Distribution of estradiol receptor and vitellogenin gene in chick liver chromatin fractions

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ABSTRACT

The distribution of estradiol receptor and vitellogenin gene was studied in estradiol stimulated chick liver chromatin fractions prepared by limited DNase II digestion and $MgCl_2$ precipitation. The receptor was found in all fractions, undigested chromatin (P1), Mg^{2+} insoluble chromatin (P2) and Mg^{2+} soluble chromatin (S2). This last fraction was rich in acidic proteins, had a high protein:DNA ratio (7.0 w/w), contained 28% of rapidly labelled RNA, 20% of the receptor, 3-5% of chromatin DNA and showed a 2 fold enrichment of vitellogenin DNA sequences over unfractionated chromatin as well as P1 and P2 DNA. On isopycnic metrizamide gradients, all chromatin fractions showed a receptor peak banding at 1.23 g/cm³, the density of nucleoproteins. Hybridization experiments showed that the DNA banding at this density in fraction S2 was enriched 4 fold in vitellogenin DNA sequences over unfractionated chromatin as well as P1 and P2 DNA. These results suggest an association of hormone receptor complex with nucleoprotein structures of an apparently active chromatin fraction.

INTRODUCTION

As steroid hormone receptors are implicated in the regulation of genetic expression (1,2), numerous attempts have been made to define their localization within the chromatin. However, considerable controversy surrounds the significance of *in vitro* experiments designed for detecting specific nuclear receptor-binding sites (3). Mechanical fractionation of chromatin, following *in vivo* translocation of receptor to the nuclei, has not resolved the question as accumulation of receptor has been reported in both fast (4) and slowly (5) sedimenting chromatin. This is not surprising, since shearing disrupts native chromatin structure, facilitating protein rearrangement (6).

The availability of relatively mild fractionation techniques which appear to maintain chromatin structure and yield fractions displaying different biochemical and physical characteristics (7, 8) has facilitated the study of receptor distribution within the chromatin. The method described by Gottesfeld et al (8), is reported to separate transcriptionally active from inactive

chromatin. In this method, chromatin is partially digested with DNase II, and inactive chromatin is separated by centrifugation and precipitation with MgCl_2 . The Mg^{2+} soluble active fraction is enriched in nascent RNA chains, consists of tissue specific subsets of middle and non repetitive DNA sequences (9) and the preferential localization of actively transcribed sequences coding for globin gene has been reported in this fraction (10). The studies reported here concern the distribution of estradiol receptor and vitellogenin gene sequences in chick liver chromatin, fractionated by the DNase II/ Mg^{2+} techniques.

MATERIAL AND METHODS

Chemicals : 2,4,6,7- (^3H) -17 β -estradiol (85-110 Ci/mmol) and (^{14}C) orotic acid (59 mCi/mmol) were obtained from the Radiochemical Centre, Amersham. DNase II (HDAC) and DNase I (RNAse free) were purchased from Worthington Biochemical, Proteinase K, RNAse I (DNase free) and RNAse TI from Serva Feinbiochemica, Heidelberg and Metrizamide (analytical grade) from Nyegaard and Co A/S, Oslo.

Animals and injection : Male Warren chicks weighing 500-700 g were stimulated during 48 h by 2 subcutaneous injections of estradiol (25 mg/kg), dissolved in propylene glycol. (^{14}C) orotic acid, dissolved in physiological saline, was administered intraperitoneally, following the 48 h stimulation.

Preparation of nuclei and fractionation of chromatin : All manipulations were performed at 0-4°C with the exception of the enzymatic digestion, which was done at 30°C. All buffers used in the preparation of nuclei and its fractionation contained 0.3 mM phenylmethylsulfonylfluoride (PMSF). Nuclei were prepared according to Blobel and Potter (11) and chromatin as described by Marushige and Bonner (12). The chromatin was suspended in 20 mM Tris-HCl pH 7.0 (12 $\text{OD}_{260}/\text{ml}$) and digested with DNase II (10 u/ OD_{260}) at 30°C. The reaction was stopped by raising the pH to 7.5 with 100 mM Tris-HCl pH 11.0 and chilling of the samples. Chromatin was separated into a first supernatant, S1 and pellet, P1 by centrifugation at 27,000 g for 30 min. Under continuous stirring, the supernatant was brought to 3 mM MgCl_2 by dropwise addition of 200 mM MgCl_2 and was left stirring for 30 min. Centrifugation at 27,000 g for 30 min resulted in a second supernatant, S2, and pellet, P2. Pellet fractions were resuspended in 20 mM Tris-HCl pH 7.5. For (^3H) estradiol binding studies, fraction P1 was solubilized by sonication.

Measurement of specific (^3H) estradiol binding : In order to remove endogenous estradiol, chromatin fractions were preincubated with 0.25% char-

coal-0.025% dextran-0.1% gelatin for 1 h at 30° C. Chromatin fractions were incubated overnight at 0°C with 1-2 nM (³H) estradiol + a 100 fold excess of unlabelled estradiol. Excess hormone was removed by a second charcoal-dextran treatment of 30 min at 0°C and aliquots were counted with Unisol (Koch Light) in an Intertechnique Liquid Scintillation Counter. All data are given as specific (³H) estradiol binding, calculated by subtracting the value for non specific binding (in presence of 100 fold excess estradiol) from the total binding obtained with (³H) estradiol alone.

Isopycnic density gradient centrifugation : Metrizamide was dissolved in 20 mM Tris-HCl pH 7.5-PMSF. Preformed gradients were prepared with 0.7 ml layers of 20,30,40,50, and 60% metrizamide and equilibrated for 24 h. The samples, 1.5 ml, were deposited and the gradients centrifuged at 4°C in a Spinco SW 50.1 rotor at 46,000 rpm for 3 h. For large scale preparations, 3.5 ml of the fractions were deposited on 9 ml gradients and centrifuged in a Spinco SW 41 rotor at 39,000 rpm for 3 h. Fractions were collected and refractive indices and radioactivity measured. Densities were calculated using the relationship $\rho \text{ (g/cm}^3\text{)} = n_{20} \text{ (refractive index)} \times 3.35 - 3.462, (13)$.

(³H) complementary DNA : (³H) cDNA was synthesized from rooster liver purified vitellogenin mRNA by P. van den Boogaart (Biochemisch Laboratorium, University of Groningen, The Netherlands). The cDNA was approximately 1000 nucleotides long, was at least 90% pure and had a specific activity of 2×10^4 dpm/ng.

Preparation of DNA : Fractions were made 0.5% SDS and incubated with Proteinase K (50-100 µg/ml) at 37° C for 2 hours. The solutions were extracted with an equal volume of Tris (50 mM pH 8.0)-saturated phenol and the phenol phase with 1/3 volume 10 mM Tris-HCl pH 8.0, 1 mM EDTA. The combined aqueous phases were extracted 3 times with chloroform-isoamyl alcohol (24:1, v/v), made 250 mM with sodium acetate and nucleic acids precipitated with ethanol. The precipitates were collected by centrifugation (90 min at 12,000 rpm), dried under vacuum and dissolved in 10 mM Tris-HCl pH 8.0, 1 mM EDTA. Pre-heated (80°C, 20 min) RNase A and RNase T1 were added to final concentrations of 50 µg/ml and 2 µg/ml, respectively and the mixtures incubated at 37°C for 1 h. The samples were made 0.5 % SDS and Proteinase K treatment, organic solvent extraction and ethanol precipitation steps repeated. The DNA precipitate was dissolved in 10 mM Tris-HCl pH 8.0, 1 mM EDTA, brought to 0.3 M NaOH and incubated at 37°C for 2 hours. Samples were then neutralized and precipitated with ethanol. The final precipitate was dissolved in redistilled H₂O. For preparing DNA of metrizamide receptor peaks, the fractions were first

concentrated by ethanol precipitation and the vacuum dried precipitates dissolved in 10 mM Tris HCl pH 8.0, 1 mM EDTA. The treatment with RNase A and RNase TI preceded those of Proteinase K and the phenol chloroform extraction steps.

DNA-excess cDNA hybridization : (^3H) cDNA - DNA hybridizations were done in 600 mM NaCl, 20 mM Tris-HCl pH 7.5, 4 mM EDTA and 0.1% SDS. The 20 μl reaction mixtures which contained 3,600 dpm (^3H) cDNA and 5 μg DNA were sealed in siliconized glass capillaries, heated in a boiling water bath for 5 min and incubated at 68°C for 72 hours. At the end of the incubation, the capillaries were emptied into tubes containing 100 units S1 nuclease in 0.5 ml S1 buffer (300 mM NaCl, 3 mM ZnCl_2 , 30 mM sodium acetate pH 4.4, 10 $\mu\text{g/ml}$ of native and denatured calf thymus DNA) and the mixtures incubated for 30 min at 45°C. At the end of the incubation, an aliquot (0.1 ml) was removed for counting and the remaining 0.4 ml was precipitated in the following manner : carrier DNA (60 μg denatured calf thymus DNA) was added, the mixtures were made 20 mM sodium pyrophosphate and precipitated with an equal volume of 20% trichloroacetic acid at 4°C for 30 min. Precipitates were collected on glass fiber filters (Whatman GF/C), washed three times with cold 6% TCA - 0.1 mM Na pyrophosphate and once with ethanol. The filters were dried and counted in Soluene - PPO - POPOP. Hybrid formation was measured as the acid precipitated radioactivity remaining after S1 treatment and is expressed as a percentage of the radioactive probe. Endogenous S1 nuclease resistance ($\leq 1\%$) was subtracted from all values.

Other methods : Polyacrylamide gel electrophoresis was performed as described by Laemmli (14). DNA was determined either by the diphenylamine reaction (15) or by its fluorescence with diaminobenzoic acid (16), RNA as described by Fleck and Munro (17) and proteins by the Folin reaction (18). The number of sites per cell was calculated on the basis of 2.3 pg DNA/cell.

RESULTS

Properties of chromatin fractions : The fractionation of chick liver chromatin by DNase II and MgCl_2 precipitation results in undigested (P1) and soluble (S1) fractions. As seen in Fig.1, there is an initial rapid rise in soluble material followed by a slow but progressive release until about 50-70% of chromatin DNA is freed. When the soluble material is further fractionated into Mg^{2+} soluble (S2) and Mg^{2+} insoluble (P2) fractions, it is apparent that the DNA of Mg^{2+} soluble fraction is very sensitive to the action of DNase II, since most of the DNA of this fraction is released within 5-10 min of

Fig. 1. Time course for the digestion of chick liver chromatin by DNase II. Estradiol stimulated rooster liver chromatin was prepared and fractionated by the DNase II/Mg²⁺ technique as described in "Material and Methods". At the indicated times, the fractions were separated and DNA measured in each fraction. Results are expressed as the percentage of total chromatin DNA found in each fraction.

digestion.

The chemical composition of chick liver nuclei, unfractionated chromatin and the fractions obtained after 15 min digestion with DNase II are given in Table 1. When compared to controls, all preparations from stimulated animals show increased ratios of protein:DNA and RNA:DNA. The most striking increase is that of the protein:DNA ratio (7.0) in fraction S2 of the stimulated ani-

TABLE I

Chemical composition of chick liver chromatin fractions

Fraction	% DNA	Protein/DNA (w/w)		RNA/DNA (w/w)		% Rapidly labelled RNA	Rapidly labelled RNA/DNA
		C	S	C	S		
Total Chromatin	100	1.4	2.6	0.26	0.46	100	1.00
P1	44	1.4	2.4	0.19	0.35	18	0.41
P2	52	1.4	2.1	0.18	0.40	54	1.04
S2	4	2.2	7.0	0.22	0.53	28	7.00

DNA, RNA and protein were measured in total chromatin and in DNase II/Mg²⁺ (15 min digestion) prepared fractions of control (C) and estradiol stimulated (S) animals. RNA was labelled for 30 min by injecting (¹⁴C) orotic acid (120 μ Ci) into stimulated animals. The percentage of rapidly labelled RNA is calculated with TCA precipitated radioactivity. The percentage of DNA in fractions P1, P2 and S2 was calculated in relation to their sum.

mals. In addition, this fraction appears to be enriched in nascent RNA as suggested by the high ratio (7.0) of rapidly labelled RNA to DNA.

Polyacrylamide gel electrophoresis showed the presence of the full histone complement in all fractions although these proteins are less abundant in fraction S2, which was enriched in acidic proteins. Fraction P2 was poor in acidic proteins and the electrophoretic pattern of P1 was similar to that of intact chromatin (data not shown).

Distribution of receptor and vitellogenin gene in chromatin fractions :

The amount of DNA and estradiol receptor in the chromatin fractions was measured after various times of digestion with DNase II. As seen in Fig. 2, the release of receptor and DNA is a biphasic phenomenon. During the first 15 min, the parallel depletion of receptor and DNA of fraction P1 coincides with their appearance in fractions S2 and P2. For a given fraction, receptor :

Fig. 2.
Time course for the release of chromatin estradiol receptor and DNA. Chromatin was fractionated and at the indicated times, receptor and DNA were measured in the fractions (see "Material and Methods"). The results are expressed as the percentage of total chromatin DNA and receptor present in each fraction. (a) fraction P1, (b) fraction P2, (c) fraction S2.

DNA ratios are constant during this period. However, this ratio is considerably higher in S2 (5.0) than in P2 (0.6). In the second period (15-60 min), the release of DNA is not correlated with an additional release of receptor. Consequently, during this period, there is a decrease in the receptor:DNA ratios of fraction S2 and to a lesser extent, fraction P2. Since the maximum release of receptor into fraction S2 was observed at 15 min digestion, this time was used for all subsequent experiments. Table II shows that after 15 min digestion, 80% of the receptor is found in the inactive chromatin fractions (P1 and P2) and the remaining 20% in the active fraction S2. As this fraction contains only 3-5% of total chromatin DNA, it displays considerable receptor enrichment when compared to total chromatin (5 fold), fraction P1 (4 fold) and P2 (8 fold).

Vitellogenin (^3H) cDNA was annealed to chromatin fractions DNA under conditions of moderate cDNA excess (11 times the estimated vitellogenin gene content in unfractionated chromatin). Table III shows that under these conditions, 20% of the cDNA is hybridized to S2 DNA of stimulated animals, compared to 8-11% for the DNA of unfractionated chromatin, for fractions P1 and P2 of control and stimulated animals, as well as that of S2 from control animals. These results suggest a two fold enrichment of vitellogenin gene sequences in fraction S2. As this fraction would appear to be enriched in both receptor and vitellogenin DNA sequences, it was necessary to ascertain if they

TABLE II
Distribution of estradiol receptor in chromatin fractions

Fraction	% DNA	% Receptor	Receptor/ DNA	Calculated sites/ nucleus
Total chromatin	100	100	1.0	1120
P1	29	38	1.3	428
P2	67	42	0.6	474
S2	4	20	5.0	218

After 15 min digestion with DNase II, DNA and receptor content were measured in total chromatin and DNase II/Mg $^{2+}$ prepared fractions as described in "Material and Methods". The number of sites/nucleus is calculated on the basis of 2.3 pg DNA/nucleus. The percentage of receptor and DNA of fractions P1, P2 and S2 was calculated in relation to their sum.

TABLE III

Hybridization of chromatin DNA to vitellogenin cDNA

Chromatin fraction	% cDNA hybridized	Fold enrichment
Total S	9	1.
S2 C	8	1
S2 S	20	2
P1 C	11	1
P1 S	11	1
P2 C	10	1
P2 S	10	1

Chromatin DNA preparations (5 μ g) from control (C) and estradiol stimulated (S) animals were annealed with 180 pg vitellogenin (^3H) cDNA (3,500 dpm) as described in "Material and Methods". The extent of hybridization was determined by S1 resistance and TCA precipitated radioactivity.

were present as free entities or as part of macromolecular complexes.

Density gradient centrifugation : Linear glycerol gradient centrifugation of fraction S2 had shown that the bulk of (^3H) estradiol labelled receptor and the material absorbing at 260 nm sedimented between 12-14 S (data not shown). However as steroid hormone receptors are known to aggregate (19), this cosedimentation suggested but did not necessarily prove an association between receptor and nucleic acids. In order to clarify this situation, chromatin fractions labelled with (^3H) estradiol were centrifuged on isopycnic non ionic metrizamide gradients. Centrifugation conditions were such that free nucleic acids and nucleoproteins were at equilibrium, while free proteins were not. Fig. 3 shows that under these conditions, a radioactive peak is found at a density of 1.23 g/cm³, the reported density of nucleoproteins (13). This peak was due to receptor-bound hormone, as the radioactivity was greatly reduced in the presence of excess non radioactive hormone. Only fractions S2 and P2 are represented in the figure ; however, P1 showed a similar peak. This density was maintained at equilibrium centrifugation (16 h), when free nucleic acids banded at 1.16 g/cm³ and free proteins, at 1.3 g/cm³. Control experiments showed that the large amount of radioactivity at the top of the

Fig. 3. Isopycnic gradient centrifugation. Fractions S2 and P2 were labelled with 2 nM (^3H) estradiol $\pm$ 2 μM cold estradiol and centrifuged on preformed 20-60% metrizamide gradients as described in "Material and Methods". Fractions (0.25 ml) were collected and radioactivity and refractive indices determined.

■—■ P2 (^3H)
□—□ S2 (^3H)

▲—▲ P2 (+ excess estradiol)
△—△ S2 (+ excess estradiol)

gradients was due to free hormone.

The recovery of receptor and DNA in the metrizamide peaks of the three fractions are given in Table IV. The receptor:DNA ratios of fraction P1 (1.4) and P2 (0.7) are approximately the same as those observed in the total fractions (see Table 2). The receptor:DNA ratio of fraction S2 is lower (3.2) than that of the total fraction (5.0). A possible explanation for an apparently low recovery of S2 DNA in the peak could be the presence of oligo-nucleotides and/or free nucleotides in the total fraction, as the DNA of this fraction is the most accessible to DNase II.

TABLE IV

Percentage receptor and DNA in chromatin metrizamide peak fractions

Fraction	% DNA	% Receptor	Receptor/DNA
S2	1	3.2	3.2
P1	16	22	1.4
P2	36	24	0.7

(³H) estradiol labelled fractions S2, P1 and P2 were centrifuged on metrizamide gradients (SW 41 rotor) as described in "Material and Methods". Fractions (0.4 ml) were collected and radioactivity and refractive indices determined. DNA was measured in the peak (1.19 - 1.27 g/cm³), after precipitation with TCA, by both the diphenylamine and fluorescence reactions. The results are expressed as the percentage of receptor and DNA in unfractionated chromatin.

The DNA present in the metrizamide receptor peaks was extracted and annealed with a moderate excess of vitellogenin cDNA. Table V shows that under conditions identical to those for the hybridization of the total fractions, 36% of the cDNA is hybridized to the DNA of S2 receptor peak. The values found

TABLE V

Hybridization of metrizamide peak DNA to vitellogenin cDNA

Chromatin fraction	% cDNA hybridized	Fold enrichment
Unfractionated	9	1
S2	36	4
P1	12	1
P2	10	1

Fractions S2, P1 and P2 were centrifuged on metrizamide gradients (SW 41 rotor) as described in "Material and Methods". In order to locate the peak, aliquots of the fractions labelled with (³H) estradiol were also centrifuged and radioactivity and refractive indices determined in the fractions of these tubes. The corresponding peaks of unlabelled fractions were pooled, DNA prepared for hybridization, 5 µg DNA of S2, P1, P2 and unfractionated chromatin annealed with 180 pg of vitellogenin (³H)cDNA (3,500 dpm) and the extent of hybridization determined (see legend of Table III).

for P1 (12%) and P2 (11%) peak DNA are similar to those observed in their respective total fractions (see Table III). A 2 fold enrichment for total S2 and 4 fold for S2 metrizamide peak was also found when hybridization was carried out with a larger excess of cDNA (~ 30 fold), confirming that the reaction had reached completion.

DISCUSSION

The DNase II digestion, Mg^{2+} precipitation procedure was used for fractionating chromatin of estradiol stimulated chick liver. The distribution of estradiol receptor and vitellogenin gene was studied in three chromatin fractions, P1 (undigested), P2 (Mg^{2+} insoluble) and S2 (Mg^{2+} soluble). This last fraction, which contained 3-5% of chromatin DNA, had a high protein : DNA ratio (7.0 w/w) and contained 28% of rapidly labelled RNA. Hybridization of chromatin fraction DNA to vitellogenin cDNA showed an approximately two fold enrichment of vitellogenin gene sequences in the DNA of fraction S2 when compared to DNA from unfractionated chromatin, from fractions P1 and P2, as well as the DNA from a S2 fraction of control animals. All these properties of fraction S2 from chick liver chromatin, similar to those previously reported for corresponding fractions from rat liver (8), hen oviduct (20 and Friend erythroleukemia cells (10), are among the basic criteria for an active chromatin fraction (21).

The distribution of estradiol receptor was unequal in the chromatin fractions and, at all time points studied, the majority of the receptor was found in fractions P1 and P2. The maximum amount of receptor (20%) measured in S2 is obtained within 15 min digestion, and at this time, the fraction contains $\sim 4\%$ of the DNA. On the basis of its receptor and DNA contents, S2 shows a 4-7 fold receptor enrichment over unfractionated chromatin, fraction P1 and fraction P2. Thus, the Mg^{2+} soluble fraction of stimulated chick liver chromatin shows an apparent enrichment of both hormone receptor and an expressed gene. In non stimulated animals there is no detectable receptor in fraction S2 (unpublished results) nor is there enrichment of vitellogenin gene. The enrichment of both receptor and vitellogenin gene in the S2 fraction from stimulated animals implies a role for the hormone-receptor complex in the modulation of specific gene transcription.

The finding that the majority of the nuclear receptor is localized in the inactive fractions P1 and P2 agrees with previous reports on the distribution of glucocorticoid and thyroid hormone receptors in cultured pituitary cells (22) and estradiol receptor in hen oviduct chromatin (20). In the former

studies, no preferential localization of receptor was observed. However with hen oviduct chromatin, a similar enrichment of estradiol receptor was reported for fraction S2. The significance of the presence of the major part of the receptor in inactive chromatin fractions is not known. It is possible that, as suggested (23), this binding represents low affinity receptor binding at non specific sites which masks a very small number of high affinity sites in the chromatin. The receptor present in the metrizamide peak of fraction S2 could be indicative of the fraction which is tightly bound to the chromatin. Studies are in progress to determine if this distribution of receptor in the chromatin reflects heterogeneity of receptor and/or distinct interactions with inactive and active chromatin fractions.

Glycerol gradient centrifugation of (^3H) estradiol labelled S2 fraction showed cosedimentation, at 12-14S, of the receptor and material absorbing at 260 nm, in agreement with reported studies on hormone receptors in chromatin fractions of other target cells (20,24-26). These results suggest but do not prove an interaction between receptor and chromatin structure, since receptors are known to aggregate easily (19). As in isopycnic gradients, free nucleic acids, free proteins, as well as nucleoproteins band at their characteristic densities, it appeared to be an appropriate method for resolving the question of aggregated receptor or receptor-nucleic acid complexes. On non ionic metrizamide gradients, in all three chromatin fractions, a receptor peak was observed at 1.23 g/cm^3 , the density of nucleoproteins (13). Based on the receptor and DNA contents of the peaks, fraction S2 shows a 2-4 fold receptor enrichment over fractions P1 and P2. Hybridization experiments carried out with the DNA which banded with receptor peaks at 1.23 g/cm^3 , showed that the DNA of fraction S2 was enriched in vitellogenin DNA sequences, approximately four fold over unfractionated chromatin and pellet fractions DNA. Thus the enrichment of receptor and vitellogenin gene observed in total S2 was also observed for S2 peak DNA obtained from metrizamide gradients. The lower receptor:DNA ratio (3.2 vs 5.0 of total S2) is quite likely due to a combination of the dissociation of hormone from the receptor and of some receptor from nucleoproteins during centrifugation on metrizamide gradients (cf 27). A similar density (1.2 g/cm^3) was reported for thyroid hormone receptor which banded with core chromatin after digestion of rat liver nuclei by micrococcal nuclease (25).

From the amount of DNA in total S2 and in S2 metrizamide peak, one might expect to find a higher enrichment of vitellogenin gene sequences in these fractions and a corresponding depletion in the pellet fraction. It is possi-

ble that DNase II digestion did not release all transcribed vitellogenin gene, due to the suboptimal pH or to difficulties inherent to the fractionation technique (10). In addition although 48 h stimulation was sufficient for maximum receptor content in the tissue, it was insufficient for maximum vitellogenin synthesis (28) and it is likely that at a given moment not all liver cells were engaged in vitellogenin transcription.

There is general agreement that steroid hormone receptors are in some way involved in gene activation (1,2). The responses of target tissues to steroid hormone administration include RNA polymerase activation, increased chromatin template capacity, RNA synthesis, specific protein synthesis and tissue growth (29). Nevertheless the nature of the nuclear binding site of the hormone receptor complex has not been defined. The receptor has been found in association with or has shown an affinity for DNA (30), nuclear membranes (31) nuclear proteins acidic (32) and basic (33), nuclear matrix proteins (34) and RNP particles (35). In addition accumulation of receptor has been reported in several chromatin subfractions (4,5,24-26). However none of these studies reported the cofractionation of a receptor and a chromatin fraction enriched in a gene induced by the hormone involved.

The comigration of receptor and vitellogenin gene sequences at the density of nucleoproteins reported here, strongly suggests that both are part of a nucleoprotein structure. As this fraction contains all components of chromatin, that is, RNA, DNA, histones and non histone proteins, (unpublished results) it remains to be determined to which of these components the hormone receptor complex is associated. Therefore, further analysis is required to ascertain whether there is a direct or indirect interaction of the hormone receptor complex and the vitellogenin gene.

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