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

pBR322 clones bearing cDNAs complementary to hCGa and hPL mRNAs (cDNAs) were isolated and purified as previously described (5, 6). The clones were digested with restriction 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 hCGa 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 3H-cDNA

The fragments were nick-translated in a 10-μl reaction mixture containing ~200 ng of fragment, 700 pmol each of [3H]dCTP and [3H]dTTP, 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 mm3), 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 stored at -20°C.

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 (1:1) and prewarmed to 45°C (26). They were dried in an upright position 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°C (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). The distribution of grains was calculated in the following way (10):

\[ MGC = \frac{\text{Number of grains}}{\text{Number of nuclei × Weeks of exposure}} \]

Because cytotrophoblasts disappear from term placenta, 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 placenta were evaluated on preparations processed in parallel.

RESULTS

Hybridization to Sections from First Trimester Placenta

Human placental villi (Villus Surface) are covered by the syncytiotrophoblast (syncytium, ST), which overlies a layer of cytotrophoblasts (Langhans cell, CT) and interstitial tissue (4). The intervillous space (IS) is the source of maternal blood. Exchange from this space with the fetal circulation occurs across the syncytiotrophoblast barrier.

In addition to this typical villous structure, there are other distinguishing histological features of the human trophoblast in first-trimester tissue. Syncytiotrophoblasts (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 syncytiotrophoblast layer (Fig. 1; also illustrated in Fig. 9).

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

Both hCGa and hPL mRNAs are abundant in first-trimester tissue (4), and the distribution of these mRNAs in the trophoblast of first-trimester placenta was examined. Sections of 7- to 12-week placenta were hybridized to nick-translated probes corresponding to hPL and hCGa 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 syncytiotum (ST) regions although more hCGa signal was seen in cytrophoblasts than was the case for hPL. Syncytiotrophoblasts 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 (X 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 (X 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 (X 200). (Compare with Fig. 1.)
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 (syncytiotrophoblastic cytoplasm) 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. 4A–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 syncytiotrophoblastic cytoplasm. 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 MGC 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 syncytiotrophoblast 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
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. 6A, 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. 6A, 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. 7A, 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.

**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. 8B) 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. 8A) 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).

**TABLE I**

| Region       | Syncytio-trophoblast | Cyto-trophoblast | Syncytio-trophoblast | Cyto-trophoblast |
|--------------|----------------------|-----------------|----------------------|-----------------|
|              | 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 × 10⁶ cpm/μg and 2.1 × 10⁷ cpm/μg, respectively.

**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 3H-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 syncytioctoplasm 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 3H-hCGα probe in syncytium is observed (B). The number of silver grains in (A) hybridized with 3H-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. x400.

| 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 x 10^7 cpm/μg and 1.3 x 10^8 cpm/μg, respectively. Background, based on pBR control, was <0.15.

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 syncytiot 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 syncytiot comprises a larger proportion of the trophoblast at term. Therefore, the contribution of syncytiotrophoblast RNA to the first-trimester RNA population results in a dilution of syncytiotrophoblast-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.
The data presented here show that the expression of hPL and hCG during placental development is quite different. Synthesis of hCG 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 syncytiotrophoblast layer, but when the cytotrophoblast population is depleted the RNA levels for hCGa decline. Thus, maximal accumulation of hCGa message may depend on the influence of cytotrophoblasts. In contrast, hPL accumulation is detectable only in the syncytiotrophoblast 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 syncytiotrophoblast. The expression of these hormonal genes could then be followed through the entire process of differentiation.

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REFERENCES

1. Ashikawa, Y., R. Nishimura, M. Takemori, and S. Tojo. 1980. Production and secretion of hCG and hCG alpha subunit by trophoblastic tissue. In: Chorionic Gonadotropin. S. J. Segal, editor. Plenum Press, NY. 147-176.
2. Biswas, S., P. Hinduja, and C. J. Dewhurst. 1972. Human chorionic somatomammotropin in amnion and urine in various stages of pregnancy: its correlation with enzymes and oestrone. J. Endocrinol. 54:251-261.
3. Boime, I., D. McWilliams, E. Szczesna, and M. Canet. 1976. Synthesis of human placental lactogen as a function of gestation. J. Biol. Chem. 251:821-825.
4. Boime, I., T. Landefeld, S. McWilliams, and D. McWilliams. 1978. The biosynthesis of chorionic gonadotropin and placental lactogen in first and third trimester human placenta. In: Structure and Function of the Gonadotropins. K. W. McKenna, editor. Plenum Press, NY. 235-257.
5. Boothby, M., R. W. Rudder, C. Anderson, D. McWilliams, and I. Boime. 1981. A single hormone or subunit gene in normal tissue and tumor-derived cell lines. J. Biol. Chem. 256:5121-5127.
6. Boothby, M., S. Daniels-McQueen, D. McWilliams, M. Zerrik, and I. Boime. 1980. Human chorionic gonadotropin alpha and beta subunit m-RNAs: translatable levels during pregnancy and molecular cloning of DNA sequences complementary to hCG. In: Chorionic Gonadotropin. S. J. Segal, editor. Plenum Press, NY. 253-275.
7. Boyd, J. D., and W. J. Hamilton. 1970. Syncytiotrophoblast. In: The Human Placenta. W. Heffler & Sons, LTD Cambridge. 157-174.
8. Bracic, M., and A. T. Haase. 1978. Detection of viral sequences of low reiteration frequency by in situ hybridization. Proc. Natl. Acad. Sci. U. S. A. 75:6125-6129.
9. Braunstein, G. D., J. L. Vaitukaitis, P. P. Carbone, and G. R. Ross. 1973. Ectopic production of human chorionic gonadotropin by neoplasms. Annu. Intern. Med. 78:39-45.
10. Caputo, V. S. Jr., and D. S. Berkley. 1971. Section techniques and grain count variations in tritium autoradiography. Stain Technol. 46:131-135.
11. Couppe, C. D., and J. R. Mc Dagget. 1976. Clonal derivatives of a herpes type 2 transformed hamster cell line: cytogenetic analysis, tumorigenicity and virus sequence detection. Int. J. Cancer 17:501-511.
12. Daniels-McQueen, E., D. McWilliams, S. Birken, R. Canfield, T. Landefeld, and I. Boime. 1978. Identification of mRNA encoding hCG and hPL subunits of human chorionic gonadotropin. J. Biol. Chem. 253:7109-7114.
13. Deswood, M., B. Susana, and R. Landesman. 1977. Human chorionic gonadotropin and its subunits in hydatidiform mole and choriocarcinoma. Obstet. Gynecol. 50:172-181.
14. Enders, A. C. 1965. Formation of syncytiotrophoblast in the human placenta. Obstet. Gynecol. 20:376-386.
15. Fieldes, J. C., and H. M. Goodman. 1979. Isolation, cloning, and sequence analysis of the subunit of human chorionic gonadotropin. Nature (London). 281:351-356.
16. Gask, H. J., L. I. Larson, and N. O. Seiberg. 1975. Immunohistochemical demonstration of chorionic gonadotropin in trophoblastic tissue. Acta Obstet. Gynecol. Scand. 54:161-163.
17. Gershon, E. J., U. J. Hustin, A. M. Reuter, R. Lambotte, and P. Francimont. 1971. Immuno-fluorescent localization of placental lactogen, chorionic gonadotropin, and its alpha and beta subunits in organ cultures of human placenta. Placenta. 1:135-144.
18. Hattori, M., H. Imura, S. Maikawa, Y. Yoshimoto, K. Sekita, T. Tomomatsu, M. Kiyokawa, and T. Kamiya. 1979. Multiple-hormone producing lung carcinoma. Cancer. 43:2429-2437.
19. Hendrickson, E. M., Y. Ashikawa, and S. Tojo. 1979. Immunohistochemical interaction on antiserum to hCG and its subunits with chorionic tissue of early gestation. Endocrinol. Jpn. 26:175-184.
20. Loke, Y. W., D. V. Wilson, and R. Borland. 1972. Localization of human chorionic gonadotropin in monolayer cultures of trophoblast cells by mixed agglutination. Am. J. Obstet. Gynecol. 113:875-879.
21. Maniatis, T., A. Jeffrey, and D. G. Kleid. 1975. Nucleotide sequence of the rightward operator of phage T. Proc. Natl. Acad. Sci. U. S. A. 72:1184-1188.
22. Midgley, A. R., and G. P. Pierce. 1962. Immunohistochemical localization of human chorionic gonadotropin. J. Exp. Med. 115:289-294.
23. McWilliams, D., and I. Boime. 1980. Cytological localization of placental lactogen messenger ribonucleic acid in syncytiotrophoblast layers of human placenta. Endocrinol. Jpn. 27:761-765.
24. Parfud, M. L., and J. G. Gall. 1973. Nucleic acid hybridization to the DNA of cytological preparations. In: Methods in Cell Biology. J. M. Prescott, editor. Academic Press, Inc., NY. 1-16.
25. Pierce, G. B., and A. R. Midgley. 1963. The origin and function of human syncytio-trophoblastic giant cell. Am. J. Path. 43:153-173.
26. Rogers, A. W. 1973. Liquid emulsion techniques for track autoradiography. In: Techniques of Autoradiography. A. W. Rogers, editor. Elsevier Scientific Publishing Co., London. 313-328.
27. Rosen, S. W., B. D. Weintraub, and S. A. Aaronson. 1980. Nonrandom ectopic protein production by malignant cells: direct evidence in vitro. J. Clin. Endocrinol. Metab. 50:834-841.
28. Rudder, R. W., C. A. Hansen, and N. J. Addison. 1979. Synthesis and processing of human chorionic gonadotropin subunits in cultured chorioncarcinoma cell. Proc. Natl. Acad. Sci. U. S. A. 76:5143-5147.
29. Rudder, R. W., C. A. Hansen, A. H. Bryan, G. J. Puttermen, E. L. White, F. Perri, K. S. Meade, and P. H. Alldenderfer. 1980. Synthesis and secretion of human chorionic gonadotropin and beta subunits by cultured human malignant cells. J. Biol. Chem. 255:1000-1007.
30. Thode, H. J., and A. W. Choate. 1963. Chorionic gonadotropin localization in the human placenta by immunofluorescent staining. II. Demonstration of HCG in trophoblast and amniotic epithelium of immature and mature placentas. Obstet. Gynecol. 22:437-443.
31. Waterke, W. B. 1978. Use of immunocytochemical techniques for the localization of human placental lactogen. J. Histochem. Cytochem. 26:268-292.
32. Weintraub, B. D., and S. W. Rosen. 1973. Ectopic production of the isolated beta subunit of human chorionic gonadotropin. J. Clin. Invest. 52:335-342.
33. Wynn, R. M. 1972. Cytotrophoblastic specialization: an ultrastructural study of the human placenta. Am. J. Obstet. Gynecol. 114:339-355.