TRANSLATION OF THE MESSENGER RNA FOR RABBIT UTEROGLOBIN IN *XENOPUS* OOCYTES

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1. Introduction

Uteroglobin is a small protein, which is detected in the uterine secretion of the rabbit during the preimplantation phase of pregnancy [1]. The secretion of this protein, which has also been called blastokinin [2], can be induced by administration of progesterone to the castrate rabbits [3], and the purified protein binds progesterone with considerable affinity and specificity [4,5]. We have recently shown that the isolated and perfused uteri can synthesize and secrete uteroglobin in quantities which are proportional to the uteroglobin content in the luminal fluid at the beginning of perfusion [6]. Treatment with estradiol for two days, followed by daily injection of progesterone for 4–5 days, leads to a high rate of amino acid incorporation into uteroglobin, representing 45% of the total incorporation into secreted proteins [6]. We therefore considered that the endometrium of these rabbits would be a suitable source for the isolation and identification of the messenger RNA (mRNA) for uteroglobin. In this paper we present evidence demonstrating the translation of this mRNA in oocytes of *Xenopus laevis*. The identification of in vitro newly synthesized uteroglobin was based on the interaction with monospecific antibodies, followed by precipitation with anti-γ-globuline and electrophoresis of the precipitate on polyacrylamide gels containing 8 M urea and 1% SDS.

2. Materials and methods

For the preparation of endometrial polysomal RNA twenty-four female rabbits of various breeds with an average weight of 3 kg were injected intramuscularly with two daily injections of estradiol-17β (100 μg) followed by four daily injections of progesterone (5 mg). Twenty four hours after the last injection the animals were killed and the uterine lumen perfused with cold saline [4]. All the buffers used for the isolation of mRNA were prepared with boiled bidistilled water and treated with 0.05% diethylpyrocarbonate at 100°C for 15 min. All the glass material was sterilized at 200°C, and special care was taken to avoid ribonuclease contamination. The endometrium was scraped and frozen in liquid nitrogen in a small volume of homogenization buffer (50 mM Tris, pH 7.5, containing 0.88 M sucrose, 25 mM NaCl, 2 mM UMP and 500 μg/ml of heparine). Immediately after thawing the endometrium, ice-cold homogenization buffer was added up to 2 ml per uterine horn, and the cells were disrupted by 5 strokes of a Teflon-glass homogenizer. One tenth the volume of a solution of 10% Triton X-100 and 10% Na-deoxycholate were then added, and homogenization continued (3 strokes, 500 rev/min). The homogenate was centrifuged at 10 000 *g* for 10 min at 0°C and 10 ml aliquots of the supernatant were layered over a discontinuous sucrose gradient consisting of 2 ml of 2.5 M and 10 ml of 1.5

M sucrose dissolved in gradient buffer (20 mM Tris–HCl, pH 7.5, containing 0.1 M NaCl, 5 mM $MgCl_2$ and 50 μg/ml of heparin). After centrifugation at 50 000 rev/min in the Spinco 60 Ti rotor the supernatant was carefully aspirated and the glassy pellet containing polysomes, monosomes, and ribosomal subunits, was washed briefly with gradient buffer and resuspended in the same buffer to a concentration of around 100 A_{260}.

The polysomal RNA was extracted by addition of SDS and Na_2 EDTA to a final concentration of 0.5% and 50 mM respectively, followed by one volume of acetate–SDS buffer (50 mM Na-acetate, pH 5.1, containing 0.5% SDS), and one volume of buffer-saturated phenol. After shaking for a few seconds one vol of chloroform was added and extraction continued by vigorously shaking for 5 min. The aqueous phase and the interphase were reextracted twice with one volume of phenol–chloroform (1:1). The interphase was discarded and the water phase was re-extracted once with phenol-chloroform (1:1) and once with chloroform alone, before addition of LiCl to a final concentration of 0.2 M and precipitation with 2 vol of ethanol at −20°C overnight.

The poly(A) rich RNA was prepared by a modification of the procedure af Aviv and Leder [7]. The RNA pellet was washed with 70%, 95% and absolute ethanol, dried and dissolved in 10 mM Tris, pH 7.6, containing 0.5 M NaCl, 0.2 mM $MgCl_2$ and 0.5% SDS. After adjusting the RNA concentration to 15 A_{260}, the polysomal RNA was applied to a column containing 1 g of oligo(dT)-cellulose (type T-2, Collaborative Research Inc., Walthman, Mass.) equilibrated with the same buffer. The column was extensively washed with this buffer until the A_{260} dropped below 0.02, and the bound RNA was eluted with bidistilled H_2O. This fraction, containing 5% of the polysomal RNA, was precipitated with LiCl and ethanol as above, washed, dried and stored in H_2O at −84°C for the oocyte injection experiments.

Microinjection into manually defoliculated oocytes [8] was carried out by the method of Gurdon et al. [9]. Two samples of thirty oocytes were injected with 50 μl of injection buffer [9] containing 20 ng endometrial RNA, and two samples of twenty oocytes were injected with globin mRNA (20 ng/50 μg) prepared as described [10]. Control samples were injected with buffer alone. Following injection, each set of oocytes was incubated for 16 h at 21°C in 200 μl of a modified Barth's solution [11] containing antibiotics (50 μg/ml streptomycin and penicillin and 40 μg/ml gentomycin) in the presence of [^{35}S] methionine (245 Ci mmol^{-1}, 400 Ci ml^{-1}). After incubation the oocytes were washed in Barth's solution, homogenized in 200 μl of 10 mM Tris–HCl, pH 7.9, and centrifuged at 10 000 *g*/10 min. The supernatants were used for determining the incorporation of [^{35}S] methionine into total protein [12]. The electrophoretic pattern of radioactive protein was analyzed by polyacrylamide gel electrophoresis in the presence of 8 M urea and 1% SDS [13]. Aliquots of the supernatant were used for immunoprecipitation with monospecific anti-uteroglobin, prepared in guinea pigs as previously described [4], and rabbit immunoglobin prepared against purified guinea pig-γ-globuline. The immunoprecipitates were washed, dissolved in 8 M urea–1% SDS, and analyzed by gel electrophoresis as indicated in the figure legends. Radioactivity in the gels was determined by cutting the gels in 1mm slices, dissolving each slice in 0.5 ml H_2O_2 at 70°C for 8 h, and adding 5 ml of a scintilation mixture (1 liter toluol containing 5 g PPO, 0.5 g POPOP, and 0.5 litre Triton X-100).

3. Results

The radioactivity profiles of the proteins sythesized by the control oocytes and by those injected with endometrial RNA are shown in fig.1. As can be seen, both in the absence and in the presence of dithioerythritol (DTE), about 10% of the total radioactive products synthesized by the oocytes injected with endometrium RNA comigrate with authentic uteroglobin whereas the control oocytes do not show radioactive peaks in this position. Around 20% of products of the oocytes injected with globin mRNA migrate with authentic rabbit globin when analyzed in identical gels (data not shown).

The results of the immunoprecipitation experiments are depicted in table 1 and fig.2. In the control and in the samples injected with globin mRNA, between 2 and 3% of the total radioactive polypeptides were located in the washed immunoprecipitate, whereas 12% of the radioactive products of the sample injected with endometrium RNA, were precipitable with a mono-

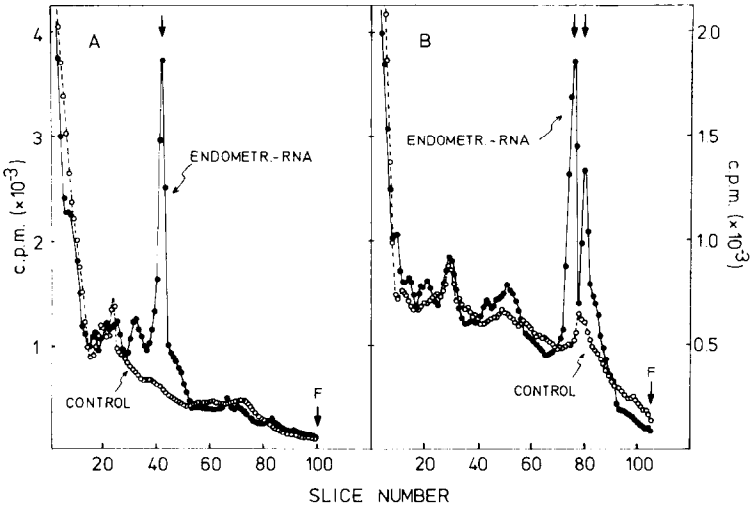

Fig.1. Gel electrophoresis of the total products synthesized by oocytes injected with either buffer or endometrial RNA. Two aliquots (10 μl) of the 10 000 *g* supernatant of oocytes injected with either buffer of (○---○) endometrium RNA (●——●) were mixed with 20 μg of authentic uteroglobin and treated at 90°C for 15 min with 50 μl of 10% TCA containing 10^{-3} M methionine. After staying at 0–2°C for 30 min the samples were centrifuged at 10 000 *g* for 10 min and washed twice with 100 μl 5% TCA, twice with acetone and dried. The precipitates were resuspended in 40 μl 10 mM Na-phosphate buffer. pH 6.8, containing 8 M urea and 1% SDS. One set of samples was brought to 10 mM DTE. All samples were incubated at 90°C for 5 min and electrophoresed in 15 cm gels containing 12.5% acrylamide and 1.2% *bis*-acrylamide according to Swank and Munkres [13]. After staining with Coomasie blue and destaining in 7.5% acetic acid –5% methanol, the gels were cut in 1 mm slices and the radioactivity determined as described in Methods. (A) Samples incubated without DTE; (B) Samples incubated with 10 mM DTE. The arrows indicate the position of authentic uteroglobin. The position of the dye is labeled with 'F'.

specific anti-uteroglobin using the double antibody technique (table 1). The immunoprecipitates were resuspended in buffer containing 8 M urea, 1% SDS and 10 mM DTE, and electrophoresed as above [13]. The radioactive profile is depicted in fig.2. As can be seen, no radioactive peak is detected in the region of uteroglobin either in the oocytes injected with buffer or in those injected with globin mRNA (fig.2A, B),

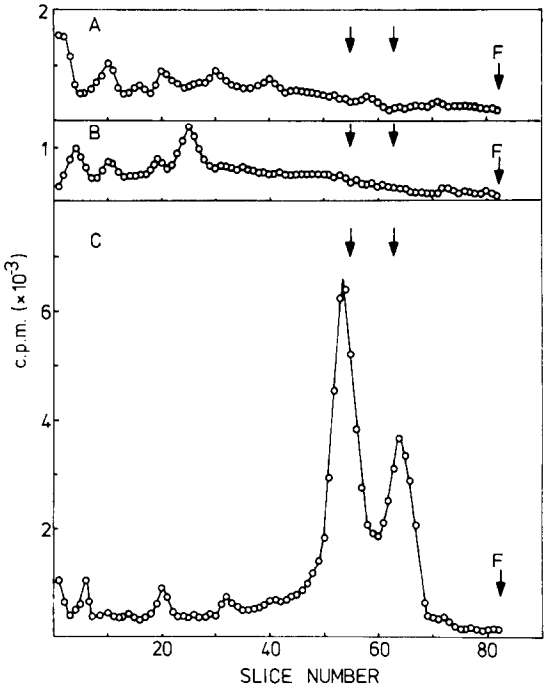

Fig.2. Gel electrophoresis of the immunoprecipitable products. The radioactively labeled products of oocytes injected with either buffer (A), globin mRNA (B), or endometrial mRNA (C), were immunoprecipitated by the double antibody technique as described in the legend to table 1. Aliquots of the washed precipitates containing between 40 and 60 000 cpm were incubated at 90°C for 5 min in 50 μl 10 mM Na-phosphate buffer, pH 6.8, containing 8 M urea, 1% SDS and 10 mM DTE, and submitted to gel electrophoresis as described in the legend to fig.1. The gels were then stained, destained, sliced and the radioactivity determined (see Methods). The position of authentic uteroglobin, added as internal marker, is indicated by arrows. The dye front is labeled 'F'.

Table 1
Immunoprecipitation of oocyte products with specific antiserum[a]

Sample		Total products (cpm × 10^{-3})	Immunoprecipitate (cpm × 10^{-3})	%
Control	1	2.317	71.8	3.1
	2	2.180	56.7	2.6
Globin mRNA	1	2.384	47.7	2.0
	2	2.691	64.6	2.4
Endometrium mRNA	1	2.985	331.3	11.1
	2	2.248	285.5	12.7

[a] The indicated amounts of radioactive products were brought to 0.15 M NaCl, and incubated at room temperature for 60 min with 200 μg of γ-globuline prepared from a monospecific anti-uteroglobin guinea pig antiserum by ammonium sulfate fractionation [4,14]. The samples were then allowed to stay at 0–2°C for 48 h before addition of 75 μl of rabbit γ-globuline purified by ammonium sulfate fractionation from an antiserum against purified guinea pig γ-globuline. Preliminary experiments have shown that this amount of anti-γ-globuline was sufficient to quantitatively precipitate the 200 μg of γ-globuline used in the assay. After staying at 0–2°C for 24 h the samples were underlayered with 300 μl of 20 mM Tris pH 7.5, containing 0.5 M sucrose, 1% Na-deoxycholate and 1% Triton X-100, and centrifuged at 10 000 *g* for 15 min. The supernatant was carefully aspirated, and the pellet washed twice with 400 μl of 10 mM Na phosphate buffer pH 7.5, containing 0.15 M NaCl. The final pellet was redissolved in 50 μl 10 mM Na-phosphate buffer, pH 6.8 containing 8 M urea and 1% SDS. Aliquots were used for the determination of radioactivity [12].

whereas over 80% of the radioactivity in the immunoprecipitate of the samples injected with endometrium mRNA comigrates with the uteroglobin marker (fig.2C).

4. Discussion

In polyacrylamide gels containing SDS native uteroglobin migrates as a single band of mol. wt 11–12 000 daltons [4]. Treatment with DTE or 2-mercaptoethanol followed by alkylation with iodoacetate, shifts the position of this band to the region of 5–6000 daltons, but still a single band is observed (unpublished observation). We therefore assume that uteroglobin is composed of two subunits of very similar, if not identical, mol. wt [4]. The two separate bands observed after treatment with DTE in gels containing urea and SDS are probably an artefact, as the relative proportion of the two bands varies considerably from experiment to experiment. Since native uteroglobin contains 4 cystein residues [15], partial reoxydation of thiol groups and the formation of intrachain disulfide bridges could account for the anomalous behaviour.

The data presented above clearly demonstrate that the poly(A)-containing RNA prepared from the endometrium of rabbits treated sequentially with estradiol-17β and progesterone contains the mRNA for uteroglobin, and that this mRNA can be faithfully translated in the oocytes of *Xenopus*. Around 10% of the total proteins synthesized by the injected oocytes represent uteroglobin as demonstrated by immunoprecipitation and gel electrophoresis, indicating that the mRNA for uteroglobin is a main component of the endometrial RNA preparation.

We have previously shown that the endometrium of rabbits treated with estradiol and progesterone synthesizes and secretes uteroglobin in vitro, in amounts proportional to the uteroglobin content of the uterine fluid [6]. It will be interesting to know, whether the endometrial content of the mRNA for uteroglobin parallels the uteroglobin synthesizing activity or whether

additional control mechanisms modulate the efficiency of specific translation. The system presented here allows the titration of the endometrial content of uteroglobin mRNA under different hormonal conditions.

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