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Steroid Hormones Differentially Induce Transcription of the Chicken Ovalbumin Gene, but Stabilize the mRNA with the Same Half-Life[1]

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The stabilization of chicken ovalbumin (OVA) mRNA by different classes of steroid hormones (estrogen, progesterone, glucocorticoid, and androgen) was studied in the oviducts of chicks treated with combinations of four steroids. The combination of estrogen with progesterone, glucocorticoid, or androgen enhanced the induction of the OVA gene more than did estrogen alone. Run-on analysis of the isolated oviduct nuclei to measure the transcription rate of the OVA gene showed that the enhanced induction of the OVA gene by the combined hormone treatments was partly caused by an increased rate of transcription. The half-life of OVA mRNA as determined using a transcription inhibitor (actinomycin D) was estimated to be about 24 h irrespective of the hormone treatment, though the half-life was about 6 h in the absence of hormones. These results suggested that the prolongation of the half-life of OVA mRNA by steroid hormones is constant irrespective of differential transcription rates of the OVA gene.

Key words: mRNA stability, nuclear receptor, post-transcriptional regulation, steroid hormones.

Gene expression is regulated at both the transcriptional and post-transcriptional levels (*1, 2*). Post-transcriptional regulation occurs at various steps, such as nuclear processing of hnRNA (*3*), mRNA transport from the nucleus to cytoplasm (*4*), and the metabolic control of cytoplasmic mRNA. A number of recent studies with post-transcriptional regulation have shown that the half-lives of some mRNAs are modified in response to various physiological conditions (*5-8*).

Steroid hormones regulate gene expression at the transcriptional and post-transcriptional levels (*1*). Transcriptional control of target genes by steroid hormones is generally accepted to mediate specific nuclear receptors which act as hormone-inducible transcription factors by binding to hormone response elements in the promoters of target genes (*9-12*). On the other hand, there is little understanding of the molecular mechanism by which these steroid hormones regulate the half-lives of the mRNAs (*13-18*). Moreover, though several lines of study have suggested the involvement of the nuclear receptors in mRNA turnover, it is still unclear at the molecular level whether the receptors are indeed associated with the control of mRNA stability in the cytosol (*19, 20*). Recent observations indicate that most species of steroid hormone receptors are principally localized in the nucleus.

To clarify the control by steroid hormones of the mRNA stability, we studied whether the nuclear receptors controlling transcription are indeed involved in the regulation of mRNA stability. We selected the gene for ovalbumin (OVA), a major egg white protein, which is expressed only in the oviduct of laying avians. Four classes of steroid hormones (estrogen, progesterone, glucocorticoid, and androgen) are known to activate cooperatively the expression of the OVA gene in the estrogen-induced chick oviducts (*21-23*). This induction by different classes of steroid hormones is speculated to be achieved by both transcriptional and post-transcriptional regulation, though only some aspects of transcriptional regulation have been clarified at the molecular level (*24, 25*). Previous studies showed that estrogen and progesterone prolonged the half-life of OVA mRNA from 6 to 24 h (*26-28*). In the present study, we examined whether the four classes of steroid hormones regulate the stabilization of OVA mRNA alone or in combination. We found that the half-life of OVA mRNA was prolonged by all steroid hormones, and neither an additive nor a synergistic effect upon OVA mRNA turnover was seen irrespective of the combination of these hormones, though the transcription of OVA gene was differentially regulated by hormone treatments. Thus, these findings suggested that the post-transcriptional regulation of OVA mRNA by steroid hormones is independent of the

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Abbreviations: OVA, ovalbumin; DES, diethylstilbestrol; PRO, progesterone; DEX, dexamethasone; DHT, dihydrotestosterone; CaM, calmodulin.

nuclear receptor-mediated transcriptional event.

MATERIALS AND METHODS

Animals and Treatments—Estrogen was administered to 3-day-old Dekalb white leghorn chicks by the subcutaneous implantation of a silicone tube (Dow Corning, MI) containing 35 mg of a synthetic estrogen, diethylstilbestrol (DES), for 14 days (primary stimulation). Withdrawal was achieved by removing the tube for 5 days. Secondary stimulation was performed by intramuscular injection of various steroid hormones [2 mg/chick of DES, progesterone (PRO), a synthetic glucocorticoid, dexamethasone (DEX), or dihydrotestosterone (DHT)] dissolved in propyleneglycol alone or combination as indicated in the figures (*28*). To inhibit the OVA gene expression, actinomycin D (3 mg/kg body weight) was given by intramuscular injection 32 h after secondary stimulation (*28*). Animals were killed at the times indicated in the figures and the magnum portion of oviducts was excised for analyses.

RNA Extraction and Quantification of mRNA—Total RNA was extracted from the oviducts by the acid guanidinium thiocyanate-phenol-chloroform method (*29*). To estimate the amounts of OVA mRNA, total RNA for each sample was blotted onto a nylon membrane (Gene Screen; NEN Du Pont, MA) using a slot blotting apparatus (*27*). The membrane was hybridized with a ^{32}P-labeled cDNA probe as previously described (*30*). Accuracy of quantification of OVA mRNA by slot blot analysis was confirmed by Northern blot analysis as previously described (*27*). The ^{32}P-labeled cDNA probes [OVA cDNA from the seventh exon (*31*), and calmodulin (CaM) cDNA from the first two-thirds of the coding sequence (*32*)] were prepared by random primer labeling (*30*). To obtained quantitative data on OVA mRNA (more than five samples), densitometric analysis of the CaM transcript on the autoradiograms was used as an internal control (*27*).

Nuclear Run-On Assay—Nuclei were isolated and run-on transcription assay was performed as described previously (*33*). Frozen oviduct was homogenized and centrifuged at 90,000×*g* at 4°C for 90 min. The nuclei were stored at −80°C until analysis. Isolated nuclei containing 30 μg of DNA were incubated at 32°C for 30 min in reaction mixture containing 25 μCi of [α-^{32}P]CTP (650 Ci/mmol; ICN). The reaction was terminated with DNase I and Proteinase K. RNA was extracted with phenol-chloroform (1:1) and precipitated by trichloroacetic acid. Radiolabeled RNA (5×10^6 cpm) was hybridized with cDNA plasmids [the full length cDNA for OVA (*34*) and the cDNA of CaM (*32*)] fixed on the membrane. The filters were exposed to X-ray film at −80°C. The relative rate of OVA transcription was measured by densitometric analysis of autoradiograms, and is shown after normalizing with respect to the level of CaM transcript (*28*).

RESULTS

The OVA Gene Was Induced by Different Classes of Steroid Hormones in Chick Oviducts—It is well established that the expression of the OVA gene is controlled by four

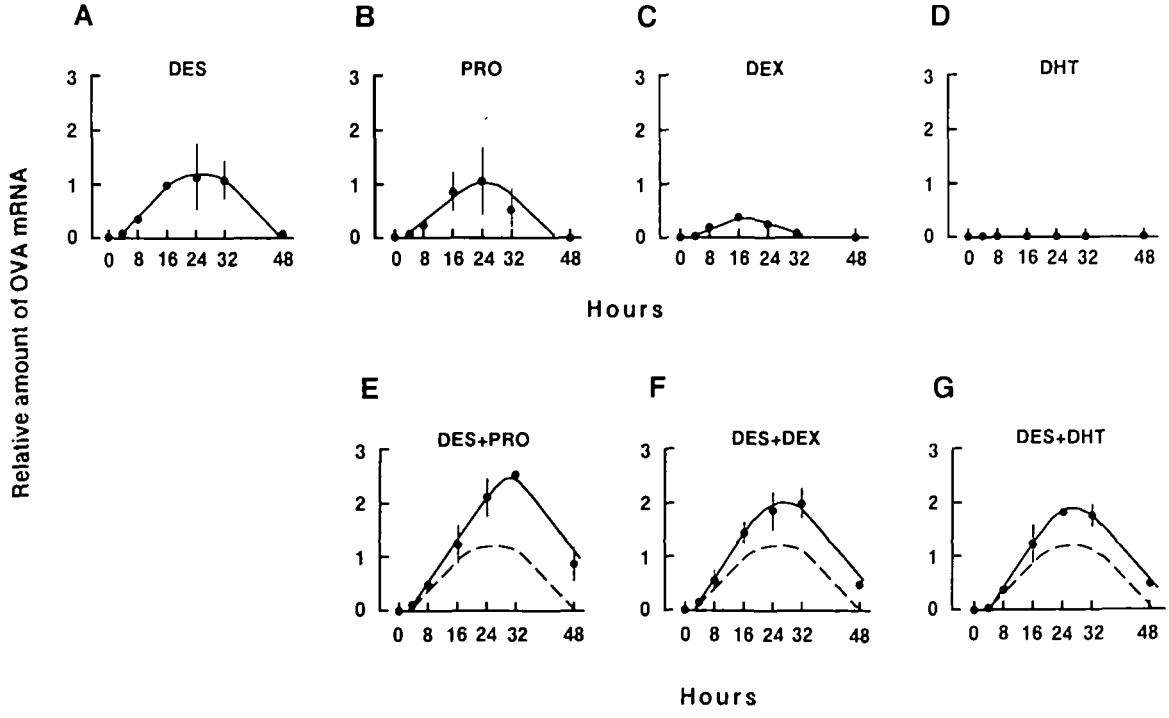

Fig. 1. **Effects of various steroid hormones on the accumulation of OVA mRNA in chick oviduct.** The chicks were given a single intramuscular injection of DES (A), PRO (B), DEX (C), DHT (D), DES plus PRO (E), DES plus DEX (F), or DES plus DHT (G) at a dosage of 2 mg each. At the indicated times, hormone-treated chicks (five birds/group) were killed and total RNA was isolated from the oviducts. OVA mRNA was quantified by slot-blot hybridization with 10 μg of total RNA. The relative amounts of OVA mRNA estimated from densitometric analyses are shown after normalization with respect to the CaM transcript as an internal control. We compared the accumulation of the OVA mRNA in the DES-treated chicks (A) with that in chicks given various hormones (E), (F), and (G); dotted lines shown in panels (E), (F), and (G) indicate DES alone (A).

distinct classes of steroid hormones (estrogen, progesterone, glucocorticoid, and androgen) in chick oviducts (*21-23*). However, there is little information as to how these steroid hormones regulate the stabilization of OVA mRNA. Therefore we focused on the effects of these steroid hormones on post-transcriptional regulation.

Withdrawn chicks were given a single intramuscular injection of 2 mg of steroid hormones (DES, PRO, DEX, or DHT) alone or in combination as indicated in Fig. 1. DES and PRO caused the linear accumulation of OVA mRNA for at least 16 h, and the maximum level was reached at 24 h (Fig. 1, A and B). The combination of DES and PRO enhanced the induction of the OVA gene more than the single treatment (Fig. 1E). The single injection of DEX hardly induced the accumulation of the OVA gene transcript (Fig. 1C), and no induction by DHT was observed (Fig. 1D), in agreement with previous observations (*22, 23*). However, combined treatment with DEX or DHT and DES increased the accumulation of OVA mRNA as compared with DES alone (Fig. 1, F and G).

Transcription Rate of the OVA Gene Induced by Steroid Hormones—Using a nuclear run-on analysis with the oviduct nuclei of the treated chicks, we measured the transcription rate of the OVA gene. These experiments continued for up to 16 h after hormone injection, because during this period the OVA mRNA accumulated linearly in the presence of any hormone tested, as shown in Fig. 1. Representative results of the run-on analysis are shown in Fig. 2 and the relative values are indicated in Table I, when the transcription rate of the OVA gene in chicks treated with DES for 8 h is taken as 1. Transcription of the OVA gene was quickly activated at 2 h and then remained almost constant until 16 h with any hormonal treatment, in agreement with the previous observations (*23, 26*). Furthermore, additive effects were seen when the chicks were treated with DES and any other hormone (PRO, DEX, or DHT) (Fig. 2 and Table I).

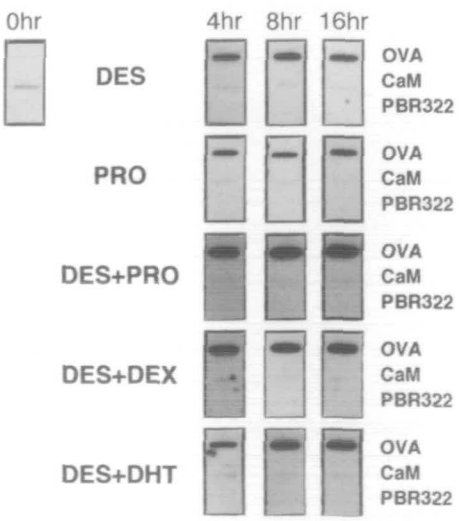

Fig. 2. **Autoradiograms of the nuclear run-on analysis of OVA gene in the hormone-treated chicks.** Chicks were treated with various steroid hormones for 0, 4, 8, and 16 h, and nuclei were prepared. *In vitro* transcription was carried out as described in "MATERIALS AND METHODS." The parent vector (PBR322) was used as a negative control.

Steroid Hormones Stabilized the OVA mRNA, but the Prolongation of the Half-Life of the mRNA Was the Same Regardless of Hormone Treatment—The half-lives of OVA mRNA stabilized by various hormones were estimated by comparison with the half-life in the chicks treated with DES. A single injection of DES prolongs the half-life of OVA mRNA from 7 to 24 h (*26, 27, 35*). The results in Fig. 1 and Table I show the relative mRNA amounts and transcription rates, when those in the DES-treated chicks 8 h after the dose are taken as 1. The accumulation curves of OVA mRNA were the same in all groups in comparison with the transcription rates. Namely, the ratios of the relative mRNA amounts divided by the relative transcription rates in the hormone-treated chicks at the indicated times were

TABLE I. **Transcription rates of the OVA gene in hormone-treated chicks.** Autoradiograms of run-on analysis were scanned by densitometry. Values are shown after normalization with respect to the transcription rates of CaM as an internal control. The results are expressed as relative amounts compared with those DES-treated chicks 8 h after the dose. Results are means ± SD of five birds.

Treatment	Relative transcription rate (mean ± SD)			
	0 h	4 h	8 h	16 h
DES	0.00	0.99 ± 0.27	1.00 ± 0.39	1.08 ± 0.12
PRO	0.00	0.76 ± 0.28	0.72 ± 0.19	0.76 ± 0.21
DES + PRO	0.00	1.37 ± 0.88	1.48 ± 0.46	1.48 ± 0.43
DES + DEX	0.00	1.37 ± 0.13	1.33 ± 0.21	1.38 ± 0.22
DES + DHT	0.00	0.70 ± 0.12	1.37 ± 0.52	1.38 ± 0.52

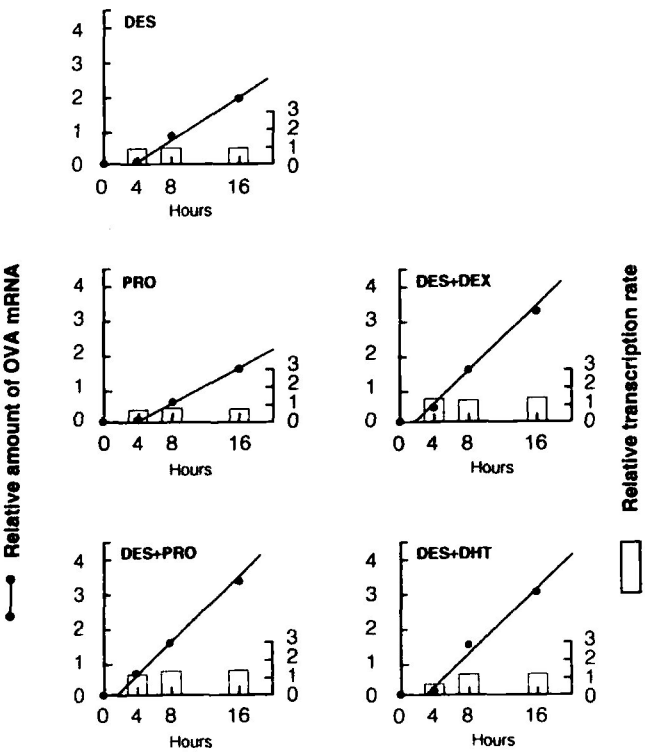

Fig. 3. **Effects of various steroid hormones on the half-lives of OVA mRNA.** The rates of transcription (Table I) and the amounts of OVA mRNA in the hormone-treated chicks are shown. The amounts of OVA mRNA were quantified by slot-blot hybridization and calculated as described in the legend to Fig. 1. The values are expressed as relative amounts compared with those in DES-treated chicks 8 h after the dose.

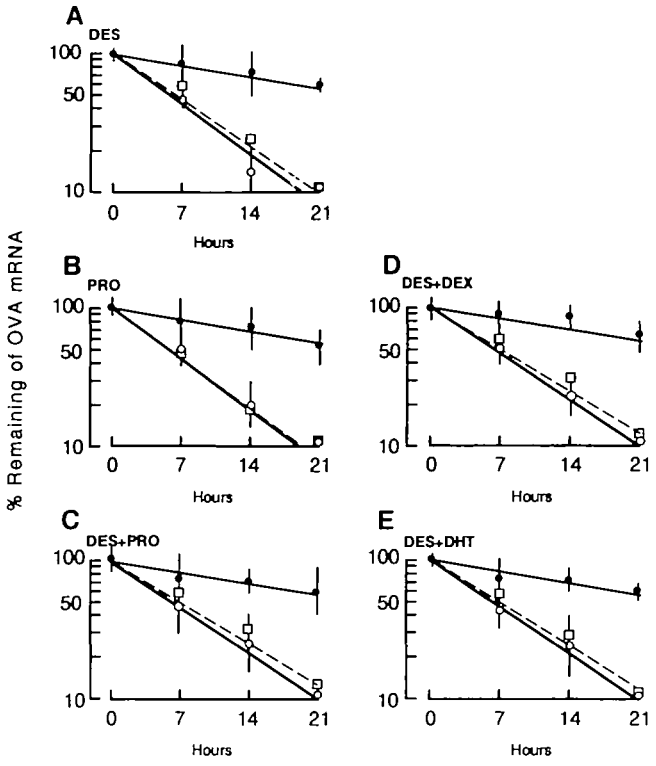

Fig. 4. **The amounts of OVA mRNA after inhibition of RNA synthesis by actinomycin D.** Chicks were injected with various hormones (DES, PRO, DES+PRO, DES+DEX, and DES+DHT). After 24 h, the chicks were treated with (●, ○) or without (□) actinomycin D. A group of the chicks (●) was simultaneously given steroid hormones. At the indicated times, chicks were killed (five birds/group) and RNA was extracted from the magnum portion of oviducts. The amounts of OVA mRNA were quantified by slot-blot hybridization analysis and calculated as described in the legend to Fig. 1.

almost identical (Fig. 3). It is therefore likely that the OVA mRNA is stabilized by any of the hormones to the same extent as by DES. To determine directly the half-life of OVA mRNA of the treated chicks, a transcription inhibitor, actinomycin D was used. In preliminary experiments, we found that this drug blocks the transcription of the OVA gene within 2 h. The amount of OVA mRNA declined slowly when the transcription was inhibited, allowing calculation of the half-life of OVA mRNA in the treated chicks. As shown in Fig. 4, A–E, a similar pattern of mRNA decay was observed in the chicks given any hormone treatment as compared to that in the case of DES treatment, and hence the half-life of OVA mRNA in all the treated chicks appeared to be 24 h. On the other hand, the half-life of OVA mRNA was decreased to about 6 h by hormone withdrawal in the absence (dotted line) or presence of actinomycin D (open circle). Thus, these results excluded the possibility that the stabilization of OVA mRNA by steroid hormone in the presence of actinomycin D is simply due to the effect of actinomycin D.

These results suggested that the increased effects of combined steroid hormones on the accumulation of OVA mRNA are mainly caused by the increase of the transcription rates, but not by the prolongation of the half-life of OVA mRNA.

DISCUSSION

Steroid hormones regulate gene expression at the transcriptional and post-transcriptional levels. Transcriptional regulation has been well characterized, giving rise to the notion that this regulation is controlled through nuclear steroid hormone receptors (*9–12*). On the other hand, there is little explanation of how the regulation of turnover of mRNAs by steroid hormones is controlled at the molecular level. Moreover, it remains unclear whether nuclear hormone receptors are associated with a post-transcriptional event.

We have shown here that various steroid hormones regulate the expression of the OVA gene at the transcriptional and post-transcriptional levels. We found that the increased accumulation of the OVA mRNA induced by combinations of these hormones is mainly regulated at the transcriptional level, and that the half-life of OVA mRNA is prolonged, but to the same extent, by any combination of steroid hormones examined. Even when all four hormones were given, the half-life of OVA mRNA was prolonged no more than by estrogen alone (Fig. 4).

One possible explanation for the stabilization of the OVA mRNA by various steroid hormones is that the loading of the mRNA on ribosomes protects it against degradation (*15*), because ligand-specific stabilization of the OVA mRNA was not evident under our conditions. As the estrogen-induced stabilization of apo VLDLII mRNA in chick liver was shown to be associated with ribosome density (*36*), OVA mRNA may be stabilized by steroid hormones in a similar manner. It would be interesting to examine how the ribosome loading and the density of ribosomes associated with OVA mRNA are affected by hormones.

The factors bound to the 3′-untranslated region (UTR) of mRNAs control the stability by protecting the mRNAs from, or by interacting with, the ribonucleases involved in mRNA turnover. For example, the mRNA of the transferrin receptor is stabilized by the binding of an iron-dependent factor to its 3′-UTR (*5, 6*), whereas the mRNA binding factor specific for the AUUUA sequence causes rapid degradation of the mRNAs of oncogene and cytokine genes, which have several AU-rich sequences in the 3′-UTRs (*37*). In *Xenopus* liver, estrogen induces the binding of a factor to the 3′-UTR of vitellogenin mRNA (*38*). As estrogen prolongs the half-life of the vitellogenin mRNA, it was speculated that this factor is involved in the mRNA turnover. Therefore, using several *in vitro*-translated 5′ and 3′-UTR fragments of the OVA mRNA, we tried to identify a cytosolic mRNA-binding factor. However, no clear binding activity was detected in the cytosols of the chicks given various hormones (data not shown).

Our recent findings suggested that the estrogen-induced stabilization of OVA mRNA is independent of the estrogen receptor-mediated transcription (*28*). In the present study, we observed the combined effects of other steroid hormones with estrogen on the transcriptional control, but there was no further modification of OVA mRNA stability. Overall, it seems unlikely that the nuclear receptors of PRO, DEX, and DHT are involved in steroid hormone-induced stabilization of OVA mRNA. Recent studies by other groups have suggested the possible existence of a cell

membrane receptor for steroid hormone receptor. Matsuda *et al.* (*39*) reported that the estrogen binds to c-erbB, which is tyrosine kinase type membrane receptor, and activates signal transduction through this receptor. Furthermore, Skoufos and Sanders (*40*) reported that phorbol ester inhibited the OVA mRNA stabilization by steroid hormone in the chick oviduct, indicating that the pathway, which is activated by this agent through protein kinase C (PKC), is associated with this regulation. One possibility is that the degradation enzyme(s) for the OVA mRNA is activated, and/or a factor(s) to protect against the degradation is inactivated by phosphorylation through PKC. Though direct evidence that phosphorylation regulates the activity of such factors has not yet been provided, it is important to test this possibility to identify the putative factor(s) and/or enzyme(s) that is phosphorylated by PKC and is responsible for the turnover of OVA mRNA.

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