Cytological Localization of Chorionic Gonadotropin α and Placental Lactogen mRNAs during Development of the Human Placenta

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ABSTRACT Probes derived from clones bearing cDNAs corresponding to the alpha subunit of human chorionic gonadotropin (hCG) and human placental lactogen (hPL) were used to localize their respective mRNAs cytologically in sections of first trimester and term human placenta. hPL mRNA was exclusively localized to the syncytial layer; hCGα mRNA was found in the syncytial layer and also in some differentiating cytotrophoblasts. Hybridization was specific because no signal was observed when labeled pBR322 was hybridized to placental sections or when the placental probes were hybridized to sections of human tonsils. In addition, RNA in placental interstitial cells did not hybridize with hCGα and hPL probes.

Hybridization with the hCGα probe was much greater in first trimester than in term sections, whereas hPL signals were comparable in both first trimester and term placentae. Syncytial formation proceeds through cellular intermediates of cytotrophoblastic origin, and the data suggest that transcription of the hCGα gene is initiated before the completion of syncytial formation. In contrast, hPL mRNA synthesis starts later in trophoblast differentiation, likely after the stage of syncytial formation. The data also suggest that hCGα mRNA synthesis becomes attenuated but that hPL is transcribed at a rather constant rate during placental development.

The human trophoblast differentiates throughout pregnancy. Progeny of mitotically active mononucleated trophoblasts (cytotrophoblasts) fuse to form and expand the mitotically inactive syncytiotrophoblast (14, 25). The ratio of cytotrophoblast to syncytiotrophoblast decreases progressively until the syncytial layer is the dominant trophoblastic component at term.

During its development, the placenta elaborates at least two peptide hormones, human placental lactogen (hPL) and human chorionic gonadotropin (hCG) (3, 4). Many histochemical studies using specific antibodies have suggested that these hormones are synthesized in the syncytiotrophoblast (16, 20, 22, 30, 31), while other studies have indicated that cytotrophoblasts may contain only the hCGα subunit (17, 19). Immunofluorescence studies, which measure only steady-state levels of the hormones, do not exclude the possibility that *de novo* synthesis of the protein and/or mRNA occurs in a cell-type distinct from that in which the highest steady-state level accumulates. Moreover, since differentiation from the germinative cytotrophoblasts to the syncytiotrophoblast proceeds through transitional cell intermediates (33), a gradient of hCG synthesis may exist in different cell types that reaches its peak in the syncytiotrophoblast. To identify the cellular location of the mRNAs encoding the hCGα subunit and hPL, we hybridized sections from human placental villi *in situ* (24) with labeled cDNA probes derived from bacterial clones bearing sequences corresponding to these mRNAs.

Here we show that hCGα mRNA is predominantly localized in the syncytiotrophoblast of placental villi; it is also present in some cytotrophoblasts. In contrast, hPL mRNA is found only in the syncytial layer. Furthermore, while the amount of hCGα signal per syncytial nucleus is much less in sections from third-trimester (term) than in sections from first-trimester placentae, the levels of hPL mRNA sequences in first- and third-trimester tissue are comparable.

MATERIALS AND METHODS

In situ hybridization was performed using techniques described by Michael Akam (personal communication) and by Copple and McDougal (11).

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Preparation of Double-stranded Complementary DNA for Alpha Subunit and hPL

pBR322 clones bearing cDNAs complementary to hCGα and hPL mRNAs (cDNAs) were isolated and purified as previously described (5, 6). The clones were digested with reduction enzyme, Pst I, and the resulting fragments were isolated by polyacrylamide electrophoresis. The following fragments were used as probes in this study: (*a*) a 440 base-pair Pst I restriction fragment which contains the complete 5′ noncoding sequence and all but the last eight amino acids of the coding region of the hCGα subunit (15); (b) a Pst I fragment of 540 base pairs that contains the information corresponding to the codons for amino acid 60 to the end of the peptide and the contiguous 3′ noncoding region of hPL. These fragments were purified as previously described (5).

Preparation of ^{3}H-cDNA

The fragments were nick-translated in a 10-μl reaction mixture containing ~200 ng of fragment, 700 pmol each of [^{3}H]dCTP and [^{3}H]TTP, and 25 pg of DNase I (21). The specific activity of the radioactive DNA was 2.1–2.5 × 10^7 cpm/μg, and its size was 60–80 bp under nondenaturing conditions. It is crucial for optimal hybridization that the fragments be reduced to this length. The labeled fragments were separated from unincorporated material by gel filtration on Sephadex G 50–80, and the excluded fraction was ethanol precipitated. The precipitates were pelleted in an Eppendorf centrifuge (Brinkmann Instruments, Inc., Westbury, NY) and dissolved in water to a final concentration of 4 ng/μl.

Tissue Preparation

Fresh placental tissue was rinsed in PBS at 4°C to remove blood and serum, then cut into small pieces (5 × 5 × 5 mm^3), avoiding calcified areas and blood vessels. The fragments were embedded in O.C.T. compound (Lab-Tek Products, Miles Laboratories, Inc., Naperville, IL), immersed in liquid nitrogen, and stored at −70°C. Human tonsil tissue was obtained immediately after tonsillectomy and processed as described above.

Embedded tissue was trimmed into 2 × 2-mm squares and excised from the O.C.T. compound just before sectioning. The tissue was cut into 5-μm sections in a cryostat (American Optical Corp., Southbridge, MA) at −20°C and placed 2–3 cm from the edge of a treated slide (see below). To evaluate possible spurious results resulting from tangential sectioning, two contiguous sections corresponding to approximately the entire volume of a cell were placed on the same slide.

After air drying for 10 min at room temperature, the sections were fixed in ethanol:glacial acetic acid (3:1) for 20 min at 4°C and rinsed in 2 × SSC. The slides were washed with two changes of 2 × SSC and dehydrated by successive 5-min periods of immersion in 75% and 95% ethanol. They were air dried and then stored at −20°C.

Slide Glass Treatment

Microscope slides were incubated for 3 h at 70°C in a solution containing 450 mM NaCl, 45 mM sodium citrate (pH 7.0), 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin (BSA). They were then dipped in water and fixed for 20 min in ethanol: glacial acetic acid (3:1). The slides were dried at 70°C in an oven overnight (8).

Cover Glass Treatment

To reduce nonspecific adsorption of the probe, cover glasses (Fisher Scientific Co., Pittsburg, PA [circle diameter 18 mm]) were immersed in Sigmacote (Sigma Chemical Co., St. Louis, MO) for 1 s and dried in an oven at 70°C overnight.

Hybridization

Before hybridization the slides with sections were immersed in 0.2 N HCl, incubated at room temperature for 20 min, and washed for 30 min with 2 × SSC (prewarmed to 70°). After rinsing in water for 5 min, the sections were dehydrated in 75% and 95% ethanol and air dried. The sections were pretreated with hybridization medium (600 mM NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA (pH 7.5), 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, 0.1% BSA, 0.1% salmon sperm DNA (sheared and denatured by boiling for 20 min in 0.2 M NaOH and subsequently reneutralized), 0.1% yeast tRNA, 0.01% poly A and poly C, 50% formamide (MCB; deionized for 2 h with 5 g of Bio Rex RG 501-X8 per 100 ml). 10 μl of this medium were applied to each section and incubated in a moist chamber for 2 h. After rinsing in water for 5 min, the sections were again dehydrated through 75% and 95% ethanol.

The sections were hybridized with 4 ng of [^{3}H]cDNA (boiled for 1 min, ~80,000 cpm) per 10 μl of medium, covered with a siliconized cover glass, and sealed with rubber cement. The covered slides were incubated in a moist chamber at room temperature overnight. After the cover glasses were carefully removed in 2 × SSC, the slides were rinsed for 6 h with several changes of 2 × SSC at 4°C, with 1 × SSC for 6 h, and the slides were dehydrated.

Autoradiography

The slides were dipped in nuclear track emulsion (Kodak NTB-2) diluted with water (1:1) and prewarmed to 45° (26). They were dried in an upright position and exposed in a light-proof box for 2–3 weeks at 4°C. The slides were developed with Kodak D-19 developer (3 min at 20°C), rinsed in cold water, fixed with Kodafix (5 min at room temperature), and stained with hematoxylin-eosin.

Mean Grain Count (MGC)

The boundary between syncytiotrophoblasts and cytotrophoblasts was outlined on enlarged micrographs (× 400) of random fields without regard to the probe used, and the number of grains over the tissue was counted. The distribution of grains over placental villi was expressed as a mean grain count (MGC) calculated in the following way (10):

$$\text{MGC} = \frac{\text{Number of grains}}{\text{Number of nuclei} \times \text{Weeks of exposure}}$$

Because cytotrophoblasts disappear from term placentae, the comparison of grain densities in sections of first-trimester and term placental sections are limited to the syncytiotrophoblast. All of the comparisons of MGC in sections from first-trimester and term placentae were evaluated on preparations processed in parallel.

RESULTS

Hybridization to Sections from First Trimester Placentae

Human placental villi (Villus Surface) are covered by the syncytiotrophoblast (syncytium, *ST*), which overlies a layer of cytotrophoblasts (Langhans cell, *CT*) and interstitial tissue (Fig. 1). The intervillous space (*IS*) is the source of maternal blood. Exchange from this space with the fetal circulation occurs across the syncytial barrier.

In addition to this typical villous structure, there are other distinguishing histological features of the human trophoblast in first-trimester tissue. Syncytial sprouts (*SP*), which represent an aggregation of syncytial nuclei within a small zone of cytoplasm, arise from the villous syncytial layer and frequently separate from their origins to become free in the intervillous space. Another common structure is composed of a collection of undifferentiated cells, the cytotrophoblastic column (*CC*). Cytotrophoblasts at the basal portion of the cell column have the greatest proliferative activity, and they are the generative cells for the multinucleated syncytial layer (Fig. 1; also illustrated in Fig. 9).

After about the fourteenth week of pregnancy, cytotrophoblastic column structures and many of the Langhans cells beneath the syncytial layer gradually disappear. By parturition the predominant structure is the villous surface, with sparse cytotrophoblasts and a layer of syncytium (33).

Both hCGα and hPL mRNAs are abundant in first-trimester tissue (4), and the distribution of these mRNAs in the trophoblast of first-trimester placentae was examined. Sections of 7- to 12-week placentae were hybridized to nick-translated probes corresponding to hPL and hCGα mRNAs. The distribution of grains as visualized under lower magnification (× 200) is shown in Figs. 1 and 2. For both probes, most of the signal was seen in the syncytial (*ST*) regions although more hCGα signal was seen in cytotrophoblasts than was the case for hPL. Syncytial sprouts were also heavily labeled (Fig. 1), and little, if any, signal was seen in the cell column region.

Examination of the sections under higher magnification in

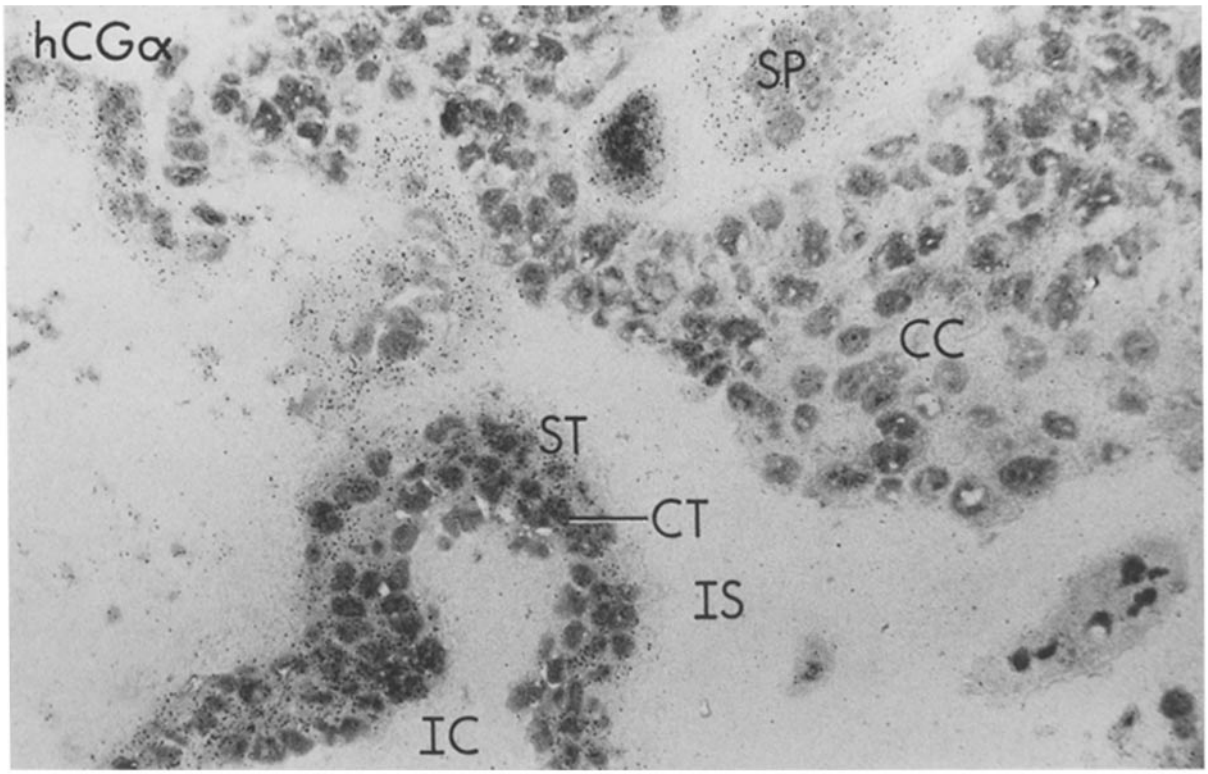

FIGURE 1 Photomicrograph of an autoradiograph of first-trimester (8 wk) placental tissue hybridized *in situ* with hCGα probe (× 200). 5-μm sections were hybridized to 80,000 cpm of nick-translated probe and exposed for 14 d (see Materials and Methods). Silver grains are observed primarily over the syncytiotrophoblast (*ST*) and syncytial sprout (*SP*) regions, and some are present over the cytotrophoblasts of the villus surface. By contrast, weak signals are seen over the cell column (*CC*) which contain undifferentiated cells (upper right). Fragments of syncytium scattered in the intervillus space (*IS*) contain hybridizable mRNA. *IC* denotes villus core (× 200). Background over interstitial tissue (*IC*) is negligible.

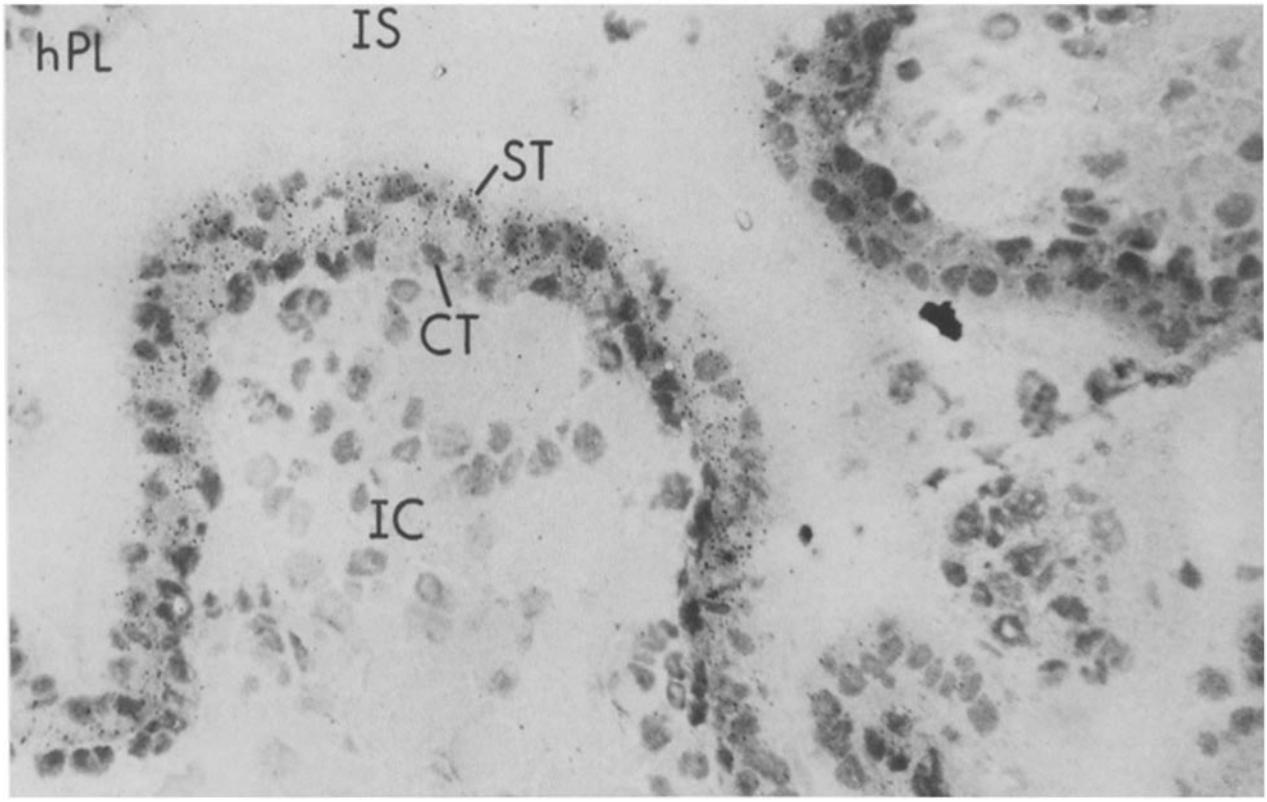

FIGURE 2 *In situ* hybridization of sections of first-trimester (8 wk) placenta with hPL probe. 5-μm sections were hybridized to 100,000 cpm of nick-translated probe and exposed for 14 d (see Materials and Methods). Specific signals are confined to syncytial region (*ST*) of villus surface although a few scattered grains are present over cytotrophoblastic region (*CT*). Background over the interstitial tissue (*IC*) is negligible (× 200). (Compare with Fig. 1.)

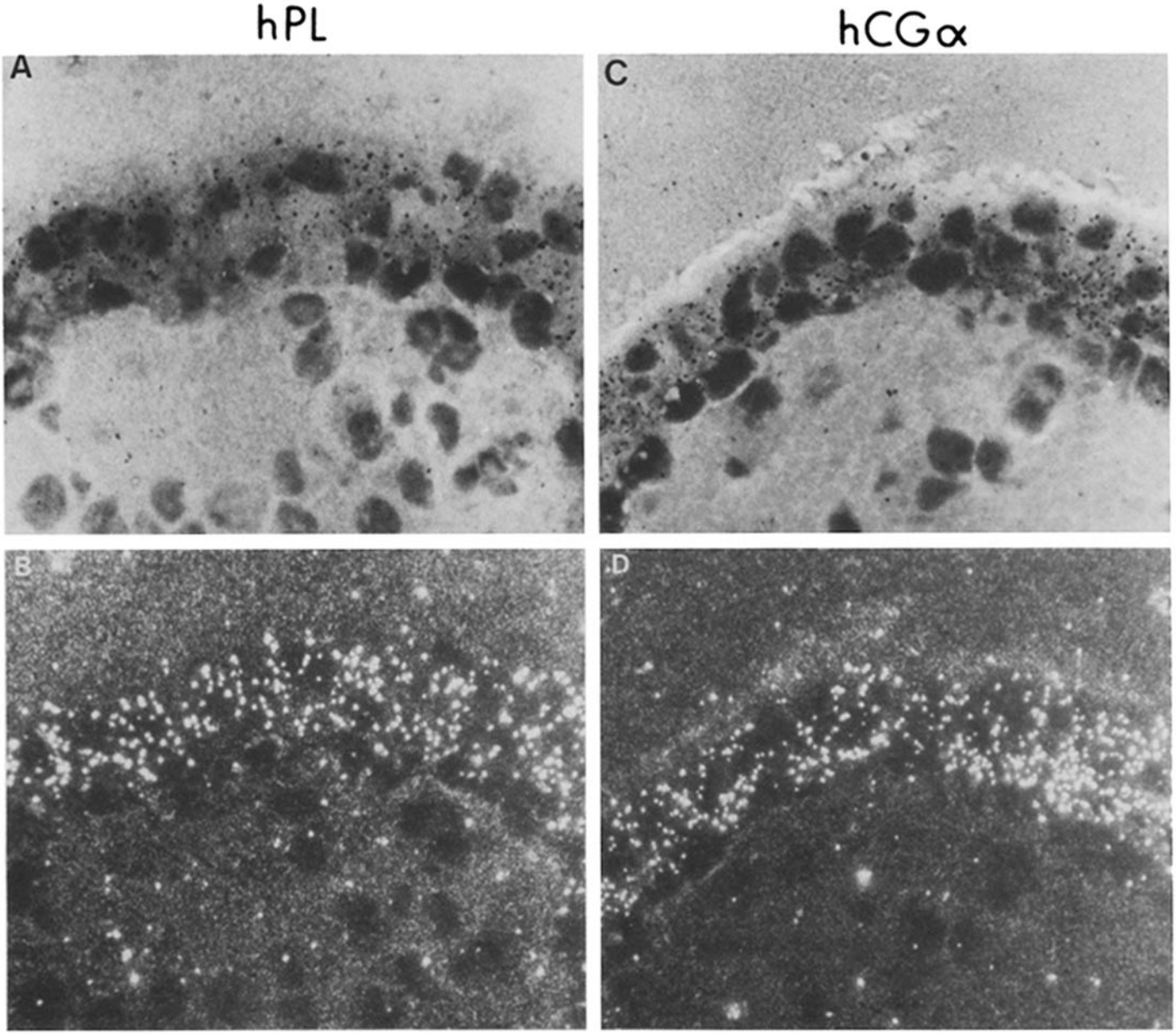

FIGURE 3 Distribution of hPL (*A, B*) and *hCGα* (*C, D*) mRNAs in first-trimester sections viewed under high magnification (× 350). Panels *B* and *D*: dark-field micrographs of the bright-field panels *A* and *C*. Note that in panels *C* and *D* the grains are closer to the *IC* region, i.e., they may overlie cytotrophoblasts.

bright- and dark-field microscopy shows in greater detail that the hPL signals are largely confined to the syncytial region (Fig. 3). In contrast, the hCGα probe revealed many more silver grains over the region of the cytotrophoblastic layer.

The hCGα signal seen in the cytotrophoblast regions could be due to tongues of syncytiotrophoblastic cytoplasm (syncytioplasm) lying over the cytotrophoblasts which could be misinterpreted in a single section. To address this point, serial sections of first-trimester tissue were hybridized to hCGα probe (Fig. 4*A-C*). Significant signals were observed in the same cytotrophoblast regions of the two adjacent sections (*A*, *B*); these sections which represent an approximate thickness of 10 μm should be enough to exclude the presence of syncytiocytoplasm. Panel *C* shows the dark-field micrograph of panel *B*. The grains seen in the intervillous space correspond to signals in syncytial sprout regions.

Using photomicrographs at higher magnification that show differences between the cytoplasmic regions of the syncytiotrophoblast and cytotrophoblast, an estimate of the mean grain count (MGC) distribution of hPL and hCGα in the different cell layers was determined. The data in Table I were obtained using sections derived from a single placenta and processed simultaneously with both probes. Cytotrophoblast to syncytiotrophoblast ratio of MCG was greater in the sections hybridized to hCGα probe (0.5) than with hPL probe (0.3). Although it is difficult to assess the contribution of grains from syncytioplasm to the surrounding cytotrophoblast cells, these data, together with those shown for the serial autoradiographs, suggest that some cytotrophoblast cells begin to express hCGα mRNA before the formation of terminal syncytium, whereas hPL is expressed later during formation of syncytium.

Specificity of Hybridization

To assess the specificity of *in situ* hybridization, several

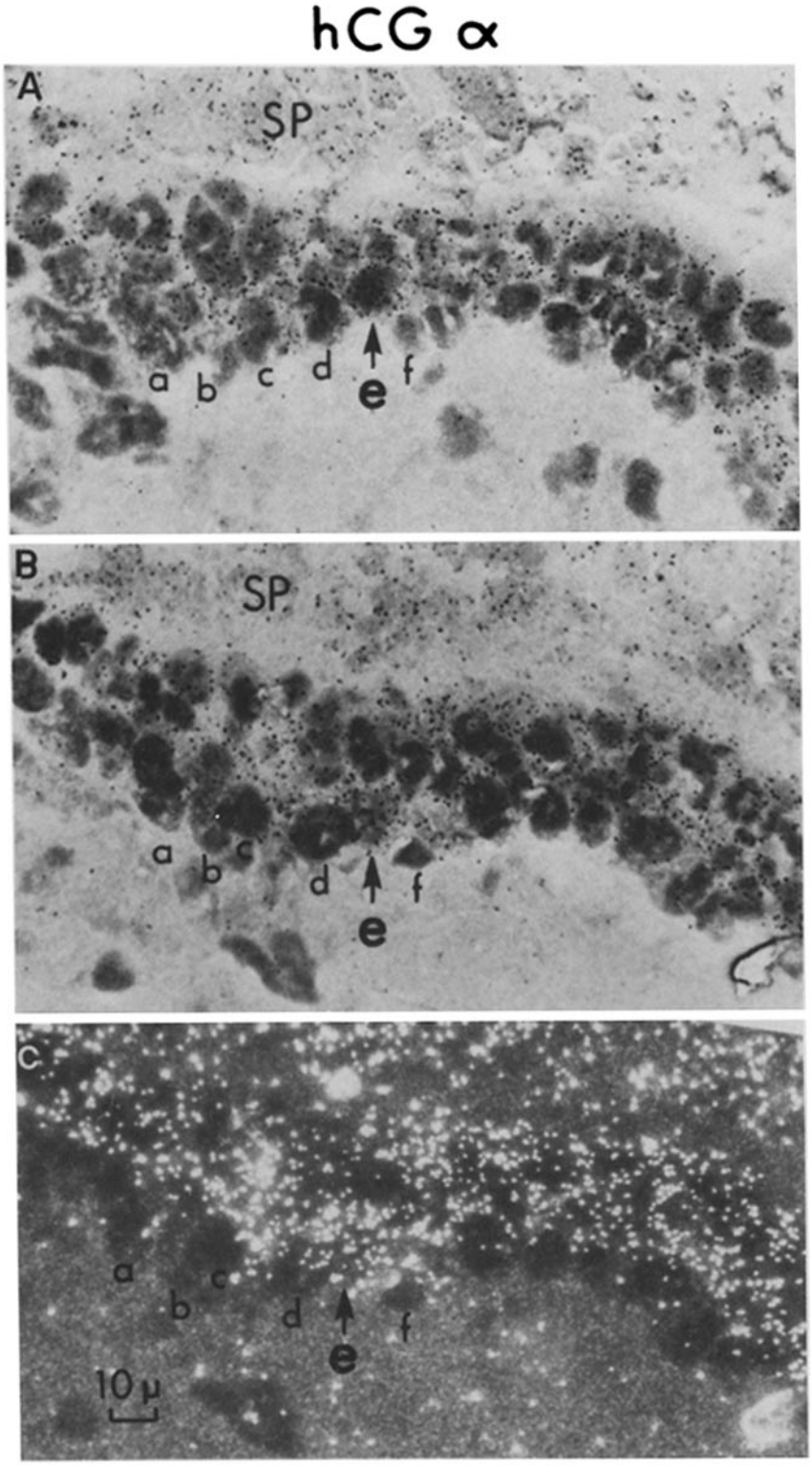

FIGURE 4 5-μm serial, adjacent sections hybridized with hCGα probe. Distribution of grains are shown in bright-field (*A*, *B*) and dark-field (*C*) photomicrographs (× 350). Panel *C* is a dark-field representation of Panel *B*. The sites designated *a–f* correspond to regions present in both sections. The arrow in area *e* represents a trophoblast region that contains many silver grains in both sections.

control experiments were performed. Placental sections (7–9 wk) were hybridized to the fragments derived from a Hha I digest of pBR322. The specific activity of this probe (7.8×10^7 cpm/μg) was more than three times greater than that of the hCGα probe (2.1×10^7 cpm/μg). The amount of radioactivity applied to the slide and the duration of exposure (2–3 wk) were identical to those used in the hybridization with placental probes. Only a few scattered silver grains were seen when placental sections were hybridized to this probe (Fig. 5).

The sensitivity of the hybridized material to RNase was examined (Fig. 6*A*, *B*; reference 22). To ensure that untreated and treated sections were from nearly identical regions of the tissue, we compared paired serial sections. Slides (sections) were treated with pancreatic RNase (100 μg/ml) or buffer by preincubating for 1 h at 37°C and washing with 2 × SSC for 6 h at 4°C. The slides were then processed for hybridization. In the absence of RNase significant hybridization to the villous surface was seen, whereas in the presence of RNase there was a marked reduction in the number of grains (Fig. 6*A*, *B*). (The efficiency of RNase treatment in these experiments was greater

than in our previous study using a single-stranded cDNA probe [23]. In each case, the RNase was incubated at 37°C for 1 h but in the earlier work the medium was 2 × SSC and here the sections were incubated in phosphate-buffered saline. Moreover, in the current study the slides were incubated with RNase with a magnetic stirrer, and after RNase incubation, the slides were washed for 6 h. In the absence of these treatments the RNase effect was reduced. In the previous paper the placental sections were hybridized at 65°C in 5 × SSC. Here, hybridization was performed at room temperature in Denhardt medium containing 50% formamide.)

As an additional control, sections of a nonplacental human tissue (tonsil) were hybridized to hCGα and hPL probes under identical conditions as described above. The typical lymphoid appearance of the tonsil tissue is clearly seen. In these sections, only a few scattered grains are observed (Fig. 7*A*, *B*). The specificity of hybridization is further shown by the lack of signal in the nontrophoblastic (interstitial) cells of the villus (Figs. 1, 2, 3, and 4). These cells serve as a convenient internal negative control for the specificity of the reaction.

Taken together, these data show that the radioactive grains in the trophoblasts were the result of specific DNA-RNA hybrids.

TABLE I

Trophoblast Distribution of the Mean Grain Count in First-Trimester Sections Hybridized to hCGα and hPL Probes

Region	Syncytio-tropho-blast	Cyto-tropho-blast	Syncytio-tropho-blast	Cyto-tropho-blast
	hPL		*hCGα*	
Number of nuclei	247	166	221	153
Mean grain count	6.7	2.0	8.4	4.3
Ratio (CT/ST)	0.30		0.51	

Background MGC based on pBR control was <0.15. Specific activities of hPL and hCGα probe were 2.5 × 10^7 cpm/μg and 2.1 × 10^7 cpm/μg, respectively.

Hybridization to Term Placental Sections

We have previously shown that the steady-state levels of hPL and hCGα mRNAs parallel the levels of these hormones in maternal serum during gestation (3, 12). The synthesis of hPL is maximal at term, whereas the peak of hCGα biosynthesis occurs in the first trimester. Accordingly, we examined the hybridization of the hCGα and hPL probes to sections from first- and third-trimester placentae (Fig. 8). It is evident that the morphology of term placental sections is much different from that in sections of first-trimester placenta. The major difference is the lack of cytotrophoblasts in term villi. In the case of hCGα, the number of grains detected at 38 wk with the hCGα probe was reduced significantly (Fig. 8*B*) compared to the hybridization observed at 8 weeks (Figs. 1, 3). Hybridization with the hPL probe showed an abundance of grains at 38 wk (Fig. 8*A*) comparable to that in 8- to 12-wk placental sections processed in parallel (Fig. 4). Quantitative estimates based on mean grain counts per syncytial nucleus in a 1-wk exposure showed an almost fourfold decrease of the hCGα mRNA in sections from term placenta, whereas with hPL there was little variation in the mean grain counts between first trimester and term (Table II).

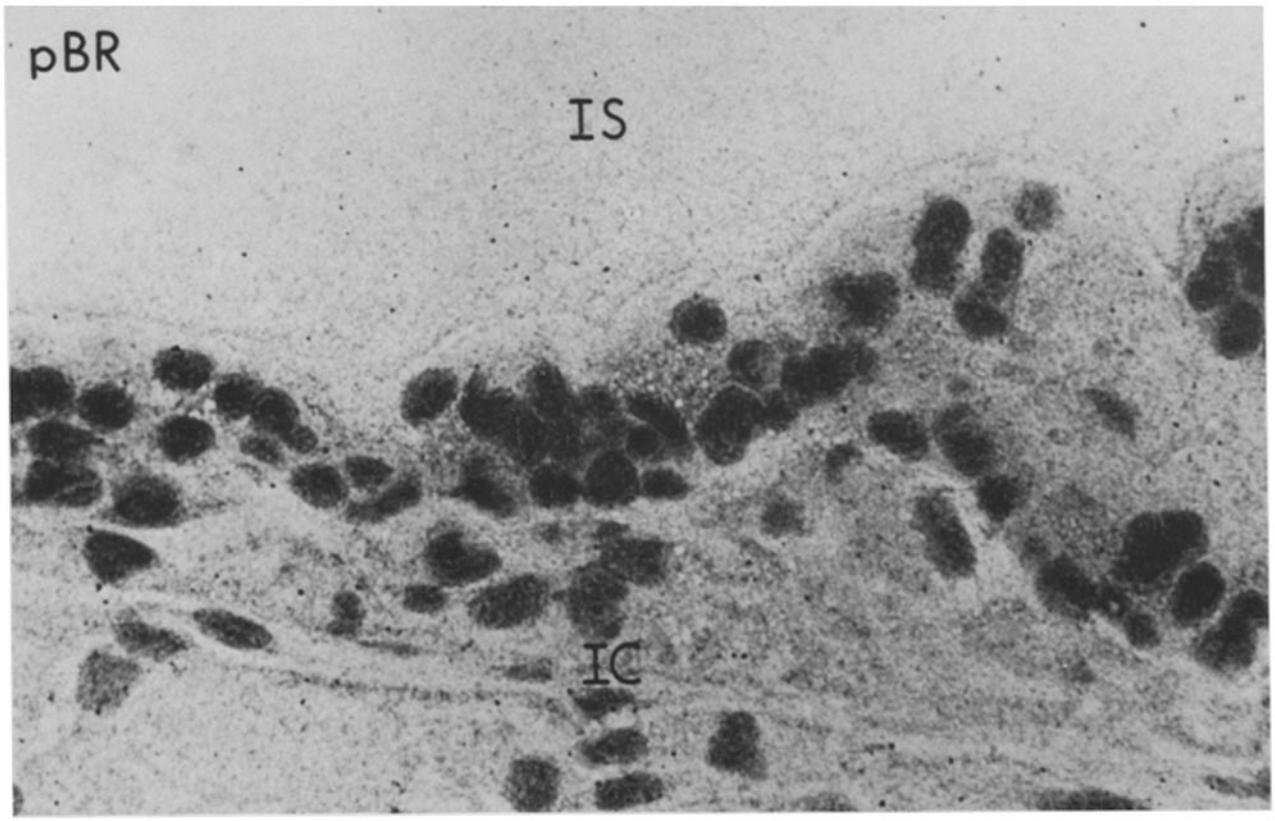

FIGURE 5 Hybridization of first-trimester (9 wk) placental sections with 300,000 cpm of a nick-translated Hha I digest of pBR322. Slides were exposed for 25 d. A few scattered grains were observed in trophoblastic region and interstitial tissue. The background, in terms of mean grain count, was <0.15.

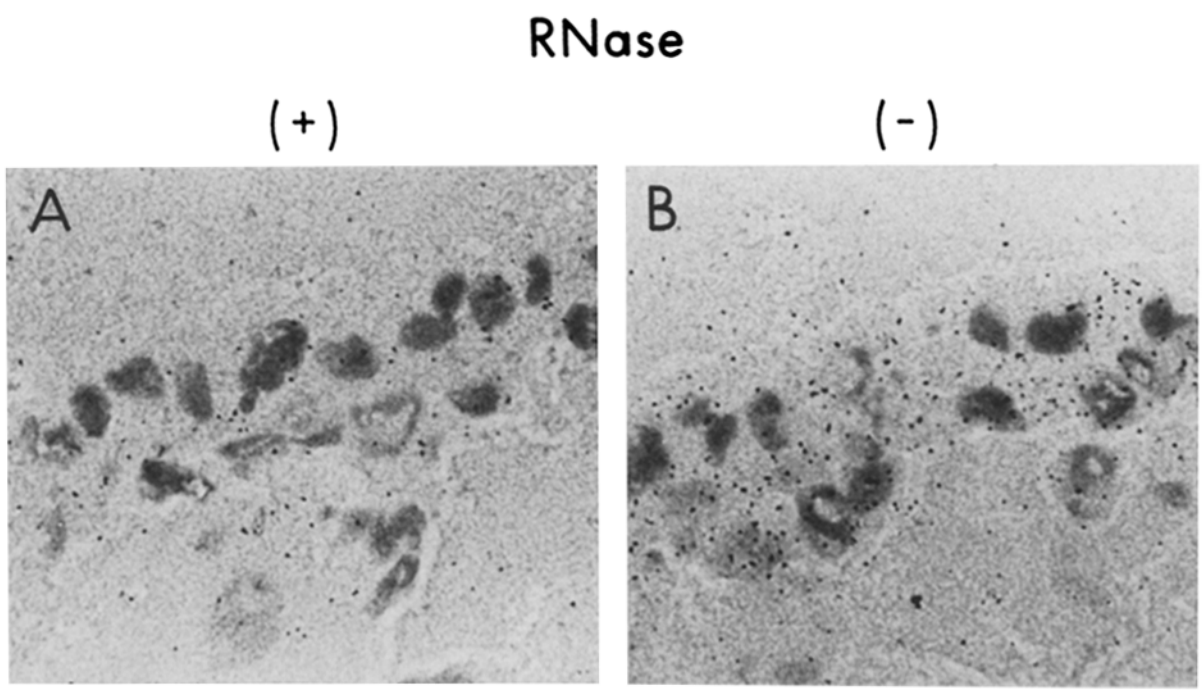

FIGURE 6 Effect of RNAse pretreatment on the hybridization of hCGα probe to first-trimester placental sections (11 wk). Before hybridization with ^{3}H-hCGα fragment, slides with tissue sections were treated with RNase (*A*) or buffer (*B*) as described in the text. The slides were exposed for 25 d. × 400.

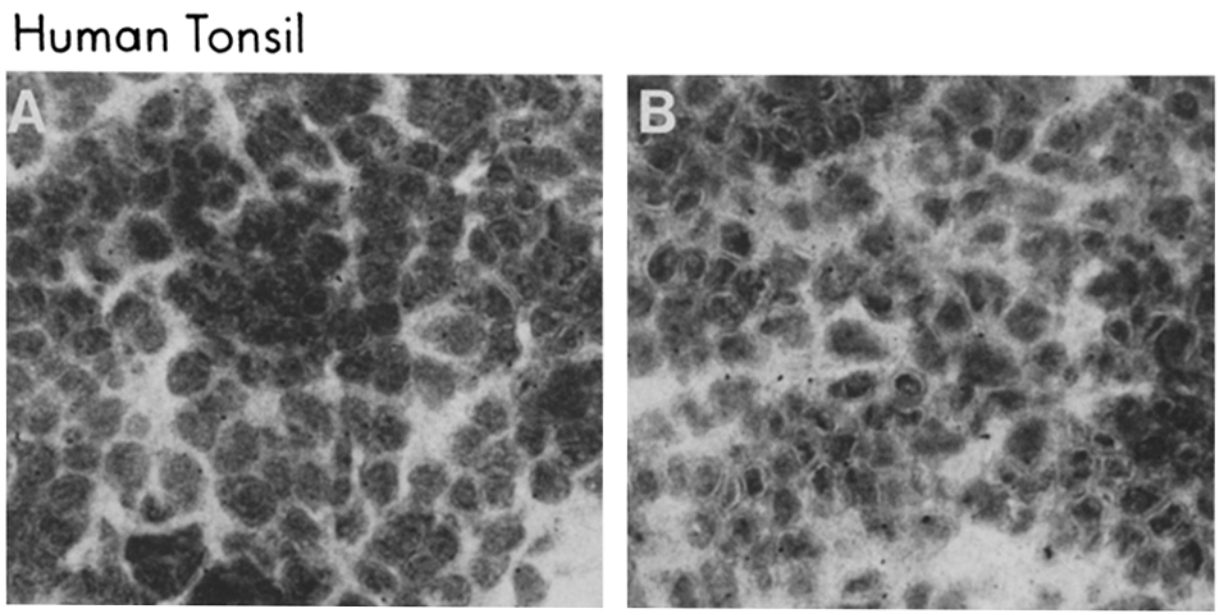

FIGURE 7 *In situ* hybridization of human tonsil tissue with 80,000 cpm of hCGα probe (*A*) or 100,000 cpm of hPL probe (*B*). Slides were exposed for 25 d (× 400). The labeling is very sparse and indistinguishable from the background.

DISCUSSION

hCGα mRNA is present not only in the syncytiotrophoblast layer but also in some cytotrophoblasts. Not all cytotrophoblasts displayed a significant signal; only those cells in direct apposition to the syncytiotrophoblast layer were positive (Figs. 1, 3, and 4). Because syncytial formation proceeds through cellular intermediates of cytotrophoblastic origin (33), the data suggest that transcription of the hCGα gene is initiated during this process but before the completion of syncytial formation. This interpretation is consistent with earlier studies which identified hCGα protein in the syncytium and cytotrophoblasts by immunohistochemical studies (14, 17).

An alternative explanation for the hCGα signals seen over the cytotrophoblasts is that they may be related to an intercalation of syncytiocytoplasm between the cytotrophoblasts which reaches the basement membrane of the cytotrophoblast layer (7). This is highly unlikely because one would expect to see a similar pattern in sections scored with the hPL probe; such a pattern was not observed in the sections hybridized with the hPL probe.

Less (three- to fourfold) hCGα mRNA was detected in term

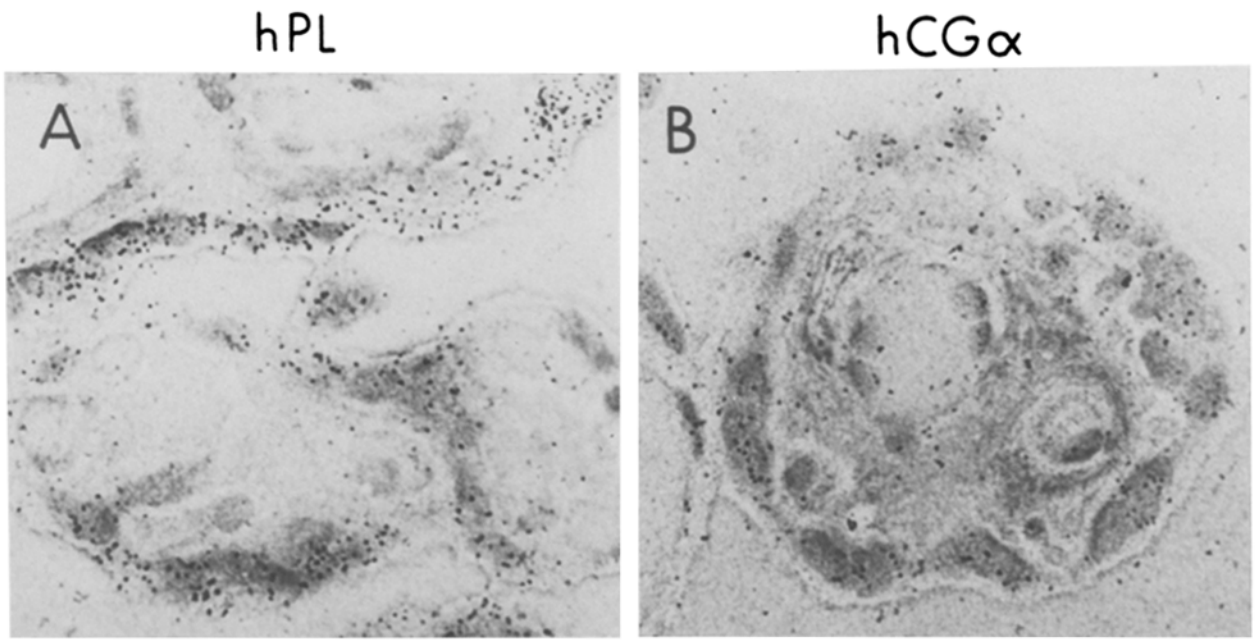

FIGURE 8 *In situ* hybridization of human term placental sections with hPL (*A*) or hCGα (*B*) probes. The major component of the villus surface of term placenta is a thin-layered syncytium. A small number of silver grains hybridized with ^{3}H-hCGα probe in syncytium is observed (*B*). The number of silver grains in (*A*) hybridized with ^{3}H-hPL probe is comparable with that of hPL mRNA signal in first trimester (Fig. 4). Although there is a marked reduction in the signal with hCGα probe (*B*), the signal elicited with the hPL probe is similar to that seen in first trimester (Fig. 4). Exposure was 21 d. × 400.

TABLE II

Change of Syncytiotrophoblast Mean Grain Count in Sections Prepared from First-Trimester and Term Placentae

Week of gestation	hPL probe	hCGα probe
8	11.9	12.3
12	12.7	6.7
38	11.1	3.3

More than 100 nuclei were counted for each probe. Specific activities of the hPL and hCGα probes were 8.4 × 10^7 cpm/μg and 1.3 × 10^8 cpm/μg, respectively. Background, based on pBR control, was <0.15.

than in first-trimester sections. Because the translatable level of hCGα mRNA declines about eightfold by the time of parturition (12), this finding was not unexpected and indicates that mRNA levels detected *in situ* reflect the overall level of message in the tissue.

Previously, we identified hPL mRNA in syncytial regions of term placental sections using a single-stranded cDNA probe synthesized from fractions of a sucrose gradient enriched for term placental mRNA (23). Because the distribution of cytotrophoblasts in term placenta is sparse, we were unable to assess their level of label easily. Using a cloned fragment of hPL cDNA, we confirm the earlier study and show that no significant hybridization was observed in cytotrophoblasts. These results would suggest that synthesis of hPL mRNA starts later in trophoblast differentiation than does hCGα mRNA synthesis, perhaps after syncytial formation (Fig. 9).

Whereas the mean grain count data for hPL mRNA reveal some grains over the cytotrophoblast region, the grains were not distributed uniformly over the cytotrophoblasts as observed with hCGα probe, but rather these hPL signals were localized near the syncytio-cytotrophoblast boundary. We attribute these signals to possible encroachment of the cytotrophoblast layer with syncytioplasm at the boundary of the two regions since the distinction between the two zones was not assessed at the electron microscopic level. Also, the spatial resolution of ^{3}H in the light microscope is ~3 μm which could result in grains being seen over the cytotrophoblastic layer, particularly at its border with the syncytiotrophoblast.

The cytological levels of hPL mRNA do not parallel the in vivo serum levels of the hormone. Although the serum concentration of hPL at term is 20-fold higher than at 10 wk of gestation, this increase parallels the growth in placental (syncytiotrophoblast) mass during gestation. Consistent with this observation, the *in situ* hybridization data reveal that the content of hPL mRNA per unit of syncytial mass remains constant during gestation.

In cell-free lysates, term RNA directed the synthesis of five times more hPL than an identical quantity of first-trimester RNA (3). We suggested that this reflected the changing composition of the trophoblast during gestation, namely that the syncytium comprises a larger proportion of the trophoblast at term. Therefore, the contribution of cytotrophoblast RNA to the first-trimester RNA population results in a dilution of syncytium-derived hPL mRNA.

Many trophoblastic and nontrophoblastic tumor lines synthesize the hCGα subunit (1, 9, 13, 18, 28, 29, 32). In most of these tumors little, if any, hPL is synthesized (27). Thus, hPL mRNA may be synthesized only in the fully differentiated trophoblast, whereas hCGα mRNA can be synthesized in less differentiated states.

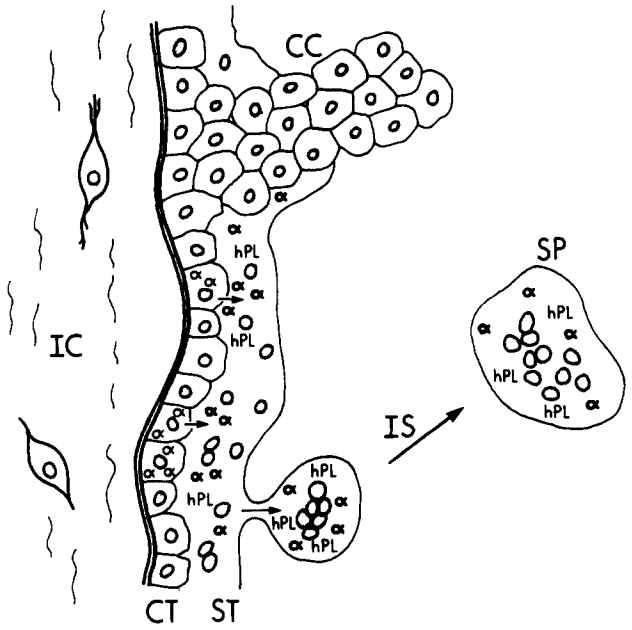

FIGURE 9 Proposed model of hCGα mRNA and hPL mRNA expression during trophoblastic differentiation. Cytotrophoblastic cell column (*CC*), which is a collection of proliferative and undifferentiated cytotrophoblasts, contains no hCGα or hPL mRNAs. Some cytotrophoblasts (*CT*) in villus surface (Langhans cells) contain hCGα mRNA but not hPL mRNA. After the completion of differentiation, both mRNAs appear in syncytiotrophoblast.

The data presented here show that the expression of hPL and hCG during placental development is quite different. Synthesis of hCGα mRNA is activated in nuclei of cells that have not reached the most differentiated stage of trophoblast development. Accumulation of this RNA is maximal within the syncytial layer, but when the cytotrophoblast population is depleted the RNA levels for hCGα decline. Thus, maximal accumulation of hCGα message may depend on the influence of cytotrophoblasts. In contrast, hPL accumulation is detectable only in the syncytium and seems largely independent of the mitotic trophoblast elements, as reflected by a level that is roughly constant on a per nuclear basis through placental development.

Further answers to the question of how hCG and hPL are differentially coupled to gestation will require a system in which isolated cytotrophoblasts can be induced to form syncytium in culture. The expression of these hormonal genes could then be followed through the entire process of differentiation.

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