Interaction of oestrogen and progesterone receptors with specific subfractions of laying-hen oviduct chromatin

Nelly MASSOL, Marie-Claire LEBEAU and Etienne-Emile BAULIEU

Unité de Recherches sur le Métabolisme Moléculaire et la Physio-Pathologie des Stéroïdes de l'Institut National de la Santé et de la Recherche Médicale (U 33, INSERM), 78 rue de Général Leclerc, 94270 Bicêtre, France

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Salt (NaCl)-extracted nuclear oestrogen receptor from hen oviduct was incubated with salt-depleted oviduct chromatin and dialysed to low salt. The oestrogen receptor (re)associated with chromatin to form a 13–14S-sedimenting fraction, as found in 'native' chromatin, and saturation of this interaction was obtained for very low receptor concentrations (approx. 0.04 nM). Similarly, purified progesterone receptor from chick oviduct cytosol associated with depleted chromatin to form an 11–12S-sedimenting fraction, as in 'native' chromatin; this interaction tended towards saturation for much higher concentrations of progesterone receptor (approx. 8 nM) than that observed for oestrogen receptor. When the two receptors were incubated with depleted chromatin from hen kidney or erythrocytes, their *s* values were as for oviduct chromatin. However, no saturation of these interactions was seen, even for high concentrations of receptor. Steroid-hormone receptors can therefore bind *in vitro* to particular subfractions of non-target-tissue chromatin, but with a much lower affinity than to target-tissue chromatin.

For many years now the idea has prevailed that steroid-hormone-receptor complexes play a role in the initiation of transcription of a number of hormone-regulated genes (O'Malley *et al.*, 1972; Palmiter, 1972; Yamamoto & Alberts, 1975). This assumption has not yet been demonstrated, however, although a number of groups have approached the problem in various ways.

When steroid-hormone target-tissue nuclei were digested mildly by either micrococcal nuclease or deoxyribonuclease II, a preferential localization of hormone receptors in certain chromatin subfractions was observed (Hemminki & Vauhkonen, 1976; Massol *et al.*, 1978; Senior & Frankel, 1978; André *et al.*, 1978; Rennie, 1979), in some cases accompanied by an enrichment in the corresponding hormone-regulated gene (Hemminki & Vauhkonen, 1976; Scott & Frankel, 1980; Alberga *et al.*, 1979).

In hen oviduct chromatin, prepared by micrococcal nuclease digestion and analysed by ultracentrifugation on sucrose gradients, two main peaks of oestrogen binding were observed, sedimenting at 13–14S, slightly faster than the mononucleosome fraction, and at 7–8S (Massol *et al.*, 1978; Senoir & Frankel, 1978; Lebeau *et al.*, 1982). In oestrogen-primed progesterone-injected immature chicks, a 12S peak of progesterone

receptor, distinguished by its *s* value from the oestrogen receptor, was also seen (Lebeau *et al.*, 1982). We have shown that the presence of these hormone-receptor peaks is correlated with biological activity (Lebeau *et al.*, 1982), and here attempt to determine the specificity and the affinities of interaction between the two hormone receptors and 'acceptor' sites in the chromatin, by inducing dissociation and reassociation of the macromolecular ligands in the presence of various salt concentrations.

Experimental

Preparation of oviduct nuclei and of large chromatin fragments

Nuclei were prepared from the magnum portion of laying-hen oviducts, essentially by the method of Hewish & Burgoyne (1973), i.e. in sucrose buffers containing buffer A (Tris, 5 mM; KCl, 60 mM; NaCl, 15 mM; spermine, 0.15 mM; spermidine, 0.5 mM; β -mercaptoethanol, 15 mM), pH 7.4, EDTA and EGTA. Phenylmethanesulphonyl fluoride (1 mM) was present in the homogenizing buffer, and all operations were carried out at 0–4°C.

Chromatin was obtained by using the solubilizing properties of micrococcal nuclease, as

described by Noll & Thomas (1975). The purified nuclei were resuspended in 0.34M-sucrose in buffer A, at a concentration of 1mg of DNA/ml; CaCl_2 was added to reach 1mM, and the nuclei were digested by micrococcal nuclease (10–15 units/ml per mg of DNA) for 10min at 20°C. The reaction was stopped by adding EDTA to 2mM final concentration and placing the tube in ice. After centrifuging for 5min at 800g, the nuclear pellet was washed in homogenizing buffer (0.34M-sucrose, buffer A, 2mM-EDTA and 0.5mM-EGTA). The digested nuclei were then lysed in an appropriate volume of 0.2mM-EDTA, pH7.5, and centrifuged at 1200g for 5min to yield a pellet of nuclear membranes and undigested nuclei, and a supernatant of soluble chromatin. This chromatin, which generally had an A_{260} of 20–30, was the starting material for the dissociation–reassociation experiments described below.

Preparation of kidney and erythrocyte nuclei and chromatin

Laying-hen kidney nuclei and chromatin were obtained by the same technique as for oviduct. Erythrocyte nuclei were prepared by the method of Zasloff & Camerini-Otero (1980), with slight modifications. Blood from laying hens was collected at the time of death into tubes containing EDTA (final concentration 20mM) to prevent coagulation. Cells were sedimented at 800rev./min during 5min, then washed three times with phosphate-saline buffer, pH7.5 (10mM-sodium phosphate, 150mM-NaCl and 3mM-KCl) until the supernatant became clear. The cells were then lysed with a solution of 0.2% Nonidet P40 in buffer A (Marshall & Burgoyne, 1976). The nuclei were sedimented at 4500rev./min for 10min, and the pellet was washed three or four times with 0.34M-sucrose in buffer A, until it became white without any apparent trace of haemoglobin. The purified nuclei were then resuspended in an appropriate volume of 0.34M-sucrose in buffer A, and incubated with micrococcal nuclease in the same manner as for oviduct and kidney. The A_{260} obtained for kidney and erythrocyte chromatin was in the same range as that obtained in oviduct preparations.

Partial dissociation of the chromatin by 0.3M-NaCl

In order to remove the oestradiol receptor from its 'acceptor' sites on the oviduct chromatin, NaCl was added to the soluble chromatin to 0.3M final concentration, a concentration known not to remove histone H1 (Thoma & Koller, 1981). After 10 strokes of a tight-fitting glass Dounce homogenizer, the preparation was centrifuged in a Beckman SW 60 rotor for 260min at 58000rev./min. The resulting pellet ($P_{0.3}$) contained nearly

all the DNA and associated proteins (mononucleosomes included), whereas the supernatant ($S_{0.3}$) contained the oestrogen receptor, which in these ionic conditions sediments as a 5S entity (Rocheffort *et al.*, 1969) (results not shown), and very little A_{260} -absorbing material.

The pellet $P_{0.3}$ was digested again by micrococcal nuclease to obtain adequate concentrations of mono-, di- and tri-nucleosomes, in order to better analyse the reassociated chromatin on 5–20% linear sucrose gradients. The pellet was resuspended in 1–2ml of 5mM-Tris/HCl, pH7.5, and blended thoroughly with a glass rod or a Pasteur pipette. CaCl_2 was added to 0.4mM final concentration, and digestion with micrococcal nuclease (20–25 units/ml per mg of DNA) was for 30s at 37°C. The reaction was stopped by adding EDTA to 0.4mM concentration and placing the tube in ice.

Reassociation experiments

Reassociation of $P_{0.3}$ with the oestrogen-receptor-containing fraction $S_{0.3}$. Equal portions (0.15–0.2ml, containing 300–600µg of DNA) of the redigested $P_{0.3}$ fraction were distributed into several tubes, to which were then added various amounts (5–200µl) of $S_{0.3}$ fraction; the maximum reassociation ratio used was $P_{0.3}/S_{0.3} = 1/0.65$. Buffer (0.3M-NaCl/0.2mM-EDTA) was added to reach an identical total volume in each tube.

The contents of each tube were then transferred into dialysis bags, and the bags were immersed in an Erlenmeyer flask containing 40vol. of 0.2mM-EDTA, pH7.5, and 10nM-[^3H]oestradiol. During this dialysis, which served two purposes, NaCl was removed progressively and the high-salt-dissociated proteins reassociated on the chromatin, and hormone-receptor binding equilibrium was re-established. All the steps up to the dialysis were performed within 1 day, and the dialysis took place overnight in the cold-room. After approx. 10h of dialysis at 5–10°C, labelling and exchange of [^3H]oestradiol on the receptor was completed by incubating the Erlenmeyer flask containing the dialysis bags at 30°C for 90min (Sutherland & Baulieu, 1976).

Reassociation of $P_{0.3}$ with cytosol progesterone receptor purified from chick oviduct. To equal portions of redigested $P_{0.3}$ in suspension in 0.3M-NaCl were added various amounts of cytosol progesterone receptor, partly purified from chick oviduct (Renoir *et al.*, 1982) and stabilized in the 8S form in 10mM-Tris/1.5mM-EDTA/50mM- Na_2MoO_4 /12mM- α -thioglycerol/10% glycerol (pH7). A small quantity (generally 100µl) of $S_{0.3}$, which does not contain progesterone receptor (Lebeau *et al.*, 1982; N. Massol, M.-C. Lebeau & E.-E. Baulieu, unpublished work), was also added in order to stabilize the progesterone receptor

during the dialysis. This did not interfere with progesterone binding on the receptor, but resulted in an increase in chromatin-associated progesterone radioactivity. Samples were dialysed overnight in the cold in 40 vol. of 0.2 mM-EDTA, pH 7.5, containing 10 nM-[³H]progesterone. The next morning exchange was continued for 2 h at 25°C (Mester & Baulieu, 1977).

Association of partly purified oestrogen receptor from calf uterus to kidney P_{0.3}. Cytosol was prepared from calf uterus in 50 mM-Tris/1.5 mM-EDTA/20 mM-Na₂MoO₄ (pH 7.4) and was partly purified on a 15 ml DEAE-Trisacryl (IBF) column in the same buffer, with a 0–0.5 M-KCl gradient (Redeuilh *et al.*, 1981). Of this solution, 55 µl, which contained 7 × 10⁶ d.p.m./ml of specifically bound [³H]oestradiol (sp. radioactivity 100 Ci/mmol), was mixed with 940 µl of kidney S_{0.3}. Various volumes of this mixture were added to a fixed quantity of kidney P_{0.3}, followed by dialysis in the presence of 10 nM-[³H]oestradiol and exchange for 90 min at 30°C.

After the exchange period, the dialysis bags were opened and the contents treated for 5 min at 0°C with 0.25% charcoal/0.025% dextran in order

to remove unbound hormone (Milgrom & Baulieu, 1969). The charcoal was removed by centrifuging for 5 min at 600g and the supernatants were layered on 5–20% sucrose gradients in 5 mM-Tris/0.2 mM-EDTA (pH 7.5). Ultracentrifugation was in a Beckman SW 60 rotor spun at 58 000 rev./min for 270 min. Two-drop fractions were collected from the bottom of the tubes after puncturing them with a needle, and the A₂₆₀ and radioactivity were determined in each fraction.

Expression of results

On the binding curves, which express bound versus total receptor (Figs. 1–4), each experimental point corresponds to a gradient tube such as those shown in Figs. 1 and 2(a)–2(d).

As bound oestrogen receptor, the sum of all the radioactivity in the peak sedimenting at 13–14S was chosen, since we have demonstrated that the main form of chromatin oestrogen receptor obtained directly in these experimental conditions from laying-hen oviduct sediments as a 13–14S entity (Massol *et al.*, 1978). As bound progesterone receptor the radioactive peak sedimenting at 11–12S was chosen for similar reasons (Lebeau *et al.*,

Fig. 1. Reassociation of oestrogen receptor containing S_{0.3} fraction with depleted oviduct chromatin (a)–(d) Various quantities of S_{0.3} fraction were dialysed overnight together with constant portions of P_{0.3} (corresponding to 370 µg of DNA) in the presence of 10 nM-[³H]oestradiol as described in the text. (a) Depleted chromatin alone; (b) 0.1 ml of S_{0.3}; (c) 0.16 ml of S_{0.3}; (d) 0.26 ml of S_{0.3}. The reassociated chromatin was analysed on sucrose gradients as described, and A₂₆₀ (---) and radioactivity (—) were measured in each fraction. Sedimentation was from right to left, and markers were bovine serum albumin (BSA; 4.2S) and catalase (cat.; 11.6S). (e) Total radioactivity in the 13–14S peak was measured for each experimental point and plotted against the concentration of oestradiol receptor added. The results of three separate experiments (○, △, ☆) are shown. Four gradients obtained in one of these experiments (☆) are shown in the top part of the Figure (a–d).

1982; N. Massol, M.-C. Lebeau & E.-E. Baulieu, unpublished work). We assume that 1 molecule of receptor binds 1 molecule of oestradiol or of progesterone. Total receptor sites are calculated from the result of charcoal/dextran analysis (Milgrom & Baulieu, 1969) of the $S_{0.3}$ fraction or of the purified oestrogen or progesterone receptor fraction.

Results

Reassociation of oestradiol-receptor-containing supernatant ($S_{0.3}$) with the 'depleted' chromatin fraction ($P_{0.3}$)

When reassociated chromatin, prepared as described above, was analysed by ultracentrifugation in sucrose gradients, the familiar profile of A_{260} absorption due to mono-, di- and tri-nucleosomes (Finch *et al.*, 1975) was observed (Fig. 1). It appeared that the sedimentation coefficients of these particles were not different from those seen in

'native' chromatin. Most of the oestradiol binding was found in a peak sedimenting at 13–14S, slightly faster than the mononucleosome fraction, just as in freshly prepared soluble chromatin directly centrifuged in low-salt conditions (Figs. 1*a–d*). Some oestradiol binding was found associated with heavier chromatin as well, especially when high concentrations of $S_{0.3}$ were added, and part of the oestrogen receptor also sedimented as a peak of approx. 7S.

Re-digested $P_{0.3}$, to which no $S_{0.3}$ was added, displayed very little oestradiol binding due to undissociated receptor (Fig. 1*a*). As increasing quantities of $S_{0.3}$ were added, there was an increase in the general level of oestradiol binding on the chromatin, the best defined fraction being the 13–14S peak. Radioactivity in this peak increased strongly when low concentrations (9–50 pM) of oestrogen receptor were added, but above this level remained fairly stable up to approx. 0.3 nM. Typical gradients are shown in Figs. 1(*a*)–1(*d*). The

Fig. 2. *Reassociation of purified oviduct progesterone receptor with depleted oviduct chromatin* (a)–(d) Various quantities of progesterone receptor were dialysed overnight together with constant quantities of $P_{0.3}$ (corresponding to 470 µg of DNA), in the presence of 10 nM-[3 H]progesterone as described in the text. (a) Depleted chromatin alone; (b) 10 µl of progesterone receptor; (c) 50 µl of progesterone receptor; (d) 150 µl of progesterone receptor. The reassociated chromatin was analysed as described in the legend to Fig. 1. (e) Total radioactivity in the 11–12S peak was measured for each experimental point and plotted against the progesterone receptor concentration added. The results of two experiments are shown. ☆, ○, Points of the same experiment; four of the gradients obtained in this experiment (☆) are shown in the top part of the Figure (a–d). Δ, Points obtained in a separate experiment.

experiment was repeated three times with a wide range of $S_{0.3}$ concentrations. The resulting binding curve (Fig. 1e) shows that saturation is obtained for very low concentrations (approx. 0.04 nM) of oestradiol receptor.

Partly purified 9S oestrogen receptor from calf uterus cytosol was also incubated with oviduct $P_{0.3}$ fraction, treated and analysed on sucrose gradient as described above. Oestradiol binding was distributed mainly as a 13–14S peak, similar to oviduct receptor, but also as a broad 5–6S peak, as expected for activated receptor (Rochefort & Baulieu, 1971). It is interesting to see that these different oestrogen receptors, purified from calf uterus cytosol or extracted from chick oviduct nuclei, both sediment as a 13–14S peak when associated with depleted oviduct chromatin (see the Discussion section).

Association of purified progesterone receptor from chick oviduct cytosol with hen oviduct $P_{0.3}$ fraction

Various quantities of molybdate-stabilized progesterone receptor, purified from oviduct cytosol of oestradiol-primed chicks, were added to a fixed quantity of redigested $P_{0.3}$ fraction from hen oviduct chromatin, and the mixture was dialysed overnight in the presence of 10 nM-[3 H]progesterone. In the absence of added progesterone receptor only trace amounts of progesterone binding were seen in the chromatin density gradients (Fig. 2a). When increasing quantities of progesterone receptor were added to the $P_{0.3}$ fraction, correspondingly increasing quantities of

radioactivity were observed, sedimenting as a 11–12S peak, slightly slower than the mononucleosome fraction, and as a peak of approx. 4S, sedimenting in approximately the same position as bovine serum albumin (see Figs. 2a–2d). The 11–12S form of the progesterone receptor was also observed in oviduct chromatin directly centrifuged in low-salt-containing sucrose gradients at 1–2h after administration *in vivo* of progesterone (3 mg/kg body wt.) to oestrogen-withdrawn or -primed chicks (Lebeau *et al.*, 1982). Therefore for the progesterone receptor, as for the oestrogen receptor, the picture of receptor binding to chromatin was the same in reassociated chromatin as in freshly prepared chromatin directly analysed in low-salt conditions. The approx. 4S form of the progesterone receptor, which increased proportionally with the total quantity of receptor added, probably corresponds to the activated form of the receptor, a result of the removal of Na_2MoO_4 during the course of the dialysis followed by 2h incubation at 25°C (Wolfson *et al.*, 1980).

When bound progesterone receptor (radioactivity in the 11–12S peak) was plotted against total added receptor, binding tended towards saturation for the largest concentration of receptor added (Fig. 2e).

Association of oestrogen and progesterone receptors with non-target-organ chromatin

Various quantities of hen oviduct $S_{0.3}$ fraction or of purified chick cytosol progesterone receptor were dialysed together with depleted chromatin

Fig. 3. Binding of oestrogen receptor to depleted kidney chromatin

Various quantities of hen oviduct $S_{0.3}$ fraction were added to a constant amount of hen kidney $P_{0.3}$ fraction (corresponding to 510 μg of DNA), dialysed and analysed on a sucrose gradient. The inset shows the result obtained when 0.52 nM receptor was added. —, Reassociation to kidney $P_{0.3}$; ----, reassociation to oviduct $P_{0.3}$ (see Fig. 1). Inset: —, radioactivity; ----, A_{260} ; 200 c.p.m. refers to [3 H]oestradiol.

Fig. 4. *Binding of progesterone receptor to depleted kidney chromatin*

Various quantities of purified progesterone receptor were added to a constant amount of hen kidney $P_{0.3}$ fraction (corresponding to $510 \mu\text{g}$ of DNA), dialysed and analysed on a sucrose gradient. The inset shows the result obtained after adding 7.7 nM -progesterone receptor. —, Reassociation to kidney $P_{0.3}$ fraction; ----, reassociation to oviduct $P_{0.3}$ fraction in the same experiment. Inset: —, radioactivity; ----, A_{260} ; values to the right of the ordinate are c.p.m. of $[^3\text{H}]$ progesterone.

Fig. 5. *Binding of calf uterus receptor to depleted kidney chromatin*

Various quantities of partly purified oestrogen receptor from calf uterus cytosol were added to a constant amount of hen kidney $P_{0.3}$ in the presence (▲) or absence (Δ) of hen kidney $S_{0.3}$ fraction. Association of oviduct $S_{0.3}$ to kidney $P_{0.3}$ (— in Fig. 3) is shown for comparison (■).

from non-target tissues, including one in which chromatin is inactive (Bellard *et al.*, 1978; Bloom & Anderson, 1979). The depleted chromatin was prepared from laying-hen kidney and erythrocyte nuclei in the same way as for hen oviduct chromatin; since these tissues do not contain oestrogen receptor (Massol *et al.*, 1978; N. Massol, M.-C. Lebeau & E.-E. Baulieu, unpublished work), the $S_{0.3}$ fraction of oviduct chromatin, prepared simultaneously from the same hen, was used as a source of oestrogen receptor for these reassociation experiments.

When analysing on sucrose gradients the reassociated chromatin after dialysis, oestradiol binding was found sedimenting mainly as a 13–14S peak for both kidney (Fig. 3, inset) and erythrocytes (results not shown), and progesterone receptor as a 12S and 4S peak for kidney chromatin (Fig. 4, inset). However, when radioactivity in the 13–14S peak (Fig. 3) or in the 12S peak (Fig. 4) was plotted against total receptor added, no saturation plateau was obtained over a wide range of receptor concentrations, indicating a lower affinity of receptor binding for chromatin from these non-target tissues than for oviduct chromatin.

In order to test the role of kidney $S_{0.3}$, various quantities of partly purified calf uterus cytosol oestrogen receptor were added to a fixed quantity of kidney $P_{0.3}$ in the presence or absence of various concentrations of kidney $S_{0.3}$ (Fig. 5). In both cases, when the reassociated chromatin was analysed on sucrose gradients, oestradiol-binding sites sedimented as two main peaks, one slightly heavier than the mononucleosome fraction, the other as a peak of approx. 7S, probably corresponding to unbound receptor. Addition of kidney $S_{0.3}$ resulted in slightly higher oestradiol binding in both peaks. However, as with oviduct $S_{0.3}$, no saturation was observed for kidney $S_{0.3}$ concentrations equivalent to those shown in Fig. 3, and for up to 1 nM receptor concentrations. This experiment also shows that, in our experimental conditions, addition of various protein concentrations does not result in artifactual saturation as described in other systems (Chamness *et al.*, 1974; Milgrom & Atger, 1975).

Discussion

Chromatin prepared by micrococcal nuclease digestion of oviduct, kidney and erythrocyte nuclei, when first dissociated by 0.3M-NaCl and then slowly reassociated by dialysis in hypo-osmotic buffer, apparently regained its original structure, as analysed by density-gradient ultracentrifugation. This suggests that, during the course of these treatments, nucleosome structure was not irreversibly altered by the high-salt conditions, as was already reported by Germond *et al.* (1976) when studying the effects of salt on nucleosome stability, and by Weisbrod & Weintraub (1979) when removing and restoring high-mobility-group (HMG) proteins, in similar experiments with various concentrations of NaCl.

When oestradiol- or progesterone-binding sites were measured in density gradients of reassociated oviduct chromatin, it was seen that the main peaks of bound oestradiol or progesterone were localized in the reconstituted chromatin similarly to chromatin constantly maintained in low-salt conditions. The receptors therefore recognize some feature in the chromatin with which they interact, much in the same way as dissociated HMG 14 and 17 recognize 'active' nucleosomes when reassociated on depleted chromatin (Weisbrod *et al.*, 1980). The sedimentation coefficients of these peaks were different for both hormone receptors (13–14S for the oestrogen receptor and 11–12S for the progesterone receptor), suggesting the presence of different chromatin 'acceptor' sites for the two receptors, or of differently conformed receptor–chromatin complexes. This difference in sedimentation coefficient was also observed in non-target-tissue chromatin, suggesting that this behaviour is due to a property of the receptors themselves.

Various quantities of chromatin salt extract containing the oestrogen receptor were incubated with stripped oviduct chromatin, presumably containing the 'acceptor' sites, and analysed on sucrose gradients. When the radioactivity in the 13–14S peak was plotted against the total receptor sites added, a saturation curve was obtained. The fact that saturation was reached for very low concentrations of oestrogen-receptor sites indicated that the affinity of the oestrogen receptor for its 'acceptor' sites sedimenting in the 13–14S fraction was very high. However, it was impossible to calculate this affinity because one could not measure the value of unbound receptor. Indeed, unbound receptor, which should sediment at 4–5S, did not appear in the gradients (owing to aggregation?), nor could one deduce it from the total number of sites, since at low concentrations of added receptor the quantity of bound receptor in the 13–14S peak was larger than the total number

of sites added (as measured in the salt extract), presumably owing to a renaturation of oestrogen-binding sites during dialysis in the presence of chromatin. Whether this was due to receptor stabilization in the presence of nuclear proteins as a result of the reduction in salt concentration or to a change in the conformation of the receptor as it binds to DNA remains to be demonstrated, and will probably be explained when the nature of the 'acceptor' sites is elucidated.

When various quantities of purified, molybdate-stabilized, cytosol progesterone receptor were added to stripped chromatin, followed by dialysis to remove the salt, two peaks of progesterone binding were seen in the density gradients, one sedimenting at 11–12S and the other at approx. 4S. When the radioactivity in the 11–12S peak was plotted against the quantity of progesterone receptor added, the curve tended towards saturation for the highest concentrations of progesterone receptor added (8nM). Multiple regression analysis showed that the best fit for these data was a second-order curve, with a probability >99% according to the *F* test of Fisher and Snedecor.

Even though these experiments do not allow calculation of the affinities of either receptor for their binding sites on oviduct chromatin, differences in receptor concentrations necessary to reach saturation (approx. 0.04nM for oestrogen receptor and approx. 8nM for progesterone receptor) give an idea of their relative affinities for the chromatin 'acceptor' sites.

When hen oviduct salt extract was dialysed in the presence of stripped chromatin from hen kidney or erythrocytes, a peak of 13–14S oestrogen-binding sites was also observed in the density gradients. However, when the radioactivity sedimenting at 13–14S was plotted against total binding sites added, no saturation was observed, including when high concentrations of receptor-containing extract were used. Similarly, when purified progesterone receptor was incubated with kidney chromatin, a peak of 11–12S progesterone-binding sites was observed, but no saturation was seen when 11–12S radioactivity was plotted against total receptor added, even at high concentrations. The same mathematical test as above was applied to these data; the best fit is a straight line, with a >99% probability. These results indicate that oestrogen or progesterone receptors can bind to non-target-cell chromatin, but with a lower affinity than to oviduct chromatin.

It appears that specificity does not reside in the salt-extractable $S_{0.3}$ fraction. For example, in Fig. 3 one can see that the same $S_{0.3}$ fraction added to two different $P_{0.3}$ fractions results in different types of binding curves. Furthermore, in the homologous system kidney $S_{0.3}$ /kidney $P_{0.3}$ (Fig. 5) the

same result was obtained as when oviduct $S_{0.3}$ was used instead of kidney $S_{0.3}$. These results do not contradict those recently published by Ruh & Spelsberg (1983), who can extract specific oestrogen-receptor 'acceptor' proteins from hen oviduct chromatin with 4–7M-guanidine hydrochloride, since it is probable that 0.3M-NaCl is insufficient to extract these proteins.

The $S_{0.3}$ fraction from both target and non-target organs does, however, appear to play a role in the association of purified receptors to chromatin, since, in the absence of this fraction, less binding of the receptor to chromatin was observed (see Fig. 5, and the Experimental section). The simplest explanation could be that purified receptors are stabilized by the addition of nuclear components.

In conclusion, when oestrogen or progesterone receptors were dialysed to low-ionic-strength conditions together with salt-depleted chromatin, they formed receptor-chromatin complexes that had the same sedimentation coefficients as in 'native' chromatin. These sedimentation coefficients differed for each receptor, but were the same whether the chromatin was from target or from non-target tissues. When oestrogen or progesterone receptor was reassociated with oviduct depleted chromatin, the resulting binding curve reached a plateau, indicating saturation, whereas when they were reassociated with non-target depleted chromatin, no saturation was seen, even for high concentrations of receptor. It appears, therefore, that something in target-tissue chromatin that is absent from non-target-tissue chromatin transforms or associates with the receptors to enhance their affinity for chromatin. These differences in affinity could also reflect physicochemical alterations of the 'acceptor' sites, which could vary according to the type of tissue and to its state of hormone-responsiveness.

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