Nuclear Binding of Progesterone in Hen Oviduct

ROLE OF ACIDIC CHROMATIN PROTEINS IN HIGH-AFFINITY BINDING

By ROBERT A. WEBSTER, GEORGE M. PIKLER and THOMAS C. SPELSBERG
Department of Molecular Medicine, Mayo Clinic, Rochester, MN 55901, U.S.A.

(Received 21 August 1975)

The multiple classes of binding sites for the progesterone–receptor complex in hen oviduct nuclei were found to be of chromatin origin. The highest-affinity, and presumably most physiologically important class, is localized in oviduct chromatin and contains approx. 6000–10000 sites per nucleus. None of these sites is detected in spleen chromatin. Two new techniques were used for assaying rapidly the binding of steroid–receptor complexes to soluble deoxyribonucleoproteins *in vitro*. The extent of high-affinity binding by the nucleic-acidic protein fraction from spleen chromatin is as great as that by the nucleic-acidic protein from oviduct chromatin. Consequently the tissue-specific nuclear binding of the progesterone receptor is found not to be a consequence of the absence of the nuclear binding sites (acceptors) from chromatin of non-target tissue (spleen), but rather a result of complete masking of these sites. In the target-tissue (oviduct) chromatin, approx. 70% of the high-affinity acceptor sites are also masked. Acidic proteins, and not histones, appear to be responsible for the masking of these acceptor sites. In addition, acidic proteins represent (or at least are an essential part of) these high-affinity sites in the oviduct nucleus. Pure DNA displays a few high- and many low-affinity binding sites. In support of previous work with immature chicks, the acidic protein fraction of the nucleic-acidic protein of hen oviduct appears to contain this high-affinity class of binding sites. Our results thus support the hypothesis that protein complexed with DNA, and not DNA alone, represents the high-affinity binding sites for the steroid–receptor complexes in nuclear chromatin. The lower-affinity classes of binding sites may represent DNA and/or other nuclear components.

Interaction of steroid hormones with the genetic material has been implicated in the molecular mechanism by which these hormones modulate target-cell metabolism. This modulation has been shown to involve significant changes in RNA and protein synthesis. Such alterations in genetic transcription and translation are preceded by the high-affinity association of the steroid with specific cytoplasmic ‘receptor’ proteins. The resulting hormone–receptor complex is then believed to be translocated to the nucleus, where it interacts with the chromatin and subsequently alters the pattern of gene expression in the target cells (Hamilton, 1968; O'Malley *et al.*, 1969; Williams-Ashman & Reddi, 1971; Jensen & DeSombre, 1972; King & Mainwaring, 1974; O'Malley & Means, 1974).

Many aspects of the mechanism of action of progesterone in the chick oviduct have been studied in some detail. The cytoplasmic receptor for progesterone has been extensively purified and characterized, and the properties of the stereospecific association with progesterone determined (Schrader & O'Malley, 1972; Schrader *et al.*, 1972, 1974, 1975; Kuhn *et al.*, 1975). The interaction of the progesterone–receptor complex with isolated nuclei *in*

vitro has also been characterized and compared with that which occurs *in vivo* (O'Malley *et al.*, 1971; Buller *et al.*, 1975a,b; Pikler *et al.*, 1976). Progesterone-induced alteration in gene expression has been monitored by examination of various aspects of the synthesis of RNA (T. C. Spelsberg & R. F. Cox, unpublished work) and protein (O'Malley *et al.*, 1969; Palmiter, 1972a,b) in oviducts from hormone-treated chicks.

Previous work has demonstrated that the binding of the progesterone–receptor complex to chick oviduct nuclei and nuclear components *in vitro* is dependent on receptor ‘activation’, is of high affinity, is ionic in nature, and is quantitatively target-tissue-specific (O'Malley *et al.*, 1971; Buller *et al.*, 1975a,b; Pikler *et al.*, 1976). Moreover, almost all of the nuclear binding seems to represent binding to nuclear chromatin (Spelsberg *et al.*, 1971b; Steggles *et al.*, 1971; O'Malley *et al.*, 1972; Schrader *et al.*, 1972) and specifically to a nucleic-acidic protein fraction obtained by selective removal of the histones and some acidic protein fractions from the chromatin (Spelsberg *et al.*, 1972). The chromatin-binding sites, or ‘acceptors’, for the progesterone receptor in the chick oviduct appear to be part of a protein fraction

AP₃ associated with the DNA in the nucleic-acidic protein (Spelsberg *et al.*, 1972). In the absence of DNA, however, this fraction binds receptor only slightly (Spelsberg, 1974). Similarly pure DNA only binds receptor slightly compared with the nucleic-acidic protein under high-ionic-strength (0.15 M-NaCl) conditions (Spelsberg *et al.*, 1972; Spelsberg, 1974). When the AP₃ fraction is re-annealed to DNA, however, the high-affinity binding (i.e. binding which occurs in the presence of 0.15 M-NaCl) is restored.

We reported in the preceding paper (Pikler *et al.*, 1976) that progesterone-receptor binding to oviduct nuclei from laying hens has the same characteristics as that to nuclei from chicks. Further, oviduct nuclei were shown to possess multiple classes of binding sites *in vivo* (Spelsberg, 1976) and *in vitro* (Pikler *et al.*, 1976) with differing affinities for the progesterone receptor. The work presented here demonstrates that in hen oviduct (1) these multiple classes of binding sites for the receptor are associated with chromatin, (2) the highest-affinity sites are associated with acidic proteins, (3) many of these sites in oviduct chromatin are masked by other fractions of the acidic proteins (and not by histones), and (4) these sites are also present in non-target-tissue chromatin but are completely masked by the acidic proteins.

Part of this work was presented at the Florida Colloquium on Molecular Biology sponsored by the Department of Biochemistry, University of Florida, March 1975 (Spelsberg *et al.*, 1975).

Materials and Methods

Tissue sources and subcellular fractions

Tissues from adult laying hens were supplied by a local produce company (Jones Produce, Rochester, MN, U.S.A.). Shortly after the hens were killed, organs were removed, cleaned, sectioned and frozen (on solid CO₂). The tissues were stored at -80°C until used. Mature erythrocytes were isolated as described by Neelin (1968). The isolation of nuclei and chromatin and the preparation of partially deproteinized chromatin were as previously described (Spelsberg *et al.*, 1971b, 1972) with slight modification. The final suspensions of native and treated chromatin were in 2.0 mM-Tris/HCl/0.1 mM-EDTA, pH 7.5 (TE buffer), at 0.2–0.8 mg of DNA/ml. Oviducts for the preparation of the progesterone-receptor complex were obtained from White Leghorn chicks that had been injected subcutaneously with 5 mg of stilboesterol for 20–25 days. Cytosol was prepared as described previously (Schrader & O'Malley, 1972).

Preparation of the [³H]progesterone-receptor complex

[1,2-³H]Progesterone (47.8 Ci/mmol) was obtained from New England Nuclear Corp. (Boston, MA,

U.S.A.) and checked for purity by t.l.c. in benzene/ethyl acetate (7:3, v/v). The cytosol progesterone-receptor complex from chick oviduct was labelled with [³H]progesterone and partially purified by (NH₄)₂SO₄ precipitation as described previously (Schrader & O'Malley, 1972). The labelled receptor preparation was stored at -80°C and dissolved in 0.01 M-Tris/HCl/0.001 M-EDTA/0.012 M-thioglycerol, pH 7.4 (TESH buffer) for use in the binding assays as described elsewhere (Pikler *et al.*, 1976).

Assays for chromatin binding of the [³H]progesterone-receptor complex

Two new methods were used to assay for steroid-receptor binding to native chromatin, partially deproteinized (soluble) chromatin and DNA. Both techniques allow many samples of soluble deoxyribonucleoprotein or DNA to be assayed together with insoluble chromatin for steroid-receptor binding capacity in a relatively short time. The samples can be washed rapidly by using low-speed centrifugation, as opposed to the time-consuming high-speed centrifugations used previously (Spelsberg *et al.*, 1972).

One method uses the antibiotic streptomycin sulphate to precipitate the DNA, together with any DNA-bound protein, including the progesterone receptor (T. C. Spelsberg, unpublished work). In this method, the binding assays are performed essentially as described in the preceding paper (Pikler *et al.*, 1976). The reaction mixtures contained in 1.0 ml final volume, 50 µg of DNA as chromatin, 'half-TESH' buffer (5 mM-Tris/HCl/0.5 mM-EDTA/6 mM-α-thioglycerol, pH 7.4), KCl as required, and various amounts of the partially purified progesterone receptor. The reactions were initiated by adding the chromatin (in a small volume of TE buffer) and mixing immediately. The reaction tubes were incubated for 90 min on ice with intermittent mixing. At the end of the incubation, approx. 1.0 mg of streptomycin sulphate (Calbiochem, La Jolla, CA, U.S.A.) in TE buffer was added to each reaction mixture, and the tube contents were again gently mixed. The DNA was allowed to precipitate for 30 min on ice and then sedimented by centrifugation at 1500 g_{av} for 5 min. The pellets were washed twice by centrifugation in 2.0 ml of TE buffer containing 0.02% (w/v) streptomycin. The chromatin material was then collected on membrane filters (0.45 µm pore size, 24 mm diameter; Millipore Corp., Bedford, MA, U.S.A.) under vacuum and washed further with the TE/streptomycin buffer. The filters were dried and counted for radioactivity in 5.0 ml of a toluene-based scintillation solution containing 6.0 g of PPO (2,5-diphenyloxazole) and 0.075 g of POPOP [1,4-bis-(5-phenyloxazol-2-yl)benzene] per litre. In all assays, complementary reaction mixtures containing the labelled receptor, but no chromatin, were

treated in the same manner to monitor background radioactivity. After radioactivity counting (30% efficiency), the filters were dried and the DNA was hydrolysed by incubating them at 90°C for 30 min in 0.3M-HClO₄. The DNA in the hydrolysate was assessed by the diphenylamine reaction (Burton, 1956). The radioactivity per filter was correlated with the amount of DNA on the same filter (d.p.m./mg of DNA).

The other method, the cellulose method, for assaying steroid-receptor binding uses an insoluble matrix to which the soluble deoxyribonucleoprotein is coupled (T. C. Spelsberg, unpublished work). Chromatin was linked to cellulose in ethanol with high-intensity u.v. light (Litman, 1968). The chromatin-cellulose preparations (2–10 mg of chromatin DNA/g of cellulose) were stored dry at room temperature (22°C) and before use were allowed to hydrate for 2 h in the TE buffer. The reactions were initiated by the addition of the chromatin-cellulose (25 µg of DNA per tube) to each assay mixture. After incubation for 90 min on ice, the chromatin material was washed with the TE buffer by the procedure described above, but without the streptomycin. In all experiments, pure cellulose was added to separate reactions and the resulting radioactivity used as background (subtracted from those of reactions containing chromatin-cellulose). Both the streptomycin and cellulose methods yielded similar results.

Results

The properties of the progesterone-receptor binding to nuclear material in these experiments were

described in the preceding paper (Pikler *et al.*, 1976). Both receptor and hormone are required for optimal binding to oviduct nuclei. Similar results have been obtained with oviduct chromatin. As shown in Fig. 1(a), the extent of binding of free [³H]progesterone (in the absence of the receptor) to oviduct chromatin is negligible compared with that of the [³H]progesterone-receptor complex. Fig. 1(b) shows that in the absence of KCl, the binding of the receptor to oviduct chromatin is not saturable within the range of receptor concentrations used. As the ionic concentration is increased, the extent of binding is decreased and saturation is readily achieved. These properties are similar to those described in the preceding paper (Pikler *et al.*, 1976) for experiments using isolated nuclei. At low ionic concentrations (0.05M-KCl), binding equilibrium is attained at 90 min, whereas at higher ionic strength (0.15M-KCl) equilibrium is reached at 20 min. Consequently, a 90-min incubation was used in all binding assays described in the present paper.

Many steroid-receptor complexes have been shown to require 'activation' by exposure to elevated temperatures (22–37°C) or to high ionic strength to achieve maximal nuclear binding [Jensen & DeSombre, 1972; King & Mainwaring, 1974; O'Malley & Means, 1974; Buller *et al.*, 1975a,b; the preceding paper (Pikler *et al.*, 1976)]. This is also true for the binding of the progesterone receptor to hen oviduct chromatin (Fig. 2). Crude receptor (whole cytosol) incubated at 4°C exhibits little binding to chromatin, whereas at 24°C extensive binding occurs. When the receptor is exposed to high salt concentrations [35%-satd. (NH₄)₂SO₄] during partial purification, a

Fig. 1. Binding of the [³H]progesterone-receptor to oviduct chromatin

Binding was assayed as described in the Materials and Methods section except that no streptomycin or cellulose was used. In (a), the binding of equivalent amounts of [³H]progesterone (●) with and (○) without receptor at 0.01 M-KCl to oviduct chromatin is shown. In (b), the effects of ionic concentration on the binding of the [³H]progesterone-receptor to oviduct chromatin is shown: ●, no KCl; □, 0.02 M-KCl; ○, 0.05 M-KCl; ■, 0.10 M-KCl. The range of three replicates of analysis for (b) is shown.

Fig. 2. Binding of the [^3H]progesterone-receptor, either as crude cytosol preparation (a) or as the partially purified preparation (b), to oviduct chromatin

The crude receptor preparation (a) represents the 100000 g_{av} . supernatant (cytosol), whereas the partially purified preparation (b) represents a 35%-(NH_4) $_2\text{SO}_4$ precipitate of this cytosol. Binding assays were carried out at 0.05M-KCl as described in the Materials and Methods section except that no streptomycin or cellulose was used. Incubations were carried out at (○) 24°C or (●) 4°C. The range of three replicates of analysis for each concentration of receptor is shown.

Fig. 3. Binding of the [^3H]progesterone-receptor to oviduct and spleen chromatin assayed by the cellulose method

Binding was assayed as described in the Materials and Methods section. ●, Oviduct chromatin-cellulose incubated in 0.05M-KCl; ■, oviduct chromatin-cellulose incubated in 0.15M-KCl; ▲, spleen chromatin-cellulose incubated in 0.05M-KCl. The range of three replicates of analysis for each receptor concentration is shown.

marked chromatin binding occurs at both 4° and 24°C, and the extent is equal to that which occurs with the crude receptor at 24°C.

We have shown in the preceding paper (Pikler *et al.*, 1976) that when oviduct nuclei are assayed under low-salt conditions by using multiple concentrations of the labelled receptor, several saturation plateaux are observed. The same phenomenon is seen with oviduct chromatin in Fig. 3. It appears that chromatin also contains several classes of binding sites with differing affinities for the progesterone receptor. The highest-affinity class of sites (i.e. binding which occurs at the lowest receptor concentrations) is absent from spleen chromatin, as was shown for spleen nuclei in the preceding paper (Pikler *et al.*, 1976), suggesting target-tissue specificity for its presence. Further, increasing the salt concentration to 0.15M-KCl eliminates all receptor binding to oviduct chromatin except that to the highest-affinity class of sites. The persistence at high salt concentration of receptor binding to the highest-affinity sites in hen oviduct chromatin and the target-tissue specificity of these sites correlate with the results obtained with nuclei (Pikler *et al.*, 1976).

Comparisons of the binding of the progesterone-receptor by oviduct nuclei and chromatin are shown in Table 1 by Scatchard-plot analysis of binding data similar to that shown in Fig. 3. The properties of the binding sites (K_d and number) in nuclei and chromatin are very similar irrespective of the assay method used. This suggests that the high-affinity progesterone-receptor binding to nuclei is mainly, if not wholly, a result of chromatin binding, and that the values for chromatin binding with respect

Table 1. Characteristics of the binding of [³H]progesterone–receptor complex to oviduct nuclei and chromatin by using different assay methods

The ‘standard’ method was described in the preceding paper (Pikler *et al.*, 1976) and differs from the other two methods described in the Materials and Methods section in that neither streptomycin nor cellulose was used. The dissociation constant, *K*_d, and number of binding sites were determined by the method of Scatchard (1949). The *K*_d was calculated by extrapolating the amount of bound receptor to a 1 litre reaction volume, thus making the *K*_d expressed in mol/litre per 25 mg of DNA. The number of binding sites was calculated by assuming 2.5 pg of DNA per oviduct nucleus. The two *K*_d values and numbers of sites for nuclei assayed in 0.05M-KCl represent the binding parameters for two separate higher-affinity binding sites, observed as two progressive saturation plateaux when increasing [³H]progesterone–receptor concentrations are used [see the preceding paper (Pikler *et al.*, 1976); see also Fig. 1].

Oviduct subcellular fraction	Assay method	[KCl]	Approx. <i>K</i> _d	Approx. no. of sites/cell
Nuclei	Standard	0.05M	1.2 × 10 ^{−9} M (first)	5300
			1.3 × 10 ^{−8} M (second)	20600
Nuclei	Standard	0.15M	6.2 × 10 ^{−9} M	10100
Chromatin	Streptomycin	0.15M	6.2 × 10 ^{−9} M	5500
Nuclei	Cellulose	0.15M	1.9 × 10 ^{−8} M	15300
Chromatin	Cellulose	0.15M	1.3 × 10 ^{−8} M	12000

Fig. 4. Binding of the [³H]progesterone–receptor in 0.15M-KCl to oviduct, erythrocyte and spleen chromatin, assayed by the streptomycin method

Binding was assayed as described in the Materials and Methods section. ●, Oviduct chromatin; ▲, erythrocyte chromatin; ■, spleen chromatin. The range of three replicates of analysis for each concentration of receptor is shown.

to the binding parameters (*K*_d and number of sites) are only moderately affected by the method used to assay receptor binding. Although the progesterone–receptor binding in 0.15M-KCl to oviduct chromatin is extensive, that to erythrocyte and spleen chromatin is minimal (see Fig. 4). This is further evidence that the high-affinity binding sites in chromatin are target-tissue specific, as found to a lesser degree for the nuclear binding.

Most of the tissue specificity of the receptor binding to chromatin, however, can be eliminated if the histones and two acidic protein fractions, AP₁ and AP₂ (Spelsberg *et al.*, 1972), are removed. Fig. 5 compares receptor binding to nuclei, chromatin and nucleic-acidic protein. An intermediate ionic strength (0.1M-KCl) was used, which allows binding to the highest-affinity sites and to one class of lower-affinity sites. Fig. 5 shows the presence of the two classes of binding sites in oviduct nuclei and chromatin. Spleen nuclei and chromatin exhibit little binding, with evidence of only one class of sites. Removal of the histones and acidic protein fractions AP₁ and AP₂, however, gives rise to extensive binding to the resulting nucleic-acidic protein from both oviduct and spleen. Apparently, the high-affinity binding sites for the receptor are present in non-target-tissue (spleen) chromatin, but they are largely masked by other proteins. Further, many of these high-affinity sites are also masked in target-tissue (oviduct) chromatin, as the receptor binding to oviduct nucleic-acidic protein is considerably greater than that to chromatin or nuclei. Fig. 5 also demonstrates that the receptor binding to pure DNA is much lower than that to nucleic-acidic protein. At higher ionic strength (~0.15M-KCl) the DNA binding is even less. These results indicate that acidic chromatin proteins determine (and may represent) the high-affinity binding sites. Alternatively, these proteins may alter the DNA structure and permit high-affinity binding of the receptor to the DNA.

To discern which proteins are responsible for masking the high-affinity sites for the progesterone receptor, chromatin cellulose was sequentially de-proteinized as shown in Scheme 1. The resulting de-histone chromatin–cellulose, nucleic-acidic protein–cellulose and DNA–cellulose were then assayed for receptor-binding capacity in 0.15M-KCl,

Fig. 5. Comparison of the $[^3\text{H}]$ progesterone-receptor binding in 0.1 M-KCl to nuclear fractions from oviduct and spleen, assayed by the streptomycin method

The assay technique is described in the Materials and Methods section. (a), Nuclei; (b), chromatin; (c), nucleic-acidic protein. ▲ represents oviduct nuclei, chromatin, or nucleic-acidic protein; ● represents spleen nuclei, chromatin, or nucleic-acidic protein; ○ represents oviduct DNA. The range of three replicates of analysis for each concentration of receptor is shown.

which eliminates all binding except that to the high-affinity sites. As shown in Fig. 6, removal of the histones fails to alter the maximum extent of receptor binding compared with whole oviduct chromatin. Subsequent removal of acidic protein fractions AP_1 and AP_2 , on the other hand, results in a significant increase in binding. Apparently, the receptor binding sites in chromatin are masked by the AP_1 and/or AP_2 acidic-protein fractions. The extent of masking of these sites in oviduct and spleen chromatin is shown in Table 2. Several interesting points are revealed: (1) oviduct chromatin displays only about 30% of its total acceptor sites for receptor binding; (2) the majority of the other sites (approx. 70%) are masked by acidic proteins, and not histones; (3) non-target-tissue chromatin (e.g. from spleen) contains as many acceptor sites as oviduct chromatin, but most of these (94% or more) are masked by protein.

Since the nucleic-acidic protein is capable of binding the progesterone receptor at high ionic strength (0.15 M-KCl), it apparently contains the high-affinity sites found in whole nuclei and chromatin. As shown in Fig. 6, most of this high-affinity binding to nucleic-acidic protein is lost, however, if the majority of the

residual acidic proteins (representing the AP_3 fraction) is removed; the resulting DNA, containing less than 4% of total chromatin, displays a greatly decreased binding compared with the nucleic-acidic protein. The degree of binding is also less than that for chromatin. In summary, pure DNA when assayed with the streptomycin or DNA-cellulose techniques displays minimal binding compared with nucleic-acidic protein. This supports the concept that the high-affinity chromatin binding sites for the progesterone receptor are contained in the AP_3 acidic-protein fraction.

Discussion

The results presented here indicate that the different classes of binding sites for the progesterone receptor in chick oviduct nuclei *in vivo* (Spelsberg, 1976) and *in vitro* (Pikler *et al.*, 1976) are of chromatin origin. In addition, the highest-affinity class of sites involves a fraction of acidic chromatin proteins bound to the DNA. The presence of more than one class of binding sites for the oestrogen receptor in kidney nuclei from the guinea-pig foetus (Sumida *et al.*, 1974)

Scheme 1. Scheme for the gross fractionation of chromatin

This procedure is based on a previously reported method (Spelsberg *et al.*, 1972). The selective removal of each group of proteins was monitored by polyacrylamide-gel electrophoresis (Panyim & Chalkley, 1969; Wilson & Spelsberg, 1973) and quantitative analysis (Lowry *et al.*, 1951).

and for the progesterone receptor in oviduct nuclei from the immature chick (Buller *et al.*, 1975b) has been suggested. By using theoretical and experimental considerations Yamamoto & Alberts (1975) argue in favour of the presence of more than one nuclear binding site for steroid-receptor complexes. The present work and that described in the preceding papers (Spelsberg, 1976; Pikler *et al.*, 1976) provide direct evidence for the existence of these different

classes of binding sites for a steroid-receptor complex in nuclei and chromatin.

The highest-affinity class of binding sites is presumed to be the one with a physiological function for the following reasons: (1) functional sites would most likely have the highest affinity, (2) the receptor binds only to the highest-affinity sites *in vitro* at ionic strengths thought to be representative of those within cells, (3) injections of progesterone into chicks

which give rise to concentrations of the hormone in serum equivalent to those in laying hens, results in nuclear binding only to the highest-affinity sites *in*

Fig. 6. Binding of the [^3H]progesterone-receptor in 0.15M-KCl to chromatin, dehistonized chromatin, nucleic-acidic protein and DNA from hen oviduct, assayed by the cellulose method

The chromatin fractions were prepared as shown in Scheme 1 and assayed for receptor binding as described in the Materials and Methods section. ●, Whole oviduct chromatin; ▲, de-histone oviduct chromatin; ■, oviduct chromatin devoid of histone and acidic proteins, AP₁ and AP₂ (this represents nucleic-acidic protein or DNA complexed with acidic proteins AP₃ and AP₄); ○, chromatin devoid of most (approx. 94%) of the protein; this is classified as pure DNA. The range of three replicates of analysis for each concentration of receptor is shown.

in vivo, and (4) the maximum alteration of nuclear RNA polymerase activity induced by progesterone treatment *in vivo* occurs when the number of nuclear-bound progesterone molecules reaches approx. 10000 (which is equivalent to the number of molecules required to saturate the higher-affinity classes; Spelsberg, 1976). Possible biological functions for the lower-affinity sites, however, cannot be ruled out. These might include (1) gene activation for enzymes involved in the metabolism of toxic concentrations of the hormone or (2) concentration of the hormone at chromosome regions near functional high-affinity sites for future interaction with the latter.

The finding that the AP₃ acidic-protein fraction gives hen oviduct chromatin the capacity to bind the progesterone receptor specifically corroborates earlier work from this laboratory using the immature-chick system (Spelsberg *et al.*, 1971b, 1972; Spelsberg, 1974). The same AP₃ fraction in the immature-chick chromatin was found to contain the high-affinity binding sites for progesterone receptor. Further, in both hen and chick, high-affinity binding sites for the receptor are present in both oviduct and spleen chromatin, but are only exposed in the oviduct target tissue.

Others have suggested that nuclear binding by steroid-receptor complexes involves the interaction of the receptor complex with DNA only, with no intervention of chromatin proteins (Baxter *et al.*, 1972; Yamamoto, 1974). The present results and others (Spelsberg *et al.*, 1971b, 1972; King & Gordon, 1972; Spelsberg, 1974), however, suggest that although DNA binds the steroid-receptor complex at low ionic strength, very little of the complex binds at high ionic strength. The acidic protein fraction AP₃, once bound to the DNA, enables the DNA to bind much greater amounts of the receptor even at high ionic strength. The receptor may bind directly to the protein or the protein may alter the DNA

Table 2. Masking of acceptor sites in hen organ chromatin

Results were taken from Figs. 5 and 6. The [^3H]progesterone had a specific radioactivity of 47.8 $\mu\text{Ci}/\text{mmol}$, and this was used, with a counting efficiency of 30%, to calculate the amount (pmol) of receptor bound. The number of molecules bound was calculated by assuming 2.5 pg of DNA per cell. The extent of binding to nucleic-acidic protein was taken as 100% of sites exposed.

Nuclear fraction	Binding of [^3H]progesterone-receptor at saturation to highest-affinity sites			
	(d.p.m./mg of DNA)	(pmol/mg of DNA)	(molecules/cell)	(% of sites exposed)
1. Oviduct				
Chromatin (DNA + histones + AP ₁ + AP ₂ + AP ₃ + AP ₄)	417000	3.93	5920	29
De-histone chromatin (DNA + AP ₁ + AP ₂ + AP ₃ + AP ₄)	52000	4.91	7390	37
Nucleic-acidic protein (DNA + AP ₃ + AP ₄)	1417000	13.37	20140	100
2. Spleen				
Chromatin	83000	0.78	1170	6
Nucleic-acidic protein	1350000	12.74	19190	100

structure and facilitate receptor binding directly to the DNA.

The results presented here also suggest that other acidic protein fractions (AP₁ and/or AP₂), and not histones, mask the receptor-binding proteins in the AP₃ fraction. This is somewhat surprising, since histones so effectively mask much of the DNA and restrict transcription. The finding that one group of acidic proteins is involved in the chromatin binding of the receptor and that another group is responsible for masking these binding sites suggests that acidic proteins may be involved in both positive and negative control of chromosome function. It also suggests that chromosomal proteins are interwoven in a complex matrix covering the DNA.

At present no role can be assigned to the large number of masked high-affinity binding sites found in non-target-cell chromatin (e.g. spleen). Their presence demonstrates that, unlike the cytoplasmic receptor, the nuclear proteins which bind the progesterone receptor are not really target-tissue-specific, but are simply masked in non-target tissues. The masking of the high-affinity binding sites in target-cell chromatin may play a role in determining the degree of cellular responsiveness to the hormone. The response could be modulated by masking or unmasking more sites under certain physiological conditions or during development. Although there is no evidence for such a mechanism, it has been shown (Spelsberg *et al.*, 1971a, 1973) that during oestrogen-induced differentiation of chick oviduct there are significant changes in the progesterone-receptor-binding capacity of chromatin and also in the concentrations of the AP₂ acidic-protein fraction (which possibly is involved in the 'masking' of acceptor sites). Also, in rat uterus, when animals receive graded doses of oestrogen, there is a positive correlation between the quantity of nuclear-bound oestrogen receptor and the degree of hormone response (Anderson *et al.*, 1972).

Characterization of the chromatin-binding sites for steroid-receptor complexes, such as that described here, seems to be one of the first steps necessary in the elucidation of the mechanism by which these hormones regulate genetic transcription in target cells.

The excellent technical assistance of Mrs. Patti Midthun, Mrs. Barbara Gosse, Mr. Paul Matthai and Mr. Bradley Syverson is greatly appreciated. This work was supported in part by research grants HD 8441, HD 9140-B and CA 14920 from the National Institutes of Health, U.S. Public Health Service, and by the Mayo Foundation. The gift of hen tissues by Jones Produce, Rochester, is sincerely appreciated.

References

Anderson, J. N., Clark, J. H. & Peck, E. J., Jr. (1972) *Biochem. Biophys. Res. Commun.* **48**, 1460-1468

- Baxter, J. D., Rousseau, G. G., Bensen, C. M., Garcea, R. L., Ito, J. & Tompkins, G. M. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1892-1896
- Buller, R. E., Toft, D. O., Schrader, W. T. & O'Malley, B. W. (1975a) *J. Biol. Chem.* **250**, 801-808
- Buller, R. E., Schrader, W. T. & O'Malley, B. W. (1975b) *J. Biol. Chem.* **250**, 809-818
- Burton, K. (1956) *Biochem. J.* **62**, 315-323
- Hamilton, T. H. (1968) *Science* **161**, 649-661
- Jensen, E. V. & DeSombre, E. R. (1972) *Annu. Rev. Biochem.* **41**, 203-230
- King, R. J. B. & Gordon, J. (1972) *Nature (London)* **240**, 185-187
- King, R. J. B. & Mainwaring, W. I. P. (1974) *Steroid-Cell Interactions*, University Park Press, Baltimore
- Kuhn, R. W., Schrader, W. T., Smith, R. G. & O'Malley, B. W. (1975) *J. Biol. Chem.* **250**, 4220-4228
- Litman, R. M. (1968) *J. Biol. Chem.* **243**, 6222-6233
- Lowry, O. H., Rosebrough, N. J., Farr, A. O. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Neelin, J. M. (1968) *Can. J. Biochem.* **46**, 241-247
- O'Malley, B. W. & Means, A. R. (1974) *Science* **183**, 610-620
- O'Malley, B. W., McGuire, W. L., Kohler, P. O. & Korenman, S. G. (1969) *Recent Progr. Horm. Res.* **25**, 105-153
- O'Malley, B. W., Toft, D. O. & Sherman, M. R. (1971) *J. Biol. Chem.* **246**, 1117-1122
- O'Malley, B. W., Spelsberg, T. C., Schrader, W. T., Chytil, F. & Steggles, A. W. (1972) *Nature (London)* **235**, 141-144
- Palmiter, R. D. (1972a) *J. Biol. Chem.* **247**, 6450-6461
- Palmiter, R. D. (1972b) *J. Biol. Chem.* **247**, 6770-6780
- Panyim, S. & Chalkley, R. (1969) *Arch. Biochem. Biophys.* **130**, 337-346
- Pikler, G. M., Webster, R. A., Spelsberg, T. C. (1976) *Biochem. J.* **156**, 399-408
- Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660-672
- Schrader, W. T. & O'Malley, B. W. (1972) *J. Biol. Chem.* **247**, 51-59
- Schrader, W. T., Toft, D. O. & O'Malley, B. W. (1972) *J. Biol. Chem.* **247**, 2401-2407
- Schrader, W. T., Buller, R. E., Kuhn, R. W. & O'Malley, B. W. (1974) *J. Steroid Biochem.* **5**, 989-999
- Schrader, W. T., Heuer, S. S. & O'Malley, B. W. (1975) *Biol. Reprod.* **12**, 134-142
- Spelsberg, T. C. (1974) in *Acidic Proteins of the Nucleus* (Cameron, I. L. & Jeter, J. R., eds.), pp. 247-296, Academic Press, New York
- Spelsberg, T. C. (1976) *Biochem. J.* **156**, 391-398
- Spelsberg, T. C., Steggles, A. W. & O'Malley, B. W. (1971a) *Biochim. Biophys. Acta* **254**, 129-134
- Spelsberg, T. C., Steggles, A. W. & O'Malley, B. W. (1971b) *J. Biol. Chem.* **246**, 4188-4197
- Spelsberg, T. C., Steggles, A. W., Chytil, F. & O'Malley, B. W. (1972) *J. Biol. Chem.* **247**, 1368-1374
- Spelsberg, T. C., Mitchell, W. M., Chytil, F., Wilson, E. M. & O'Malley, B. W. (1973) *Biochim. Biophys. Acta* **312**, 765-778
- Spelsberg, T. C., Webster, R. A. & Pikler, G. M. (1975) in *Chromosomal Proteins and their Role in the Regulation of Gene Expression* (Stein, G. S. & Kleinsmith, L. J., eds.), pp. 153-186, Academic Press, New York

- Steggles, A. W., Spelsberg, T. C., Glasser, S. R. & O'Malley, B. W. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1479-1482
- Sumida, C., Gelly, C. & Pasqualini, J. R. (1974) *C. R. Acad. Sci. Ser. D (Paris)* **279**, 1793-1796
- Williams-Ashman, H. G. & Reddi, A. H. (1971) *Annu. Rev. Physiol.* **33**, 31-82
- Wilson, E. M. & Spelsberg, T. C. (1973) *Biochim. Biophys. Acta* **322**, 145-154
- Yamamoto, K. R. (1974) *J. Biol. Chem.* **249**, 7068-7075
- Yamamoto, K. R. & Alberts, B. (1975) *Cell* **4**, 301-310