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A REVIEW OF REGULATION OF GENE EXPRESSION BY STEROID HORMONE RECEPTORS

BERT W. O'MALLEY, ROBERT J. SCHWARTZ and WILLIAM T. SCHRADER
Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030, U.S.A.

SUMMARY

We have investigated the manner by which progesterone receptors act to induce initiation of RNA synthesis in a cell free system derived from chick oviduct. A method utilizing rifampicin enabled us to measure the formation of binary initiation complexes between RNA polymerase and chick oviduct chromatin and allowed for the quantitative assessment of RNA chain initiation sites, RNA chain propagation rates, and RNA chain size under conditions which prevent secondary chain reinitiations. Purified progesterone–receptor complex stimulated transcription of oviduct chromatin *in vitro* by promoting an increase of 3000 to 5000 additional sites for RNA chain initiation. These data showed that progesterone receptor can directly increase the number of RNA polymerase initiation sites in the chromatin template in the absence of a detectable change in either the rate of RNA propagation or the size of the RNA product. The stimulatory effect of the purified progesterone receptor was time dependent ($T_{1/2}$ of 15 min) and the concentration of receptor for half maximal stimulation of RNA chain initiation was $\sim 5 \times 10^{-9}$ M, a value which correlates directly with the receptor affinity for binding to chromatin. The stimulatory effect of the purified progesterone receptor appeared to be relatively specific for oviduct chromatin in comparison to non-target tissue chromatins or DNA. Utilizing a copy DNA probe to ovalbumin mRNA, a greater than ten-fold increase in transcription of the ovalbumin gene was detected after the incubation of progesterone receptor with oviduct chromatin. The data presented here show that steroid hormone receptor complexes can directly regulate gene transcription *in vitro* in a manner which mimics the events observed *in vivo* in target cells.

INTRODUCTION

Previous studies from this laboratory have shown that estrogen-mediated growth and differentiation of the chick oviduct involve significant alterations in gene transcription. Competition hybridization experiments have demonstrated qualitative changes in the repetitive sequences of nuclear RNA synthesized during estrogen-mediated differentiation [1]. More recent studies have also demonstrated increased transcription of unique sequence DNA during oviduct growth [2]. Concomitant with steroid-mediated oviduct differentiation, increased levels of nuclear RNA [3], RNA polymerase activity [4, 5], chromatin template capacity, and changes in chromatin nonhistone proteins [6, 7] were also observed in the oviduct. Preceding the well documented specific changes in protein synthesis [8, 9], previous studies have demonstrated an unequivocal net accumulation of specific, biologically active messenger RNA, coding for ovalbumin [10–12].

When estrogen treatment of the chicks is discontinued, a reduction in the overall level of RNA and protein synthesis occurs and the cells ability to synthesize specific proteins such as ovalbumin decreases [10, 11, 13]. If either estrogen or progesterone is re-administered (secondary stimulation) there is a rapid increase in the production of ovalbumin mRNA and the induction of other egg-white proteins begins again [13]. Therefore, the chick oviduct provides a system to study a specific endocrine response in which overall gene expression can be dramatically altered while a specific marker for measuring changes in the transcription rate of a single gene is also available.

To define the mechanism of action of a steroid hormone, it is necessary to examine the process by which biochemical information held by the steroid receptor complex is transferred to the transcriptional apparatus. Of the many possible approaches to this problem, our recent efforts have centered on direct examination of the effects of steroid receptors on chromatin transcription in cell-free systems. Since the initial identification and characterization of the oviduct progesterone receptor by Sherman *et al.* [14] we have sought to obtain this important regulatory molecule in pure form. Our recent success in this venture [15] now allows us to directly assess the effect of a pure steroid hormone–receptor complex on gene expression under cell-free conditions. Initial efforts in our laboratory have been to characterize the interaction of purified RNA polymerase with intact chromatin. The process of initiation of RNA synthesis has received special attention, since this step appears to be regulated by steroids [16]. In this review, we will discuss our recent evidence on the progesterone dependent transcription of chromatin in the chick oviduct.

EXPERIMENTAL AND RESULTS

Formation of initiation complexes on chick DNA and chromatin

The capacity of oviduct chromatin to serve as a template for *E. coli* RNA polymerase has previously

been shown to increase following estrogen administration to the immature chick [7, 17]. While chromatin template activity measurements may generally reflect the amount of DNA sequences made available to RNA polymerase, the components of such a reaction are so complex that these experiments shed little light on the biochemical mechanisms of hormone-induced alterations in gene transcription. In order to determine the effect of steroids on gene transcription in the chick oviduct it appeared necessary to monitor and control all of the parameters involved in RNA synthesis.

Procedures for measuring the initiation of RNA chains *in vitro* were adopted from studies initially carried out in bacterial and bacteriophage systems [18, 19]. The initiation of RNA synthesis can be divided into 2 basic processes. First, RNA polymerase binds randomly and reversibly to DNA to form a series of nonspecific complexes. However, after sufficient time and at a proper temperature RNA polymerase binds proximal to an initiation region and forms a stable binary complex with DNA [20, 21]. This complex has undergone a transition involving the local destabilization of the DNA duplex structure [21], and is now capable of rapidly initiating an RNA chain [22]. The second process is the actual initiation step in which RNA polymerase catalyzes the formation of the first phosphodiester bond between 2 nucleoside triphosphates.

The existence of stable RNA polymerase–DNA initiation complexes was elucidated through the use of the drug rifampicin [24, 25]. Rifampicin is a competitive inhibitor of RNA synthesis which acts prior to the formation of the first phosphodiester bond, but which has no effect on RNA chain elongation. RNA chain initiation by RNA polymerase bound to DNA in a stable initiation complex is so rapid that it can occur in the presence of the drug [23]. The fraction of RNA polymerase in stable complexes can be determined following the simultaneous addition of a mixture of rifampicin and the four ribonucleoside triphosphates [26]. RNA polymerase molecules which are free in solution or randomly bound to DNA will be inhibited by rifampicin. Under these conditions reinitiation of RNA transcription will be completely inhibited and thus each RNA polymerase at a stable initiation site can synthesize one and only one RNA chain. By measuring the number of RNA chains made, the rifampicin challenge assay provides a method for quantitating the number of RNA polymerase initiation sites for a given template [5, 16, 27, 29].

Acute progesterone stimulation of initiation of RNA synthesis

Although incapable of inducing egg-white protein synthesis in the oviduct during primary stimulation, progesterone not only reestablishes ovalbumin protein synthesis but appears generally to mimic estrogen as a secondary stimulant to hormonally-withdrawn chicks [13, 28]. Furthermore, the similarity in the extent of egg-white protein synthesis induced by progesterone suggests that the time course of induction of RNA synthesis during secondary stimulation is similar to estrogen and not influenced by the cytodifferentiation of new tubular gland cells [13]. Therefore, it was of interest to understand the manner by which progesterone can substitute for estrogen in hormone-withdrawn chicks.

Estrogen-treated chicks were withdrawn from hormone treatment for 12 days and then restimulated with a single injection of progesterone (2.0 mg). Oviduct chromatin was isolated following secondary stimulation and assayed by the rifampicin-challenge technique. The number of initiated RNA chains was calculated as described above. As shown in Table 1, withdrawn chromatin had the capacity to support the initiation of 8600 RNA chains/pg of DNA. Following a single injection of progesterone, a rapid increase in the number of initiation sites was found. Within a half-hour of hormone treatment, the number of initiation sites had nearly doubled to a level of 15,900 sites. After 1 h of progesterone stimulation a maximum of 23,000 initiation sites was detected. There-

Table 1. The effect of progesterone administration *in vivo* on RNA polymerase transcription of withdrawn oviduct chromatin *in vitro*

Hours after progesterone administration*	Size of RNA product (nucleotides)	Initial elongation rate (nucleotides/s)†	pmol of RNA chains initiated/5 μg chromatin‡	Initiation sites/pg DNA
0	820	7.2	0.072	8,600
0.5	730	7.0	0.132	15,900
1.0	750	7.8	0.191	23,000
2.0	750	6.7	0.134	16,100
6.0	800	8.0	0.123	14,800
24.0	750	8.0	0.112	13,500

* Chicks were stimulated for 12 days with DES, withdrawn from hormone treatment for 10 days, and then injected with 2 mg of progesterone.

† Initial elongation rate was determined for 1 min of chain propagation as previously described [16].

‡ The amount of initiated chains was calculated from the total nucleotides incorporated at the transition point of RNA polymerase titration curves assuming that RNA contained 25% UMP, and divided by the number average chain size of 770 nucleotides as described [16].

Table 2. Effect of the combined administration of progesterone and diethylstilbestrol on initiation sites for RNA synthesis in oviduct chromatin

Dose of hormone*	RNA chains initiated (pmol/5 μg DNA)	Initiation sites/pg DNA†
Control	0.076	9100
0.25 mg Progesterone	0.135	16,200
1.25 mg Progesterone	0.187	22,500
2.0 mg Progesterone	0.196	23,600
1.25 mg DES	0.191	23,000
1.25 mg Progesterone + 1.25 mg DES	0.180	21,600

* Chicks were stimulated for 12 days with DES, withdrawn from hormone treatment for 10 days and then injected with various doses of hormone.

† The amount of initiated chain was calculated as described in the legend to Table 1.

after, the number of initiation sites declined so that by 24 h after progesterone administration, 13,500 sites were observed. During this time of intense transcriptional activity the parameters of RNA chain propagation rate (~7.5 nucleotides/s) and the number average chain length of the RNA product (~770 nucleotides) did not vary significantly from withdrawn oviduct chromatin (Table 1). Therefore, the progesterone-induced increase in chromatin transcription in the oviduct was mainly due to a modulation in the number of available RNA polymerase initiation sites.

To investigate further the mechanism by which progesterone augments increased chromatin transcription in withdrawn chicks, we tested the possibility that nuclear progesterone receptors acted at sites on chromatin which were identical to those affected by estrogen. Withdrawn chicks were given single injections containing increasing doses of progesterone. Chromatin was then isolated from oviduct tissue at 1 h following restimulation and assayed for the number of RNA polymerase initiation sites (Table 2). The number of initiation sites increased in a dose-dependent fashion from a control value of about 9100 (sites per pg DNA) to a maximum value of ~22,500 sites at a dose of 1.25 mg of progesterone. We have previously reported a dose-dependent estrogen stimulation of RNA polymerase initiation sites [27]. In the present experiments, a maximal response occurred 1 h after the *in vivo* injection of 1.25 mg of DES and resulted in the formation of ~23,000 initiation sites (Table 2). Thus the same dose of either steroid was effective in stimulating the same number of initiation sites.

We then tested the possibility that these steroids induced the initiation of transcription at the same sites. Both DES (1.25 mg) and progesterone (1.25 mg) were administered together to withdrawn chicks. If estrogen and progesterone acted at different chromatin RNA initiation sites then the stimulation of RNA synthesis by the two hormones would be additive. The results in Table 2 show that the number of RNA chain initiations was not additive when both hormones were simultaneously administered. These data suggest that in the withdrawn chick oviduct, estrogen and progesterone may act at similar chromatin sites to enhance transcription.

A quantitative measure of the intracellular levels of ovalbumin mRNA was obtained by hybridizing [^{3}H]-DNA complementary to ovalbumin mRNA ($cDNA_{ov}$) with RNA extracted from chick oviducts. From the kinetics of the $cDNA_{ov}$–$mRNA_{ov}$ reassociation reaction at various RNA concentrations, the $mRNA_{ov}$ concentration in the extract can be determined [30, 31, 41]. In withdrawn chicks there is little or no detectable synthesis of ovalbumin protein and only 10 ovalbumin mRNA molecules were present per tubular gland cell (Table 3). Within 4 h after a single injection of progesterone (2 mg) to withdrawn chicks, we observed an accumulation of 1200 additional ovalbumin messenger RNA molecules per gland cell in the oviduct. The number of messenger RNA molecules continued to increase reaching a level of 5400 molecules by 24 h as shown in Table 3. Thus, like estrogen, progesterone induces accumulation of ovalbumin mRNA in oviduct cells of withdrawn chicks. This increase in the level of ovalbumin mRNA follows temporarily the steroid-induced increase in chromatin initiation sites for RNA synthesis. These experiments demonstrate that during secondary hormonal stimulation of withdrawn chicks, progesterone is an adequate substitute for estrogen in respect to its effect on gene expression.

The purified progesterone receptor stimulates initiation of transcription of oviduct chromatin in vitro

During the course of investigating the mechanism of action of steroid hormone we have identified the

Table 3. Effect of progesterone administration on levels of ovalbumin mRNA content in oviducts of withdrawn chicks

Hours after progesterone administration	Number of molecules $mRNA_{ov}$/ tubular gland cell
0	5–10
4	1200
10	2400
24	5400

progesterone receptor proteins in the chick oviduct [12, 32, 33]. We have recently purified the cytoplasmic progesterone receptor to homogeneity [15]. The receptor appears to function by complexing with progesterone in the cytoplasm followed by translocation of the steroid–receptor complex to the nuclear compartment [32, 34, 35]. The steroid–receptor complex binds then to a limited number of nuclear acceptor sites which are present in greater numbers in oviduct nuclei than in nontarget cell nuclei [35]. Although the subsequent events are not yet completely defined, stimulation of nuclear RNA synthesis occurs and leads to mRNA synthesis and ultimately to the induction of synthesis of cell-specific proteins. Since an increase in RNA chain initiation sites was detected within 30 min of progesterone administration to withdrawn chicks (Table 1), these results appeared to be consistent with our hypothesis that steroid–receptor complexes enter target cell nuclei and modulate gene transcription. Nevertheless, there has previously been no proof that the steroid receptor complex could act directly on nuclear chromatin to stimulate transcription. Toward this end, we tested the effect of purified progesterone–receptor complexes on all parameters of transcription using a reconstituted cell-free system which contained the following purified components: progesterone–receptor complex, *E. coli* RNA polymerase, ribonucleotides, cofactors, heparin and chromatin from withdrawn chicks [37].

A fixed concentration of withdrawn oviduct chromatin [5 μg] was preincubated with increasing quantities of purified progesterone–receptor complex (up to 1.1×10^{-8} M) for 30 min at 22°C. The cytoplasmic progesterone receptor complex contains mainly 6S dimers which are composed of A (80,000 MW) and B (117,000 MW) subunits. The intact 6*S* dimer was purified by the affinity chromatography procedure of Kuhn *et al.* [15]. The chromatin receptor complexes were next incubated for an additional 30 min with a saturating concentration of RNA polymerase (15 μg) to allow for the formation of stable initiation complexes. Finally, rifampicin, heparin and nucleotides were added as before for a 15 min period of RNA synthesis. RNA synthesis in the presence of rifampicin increased in a receptor dose-dependent manner in which the half maximal stimulation occurred at a progesterone receptor concentration of 0.5×10^{-8} M [Fig. 1]. In this experiment, RNA synthesis was stimulated to a maximum of ~75% over control values at 1.1×10^{-8} M of purified progesterone–receptor complex. Shown also in Fig. 1, are results of an experiment in which the progesterone receptor was presaturated with nonradioactive progesterone prior to application to the affinity column used to purify the molecule. Under these conditions all available steroid–receptor binding sites were saturated with progesterone and thus the progesterone receptor was prevented from subsequently interacting with the steroid which was covalently linked to the affinity resin. The cluted material from the affinity preparation which was pretreated with an excess of progesterone did not contain any detectable [^{3}H]-progesterone–receptor complex and was also incapable of causing any increase in RNA chain initiation over those values obtained by control chromatins. This experiment strongly indicated that the stimulation in chromatin transcription was due to the progesterone receptor and not to a minor contaminant which was nonspecifically bound to the affinity column.

We have previously reported that crude ammonium sulfate precipitates of cytosol fractions of eucaryotic cells can often spuriously stimulate RNA synthesis by a template independent process which is unrelated to hormone receptor [38]. To rule out such an effect in the present study, we investigated the sensitivity of rifampicin-resistant incorporation of [^{3}H]-UMP into RNA under various conditions. Table 4 shows that RNA synthesized in the presence of receptor and chromatin was completely dependent upon template and inhibited by actinomycin D. Furthermore, none of the components alone contained significant synthetic activity, and hence neither the chromatin, polymerase or receptor were contaminated with other enzymes which were capable of incorporating

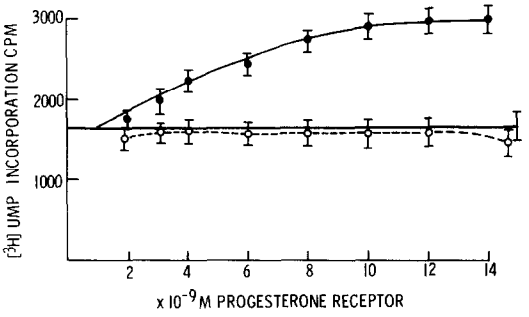

Fig. 1. Effect of purified progesterone–receptor complexes on oviduct chromatin RNA synthesis *in vitro*. Chick oviduct ammonium sulfate cytosol [15] was divided into two parts. Progesterone was added to one half (○---○), while the other half remained untreated (●---●). Both fractions were separately incubated with a deoxycorticosterone hemmisuccinate B.S.A. Sepharose affinity resin and eluted as described [16]. The eluted fraction pretreated with progesterone did not contain any progesterone receptors while the untreated fraction contained progesterone receptor (1.5×10^{-8} M). Various amounts of the eluted fractions in 10 mM Tris–HCl (pH 7.4), 1 mM Na_2EDTA, 12 mM 1-thioglycerol and 50 mM KCl were added in a maximum vol. of 100–25 μl of a withdrawn oviduct chromatin suspension (5 μg of DNA) and incubated for 30 min at 22°C. A saturating amount of *E. coli* RNA polymerase (15 μg) was then added together with the following components with 20 μmol Tris–HCl (pH 7.9), 1.0 μmol $MnCl_2$, 0.5 μmol β-mercaptoethanol and 100 μg B.S.A. and incubated for an additional 30 min at 22°C. At the end of the preincubation period, RNA synthesis was started by the addition of 37.5 nmol of ATP, GTP, CTP, and UTP and 10 μCi [^{3}H]-UTP, 10 μg of rifampicin, 0.1 μmol potassium phosphate (pH 7.0) and 200 μg heparin in a final volume of 250 μl for 15 min at 37°C. Synthesized RNA was precipitated with 5% trichloroacetic acid, collected on glass fiber filters and counted in scintillation fluid at an efficiency of 19.5%. The straight line represents the level of constitutive RNA synthesis in the absence of added receptors.

Table 4. Effect of progesterone–receptor complexes on RNA synthesis from oviduct chromatin *in vitro*

Components added to assay	[^{3}H]-UMP incorporated into RNA/5 μg chromatin DNA* c.p.m.	% activity
Background:		
RNA polymerase only	230	8
Progesterone–receptor complex plus RNA polymerase	400	14
Chromatin alone	200	7
Control:		
Chromatin plus RNA polymerase	2730	100
plus boiled progesterone–receptor complex	2860	105
plus 10^{-8} M progesterone alone	2430	89
Experimental:		
Chromatin plus RNA polymerase plus 10^{-8} M Progesterone–receptor complex	4100	150
plus α-Amanitin (10 μg)	3740	137
plus Actinomycin D (10 μg)	320	12

* RNA synthesis was assayed in the presence of nucleotides and rifampicin as described in Fig. 1.

[^{3}H]-UTP into acid insoluble material. In these experiments, the purified progesterone receptor preincubated with chromatin in the presence of *E. coli* RNA polymerase increased rifampicin-resistant RNA synthesis 50% over control values. With the addition of α-amanitin, a potent inhibitor of oviduct RNA polymerase II, there was little effect on the progesterone-receptor directed stimulation of RNA transcription. Thus the stimulation of RNA synthesis was not due to the activation of endogenous RNA polymerase in the chromatin preparations but rather was due to transcription by the added *E. coli* enzyme. Nevertheless, it should be noted that receptor-mediated stimulation of RNA synthesis also occurred in the presence of exogenously added oviduct RNA polymerase II [39]. Moreover, Table 4 also shows that the increased RNA synthesis was dependent upon the native structure of the hormone–receptor complex since neither free progesterone nor boiled receptor was effective in stimulating RNA synthesis. Importantly, the purified progesterone receptor does not contain any detectable proteolytic or nuclease activity which would spuriously affect the chromatin template [37]. Thus, the stimulation of [^{3}H]-UMP incorporation is derived through a chromatin template-dependent process which is directly mediated by an intact progesterone–receptor complex.

Quantitation of RNA initiation sites induced by receptor–steroid complexes was determined by measuring the total incorporation of nucleotides in the presence of rifampicin and dividing this value by the number average chain length of the RNA product. From the data in Table 5, it was calculated that withdrawn chromatin, in this experiment, had the capacity to code for 10,000 RNA chains per pg of DNA. After an *in vitro* incubation with progesterone–receptor complexes, the capacity to initiate RNA synthesis was increased to 15,200 sites at 1×10^{-8} M receptor. There were no significant differences in either the size of the RNA product or in the initial rate of elongation. Thus, the purified receptor preparation had stimulated the RNA initiation events, rather than the steps required for RNA chain elongation.

The stimulatory effect of the progesterone receptor on RNA chain initiation on chromatin was next verified by a totally independent assay. Quantitation of the number of 5′-termini present in the population of nascent RNA chains was measured by the incorporation of γ[^{32}P]-GTP into RNA [48]. We have previously shown that 57% of the initial 5′-tetraphosphate nucleosides (A + G) from the RNA synthesized from oviduct chromatin, is guanosine [16]. As shown in Table 6 the number of 5′-tetraphosphate nucleosides incorporated into nascent RNA chains also in-

Table 5. Effect of progesterone receptor on the size and rate of RNA chain synthesis on oviduct chromatin

Concentration of progesterone receptor	Total incorporation of nucleotide* (pmol)	Initial elongation rate† (nucleotides/s)	Number average chain size‡ (nucleotides)	RNA chains initiated (pmol/5 μg) chromatin DNA	Initiation sites/pg DNA
No receptor	62	7.9	739	0.083	10,000
1×10^{-8} M	91	7.8	720	0.126	15,200

* RNA was synthesized in the presence of rifampicin and nucleotides as in Fig. 1 and corrected for base composition of RNA assuming 25% UMP.

† Measured in first min of synthesis.

‡ Measured by sucrose gradient analysis of RNA synthesized in 15 min.

Table 6. Effect of progesterone receptor on the incorporation of $\gamma[^{32}P]$-GTP at the 5'-end of rifampicin resistant RNA chains

Treatment	[^{32}P]-GTP (pmol)*	5' Termini (pmol)†	RNA chains/pg of DNA
Control	0.048 ± 0.002	0.086	10,300
1×10^{-8} M Progesterone receptor	0.073 ± 0.003	0.131	15,700

* Withdrawn chick oviduct chromatin (5 μg) and 1×10^{-8} M progesterone receptor complex were preincubated for 30 min at 22°C. RNA polymerase (15 μg) and the assay component described previously in Fig. 1 were added for an additional 30 min. RNA synthesis was then started by the addition of 85.2 μCi of $\gamma[^{32}P]$GTP (5000 c.p.m./pmol), together with nucleotides, rifampicin and heparin. After 10 s at 37°C the transcription reaction was stopped by pipetting a 125 μl sample on DEAE filter paper and the filters were washed according to the procedure of Roeder [47]. The incorporated $\gamma[^{32}P]$-GTP was identified as guanosine tetraphosphate after degradation of the RNA product with alkali [37, 48].

† Total number of initiated chains was estimated by the value of guanosine 5'-tetraphosphate nucleosides multiplied by 1.8, a factor for total chain starts.

creased 50% in the presence of progesterone receptor. The incorporation of $\gamma[^{32}P]$-GTP into the 5'-termini of RNA substantiates our observations that the progesterone receptor stimulates initiation of transcription in the cell-free assay by increasing the number of chromatin initiation sites for RNA synthesis available to RNA polymerase.

The kinetics for the stimulation of chromatin transcription *in vitro* revealed a $T_{1/2}$ of 15 min [37]. This value is close to the optimal time required for receptor binding to chromatin at 22°C and similar to the kinetics of receptor appearance in nuclei *in vivo* and *in vitro* following progesterone administration [32, 35]. A maximal increase in transcription was found to occur within 30 min after the addition of receptor. Previous studies have demonstrated a quantitative tissue specificity for binding of oviduct progesterone receptor complexes to nuclei and to chromatin [35, 40].

We also investigated the ability of the receptor to modify the transcriptional response of various chromatins. At receptor concentrations which produced maximal stimulation, there was an increase of 4700 initiation sites per pg of DNA in oviduct chromatin but only 750 and 300 additional receptor-induced sites in liver and erythrocyte chromatin respectively. It thus appears that the progesterone receptor-mediated effect is at least partly dependent on the presence of tissue-specific proteins in the oviduct chromatin. These proteins may be related to the nonhistone chromosomal proteins which convey quantitative tissue specificity for receptor binding and comprise a vital part of the chromatin 'acceptor sites' for steroid hormone–receptor binding [6, 36].

The progesterone receptor stimulates synthesis of ovalbumin mRNA sequences from chromatin in vitro

The data presented above strongly support our proposal that direct transcriptional control is the primary locus of steroid hormone action. Since progesterone can induce ovalbumin in withdrawn chicks, it was conceivable that the purified progesterone receptors could induce transcription of the ovalbumin gene in isolated oviduct chromatin. Such a demonstration would strongly reaffirm the notion that the *in vitro* receptor–chromatin interaction mimics the events *in vivo*. To test this assumption we transcribed oviduct chromatin *in vitro* in the presence of bacterial RNA polymerase. The RNA synthesized was isolated and reacted with [^{3}H]-cDNA$_{ov}$ [41]. We have previously reported that chromatin from withdrawn chick oviduct was a poor template for the *in vitro* synthesis of ovalbumin mRNA [41]. However, within 2 h after a single *in vivo*

Table 7. *In vitro* synthesis of ovalbumin mRNA from chick oviduct chromatin in the absence or presence of purified progesterone–receptor complexes

Source of chromatin	Progesterone receptor (1×10^{-8} M)	Chromatin in reaction (μg DNA)	RNA synthesized (μg)	Percent mRNA$_{ov}$ in RNA	pg of mRNA synthesized ($\times 10^{-3}$)	pg mRNA$_{ov}$/μg DNA
Withdrawn oviduct	–	400	125	0.0015	1.9	0–4.8
Withdrawn oviduct	+	400	135	0.015	20.9	50.0

Chromatin was preincubated with progesterone receptor for 30 min at 22°C. Bulk RNA synthesis was performed at 22°C as described by Chang *et al.* [31]. The purified RNA was hybridized with cDNA$_{ov}$ (1.5 μg as described previously [41]).

injection of progesterone to withdrawn chicks, oviduct chromatin was capable of supporting the synthesis of ovalbumin mRNA sequences [31]. These results implied that the ovalbumin gene in chromatin is inaccessible to RNA polymerase or 'repressed' following hormone withdrawal and prior to hormonal stimulation. In contrast, stimulation by steroid hormones alters the chromatin template of the oviduct target cells in such a manner that the ovalbumin gene is 'open' or available to be transcribed by RNA polymerase.

To extend these studies further, we asked whether the purified progesterone receptor complex could directly stimulate the transcription of the ovalbumin gene in a cell-free system. The results of this experiment are shown in Table 7. Bulk amounts of RNA were synthesized from both withdrawn chromatin and from withdrawn chromatin incubated in the presence of progesterone receptor (1×10^{-8} M). Both RNA preparations were assayed for complementary sequences to [^{3}H]-cDNA$_{ov}$. The RNA synthesized in the presence of receptor-hormone complex contained at least a 10-fold enrichment of mRNA sequence as compared to that found in untreated, withdrawn chromatin controls [29]. It thus appears that a steroid–receptor complex may act directly on chromatin to both enhance the number of initiation sites for RNA synthesis and also to induce the synthesis of specific mRNAs for induced proteins.

DISCUSSION

The mechanism of steroid hormone action may be related to the distinctive properties of the progesterone receptor [42, 43]. Our current working hypothesis for progesterone action in chick oviduct is summarized in Fig. 2. The progesterone receptor is a dimer composed of A and B subunits which have different and unique properties. The intact dimer (6*S*) appears to be located in the cytoplasm of the target cell in the absence of hormone stimulation and translocates to the nuclear compartment upon administration of progesterone. Both the A and B subunits bind a molecule of hormone. The B subunit binds to the nonhistone protein–DNA 'acceptor sites' of oviduct chromatin but not to pure DNA, while the A subunit binds to pure DNA, but poorly to chromatin [33, 42]. Accordingly these observations have led to the suggestion that the A subunit could be the actual gene regulatory protein and the B subunit could specify where in chromatin the A protein is to localize [33, 42]. In the absence of the B component of the dimers, the A subunit alone should encounter difficulty in locating the specific initiation sites [genes] it is to regulate, while the B subunit alone should be totally inactive as a transcriptional stimulant. Consistent with these predictions, we have found that purified A subunit protein [44] was capable of stimulating transcription on withdrawn chromatin, but only at much higher concentrations (~10–50-fold) than that required for the intact dimer [45]. The isolated B subunit [46] was ineffective in stimulating transcription from oviduct chromatin at any concentration tested [45]. These observations are consistent with a model in which one part of the progesterone receptor dimer (B-subunit) acts as a binding site specifier to localize the dimer in certain regions of chromatin, while the DNA-binding subunit (A-subunit) is effective in destabilizing a portion of the chromatin

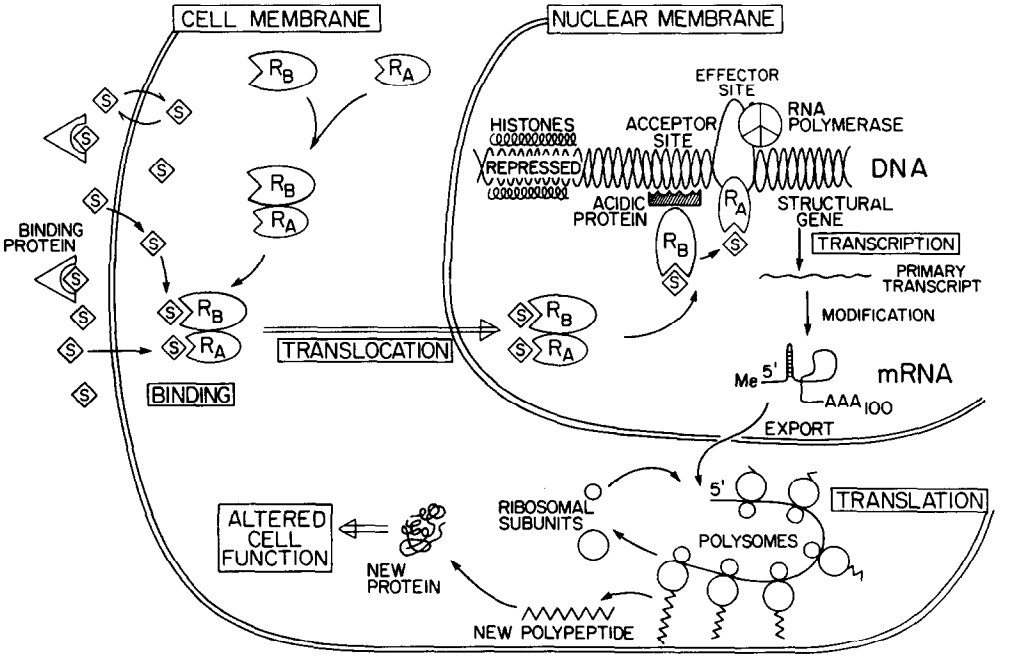

Fig. 2. A hypothetical mechanism for steroid hormone action in the chick oviduct.

DNA so that RNA polymerase can initiate RNA synthesis. Following this process, the translation of these nascent, hormone-induced mRNAs provides the intracellular proteins responsible for mediating the target cell functions inherent to the steroid hormone (Fig. 2).

In summary, the results presented in this manuscript are inconsistent with models of steroid hormone action which postulate a requirement for RNA or protein intermediates to be induced which then exert secondary or feedback effects on transcription. Rather, the data presented here support our proposal that direct, positive regulation of nuclear gene transcription is a viable mechanism for steroid hormone action in eucaryotic cells.

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DISCUSSION

Rousseau. In the estradiol-withdrawn chicks, is ovalbumin produced by the oviduct in response to progesterone alone, as expected from the effect of progesterone receptor *in vitro*?

O'Malley. Yes you get just as great a quantitative response as with estrogen.

Tuohimaa. Assuming that a steroid-specific transcription is required for ovalbumin and avidin synthesis and

that this is a limiting step of the protein synthesis, I would like to know how do you explain the recent finding of Sharma *et al.* published in *Nature* (**259** (1976) 588) which shows that ovalbumin can be induced by a nonsteroidal compound, ethione. Similarly, our results show that avidin can be induced by several nonspecific factors. We can induce avidin in an immature chick oviduct by transcription blockers, like actinomycin-D, without progesterone administration (Elo *et al. Molec. Cell. Endocr.* **2** (1975) 203). In addition, avidin can be induced by a mechanical trauma or prostaglandin F2α. How do these results fit to the hypothesis of transcription regulation?

O'Malley. We have seen no induction to these agents.

Pasqualini. What is the optimal condition to stabilize the receptor protein before binding to the tritiated progesterone?

O'Malley. We just use nothing special. We need about 0.05 to 0.1 molar salt, neutral pH, a little DTP.

Notides. Your work, Dr. O'Malley, is impressive. When you isolate the A and B subunits separately can you recombine them and see the effect on transcription? Also, why do you think both subunits bind progesterone when progesterone-binding by one of the subunits could be sufficient for receptor activation?

O'Malley. I cannot answer your question but I might remind you that the lac repressor contains 4 identical subunits and each must bind one molecule of inducer for activation.

Clark. This is an elegant work and I think it should be pointed out that five years ago everyone would have agreed that it would not be possible to do these experiments in 1976. I predict in five more years Colonel Sanders will be serving cloned chicken breasts.