The Translation of a Human Placental Lactogen mRNA Fraction in Heterologous Cell-Free Systems: The Synthesis of a Possible Precursor.

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Received October 19, 1974

Summary: RNA extracted from term placental homogenates directed the synthesis of human placental lactogen (HPL) in a heterologous cell-free system derived from Krebs II ascites tumor cells. HPL was one of the major products synthesized. It was identified on SDS-polyacrylamide gels and by trypsin fingerprinting.

Translation of placental RNA in a cell-free system from wheat germ resulted in the synthesis of a protein that migrated slower than HPL on SDS-polyacrylamide gels; no labeled protein was observed that comigrated with authentic HPL. This heavier protein had a molecular weight of about 25,000. It contained the same overlapping HPL tryptic peptides seen when the RNA was translated in the ascites cell-free system. This raises the possibility that a HPL precursor was synthesized in the wheat germ system.

One of the unique hormones elaborated by the human placenta is human placental lactogen (HPL). Its synthesis is apparently required for sustaining a successful pregnancy. Several reports indicate that low blood levels of HPL are associated with many clinical dysfunctions during pregnancy (1-4).

HPL may also reflect the degree of differentiation of the placenta during gestation (5-6), since at term when the proportion of differentiated mass is greatest, the blood levels of hormone *in vivo* and the amount synthesized *in vitro* are maximal (7).

In order to study the synthesis of HPL as a function of gestation it would be useful to measure the corresponding mRNA activity. Here the preparation of a placental RNA fraction and its translation in heterologous cell-free systems is described. It was found that a major protein synthesized in ascites extracts was HPL. In contrast, translation in a wheat germ cell-free system resulted in a protein containing HPL tryptic peptides but heavier than HPL. The latter suggests the synthesis of a precursor for the hormone.

METHODS

Isolation of Cell-Free Extracts:

Preincubated cell-free extracts from Krebs II ascites tumor cells and non-

preincubated 30,000 x g supernate (S-30) derived from wheat germ were prepared according to previously published procedures (8-9).

Preparation of Placental RNA:

Term placental tissue (obtained by Cesarean Section) was prepared as described elsewhere (7). The tissue was homogenized in 1 volume of buffer containing 50 mM Tris HCl (pH 7.7), 5 mM KCl, 5 mM $MgCl_2$, 7 mM β-mercaptoethanol, 880 mM sucrose, and 0.5 mM EDTA. Homogenization was carried out in the cold for about 3 min with a motor driven stainless steel pestle (Kontes Glass Co.) and glass vessel. The homogenate was centrifuged at 8500 x g for 10 min and the composition of the supernate was adjusted to 0.1 M Tris HCl (pH 8.5), 0.1 M NaCl and 1% sodium dodecyl sulfate. To this was added an equal volume of a cold mixture of phenol: chloroform: isoamyl alcohol (50:50:0.5) saturated with 0.1 M Tris HCl (pH 8.5). The supernate was extracted for 20 min at room temperature and the phases were separated by centrifuging at 7000 x g for 10 min. The aqueous phase was reextracted twice more with an equal volume of this phenol mixture. The aqueous phase was made 2% in potassium acetate (pH 5.0) and the RNA precipitated overnight with 2 volumes of ethanol (prechilled to -20°). The RNA suspension was centrifuged at 5000 x g for 10 min and the pellets were washed as described by Shapiro et. al. (10). The RNA was dissolved in sterile H_2O; the yield of RNA per gm of placenta was 0.25 - 0.4 mg.

Assays for Protein Synthesis:

Protein synthesis in ascites extracts was assayed in 0.06 ml reaction

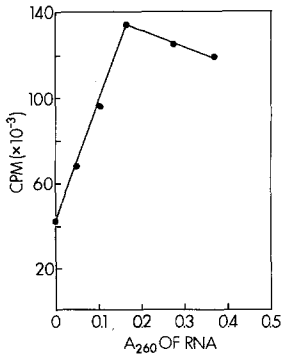

Figure 1. Protein synthesis by an S-30 extract from ascites tumor cells in response to RNA from term placenta. The incubations were for 90 min at 32°.

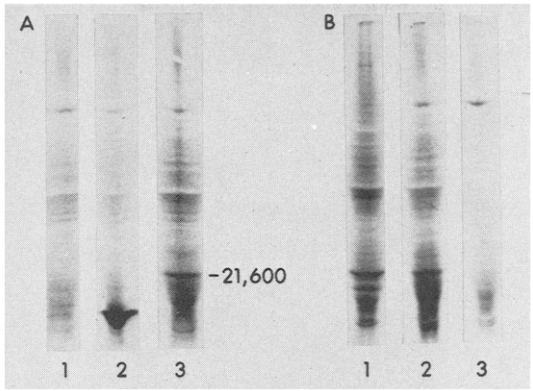

Figure 2. SDS-polyacrylamide gel electrophoresis of proteins synthesized in response to placental RNA. The ascites S-30 extract was incubated in the absence of RNA (A-1, B-3), in the presence of reticulocyte RNA (A-2), and with term placental RNA (A-3, B-2). Also included are proteins synthesized in an endogenous term placental cell-free system, (B-1). The amount of radioactivity added was about 50,000 cpm for the RNA dependent samples and about 15,000 cpm for those without added RNA.

mixtures as previously described (8).

Assays in wheat germ extracts (S-30) contained in a final volume of 50 µl, 10 µl of S-30, 20 mM HEPES (pH 7.6), 2.5 mM dithiothreitol, 1.5 mM ATP, 1.5 mM P-creatine, 2 µg of creatine kinase, 0.3 mM GTP, 20 µM of unlabeled amino acids, 54 mM KCl, 3.5 mM magnesium acetate, and 0.5 µM [^{35}S] methionine (Amersham/Searle, specific activity 200 Ci/mmole). The reactions were then processed as described for the ascites extracts (8).

Analyses of [^{35}S] Methionine Labeled HPL:

The product synthesized in vitro was examined on linear gradient (7-28%) polyacrylamide slab gels and by tryptic fingerprinting as previously described (7).

RESULTS

Term placental RNA stimulated amino acid incorporation 3-4 fold in an ascites S-30 (Fig. 1). Optimal incorporation required about 15 µg of RNA per 60 µl, 3.3 mM Mg^{++} and 70 mM KCl.

The products were examined using SDS-polyacrylamide gels (Fig. 2). A major discrete protein comigrated with authentic HPL as well as with the protein

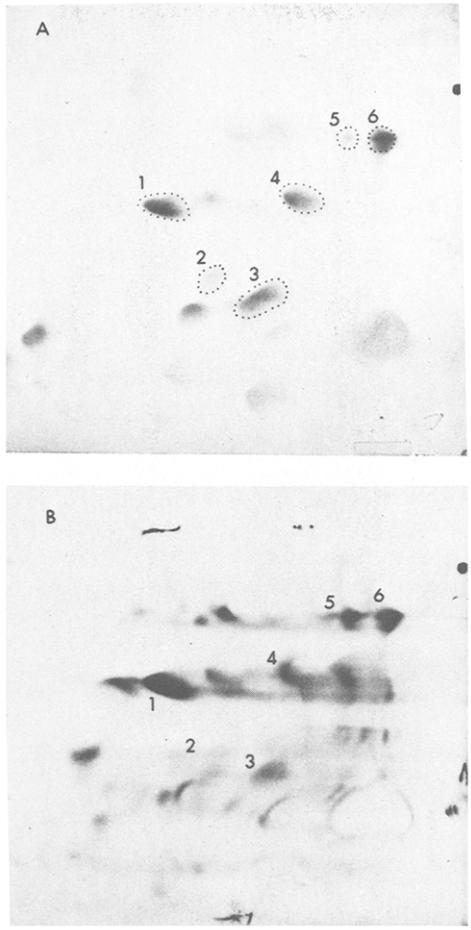

Figure 3. Two dimensional tryptic fingerprint analysis of a mixture of 7 mg of unlabeled carrier HPL and labeled proteins, synthesized in response to term placental RNA. About 400,000 cpm eluted from the region corresponding to HPL in an SDS gel was mixed with the carrier. Panel B is the autoradiograph (8 day exposure) of Panel A which has been stained with ninhydrin. The ninhydrin positive peptides of HPL which show the same mobility as the labeled peptides are denoted by the dotted rings. The other labeled peptides do not have ninhydrin counterparts and must reflect peptides derived from other proteins eluted from the gel.

corresponding to HPL synthesized by an endogenous system composed of placental ribosomes and ascites cell-sap. This protein was not seen in the absence of RNA. As expected, globin mRNA directs the synthesis of proteins not corresponding to HP

HPL contains six tryptic peptides which contain a methionine residue (11-12) Tryptic peptide analysis of the products synthesized in response to the placental RNA revealed 6 labeled peptides (Fig. 3, dotted rings 1-6). These peptides were

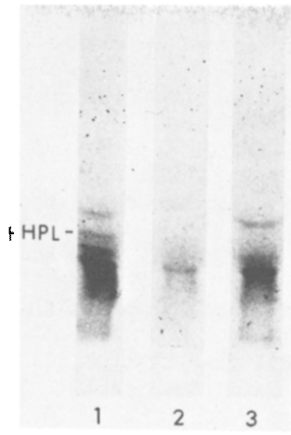

Figure 4. SDS gel electrophoresis of protein synthesized in wheat germ extracts programmed with placental RNA. The S-30 fraction was incubated in the absence of RNA (2), in the presence of term placental RNA (3), and a mixture of placental RNA and labeled HPL synthesized from term placental ribosomes (1). The amount of radioactivity added was approximately 20,000 cpm for the RNA dependent samples and about 8000 cpm in the absence of mRNA.

not present on maps containing carrier HPL and the proteins synthesized in the absence of mRNA or in the presence of globin mRNA.

As can be seen in Figure 2, the primary product synthesized in the ascites extracts was HPL. When placental RNA was translated in an S-30 from wheat germ, a different distribution of proteins was observed (Figure 4). A major protein migrated slower than the HPL standard, and it had a molecular weight of about 25,000. We suspected that this protein might be a precursor. Consistent with this observation, a fingerprint of the protein shows that it contains the 6 [^{35}S] methionine-labeled tryptic peptides seen in the fingerprint of the protein synthesized in the ascites system (Figure 5). Thus, in the presence of placenta RNA the wheat germ system synthesizes a protein containing HPL sequences, but larger than HPL.

DISCUSSION

RNA from term placental homogenates directs the synthesis of HPL in a cell-free system derived from ascites tumor cells. However, translation of this RNA in a comparable system from wheat germ resulted in the synthesis of a larger protein containing HPL sequences. No protein comigrating with HPL (21,600) was

seen. This difference in product formation with different cell-free systems has been observed by others. Milstein et al. (13) showed that when a myeloma light chain mRNA is translated in a cell-free system from rabbit reticulocytes a heavier protein containing light chain sequences was observed; translation in the ascites cell-free system yielded mostly finished product, i.e. light chain. This difference was also observed when myeloma light chain mRNA was translated in the wheat germ and ascites cell-free systems (T. Honjo, D. Swan, M. Nau and P. Leder, Personal Communication).

Although it appears that the wheat germ system lacks the cleavage activity

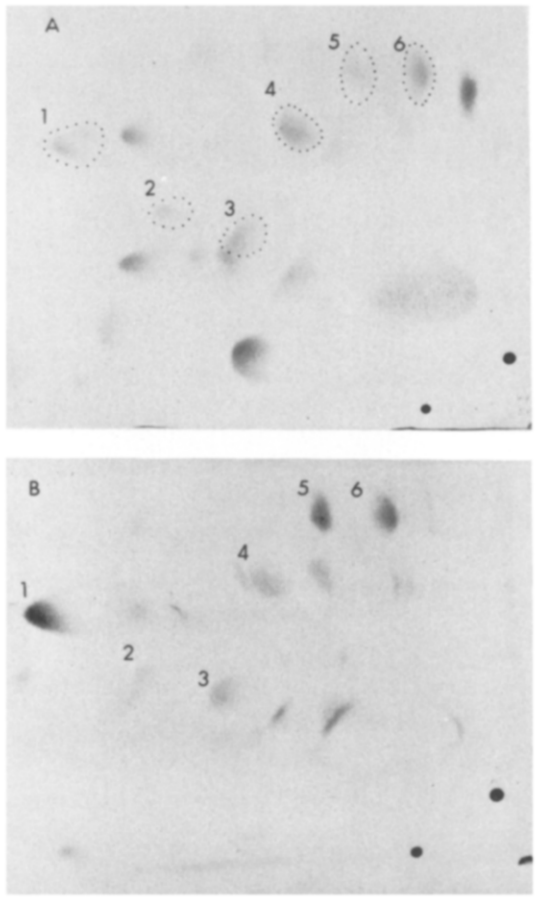

Figure 5. Two dimensional tryptic fingerprint analysis of a mixture of carrier HPL and labeled protein synthesized in a wheat germ S-30. The equivalent of about 200,000 cpm was eluted from 2 gels and treated as described in the legend to Figure 3.

seen in ascites extracts, it is not clear if the protein generated in the wheat germ represents a physiological precursor of HPL, since no protein of this molecular weight has been characterized in vivo; although often a minor discrete protein of that size was observed in the endogenous placental system.

At term the placenta secretes larger quantities of HPL into maternal serum than in the first trimester. This probably reflects an enhanced synthesis of the hormone at term since more endogenous HPL is synthesized by term extracts than by comparable cell-free extracts from first trimester tissue (7). It was postulated that this reflected an increased differentiation of the placenta in which the proportion of placental syncytium increases at term; the syncytium appears to be the site of synthesis of HPL (6). This would likely result in more HPL mRNA at term. Consistent with this we have preliminary evidence that RNA from term tissue directs the synthesis of significantly more HPL than a comparable quantity of RNA isolated from first trimester tissue.

ACKNOWLEDGEMENT

We are grateful to Mrs. Marion Nau and Dr. P. Leder for helping us with the wheat germ cell-free system. This work was aided in part by a grant from the Population Council (#M73, 135) and by a Departmental Grant from the Ford Foundation.

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