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ESTROGEN RECEPTORS AND OVALBUMIN GENES IN HEN OVIDUCT CHROMATIN FRACTIONS

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SUMMARY

Hen oviduct nuclear lysates were digested with DNase II and three fractions were isolated: 10,000 *g* pellet, $MgCl_2$ insoluble chromatin and $MgCl_2$ soluble chromatin. The 10,000 *g* pellet contained DNA bound to nuclear membranes and unfractionated chromatin. The $MgCl_2$ insoluble chromatin was characterized by a prominent 11S component in sucrose gradients and a 180 base pair DNA component in polyacrylamide gels in agreement with data on nucleosomes. The $MgCl_2$ soluble chromatin displayed 4 S and 14 S peaks in sucrose gradients and 175 and 90 base pair peaks in DNA gels. The concentration of estrogen receptors was about 4.5-fold higher in the $MgCl_2$ soluble chromattn as compared to that of the $MgCl_2$ insoluble chromatin as revealed by the steroid exchange assay. The concentration of ovalbumin gene sequences was measured with [^{3}H]-complementary DNA to ovalbumin mRNA. The $MgCl_2$ soluble chromatin was 1.9-fold richer in ovalbumin genes sequences than the $MgCl_2$ insoluble chromatin. The results suggest that both estrogen receptors and ovalbumin genes sequences are concentrated in a minor subfraction of hen oviduct chromatin. Ovalbumin gene sequences were also concentrated in the 10,000 *g* pellet, but its receptor concentration could not be assayed.

INTRODUCTION

Chromatin of eucaryotic cells is composed of bead-like structures (*v*-bodies or nucleosomes) connected by extended stretches of DNA [1–5]. The beads contain 120 to 200 base pairs of DNA [4] and sediment as 11 S particles in sucrose gradients [5]. The filaments connecting the beads may be structurally more heterogenous; DNA chains varing from 20 to 4000 base pairs have been described [5–7]. Bonner and coworkers have digested liver chromatin with DNase II and fractionated the products according to their solubility in $MgCl_2$ [8]. The $MgCl_2$ insoluble fraction resembled nucleosomes while the $MgCl_2$ soluble fraction contained particles other than nucleosomes which were suggested to derive from transcriptionally active chromatin [9].

In this study hen oviduct chromatin is digested with DNase II and fractionated with the $MgCl_2$ precipitation as described by Bonner and coworkers [8]. The $MgCl_2$ insoluble and soluble chromatin fractions are characterized. The concentration of estrogen receptors in the fractions is measured and correlated to the presence of ovalbumin gene sequences assayed with radioactive DNA complementary to ovalbumin mRNA [10].

MATERIALS AND METHODS

Isolation and characterization of chromatin fractions

Preparative and analytical methods have been described in detail in our previous articles [11–12]. Oviduct tissue was removed from a laying hen and homogenized in 1 mM $MgCl_2$ using 15 strokes of a loosely fitting Teflon–glass homogenizer. The homogenate was centrifuged. The pellet was resuspended in 0.1 M NaCl containing 1 mM $MgCl_2$, homogenized and passed through 4 layers of cheesecloth. Homogenization and filtration were repeated once more and nuclei were purified in 2 M sucrose gradients at 55,000 *g* for 30 min. The nuclear pellet was washed three times with 0.15 M NaCl. The first wash contained 0.1% Triton X-100 and the two other ones 1 mM EDTA pH 7.6.

Purified nuclei were lysed and the chromatin was digested with DNase II (HDAC, Worthington, about 10 units/A_{260} unit of chromatin) for 20 min at 37°. Digestion was terminated by adding 50 mM Tris pH 8.0 and nuclear membranes and unfractionated chromatin were removed at 10,000 *g* for 10 min. The pellet was designated as the 10,000 *g* pellet. The supernatant was made 3 mM with respect to $MgCl_2$ and the $MgCl_2$ insoluble chromatin was collected at 10,000 *g* for 10 min. 0.1 M NaCl was added into the supernatant and the $MgCl_2$ soluble chromatin was collected at 100,000 *g* for 16 h.

Endogenous estrogen receptors in the chromatin fractions were labelled by the estrogen exchange assay of Anderson *et al.* [13] using [^{3}H]-estradiol as described [11].

Isokinetic sucrose gradient centrifugation, deproteinization of DNA samples and polyacrylamide gel electrophoresis of DNA have also been described elsewhere [11].

Determination of ovalbumin gene sequences

Ovalbumin gene sequences in the chromatin fractions were quantitated using RNA-dependent DNA polymerase catalyzed [^{3}H]-DNA complementary to ovalbumin mRNA as detailed previously [10]. The complementary DNA probe was about 1000 base pairs long. It was used in 20-fold excess over ovalbumin genes assuming unfractionated chick genome. The hybridizations were carried out in 10 μl capillaries containing 0.5 M NaCl, 25 mM HEPES, pH 6.8, 0.5 mM EDTA, 100 μg of hen DNA and 1 ng of complementary DNA at 68°C for 14 days. The annealing time should be sufficient (complementary DNA $C_0t = 3.66 \times 10^{-1}$) to ensure complete hybridization. Duplex formation was assayed by single-strand specific nuclease digestion [10]. The percentage of complementary cDNA resistant to the nuclease digestion was used as a measure of the amount of ovalbumin sequences in the DNA fractions.

RESULTS

Hen oviduct chromatin was digested with DNase II and fractionated according to the $MgCl_2$ precipitation method of Bonner *et al.*[8]. Endogenous chromatin-bound estrogen receptors were labelled with [^{3}H]-estradiol using the steroid exchange assay [13]. The concentration of estrogen receptors was about 4.5 times higher in the $MgCl_2$ soluble chromatin than that in the $MgCl_2$ insoluble chromatin (Table 1).

The amount of ovalbumin gene sequences was titrated with [^{3}H]-DNA complementary to ovalbumin mRNA. The [^{3}H]-DNA probe hybridized 2 times more efficiently to DNA of the 10,000 *g* pellet and 1.9 times more efficiently to DNA of the $MgCl_2$ soluble fraction as compared to the $MgCl_2$ insoluble fraction. It is of interest that both estrogen receptors and ovalbumin gene sequences were enriched in the $MgCl_2$ soluble chromatin fraction.

The chromatin fractions were characterized further in isokinetic sucrose gradients, were absorbance at 254 nm and the specific radioactivity of [^{3}H]-estradiol was monitored (Fig. 1). The $MgCl_2$ insoluble chromatin was characterized by a major peak of 11 S and smaller peaks of 15 S and 18 S, respectively. The 11 S particles resemble nucleosomes or *v*-particles and the 15 S and 18 S peaks multimers of them described

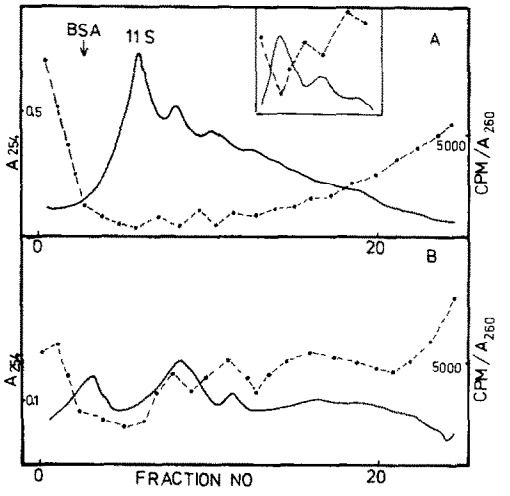

Fig. 1. Sucrose gradient sedimentation of the $MgCl_2$ insoluble (A) and the $MgCl_2$ soluble chromatin (B). The chromatin fractions were suspended in 10 mM $NaHSO_3$ containing 1 mM EDTA pH 7.2 and layered over 5–24% sucrose gradients. The gradients were run at 63,000 *g* for 18 h. (——) absorbance at 254 nm, (· · · · ·) [^{3}H]-estradiol radioactivity/A_{260} unit in gradient fractions. The insert of panel A details the relations of A_{254} and specific radioactivity around the 11 S peak in an independent run.

for a number of chromatins [1–5]. The specific radioactivity (c.p.m./A_{260} unit) of the chromatin fraction was high in the large molecular weight region of the gradient (over 20 S) and very low in the 11 S monomer region. The low specific radioactivity of nucleosomes is demonstrated in the insert of Fig. 1A.

The $MgCl_2$ soluble chromatin displayed two major peaks at about 4 S and 14 S (Fig. 1B). The profile resembled that of the presumed transcriptionally active liver chromatin described by Gottesfeld *et al.*[9]. The specific radioactivity of the $MgCl_2$ soluble chromatin was high in the large molecular weight region of the gradient (over 20 S). However, two peaks of radioactivity could be observed at about 14 S and 20 S.

DNA was purified from the chromatin fractions and subjected to polyacrylamide gel electrophoresis (Fig. 2). DNA from the 10,000 g pellet produced a single peak of about 2000 base pairs. The $MgCl_2$ insoluble chromatin displayed peaks of 180 and 420 base pairs in agreement with the results of other

Table 1. Distribution of estrogen receptors and ovalbumin gene sequences in the chromatin fractions

Fraction	Specific radioactivity of [^{3}H]-estradiol (c.p.m./mg DNA)	Ovalbumin cDNA hybridization (%/μg DNA)
A. 10000 g pellet	n.g.	0.32 ± 0.21
B. $MgCl_2$ insoluble chromatin	26000 ± 5100	0.16 ± 0.03
C. $MgCl_2$ soluble chromatin	119000 ± 4700	0.31 ± 0.08

n.g. = not given because of very high non-specific binding in the steroid exchange assay.

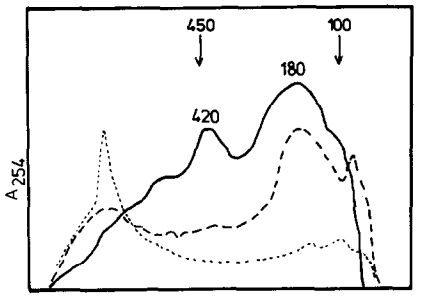

Fig. 2. Polyacrylamide gels of DNA samples purified from the 10,000 pellet (· · · · ·), $MgCl_2$ insoluble chromatin (——) and $MgCl_2$ soluble chromatin (– – –). The gels were calibrated with xylene cyanol FF (450 base pairs) and bromphenol blue (100 base pairs). The figures refer to the number of base pairs calculated from the distance of migration.

workers [3–7]. The $MgCl_2$ soluble chromatin produced a different profile of DNA. The two major peaks corresponded to 175 and 90 base pairs of DNA; the minor large molecular weight peak contained about 2000 base pairs of DNA.

DISCUSSION

Three fractions were isolated from hen oviduct chromatin using DNase II digestion and $MgCl_2$ precipitation according to Bonner[8] as summarized in Table 2. The 10,000 *g* pellet contained about 15% of the total DNA, which were mainly large size DNA fragments. The $MgCl_2$ insoluble chromatin contained up to 45% of the total nuclear DNA. By chemical composition, sedimentation properties and size of DNA the fraction resembles nucleosomes described for other eucaryotic cells.

The $MgCl_2$ soluble chromatin contained only about 5% of the total nuclear DNA. The fraction contains slightly less histones but much more non-histones per unit DNA than the $MgCl_2$ insoluble chromatin [12]. The $MgCl_2$ soluble chromatin resembles the properties of the presumed transcriptionally active chromatin of Bonner and coworkers also by the high specific radioactivity of nascent RNA and by sedimentation properties.

It is of particular interest that estrogen receptors were concentrated in the $MgCl_2$ soluble fraction, also rich in the ovalbumin gene sequences. However, the relative enrichment of the ovalbumin gene sequences is smaller than that of the receptors. This may indicate that a segment of the gene is localized in the nucleosome fraction. In the case of the globin gene it has been shown that the sequences are almost completely represented both in the nuclease resistant (covered) and in the polylysine accessible (open) region of DNA [14].

It has been shown elsewhere that the concentration of estrogen receptors in chick oviducts preferentially increases in the $MgCl_2$ soluble chromatin in the course of estrogen stimulation [12]. Thus hormone stimulation may involve removal of nucleosomes from the steroid specific region of the genome and transform a segment from the $MgCl_2$ insoluble fraction into the $MgCl_2$ soluble fraction.

The enrichment of nascent RNA, estrogen receptors and ovalbumin gene sequences in the $MgCl_2$ soluble fraction may indicate the isolation of an active biological unit related to hormone action. However, as the fraction contains a small proportion of the total chromatin, it also contains a minor fraction of the total nascent RNA, estrogen receptors and ovalbumin gene sequences. Moreover, there is no direct evidence that the fraction would be involved in the action of steroid hormone. Recent work on thyroid and glucocorticoid hormone receptors in chromatin fractions of cultured cells have established no clear correlation between the receptor concentration and nascent RNA [15].

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Table 2. Properties of the chromatin fractions from hen oviduct

Property (reference)	10,000 *g* pellet	$MgCl_2$ insoluble chromatin	$MgCl_2$ soluble chromatin
% of nuclear DNA [11]	15	45	5
Protein/DNA ratio [11]	7.6	1.6	3.7
Major components in sucrose gradients, Svedberg units	—	11	4, 14
Major DNA components, base pairs	2000	180	175, 90
Labelling of nascent RNA, relative units [12]	—	1	4.5
Enrichment of estrogen receptors, relative units	—	1	4.5
Estrogen receptors in an oviduct cell [12]	—	1400	960
Enrichment of ovalbumin gene sequences, relative units	2.0	1	1.9

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DISCUSSION

Spelsburg. One word of warning is to be careful in that some of these chromatin fractions contain nuclear envelope, especially those in the upper fraction. You should at least make sure you have intact receptors in that region and not free steroid adsorbed on the envelope.