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SUPPORT FOR CHROMATIN ACIDIC PROTEINS AS ACCEPTORS FOR PROGESTERONE IN THE CHICK OVIDUCT

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SUMMARY

Studies in this laboratory have supported the role of chromosomal protein–DNA complexes as the nuclear acceptor sites for progesterone in the avian oviduct. The protein coacceptor appears to be a low molecular weight acidic protein(s) which when removed from the DNA results in a marked loss of binding by the activated progesterone-receptor complex. When the protein is reannealed back to the pure DNA, the binding capacity is restored. During studies on these nuclear binding sites for progesterone in the hen oviduct, a seasonal variation in the level and function of the progesterone receptor (P-R) was detected. Cytosol preparations obtained from the chick oviducts during the winter/spring period between January and May display reduced receptor levels as well as a loss of the capacity of the receptor to bind to nuclear "acceptor" sites *in vitro*. The binding of [^{3}H]-P-R to whole chromatin or purified acceptor proteins reannealed to DNA display the same rhythm. No such rhythm is detected for the binding of P-R to pure DNA. The nuclear binding *in vivo*, achieved by injecting [^{3}H]-progesterone into the wing vein and analyzing the radioactivity localized in the oviduct nuclei, also displays a similar rhythm. These results indicate that the native nuclear acceptor sites for progesterone in the chick oviduct are protein-DNA complexes and not pure DNA. The failure of P-R to bind the nuclear acceptor sites *in vivo* and *in vitro* during this period can be explained by the two subunit hypothesis of Schrader and O'Malley, whereby one of the two subunits is absent or inactive during this period.

INTRODUCTION

Steroids enter cells from the vascular system and interact with high affinity and specificity with soluble protein receptors which are found only in target cells of those steroids [1–5]. The interaction of the receptor with the steroid results in binding and nuclear translocation of the receptor (as a complex with the steroid) by an undefined mechanism called "activation". The nuclear binding by steroid receptor complexes has been speculated to be due to diffusion/equilibrium [3, 4, 6] or, more popularly, to the presence of high affinity nuclear sites, called "acceptors", on the chromatin [1, 2, 5, 7–12]. The result of this nuclear binding is an immediate alteration of DNA-dependent RNA synthesis, the expression of messenger RNA, and the translation of these mRNA into proteins [1–5]. Thus, the nuclear interactions of steroid receptor complexes represent the first nuclear event resulting in alterations in gene expression.

Virtually every component in the nucleus has been suggested as the acceptor which binds the S-R. Examples are the nuclear envelope [13, 14], the ribonucleoproteins [15], histone proteins [16, 17], basic nonhistone proteins [8–10], acidic nonhistone proteins [18–20], DNA [21–24], and combinations of acidic proteins and DNA [2, 5, 11, 12, 25–33]. There have also been reports of a specific binding of free steroids (not bound to receptor) to nuclear material [14, 19, 34] suggesting the presence of unbound nuclear receptors. Nevertheless, the chemical identity of the nuclear acceptors has yet to be determined.

This laboratory became interested in the role of nonhistone proteins and DNA in the nuclear acceptor activity for P-R from chick oviduct in 1971 when it was found that these entities displayed the greatest acceptor activity among all the components of chromatin [2,7, 12, 25–33]. The protein fraction containing this acceptor activity (1) is tightly bound to DNA [2, 12, 25–27]; (2) expresses greater activity in target as opposed to non-target tissues [26, 27, 30, 35–37]; (3) requires reannealing to DNA for activity [27, 29]; (4) is destroyed by proteases but not nucleases [2, 25]; (5) behaves like a low molecular weight acidic chromatin protein and not a histone [2, 7, 12, 25, 29, 33]; and (6) using immunofluorescence technique, is found to be localized within the nucleus (nucleoplasm) and not associated with the nuclear envelope or the nucleolus [38]. Interestingly, when the histones and some nonhistones are removed from the chromatin, the levels of acceptor activity are enhanced indicating the presence of repressed sites in intact chromatin [2, 12, 25–30]. Subsequent removal of the remaining acidic protein from the DNA results in a loss of much (but not all) of the "acceptor" activity. The dissociated proteins (called AP_3) which when removed from DNA cause an 80% loss of acceptor activity, also

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restore acceptor activity to the DNA when reannealed back to the DNA [25–27, 30]. This AP_3 fraction, when selectively removed from or reinstated to whole chromatin, causes a loss or restoration respectively of the acceptor activity in that chromatin [25, 27, 30]. This previous work implicated a nonhistone acidic protein–DNA complex as the high affinity nuclear binding component for steroid receptors.

Evidence is presented in this paper for a seasonal variation in the binding of progesterone to these nuclear acceptor sites. This variation was found to correlate with the date on which the receptor preparations were isolated. The seasonal variation in nuclear binding occurs *in vivo* as well as *in vitro*. It occurs when incubating the isolated progesterone–receptor complex with the isolated acceptor protein–DNA complex but not with DNA. These results support the role of nucleoprotein and not pure DNA as the acceptor site for progesterone receptor.

METHODS

The methods utilized in these studies have been reported previously [26–32, 39, 40]. The *in vitro* binding of the isolated progesterone-receptor complex (P-R) from chick oviduct with the nuclear acceptor sites (i.e. the nuclear binding sites for P-R) obtained from hen oviduct have been described elsewhere [12, 26, 27, 40].

Briefly, the P-R is obtained from the 100,000 *g* supernatant of homogenized oviducts of immature chicks. The chicks represent 5-week-old birds injected for 4 weeks with diethylstilbestrol (DES) to obtain fully developed oviducts. The cytosol is labelled with tritiated progesterone during a 2-h incubation and the steroid–receptor complex is partially purified via ammonium sulfate fractionation. The receptor preparations can then be used immediately or stored at −80°C for more than a year [31, 32, 39, 40]. The chromosomal components are from hen oviduct obtained at a slaughter house in Minneapolis, Minnesota. The nuclear chromatin is then extracted with various solvents to remove selective chromosomal protein and RNA fractions from the DNA [12, 28, 29, 32]. This partially deproteinized chromatin, called nucleoprotein (NAP), still contains the acceptor sites for the P-R. The binding assay is carried out using the streptomycin procedure [32] to separate the nuclear acceptors bound with the P-R from the unbound P-R. The bound nucleoproteins are collected on Millipore filters, counted in a liquid scintillation spectrometer, and subsequently the filters are analyzed for content of DNA using the diphenylamine procedure. The counts per minute bound per milligram of DNA are then calculated. The binding to pure DNA (representing the background) is subtracted from the binding to the NAP for measuring the true protein acceptor binding.

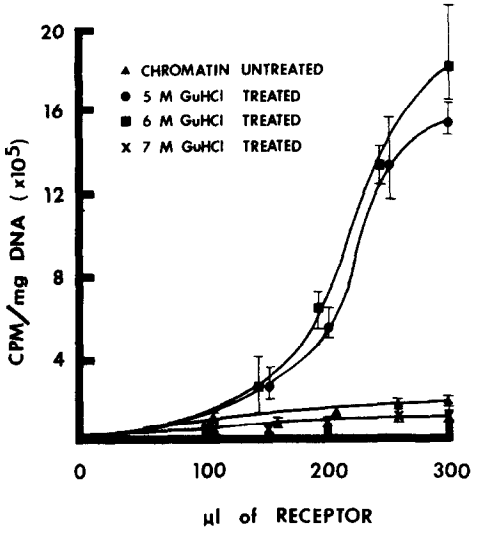

Fig. 1. Comparison of the levels of binding of [^{3}H]-P-receptor to untreated and extracted chromatin. The chromatin and DNA are prepared as described in the Methods. (▲) Represents untreated chromatin. The chromatin was attached to cellulose and extracted with (●) 5 M GuHCl, (■) 6 M GuHCl or (×) 7 M GuHCl as described elsewhere [12]. The residual nucleoprotein was assayed for acceptor activity using the cellulose method as described elsewhere [12, 23, 29, 32]. The mean and S.D. of 5 replicate analyses of binding for each receptor level are given.

RESULTS

As reported previously, 80% of the nuclear "acceptor" sites for progesterone in the chick oviduct are masked [12, 27, 28, 32]. As shown in Fig. 1, these masked sites are "unmasked" when histones and a select fraction of nonhistone proteins are removed from the chromatin resulting in the NAP (residual protein–DNA complex). This binding of the P-R to the NAP is at least 5-fold greater than that to whole chromatin and results in a more sensitive analysis of the nuclear binding capacities of the steroid-receptor complexes [12, 28, 29]. The binding to these previously "masked" acceptor sites *in vitro* requires intact activated receptor bound with progesterone as does binding to the normally "unmasked" sites [11, 12, 28, 32]. Thus, the requirements for *in vivo* nuclear binding of steroid-receptor complexes are found with our *in vitro* conditions [1, 2, 5, 12, 26–32]. The protein co-acceptor has been dissociated from DNA, purified, and reconstituted back to the purified DNA. As shown in Fig. 2, binding to this reconstituted NAP requires the activated receptor and displays the same high capacity for binding P-R as the native (undissociated) NAP. The free [^{3}H]-progesterone and heat denatured [^{3}H]-P-R display little or no binding to the NAP, while activated intact P-R does bind markedly. Figure 3 shows that this acceptor activity in this same fraction of chromosomal protein (using select GuHCl extracts) has an apparent isoelectric point (pI) of ~5 using acrylamide as a media. When the acceptor activity is isolated by another method and solvent, an apparent pI of 6 is observed. Either

more than one species of acceptor protein exists naturally or there is a breakdown of the native species or the different isolation methods result in changes in the apparent pI of this protein. Nevertheless, this acceptor activity appears to be an acidic protein.

Every winter for the past few years, we noticed a loss in the binding of the P-R to our native or reconstituted acceptor protein–DNA complexes. Figure 4 shows the P-R binding to the NAP and DNA using the winter (February) and summer (August) receptor preparations. It can be seen that the NAP binding by the winter receptor is much less than that by the summer receptor. The level of binding to DNA by these receptors is equivalent. Using 600 μl of P-R preparations from 1975 to 1977, the levels of binding to acceptor protein reconstituted to DNA were analyzed (Fig. 5). The data represent binding to the reconstituted NAP over that to pure DNA. A circannual rhythm is substantiated since computer analysis using the method of least squares to fit the data to cosine curves of varying periodicities revealed that ~90% of the points could be fit to a cosine curve (Spelsberg and Halberg, in preparation). The acrophase (peak period of binding) centers in September

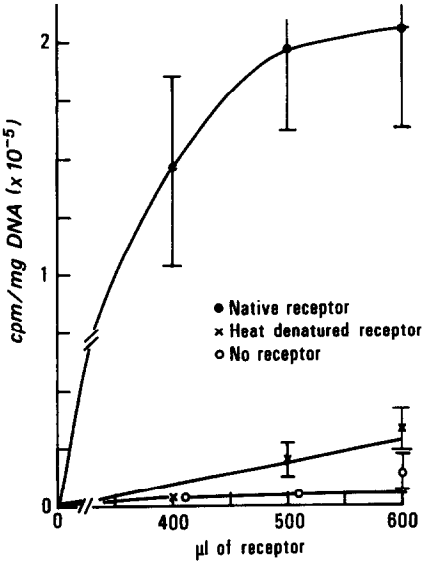

Fig. 2. Binding of [^{3}H]-progesterone to nucleoacidic protein (NAP): Dependence of intact receptor. The P-R was obtained from the oviducts of immature chicks injected with 5 mg diethylstilbestrol/chick/day for 4 weeks as described previously [12, 29, 39]. The receptor was isolated, partially purified by $(NH_4)_2SO_4$ precipitation, and stored at −80°C until needed. The nuclear binding assays were conducted as described previously [12, 28, 29, 32]. The NAP was prepared as described elsewhere [12, 26–29, 32]. (●) Binding to NAP using an intact, activated P-R: (×) binding to NAP using a heat denatured (30 min at 50°C) P-R preparation; (○) binding to NAP using free [^{3}H]-P. All bindings were performed by the streptomycin method as described previously [12, 29, 32]. The nuclear material was collected on Millipore filters and dried. The filters were counted in a scintillation spectrometer using PPO-POPOP-toluene based fluor. The DNA per filter was quantitated and the CPM per mg DNA calculated [12, 31 32].

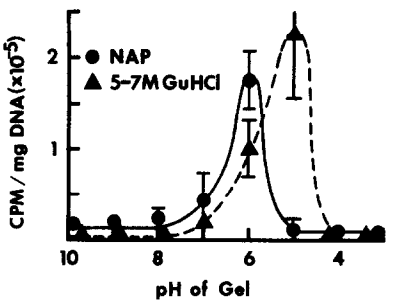

Fig. 3. Isoelectric focusing of acceptor activity in acrylamide gels. The nucleoacidic protein (NAP) was isolated from whole chromatin using 2 M NaCl + 5 M urea (pH 6.0) as described previously [26, 27]. The acceptor protein was isolated using a 7-M GuHCl extract of chromatin previously treated with 5 M GuHCl as described elsewhere [12, 29]. (●) NAP and (▲) 5–7 M GuHCl fractions were resuspended in 6 M GuHCl solution, dialyzed versus 8 M urea solution and then added to the monomer acrylamide solutions containing 5% acrylamide and 5 M urea and ampholines (pH 3–10) from LKB. These solutions were then poured into the slab gel holder, allowed to polymerize and focusing performed at 4°C using 1 mA for 1 h, then at 100 V for 1 h and then at 200 V overnight (14 h). Afterwards the slab gel was frozen and sectioned in 0.3 cm sections. Part of the sections were extracted with 0.01 M NaCl and the pH gradient determined. The remaining sections were pulverized and extracted with 30% formic acid. The extracts were dialyzed versus H_2O, lyophilized, and the protein in each fraction quantitated. The protein was reannealed to DNA and the acceptor activity analyzed as described elsewhere [12, 29]. The binding of [^{3}H]-progesterone receptor complex to these reconstituted NAP is plotted as c.p.m./mg DNA (corrected for DNA binding) versus the average pH of that section. (Knowler and Spelsberg, unpublished results.)

with a bathophase (trough) around April. Figure 6(A) shows similar variations in binding to undissociated NAP. However, there is no such rhythm in the binding to pure DNA (Fig. 6B). Interestingly, the levels of P-R in the cytosol, the amounts of protein in the

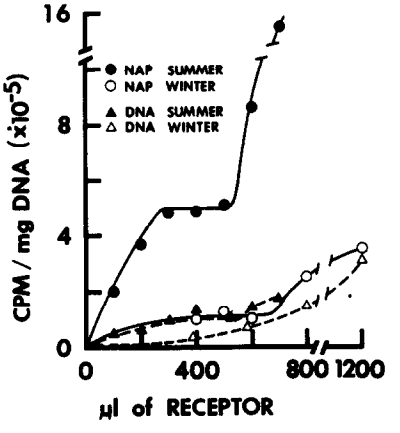

Fig. 4. Binding of two preparations of [^{3}H]-P-R to hen oviduct NAP of DNA. The assays were carried out essentially as described in the Methods and the legend of Fig. 2. (●) Binding to NAP using [^{3}H]-P-R from August; (○) binding to NAP using a [^{3}H]-P-R from February (▲) binding to pure DNA using a [^{3}H]-P-R from August; (△) binding to pure DNA using [^{3}H]-P-R from February.

receptor preparations and oviduct weights display circannual rhythms (Fig. 6C). Therefore, once each year, the oviduct weights and progesterone-receptor levels decrease to about half their usual levels. This is accompanied by a complete loss of the binding of P-R to the nucleoprotein acceptor sites (over that to DNA). As found in the above studies, no rhythm in the binding to pure DNA occurs. The binding to NAP decreases to that of pure DNA during the winter. Thus, our probe (the P-R) for monitoring the purification of the acceptor proteins is varying in amounts and function.

Preliminary analysis of changes in nuclear binding of [^{3}H]-P were performed *in vivo*. Injections of [^{3}H]-P (200 μCi/bird) into the wing vein were followed in 0.5 h by sacrifice of the bird, removal of the oviducts and isolation of the nuclei as described previously [41]. Blood samples were also obtained from the birds. Table 1 shows that while little change occurred in the levels of [^{3}H]-P in the blood, marked changes in the nuclear binding of the steroid *in vivo* occurred during the winter/spring period. Thus, the rhythms of nuclear binding of [^{3}H]-P-R to whole chromatin and to the partially purified acceptor protein (reannealed to DNA) performed *in vitro*, display the same periodicity as that of the native (*in vivo*) nuclear binding. The binding of [^{3}H]-P-R to pure DNA *in vitro* shows no rhythm.

Further studies on the receptors revealed that the seasonal difference in nuclear binding capacities are an indigenous property of the receptor preparations.

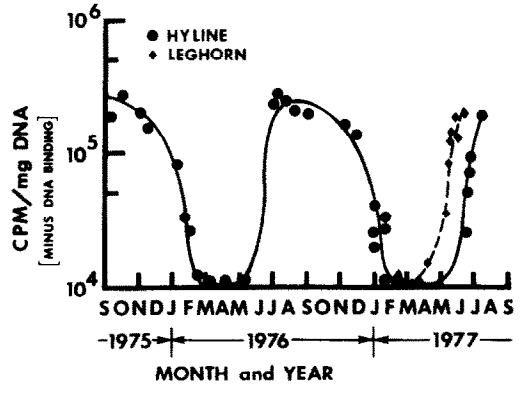

Fig. 5. Binding to reconstituted NAP using [^{3}H]-progesterone receptor preparations isolated at various periods of the year. The binding assays were carried out by both the streptomycin and the cellulose methods [12–28, 29, 32]. The points are plotted with respect to the date of the receptor isolation. The receptor preparations were isolated from both the Leghorn (◆) and Hyline (●) strains of chicks and assayed for NAP and DNA binding as described in the Methods and in the legend of Fig. 2. The receptor preparations were stored as ammonium sulfate precipitates at −80°C for 1–2 days before assaying for nuclear binding. The reconstituted NAP was prepared by reannealing a partially purified acceptor protein to DNA followed by removal of unbound protein as described previously [12, 28, 29, 32]. The ability of each receptor to bind to the NAP is plotted as c.p.m./mg DNA as described in the legend of Fig. 1 except that the binding values were corrected for DNA binding.

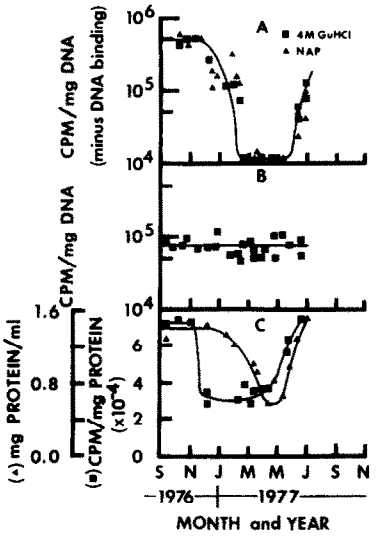

Fig. 6. Binding to DNA and undissociated NAP using [^{3}H]-progesterone receptor preparations isolated throughout the year. The assays were carried out as described in Methods and in the legend of Fig. 2. (A) Binding to undissociated NAP representing residual nucleoprotein after extraction of chromatin with (■) 4.0 M GuHCl, pH 6.0 or (▲) 2.0 M NaCl + 5.0 M urea, pH 6.0. (B) Binding to pure DNA. (C) Levels of (■) [^{3}H]-P-R and (▲) protein in the receptor preparation.

Steroid receptor preparations isolated at various periods of the year were assayed for binding to the same acceptor protein–DNA complex or to whole chromatin. Similar rhythms of binding were observed and were verified as circannual rhythms via cosinor analysis (described above) [42]. Analysis of the progesterone receptor using isoelectric focusing in a Sephadex medium using an LKB Multiphor allowed

Table 1. Seasonal variations in nuclear binding of [^{3}H]-progesterone *in vivo**

	Nuclear binding (c.p.m./mg DNA)	Blood levels (c.p.m./ml)
December	8192 ± 1728	44,000
January	1141 ± 200	40,000
February	475 ± 76	—
March	698 ± 45	39,025
April	1628 ± 193	58,563
May	2125 ± 38	32,752
June/July	5254 ± 394	40,127

* The extent of nuclear binding of [^{3}H]-progesterone ([^{3}H]-P) to fully developed oviducts of immature chicks. DES treated chicks were injected with 200 μCi of [^{3}H]-P in 50 μl (ethanol–H_2O, 1:1) in the wing vein. Evans-Blue dye is included as a marker for the accuracy of the injection. One-half hour after injection, samples of blood were withdrawn from the vein of the other wing, the birds sacrificed and the oviduct quickly excised. The nuclei were then immediately isolated, quantitated for DNA, and the radioactivity in the nuclei and blood measured in a liquid scintillation spectrometer (as described in Methods). The values represent the c.p.m./mg DNA for nuclear binding and the c.p.m./ml for blood levels of the [^{3}H]-P. The nuclear bindings show the mean ± S.D. from three groups. The blood levels show the mean from six samples of blood.

the separation and quantitation of two molecular species (A and B species) of this receptor [45]. These receptor species were first identified by DEAE chromatography by Schrader and O'Malley [39]. Preliminary analysis of the various receptor preparations throughout the year has revealed that the levels of the A receptor species follow a similar pattern as the nuclear binding, that is, markedly decreasing between January and April and then rising between April and June. Loss of this one species of the receptor explains the overall decrease by half of the total receptor level. Further, the correlation between the levels of this receptor species and the degree of binding of the [^{3}H]-P-R to nuclear acceptor sites *in vivo* and *in vitro* suggests that the A receptor species must play a key role in the biological action of progesterone.

DISCUSSION

In the past decade, levels and functions of steroid receptors have largely been considered to be relatively constant. Exceptions are those receptors whose synthesis are known to be dependent on the action of other steroids, such as in the case of the oestrogen dependency of the progesterone receptor [1, 2]. There have been cases identifying the possible mutated or defective receptors in certain cloned cell lines or in the hereditary testicular feminization syndrome in a certain strain of mice [43] wherein the steroid receptor displays either an inability to become activated and/or bind to the nuclear acceptor sites. Although in the latter model, the levels of the androgen receptors in target cells are reduced, the nuclear binding is almost completely abolished. Recently, it has been reported that oestrogen receptors in a variety of animal tissues display a seasonal variation [44]. What these changes in levels of receptor mean in terms of tissue response to the hormones remains undetermined. We have been monitoring for the past 2 years a seasonal variation in the levels and functions of the progesterone receptor in the chick oviduct. Through studies of the nuclear acceptor proteins for the progesterone receptor in the chick oviduct, a seasonal variation in the capacity of the receptor to bind to whole chromatin and to the purified acceptor protein reannealed to DNA was detected. The data presented here represent preliminary results of these rhythms.

Circannual rhythms have been observed for oviduct weight, soluble protein, molecular species of receptor, and receptor levels in the fully developed oviducts of immature chicks treated with oestrogen. Correlating with these rhythms are similar rhythms in the ability of the P-R to bind to nucleoprotein (protein–DNA) but not pure DNA binding sites. As the level of receptor in the low period or bathophase (late winter–early spring) decreases to 50% of the level measured in the high period or acrophase (fall), the P-R loses its capacity to bind to the NAP "acceptor" sites over that to pure DNA. The extent of binding to DNA, although somewhat variable, displays no rhythm. These results suggest that steroid-receptor complexes in target cells are not always at a constant level or function and that changing levels of receptors in target cells may reflect major changes in the functions of the receptor.

Preliminary studies *in vivo* reveal a marked reduction in the nuclear translocation and binding of [^{3}H]-P-R in oviduct of chicks during the late winter (bathophase) as compared to those in the fall (acrophase) [45–47]. Thus, the patterns of nuclear binding observed *in vitro* with the isolated receptor and partially purified nuclear acceptor (NAP) are mimicked *in vivo*. The fact that we observe similar seasonal patterns in nuclear binding both *in vitro* and *in vivo* supports the nativeness of our *in vitro* binding assay and also that the NAP (acceptor protein–DNA complex), not pure DNA, represents the native acceptor site for progesterone-receptor in the chick oviduct.

These results indicate that at one period of a year the chick oviduct is not responsive to progesterone, whereas in other periods of the year it is highly responsive. It is not known currently if this phenomena also occurs in chicks in other climates, in adult hens or in other animals. The biological significance of these rhythms is obscure. It is tempting to relate such rhythms to the reproductive cycles in birds. As a result of selection pressures, birds and many other animals exhibit restricted breeding seasons achieving optimal conditions for reproduction resulting in better survival. In most species of birds, these breeding seasons generally occur once a year. They require the bird to recognize certain clues to predict the oncoming time of year for optimal conditions for reproduction. Physiological studies of these birds (and some mammals) suggest the presence of an endogenous mechanism which controls the circannual rhythms of reproduction [48–51]. In these particular animals, the photoperiod, temperature and other environmental conditions appear to synchronize the annual reproductive cycles. Circannual rhythms in molting, body weight, fat deposition, daily activity, gonadal weight and development, and egg laying are documented [52–54]. In general, once a year adult chicks (hens) will molt (the shedding and renewal of feathers) for a month or more, generally with an accompanying reduction or loss of egg production [55, 56]. The timing of a molt occurs every 12–15 months after hatching even under controlled photoperiods, temperature, humidity, etc. Thus, an endogenous system controlling the molt of domestic fowl is supported.

The results presented here indicate that: (1) steroid-receptor complexes are not constant entities but vary seasonally; (2) although the rhythms in the overall level, the molecular species, and the function of the progesterone receptor may be due to changes in the water or food content, it is equally probable that they are due to an endogenous mechanism or to a physical environmental stimuli (temperature, photoperiod,

etc.); (3) the regulation of steroid hormone action at the receptor level of target cells may explain the seasonal (or stress induced) molt and reproductive patterns of chickens; (4) regulation of the target cell responsiveness to steroids may represent another level of endocrine control in many animals; (5) these apparent endogenous regulations of the molecular species of the progesterone receptor provide an opportunity to study in greater detail the mechanism of action of the two molecular species of the progesterone receptor.

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REFERENCES

1. Jensen E. V. and DeSombre E. R.: Mechanism of action of the female sex hormones. *A. Rev. Biochem.* **41** (1972) 203–230.
2. Spelsberg T. C. and Toft D. O.: The mechanism of action of progesterone. In *Receptors and the Mechanism of Action of Steroid Hormones* (Edited by J. R. Pasqualini). Marcel Dekker, New York Part I (1976) pp. 261–309.
3. Gorski J. and Gannon F.: Current models of steroid hormone action: a critique. *A. Rev. Physiol.* **38** (1976) 425–450.
4. Yamamoto K. R. and Alberts B. M.: Steroid receptors: elements of modulation of eukaryotic transcription. *A. Rev. Biochem.* **45** (1976) 721–746.
5. O'Malley B. W. and Means A. R.: Female steroid hormones and target cell nuclei. The effects of steroid hormones on target cell nuclei are of major importance in the induction of new cell functions. *Science* **183** (1974) 610–620.
6. Williams D. and Gorski J.: Equilibrium binding of estradiol by uterine cell suspensions. *Biochemistry* **13** (1974) 5537–5542.
7. Stein G. S., Spelsberg T. C. and Kleinsmith L. J.: Nonhistone chromosomal proteins and gene regulation. Nonhistone chromosomal proteins may participate in the specific regulation of gene transcription in eukaryotes. *Science* **183** (1974) 817–824.
8. Puca G. A., Sica V. and Nola E.: Identification of a high affinity nuclear acceptor site for estrogen receptor of calf uterus. *Proc. natn Acad. Sci., U.S.A.* **71** (1974) 979–983.
9. Puca G. A., Nola E., Hibner U., Circala G. and Sica V.: Interaction of the estradiol receptor from calf uterus with its nuclear acceptor sites. *J. biol. Chem.* **250** (1975) 6452–6459.
10. Mainwaring W. I. P., Symes E. K. and Higgins S. J.: Nuclear components responsible for the retention of steroid-receptor complexes especially from the standpoint of the specificity of hormone responses. *Biochem. J.* **156** (1976) 129–141.
11. Klyzsejko-Stefanowicz L., Chui J. F., Tsai Y. H. and Hnilica L. S.: Acceptor proteins in rat androgenic tissue chromatin. *Proc. natn Acad. Sci., U.S.A.* **73** (1976) 1954–1958.
12. Spelsberg T. C., Webster R. A., Pikler G. M., Thrall C. L. and Wells D. J.: Nuclear binding sites (acceptors) for progesterone in avian oviduct: characterization of the higest affinity sites. *Ann. N.Y. Acad. Sci.* **286** (1977) 43–63.
13. Jackson V. and Chalkley R.: The binding of oestradiol-17β and the govine endometrial nuclear membrane. *J. biol. Chem.* **249** (1974) 1615–1626.
14. Jackson V. and Chalkley R.: The cytoplasmic estradiol receptors of bovine uterus. Their occurrence, interconversion, and binding properties. *J. biol. Chem.* **249** (1974) 1627–1636.
15. Liao S., Liang T. and Tymoczko J. L.: Ribonucleoprotein binding of steroid-"receptor" complexes. *Nature, New Biol.* **241** (1973) 211–213.
16. Monder C. and Walker M. C.: Interactions between corticosteroids and histones. *Biochemistry* **9** (1970) 2489–2497.
17. Sluyser M.: Interaction of steroid hormones with histones *in vitro*. *Biochem. biophys. Acta* **182** (1969) 235–244.
18. King R. J. B. and Gordon J.: Involvement of DNA in the acceptor mechanism for uterine oestradiol receptor. *Nature, New Biol.* **240** (1972) 185–187.
19. Liang T. and Liao S.: Interaction of oestradiol- and progesterone-receptors with nucleoprotein: heat-labile acceptor factors. *Biochim. biophys. Acta* **277** (1972) 590–594.
20. Defer N., Dastugue B. and Kruh J.: Direct binding of corticosterone in estradiol to rat liver nuclear nonhistone proteins. *Biochimie* **56** (1974) 559–566.
21. Baxter J. D., Rousseau G. G., Bensen M. C., Garcea R. L., Ito J. and Tomkins G. M.: Role of DNA and specific cytoplasmic receptors in glucocorticoid action. *Proc. natn Acad. Sci., U.S.A.* **69** (1972) 1892–1896.
22. Andre J. and Rochefort H.: *In vitro* binding of the oestrogen receptor to DNA: absence of saturation at equilibrium. *FEBS Lett.* **50** (1975) 319–323.
23. Toft D. O.: The interaction of oestrogen receptors with DNA. *J. steroid Biochem.* **3** (1972) 515–522.
24. Yamamoto K. R. and Alberts B. M.: On the specificity of the binding of the estradiol receptor protein to deoxyribonucleic acid. *J. biol. Chem.* **249** (1974) 7076–7086.
25. Spelsberg T. C.: The role of nuclear acidic proteins in binding steroid hormones. In *Acidic Proteins of the Nucleus* (Edited by I. L. Cameron and J. R. Jeter). Academic Press, New York (1974) pp. 247–296.
26. Spelsberg T. C., Steggles A. W. and O'Malley B. W.: Progesterone-binding components of chick oviduct—III. Chromatin acceptor sites. *J. biol. Chem.* **246** (1971) 4188–4197.
27. Spelsberg T. C., Steggles A. W., Chytil F. and O'Malley B. W.: Progesterone-binding components of chick oviduct—V. Exchange of progesterone-binding capacity from target to nontarget tissue chromatins. *J. biol. Chem.* **247** (1972) 1368–1374.
28. Spelsberg T. C., Webster R. A. and Pilker G. M.: Chromosomal proteins regulate steroid binding to chromatin. *Nature, Lond.* **262** (1976) 65–67.
29. Spelsberg T. C., Webster R., Pikler G., Thrall C. and Wells D.: Role of nuclear proteins as high affinity sites ("acceptors") for progesterone in the avian oviduct. *J. steroid Biochem.* **7** (1976) 1091–1101.
30. O'Malley B. W., Spelsberg T. C., Schrader W. T., Chytil F. and Steggles A. W.: Mechanisms of interaction of a hormone-receptor complex with the genome of a eukaryotic target cells. *Nature, Lond.* **235** (1972) 141–144.
31. Pikler G. M., Webster R. A. and Spelsberg T. C.: Nuclear binding of progesterone in hen oviduct. Binding to multiple sites *in vitro*. *Biochem. J.* **156** (1976) 399–408.
32. Webster R. A., Pikler G. M. and Spelsberg T. C.: Nuclear binding of progesterone in hen oviduct. Role of acidic chromatin proteins in high-affinity binding. *Biochem. J.* **156** (1976) 409–418.
33. Spelsberg T. C., Thrall C. L., Webster R. A. and Pikler

G. M.: Isolation and characterization of the nuclear acceptor that binds the progesterone-receptor complex in hen oviduct. *J. Toxicol. Environ. Health* **3** (1977) 309–337.
34. Sumida C., Gelly C. and Pasqualini J. R.: Etude des complexes ^{3}H-oestradiol-macromolecules dans le noyau du rein de foetus de cobaye. Liaison directe de l'oestradiol a la fraction chromatingraph. *C.r. hebd Séanc. Acad. Sci., Paris* Ser. D **279** (1974) 1793–1796.
35. Steggles A., Spelsberg T. C. and O'Malley B. W.: Tissue specific binding *in vitro* of progesterone-receptor to the chromatins of chick tissues. *Biochem. biophys. Res. Commun.* **43** (1971) 20–27.
36. Steggles A. W., Spelsberg T. C., Glasser S. R. and O'Malley B. W.: Soluble complexes between steroid hormones and target-tissue receptors bind specifically to target-tissue chromatin. *Proc. natn Acad. Sci., U.S.A.* **68** (1971) 1479–1482.
37. Spelsberg T. C., Pikler G. M. and Webster R. A.: Progesterone binding to hen oviduct genome: specific versus nonspecific binding. *Science* **194** (1976) 197–199.
38. Chytil F.: Immunochemical characteristics of chromosomal proteins. *Meth. Enzym.* **40** (1975) 191–198.
39. Schrader W. T. and O'Malley B. W.: Progesterone-binding components of chick oviduct—IV. Characterization of purified subunits. *J. biol. Chem.* **247** (1972) 51–59.
40. Schrader W. T., Toft D. O. and O'Malley B. W.: Progesterone-binding protein of chick oviduct—VI. Interaction of purified progesterone-receptor components with nuclear constituents. *J. biol. Chem.* **247** (1972) 2401–2407.
41. Spelsberg T. C.: Nuclear binding of progesterone in the chick oviduct: multiple binding sites *in vivo* and transcriptional response. *Biochem. J.* **156** (1976) 391–398.
42. Spelsberg T. C. and Halberg F. (in preparation).
43. Bardin C. W. and Bullock L. P.: Testicular feminization: studies of the molecular basis of a genetic defect. *J. invest. Derm.* **63** (1974) 75–84.
44. Hughes A., Jacobson H. I., Wagner R. K. and Jungblut P. W.: Ovarian-independent fluctuations of oestradiol receptor levels in mammalian tissues. *Molec. cell. Endocr.* **5** (1976) 379–388.
45. Boyd P. A. and Spelsberg T. C. (in preparation).
46. Boyd P. A. and Spelsberg T. C.: Characterization of ^{3}H-progesterone receptors of chick oviduct using isoelectric focusing. *Annual Endocrine Soc. Meeting* (1978) Abst. No. 519.
47. Spelsberg T. C.: (in preparation).
48. Gwinner E.: Photoperiodic synchonization of circannual rhythm in the European starling (Sturnusvulgaris). *Naturwissenschaften* **64** (1977) 544–45.
49. Davis D. E.: The annual rhythm of fat deposition in wood chucks (*Marmota monax*). *Physiol. Zool.* **40** (1967) 491–402.
50. Follett B. K.: Photoperiodism and seasonal breeding in birds and mammals. In *Control of Ovulation* (Edited by D. B. Crighton, N. B. Haynes, C. R. Foxcroft and G. E. Lamming) Butterworths, London (1978) pp. 267–293.
51. Pengelley E. T.: *Circannual Clocks.* Academic Press, New York (1974).
52. Farner D. S. and Lewis R. A.: Photoperiodism and reproductive cycles in birds. In *Photophysiology* (Edited by A. C. Giese). New York Academy of Science, Vol. 6 (1971) pp. 325–370.
53. Farner D. S. and Follett B. K.: Light and other environmental factors affecting avian reproduction. *J. Anim. Sci.* **25** (1966) 90–118.
54. Winchester C. F.: Growth and development. Seasonal metabolic and endocrine rhythms in the domestic fowl. *Univ. Missouri Agric. Exp. Station Res. Bull.* **315** (1940) 5–52.
55. Card L. E. and Nesheim M. C.: *Poultry Reproduction.* Lea & Febiger, Philadelphia (1972) pp. 29–122.
56. Assenmacher I.: Reproductive endocrinology: the hypothalamo-hypophysial axis. In *Breeding Biology of Birds* (Edited by D. S. Farner). National Academy of Science, Washington (1973) pp. 158–208.