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Temporal Regulation of the Expression of *Syncytin* (*HERV-W*), Maternally Imprinted *PEG10,* and *SGCE* in Human Placenta[1]

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ABSTRACT

Maternally imprinted *PEG10* and *SGCE,* separated by ~2.15 Mb from *Syncytin (HERV-W)* gene at 7q21.3, are implicated in choriocarcinoma and Silver-Russell syndrome. Here we have analyzed the temporal regulation of mRNA expression of these genes in placenta and demonstrate that *Syncytin* gene activation is highest in term placenta, *PEG10,* downregulated at early hypoxic phase, and highly activated at 11–12 wk of gestation. In contrast, transcription from *SGCE* remained unchanged throughout pregnancy, suggesting two neighboring imprinted genes are differentially regulated at very early pregnancy. Additionally, accumulation of two major species of mRNA (8 kb and 3.1 kb) encoded by *HERV-W* in placenta is regulated: 3.1 kb mRNA level remained unchanged throughout pregnancy, whereas the production of 8 kb species was highest in term placenta. Western blot and immunohistochemical staining of placental tissues with monoclonal antibodies revealed a marked reduction of syncytin glycoprotein synthesis in late pregnancy. Therefore, the relative levels of 3.1 kb and 8 kb mRNAs in trophoblasts could regulate syncytin protein synthesis, possibly by competition of the two mRNA species for translational apparatus.

gene regulation, placenta, pregnancy, syncytiotrophoblast, trophoblast

INTRODUCTION

Early placental growth (8–10 wk of gestation), characterized by extensive proliferation of stem cytotrophoblasts and differentiation to multinucleated syncytotrophoblasts (STs) and invasive cytotrophoblasts (iCT), is regulated by transcriptional activation as well as silencing of a variety of trophoblast-specific genes [1]. About 1% of the human genome harbors replication defective endogenous retroviruses (*HERV*) that are, in general, transcriptionally silent. In exceptional circumstances, activation of certain retroelements is essential for physiological growth and development of human embryos [2]. One such element is the recently discovered *HERV-W* (*Syncytin*), which codes for envelope glycoprotein and is exclusively synthesized in human placenta and testis [3, 4]. *HERV-W* could be transcribed to produce unspliced 8-kb and spliced 3-kb mRNAs. In testis, only the 8-kb mRNA species is transcribed and translated to produce syncytin glycoprotein. This species, therefore, is defined here as SynT or testis-syncytin mRNA. Although both species of mRNAs (8 kb and 3.1 kb) are produced in trophoblasts, the 3.1-kb species is translated to produce syncytin, and we therefore define the latter as SynP or placenta-syncytin mRNA. The name *syncytin* is derived from its proposed fusogenic properties in spermatogenesis and placental morphogenesis [3]. The paternally expressed gene, *PEG10*, is also a functional retrotransposon located ~2.15 kb away from *syncytin* (*HERV-W*), (Fig. 1, Ai) at 7q21.3. Unlike *HERV-W*, *PEG10* contains no long terminal repeat sequences (Fig. 1A, ii). *PEG10* mRNA is predominantly expressed in placenta and testis and to a lesser extent in brain and lung [5] and codes for a protein homologous to mouse MyEF-3 (myelin expression factor 3), which is presumed to be necessary for producing myelin-binding protein (MBP) and has been shown to participate in myelinating neurons [6].

SGCE, the epsilon (ϵ) member of the sarcoglycan family, is a component of the transmembrane dystrophin-glycoprotein (DGC) complex. It mediates muscle cytoskeleton-extracellular matrix communications by stabilizing membranes [7]. The transmembrane DGC complex often contains subcomplexes containing dystroglycans and sarcoglycans. ϵ-Sarkoglycan exhibits strong homology to α-sarkoglycan [8, 9]; however, unlike α-, ϵ-sarkoglycan is highly activated in placenta in addition to heart and skeletal muscle [10]. Although the abundance of *HERV-W*, *PEG10,* and *SGCE* transcripts in human placenta indicated their functional role, very little is known about their regulation during pregnancy, primarily because these studies were often carried out with late or full-term placental materials [3–5, 10–12]. Nevertheless, a specific function of syncytin in placental morphogenesis became apparent from recent work on aberrant pregnancies. For instance, reduced syncytin expression has been directly correlated with insufficient or late differentiation of STs in Down syndrome pregnancies [13–16], suboptimal invasion of trophoblast in preeclampsia, and hypertensive pregnancy disorders [11, 12]. Furthermore, two neighboring genes at 7q21.3 (*SGCE* and *PEG10*), implicated in Silver-Russell syndrome (SRS), characterized by intrauterine growth restriction [17–19] and

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TABLE 1. Primer sets used to amplify cDNAs and generating hybridization probes.*

Gene	Protein	Accession no.	Product size	Primer sequences
HERV-W	Syncytin (SynPT)	NM014590	400 bp	F-ccg ctg aaa gag ggg gaa R-tag agg ttg tgc agc tga ga
HERV-W	Syncytin (SynT)	NM014590	640 bp	F-tca ggg ata gcc ccc atc tat R-aac cct ttg cca cta cat caa ttt
PEG10	Paternally expressed 10	AB049834	625 bp	F-cac atc tga tga gac tga aag ttc R-ttg taa cag caa tgc aat att agc
SGCE	ϵ-sarcoglycan	AF397424	322 bp	F-cgc acc ttt gag act gca a R-tct cca cga atc act tcc tga
ACTB	β-actin	XM037135	307 bp	F-ctt cta caa tga gct ggg tg R-tca tga ggt agt cag tca gg

* SynPT amplifies both 8-kb and 3.1-kb syncytin cDNA, whereas SynT set amplifies only 8-kb syncytin cDNA.

choriocarcinoma [20, 21], are maternally imprinted [22, 23]. Therefore, future experiments investigating their functional link to placental and fetal development might be facilitated by understanding the regulation of these genes during normal pregnancies. Here we demonstrate that two neighboring imprinted genes *(PEG10* and *SGCE)* are differentially regulated in placenta and that despite increased transcriptional activation of *HERV-W*, syncytin protein synthesis is markedly reduced in late pregnancy.

MATERIALS AND METHODS

Placental Tissues, Embryo, Decidua, and RNA Extraction

This study was approved by the local ethics committees of St. George's and King's College Hospitals, London, and written consent was obtained from patients before the collection of samples. Placental tissue was taken from patients undergoing termination of pregnancy with gestational age range of 7–12 wk. Full-term placental tissues were obtained after vaginal delivery or after cesarean section. Tissue was dissected and washed in PBS (Ca^{2+}/Mg^{2+}-free) until clean and villous material isolated by further dissection in a sterile Petri dish placed under a stereomicroscope. Embryonic tissue from a 12-wk pregnancy and decidual tissue were carefully dissected and repeatedly washed in PBS. Samples were stored in RNA Later (Ambion, Austin, TX) overnight at 4°C before transfer to storage at −20°C. Women undergoing chorionic villus sampling (CVS), for ultrasonographic indications, were asked to allow tissue in excess of that needed for cytogenetic analysis to be used for the study. These samples were stored and only materials from chromosomally normal pregnancies were used. Tissues were homogenized in TRIzol (Invitrogen, Carlsbad, CA) using the manufacturer's standard protocol for total RNA preparation. RNAs were stored as ethanol precipitates at −70°C.

Genetic Map, mRNAs, and cDNA Amplification by Reverse Transcription-Polymerase Chain Reaction

The relative positions of *HERV-W*, maternally imprinted neighboring *PEG10*, and *SGCE* at 7q21.3 and their corresponding mRNAs are shown in Figure 1A. *HERV-W* codes for two major species of mRNA, 8 kb and 3.1 kb, but in testis, only the 8-kb species is transcribed to produce syncytin glycoprotein and therefore defined as testis syncytin (SynT). Although both 8-kb and 3.1-kb mRNAs are produced in human placenta, the latter is translated to produce the functional envelope glycoprotein, syncytin [4]. To distinguish 8-kb from 3.1-kb mRNA, the latter is described as placental syncytin mRNA (SynP).

RNAs were treated with DNase I (Sigma, St. Louis, MO) in 30 mM Tris-Cl (pH 7.8), 50 mM NaCl, and 10 mM $MgCl_2$, in the presence of 40 U RNaseOUT (Invitrogen) for 30 min before phenol-chloroform extraction and ethanol precipitation. First-strand cDNA was synthesized from 5 μg of total RNA using Superscript II reverse transcriptase (RT) (Invitrogen), primed with oligo $(dT)_{12–18}$ (Invitrogen) according to the manufacturer's recommended protocol, and then treated with RNase H (Invitrogen) for 30 min prior to heat inactivation (70°C for 15 min) and cDNA amplification. Standard polymerase chain reaction (PCR) profile for all reactions: 94°C, 5-min initial denaturation followed by 94°C, 45 sec, 50–58°C (depending upon primer set; Table 1) annealing, 30 sec, 72°C extension, 90 sec for 15 cycles (to maintain exponential amplification), and a final extension cycle at 72°C for 10 min. Unlike Northern blot, RT-PCR analysis in a multiplex allows quantitative analysis of several mRNA species in a single exponential amplification reaction under defined experimental conditions in which the yield of amplified products in the multiplex is comparable to that of each single species alone (data not shown). All multiplex experiments were optimized so that the yield of each product in the multiplex reaction matched the yield of individual primer sets under the same conditions (data not shown). PCR products were resolved on 1.5%–2.0% agarose gels before Southern blotting by alkaline transfer to nylon membranes (Nytran-Plus, Schleicher and Schuell, Keene, NH).

RNA and DNA Blot Hybridization

Approximately 15 μg of total RNA was resuspended in loading buffer containing 1× MOPS, 50% deionized formamide, and 2.2 M formaldehyde and separated on agarose gels containing formaldehyde (2.2 M) buffered with MOPS (40 mM MOPS, 10 mM sodium acetate, 1 mM EDTA, pH 7.2), transferred to a nylon membrane (Nytran-Plus, Schleicher and Schuell), washed in 2× saline sodium citrate, and baked to reverse the formaldehyde reaction.

The *syncytin* probe was a 1.7-kb *Eco*RI fragment from a cDNA clone (kindly provided by Dr. F. Mallet). SynP, SynT, *PEG10*, *SGCE*, and β-actin probes were generated from RT-PCR products. All probes were gel purified by electroelution and concentrated by Elutip-D columns as described previously [24]. Two hundred nanograms of probe was radiolabeled using Megaprime kit (Amersham, Bucks, U.K.), column purified (NAP5, Pharmacia, Bucks, U.K.), denatured, and added to the hybridization solution. For multiplex analysis, SynP plus SynT was cohybridized after stripping, with β-actin. *SGCE* was hybridized before stripping and subsequent cohybridization of *PEG10* and β-actin. Following overnight hybridization at 65°C, membranes were washed extensively (1% SDS, 50 mM PO_4 at room temperature) and autoradiographed. For sequential hybridization, filters were stripped by boiling in 1% SDS solution.

Densitometry and Data Analysis

At least three exposures of each autoradiogram were scanned from each experiment, and only those scans exhibiting linearity were used to generate data points. Densitometry was carried out using a 1D-Multi Lane Densitometry program in an AlphaImager (1220 version 5.5, Alpha Innotech Corp., San Leandro, CA). For autodetection of pixel density in a peak, the scan width as well as baseline values were maintained constant for each exposure. Scan data (experimental and control) were transferred to Microsoft Excel (Redlands, WA) in which pixel density of each experimental lane was normalized to its corresponding β-actin value. Each experiment was repeated at least twice, and average values for each data point were plotted.

Western Blot and Immunohistochemistry

Proteins from placental villous tissue, dissected as described above, were extracted in T-Per (Pierce, Rockford, IL) following the recommended protocol. Protein concentrations were measured (Bradford assay, BioRad, Hercules, CA), and 10 μg of extract was resolved in 8% SDS-polyacrylamide gel and transferred to polyvinyl difluoride membrane. Immunostaining was carried out with primary 6A2B2 monoclonal antibody (1 in 3000 dilution) and anti-mouse secondary antibody. Reactions were detected using enhanced chemiluminescence reagents (Roche, Nutley, NJ) and fluorography. Subsequently, blots were stained with 0.25% Coomassie brilliant blue R in 40% methanol, destained, and photographed.

Placental tissue from 7-, 8-, and 12-wk and full-term pregnancies was fixed in 4% (v/v) freshly prepared paraformaldehyde in PBS (Ca^{2+}/Mg^{2+}-

free) for 4 h at room temperature. The samples were cryoprotected, embedded in paraffin, and frozen in liquid nitrogen. Multiple sections (7 μm width) were cut and immunostained using ChemMate Detection kit (DAKO, Glostrup, Denmark). Following deparaffinization, slides were immersed in MS unmasker buffer (Microm Microtech, Francheville, France) and placed in a microwave (800 W) for three cycles (5 min each). Endogenous peroxidase activity was blocked (3% H_2O_2, 10 min) prior to incubation with monoclonal mouse anti-human *syncytin* (6A2B2, 1:100) followed by biotinylated goat anti-mouse immunoglobulin (30 min) and streptavidin peroxidase (30 min). The slides were developed by adding substrate-chromogen mixture (DAKO) for 7 min at room temperature. Reactions were stopped by repeated washing in distilled water followed by brief counterstaining (1 min) with freshly prepared and filtered Mayer hematoxylin (Merck, Middlesex, UK) followed by rinsing in 1% acid-alcohol. The slides were dehydrated in ascending ethanol series prior to mounting. For negative controls, the primary antibody was replaced with nonspecific isotype controls (negative control reagents, IgG_1, monoclonal; DAKO). The sections were visualized in a Nikon E400 microscope and images were captured using Lucia version 4.21 system for image processing and analysis.

Statistical Analysis

Densitometric data were transferred to Microsoft Excel Worksheet and means, SDs, and variance (analysis of variance) for each data set were computed using Analysis ToolPak software. Values are shown as mean ± SEM. To compare gene expression in early and late placenta, the average value for early samples was compared with the average value for the full-term samples. A value for the level of significance (*P* value) was calculated using Poisson statistic. $P < 0.05$ was considered significant.

RESULTS

Total RNA extracted from early and term placentae was resolved in formaldehyde-agarose gels and hybridized with a *syncytin*-specific cDNA probe. The results shown in Figure 1B demonstrated that both the 8.0-kb and 3.1-kb mRNAs were expressed and accumulated in early as well as term placenta. However, in term placenta, the 8.0-kb testis-specific mRNA (SynT) production was at its highest. Densitometric scanning of this blot normalized to β-actin showed that SynT expression in term placenta was significantly increased ($P < 0.01$), compared with that in early pregnancies (compare lanes 1–8 with 9–10). This relative expression pattern of SynP and SynT was virtually identical to that of published data on cDNA hybridization to term placental mRNA (see [3, 4]). Hybridization with a *PEG10*-specific probe (Fig. 1A, ii) revealed that increased activation of *PEG10* occurred at ~11 wk, and this high level of expression was maintained and was significantly increased ($P < 0.05$) in term placenta, compared with that in early pregnancy (Fig. 1C). In addition to the 6.3-kb full-length *PEG10* mRNA, a 2.1-kb species was detected in all stages of pregnancy. The transcription of the 2.1-kb band increased as the pregnancy progressed (Fig. 1C). This transcript also hybridized to another *PEG10* probe (Fig. 1A, ii and data not shown) and we suspect that it could have originated from a cryptic splice site within the full-length 6.3-kb mRNA or that its transcription was initiated at an internal site in *PEG10*. Rehybridization of the same blot (Fig. 1C) with *SGCE* cDNA probe (Fig 1A, iii) showed that this gene was active at all stages of pregnancy.

To verify the Northern blot hybridization data, we examined the relative expression of SynP, SynT, *PEG10*, *SGCE*, and β-actin mRNAs by semiquantitative RT-PCR. To achieve accurate estimates, cDNAs were amplified exponentially together, using two or three pairs of gene-specific primers (multiplex) for only 15 cycles before the products were resolved in agarose gels and identified by sequential or cohybridization with specific probes followed by densitometric measurement. Representative examples of these experiments for each probe are shown in Figure 2, A, B, and E. To estimate total syncytin expression and accumulation, SynP plus SynT (SynPT), cDNAs from placenta at various stages of pregnancy were amplified with primers at the 3′ end of *HERV-W* (see Table 1 and Fig. 1A, i) and β-actin primers in multiplex (Fig. 2A). Notably, the *HERV-W* primer set, by virtue of its location at the 3′ end of the gene, will amplify both SynP (3.1 kb) and SynT (8 kb) cDNAs; therefore, the amplified products are called SynPT. As expected, the cDNA from whole embryo failed to amplify SynPT (Fig. 2A, lane 12). The total *syncytin* expression was highest in term placenta and was significantly higher ($P < 0.01$) when compared with the yield at early stages of pregnancy (Fig. 2C). We suspected that SynPT level in early placenta was due primarily to the amplification of SynP cDNA, whereas in late placenta, the products were contributed by both SynP and SynT cDNAs. Because both SynP and SynT could be translated to produce syncytin protein in placenta and testis, respectively [3, 4], and could potentially compete with each other for protein synthesis apparatus, we examined the relative level of translatable SynP mRNA available in early and term placentae. Such measurement is possible if one could estimate the contribution by SynT cDNA to the total amplified products (SynPT). To achieve this, a set of primers (Table 1 and Fig. 1A, i) was used where the forward and reverse primers were placed at a region that could amplify only 8 kb (SynT) cDNA. To examine the relative level of translatable SynP mRNA synthesis, cDNAs were coamplified (Fig. 2B). The relative level of translatable SynP mRNA synthesis was determined by subtracting SynT from SynPT for each experimental time point (Fig. 2B) and is shown in Fig. 2D. Assuming that two mRNA species compete, the level of SynP mRNA available to produce syncytin protein significantly decreased ($P < 0.01$) in term placenta, compared with that in early pregnancy (Fig. 2, B and D).

These results are consistent with the data obtained by Northern blot hybridization (Fig. 1B). A similar analysis of *PEG10* and *SGCE* mRNA expression at different stages of gestation revealed that activation of *PEG10* began to increase at the end of the first trimester and was highest in term placenta, but *SGCE* transcription remained unchanged throughout pregnancy (Fig. 2, E and F). Placental tissues are difficult to obtain from voluntary termination of normal pregnancies following 12 wk of gestation. Therefore, clinically normal CVS were used for analysis (12–14 wk) in these experiments. Together, these results show that production of *HERV-W* mRNAs (SynP plus SynT) is maximum in full-term placenta and *PEG10* gene is downregulated during very early hypoxic development, and the stage of pregnancy has no significant effect on *SGCE* regulation.

To investigate whether the syncytin mRNA data correlate with the synthesis/accumulation of syncytin protein, Western blots and immunohistochemistry were performed. Proteins extracted from early (7–12 wk) and term placentae (late, both cesarean and vaginal deliveries) were immunoblotted with a mouse monoclonal antibody [4] against syncytin (see *Materials and Methods*). Following antibody staining, membranes were briefly stained with Coomassie brilliant blue R to estimate the relative quantity of protein transferred in each lane (Fig. 3A). A visual examination of the blot suggests that unglycosylated (M_r, $\sim 55 \times 10^3$; 55, ugp), partially glycosylated ($\sim 60 \times 10^3$; 60, pgp), and fully glycosylated (M_r, $\sim 85 \times 10^3$; 85, gp) syncytin precursor proteins were present in each lane (Fig. 3A). However, quantitative analysis of the bands revealed that the produc-

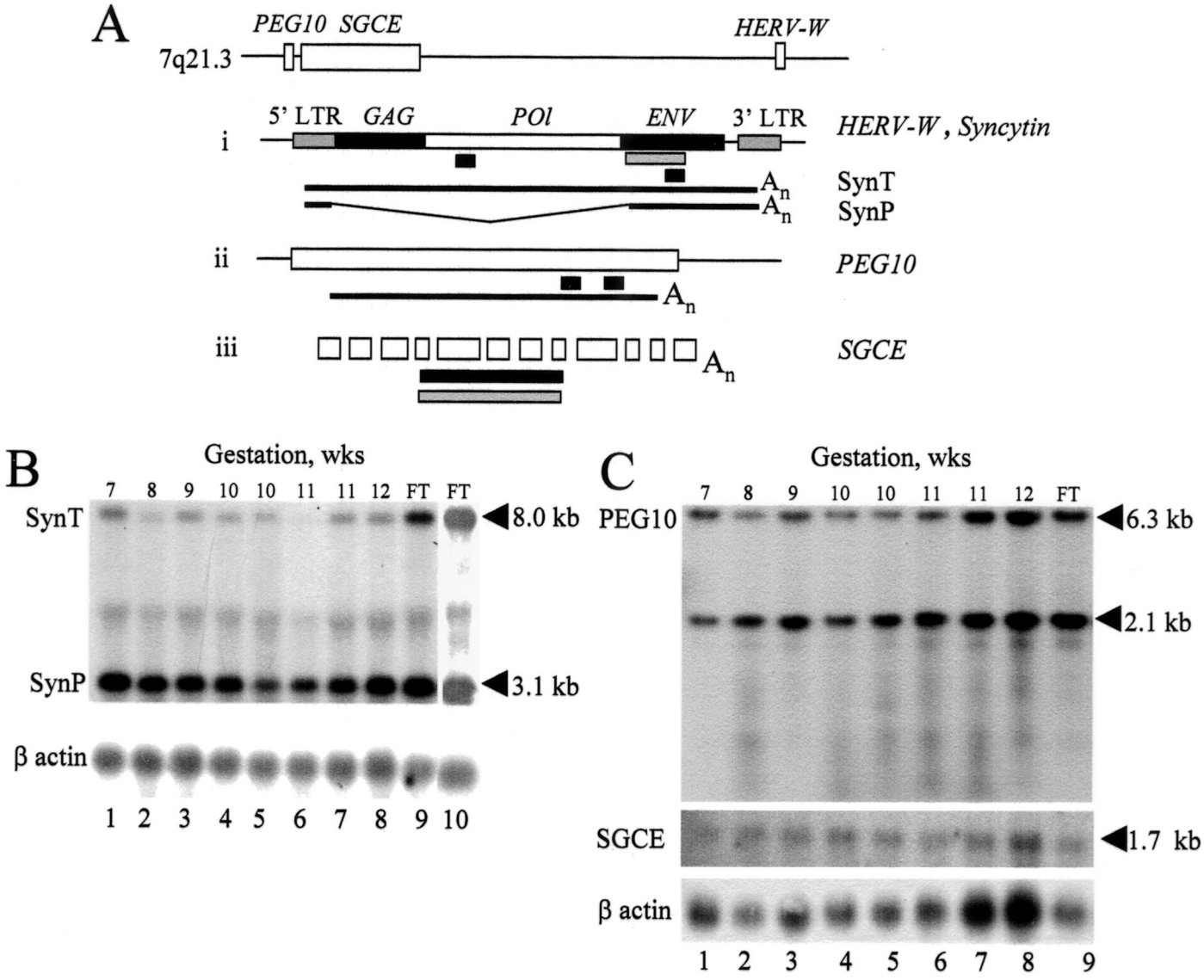

FIG. 1. Expression of *syncytin (HERV-W), PEG10,* and *SGCE* mRNA in early and late pregnancies. **A**) The chromosomal position, physical map, and mRNA species transcribed from *HERV-W* (i), *PEG10* (ii), and *SGCE* (iii) genes; the region of cloned and RT-PCR-generated cDNA probes used for Northern blot hybridizations and cDNA amplifications are shown in closed boxes; **B** and **C**) Blots representing placental mRNA from various stages of pregnancy as indicated were hybridized with *HERV-W* (**B**), *PEG10,* and *SGCE*-specific cDNAs (**C**), respectively. FT, Full term.

tion of all three species of synctin was significantly increased ($P < 0.01$) in early pregnancy, compared with that in term (late) placentae (Fig. B). The 55–60 $\times$ 10^3 bands migrate as a single band in shorter runs or in 12% gels (data not shown). To further verify these results, immunohistochemistry was performed, using the same monoclonal antibody against syncytin, on placental sections from 7-wk, 8-wk, 12-wk, and term pregnancies. The intensity of staining was highest in 7- and 8-wk sections, somewhat reduced in 12-wk placenta, and was very weak in full-term placenta, compared with that in early pregnancy (Fig. 3C). These data are in agreement with the results obtained by Western blotting. Notably, both extravillous trophoblasts and STs reacted equally with the antibody. Although faint staining of intravillous cytotrophoblasts is detectable in 12-wk placental tissues, we did not detect immunostaining of inner villous vascular cells. Therefore, despite a quantitative increase in total syncytin mRNA (SynPT) expression in late placenta (Figs. 1A and 2, A, B, and E), syncytin protein synthesis was inhibited at this stage of pregnancy. Antibodies against PEG10 and human SGCE are currently unavailable. Therefore, expression of these proteins in human placenta would require future investigation.

DISCUSSION

Human placental development begins with an extensive proliferation of stem cytotrophoblasts in hypoxic environment followed by differentiation into multinucleated STs and iCTs. This developmental transition of placenta is marked by downregulation and simultaneous activation of a plethora of genes, some of which are trophoblast specific and a few are imprinted [1, 25]. This study was undertaken as a first step toward understanding the functional role of *Syncytin*, *PEG10*, and *SGCE* in placental morphogenesis by examining their transcriptional regulation in early and late human pregnancies. The significance of these genes in human pregnancy is underscored by the fact that the chromosomal region harboring these genes is implicated in primordial growth restriction observed in SRS [17–19], cho-

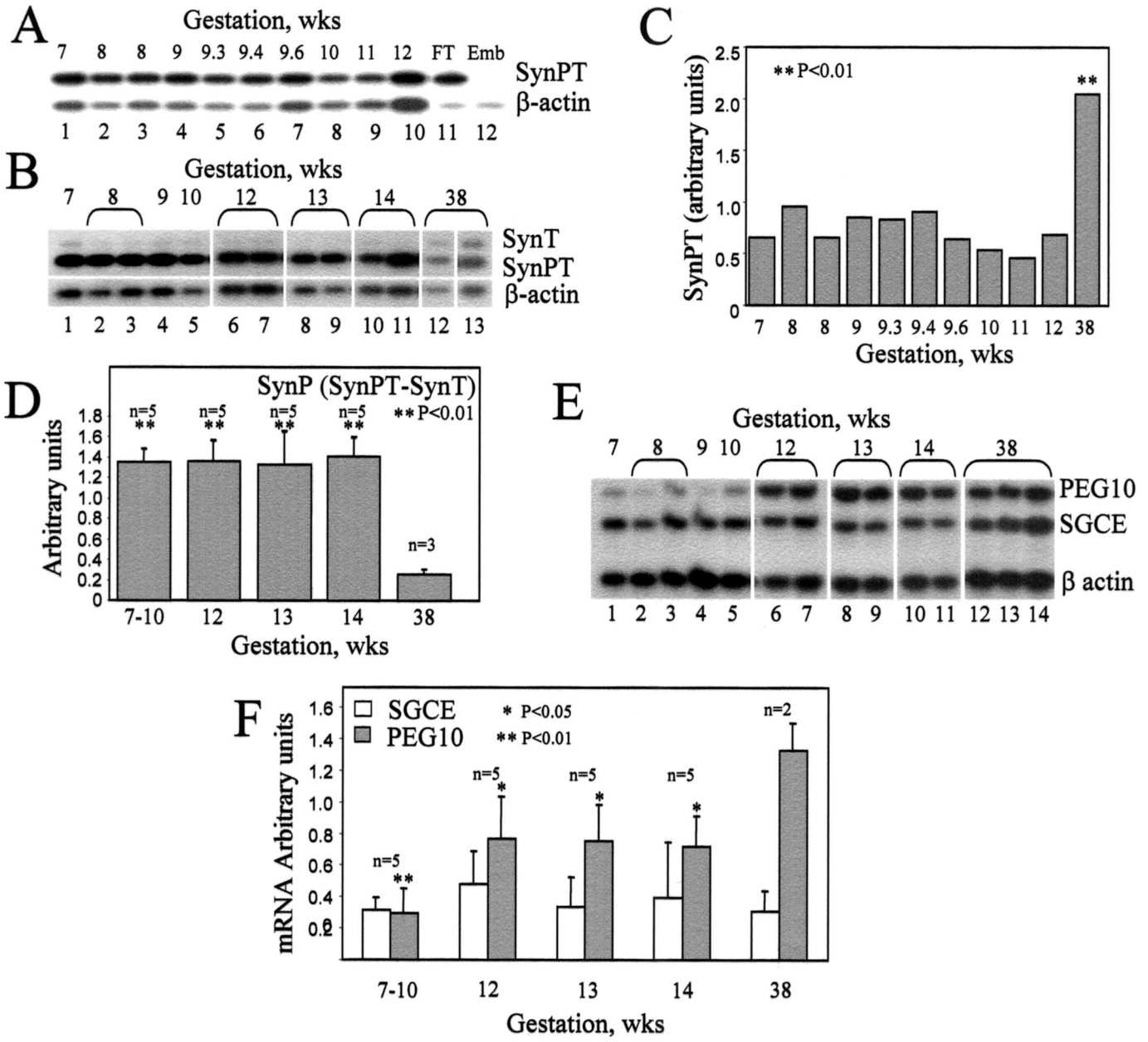

FIG. 2. Multiplex RT-PCR analysis of SynPT and SynT, *PEG10,* and *SGCE* transcription in early and late placenta. **A**) RT-PCR amplification of *HERV-W* 3.1-kb and 8-kb mRNAs with primers common to both species (SynPT) together with β-actin and hybridized sequentially. Lane 12 shows amplified products from 12-wk embryo. **B**) Representative lanes from a multiplex amplifying SynPT, SynT, and β-actin together cohybridized with SynPT- and SynT-specific probes and subsequently with β-actin; lanes 6–11 are CVS. **C**) Densitometry data from the experiment shown in **A** in which cDNAs (SynPT and β-actin) were hybridized independently and SynPT values were plotted; the *P* value was estimated by comparing full-term (FT) value with mean of early expressions. **D**) Expression of SynPT and SynT was analyzed from the data shown in **B** and similar experiments, each data point normalized to β-actin giving an estimate of species-specific mRNA transcription, and SynP values were obtained by subtracting SynT from SynPT. Each data point is expressed as mean ± SD; *P* values were determined by comparing mean values of 23 early samples with that of 38 wk. **E**) Representative lanes from a multiplex coamplifying *SGCE, PEG10,* and β-actin together cohybridized with specific probes. **F**) Histogram showing expression profile of *PEG10* and *SGCE* during early and late human pregnancies; data are presented as mean ± SD from several experiments. *P* values were determined by comparing mean values of early samples with that of 38 wk.

riocarcinoma [20, 21], pre-eclampsia [11, 12], and Down syndrome [13–16]. Additionally, neighboring imprinted genes such as *IGF2/H19* (11p15.5), *DLK/GTL2* (14p13), and *HYMAI/PGLAI* (6q24) are co-ordinately regulated by common differentially methylated imprinting control region [24, 26]. Therefore, the data on transcriptional regulation of *PEG10* and *SGCE* will facilitate identification of the regulatory elements controlling their imprinted expression in human placenta. Our long-term goal is to establish whether the physiological expression patterns of these genes are affected in a variety of genetically compromised pregnancies that are marked by aberrant placental morphology and intrauterine growth restriction.

The critical finding of this study is that syncytin mRNA production (which includes both placenta and testis-specific species, SynP and SynT) is highest in term placenta, but the protein synthesis is lowest, compared with that in early pregnancy. Moreover, steady-state SynP mRNA level remained unchanged throughout pregnancy and SynT is overproduced in term placenta. Given that SynP mRNA is gen-

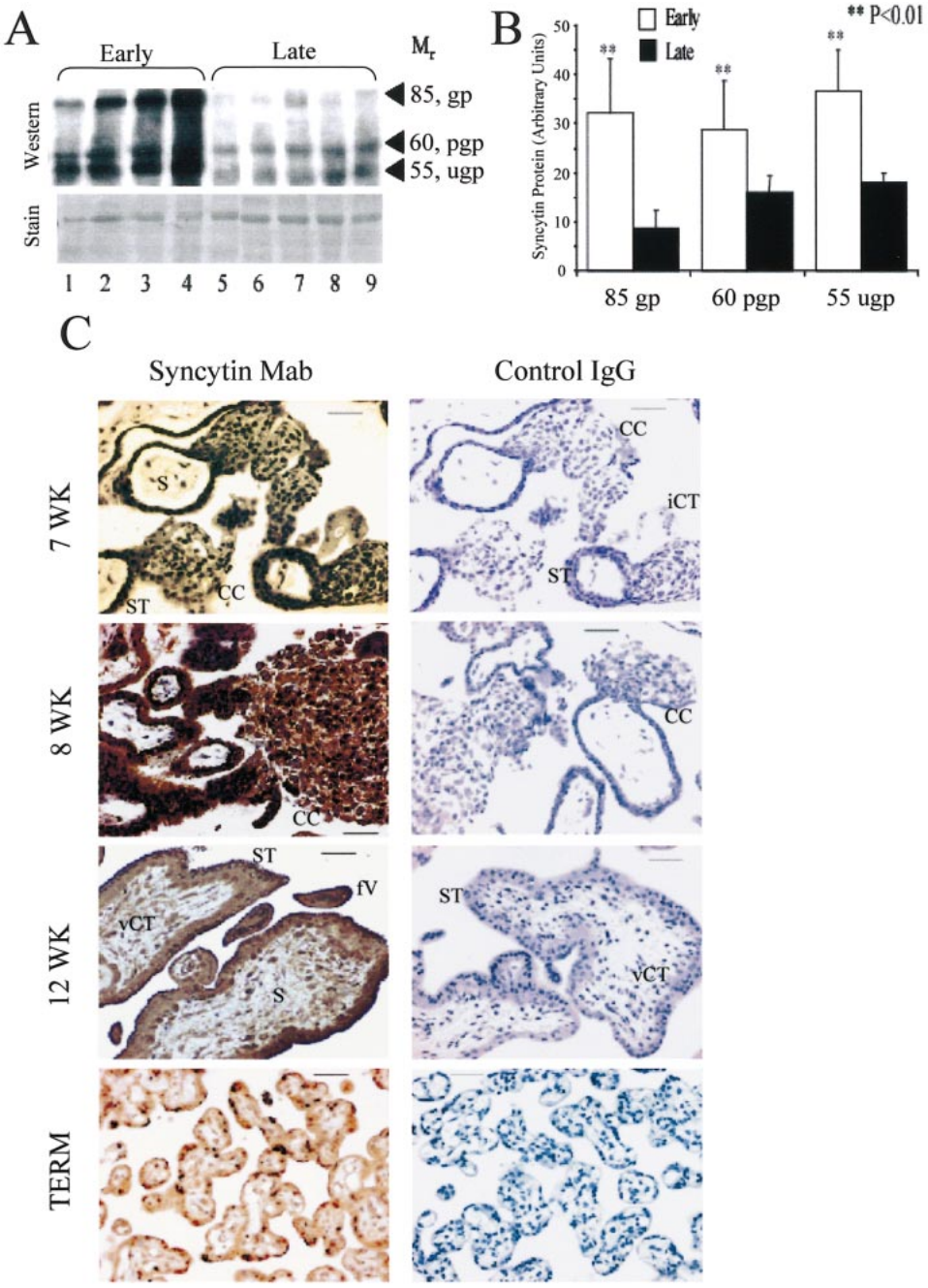

FIG. 3. Syncytin protein is most abundant in early placenta and localized in villous and extravillous trophoblasts and STs. **A**) Western blot of proteins extracted from placenta at early (7, 8, 9, and 12 wk, lanes 1-4, respectively) and late pregnancies (lanes 5–9). Unglycosylated (ugp; M_r $\sim 55 \times 10^3$), partially glycosylated (pgp, M_r $\sim 60 \times 10^3$) and glycosylated (gp; M_r $85–90 \times 10^3$) syncytin envelope proteins are indicated. A region of the Coomassie-stained blot is shown below the Western blot; **B**) A quantitative analysis of the data shown in **A**. The intensity of Western staining for each band was measured, and the values were normalized to the prominent band in the stained gel to establish quantitative difference in syncytin protein synthesis between early and late pregnancies. *P* values were determined by comparing the means of early and late samples for each band. **C**) Placental sections from various stages of pregnancy (indicated) stained with mouse anti-human syncytin monoclonal or nonspecific isotype control. cc, Cell column; s, stroma; vCT, villous cytotrophoblasts; fST, floating villous. Bar = 25 μM.

erated by splicing of SynT precursors [3, 4], it is possible that SynT is overexpressed or stabilized in late placenta, compared with that in early pregnancy. Marked reduction of envelope glycoprotein synthesis most likely was due to the competition between placenta (3.1 kb)- and testis (8.0 kb)-specific mRNAs for ribosomes [27]. Such posttranscriptional regulation of a fusogenic surface glycoprotein in trophoblasts could be a physiological necessity to sustain pregnancy following implantation. Excessive accumulation of STs could lead to uncontrolled invasion, a phenomenon (placenta percreta) quite opposite to the superficial implantation in pre-eclampsia. Two independent reports [11, 12] have demonstrated a marked reduction of *syncytin* expression in pre-eclamptic trophoblasts, leading to suboptimal invasion, compared with that of gestational age-matched normal pregnancies. Whether the low level of syncytin protein in pre-eclampsia is due to hyperactivation of SynT transcription (mRNA competition for translational apparatus) remains to be determined. Interestingly, increased production of superoxide dismutase 1 and reduced *syncytin* expression in Down syndrome early pregnancies have been postulated to be associated with poor differentiation of STs, hypoplasia, and hypovascularity of the placenta [13–16, 28].

Unlike *Syncytin*, very little is known about the *PEG10* gene products. The predicted amino acid sequences from *PEG10* (ORF1) have significant homology to mouse MyEF-3, an activator of MBP, which participates in myelinating neurons [6]. Because database searches for *PEG10* genomic sequences show homology only to a BAC (AC069292) containing human *PEG10* and *SGCE*, it is highly unlikely that extra bands (Fig. 1C) were due to cross-hybridization. Notably, two open reading frames (ORF1 and ORF2), encoded by *PEG10* mRNA, have some

amino acid sequence homology to gag (ORF1) and pol (ORF2) proteins of Sushi-ichi, a retrotransposon from pufferfish [5]. *PEG10* is downregulated at early hypoxic phase of placental growth and is induced at 11–12 wk of gestation (Figs. 1 and 2), suggesting that the gene product could be essential for trophoblast differentiation and uterine implantation.

SGCE is constitutively expressed in early and late human placenta, indicating that the gene product could be essential for cell-to-cell signal transduction during proliferation, differentiation, and uterine invasion of trophoblasts. This conjecture is consistent with the observation that mice carrying homozygous deletion (dystroglykin$^{-/-}$) die prior to implantation (d6.5) because of disruption of Reichert membrane [29], whereas in adults, heterozygous loss-of-function mutations in *SGCE* are linked to Myoclonus-dystonia syndrome characterized by myoclonic jerks of arms (writer's cramp) and axial muscles. Notably, *SGCE* and *PEG10* exhibit very little coordinated activation at the hypoxic phase of placental growth (6–10 wk) (Fig. 2, E and F). Because the two genes are organized in a head-to-head (opposite) orientation and are differentially activated at early hypoxic phase of placental growth, it is possible that they share common regulatory elements capable of interacting with gene-specific transactivator/inhibitor [30].

Genomic imprinting in mammals is proposed to have evolved to maintain balanced growth and development through monoallelic expression of the genes in the placenta as well as embryo [25]. The early studies on the developmental potential of androgenetic and gynogenetic mouse embryos have shown that paternal and maternal haploid chromosomes, in general, contribute to the development of extraembryonic tissues (placenta) and the embryo proper, respectively [31, 32]. Partial hydatiform moles in human triploid pregnancies with paternal (diandy) or maternal (digyny) duplication of haploid chromosomes have two distinct developmental phenotypes, suggesting their parent-of-origin effect on growth and differentiation of human placenta [33]. Despite a catalogue of imprinted genes identified through genetic and molecular analysis of uniparental disomies in mouse [34, 35] we know very little about their regulation as well as specific role in human placental growth, embryogenesis, or both. *HERV-W* maps very close to two neighboring maternally imprinted (paternal expression) genes, *SGCE* and *PEG10* at 7q21 [5]. Because imprinted genes are clustered [26], coordinately regulated through neighboring chromatin domains [36], and both *PEG10* and *HERV-W* are retroelements, it is possible that *Syncytin* could be paternally expressed. Genetic polymorphism analysis of placental cDNAs from pregnancies with maternal iso- and heterodisomy of chromosome 7 [17–19] and triploidy might help to settle this issue. Our recent analysis revealed relaxation of imprinting of SGCE and PEG10 in placenta from a variety of genetically compromised pregnancies including Down syndrome (unpublished results).

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