Note
Steroid Hormone-induced Expression of the Chicken Ovalbumin Gene and the Levels of Nuclear Steroid Hormone Receptors in Chick Oviduct

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Received July 10, 1995

The induction of the chicken ovalbumin (OVA) gene by different classes of steroid hormones and the mRNA levels of estrogen (ER), progesterone (PR), and glucocorticoid (GR) receptors were studied in chick oviducts. Combined treatment with two hormones increased the induction of the OVA gene more than single treatment, when the levels of OVA mRNA were measured with Slot blot analysis. To discover the role of nuclear steroid hormone receptors as transcriptional factors in the OVA gene induction, we analyzed the levels of ER (with RT-PCR), PR, and GR mRNAs (with Northern blotting). The level of PR mRNA was increased only by estrogen, while no steroid hormone affected the levels of ER and GR mRNAs. Thus, these findings show that the levels of nuclear receptors do not reflect the OVA mRNA level in the oviduct of steroid hormone-treated chicks.

Key words: transcriptional factor; nuclear receptor; steroid hormone; ovalbumin mRNA; chick oviduct

The gene expression of ovalbumin (OVA), a major egg white protein, is strictly controlled under estrogen and other steroid hormones (progesterone, glucocorticoid, and androgen) in chicken oviduct tubular gland cells. Especially, estrogen plays a primary role in OVA gene expression. Furthermore, four classes of these steroid hormones cooperatively activate the expression of the OVA gene, and this regulation mainly occurs at the transcriptional level. It is generally accepted that transcriptional regulation by steroid hormones is controlled by nuclear receptors, which belong to a nuclear receptor superfamily and act as ligand-inducible transcriptional factors, binding to a hormone responsive element in the 5′ or 3′ flanking regulatory regions of target genes. Therefore, the level of the nuclear hormone receptor, like many transcriptional factors, may reflect steroid hormone-induced transcription. For example, in Xenopus liver, estrogen drastically induces the expression of the vitellogenin (VT) gene, which is a precursor of egg yolk protein, and during this induction the mRNA level of Xenopus estrogen receptor (ER) is up-regulated. Furthermore, Corthesy et al. suggested that the mRNA level of ER is correlated with the level of the VIT mRNA in the liver of estrogen-treated Xenopus.

In this study, we analyzed the correlation between the mRNA levels of nuclear hormone receptors and the level of the OVA mRNA in the oviduct of steroid hormone-treated chicks. Figure 1 indicates the effects of various steroid hormones on the induction of the OVA gene in estrogen-induced chick oviducts estimated by Slot blot analysis. Chicks that had been withdrawn for 5 days from primary estrogenic stimulation were given a single intramuscular injection of 2 mg of each steroid hormones (a synthetic estrogen, diethylstilbestrol (DES), progesterone (PRO), a synthetic glucocorticoid, dexamethasone (DEX), or dihydrotestosterone (DHT) dissolved in propylene glycol alone or combination). After 16 h, the chicks were killed and total RNA was extracted by the AGPC method. The relative amounts of OVA mRNA were measured by Slot blot analysis and hybridized with [32P]labeled OVA cDNA. We used densitometry of autoradiograms of the CaM mRNA as an internal control. Treatment with DES, PRO, or DEX alone induced the accumulation of OVA mRNA, while DHT had no effect. Furthermore, combined treatment with those hormones (DES + PRO, DES + DEX, and DES + DHT) increased the induction of the OVA gene more than a single hormone treatment (Fig. 1).

Additive effects of these hormones on the accumulation of OVA mRNA are mainly caused by increasing the level of transcription, but not by the prolonged half-life of OVA mRNA (Y.A., N. Miyatake, H. Ushiku, E.Y., A. Matsuura, Y. N., S. M., T.H.).

Abbreviations: OVA, ovalbumin; ER, estrogen receptor; PR, progesterone receptor; GR, glucocorticoid receptor; DES, diethylstilbestrol; PRO, progesterone; DEX, dexamethasone; DHT, dihydrotestosterone; CaM, calmodulin; RT-PCR, reverse transcription polymerase chain reaction; AGPC, acid guanidinium-phenol-chloroform.

Fig. 1. Differential Induction of the Chicken Ovalbumin Gene by Four Distinct Classes of Steroid Hormones.

Chicks were killed 16h after the dose of various steroid hormones and the total RNA was isolated by AGPC method from five birds for each treatment. OVA mRNA was analyzed by Slot blot analysis. Total RNA (2 μg) was put onto a nylon membrane and hybridized with OVA or CaM cDNAs. Only representative results of the OVA and CaM mRNAs are shown in (A). The relative abundance of the transcript estimated from densitometric analysis is shown after normalization with the CaM mRNA in (B) and expressed as the mean ± S.D. of more than five samples from different chicks. ND, not detectable.

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and S.K., unpublished results). Then we analyzed the amounts of steroid hormone receptors [estrogen receptor (ER), progesterone receptor (PR), and glucocorticoid receptor (GR)] which play a central role in ligand-inducible transcription. However, little information is available about the gene expression of ER in chick oviduct. Because of the low level of ER mRNA in chick oviduct, we couldn't detect the transcript of ER by Northern blot analysis. Therefore, we have developed a sensitive assay using reverse transcription-polymerase chain reaction (RT-PCR) to measure the low levels of ER transcript.\(^{18}\) The PCR product was detected by hybridization with the \(^{32}\)P labeled full-length of chicken ER cDNA.\(^{19}\) Using this RT-PCR method, we analyzed the relative amount of ER mRNA. Data was expressed by normalization with \(\beta\)-actin mRNA.\(^{18}\) As shown in Fig. 2, the level of ER mRNA was constant among the chicks treated with various steroid hormones. When chicks were treated with two distinct hormones, the mRNA levels of OVA were increased additively (Fig. 1). However, the level of ER mRNA did not increase in proportion to the levels of OVA mRNA.

The relative level of the progesterone receptor (PR) transcript was measured by Northern blot analysis.\(^{20}\) Fractionated poly(A)+ RNA was hybridized with \(^{32}\)P labeled full length of chicken PR cDNA.\(^{21}\) Data were normalized with CaM mRNA. The amount of PR mRNA was increased about two fold by DES, but not by PRO or the other hormones (Figs. 3A and 3B). This result was in agreement with the previous observations,\(^{22,23}\) in which only estrogen, out of four steroid hormones, induces the PR gene expression. Thus, the increased rates of OVA gene transcription by the combined treatment of DES with another hormone may be due, at least in part, to the increased level of the PR.

In the same way, the levels of GR mRNA were estimated by Northern blot analysis.\(^{24}\) As shown in Figs. 3A and 3C, no significant modulation of GR gene expression by steroid hormones was observed.

These results indicated that the mRNA levels of ER, PR, and GR did not reflect the OVA mRNA levels.

Generally, in transcriptional control of gene promoters, many transcriptional factors are involved in positive and negative ways.\(^{25,26}\) Furthermore, synergism among these factors bound upon a certain promoter is well known.\(^{27,28}\) Though no synergism among the nuclear receptors was found on the OVA gene induction in this study. For the chicken OVA gene promoter, in

The transcripts of PR and GR in chicks at 16 h after the dose were analyzed by Northern blot analysis. Thirty \(\mu\)g of poly(A)+ RNA was separated on 1% agarose-formaldehyde gel. RNA was transferred onto a nylon membrane and hybridized with \(^{32}\)P labeled chicken PR, human GR, or chicken CaM cDNA. Blots were exposed to X-ray film for 12 h (PR, CaM) or 10 days (GR). Only representative results are shown in (A). The relative abundance of the transcripts of PR (B) and GR (C) estimated from densitometric analysis are shown after normalization with the CaM mRNA. These results are expressed as the mean ± S.D. of more than five samples from different chicks.

### Fig. 2. The Levels of ER mRNA in Chick Oviduct Was Constant Irrespective of Steroid Hormone Treatments.

The transcript of ER in chicks at 16 h after the dose was analyzed by RT-PCR method. ER and \(\beta\)-actin cDNAs were generated from total RNA using specific primers for ER and \(\beta\)-actin. Specific cDNAs were amplified by PCR (24 cycles for ER, 19 cycles for \(\beta\)-actin). PCR products were electrophoresed in 1% agarose gel and transferred onto a nylon membrane. Blots were hybridized with \(^{32}\)P labeled chicken ER and mouse \(\beta\)-actin. Only representative results are shown in (A). The relative abundance of the transcript estimated from densitometric analysis is shown after normalization with the \(\beta\)-actin mRNA in (B) and expressed as the mean ± S.D. of more than five samples from different chicks.

### Fig. 3. The Levels of PR and GR mRNAs Were not Positively Regulated by Respective Ligands.

The transcripts of PR and GR in chicks at 16 h after the dose were analyzed by Northern blot analysis. Thirty \(\mu\)g of poly(A)+ RNA was separated on 1% agarose-formaldehyde gel. RNA was transferred onto a nylon membrane and hybridized with \(^{32}\)P labeled chicken PR, human GR, or chicken CaM cDNA. Blots were exposed to X-ray film for 12 h (PR, CaM) or 10 days (GR). Only representative results are shown in (A). The relative abundance of the transcripts of PR (B) and GR (C) estimated from densitometric analysis are shown after normalization with the CaM mRNA. These results are expressed as the mean ± S.D. of more than five samples from different chicks.

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addition to nuclear receptors, the involvement of other transcriptional factors is suggested from previous reports. Our results, showing no clear relationship between the amounts of the nuclear receptors and the effects of hormones on OVA gene induction, imply that the induction of the chicken OVA gene by steroid hormones may be due to cooperative functions of the nuclear receptors with the other transcriptional factors, which have not been identified so far. Furthermore, the co-factors, which modulate the transactivation function of the nuclear receptors without binding to the gene promoter, are supposed to be involved in this induction. To understand the induction of the chicken OVA gene at the molecular level, the identification and characterization of transcriptional factors as well as co-factors involved in the function of the OVA promoter are thus clearly required.

Acknowledgments. We thank Professor P. Chambon for the generous gifts of chicken OVA, ER, PR, and human GR cDNAs, Dr. C.D. Rasmussen for the generous gift of chicken CaM cDNA, and Dr. K. Agata for the generous gift of mouse β-actin cDNA. We thank Mr. Y. Umehara for technical assistance. This work was supported in part by a grant from the Hayashida Foundation of Tokyo University of Agriculture (S.K.) and by a Grant-in-Aid for Priority Areas (No. 05273220) from the Ministry of Education, Science, and Culture of Japan (S.K.).

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