and differentiation. However, the co-localization of syncytin1 and its receptor in trophoblastic cells that do not fuse (extravillous trophoblast) (Muir *et al.*, 2006) suggests that syncytin1 and its receptor appear to be required but not sufficient for trophoblastic cell fusion.

We then studied the expression of syncytin2 in human placenta. *In situ,* syncytin 2 immunolocalization is restricted to some CT of the villous trophoblast. Furthermore, this localization highlights the modification of CT shape from cuboïdal in early placenta to flat with cytoplasmic processes in term placenta (Malassine *et al.*, 2008). *In vitro*, syncytin 2 is only observed in some aggregated CTs, with stronger staining at intercellular sites (Fig. 2C). Syncytin 2 transcript levels decrease significantly upon CT fusion into ST in culture (Fig. 2D). Syncytin 2 is highly fusogenic when overexpressed by transfection in various cultured cells, however its role in villous trophoblastic fusion has not been yet demonstrated.

Kinetics of expression of Cx43, ZO-1 and syncytins during syncytiotrophoblast formation

Expression of these proteins at the right time to correct place is a challenge for the CT fusion. Therefore the evolution of Cx43, ZO-1 as well as syncytin 1 and 2 expression was analyzed during *in vitro* trophoblast differentiation.

As shown in Fig. 2C it appears that syncytin 2 and ZO-1 are highly expressed in isolated CTs and their expression rapidly decrease during ST formation. On the other hand, syncytin1 and Cx43 mRNA increase with cell aggregation and fusion and then slightly decrease. Proteins expression follows the same pattern (data not shown). These results are in agreement with the fact that Cx43 and ZO-1 are mainly co-localized during the aggregation stage at 48 hours of culture. In addition, these results illustrate the striking difference in syncytin1 and 2 localization: syncytin 2 only in some CT and syncytin 1 in all CT and ST.

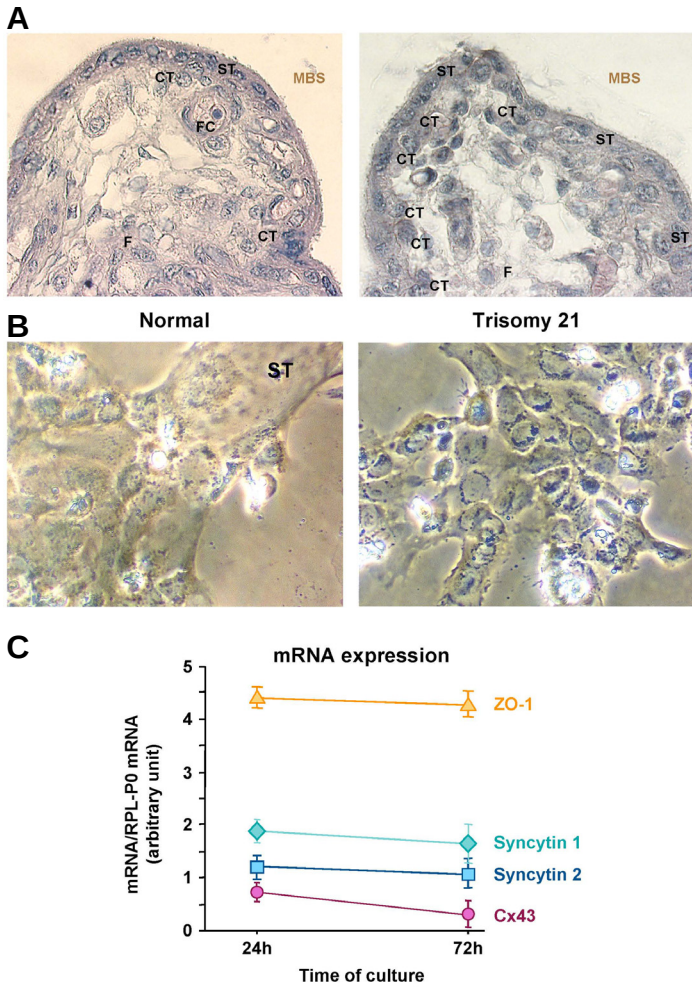


Fig. 3. Evolution of membrane protein expression during *in vitro* differentiation of trophoblastic cells isolated from trisomy 21-affected placenta. (A) *Microscopic morphology of second trimester chorionic villi of normal placentas (19 weeks of amenorrhea) and trisomy 21-affected placentas (18 weeks of amenorrhea). In normal placenta, a large amount of cytotrophoblastic cells (CT) have fused into a thin multinucleated syncytiotrophoblast (ST). In trisomy 21 placenta, many cuboidal cytotrophoblastic cells (CT) are still present beneath the syncytiotrophoblast (ST) increasing the thickness of the trophoblastic layer. Scale bar = 10 μm.* **(B)** *Morphological differentiation during* in vitro *culture of normal and T21 trophoblastic cells. Cytotrophoblastic cells were purified from three distinct age matched (second trimester) normal and T21-affected placentas and separately cultured. The cells were visualized under phase contrast light microscopy (Scale bar = 10 μm). At 72 h, normal cytotrophoblastic cells had fused resulting in the formation of a large syncytium containing numerous nuclei. In contrast, T21 cytotrophoblasts were still aggregated and had not fused.* **(C)** *Real-time RT-PCR analysis of* syncytin 1, syncytin 2, ZO-1 *and* Cx43 *mRNA during* in vitro *differentiation of T21 trophoblastic cells. Total mRNA were extracted after 24 and 72 h of culture. Data are expressed as the level of each mRNA normalized to that of* RPL-P0 *mRNA.*

TABLE 1

MAIN FACTORS MODULATING TROPHOBLASTIC CELL FUSION AND DIFFERENTIATION *IN VITRO*

Stimulation action	Inhibiting action
EGF (Alsat *et al.*, 1993; Morrish *et al.*, 1987)	TGF-β1 (Cronier *et al.*, 1997a; Morrish *et al.*, 1991)
hCG (Cronier *et al.*, 1994; Shi *et al.*, 1993)	
cAMP (Cronier *et al.*, 1997b; Keryer *et al.*, 1998)	LIF (Nachtigall *et al.*, 1996)
GM-CSF (Garcia-Lloret *et al.*, 1994)	Hypoxia, SOD-1 (Alsat *et al.*, 1996; Frendo *et al.*, 2001; Frendo *et al.*, 2000a; Levy *et al.*, 2000)
Macrophages and macrophage-conditionned media (Cervar *et al.*, 1999; Khan *et al.*, 2000)	Endothelin (Cronier *et al.*, 1999a)
Dexamethasone (Cronier *et al.*, 1998)	15ΔPGJ2 (Levy *et al.*, 2000)
Estradiol (Cronier *et al.*, 1999b)	TNFα (Leisser *et al.*, 2006)

Trisomy 21 as a model of abnormal human trophoblast fusion and differentiation

Trisomy of chromosome 21 (T21), which causes the phenotype known as Down syndrome, is the major known genetic cause of mental retardation and is found in around 1:800 live births. Little is known about placental development in this aneuploid condition. However, a defect in syncytiotrophoblast formation in T21-affected placentas is observed. Cultured cytotrophoblasts, isolated from T21-affected placentas, aggregate but fuse poorly or belatedly (Frendo *et al.*, 2000b; Massin *et al.*, 2001). This is in agreement with previous histological observations pointing to an increased percentage of two layered trophoblast in T21 placentas (Oberweiss *et al.*, 1983; Roberts *et al.*, 2000) (Fig. 3A). In addition, we demonstrated that this *in vitro* defect or delay in syncytiotrophoblast formation is characterized by a dramatic decrease in the synthesis of syncytiotrophoblastic pregnancy-associated hormones (Pidoux *et al.*, 2004a) and by the secretion of an hyperglycosylated hCG with low bioactivity (Frendo *et al.*, 2004). This abnormal trophoblast fusion implicates at least in part overexpression of SOD-1 (Frendo *et al.*, 2002). In addition we recently showed that during the second trimester of pregnancy, syncytin 2 is immunolocalized in some cuboidal CTs in T21 placentas, whereas in normal placentas it is observed in flat CTs extending into their cytoplasmic processes. These results highlight the abnormal trophobalst differentiation observed in trisomy 21 affected placentas.

As shown in figure 3B, CT isolated from T21 affected placenta, aggregate normally but do not fuse or fuse poorly. In these cells, transcript levels of Cx 43, ZO-1, syncytin1 and 2 do not vary with time in culture (Fig. 3C). Proteins expression follows the same pattern (data not shown).

In contrast, in normal CT the decrease of ZO-1 and syncytin1 and the increase of Cx43 and Syncytin 2 expression are associated with cell-cell fusion. This highlights that the timely regulated expression of these proteins seems to be required for normal trophoblast fusion.

Other factors involved in trophoblastic cell fusion

During last years, in different *in vitro* models such as BeWo cells and explants culture other factors have been described to be directly involved in trophoblastic cell fusion: the phosphatidylserine (Adler *et al.*, 1995), cadherin 11 (Getsios and MacCalman, 2003), CD 98 (Dalton *et al.*, 2007), caspase 8 (Black *et al.*, 2004), ADAMs12 (Huppertz *et al.*, 2006). Phosphatidylserine flip is a phospholipid normally confined to the inner layer of the plasma membrane but prior to fusion this translocates to the outer layer and facilitates inter-membrane fusion. According to Huppertz and Kingdom (Huppertz and Kingdom, 2004), this phosphatidylserine flip is a consequence of activation of initiator caspase (caspase 8) leading to the concept that the molecular machinery of early apoptosis is involved in the fusion process. ADAM 12 (meltrin alpha) is a disintegrin and metalloprotease involved in myoblast fusion and CD 98 is an integral membrane protein with N-linked glycosylation sites known to be important for adhesion, aminoacid transport and cell-cell fusion as well as virus induced cell fusion. Manipulations of CD98 expression by antisense oligonucleotide and small interfering RNA affect both amino acid transport and cell fusion in BeWo cells (Kudo *et al.*, 2003). Furthermore, primary CTs cultured in the presence of antisense oligonucleotides specific for cadherin-11 a cell adhesion molecule, do not undergo terminal differentiation and fusion with time in culture (Getsios and MacCalman, 2003). In the present study we show for the first time that the expression of some factors required for trophoblast fusion are timely regulated. Indeed it is conceivable that cells must be joined by tight junctions and adherens junctions prior to establish gap junctions, gap junctionnal communication and to initiate membrane fusion. This latest process is facilitated by the syncytins which might form bundles of alpha-helices similar to the class 1 viral fusion protein that bring membranes close together (Larsson *et al.*, 2008).

On the other hand, an increasing number of factors promoting or inhibiting trophoblastic cell fusion and differentiation is described through the literature and summarized in Table 1. They are described to modulate *in vitro* the ST formation in primary cultures of villous CT, and they illustrate the numerous signaling pathways implicated in trophoblast differentiation. However little is known on the role of these soluble factors on the expression, the membrane localization and /or the interaction of the different identified partners of cell fusion.

Among these factors, hCG might be the major one. Since the study of Shi *et al.* (Shi *et al.*, 1993) demonstrating that hCG has a direct role in trophoblast differentiation, different studies have confirmed the importance of hCG and its membrane receptor in syncytiotrophoblast formation (Pidoux *et al.*, 2007a; Pidoux *et al.*, 2007b; Shi *et al.*, 1993). The hCG receptor, which has seven transmembrane domains, belongs to the superfamily of G protein-coupled receptors (Loosfelt *et al.*, 1989; McFarland *et al.*, 1989; Minegishi *et al.*, 1990; Pierce and Parsons, 1981). HCG binding to its receptor activates adenylate cyclase, phospholipase C and ion channels, which in turn control cellular cAMP, inositol phosphates, Ca^{2+} and other secondary messengers (Gudermann *et al.*, 1992; Hipkin *et al.*, 1992). Agents increasing

cellular levels of cAMP promote cytotrophoblast fusions *in vitro* (Keryer *et al.*, 1998) and also elevate mRNA levels of syncytin1 in cultured trophoblasts (Frendo *et al.*, 2003b). Interestingly syncytin 1 was shown to be a target gene of GCMa, a placenta specific transcription factor that is required for placental development in mouse (Schreiber *et al.*, 2000). GCMa, which possesses two PKA phosphorylation sites might be phosphorylated by PKA inducing a stimulation of its transcriptional activity and therefore syncytin1 expression (Knerr *et al.*, 2005).

Interestingly, we recently demonstrated that Trisomy 21 affected trophoblastic cells produce abnormally glycosylated forms of hCG with low biological activity (Frendo *et al.*, 2004). Furthermore, these cells express a low number of hCG/LH receptors (Pidoux *et al.*, 2007a). Endly, we demonstrated that the *in vitro* defect of ST formation in T21 is reversible when CT are treated with biosynthetic hCG (Pidoux *et al.*, 2007a). These results confirm in a pathological model the major role of hCG and its receptor in trophoblast fusion and differentiation.

Concluding remarks

Trophoblast fusion is one essential step of the trophoblast differentiation pathway and is a multifactorial and dynamic process finely regulated and still poorly known. We and others are just at the beginning of the identification of possibly a large number of prerequisite factors for this process. The recently identified retroviral envelop glycoproteins (syncytins), highly fusogenic proteins, appear to play a major role. Identification of syncytin 2 receptor will allow us a better understanding of its exact role in placental development. In addition our results point to the fact that abnormal trophoblast fusion and differentiation in T21-affected placenta is reversible *in vitro*.

References

ADLER, R., NG, A. and ROTE, N. (1995). Monoclonal antiphosphatidylserine antibody inhibits intercellular fusion of the choriocarcinoma line, JAR. *Biol Reprod* 53: 905-910.

ALSAT, E., HAZIZA, J. and EVAIN-BRION, D. (1993). Increase in epidermal growth factor receptor and its messenger ribonucleic acid levels with differentiation of human trophoblast cells in culture. *J Cell Physiol* 154: 122-128.

ALSAT, E., WYPLOSZ, P., MALASSINÉ, A., GUIBOURDENCHE, J., PORQUET, D., NESSMANN, C. and EVAIN-BRION, D. (1996). Hypoxia impairs cell fusion and differentiation process in human cytotrophoblast, *in vitro*. *J Cell Physiol* 168: 346-353.

ANDERSON, J.M., STEVENSON, B.R., JESAITIS, L.A., GOODENOUGH, D.A. and MOOSEKER, M.S. (1988). Characterization of ZO-1, a protein component of the tight junction from mouse liver and Madin-Darby canine kidney cells. *J Cell Biol* 106: 1141-1149.

BANI-YAGHOUB, M., BECHBERGER, J., UNDERHILL, T. and NAUS, C. (1999). The effects of gap junction blockage on neuronal differentiation of human NTera2/clone D1 cells. *Exp Neurol* 156: 16-32.

BARKER, R.J, PRICE, R.L and GOURDIE, R.G. (2002). Increased association of ZO-1 with connexin43 during remodeling of cardiac gap junctions. *Circ Res* 90: 317-324.

BEATCH, M., JESAITIS, L.A., GALLIN, W.J., GOODENOUGH, D.A. and STEVENSON, B.R. (1996). The tight junction protein ZO-2 contains three PDZ (PSD-95/Discs-Large/ZO-1) domains and an alternatively spliced region. *J Biol Chem* 271: 25723-25726.

BENIRSCHKE, K. and KAUFMANN, P. (2000). Pathology of the human placenta. New-York. Springer-Verlag. pp 22-70.

BLACK, S., KADYROV, M., KAUFMANN, P., UGELE, B., EMANS, N. and HUPPERTZ, B. (2004). Syncytial fusion of human trophoblast depends on caspase 8. *Cell Death Differ* 11: 90-98.

BLAISE, S., DE PARSEVAL, N., BENIT, L. and HEIDMANN, T. (2003). Genomewide screening for fusogenic human endogenous retrovirus envelopes identifies syncytin 2, a gene conserved on primate evolution. *Proc Natl Acad Sci USA* 100: 13013-13018.

BLOND, J-L., LAVILLETTE, D., CHEYNET, V., BOUTON, O., ORIOL, G., CHAPEL-FERNANDES, S., MANDRAND, B., MALLET, F. and COSSET, F.L. (2000). An envelope glycoprotein of the human endogenous retrovirus HERV-W is expressed in the human placenta and fuses cells expressing the type D mammalian retrovirus receptor. *J Virol* 74: 3321-3329.

CERVAR, M., BLASCHITZ, A., DOHR, G. and DESOYE, G. (1999). Paracrine regulation of distinct trophoblast functions *in vitro* by placental macrophages. *Cell Tissue Res* 295: 297-305.

CRONIER, L., ALSAT, E., HERVE, J-C., DELEZE, J. and MALASSINE, A. (1997a). Transforming growth factor beta1 (TGFb1) inhibits gap junctional communication during human trophoblast differentiation. *Trophobl Res* 10: 377-391.

CRONIER, L., ALSAT, E., HERVÉ, J-C., DELÈZE, J. and MALASSINÉ, A. (1998). Dexamehasone stimulates Gap junctional communication peptide hormone production and differentiation in human term trophoblast. *Trophobl Res* 11: 35-49.

CRONIER, L., BASTIDE, B., HERVÉ, J-C., DELÈZE, J. and MALASSINÉ, A. (1994). Gap junctional communication during human trophoblast differentiation: influence of human chorionic gonadotropin. *Endocrinology* 135: 402-408.

CRONIER, L., DUBUT, A., GUIBOURDENCHE, J. and MALASSINÉ, A. (1999a). Effects of endothelin on villous trophoblast differentiation and free intracellular calcium. *Trophobl Res* 13: 69-86.

CRONIER, L., GUIBOURDENCHE, J., NIGER, C. and MALASSINE, A. (1999b). Oestradiol stimulates morphological and functional differentiation of human villous cytotrophoblast. *Placenta* 20: 669-676.

CRONIER, L., HERVE, J., DELEZE, J. and MALASSINE, A. (1997b). Regulation of gap junctional communication during human trophoblast differentiation. *Microsc Res Tech* 38: 21-28.

DALTON, P., CHRISTIAN, H.C., REDMAN, C.W., SARGENT, I.L. and BOYD, C.A. (2007). Membrane trafficking of CD98 and its ligand galectin 3 in BeWo cells--implication for placental cell fusion. *FEBS J* 274: 2715-2727.

DE PARSEVAL, N. and HEIDMANN, T. (2005). Human endogenous retroviruses: from infectious elements to human genes. *Cytogenet Genome Res* 110: 318-332.

DE PARSEVAL, N., LAZAR, V., CASELLA, J-F., BENIT, L. and HEIDMANN, T. (2003). Survey of human genes of retroviral origin: identification and transcriptome of the genes with coding capacity for complete envelope proteins. *J Virol* 77: 10414-10422.

DEFAMIE, N., MOGRABI, B., ROGER, C., CRONIER, L., MALASSINE, A., BRUCKER-DAVIS, F., FENICHEL, P., SEGRETAIN, D. and POINTIS, G. (2001). Disruption of gap junctional intercellular communication by lindane is associated with aberrant localization of connexin43 and zonula occludens-1 in 42GPA9 Sertoli cells. *Carcinogenesis* 22: 1537-1542.

DUFFY, H.S., ASHTON, A.W., O'DONNELL, P., COOMBS, W., TAFFET, S.M., DELMAR, M. and SPRAY, D.C. (2004). Regulation of connexin43 protein complexes by intracellular acidification. *Circ Res* 94: 215-222.

EATON, B. and CONTRACTOR, S. (1993). *In vitro* assessment of trophoblast receptors and placental transport mechanisms. In *The human placenta.* (Eds. Redman CW, Sargent IL, Starkey PM). London: Blackwell Scientific Publication. p 471-503.

FRENDO, J-L., CRONIER, L., BERTIN, G., GUIBOURDENCHE, J., VIDAUD, M., EVAIN-BRION, D. and MALASSINE, A. (2003a). Involvement of connexin 43 in human trophoblast cell fusion and differentiation. *J Cell Sci* 116: 3413-3421.

FRENDO, J-L., GUIBOURDENCHE, J., PIDOUX, G., VIDAUD, M., LUTON,nD., GIOVANGRANDI, Y., PORQUET,D., MULLER, F. and EVAIN-BRION, D. (2004). Trophoblast Production of a Weakly Bioactive Human Chorionic Gonadotropin in Trisomy 21-Affected Pregnancy. *J Clin Met Endocrinol* 89: 727-732.

FRENDO, J-L., OLIVIER,nD., CHEYNET, V., BLOND, J-L., VIDAUD, M., RABREAU, M., EVAIN-BRION, D. and MALLET, F. (2003b). Direct involvement of HERV-W Env glycoprotein in human trophoblast cell fusion and differentiation. *Mol Cell Biol* 23: 3566-3574.

FRENDO, J-L., THEROND, P., GUIBOURDENCHE, J., VIDAUD, M. and EVAIN-BRION, D. (2002). Implication of copper zinc superoxide dismutase (SOD-1) in human placenta development. *Ann N Y Acad Sci* 973: 297-301.

FRENDO, J-L., THÉROND, P., BIRD, T., MASSIN, N., MULLER, F., GUIBOURDENCHE, J., LUTON, D., VIDAUD, M., ANDERSON, W. and EVAIN-BRION, D. (2001). Overexpression of copper zinc superoxide dismutase impairs human trophoblast cell fusion and differentiation. *Endocrinology* 142: 3638-3648.

FRENDO, J-L., THÉROND, P., GUIBOURDENCHE, J., BIDART, J-M., VIDAUD, M. and EVAIN-BRION, D. (2000a). Modulation of copper/zinc Superoxide Dismutase expression and activity with *in vitro* differentiation of human villous cytotrophoblast. *Placenta* 21: 773-781.

FRENDO, J-L., VIDAUD, M., GUIBOURDENCHE, J., LUTON, D., MULLER, F., BELLET, D., GIOVANGRANDI, Y., TARRADE, A., PORQUET, D., BLOT, P. and EVAIN-BRION, D. (2000b). Defect of villous cytotrophoblast differentiation into syncytiotrophoblast in Down syndrome. *J Clin Endocrinol Metab* 85: 3700-3707.

GARCIA-LLORET, M., MORRISH, D., WEGMANN, T., HONORE, L., TURNER, A. and GUILBERT, L. (1994). Demonstration of functional cytokine-placental interactions: CSF-1 and GM-CSF stimulate human cytotrophoblast differentiation and peptide hormone secretion. *Exp Cell Res* 214: 46-54.

GETSIOS, S. and MACCALMAN, C. (2003). Cadherin-11 modulates the terminal differentiation and fusion of human trophoblastic cells *in vitro*. *Dev Biol* 257: 41-54.

GIEPMANS, B.N., HENGEVELD, T., POSTMA, F.R. and MOOLENAAR, W.H. (2001). Interaction of c-Src with gap junction protein connexin-43. Role in the regulation of cell-cell communication. *J Biol Chem* 276: 8544-8549.

GIEPMANS, B.N. and MOOLENAAR, W.H. (1998). The gap junction protein connexin43 interacts with the second PDZ domain of the zona occludens-1 protein. *Curr Biol* 8: 931-934.

GUDERMANN, T., BIRNBAUMER, M. and BIRNBAUMER, L. (1992). Evidence for dual coupling of the murine luteinizing hormone receptor to adenylyl cyclase and phophoinositide breakdown and Ca2+ mobilization. Studies with the cloned murine luteinizing hormone receptor expressed in L cells. *J Biol Chem* 267: 4479-4488.

HERVE, J-C., BOURMEYSTER, N. and SARROUILHE, D. (2004). Diversity in protein-protein interactions of connexins: emerging roles. *Biochim Biophys Acta* 1662: 22-41.

HIPKIN, R., SANCHEZ-YAGUE, J. and ASCOLI, M. (1992). Identification and characterization of a luteinizing hormone/chorionic (LH/CG) receptor precursor in a human kidney cell line stably transfected with the rat luteal LH/CG receptor complementary DNA. *Mol Endocrinol* 6: 2210-2218.

HUNTER, A.W., BARKER, R.J., ZHU, C. and GOURDIE, R.G. (2005). Zonula occludens-1 alters connexin43 gap junction size and organization by influencing channel accretion. *Mol Biol Cell* 16: 5686-5698.

HUPPERTZ, B., BARTZ, C. and KOKOZIDOU, M. (2006). Trophoblast fusion: fusogenic proteins, syncytins and ADAMs, and other prerequisites for syncytial fusion. *Micron* 37: 509-517.

HUPPERTZ, B. and KINGDOM, J.C. (2004). Apoptosis in the trophoblast—role of apoptosis in placental morphogenesis. *J Soc Gynecol Investig* 11: 353-362.

ITOH, M., NAGAFUCHI, A., YONEMURA, S., KITANI-YASUDA, T., TSUKITA, S. and TSUKITA, S. (1993). The 220-kD protein colocalizing with cadherins in non-epithelial cells is identical to ZO-1, a tight junction-associated protein in epithelial cells: cDNA cloning and immunoelectron microscopy. *J Cell Biol* 121: 491-502.

KERYER, G., ALSAT, E., TASKEN, K. and EVAIN-BRION, D. (1998). Cyclic AMP-dependent protein kinases and human trophoblast cell differentiation *in vitro*. *J Cell Sci* 111: 995-1004.

KHAN, S., KATABUCHI, H., ARAKI, M., NISHIMURA, R. and OKAMURA, H. (2000). Human villous macrophage-conditioned media enhance human trophoblast growth and differentiation *in vitro*. *Biol Reprod* 62: 1075-1083.

KING, A., THOMAS, L. and BISCHOF, P. (2000). Cell culture models of trophoblast II: trophoblast cell lines—a workshop report. *Placenta* 21 Suppl A: S113-S119.

KLIMAN, H., NESTLER, J., SERMASI, E., SANGER, J. and STRAUSS III, J. (1986). Purification, characterization, and *in vitro* differenciation of cytotrophoblasts from human term placentae. *Endocrinology* 118: 1567-1582.

KNERR, I., SCHUBERT, S.W., WICH, C., AMANN, K., AIGNER, T., VOGLER, T., JUNG, R., DOTSCH, J., RASCHER, W. and HASHEMOLHOSSEINI, S. (2005). Stimulation of GCMa and syncytin via cAMP mediated PKA signaling in human trophoblastic cells under normoxic and hypoxic conditions. *FEBS Lett* 579: 3991-3998.

KUDO, Y., BOYD, C.A., MILLO, J., SARGENT, I.L. and REDMAN, C.W. (2003). Manipulation of CD98 expression affects both trophoblast cell fusion and amino acid transport activity during syncytialization of human placental BeWo cells. *J Physiol* 550: 3-9.

LANDER, E.S., LINTON, L.M. and BIRREN, B., *et al*. (2001). Initial sequencing and analysis of the human genome. *Nature* 409: 860-921.

LANGBEIN, M., STRICK, R., STRISSEL, P.L., VOGT, N., PARSCH, H., BECKMANN, M.W. and SCHILD, R.L. (2008). Impaired cytotrophoblast cell-cell fusion is associated with reduced Syncytin and increased apoptosis in patients with placental dysfunction. *Mol Reprod Dev* 75: 175-183.

LARSSON, L.I., BJERREGAARD, B. and TALTS, J.F. (2008). Cell fusions in mammals. *Histochem Cell Biol*. 129: 551-561.

LECANDA, F., TOWLER, D., ZIAMBARAS, K., CHENG, S., KOVAL, M., STEINBERG, T. and CIVITELLI, R. (1998). Gap junctional communication modulates gene expression in osteoblastic cells. *Mol Biol Cell* 9: 2249-2258.

LEISSER, C., SALEH, L., HAIDER, S., HUSSLEIN, H., SONDEREGGER, S. and KNOFLER, M. (2006). Tumour necrosis factor-alpha impairs chorionic gonadotrophin beta-subunit expression and cell fusion of human villous cytotrophoblast. *Mol Hum Reprod* 12: 601-609.

LEVY, R., SMITH, S.D., CHANDLER, K., SADOVSKY, Y. and NELSON, D.M. (2000). Apoptosis in human cultured trophoblasts is enhanced by hypoxia and diminished by epidermal growth factor. *Am J Physiol Cell Physiol* 278: 982-988.

LOEWENSTEIN, W. (1981). Junctional intercellular communication: the cell-to-cell membrane channel. *Physiol Rev* 61: 829-913.

LOOSFELT, H., MISRAHI, M., ATGER, M., SALESSE, R., VU HAI-LUU THI, M., JOLIVET, A., GUIOCHON-MANTEL, A., SAR, S., JALLAL, B. and GARNIER, J. (1989). Cloning and sequencing of porcine LH-hCG receptor cDNA: variants lacking transmembrane domain. *Science* 245: 525-528.

MALASSINE, A. and CRONIER, L. (2005). Involvement of gap junctions in placental functions and development. *Biochim Biophys Acta* 1719: 117-124.

MALASSINE, A., FRENDO, J-L., BLAISE, S., HANDSCHUH, K., GERBAUD, P., TSATSARIS, V., HEIDMANN, T. and EVAIN-BRION, D. (2008). Human endogenous retrovirus-FRD envelope protein (syncytin 2) expression in normal and trisomy 21-affected placenta. *Retrovirology* 5: 6.

MALASSINE, A., HANDSCHUH, K., TSATSARIS, V., GERBAUD, P., CHEYNET, V., ORIOL, G., MALLET, F. and EVAIN-BRION D. (2005). Expression of HERV-W Env glycoprotein (syncytin) in the extravillous trophoblast of first trimester human placenta. *Placenta* 26: 556-562.

MANGENEY, M., RENARD, M., SCHLECHT-LOUF, G., BOUALLAGA, I., HEIDMANN, O., LETZELTER, C., RICHAUD, A., DUCOS, B. and HEIDMANN, T. (2007). Placental syncytins: Genetic disjunction between the fusogenic and immunosuppressive activity of retroviral envelope proteins. *Proc Natl Acad Sci USA* 104: 20534-20539.

MASSIN, N., FRENDO, J-L., GUIBOURDENCHE, J., LUTON, D., GIOVANGRANDI, Y., MULLER, F., VIDAUD, M. and EVAIN-BRION, D. (2001). Defect of syncytiotrophoblast formation and hCG expression in Down syndrome. *Placenta* 22: Suppl A: S93-S97.

MCFARLAND, K., SPRENGEL, R., PHILLIPS, H., KOHLER, M., ROSEMBLIT, N., NIKOLICS, K., SEGALOFF, D. and SEEBURG, P. (1989). Lutropin-choriogonadotropin receptor: an unusual member of the G protein-coupled receptor family. *Science* 245: 494-499.

MI, S., LEE, X., LI, X-P., VELDMAN, G.M., FINNERTY, H., RACIE, L., LAVALLIE, E., TANG, X-Y., EDOUARD, P., HOWES, S., KEITH, J.C. and MCCOY, J.M. (2000). Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* 403: 785-789.

MINEGISHI, T., NAKAMURA, K., TAKAKURA, Y., MIYAMOTO, K., HASEGAWA, Y., IBUKI, Y., and IGARASHI, M. (1990). Cloning and sequencing of human LH/hCG receptor cDNA. *Biochem Biophys Res Commun* 172: 1049-1054.

MORRISH, D., BHARDWAJ, D., DABBAGH, L., MARUSYK, H. and SIY, O. (1987). Epidermal growth factor induces differentiation and secretion of human chorionic gonadotropin and placental lactogen in normal human placenta. *J Clin*

Endocrinol Metab 65: 1282-1290.

MORRISH, D., BHARDWAJ, D. and PARAS, M. (1991). Transforming growth factor β1 inhibits placental differentiation and human chorionic gonadotropin and human placental lactogen secretion. *Endocrinology* 129: 22-26.

MUIR, A., LEVER, A.M. and MOFFETT, A. (2006). Human endogenous retrovirus-W envelope (syncytin) is expressed in both villous and extravillous trophoblast populations. *J Gen Virol* 87: 2067-2071.

NACHTIGALL, M.J., KLIMAN, H.J., FEINBERG, R.F., OLIVE, D.L., ENGIN, O. and ARICI, A. (1996). The effect of leukemia inhibitory factor (LIF) on trophoblast differentiation: a potential role in human implantation. *J Clin Endocrinol Metab* 81: 801-806.

OBERWEISS, D., GILLEROT, Y., KOULISCHER, L., HUSTIN, J. and PHILIPPE, E. (1983). Le placenta des Trisomie 21 dans le dernier trimestre de la gestation. *J Gynecol Obstet Biol Reprod* 12: 345-349.

OGREN, L. and TALAMENTES, F. (1994). The placenta as an endocrine organ: polypeptides. In *Physiology of reproduction.* (Eds. Knobil E. and Neill J.) New-York: Raven Press. p 875-945.

PIDOUX, G., GERBAUD, P., LAURENDEAU, I., GUIBOURDENCHE, J., BERTIN, G., VIDAUD, M., EVAIN-BRION, D. and FRENDO, J-L. (2004a). Large variability of trophoblast gene expression within and between human normal term placentae. *Placenta* 25: 469-473.

PIDOUX, G., GERBAUD, P., MARPEAU, O., GUIBOURDENCHE, J., FERREIRA, F.,nBADET, J., EVAIN-BRION, D. and FRENDO, J-L. (2007a). Human Placental Development Is Impaired by Abnormal Human Chorionic Gonadotropin Signaling in Trisomy 21 Pregnancies. *Endocrinology* 148: 5403-5413.

PIDOUX, G., GERBAUD, P., TSATSARIS, V., MARPEAU, O., FERREIRA, F., MEDURI, G., GUIBOURDENCHE, J., BADET, J., EVAIN-BRION, D. and FRENDO, J-L. (2007b). Biochemical characterization and modulation of LH/CG-receptor during human trophoblast differentiation. *J Cell Physiol* 212: 26-35.

PIDOUX, G., GUIBOURDENCHE, J., FRENDO, J-L., GERBAUD, P., CONTI, M., LUTON, D., MULLER, F. and EVAIN-BRION, D. (2004b). Impact of trisomy 21 on human trophoblast behaviour and hormonal function. *Placenta* 25 Suppl A: S79-S84.

PIERCE, J. and PARSONS, T. (1981). Glycoprotein hormones: structure and function. *Annu Rev Biochem* 50: 465-495.

ROBERTS, L., SEBIRE, N., FOWLER, D. and NICOLAIDES, K. (2000). Histomorphological features of chorionic vill at 10-14 weeks of gestation in trisomic and chromosomally normal pregnancies. *Placenta* 21: 678-683.

SAEZ, J., BERTHOUD, V., MORENO, A. and SPRAY, D. (1993). Gap junctions. Multiplicity of controls in differentiated and undifferentiated cells and possible functional implications. *Adv Second Messenger Phosphoprotein Res* 27: 163-198.

SCHREIBER, J., RIETHMACHER-SONNENBERG, E., RIETHMACHER, D., TUERK, E.E., ENDERICH, J., BOSL, M.R. and WEGNER, M. (2000). Placental failure in mice lacking the mammalian homolog of glial cells missing, GCMa. *Mol Cell Biol* 20: 2466-2474.

SEGRETAIN, D., FIORINI, C., DECROUY, X., DEFAMIE, N., PRAT, J.R. and POINTIS, G. (2004). A proposed role for ZO-1 in targeting connexin 43 gap junctions to the endocytic pathway. *Biochimie* 86: 241-244.

SHI, Q., LEI, Z., RAO, C. and LIN, J. (1993). Novel role of human chorionic gonadotropin in differentiation of human cytotrophoblasts. *Endocrinology* 132: 1387-1395.

SORGEN, P.L., DUFFY, H.S., SAHOO, P., COOMBS, W., DELMAR, M. and SPRAY, D.C. (2004). Structural changes in the carboxyl terminus of the gap junction protein connexin43 indicates signaling between binding domains for c-Src and zonula occludens-1. *J Biol Chem* 279: 54695-54701.

STEVENSON, B.R., SILICIANO, J.D., MOOSEKER, M.S. and GOODENOUGH, D.A. (1986). Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. *J Cell Biol* 103: 755-766.

TOYOFUKU, T., AKAMATSU, Y., ZHANG, H., KUZUYA, T., TADA, M. and HORI, M. (2001). c-Src regulates the interaction between connexin-43 and ZO-1 in cardiac myocytes. *J Biol Chem* 276: 1780-1788.

WILLECKE, K., EIBERGER, J., DEGEN, J., ECKARDT, D., ROMUALDI, A., GULDENAGEL, M., DEUTSCH, U. and SOHL, G. (2002). Structural and functional diversity of connexin genes in the mouse and human genome. *Biol Chem* 383: 725-737.

WILLOTT, E., BALDA, M.S., FANNING, A.S., JAMESON, B., VAN ITALLIE, C. and ANDERSON, J.M. (1993). The tight junction protein ZO-1 is homologous to the *Drosophila* discs-large tumor suppressor protein of septate junctions. *Proc Natl Acad Sci USA* 90: 7834-7838.

Further Related Reading, published previously in the *Int. J. Dev. Biol.*

See our recent Special Issue ***Epigenetics & Development*** edited by Saadi Khochbin and Stefan Nonchev at:
http://www.ijdb.ehu.es/web/contents.php?vol=53&issue=2-3

See Special Issue ***Pattern Formation*** edited by Michael K. Richardson and Cheng-Ming Chuong at:
http://www.ijdb.ehu.es/web/contents.php?vol=53&issue=5-6

The influence of the intrauterine environment on human placental development
Graham J. Burton, Eric Jauniaux and D. Stephen Charnock-Jones
Int. J. Dev. Biol. (2010) 54: 303-312 (doi: 10.1387/ijdb.082764gb)

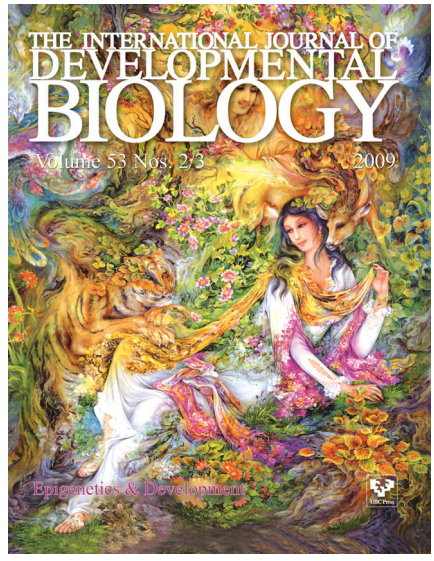
Spatiotemporal expression of the selenoprotein P genein postimplantational mouse embryos
Se-Ra Lee, Jung-Min Yon, In-Jeoung Baek, Mi-Ra Kim, Chun-Gui Park, Beom-Jun Lee, Young-Won Yun and Sang-Yoon Nam
Int. J. Dev. Biol. (2008) 52: 1005-1011

A simple in vivo approach to investigate invasive trophoblast cells
Juan A. Arroyo, Toshihiro Konno, Darya C. Khalili and Michael J. Soares
Int. J. Dev. Biol. (2005) 49: 977-980

Programmed cell death is not a necessary prerequisite for fusion of the fetal mouse palate.
Sachiko Takahara, Toshiya Takigawa and Kohei Shiota
Int. J. Dev. Biol. (2004) 48: 39-46

Chromosome 21: a small land of fascinating disorders with unknown pathophysiology.
Stylianos E Antonarakis, Robert Lyle, Samuel Deutsch and Alexandre Reymond
Int. J. Dev. Biol. (2002) 46: 89-96

Changes in the placenta and in the rat embryo caused by the demethylating agent 5-azacytidine.
M Vlahovic, F Bulic-Jakus, G Juric-Lekic, A Fucic, S Maric and D Serman
Int. J. Dev. Biol. (1999) 43: 843-846



5 yr ISI Impact Factor (2008) = 3.271