SYNTHESIS AND PROCESSING OF PRE-α_{2u}-GLOBULIN IN DIFFERENT TRANSLATIONAL SYSTEMS

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Received 26 August 1981

1. Introduction

Several proteins synthesized by the rat liver display some degree of sex-specificity. One of the best known examples is α_{2u}-globulin, a protein synthesized by the adult male rat liver and secreted into the blood. α_{2u}-Globulin is rapidly cleared by the kidney and represents a major protein in urine of male rats. Its synthesis is under complex hormonal control involving the participation of sex hormones, glucocorticoids, thyroid hormone and pituitary hormones. Here we have studied the production of sex-specific proteins in rat liver by comparing the translation products of mRNA extracts in different translation systems. Major sex-specific proteins could be demonstrated in male as well as in female liver. The predominant sex-specific protein coded by male liver mRNA could be precipitated quantitatively by α_{2u}-globulin antiserum. Comparison of its size in 3 heterologous systems (wheat germ, reticulocyte lysate and *Xenopus* oocytes) demonstrates that the corresponding mRNA codes for a precursor protein that is slightly larger than the α_{2u}-globulin found in rat urine. Partial processing occurs in the wheat germ system. Oocytes assure complete processing and even secrete α_{2u}-globulin.

2. Materials and methods

2.1. *Preparation of mRNA*

Wistar rats (90-day-old) were fasted for 24 h. The livers were removed under ether anesthesia. Polysomes were prepared as in [1]. The resulting polysomal pellets were dissolved in RNA buffer (30 mM Tris–HCl (pH 7.5); 2 mM Mg^{2+}; 100 mM NaCl; 0.5% SDS) at a final concentration of 40 A_{260} units/ml. Polysomes were incubated for 30 min at 37°C in the presence of self-digested proteinase K (0.5 mg/ml). After this incubation the solution was adjusted to 0.2 M NaCl and RNA was precipitated at −20°C with 2 vol. ethanol. Separation of mRNA from total polysomal RNA was performed by affinity chromatography on oligo(dT)-cellulose (type 2, Collaborative Research) as in [2].

2.2. *Protein synthesis in vitro*

The wheat germ system was prepared as outlined [3] but with omission of the preincubation step. The assay mixture contained in 100 μl: 40 μl wheat germ S-30 fraction; 12 mM Hepes buffer (pH 7.6); 2 mM dithiothreitol; 1 mM ATP; 20 μM GTP, 8 mM creatine phosphate; 4 μg creatine phosphokinase; 200 μM of each of the 19 unlabelled amino acids; 30 mM K-acetate, 1.8 mM Mg-acetate; 80 μM spermine; 20 μCi [^{35}S]methionine; and 5 μg mRNA. Routinely translation was performed in a 140 μl reaction mixture during 2 h at 30°C. Incorporation of radioactivity into trichloroacetic acid-insoluble material was monitored on a 5 μl sample using Whatman 3 MM filter papers as in [4]. Earlier experiments revealed that optimal stimulation of methionine incorporation (±40-fold) was obtained using a final RNA concentration of 5 μg/100 μl reaction mixture. Translation in nuclease-treated reticulocyte lysate (Radiochemical Centre, Amersham) was performed as in [5]. Routinely, 7 μg mRNA was added to a 140 μl reaction mixture containing 56 μCi [^{35}S]methionine. The reaction was allowed to proceed for 2 h at 30°C. Incorporation of radioactivity in trichloroacetic acid-insoluble products was determined on a 5 μl sample as in [5]. Microinjection and culture of oocytes of

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00145793/81/0000–0000/$02.75

Xenopus laevis was performed as in [6]. Groups of 10 oocytes were injected either with 50 nl phosphate-buffered saline (control) or with 50 nl poly(A)-RNA. The male RNA preparation contained 0.8 mg RNA/ml. The female preparation 1 mg/ml. Oocytes were cultured in 100 μl medium. Three hours after injection, 75 μCi [^{35}S]methionine was added and incubation was continued for 3 days at 19°C. After this period the medium was carefully removed and the oocytes were homogenised. The homogenate was centrifuged for 5 min at 9000 × *g* in an Eppendorf microfuge and the incorporation of radioactivity was monitored by trichloroacetic acid precipitation [4].

2.3. *Product analysis*

Products of synthesis in vitro were analysed by polyacrylamide gel electrophoresis of trichloroacetic acid precipitates or immunoprecipitates. Immunoprecipitation was performed as in [7] using a specific anti-α_{2u}-globulin serum raised in rabbits. To 100 μl aliquots of the incubated reaction mixture, 5 μg carrier, α_{2u}-globulin was added and sufficient α_{2u}-globulin antiserum (40 μl) to quantitatively precipitate the added antigen.

Trichloroacetic acid precipitates and immunoprecipitates were dissolved by boiling at 100°C in 40 μl sample buffer (10 mM Na_2PO_4 (pH 7.2), 10% glycerol, 2% sodium dodecyl sulfate (SDS) and 2% β-mercaptoethanol). Samples were run on a 15% SDS–polyacrylamide slab gel for 15 h at 6 mA as in [8]. The concentration of bisacrylamide in the gel was 0.1%. Gel slabs were dried and autoradiographed on Agfa X-ray films after treatment with Enhance (New England Nuclear).

3. Results and discussion

A representative analysis of the [^{35}S]methionine-labelled products synthesized in a wheat germ cell-free system programmed with liver mRNA derived from male and female rats is shown in fig.1. Both mRNA preparations stimulated methionine incorporation to a similar degree but SDS–polyacrylamide gel electrophoresis reveals marked differences in the proteins responsible for this incorporation. Trichloroacetic acid precipitates from wheat germ programmed with male mRNA reveal a prominent sex specific reaction product (protein M) in the 22 000 M_r region. Translation of female mRNA reveals a prominent sex-specific protein (protein F) in the 33 000 M_r region. Since it is known that one of the major proteins synthesized by male rat liver is α_{2u}-globulin we investigated whether protein M could be precipitated using anti-α_{2u}-globulin. As shown in fig.1 this was in fact the case. Nevertheless it may be noticed that protein M is slightly larger than native α_{2u}-globulin derived from rat urine. The latter protein migrates on the same gel in the 19 500 M_r region. Protein F has not been identified yet. Anti-α_{2u}-globulin precipitates only a small fraction of the female translation products. On autoradiography the immunoprecipitate displays a weak band located in the protein F region. It cannot be excluded that this represents a non-specific interaction with the antiserum. In fact, the immunoprecipitated material represents only a minor fraction of the material migrating in the protein F region.

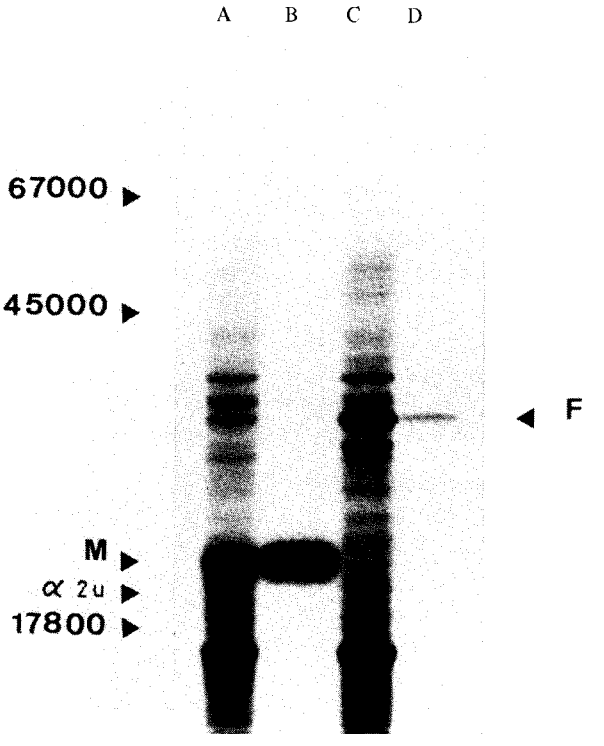

Fig.1. Translation in a wheat germ cell free system of liver mRNA derived from male and female rats, respectively: (A,B) trichloroacetic acid and immunoprecipitate of male liver mRNA translation products; (C,D) trichloroacetic acid and immunoprecipitate of female liver mRNA translation products. The marker proteins were: bovine serum albumin (6700), ovalbumin (45 000) and myoglobin (17 800).

To further analyse the relationship between pro-

tein M and α_{2u}-globulin, the translation of liver mRNA was compared in 3 systems wheat germ, reticulocyte lysate and *Xenopus* oocytes. Table 1 shows that poly(A)-RNA derived from male or female rat liver stimulates [^{35}S]methionine incorporation some 20 times in reticulocyte lysate and 30 times in wheat germ. Incorporation of amino acids in *Xenopus* oocytes was doubled after injection of male mRNA whereas female mRNA preparations consistently tended to inhibit incorporation in this system. In all 3 translational systems immunoprecipitation with anti-α_{2u}-globulin antiserum yields more precipitable material in reaction mixtures programmed with male mRNA. An analysis of the trichloroacetic acid-precipitable material derived from oocytes and wheat germ is shown in fig.2. In homogenates of oocytes programmed with male mRNA a major sex-specific reaction product may be noticed. This protein migrates in exactly the same region as native α_{2u}-globulin. In the medium derived from the same oocytes a slight spot is observed in the same region. No specific incorporation products can be identified in oocytes programmed with female mRNA. The wheat germ system again reveals protein M when programmed with male mRNA and protein F when programmed with female mRNA. In contrast with the former experiment, however, protein M is accompanied by a slightly smaller protein that migrates in the α_{2u}-globulin region. Partial processing in the wheat germ system has been described for several other secretory proteins [7,9]. Analysis of the immunoprecipitable material in fig.3 confirms that wheat germ programmed with male mRNA syn-

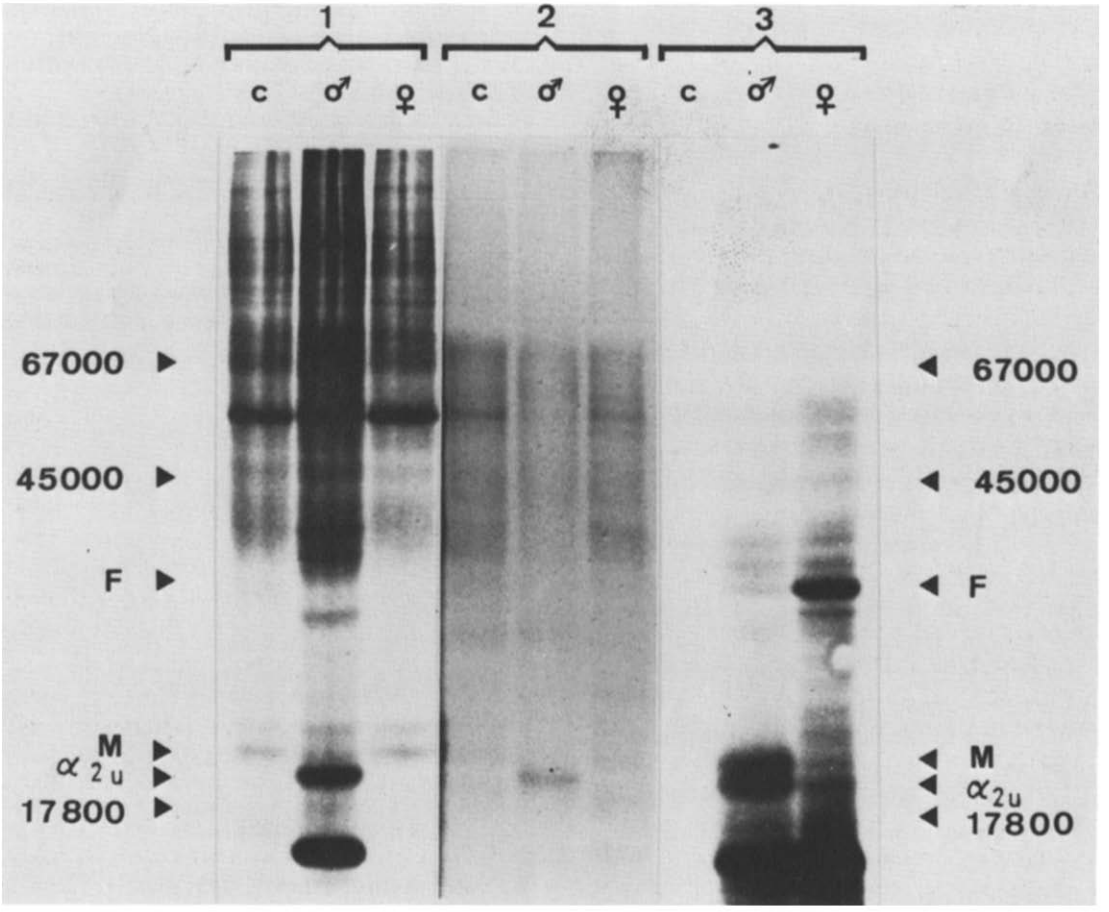

Fig.2. Comparison of the translation of liver mRNA in wheat germ and *Xenopus* oocytes: (1) oocyte homogenate; (2) oocyte medium; (3) wheat germ; (♂) male mRNA; (♀) female mRNA; (c) control (no mRNA added).

Table 1
Incorporation of [^{35}S]methionine in trichloroacetic acid-precipitable material and α_{2u}-globulin antiserum immunoprecipitates in various translational systems programmed with male or female rat liver mRNA

System	mRNA prep.	cpm in total protein ($\times 10^{-3}$)	cpm in immunoprecipitate ($\times 10^{-3}$)
	–	74	32
Reticulocyte lysate	Male	1742	342
	Female	1515	135
	–	7140	90
Xenopus oocytes cells homogenate	Male	16 411	756
	Female	5230	50
	–	529	28
medium	Male	656	174
	Female	873	144
	–	234	12
Wheat-germ	Male	6827	748
	Female	7589	185

Male and female rat liver poly(A)-RNA was prepared from polyribosomes as in section 2. The incorporation of [^{35}S]-methionine in total trichloroacetic acid-precipitable material and in the α_{2u}-immunoprecipitates was compared in 3 translational systems: wheat germ, reticulocyte lysate and *Xenopus* oocytes, respectively

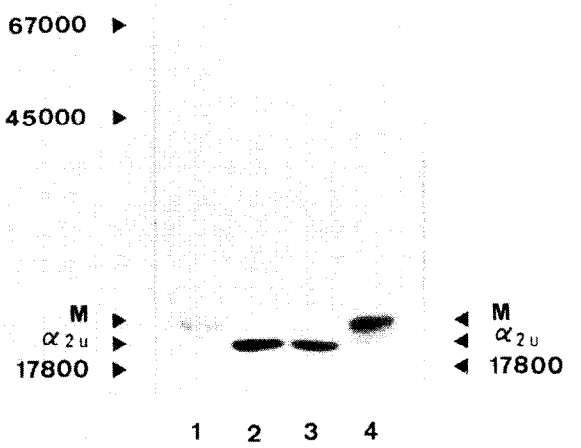

Fig.3. Incorporation of [^{35}S]methionine in immunoprecipitable translation products from reticulocyte lysate, *Xenopus* oocytes and wheat germ, respectively, programmed with male and female mRNA. Poly(A)-RNA from male and female rat liver was translated in various systems. After immunoprecipitation with α_{2u}-globulin antiserum as in section 2 translation products were analysed as in fig.2: (1) reticulocyte lysate; (2) oocyte homogenate; (3) oocyte medium; (4) wheat germ.

thesize both forms of immunoprecipitable material but predominantly the larger M_r species. Only small amounts of immunoprecipitable material were present in the corresponding samples of systems programmed with female mRNA and those migrated in the protein F region (not shown). Taken together these data suggest that male rat liver mRNA contains a messenger that codes for pre-α_{2u}-globulin. The ability of *Xenopus* oocytes to convert pre-α_{2u}-globulin into α_{2u}-globulin and to secrete this protein into the medium strongly suggests that the larger size of pre-α_{2u}-globulin is due to the presence of an N-terminal signal peptide related to its secretion. During the preparation of this manuscript a signal peptide containing 19 predominantly hydrophobic aminoacids has in fact been identified at the amino terminal portion of the α_{2u}-globulin mRNA translation product in reticulocyte lysate [10]. The presence of such a signal sequence is compatible with studies indicating that α_{2u}-globulin is synthesized on membrane bound polysomes and offers a further point of analogy between this protein and the major urinary proteins (MUPs) of the mouse [11].

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