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37. Requests for reprints should be addressed to: R. J. Grand, M.D., 300 Longwood Ave., The Children's Hospital Medical Center, Boston, Mass. 02115 (USA).
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Developmental biochemistry
placenta

protein synthesis
ribosomes

Protein Synthesis in Cell-free Extracts from First and Third Trimester Human Placenta

IRVING BOIME[25]
Departments of Obstetrics and Gynecology and Pharmacology, Washington University School of Medicine, Saint Louis, Missouri, USA

LAURENCE CORASH AND ERHARD GROSS
Section on Molecular Structure, Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA

Extract

Ribosomal and cell sap fractions were prepared from first and third trimester human placentas. High endogenous activity was observed with ribosomes prepared from first trimester or third trimester placenta when incubated either in the presence of cell sap derived from first trimester placenta tissue or from Krebs II ascites tumor cells. The homologous placental system is capable of translating poly(U) and globin mRNA, although the latter is translated at about 10% of the efficiency as observed in the homologous ascites tumor system. The placental cell sap may be deficient in a factor necessary for the translation of globin mRNA. The preparation of active ribosomes from first and third trimester polysomes shows that it will be possible to study the endogenous synthesis of placental proteins throughout pregnancy.

Speculation

These results indicate the feasibility of studying the cell-free synthesis of placental peptide hormones. Human chorionic gonadotropin and human placental lactogen reach peak levels of synthesis at different gestational periods and thus the relation between the synthesis of the hormones and their secretion can be elucidated.

Although the secretion of various placental peptide hormones during gestation is well documented (16), little is known about the biosynthesis of these hormones. It is not clear as to whether or not human chorionic gonadotropin and human placental lactogen are synthesized in the form of precursor molecules as are some other hormones (15). Furthermore, although peak levels of human chorionic gonadotropin are observed during the first trimester of pregnancy, it has not been established whether there is an increase in (*1*) the *de novo* synthesis of the hormone (*2*) the rate of mobilization of a precursor, or (*3*) the cellular secretion of the hormone. In order to answer these and other questions regarding the regulation of placental protein synthesis, it is necessary to have an active, cell-free, protein-synthesizing system from placental tissue taken at various stages of gestation.

We wish to report the preparation of such a system from the first and third trimester human placenta. These consist of ribosomes from placenta and ribosome-free supernatant fractions prepared from the same tissue or from ascites tumor cells.

MATERIALS AND METHODS

Rat liver tRNA was a gift from Dr. Dolph Hatfield and crude rabbit globin mRNA was a gift from Dr. Philip Leder. (^{3}H)Leucine was purchased from the New England Nuclear Company (18) and sucrose was obtained from Schwarz-Mann (19).

PREPARATION OF PLACENTA RIBOSOMES

Normal placentas of gestational ages from 8 to 12 weeks were obtained by vacuum curettage. Immediately after evacuation the tissue was immersed in a sterile cold buffer solution containing 30 mM Tris, pH 7.5, 120 mM KCl, 7 mM 2-mercaptoethanol, and 5 mM magnesium acetate. Placentas were pooled in order to obtain at least 30 g tissue and were stored at 4° for no more than 2–3 hr before further treatment. (Fluoresence and light microscopy have shown that fetal and maternal tissue can be separated from placental villi (6).)

The placental tissue was rinsed in the above buffer to remove the blood. The tissue was then centrifuged at 100 × *g* for 10 min. The pellet was minced with scissors and homogenized on a 1:1 (v/w) basis with the above buffer containing 0.5 mM EDTA (10, 13). Homogenization was carried out in the cold for about 3 min with motor-driven Teflon and glass homogenizers (20). The homogenate was then centrifuged at 8,500 × *g* for 10 min at 4°.

The supernatant fluid was brought to 1% deoxycholate concentration with a 10% solution. Twenty milliliters of this suspension was then layered over a discontinuous gradient made from 4 ml of a 1.30 M and a 4 ml of a 1.17 M sucrose solution prepared in the homogenizing buffer. The gradients were placed in a Beckman (21) *type 60* titanium rotor and centrifuged at 200,000 × *g* for 3 hr at 4°.

The top layers were then aspirated and the tubes containing the pellets were rinsed gently with homogenizing buffer to remove any residual deoxycholate. Occasionally, white fluffy material collected around the ribosome pellets but much of this material could be removed with a stirring rod and rinsed away. The pellets were resuspended in homogenizing buffer with the aid of a small hand homogenizer.

Term placentas obtained from uncomplicated cesarean sections, were treated in the same manner with minor modifications. Tissue was collected in sterile buffer at 4° and 1-inch cubes of noninfarcted and noncalcified tissue were selected. The cubes were washed free of blood, minced with scissors, and pressed through a 1.5-mm grid to remove connective tissue and vasculature. The tissue was then treated as described above. The yield of ribosomes from first and third trimester tissue was of the order of 4 A_{260} (0.3 mg)/g compacted placental villi.

PREPARATION OF RIBOSOME-FREE SUPERNATANT FRACTIONS (CELL SAP)

The preparation of the cell sap fraction from placenta was carried out as described above except for the following: (*1*) there was no EDTA in the homogenizing buffer, (*2*) the postmitochondrial supernatant fluid was not treated with deoxycholate and it was centrifuged at 250,000 × *g* for 2 hr in the absence of sucrose solutions. The cell sap fraction so obtained was dialyzed overnight against the homogenization buffer without EDTA and stored in 200-μl aliquots in liquid nitrogen. The ribosomal and cell sap fractions derived from Krebs II ascites tumor cells were prepared as described elsewhere (4).

ASSAY FOR PROTEIN SYNTHESIS

Endogenous or exogenous mRNA directed protein synthesis was assayed in 0.06-ml reaction mixtures composed of 30 mM Tris-HCl (pH. 7.5), 3.3 mM magnesium acetate (10 mM for poly(U)), 70 mM KC1, 7 mM 2-mercaptoethanol, 1 mM ATP, 0.1 mM GTP, 0.6 mM CTP, 10 mM creatine phosphate, 0.16 mg/ml creatine kinase, 40 μM each of 19 nonradioactive amino acids, and 2 μM (^{3}H)leucine (specific activity 20–30 Ci/mmol). In addition, 3 μg rat liver or ascites tRNA (1) were added to all reactions as it was found that the endogenous and exogenous activities were stimulated two- to fourfold upon tRNA addition. The amount of ribosomes and cell sap added will be noted in the appropriate experiment. Incubation was at 33° for 60 min. The reactions were stopped by the addition of 0.2 ml 0.1 M KOH. Incubation was continued for 20 min and 1 ml 10% cold CCl_3COOH was then added. This mixture was cooled at 0° for 5 min and the precipitate was collected on a 0.45-μm pore size Millipore filter, washed three times with 3 ml each of 5% CCl_3COOH, dried and counted in a Packard liquid scintillation counter. All experiments were performed in duplicate.

SODIUM DODECYL SULFATE-2-MERCAPTOETHANOL POLYACRYLAMIDE GEL ELECTROPHORESIS

Three- to fivefold scaled-up reaction mixtures containing (^{3}H)leucine were treated with 0.2 ml 0.1 N KOH/0.06 ml as described above. After incubation, the mixture was adjusted to 10% in CCl_3COOH and precipitation was continued for 30 min at 4°. The precipitated proteins were then centrifuged at 12,000 rpm in an Eppendorf (22) centrifuge for 2 min. The precipitate was washed, once with cold 5% CCl_3COOH and twice with acetone to remove residual CCl_3COOH. The samples were prepared for analysis and then subjected to acrylamide gel electrophoresis in a linear 7–30% acrylamide gradient as described previously (3). Appropriate markers were included in each experiment and in the case where globin was analyzed, ^{14}C-labeled rabbit globin was mixed with the ^{3}H-labeled *in vitro* mixture. The gels were stained with 0.2% Coomassie blue in 5% methanol and 7% acetic acid. Appropriate lanes were cut out vertically, sliced horizontally in 1-mm segmants, and placed in scintillation vials containing 0.2 ml 2% NH_4OH in 30% H_2O_2. The vials were heated at 60° overnight to solubilize the gel and 0.8 ml Protosol and 10 ml of Omnifluor scintillation fluid were added.

RESULTS

ENDOGENOUS ACTIVITY OF PLACENTAL RIBOSOMES

Studies by Laga *et al.* (13) on third trimester placenta demonstrated that the inclusion of 0.5 mM EDTA in the homogenization medium preserved polysomes, as determined by sucrose density gradient analyses and stimulated endogenous amino acid incorporation. Consistent with this, we have found ribosomes prepared in the presence of 0.5 mM EDTA to have considerably higher endogenous activity than if EDTA is omitted.

High endogenous activity was observed with ribosomes prepared from first trimester placenta and incubated either in the presence of cell sap derived from first trimester placenta tissue or from Krebs II ascites tumor cells (Fig. 1), although the cell sap from Krebs II ascites tumor was somewhat more active. The activity with the cell sap from third trimester tissue was about 60% of that of the first trimester preparation.

The time course of incorporation in the homologous system was linear for at least 15 min and some residual synthesis was still observed after 60 min (Fig. 2).

The endogenous activities of ribosomes prepared from first trimester and term placenta were comparable when tested with ascites or placental cell sap (Fig. 3). Amino acid incorporation has not saturated with respect to the concentration of ribosomes, even more than 1 mg/ml ribosomes.

RESPONSE TO EXOGENOUS mRNA's

The homologous placental system is capable of translating

poly(U) at about 40% of the activity of a homologous ascites preparation (Table 1). The placental cell sap is highly active in supporting poly(U)-dependent poly-Phe synthesis with ascites ribosomes. These results and those of the experiments measuring endogenous activity show that at least in terms of the availability of factors required for the elongation reactions, the placental system is very active.

A natural messenger, globin mRNA isolated from rabbit reticulocytes is translated in the homologous placental system, although the activity in the presence of ascites cell sap is about eightfold greater (Table 2). The translation of globin mRNA in both systems is completely dependent on the presence of ribosomes and cell sap and the activity is totally abolished by RNase or puromycin.

The nature of the product synthesized in response to the added globin mRNA in the homologous placental system was investigated by polyacrylamide gel electrophoresis. Authentic rabbit globin labeled with (^{14}C)leucine was mixed with the labeled proteins synthesized in the homologous cell-free system (Fig. 4). The ^{3}H radioactivity comigrated with the ^{14}C-labeled globin and the distribution of the radioactivity of protein synthesized in the presence of globin mRNA is discrete. Although there is only a 2.5-fold stimulation of incorporation into acid-insoluble protein, there was more than a 10-fold difference in counts in the globin region compared with the same region observed in the absence of mRNA. This indicates that essentially all of the message-dependent radioactivity was incorporated into globin.

The [Mg^{++}] optimum for translating globin mRNA is about 3.3 mM, consistent with the results obtained for this mRNA in other cell-free systems (2, 9). The [KCl] optimum for both endogenous synthesis and the globin mRNA-dependent activity are the same, about 70 mM. These data suggest that the homologous placental system can initiate and accurately translate an added natural mRNA.

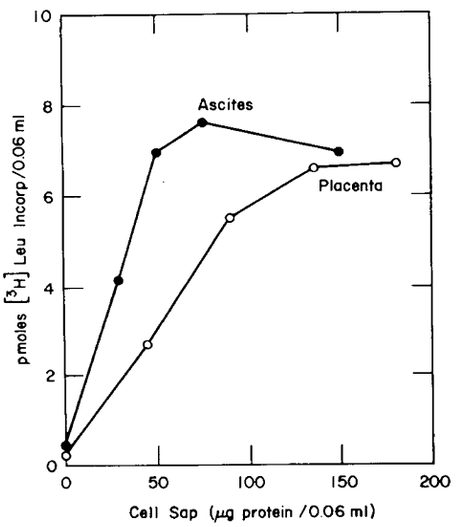

Fig. 1. Endogenous activity of first trimester ribosomes in the presence of ascites or placental cell sap. All reaction mixtures contained 50 μg placental ribosomes and were incubated for 60 min. The reactions were assayed as described in *Materials and Methods.* One picomole is equivalent to about 25,000 cpm. *Incorp:* incorporated.

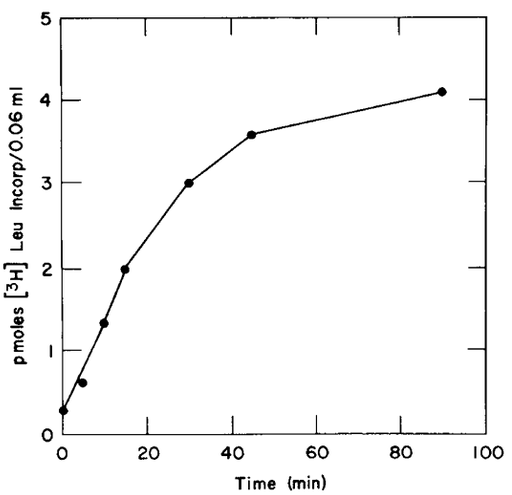

Fig. 2. Time course of endogenous amino acid incorporation by the homologous cell-free system derived from 1st trimester tissue. Each reaction mixture contained 20 μg ribosomes and placental cell sap equivalent to 130 μg protein. Aliquots (0.01 ml) were sampled at the times indicated and the results are expressed in terms of 0.06 ml reaction mixture. *Incorp:* incorporated.

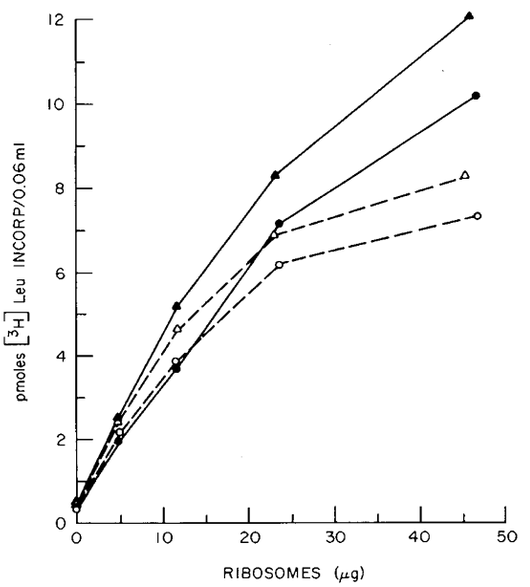

Fig. 3. Endogenous amino acid incorporation by ribosomes derived from 1st and 3rd trimester placentas. Reactions contained either cell sap prepared from first trimester tissue (– – –) or from ascites tumor cells (——) equivalent to 130 μg and 100 μg of protein, respectively. (△, ▲ denote 3rd trimester ribosomes; ○, ● denote 1st trimester ribosomes). *Incorp:* incorporated.

Table 1. *Translation of poly(u) in cell-free systems prepared from human first trimester placenta and mouse ascites tumor cells*[1]

Ribosomes	Cell sap	(^{14}C)Phe incorporated, pmol
Ascites	Ascites	74
Placenta	Placenta	28
Placenta	Ascites	40
Ascites	Placenta	47

[1] Each reaction mixture contained 7 μg ribosomes, 50 μg poly(U) and 100 pmol (^{14}C)Phe. The Mg^{++} concentration was 10 mM. Where indicated, the equivalent of 100 μg protein for ascites cell sap and 130 μg protein for the first trimester placenta cell sap was added. The mixtures were incubated for 45 min and samples processed as described in *Materials and Methods.* The background of incorporation (*i.e.*, in the absence of poly(U)) was 0.7–0.8 pmol.

Table 2. *Translation of globin mRNA in extracts derived from human placenta and mouse ascites tumor cells*[1]

Additions of mRNA	Ribosomes	Cell sap	(^{3}H)Leucine incorporated, pmol
+	Placenta	Placenta	0.71
–	Placenta	Placenta	0.30
+	Placenta	Ascites	4.70
–	Placenta	Ascites	0.69
+	Ascites	Ascites	6.1
–	Ascites	Ascites	0.5

[1] Each reaction mixture contained, where indicated, the following: 6 μg crude reticulocyte mRNA, the equivalent of 3 μg ribosomes, placental cell sap (130 μg protein) and ascites cell sap (170 μg protein). Placenta ribosomes and cell sap were derived from first trimester tissue.

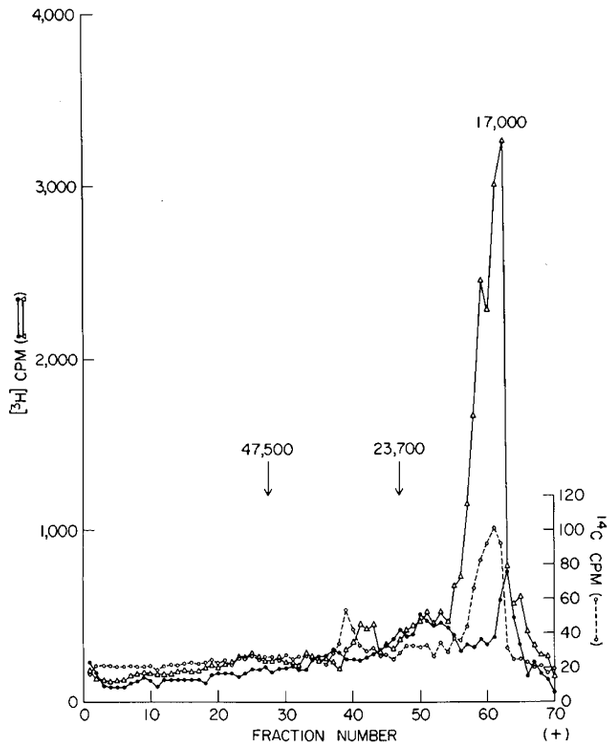

Fig. 4. Sodium dodecyl sulfate-polyacrylamide electropheretogram of products synthesized in response to globin mRNA by a homologous placental cell-free system. A 7–28% polyacrylamide gel was prepared as described previously. Included in the gel were 15,000 cpm of (^{3}H)labeled material synthesized in response to globin mRNA (△) and in another lane 7,500 cpm of ^{3}H-labeled material synthesized in the absence of mRNA (●). In addition ^{14}C-labeled rabbit globin (○) was added to both samples (only one shown) and electrophoresis performed for 3 hr at 200 v. The direction of migration of material is to the right. Heavy (*47,500*) and light chain (*23,700*) immunoglobulin standards were included in the experiment.

DISCUSSION

The results presented indicate a relatively high endogenous activity for the cell-free protein synthesis systems prepared from first and third trimester tissue and that the homologous placental cell-free system is capable of initiating and translating natural message.

Despite the low activity of the homologous placental system to support globin mRNA translation, globin is produced even at this level of stimulation (Fig. 4). This low activity may be the result of (*1*) some host and/or tissue specificity in which there is a deficiency in one or more factors required for globin synthesis, (*2*) inactivation of a factor or factors during the preparation of the extracts, and (*3*) the presence of ribonuclease or an inhibitor of globin mRNA translation. The last is unlikely, inasmuch as globin mRNA translation in the homologous ascites system is unaffected by the addition of placental cell sap over the concentration range employed in the experiment.

The placental cell sap may be deficient in a factor necessary for the translation of globin mRNA. This is likely since placental ribosomes incubated with cell sap from ascites tumor cells can translate globin mRNA with an activity nearly as great as that of the homologous ascites system (Table 2). The relatively high endogenous activity and the poly(U)-dependent stimulation of the homologous placental system show that the low level of translation of globin mRNA is not the result of an impairment of the elongation reactions. Possibilities (*1*) and (*2*) described above could be further elucidated with reticulocyte factors obtained from a KCl wash of reticulocyte ribosomes. In this connection, it has been shown that specific eukaryotic factors may be required for the translation of specific classes of mRNA's (8, 17, 12).

The endogenous activities of the first and third trimester ribosomes are comparable and the latter are much more active in this respect than previously reported (5, 11, 13). It appears then that the protein synthetic capacity of isolated ribosomes does not change during the late stages of pregnancy. This is consistent with the finding that the peak synthesis of human placental lactogen occurs in the third trimester of pregnancy (7, 14). The preparation of active ribosomes from first and third trimester tissue shows that it will be possible to study endogenous synthesis of placental proteins throughout pregnancy. In this regard, preliminary data from both first and third trimester polysomes shows the *in vitro* synthesis of discrete proteins ranging in molecular weight from 15,000 to 50,000. These results indicate the feasibility for studying cell-free synthesis of human chorionic gonadotropin and human placenta lactogen at different gestational periods. Peak levels of these hormones occur at different times and an investigation of the relation between the synthesis of the hormones and their secretion should be rewarding.

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25. Requests for reprints should be addressed to: I. Boime, Ph.D., Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, Mo. 63110 (USA).
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Acid hydrolases
lysosomes
placenta

Subcellular Constituents of Human Placenta. II. Isolation and Density Distribution of Lysosomes from First Trimester Tissue

LAURENCE CORASH AND ERHARD GROSS

Section on Molecular Structure, Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA

Extract

First trimester placental lysosomes may play an important role in specialized physiologic events which occur in early gestation: endometrial invasion, cellular differentiation, and rapid organ growth. Placental tissue is readily separated from contaminating tissues and the syncytium is a source rich in lysosomes. Based on sedimentation velocity analysis, two lysosome "populations" are separated, each of which shows bimodal density distribution after isopycnic ultracentrifugation. Compared with term placental lysososmes, first trimester lysosomes are of lower buoyant density. First trimester placentas yield lysosome fractions with greater specific activities for acid hydrolases than comparable subcellular fractions from term placenta. This correlates well with the postulated participation of the organelle in early placental processes.

Speculation

Placenta participates in many highly specialized processes in which the lysosome is hypothesized to be critical. Investigations on different tissues indicate that the incorporation of estrogens into lysosomal membranes alters organelle stability, thus possibly constituting a control mechanism of organelle function. The lower buoyant density of first trimester placental lysosomes may be indicative of differences in the lipid composition, possibly in membranes, and hence stability. The increased acid hydrolase activities of first trimester placental lysosomes point to similar control mechanisms in the trophoblast. Elucidation of these lysosomal functions will contribute to the understanding of processes operative in normal and neoplastic cellular growth.

First trimester human placenta is a unique biologic environment for the study of lysosome function. The organelle is postulated to play a critical role in a number of physiologic events: endometrial invasion, cellular differentiation and growth, and fetal-maternal transport. In addition, the similarity between first trimester trophoblastic tissue and neoplasia has been emphasized (17). Elucidation of lysosomal control mechanisms in early gestation may contribute to the understanding of lysosome function in neoplastic growth. It has recently been demonstrated for rat preputial sex glands that sex hormones affect lysosomes and that the organelle may act as a "tertiary messenger" between plasma membrane and