STIMULATION OF RNA POLYMERASE I ACTIVITY BY 17β-ESTRADIOL–RECEPTOR COMPLEX ON CHICK LIVER NUCLEOLAR CHROMATIN

Françoise BIERI-BONNIOT*, Urs JOSS* and Christa DIERKS-VENTLING
Friedrich Miescher-Institut, PO Box 273, CH-4002 Basel, Switzerland

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1. Introduction

When an immature chick is injected with 17β-estradiol the first detectable biochemical event is the appearance of estradiol receptor in nuclei [1] and on chromatin [2]. This occurs approximately 6 h prior to the increase in total RNA synthesis [3,4] and ribosomal RNA production [4] and approximately 12 h before vitellogenin is synthesized [3,5]. Whether the appearance of the estradiol receptor is the key event which triggers off the observed sequence of biochemical changes is an intriguing but unresolved question. Evidence is accumulating however that hormone receptors play a definite role in the process of transcription of specific genes [6–8]. Thus, the progesterone receptor of chick oviduct reportedly increases in chromatin the number of initiation sites for *Escherichia coli* RNA polymerase [7]. In a recent study [9,10] we showed that an estradiol receptor fraction added to chick liver chromatin increased its endogenous transcriptional capacity proportionally to the amount of receptor present. Since chromatin from nuclei contains all RNA polymerase species, we were not able to demonstrate which RNA polymerase was specifically stimulated. For this reason we resorted to nucleoli and nucleolar chromatin which contain almost only RNA polymerase I. Nucleoli from estrogen-treated chicks were found to have twice as much RNA polymerase I activity than those from controls [11]; in this paper we show that they also contain estradiol receptor. Furthermore, we provide evidence that RNA polymerase I can be stimulated by estradiol receptor when the latter is added to nucleolar chromatin from untreated chicks and we conclude that as a result of this stimulation transcription of ribosomal RNA is increased as observed in vivo [3,4].

* Present address: Research Department, Pharmaceutical Division, Ciba-Geigy Limited, CH-4002 Basel, Switzerland

Address correspondence to Christa Dierks-Ventling

2. Experimental

2.1. *Materials*

α-Amanitin was obtained from Boehringer, Ingelheim, FRG and [5-^{3}H]CTP (spec.act. 23 Ci/mmol) was from The Radiochemical Centre, Amersham, England. All other chemicals were the same as described in a previous communication [10]. Chicks weighing 200 g were used throughout. They were injected intramuscularly with 17β-estradiol in propylene glycol, 2 mg estrogen/0.1 ml propylene glycol/100 g body wt [3].

2.2. *Isolation of nuclei and nucleoli*

Preparation of nuclei at pH 5.8 and their physical properties have been reported [12]. Preparation of nucleoli from these nuclei was done by combining two existing methods [13,14] for the purpose of improved purity and yield [15].

2.3. *Preparation of chromatin from nuclei or nucleoli*

Nuclei or nucleoli were exposed for 3 min to 0.01 M Tris buffer, pH 7.5–0.15 M NaCl in amounts corresponding to 0.5 g liver/ml. After a 3 min centrifugation at 800 × *g* (HB-4 rotor, Sorvall centrifuge) the pellet was washed twice with distilled water.

Chromatin was resuspended in distilled water and the preparation continued according to [16]. DNA, RNA and protein determinations were done as before [3].

2.4. *Preparation and assay of estradiol receptor*

From chicks injected with estrogen 24 h earlier, liver nuclei were purified from which the estradiol–receptor complex was extracted as described [1,10]. The resulting fraction was 20-fold purified and contained usually 120–150 fmol estradiol receptor/mg protein. It did not contain any DNA, nor RNA polymerase activity nor hydrolytic activity (F1 histone of chromatin remained intact during incubation) and its nuclease content was carefully assessed: DNAase activity was absent and RNAase activity was moderately present (for details see ref. [10]). Preparations of nuclei, nucleoli and their respective chromatins were all assayed for estradiol receptor content by our modification [1] of the method of Mester and and Baulieu [17].

2.5. *Determination of RNA polymerase activities*

The incubation mixture consisted of 12% glycerol, 80 mM Tris buffer, pH 7.9, 4 mM thioglycerol, 0.1 mM EDTA, 0.1 mM dithiothreitol, 1 mM each of ATP, GTP and UTP, 0.2 mM CTP, 1 μCi [5-^{3}H]CTP (spec.act. 23 Ci/mmol) and 2 mM $MnCl_2$ in final vol. 0.250 ml. The reaction was started at 0 time by the addition of 50 μl of nuclei (0.5 mg protein) or nuclear chromatin (25 μg DNA). The assay was done in the presence of α-amanitin (1 μg/assay) to differentiate RNA polymerase I activity from I + II. When assaying endogenous activity of nucleoli or nucleolar chromatin, the procedure was exactly the same except that 10 mM $MgCl_2$ replaced the $MnCl_2$ in the incubation mixture. Incubations, 10 min at 30°C, were in triplicate, unless mentioned otherwise. Trichloroacetic acid-precipitable counts were determined as described [10]. Results are expressed as pmol CMP incorporated/μg DNA or /mg protein.

3. Results

3.1. *Estradiol treatment of chicks induced estradiol receptor simultaneously with RNA polymerase I activity*

After chicks received 17β-estradiol, the estradiol receptor content in liver nuclei started to increase immediately, reaching a maximum at 24 h; RNA polymerase I activity followed an almost identical pattern (fig.1). We therefore chose chicks which had been estradiol-treated 24 h, previously for further experiments.

3.2. *Intranuclear localization of RNA polymerase I activity and estradiol–receptor complex*

The concomitant rise in estradiol receptor content and RNA polymerase I activity seen after estradiol treatment led us to ask whether the two components were localized in the same compartment. We therefore measured these activities in isolated nucleoli and nucleolar chromatin and compared them to their activities in whole nuclei and nuclear chromatin (table 1). In nucleolar chromatin almost 95% of RNA polymerase activity is polymerase I, as measured in the presence of α-amanitin; this activity was 3-fold

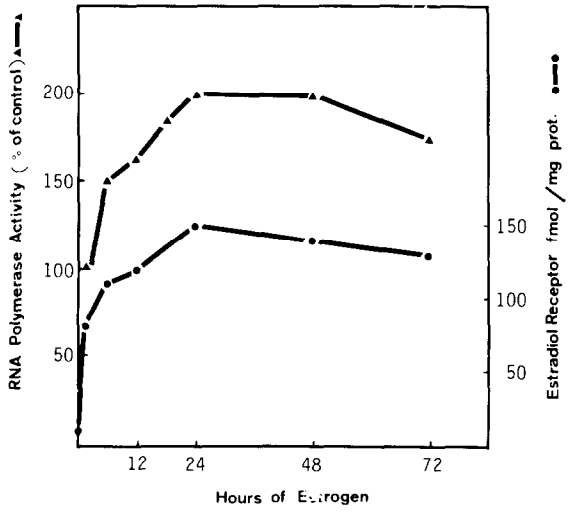

Fig.1. RNA polymerase I activity and estradiol receptor appearance as a function of time after in vivo estrogen treatment. Chicks (200 g body wt) received 17β-estradiol dissolved in propylene–glycol (2 mg/0.1 ml/100 g body wt) intramuscularly at time 0. From these birds nuclei were prepared at the times indicated. Nuclear RNA polymerase I activity was measured as described in section 2 and the estradiol receptor was assayed in nuclear extracts as indicated in the experimental section. Each point represents at least 3 different birds; all assays were done in triplicates. Mean values ± SE are given.

Table 1
RNA polymerase activities and estradiol receptor content of subfractions of chick liver nuclei

	RNA polymerase activity[a]				Estradiol receptor complex		
	Control (pmol/mg protein)		E 24 (pmol/mg protein)		Control (fmol/mg protein)	E 24 (fmol/mg protein)	(Total activity/g liver)
Nuclei	70 ± 3	I + II	113 ± 3.5	I + II	20	120	1800
	25 ± 1	I	55 ± 8	I			
Nuclear chromatin	168 ± 29	I + II	368 ± 67	I + II	0	125	1250
	84 ± 12	I	180 ± 20	I			
Nucleoli	72 ± 5	I + II	163 ± 12	I + II		n.d.	n.d.
	78 ± 1	I	148 ± 5	I			
Nucleolar chromatin	300 ± 120	I + II	901 ± 102	I + II	0	490	200[b]
	285 ± 60	I	856 ± 51	I			

[a] Tested in the presence and absence of α-amanitin; RNA polymerase I activity was therefore obtained by difference
[b] The yield of nucleoli from nuclei being very poor (less than 30%), this figure is not representative of the true total activity

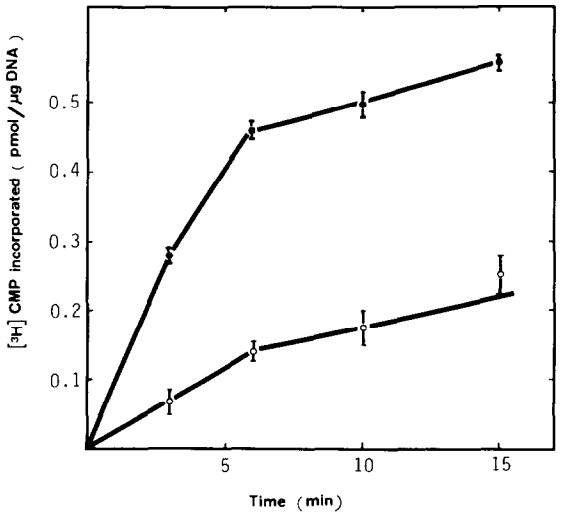

Fig.2. Time course of endogenous RNA polymerase I activity of untreated and estrogen-treated chick liver nucleolar chromatin. Nucleolar chromatin was prepared from purified nuclei from control (○—○) or from 24 h estrogen-treated chicks (●—●). Chromatin (25 μg DNA) was incubated at 30°C under optimal conditions for RNA polymerase I as described in section 2. Each point is the mean value from three different experiments ± SE.

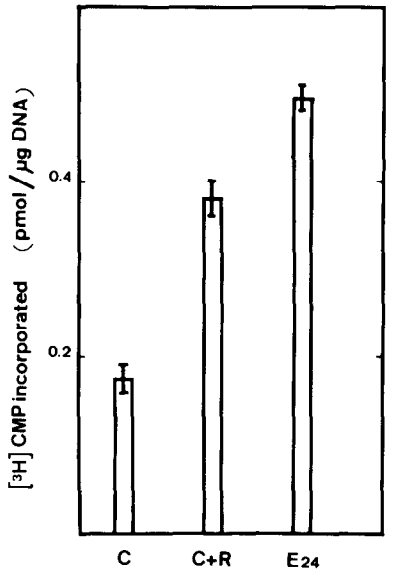

Fig.3. Effect of estradiol–receptor complex on RNA polymerase I activity of chick liver nucleolar chromatin. Nucleolar chromatin from control (C) and from 24 h estrogen-treated chicks (E 24) was prepared as for fig.1 and its endogenous RNA polymerase activity was determined. Amounts of chromatin corresponding to 25 μg DNA were incubated according to the conditions described in the experimental section. Addition of estradiol–receptor complex to chromatin (C + R) was done according to ref. [7]; e.g., a saturating amount of estradiol receptor (6 pmol/μg DNA) was added to chromatin.

increased after estrogen treatment (see also fig.2 for time course of this activity in nucleolar chromatin from controls and estrogen-treated animals; the 10 min time-point compares with the data in table 1). The highest content of estradiol receptor was also found in the nucleolar chromatin fraction. Although in control nuclei a baseline level of estradiol receptor could be detected, in its corresponding chromatin preparation estradiol receptor was absent. These observations led us to ask whether the estradiol–receptor complex caused the increase in endogenous transcriptional activity of chromatin, e.g., whether it stimulated or activated RNA polymerase I. This hypothesis was tested in the following reconstitution experiments.

3.3. *Estradiol–receptor complex stimulates endogenous RNA polymerase I of nucleolar chromatin*

The transcriptional activity of nucleolar chromatin from untreated chicks was measured in the presence or absence of added estradiol–receptor complex (fig.3). Addition of estradiol receptor resulted in an almost two-fold increase of the endogenous RNA polymerase activity reaching a value comparable to that in chromatin from estrogen-treated chicks.

4. Discussion

A few years ago it was reported that RNA polymerase I is associated with the estradiol receptor in preparation from calf uterus [18,19] and quail oviduct [20]. In what way this association took place, whether it produced a change in the polymerase activity etc., was neither hypothesized nor tested. When we demonstrated that within 24 h of a single estradiol injection into chicks the RNA polymerase I and II activities of liver nuclei were doubled and that estradiol receptor content in chromatin from these nuclei was very large compared to controls [10], we

postulated that the observed increase of the polymerase activities was caused by the hormone receptor. We proved this by 'reconstitution' of the situation seen after hormone treatment; e.g., we measured the endogenous transcriptional capacity of nuclear chromatin prepared from untreated chicks before and after addition of semi-purified estradiol–receptor complex [9,10], the addition resulted in the transcription capacity being more than doubled, approaching values seen in chromatin from estradiol-treated chicks. We did not distinguish between increased activities of RNA polymerase I or II, although experiments with α-amanitin suggested an involvement of both enzymes. To test which, if any, of the RNA polymerases was preferentially stimulated, a preparation containing only one RNA polymerase is required: nucleolar chromatin which contains almost exclusively RNA polymerase I is ideally suited as a system. In this paper we show a correlation of estradiol receptor levels and RNA polymerase I activities in nuclei and nucleoli and in their respective chromatin preparations (table 1) and then proceed to the type of reconstitution experiment with nucleolar chromatin which we had previously done with nuclear chromatin [9,10]. Addition of estradiol–receptor complex to nucleolar chromatin increased RNA polymerase I activity by 100%, the result again approached the value normally obtained with nucleolar chromatin from 24 h estradiol-treated chicks (fig.3). We now wish to recall that purified RNA polymerase I can be resolved into two forms I_A and I_B which seem to coexist in equilibrium [15]. Form I_A is predominant in nucleoli and increases after hormone treatment of chicks [15]. Whether the estradiol receptor caused the increase of form I_A could of course not be tested in our experimental conditions, but indirect evidence would suggest this. The mechanism of this increase, however, remains unknown. It is tempting to suggest that the stimulation of the RNA polymerase may require the intermediary action of an unknown protein factor located on chromatin, because we found no stimulation of the polymerases by estradiol receptor in the presence of naked DNA [15]. The suggestion of Schwartz et al. [7] that the progesterone receptor of chick oviduct opened up more initiation sites on chromatin for *E. coli* polymerase to transcribe may also explain our results, but presently we have no way of proving this with endogenous RNA polymerase. To conclude, our data suggest that estradiol–receptor complex activates ribosomal transcription just as it activates mRNA transcription [7,8], thus possibly coordinating the observed increase of translation [4] and the increase of the translational machinery itself necessary for optimum production of vitellogenin.

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