THE ISOLATION OF mRNA ENCODING THE ALPHA SUBUNIT OF HUMAN CHORIONIC GONADOTROPIN

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SUMMARY: MRNA was prepared from first trimester placentae and translated in a wheat germ cell-free system. A major synthesized product had an apparent molecular weight of about 16,000. Tryptic fingerprint analysis revealed that this protein contained peptides overlapping with peptides from authentic alpha subunit of hCG. Since the protein portion of alpha has a molecular weight of 10,600, the data suggest the synthesis of a precursor. No synthesis of the beta subunit was detected.

With first trimester RNA, 4-5 times more of the alpha protein was synthesized than with term RNA. Thus, it appears that the larger amounts of hCG-alpha produced in first trimester placenta is due to an increased proportion of the corresponding mRNA.

Human chorionic gonadotropin (hCG) is the major peptide hormone produced by the placenta during early pregnancy. It first appears in maternal serum soon after conception, reaching maximal levels at 10-12 weeks, and then declining to basal levels that remain fairly constant throughout pregnancy.

Although the chemistry of hCG has been elucidated (1-3), little information is available regarding its biosynthesis. Since it consists of two nonidentical subunits, a question that arises is whether the subunits are synthesized from separate mRNAs or if they are synthesized in tandem from one mRNA. This question may also apply to the biosynthesis of the subunits for the pituitary hormones, FSH, TSH and LH, since they are structurally very similar to hCG subunits. To examine this point and the control of hCG synthesis as a function of gestation, it is important to measure the corresponding mRNA activity.

It was previously shown that 5 times more of the alpha subunit of hCG was synthesized in cell-free extracts from first trimester tissue than

Abbreviations used: SDS, sodium dodecyl sulfate; FSH, follitropin; LH lutropin; TSH, thyrotropin.

from term tissue (4). Here we show that this enhanced synthesis is largely the result of an increased proportion of the corresponding mRNA. Also, first trimester mRNA directs the synthesis of a protein in the wheat germ cell-free system that is heavier than the protein portion of the alpha subunit, but containing authentic alpha tryptic peptides. This suggests the synthesis of a precursor to the alpha subunit.

METHODS

[^{35}S] Methionine (sp. act. 300 Ci/mM) was purchased from New England Nuclear and Amersham/Searle. Density gradient pure sucrose was obtained from Schwarz-Mann and wheat germ from ADM Mill (Shawnee Mission, Kansas).

Preparation of cell-free extract: The 30,000 x g supernate (S-30) was prepared according to Roberts and Paterson (5), with the ommission of the preincubation step.

Isolation of placental RNA: First trimester tissue was homogenized directly in a glass (stainless steel pestle) homogenizer with 2 volumes of a buffer containing 50 mM Tris-HCl, pH 7.8, 25 mM KCl, 5 mM $MgCl_2$, 7 mM β-mercaptoethanol, 880 mM sucrose and 0.5 mM EDTA. The RNA was extracted as described previously (6).

RNA was prepared from term placenta as described above, except that the tissue was taken through a tissue press before homogenization (6). Crude first trimester and term RNA were purified further on an oligo (dT)-cellulose affinity column (7).

Assay for protein synthesis: The translation of placental RNA was assayed in the wheat germ cell-free system (6).

Product analysis: The *in vitro* synthesized products were analyzed using SDS-polyacrylamide gel electrophoresis or tryptic fingerprinting (6).

Sucrose gradient analysis of RNA: RNA was examined on a 5-20% sucrose gradient containing 0.1 M Tris-HCl (pH 7.8) and 0.1 mM EDTA (8). The RNA was heated at 60° for 5 min, chilled immediately and then layered on the gradient. The gradients were centrifuged at 32,000 rpm for 18 hours in a Beckman SW-41 rotor at 17°. Fractions of 0.36 ml were collected and the absorbance at 260 nm was determined.

RESULTS

First trimester placental mRNA was purified using an oligo (dT)-cellulose affinity column. The adsorbed mRNA fraction was very active in stimulating radioactive amino acid incorporation into acid precipitable protein (Table I).

The products synthesized were then analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 1). A prominent component migrated as a doublet consisting of a major protein with a molecular weight of about 16,000 (Fig. 1, arrow) and a minor one having a molecular weight of about 14,000. In addition, the synthesis of the human placental lactogen precursor (prehPL, m.wt. 25,000)

TABLE I. Protein synthesis in response to first trimester placental RNA fractions prepared by oligo (dT)-cellulose chromatography.[a]

RNA (µg/.05ml)	[^{35}S] Met incorp. (cpm/.05ml)
NONE	12,000
Crude	
(2.0)	25,000
(5.0)	50,000
Adsorbed	
(1.0)	85,000
(2.0)	148,000
(3.0)	162,000
Unadsorbed	
(5.0)	14,000

[a] Incubations were performed at 25° for 60 min. Adsorbed and unadsorbed are column bound and unbound RNA fractions, respectively.

TABLE II. Level of methionine labeled hCG tryptic peptides synthesized by first trimester and term RNA. [a]

RNA (µg/.25ml)	Radioactivity applied to map (cpm)	Cpm recovered Peptide 1 (%)	Peptide 2 (%)
F.T. Crude (55)	325,647	3970 (1.22)	5225 (1.61)
Term Crude (57.5)	344,610	890 (.26)	1335 (.37)
RATIO: F.T. (%)/term (%)		4.69	4.13
F.T. Purified (8.5)	165,555	1895 (1.15)	2575 (1.56)
Term Purified (6.1)	196,317	510 (.26)	750 (.38)
RATIO: F.T. (%)/term (%)		4.42	4.11

[a] Purified RNA is the bound fraction from the oligo dT column. Tryptic peptides 1 and 2 (Fig. 2) were cut out from fingerprints and counted.

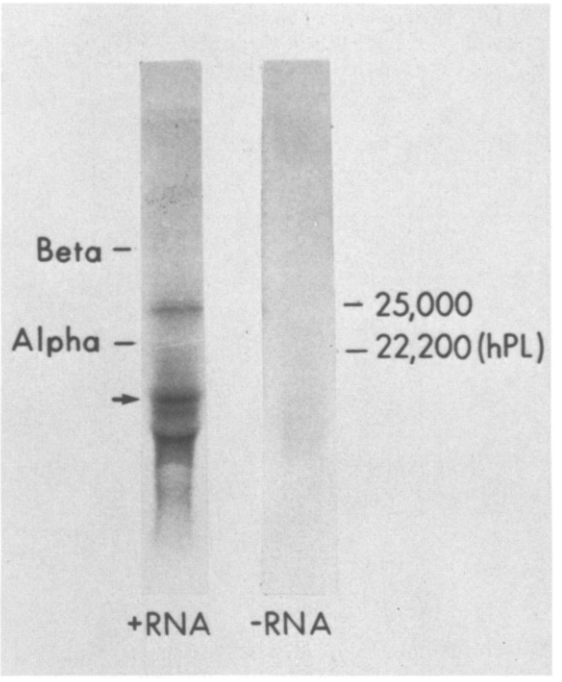

Figure 1. Autoradiograph of an SDS-polyacrylamide gel of proteins synthesized in wheat germ S-30 containing [^{35}S] methionine and first trimester RNA. The radioactivity applied to each lane was: minus RNA, 11,238 cpm; plus RNA, 34,440 cpm.

was observed. It is difficult to compare alpha and beta subunit standards here, since their sugar content would markedly retard their migration on SDS gels and hence migration would not correspond to protein molecular weight (9). Purified alpha subunit has a molecular weight of 16,000 and yet it migrates on SDS gels with an apparent molecular weight of 23,000 (Fig. 1). Since the proteins synthesized in the wheat germ system would unlikely be glycosylated, the bands observed would represent the protein portions of the molecule.

More direct identification of the two proteins was obtained as follows: the bands were eluted from a preparative gel, mixed with purified hCG, aminoethylated (10), digested with trypsin and the peptides analyzed by two dimensional chromatography and electrophoresis (6). The fingerprints were sprayed with ninhydrin to localize peptides derived from the carrier and then autoradiographed.

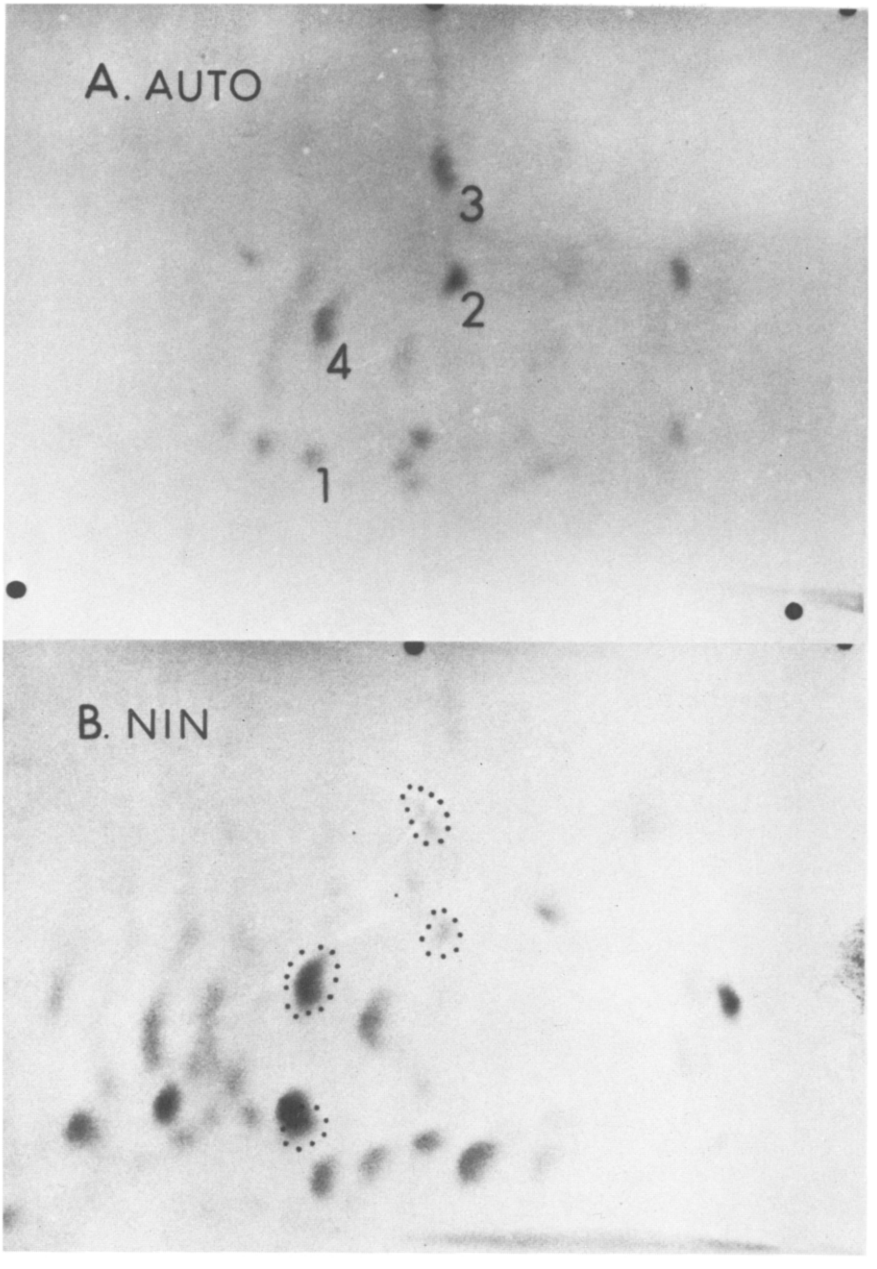

Figure 2. Two dimensional tryptic fingerprint analysis of a mixture of purified hCG and labeled protein synthesized in a wheat germ S-30 containing placental RNA. The labeled doublet proteins were eluted from a preparative gel. Panel A is the autoradiograph of ninhydrin stained Panel B. Approximately 300,000 cpm were applied. Ninhydrin peptides from carrier hCG overlapping with the synthesized peptides are denoted by the dotted rings.

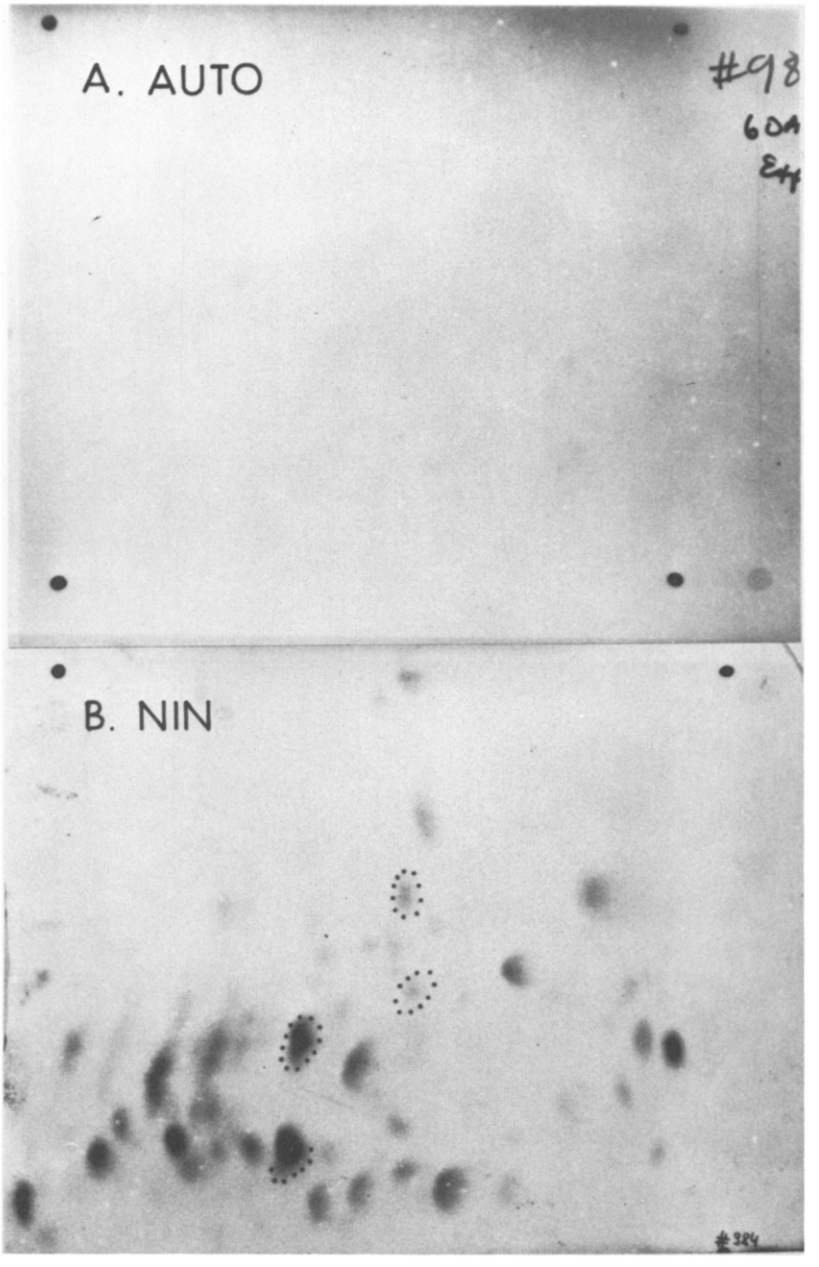

Figure 3. Two dimensional tryptic fingerprint of carrier hCG and proteins synthesized in the wheat germ without RNA. Approximately 100,000 cpm was applied.

Based on the amino acid sequence, tryptic hydrolysis of amino-ethylated-treated hCG should yield about 40 peptides. The ninhydrin stained

map displays about 25 major peptides and several minor ones (Fig. 2). There are 3 methionine tryptic peptides in the alpha subunit and 1 in the beta. By aminoethylating the protein, these tryptic peptides should be resolvable in the 2 dimensional system used here (4). There were 4 methionine labeled tryptic peptides that coincided with ninhydrin stained tryptic peptides from carrier hCG. In the region of peptide number one, there were two ninhydrin stained peptides that migrated very close to each other. When this region was eluted and electrophoresed at pH 6.5, the two ninhydrin peptides were better separated and the radioactivity corresponded to one of them. The 4 labeled peptides were not seen when the total product was examined from a reaction mixture incubated without RNA (Fig. 3). These peptides were not present on maps containing hCG and the proteins synthesized in the presence of globin mRNA.

When labeled protein was mixed separately with individual subunits, the 4 methionine tryptic peptides corresponded to peptides derived from the alpha subunit. No methionine labeled tryptic peptide corresponding to a peptide from the beta subunit was detected. Thus, it is possible that one of the overlapping peptides represents a non-specific tryptic cleavage product. Based on fingerprint analysis the 14,000 molecular weight protein appears to be a prematurely released protein.

Total placental RNA was resolved on a 5-20% sucrose gradient (Fig. 4). Fractions from the 4S-18S region were translated in the wheat germ system and the products examined on SDS gels. The peak activity for the 16,000 molecular weight protein (arrow) sedimented at about 10S.

For comparing the levels of hCG alpha-mRNA in first trimester and term placentae, both total cellular RNA and oligo-dT purified mRNA were used. Total RNA was used to eliminate artefacts arising from a difference in recovery of poly (A) containing mRNA. Tryptic peptides 1 and 2 were used to assess the amount of translatable RNA present. About 4 times more of these peptides were synthesized with first trimester RNA than with term RNA (Table II). The total methionine incorporation per μg of RNA was comparable with first trimester

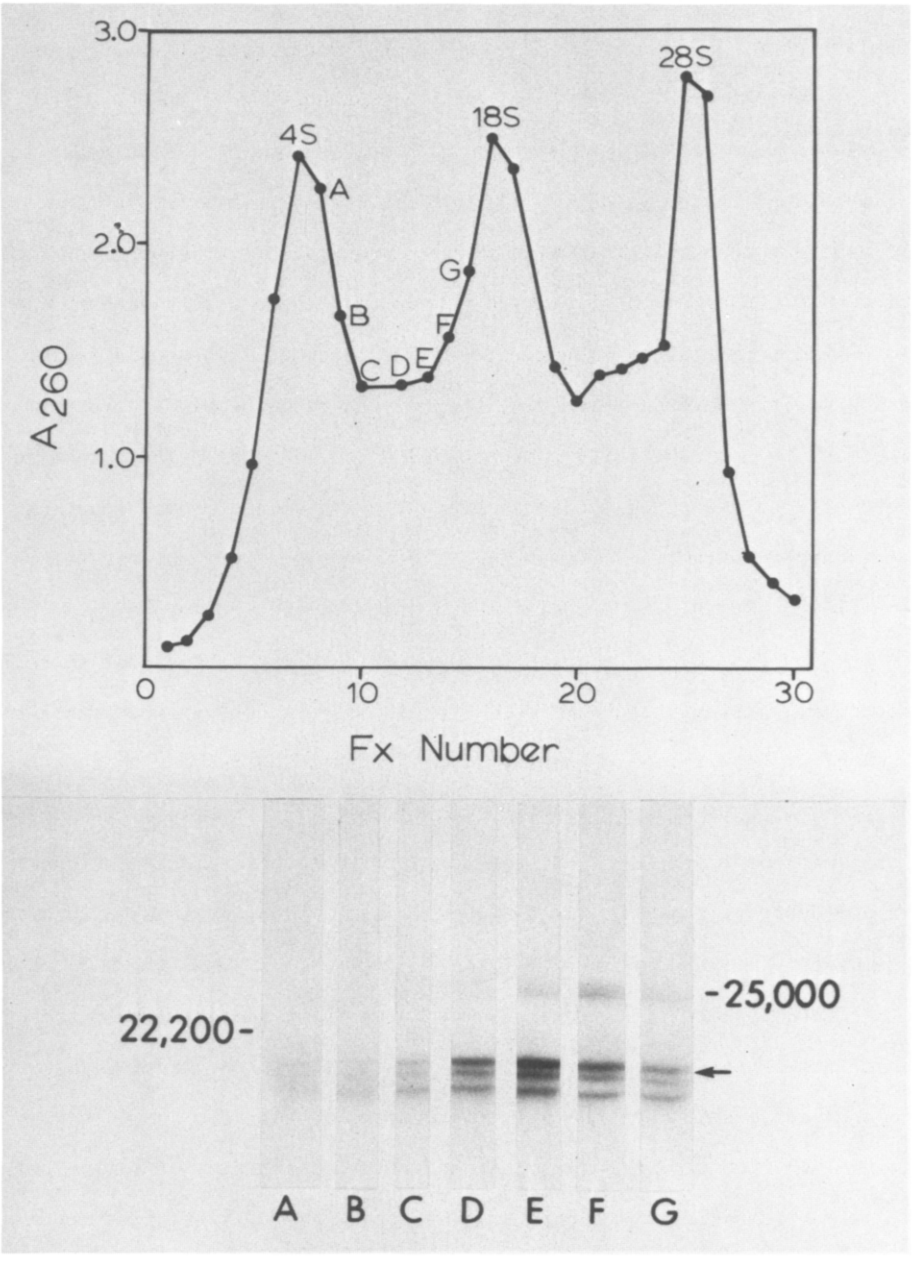

Figure 4. Sucrose gradient centrifugation of total first trimester placental RNA. The RNA in fractions A-G were ethanol-precipitated and equal amounts of RNA were translated in wheat germ S-30. Equivalent protein was applied to the gel.

and term RNAs and thus the lower levels seen at term were not a result of a decrease in the efficiency of protein synthesis.

DISCUSSION

RNA derived from first trimester placenta directs the synthesis of a protein (m.wt. 16,000) larger than the protein portion of the alpha subunit (m.wt. 10,600) of hCG but containing alpha tryptic peptides. This suggests a precursor to the alpha subunit was synthesized, as has been demonstrated for other secretory hormones (6,11-15). However, it is possible that some carbohydrate was coupled to the protein. Although unlikely, if the wheat germ S-30 glycosylated the nascent chain, the completed protein would migrate slower on SDS gel and would not accurately reflect the molecular weight of the protein portion of the molecule (9).

The data suggest that the subunits are synthesized from separate mRNAs. First, the total protein molecular weight of the two subunits is about 27,000 and yet the band containing alpha tryptic peptides has a molecular weight of about 16,000. This protein may have arisen from a larger protein but this is unlikely since wheat germ extracts apparently lack the cleavage activity required for the processing of a variety of precursor proteins (11-15). Also, the appearance of a protein containing beta peptides would have been expected and this was not observed. Secondly, the mRNA encoding for the alpha protein sedimented at about 10S, too small to encode both of the subunits (or their possible precursors) in tandem. Definitive proof concerning this point can only be established when the beta mRNA is isolated.

The lack of detectable beta mRNA suggests that it is present in much smaller quantities than alpha mRNA. This is consistent with the findings that in placental and pituitary tissue, the amount of free alpha subunit greatly exceeds free beta subunit (16-21). Perhaps synthesis of beta mRNA is under stringent control and that its levels constitute a rate limiting step in the expression of hCG in vivo. That the synthesis of the beta subunit may be limiting has been suggested, based on the levels of free subunits observed in gonadotropin-synthesizing tissues (18-20).

The differences in the level of the alpha subunit protein synthesized

by first trimester and term mRNAs parallels the in vivo blood levels of hCG. This strongly suggests that the rate of mRNA synthesis contributes to the amount of the hormone seen in vivo, as previously discussed (4,22).

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