LIPOVITELLIN SYNTHESIZING POLYSOMES: SPECIFIC AND QUANTITATIVE ISOLATION

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1 Introduction

Lipovitellin, a phosphoprotein with a molecular weight of about 160,000, is a major egg-yolk protein. It is synthesized in the liver of the laying hen, together with another phosphoprotein, phosvitin [1]. Immediately after synthesis, they are secreted into the blood as a 1:1 complex. In roosters, the synthesis of lipovitellin and phosvitin can be induced by an injection of estradiol [2].

The present experiments were initiated to study the fate of the messenger RNA of a hormone-induced protein. As a first step, the polysomes involved in lipovitellin synthesis were isolated by precipitation with specific antisera to lipovitellin. Good evidence exists that the synthesis of secretory proteins takes place on membrane-bound polysomes exclusively [3–7]. As expected lipovitellin is synthesized on this class of polysomes.

In our laboratory the kinetics of lipovitellin synthesis were studied. After hormone induction the rate of synthesis increases linearly with time during several days. The present results, obtained by quantitative immunoprecipitation of the lipovitellin-synthesizing polysomes, show that this increase parallels closely the amount of liver ribosomes engaged in lipovitellin synthesis.

2. Materials and methods

2.1. *Materials*

Estradiol was a generous gift from Organon, Oss, The Netherlands. Freund's complete adjuvant was purchased from Difco Laboratories, Detroit, Michigan, USA and Nonidet P40 from Shell Nederland Chemie, Rotterdam, The Netherlands. Ovalbumin was obtained from Worthington Biochemical Corp., Freehold, New Jersey, USA and anti-ovalbumin from Calbiochem, Los Angeles, Calif., USA. Carrier-free ^{32}P-orthophosphate in saline was bought from Philips-Duphar, Petten, The Netherlands.

2.2. *Purification of lipovitellin and preparation of the antiserum*

Lipovitellin, isolated from egg-yolk according to Bernardi and Cook [8], was purified and separated into its α- and β-components by TEAE-cellulose chromatography [9]. A solution of α-lipovitellin (8 mg/ml) was emulsified in an equal volume of

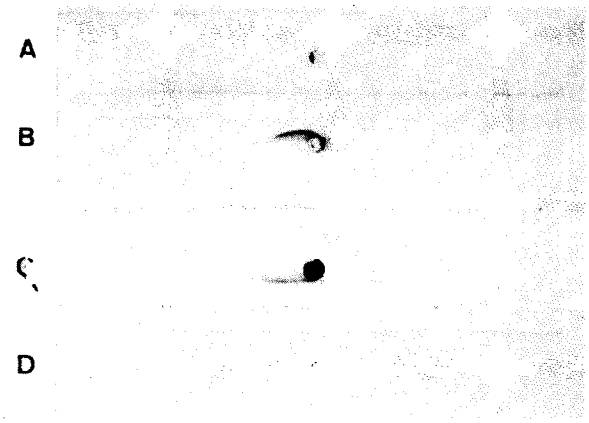

Fig. 1. Micro-immunoelectrophoretic tests of the anti-lipovitellin serum. In the trough: 80 μl of the undiluted antiserum. In the wells 2.5 μl of the following solutions: A) Livetins 10 mg/ml; B) Lipovitellin (8 mg/ml); C) Serum of an estrogenized rooster; D) Serum of a control rooster.

Freund's complete adjuvant. One ml-doses of this emulsion were injected into rabbits intradermally, 3 times at 10 day intervals. Eleven days after the last injection, the rabbits were bled by heart puncture.

The antiserum was tested in a micro-scale immunoelectrophoresis [10]. With α- and β-lipovitellin a single precipitation arc was formed (fig. 1B). No reaction occurred with the livetins, which are normal serum proteins present in egg-yolk, or with rooster serum (fig. 1A and 1D). The serum of an estrogenized rooster gave a precipitation line, typical for lipovitellin (fig. 1C). Evidently, our anti-lipovitellin serum does not contain antibodies to other serum proteins.

2.3. *Isolation of rat liver cell sap as a ribonuclease inhibitor*

Albino rats of an inbred Wistar strain, weighing about 250 g, were starved overnight. After decapitation the livers were excised, chilled and homogenized in ice-cold homogenization medium (1 ml/g liver; 0.25 M sucrose in TKM-buffer: 50 mM Tris, 25 mM KCl and 5 mM $MgCl_2$, pH 7.5 at 20°. Just before use dithiothreitol was added to a concentration of 1 mM). The homogenate was centrifuged at 30,000 rpm in the Spinco rotor 30 during 7 hr at 5°. The supernatant, rat liver cell sap, was stored at −90°.

2.4. *Labelling and isolation of the polysomes*

The experiments were performed with 2–4 week old hybrids of White Leghorn roosters and Rhode Island Red hens, obtained from the Spelderholt Institute for Poultry Research, Beekbergen, The Netherlands. The animals were fed at libitum.

Part of the animals received a subcutaneous injection of estradiol (25 mg/ml propylene glycol) in a dose of 35 mg/kg body weight. In order to label the ribosomal RNA of the polysomes, the estrogenized and the control roosters received an intravenous injection of ^{32}P-orthophosphate (35 mCi/kg body weight) 48 hr before death.

At the end of this period the animals were killed, the livers excised and chilled in ice-cold sucrose medium. 4 g of liver were homogenized in 28 ml rat liver cell sap in a Potter–Elvehjem type homogenizer with ten strokes of a tightly fitting Teflon pestle. The homogenate was centrifuged at 10,000 rpm for 10 min at 5°. The post-mitochondrial supernatant was collected and from this the free, membrane-bound, and total polysomes were isolated according to Blobel and Potter [11]. Nonidet P40 was used as the detergent instead of Triton X-100. Discontinuous gradients were centrifuged for 12 hr at 50,000 rpm in the Spinco rotor 50 Ti at 5°.

2.5. *Immunoprecipitation of the polysomes*

Polysome pellets were suspended in rat liver cell sap to a concentration of 0.75 mg ribosomal RNA/ml. Insoluble aggregates were removed by 10 min centrifugation at 2,000 *g*. Aliqots of 125 μl were mixed with 250 μl of undiluted antiserum. The mixtures were left for 1 hr in ice. Then 125 μl of antigen solution was added with concentrations found to be optimal for precipitation. After 60 min at 0°, the solutions were centrifuged at 2,000 *g* for 20 min. The supernatants were discarded and the precipitates were washed twice with 1 ml 0.5 M NaCl in TKM-buffer. 300 μl 6 M sulfuric acid and 150 μl 70% perchloric acid were added. After heating for 6 hr at 180° a clear, colourless solution was obtained. This was diluted with 4 ml water and the radioactivity was measured by Cerenkov radiation in a liquid scintillation counter (Nuclear Chicago Mark I).

3. Results and discussion

Free, membrane-bound, and total polysomes were isolated 88 hr after estradiol injection. Fig. 2 shows the precipitation of these polysomes with anti-lipovitellin serum at different lipovitellin concentration. From these curves the maximum percentage of radioactive RNA in the immunoprecipitate was determined. The results of four experiments are presented in table 1.

Polysomes from control roosters do not precipitate specifically with the anti-lipovitellin serum. The small amount of radioactivity found in the precipitate does not exceed the aspecific adsorption to a heterologous immunoprecipitate (ovalbumin–antiovalbumin).

Polysomes from the estrogenized rooster, on the other hand, react specifically: 10% of the ribosomes precipitate with anti-lipovitellin (table 1–total polysomes). The lipovitellin-synthesizing polysomes are largely restricted to the membrane-bound class. The presence of some specific polysomes in the free polysome fraction of the estrogenized rooster is probably

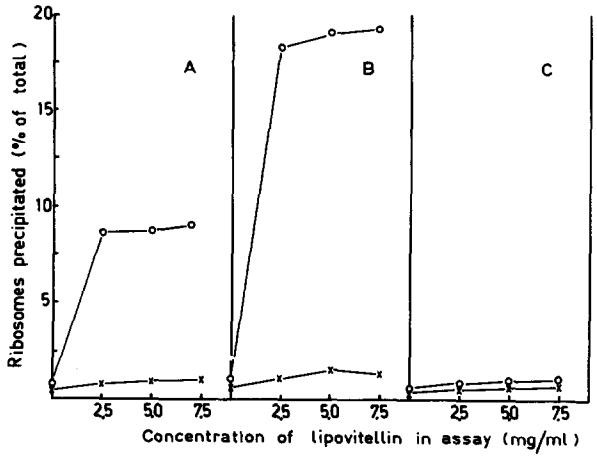

Fig. 2. Precipitation of the polysomes with anti-lipovitellin serum. Polysomes were treated as described under Methods. The radioactivity in the immunoprecipitate was expressed as the percentage of the total amount of radioactivity in the test tube (at least 10^4 counts/min). A) Total polysomes; B) membrane-bound polysomes; C) free polysomes. (o—o—o): estradiol treated rooster; (x—x—x): control rooster.

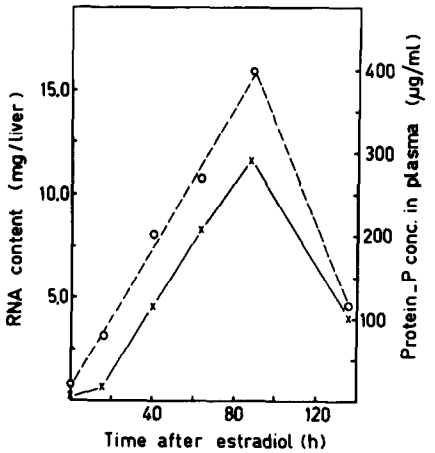

Fig. 3. Number of ribosomes, engaged in lipovitellin synthesis at different intervals after estradiol administration. At different times the roosters were killed after taking 1 ml blood from the wing vein. The protein-phosphate content of the plasma, which reflects the lipovitellin concentration [2], was determined (x—x—x). The RNA was extracted by a modified Schmidt–Thannhauser method [13] and determined by an orcinol reaction [14]. Lipovitellin synthesizing polysomes were determined as described in the text (o—o—o).

Table 1
Precipitation of ribosomes engaged in lipovitellin synthesis.

Estradiol	Polysomes	Lipovitellin Anti-lipovitellin	Ovalbumin Anti-ovalbumin
–	Total	0.7 ± 0.2	0.1
–	Bound	1.2 ± 0.3	1.6
–	Free	0.7 ± 0.2	0.2
+	Total	9.8 ± 0.6	0.2
+	Bound	17.6 ± 1.6	1.2
+	Free	2.3 ± 0.8	0.1

The results are expressed as percentage of the ribosomes present in each fraction. For the lipovitellin–antilipovitellin system the average of four experiments ± standard error is given.

due to the admixture of some bound polysomes to a degree varying from one experiment to the other (fig. 2). Therefore, we conclude that only membrane-bound polysomes synthesize lipovitellin. Our results clearly demonstrate, that the method employed is specific for lipovitellin-synthesizing polysomes. It is probably also quantitative, as will be shown below.

It has been shown in our laboratory (E.W. Bergink, quoted in [12]), that the rate of lipovitellin synthesis increases linearly with time, beginning at about 4 hr after estradiol. This was calculated from the accumulation of the phosvitin–lipovitellin complex in the blood and its turnover. The increased in rate should be due to an increase in the number of ribosomes engaged in lipovitellin synthesis. Therefore, the percentage of ribosomes synthesizing lipovitellin was determined at different intervals after hormone administration. The total amount of ribosomes in the liver was determined by measuring the total RNA-content of the liver. From these data the number of lipovitellin-synthesizing ribosomes was calculated and expressed as mg RNA per liver.

As can be seen from fig. 3, the amount of lipovitellin synthesizing polysomes increases linearly with time for at least three days. We prefer to interpret these results in terms of a long-lived messenger RNA for lipovitellin, synthesized at a constant rate for a few days.

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