Synthesis of Human Placental Lactogen Messenger RNA as a Function of Gestation

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It was shown previously that 4 to 5 times more human placental lactogen (hPL) was synthesized in cell-free extracts from term placenta than in comparable extracts prepared from first trimester tissue. In an attempt to define what accounts for this differential rate of synthesis RNA was prepared from first trimester and term placentae. Following purification through an oligo(dT)-cellulose column, these RNA preparations were tested for their ability to direct the synthesis of the hPL precursor in the wheat germ cell-free system. With similar amounts of first trimester and term mRNA, the overall efficiency as defined by the stimulation of total amino acid incorporation was comparable. However, there was approximately 4 times more hPL synthesized in the presence of term RNA. This was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and tryptic fingerprinting.

The peak of the hPL precursor messenger activity sediments at 12 to 13 S on sucrose gradient. Analysis of the RNA by formamide-polyacrylamide gel electrophoresis further supported this value.

The data indicate that the increased synthesis of hPL at term reflects greater levels of hPL mRNA in term tissue than in first trimester tissue. The data also show that the overall in vivo levels of hPL can be correlated not only with the increase in placental syncytial mass during pregnancy but also in the greater proportion of hPL synthesized per g of tissue. The latter results from the continual differentiation of the placenta occurring throughout gestation.

One of the major peptide hormones released by the human placenta is human placental lactogen (hPL). Although its precise role during gestation has not been clearly defined, its synthesis reflects the normal function of the placenta.

Third trimester (term) placenta secretes greater amounts of hPL into maternal serum than does first trimester placenta. Most investigators have agreed that the enhanced levels of the hormone at term reflect a proportional increase in the mass of placental tissue (1-4). Placental mass is approximately proportional to the levels of hPL in maternal sera and in one study it was shown that in tissue slices from first trimester and term placentae the fraction of hPL in newly synthesized proteins is constant (5). One might therefore have expected that in cell-free extracts from the same amount of tissue the level of hPL synthesized would be the same. Instead, from the same

*This work was aided in part by Grant M74.114 from the Population Council and Grant AM-16865 from the National Institutes of Health.

The abbreviations used are: hPL, human placental lactogen; Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.
from regions of the gradient were tested for total protein and RNA on a 5 to 20% sucrose gradient (Fig. 1). Five fractions of wheat germ cell-free extracts (15). The approximate size of the heavier than hPL but containing hPL sequences is formed in extracts of ascites tumor cells, while a protein adsorbed RNA was 1 to 2% of the input total cellular RNA. 

We have recently found that more hPL mRNA is recovered when the placenta is obtained from cesarean section than from normal delivery. Thus, all term placentae used in this study were from cesarean section. Also the amount of mRNA recovered is much less when polysomes are extracted instead of the total cytoplasmic extract. All mRNA was extracted from cytoplasmic extracts for the experiments described. The crude placental RNA was purified further on an oligo(dT)-cellulose affinity column as described previously (11). The yield of associated mRNA was 1 to 2% of the input total cellular RNA.

Assays for Protein Synthesis: Wheat Germ—Assays contained in a final volume of 50 μl, approximately 10 μl of nonprecipitated S-30 (10), 29 mM Hepes (pH 7.6), 2.5 mM diethiothreitol, 1.5 mM ATP, 1.5 mM phosphocreatine, 2 μg of creatine kinase, 0.3 mM GTP, 20 μM unlabeled amino acids, 64 mM KCl, 4.0 mM magnesium acetate, and 0.5 mM [35S]methionine. The reactions were then processed as previously described (15).

Analysis of [35S]Methionine-labeled hPL—Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and tryptic peptide analyses of hPL were performed essentially as previously reported (6). However, instead of a 2 to 8% gradient of acrylamide, 20% acrylamide gels were used.

Sucrose Gradient Analysis of RNA—Total placental RNA was resolved on 5 to 20% sucrose gradients containing 0.1 M Tris-HCl (pH 7.8)/0.1 mM EDTA as described by Honjo et al. (12). The RNA was dissolved in sterile water, heated for 5 min at 85°, rapidly chilled, and then the RNA was layered on the gradient. The gradients were centrifuged in a Beckman SW-41 rotor at 35,000 rpm for 18 hours. Fractions of 0.3 ml were collected and the absorbance at 260 nm was determined.

Formamide-Acrylamide Gel Electrophoresis of RNA—Placental RNA was further resolved by acrylamide gel electrophoresis in formamide according to modifications by Orkin et al. (13) of the procedures of Pinder et al. (14). Formamide (100 ml) which was deionized with 5 g of Dowex RG-50X-8 mixed bed resin was made 20 mM in phosphate with solid NaH2PO4 and Na2HPO4 and the pH brought to 6.5. This buffered formamide was mixed with acrylamide-bisacrylamide polymerizing solution and the pH of the resulting mixture brought to 6.5. This solution was placed in Plexiglas tubes to polymerize, and 20 mM phosphate-buffered formamide was added over the acrylamide; then the top of the tube was covered with parafilm. The tubes could then be stored in the cold room for up to 3 weeks. Aliquots of RNA solutions which had been dialyzed to remove salt were lyophilized. The samples were dissolved in 10 to 15 μl of 5 mM barbitosal-buffered formamide containing 20% sucrose and 0.05% bromphenol blue. The gels were placed in an electrophoresis apparatus containing 20 mM phosphate buffer in the anodal and cathodal chambers. The gels were run initially at 50 volts for 0.5 hour and then at 100 volts for 5 to 10 hours. Gels were removed from the tubes and stained overnight in Stainsall (Eastman Catalog No. 2718) and destained in water.

RESULTS

Crude RNA isolated from term placenta has been shown to direct the synthesis of hPL in cell-free extracts. hPL itself is formed in extracts of ascites tumor cells, while a protein heavier than hPL but containing hPL sequences is formed in wheat germ cell-free extracts (15). The approximate size of the hPL mRNA was determined by resolving the total placental RNA on a 5 to 20% sucrose gradient (Fig. 1). Five fractions from regions of the gradient were tested for total protein synthesis activity in wheat germ S-30 (closed circles), and each fraction of these was run on an acrylamide gel to assess synthesis of the precursor (arrow). The peak of the hPL precursor messenger activity sedimented at 12 to 13 S. When the RNA in this region was combined and centrifuged again in a 5 to 20% sucrose gradient, the hPL mRNA retained the same sedimentation rate.

Analysis of RNA by formamide-polyacrylamide gel electrophoresis further supported the size of the RNA as 12 to 13 S (Fig. 2). The appearance of a band corresponding to this molecular weight was associated with peak Fraction III from the sucrose gradient shown in Fig. 1, whereas this band was absent in Fraction I which contained no detectable hPL mRNA activity (Fig. 2B). Furthermore, when placental RNA was purified twice through an oligo(dT)-cellulose column, the major discrete band migrated at about 13 S (Fig. 2A). The gel data suggested that this RNA band had the mRNA activity. To confirm this, the (dT)-purified RNA was fractionated on a 5 to 20% sucrose gradient. Again, a major peak was observed sedimenting at about 13 S. All hPL mRNA activity was associated with this fraction.

Assuming that the molecular weight of the mRNA can be estimated by comparing sedimentation values and molecular weights for similarly analyzed RNAs, the size of hPL mRNA is approximately 300,000. This suggests that the information encoded in this mRNA is more than adequate to code for the hPL precursor (Mr = 25,000).

For comparing the levels of hPL mRNA in first trimester and term placentae the poly(A)-containing RNA isolated from the tissue was purified on an oligo(dT)-cellulose column (11).
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Ordinarily, 25 mg of crude RNA were applied to the column, and the recovery of the adsorbed fraction (poly(A)RNA) represented 1 to 2% of the input RNA. Similar amounts of the adsorbed mRNA fractions from the two gestational periods stimulated total amino acid incorporation in the wheat germ S-30 to about the same extent (Fig. 3). Therefore, the efficiency of the two RNAs is comparable. In addition, the sucrose gradient profiles of the total cellular RNA derived from first trimester and term placentae were very similar.

The products synthesized in response to these adsorbed RNA fractions were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 4). Although the same amount of radioactivity from each preparation was applied to the gel, it is clear that much more of the hPL precursor is synthesized in response to term mRNA than to first trimester mRNA. The bands corresponding to the precursor were cut out of the gels and the radioactivity was determined. In the case of the term preparation the band contained approximately 20% of the total radioactivity applied to the gel; in the case of the first trimester preparation the figure was only about 5%.

The region of the gel containing the labeled hPL precursor was eluted from a preparative gel, mixed with purified unlabeled hPL, digested with trypsin, and the resulting peptides analyzed by two-dimensional chromatography and electrophoresis. The fingerprint was sprayed with ninhydrin in order to localize the peptides from the purified carrier and exposed to x-ray film (Fig. 5).

hPL contains six tryptic peptides each of which contains 1 methionine residue (16, 17). The product of the wheat germ system programmed with term RNA contained methionine-labeled tryptic peptides that were coincident with authentic hPL peptides (Panels A and B, Fig. 5). Panel B is the autoradiograph of Panel A. These labeled peptides were not present on maps containing carrier hPL and the proteins synthesized in the absence of mRNA (Fig. 5, Panel D) or in the presence of globin mRNA (data not shown).

Of the six methionine tryptic peptides, peptide 1 is closest to the NH₂ terminus of hPL (6). The methionine in this peptide represents the 14th amino acid residue from the NH₂-terminal end (16, 17). (It is not clear why there is an asymmetric distribution of radioactivity in the overlapping peptides. It appears that there is a progressive dilution of radioactivity from the amino to carboxy end of the protein, since from amino acid analyses, peptides 2 and 3 are located near the carboxy end of hPL. This effect is probably related to the efficiency of the wheat germ system because in highly active extracts the asymmetry of the labeled peptides is reduced. Possibly some of the asymmetry can be attributed to the inclusion in the protein band of some incomplete prematurely released peptides from ribosomes in less efficient extracts (24).) Thus, the level of peptide 1 will probably best reflect the number of RNA molecules that have initiated and thus the proportion of hPL mRNA. Furthermore, since the RNA is translated in a heterologous cell-free system, any differences between first trimester and...
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FIG. 5. Two-dimensional tryptic fingerprint analyses of a mixture of carrier hPL and labeled protein synthesized in the presence (A and B) and absence (C and D) of term RNA. The reaction product was mixed with carrier. Panels B and D are the corresponding autoradiographs of Panels A and C which have been stained with ninhydrin. The term mRNA activities would not likely be related to levels of the various cytoplasmic protein synthesis factors. Peptide 1 was cut out from two-dimensional fingerprints and counted. More of the labeled peptide was synthesized when the wheat germ S-30 was programmed with term mRNA than with first trimester RNA (Table I). The radioactivity seen in the absence of RNA was the result of nonspecific smearing of endogenous peptides. There was no discrete radioactive spot corresponding to peptide 1 observed in the absence of RNA (Fig. 5).

It was conceivable that the lower level of hPL mRNA in first trimester tissue may be related to a selective loss of hPL mRNA during isolation. To test this point equal quantities of first trimester and term tissue were mixed and the total RNA extracted from the resulting homogenate. The RNA was also extracted from the corresponding individual tissues. Equivalent subsaturating amounts of the unfractionated RNA were added to the wheat germ system (Fig. 6). The bands corresponding to the precursor were cut out of the gels and the radioactivity determined. In the term and first trimester preparations the band contained approximately 18% and 2%, respectively, of the total radioactivity applied to the gel. In the mixed preparation the figure was approximately 11%. Therefore, the presence of first trimester tissue did not appreciably alter the recovery of total hPL mRNA activity (taking into account dilution by first trimester RNA). Thus, the lower level of hPL mRNA recovered from first trimester tissue was not an artifact of preparation. Also this experiment shows that: (a) there were no specific translational inhibitors in the first trimester RNA sample which would lower the translation efficiency of hPL mRNA; and (b) the difference in hPL mRNA activity in unfractionated RNA from term and first trimester tissue is comparable to that seen in oligo(dT)-purified mRNA. Thus, the results with the latter were not related to differences in recovery of poly(A)-containing RNA.

DISCUSSION

It had been shown earlier that 4 to 5 times more hPL was synthesized in cell-free extracts from term tissue than from the...
TABLE I

Level of methionine tryptic peptide 1 synthesized in wheat germ S-30 in presence of oligo(dT)-purified placental RNA

| Experiment | RNA added | Counts per min applied to map | Counts per min in peptide 1 | % |
|------------|-----------|-------------------------------|----------------------------|---|
|            | µg/0.25 ml |                               |                            |   |
| I          | None       | 275,000                       | 200                        | 0.65 |
|            | First trimester | 505,000             | 3,270                      | 3.0 |
|            | (8.0)   | 990,000                       | 27,100                     | 2.7 |
|            | Term (8.0) | 330,000                       | 7,642                      | 2.3 |
| II         | None       | 30,000                        | 72                         | 0.68 |
|            | First trimester | 260,000             | 1,770                      | 0.68 |
|            | (8.0)   | 330,000                       | 7,642                      | 2.3 |
|            | Term (8.0) | 330,000                       | 7,642                      | 2.3 |

same amount of first trimester tissue; but it was not clear if this increase was the result of a translational control mechanism or an increase in specific hPL mRNA. The data presented here suggest that the enhanced synthesis of hPL in term cell-free systems is largely the result of an increased proportion of the corresponding mRNA. This is shown with mRNA purified from oligo(dT)-cellulose chromatography which produced both an increased amount of labeled hPL and an increased level of the methionine tryptic peptide closest to the NH₂ terminus.

Both by sucrose gradient and formamide acrylamide gel analyses, the size of the hPL mRNA from term tissue appears to be about 13 S. When RNA derived from first trimester tissue was resolved on a 5 to 20% sucrose gradient, the hPL activity also sedimented at about 13 S (data not shown). Thus, the apparent size of the mRNA remained unchanged between first trimester and term.

It appears that the differential serum level of hPL seen during gestation are largely the result of changes in synthesis rate of the hormone, although these data cannot exclude some additional regulatory component exerted on the secretion of hPL. The data presented are not compatible with earlier observations that the proportion of hPL synthesized in first trimester and term placental slices was similar (5). It is possible that the sensitivity and specificity of the radioimmunoassay used in the earlier study were insufficient to detect the difference shown here.

The enhanced synthesis of hPL at term is consistent with the hypothesis that the placenta is continually differentiating. The relatively immature placental cell, the cytotrophoblast, is the generative cell for the more differentiated syncytiotrophoblast, and the latter is apparently the region where hPL is synthesized (22). During gestation there is an increase in the proportion of syncytiotrophoblast per g of placental villi (23). Thus, a greater synthesis of the hormone per g of tissue at term probably reflects this increase. In other words, the overall in vivo levels of hPL can be correlated not only with the increase in placental syncytiotrophoblast mass during pregnancy but also in a greater proportion of hPL synthesized per g of tissue.

Acknowledgments—The authors are grateful to Dr. David Swan for helping us with the formamide gels. We would also like to thank Miss Kathy Neely for her excellent typing assistance.

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Synthesis of human placental lactogen messenger RNA as a function of gestation.
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J. Biol. Chem. 1976, 251:820-825.

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