ESTRADIOL-INDUCED ENHANCEMENT OF THE PROCESSING OF THE 32S RIBOSOMAL PRECURSOR IN ROOSTER LIVER

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1. Introduction

In birds the liver synthesizes specific yolk proteins [1]. Normally, this synthesis starts at the onset of laying, but administration of 17β-estradiol induces yolk protein synthesis also in immature pullets and in cocks [2]. With high doses of the hormone a synthetic rate can be obtained which is of the same order of magnitude as that in laying hens [3]. It can be estimated that the amount of secretory proteins synthesized by the liver of estradiol-induced cocks is increased three times compared to that of non-induced cocks [4]. Protein synthesis by the liver will therefore be increased. Van den Berg et al. [5] and Jost et al. [6] have shown a considerable increase in hepatic RNA under conditions of estradiol-induced yolk protein synthesis. This increase is, at least partially, a result of the enhanced activity of the nucleolar RNA polymerase [5]. In this paper we describe the effect of estradiol on the various steps of ribosomal RNA processing in cocks.

2. Materials and methods

2.1. *Animals and injections*

Roosters were 2 to 3 weeks old hybrids of White Leghorn roosters and Rhode Island Red hens obtained from Van der Sterren, Venray, The Netherlands. 17β-estradiol, a gift from Organon, Oss, The Netherlands, was dissolved in propanediol-1,2 (25 mg/ml) and injected subcutaneously (25 mg/kg body weight). Carrier-free [^{32}P]orthophosphate in dilute HCl was obtained from Philips-Duphar, Petten, The Netherlands. It was neutralized before use and injected intravenously (25 mCi/kg).

2.2. *Isolation of nucleoli*

The procedure used was essentially as described by Busch [7]. Roosters were anaesthesized with ether. About 4 g of liver were quickly removed and cooled in ice-cold solution A (0.32 M sucrose, 3.3 mM $CaCl_2$). All following steps were carried out at 0–4°C. To prevent RNA degradation the solutions mentioned in this section were supplied with polyvinylsulfate to a concentration of 100 μg/ml. The liver was cut into small pieces and washed several times with solution A. The liver was homogenized in 10 volumes of solution B (2.25 M sucrose, 3.3 mM $CaCl_2$) with 3–5 strokes of a loose Teflon–glass Potter-Elvehjem homogenizer. Connective tissue was removed by filtration through 4 layers of surgical gauze. The homogenate was diluted with 5 volumes of solution B. The nuclei were spun down by centrifugation at about 50 000 *g* in a SW 25.2 or SW 25.3 rotor, Spinco, for 60 min. The pinkish pellet consisted of almost pure nuclei. In the course of our investigations it turned out that equally well-purified nuclei could be isolated by centrifuging the undiluted homogenate over a layer of solution C (2.16 M sucrose, 3.3 mM $CaCl_2$). The nuclear pellet was suspended in 8 ml 0.32 M sucrose. Diethylpyrocarbonate was added to a concentration of 0.1% to inhibit ribonuclease activity [8]. The nuclei were then disrupted in 5 ml portions by sonication with a Branson Sonifier B_{10} at an input of 70–75 W 5 times for 10 sec with intermittent cooling for 50 sec. The disruption of the nuclei was followed by phase-contrast microscopy. Nucleoli were purified by sedimentation through a 0.88 M sucrose layer at 2000 *g* for 30 min. From the supernatant nucleoplasmic material was precipitated by adding two volumes of ethanol at −20°C.

2.3. *RNA extraction*

A pellet of nucleoli or nucleoplasmic material was suspended in an adequate volume (one ml for one g of liver) of acetate buffer (10 mM sodium acetate pH 5.1, 0.14 M NaCl, 1 mM EDTA). Then, one-tenth volume of freshly prepared detergent solution (2% sodium dodecyl sulfate and 1% sodium deoxycholate) was added under vigorous stirring. This solution was then added slowly with stirring to a phenol mixture [9] (phenol 500 g, cresol 70 ml, 8-hydroxyquinolin 0.5 g and water 150 ml) at 55°C. This solution was stirred at 55°C for 10 min and subsequently at 20°C for 15 min. The layers were separated by centrifugation at 2000 *g* for 10 min. The water layer and the interface were made 0.5 M NaCl and stirred for 15 min at 20°C with another volume of phenol mixture. After centrifugation, the water layer was stirred with chloroform (chloroform with 1% amyl alcohol) for another 15 min at 20°C. In this way, good extraction was achieved with little contamination of DNA.

Nucleoplasmic RNA was precipitated with 2 volumes of 4.2 M sodium acetate to remove DNA, whereas nucleolar RNA was precipitated with 2 volumes of absolute ethanol at 0°C. RNA was reprecipitated twice from 0.15 M sodium acetate pH 6.0 containing 0.5% SDS.

2.4. *Analyses of RNA*

Nucleolar RNA was analyzed by polyacrylamide electrophoresis.

The procedure was essentially that of Loening [10]. The E-buffer was used and glycerol was present in all solutions at a concentration of 8.7% to facilitate slicing [9]. The ribosomal precursor RNAs were separated on 2.2% gel of 7.5 cm length. A 2.0% gel of 0.5 cm was prepared on top of the 2.2% gel to separate the contaminating DNA from the precursor molecules. Good separation was achieved by electrophoresis during 16 h at 2 mA per gel (0.6 cm diameter). About 50 μg of RNA in 100 μl of E-buffer, containing 17% glycerol, could be separated. After electrophoresis the gels were scanned at 260 nm with a Gilford gel scanner attached to a Zeiss PMQ II spectrophotometer.

For the determination of the radioactivity in the RNA molecules, the gels were frozen at −90°C and sliced in a home-made gel slicer at −25°C. Slices of about 0.8 mm were counted with 3 ml of scintillation solution (toluene 3 1, 2-methoxy-ethanol 1 l, PPO 4 g/l, bis-MSB 80 mg/l) in a Philips liquid scintillation counter.

Nucleoplasmic RNA was dissolved in 0.3 ml 50 mM sodium acetate pH 6.0, containing 0.2% SDS, and layered carefully over a linear 5–20% sucrose gradient in 0.1 M sodium acetate pH 6.0, including 0.2% SDS.

Ribosomal RNAs were separated by centrifugation at 21 000 rev/min for 16 h at 22°C in a SW 25.3 rotor. The absorption at 260 nm was monitored by pumping the gradient through a home-made flow cell, attached to a Zeiss PMQ II spectrophotometer. Then, the gradient was divided in about 25 fractions of about 0.7 ml. Radioactivity was counted in a Philips liquid scintillation solution (toluene: Triton X-100, 2:1 (v/v), PPO 4 g/l, bis-MSB 80 mg/l).

3. Results

The analyses of rooster liver nucleolar RNA on polyacrylamide gels are shown in fig.1. The mature ribosomal RNA species were localized by comigration with cytoplasmic ribosomal RNA. Their mol. wts. were taken as 1.58×10^6 and 0.7×10^6 respectively, according to Loening [12]. In addition, two major and one minor precursor molecule can be detected. Linear extrapolation of log mol. wt. against electrophoretic mobility leads to 3.6, 2.2, and 1.9×10^6 dalton for the precursor molecules, in good agreement with the molecular weights found by Perry et al. [13] in cultured chicken cells.

The incorporation of ^{32}P into the precursor molecules of controls and estradiol-treated roosters is shown in fig.1. The pulse was given 26 h after estradiol administration since the major increase of hepatic RNA begins at that time [5]. A 15 min pulse only labelled the high mol. wt. precursor of 3.6×10^6. After longer intervals the other molecules are also labelled.

The effect of estradiol treatment on the specific activity of the 3.6×10^6 and 1.9×10^6 precursor is presented in fig.2. The specific activity of both precursor molecules increases linearly in time, reflecting the still incomplete saturation of the nucleotide precursor pools (data not shown). Estradiol induces only a slight and similar increase in the specific activity of both rRNA precursors.

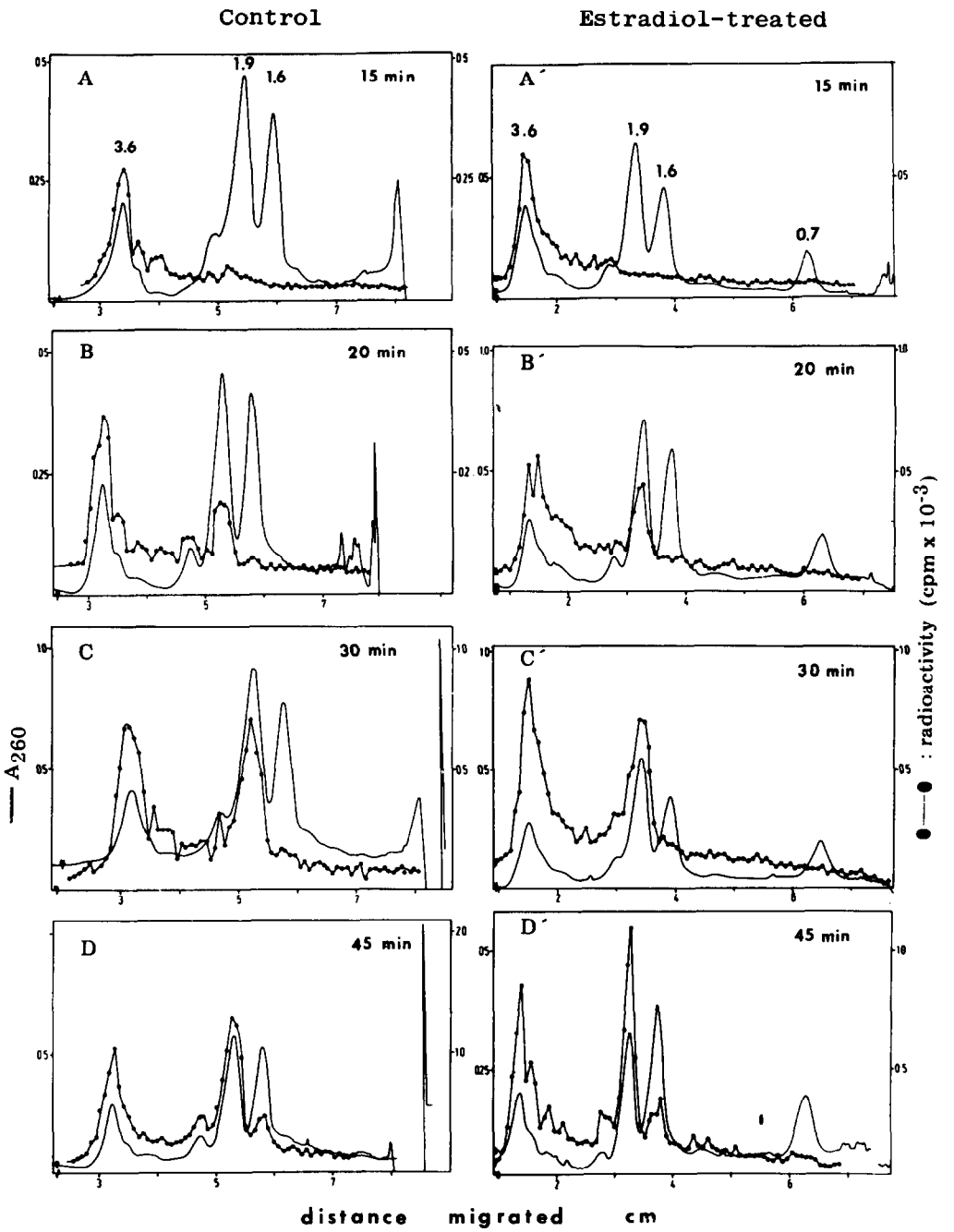

Fig.1. Effect of estradiol on the phosphate-labelling of nucleolar RNA. $^{32}P_i$ was injected intravenously in control roosters (A–D) and roosters treated for 26 h with estradiol (A′–D′). Nucleolar RNA was prepared from 3 pooled livers and analyzed on PAA gels after labelling for 15 min (A,A′), 20 min (B,B′), 30 min (C,C′) and 45 min (D,D′).

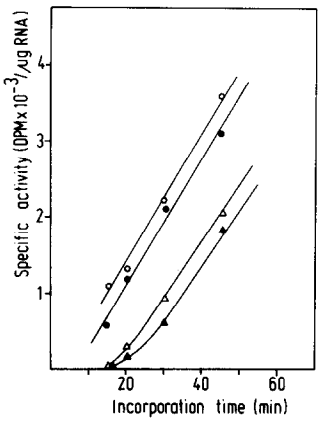

Fig.2. Effect of estradiol on the specific activity of pulse-labeled ribosomal precursors. The specific activities of the 3.6 X 10^6 dalton (○——○, ●——●) and the 1.9 X 10^6 dalton (△——△, ▲——▲) precursors were calculated from the analyses of fig.1. Controls: closed symbols. Estradiol treated for 26 h: open symbols.

The hormone induces, however, a large change in the relative amount of precursor molecules present in the nucleolus (table 1). The amount of the 3.6 X 10^6 precursor is increased about 40% relative to the 1.9 X 10^6 precursor. The amount of the 1.58 X 10^6 rRNA is somewhat variable, probably owing to nucleoplasmic contamination.

3.1. *Effect of estradiol on nucleoplasmic ribosomal RNA*

Sedimentation profiles of nucleoplasmic RNA obtained from the supernatant of sonicated nuclei are shown in fig.3. At 26 h after estradiol the 28S RNA of the nucleoplasm becomes clearly labelled with a 1 h pulse of ^{32}P, whereas in control animals the labelling of the 28S RNA species is much less. We interpret this as a much more rapid turn-over of the ribosomal precursors in estradiol-treated roosters.

4. Discussion

Estradiol induces an increase of total RNA content in rooster liver [5,6]. In the first 10–20 h after hormone administration the RNA content increases about 15% over that of the control. At 24–26 h a sharp increase in RNA content starts, leading to values 90% over those of the controls [5]. The higher rate of synthesis can be wholly, and will at least partly be, due to an enhanced level [5] of nucleolar RNA polymerase(s).

From the labelling kinetics a very fast processing of the 3.6 X 10^6 precursor can be calculated. The time lag for the specific activity between the 3.6 and 1.9 X 10^6 precursor is about 20 min. Since the pool size of the 3.6 X 10^6 precursor is about one quarter of that of the 1.9 X 10^6 precursor in the controls, and about 40% in the hormone-treated birds, a turn-over time of only several minutes can be calculated.

This rapid turn-over will result in a low ratio of the pool size to newly synthesized RNA, even in the first minutes. Consequently, only large changes in turn-over time will be sufficiently reflected in specific activity differences to become experimentally detectable. The continuing increase in (nucleotide) precursor specific activity also encumbers the analysis.

Since our measurements were done after the change of rRNA synthetic activity to a higher level [5], and the ratio of the precursor pool sizes remains

Table 1
Effect of estradiol on the molar ratio of the precursor molecules in the nucleolus

	Ratio of 3.6 X 10^6/1.9 X 10^6 RNA (± S.D.)	Number of determinations	
Control	0.27 ± 0.05	4	$P < 0.05$
Estradiol-treated for 26 h	0.38 ± 0.04	5	

The molar amounts of the 3.6 X 10^6 and the 1.9 X 10^6 precursor present in a liver sample of control and estradiol-treated roosters were determined from the absorbances in fig.1. Statistical significance was determined by Student's t-test.

constant during our experiment we may assume steady state conditions to hold for the time of our analysis. Then, changes in turn-over rate constants are directly reflected in the amounts of the different precursors present, i.e. in the pool size. If zero-order, and first-order kinetics for the synthesis of the first precursor, and for the processing reactions respectively, are taken the following equations hold:

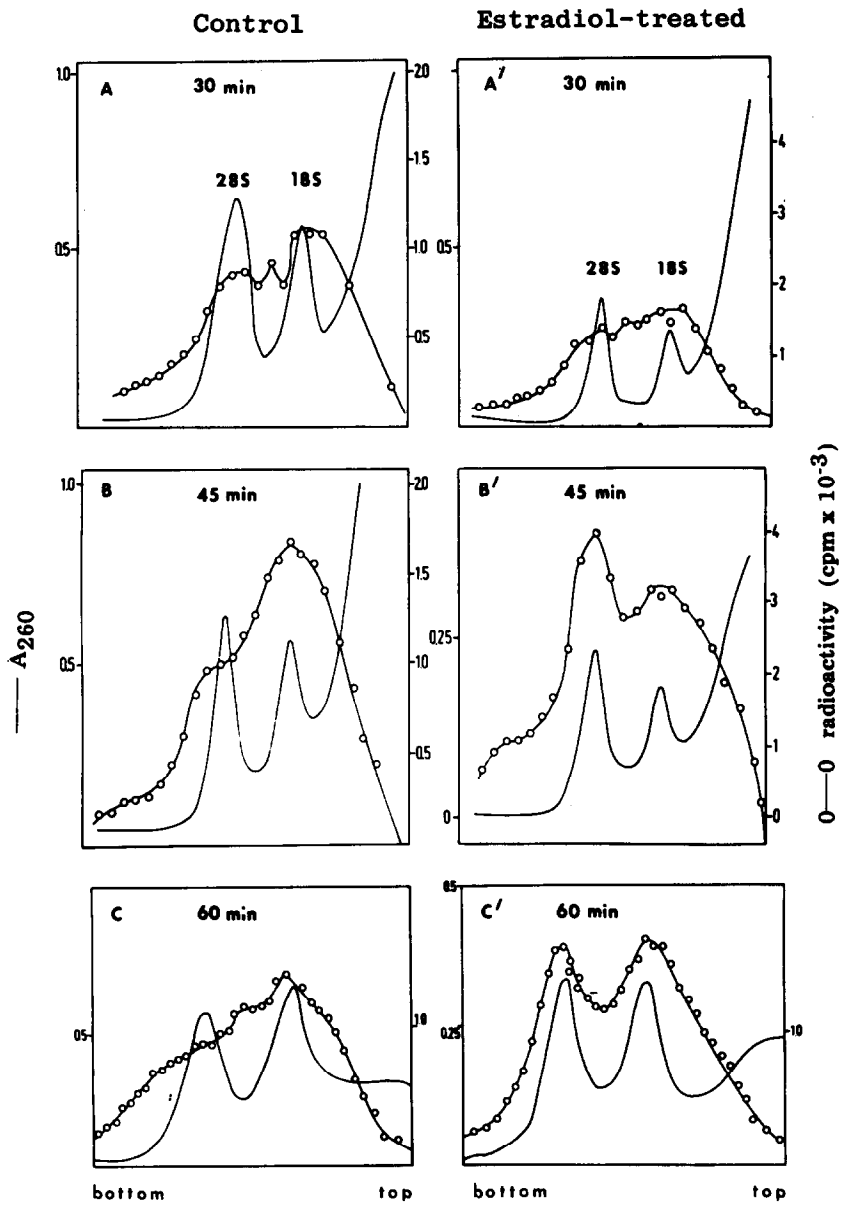

Fig.3. Effect of estradiol on the phosphate-labeling of nucleoplasmic RNA. $^{32}P_i$ was injected intravenously into control roosters (A–C) and roosters treated for 26 h with estradiol (A'–C'). Nucleoplasmic RNA was prepared from 3 pooled livers and analyzed on sucrose gradient after labeling for 30 min (A,A'), 45 min (B,B') and 60 min (C,C'), respectively.

$$\frac{d[RNA_1]}{dt} = 0 = k_s - k_1[RNA_1]$$

$$[RNA_1] = \frac{k_s}{k_1}$$

$$\frac{d[RNA_2]}{dt} = 0 = k_1[RNA_1] - k_2[RNA_2]$$

$$[RNA_2] = \frac{k_1[RNA_1]}{k_2} = \frac{k_s}{k_2}$$

$$\frac{[RNA_1]}{[RNA_2]} = \frac{k_2}{k_1}$$

Hence, the ratio of the molar concentrations of the precursor molecules in the nucleolus is inversely related to the ratio of their processing rate constants.

We therefore can conclude from the data in table 1 that estradiol enhances the processing of the 1.9×10^6 precursor relative to that of the first transcription product. The earlier appearance of 28S RNA in the nucleoplasm is in agreement with this conclusion.

The processing of the 32S precursor to the 28S mature rRNA appears to have a specific sensitivity towards a number of agents or treatments in different tissues [15–20]. A selective hormonal effect on this processing step is therefore quite possible.

Anyhow, besides stimulating synthesis of the rRNA precursor, estradiol also influences at least one of the processing steps. Further experiments are needed to study the mechanisms of the enhancement observed.

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