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Modulation of Vitellogenin II Gene by Estradiol and Progesterone in the Japanese Quail

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Estrogen and progesterone receptors are reported to functionally cooperate in gene activation if their cognate binding sites are close to one another in the gene. Our studies show that the expression of the vitellogenin (VTG) gene is induced by estradiol alone or along with progesterone. Progesterone alone inhibits the expression completely. Methylation status of the VTG gene remains unaltered by steroid hormones. Gel mobility shift assay shows that qualitative and quantitative changes occur in the *trans*-acting factor(s) that bind to estradiol and progesterone responsive elements (ERE and PRE) after administration of these steroid hormones. We, therefore, conclude that the interaction of *trans*-acting factors that bind to ERE and PRE play a role in the regulation of VTG gene expression.

Vitellogenin (VTG) is a precursor of egg yolk proteins, phosvitin and lipovitellin. It is synthesized in the liver of mature egg-laying vertebrates (1,2). Upon administration of 17β-estradiol, males and immature chickens also synthesize VTG which is subsequently secreted from the liver into the blood stream (3). The hormone-dependent synthesis of VTG thus offers an excellent model system to study the regulation of transcription of a specific gene by steroid hormones in terminally differentiated cells.

The VTG II gene promoter has two TATA boxes at −26 and −59. There are also two CAAT boxes, at −68 and −100 (4). There are several other potential *cis*-acting elements in the promoter and coding region of the gene. Three sequence elements resembling estrogen responsive element (ERE) are present around −620, −348 and −292 in the promoter region (5). The consensus ERE at −620 is 5′-GGTCANNNTGACC-3′. The two EREs at −292 and −348 are imperfect, and the ERE at −292 is apparently non-functional (6,7). A progesterone responsive element (PRE) is located between −610 to −590, a few nucleotides down-stream from the perfect ERE at −620 (8). A -CCGG- sequence is present between the consensus ERE and PRE. The true ERE and PRE have been shown to act synergistically when placed upstream from the TK promoter (9). However, such synergism between ERE and PRE *in vivo* has not been shown. Kraus et al (10) have shown by cell culture and transfection studies that PR can act as a potent ligand-dependent repressor of ER activity.

Here, we report the changes in the level of *trans*-acting factors that bind to ERE and PRE of the VTG gene of the bird, Japanese quail, after administration of estradiol and progesterone. ERE and PRE are shown to act as transcriptional regulators for the expression of VTG gene.

MATERIALS AND METHODS

Animal. Japanese quails (*Coturnix coturnix japonica*) were used for the study. The female begins to lay eggs from 8–10 weeks of age. Peak laying occurs between 20–30 weeks. Egg laying declines from ∼30 weeks and stops at around ∼60 weeks. Following experiments were carried out with adult (20–25 week) female birds.

Administration of steroid hormones. The birds were administered 17β-estradiol and progesterone, 2.5 mg/100 gm body wt., in propylene glycol intramuscularly. The birds were tagged and kept with other birds for 48 hr in standard conditions.

RNA isolation and northern hybridization. Total RNA from the liver was isolated according to Auffray and Rougeon (11)

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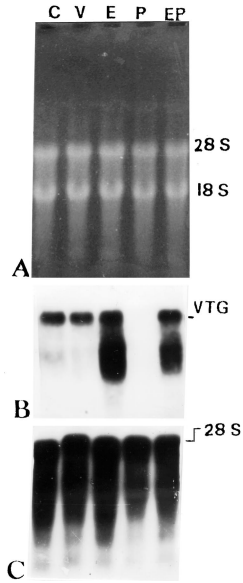

FIG. 1. Formaldehyde denaturing gel electrophoresis of total RNA (15 ug) purified from liver of Japanese quail, either with or without administration of estradiol, (E), progesterone (P) or E+P. (A) EtBr staining (B) Northern blot hybridization with 32P-labelled VTG cDNA, and (C) with 32P-labelled 28S rRNA cDNA. C—control, V—vehicle.

as modified by Kuhl *et al* (12), resolved on 1% denaturing formaldehyde agarose gel and blotted to nytran membrane (13). ^{32}P-Labelled probe of VTG cDNA was prepared by nick translation (14) and used for hybridization.

DNA isolation and Southern blot hybridization. High m.w. DNA was isolated from liver (15), digested first with Eco RI to completion, and then either with Msp I or Hpa II to completion. DNA (25 ug) digested by Eco RI, Eco RI + Msp I, and

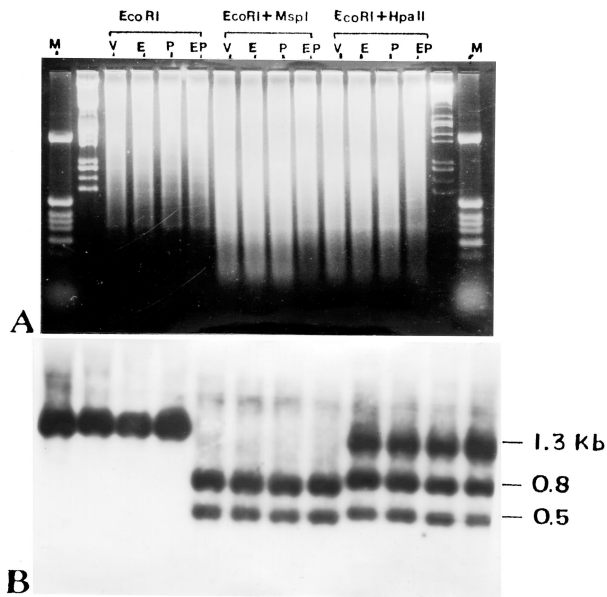

FIG. 2. Methylation status of -CCGG- sequence in the 1.3 kb promoter region of the VTG gene after administration of estradiol or progesterone alone or together. (A) EtBr staining of DNA fragments resolved on 1.5% agarose gel. (B) Southern blot hybridization with 32P-labelled 1.3 kb VTG fragment. V—control, E—estradiol, P—progesterone, EP—E + P, M—pBR322 plasmid digested by HinfI.

Eco RI + Hpa II was resolved on 1.5% 1× TBE agarose gel and transferred to nytran membrane. The membrane was hybridized with the ^{32}P-labelled 1.3 kb promoter region (−1484 to −75) of the VTG gene.

Preparation of nuclear extract. Liver nuclear extract (NE) was prepared according to Gorski *et al* (16). The protein content of NE was determined according to Lowry (17).

Gel mobility shift assay. One and 4 ug of NE were incubated with 0.1ng of probe labelled with α-^{32}P dCTP using the Klenow fragment of DNA polymerase (18). Protein-DNA complexes were separated from protein free DNA by 5% non-denaturing polyacrylamide gel electrophoresis.

RESULTS AND DISCUSSION

Modulation of expression of the VTG gene was carried out after administration of 17β-estradiol and progesterone. Fig. 1a shows 28S and 18S rRNA at a ratio of 2:1. Fig. 1b shows that the expression of the VTG gene is (i) stimulated by 17β-estradiol, (ii) inhibited by progesterone completely, and (iii) stimulated by estradiol and progesterone when given together. The average size of the VTG transcript is ~6.6 kb. The increase in transcription of VTG gene may either be due to an increase in the rate of transcription, or an increase in the half-life of VTG mRNA mediated through the ER, or the mRNA binding proteins. Stabilization of hepatic *X.laevis* VTG mRNA is reported to be mediated through the 3′ untranslated region and estrogen. Seventy one and 141 kd proteins bind specifically to the short sequence (27-nucleotide) of the 3′ end of the VTG mRNA. These proteins are induced by estrogen (19). Progesterone completely inhibits transcription of the VTG gene. The peak level of progesterone that coincides with egg laying in the quail has a critical role in the egg laying process. It may down-regulate/inhibit the rate of transcription. Progestins have been shown to inhibit the expression of estrogen-induced genes (20). Progesterone is reported to (i) rapidly and selectively reduce the level of estradiol receptor (21,22), and (ii) decrease the affinity of nuclear ER and PR for the DNA. An increase in transcription, when both the steroid hormones are administered simultaneously may be due to (i) synergistic action of steroid hormones, and (ii) overlapping sites for binding of both the hormone-receptor (H-R) complexes, ER and PR, in the promoter of the gene. Thus, the level or affinity of *trans*-acting factor(s) that bind to *cis*-acting element(s) is essential for determining the response of a cell to a hormone.

A -CCGG- sequence is present between the ERE and PRE of the gene (23). Fig. 2 shows that no changes occur in the methylation status of the -CCGG-sequence in response to estradiol and progesterone, alone or together.

Fig. 3 is a restriction map of the 1.3 kb (−75 to −1423) VTG gene. The DNA fragments

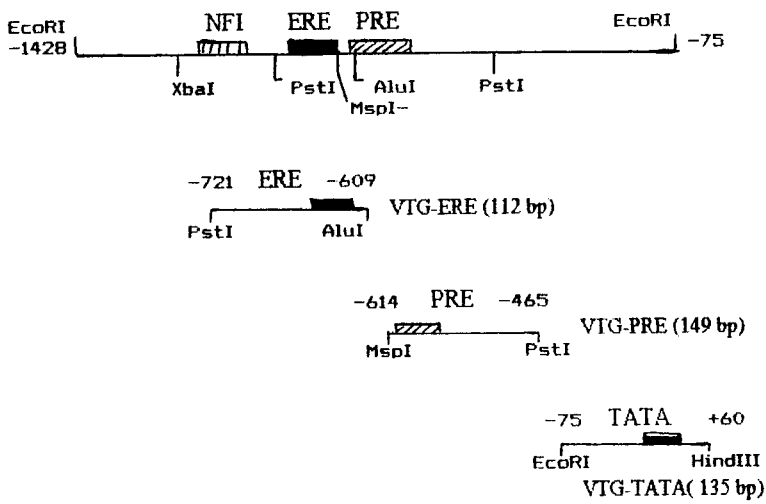

FIG. 3. Map of the 1.3 kb fragment (−1428 to −75) of the VTG II gene promoter and the probes used for gel mobility shift assay. ERE—Estrogen responsive element; PRE—Progesterone responsive element; TATA—TATA box.

containing ERE (112 bp), PRE (149 bp) and TATA (135 bp) were end-labelled by ^{32}P and used for gel mobility shift assay. Three DNA-protein complexes (E1, E2 and E3) are seen with the nuclear extract (NE) of all four sets (Fig. 4a). Administration of estradiol alone or along with progesterone increases the level/binding affinity of *trans*-acting factors which may be responsible for higher expression of VTG gene. Estradiol is reported to induce DNase I hypersensitive sites in the VTG gene promoter (24). Thus, the chromatin is more relaxed in the liver of estradiol primed birds than in control birds. The hypomethylated -CCGG- sequence (Fig. 2) and the relaxed chromatin of birds make the ERE readily accessible to *trans*-acting factors. The ER is reported to bind to the coding strand of ERE with a 60-fold higher affinity than to a dsERE sequence (25). Cato *et al* have shown by *in vitro* studies that E + P act synergistically to induce CAT activity in the chimeric VTG II TK-CAT construct (pERE^{+}PRE^{+}) (9). This agrees with our *in vivo* finding that VTG transcription is induced in birds by E + P. Progesterone increases the level of DNA-binding proteins, but the DNA protein complexes are upshifted. The upshift may be due to the appearance of a repressor

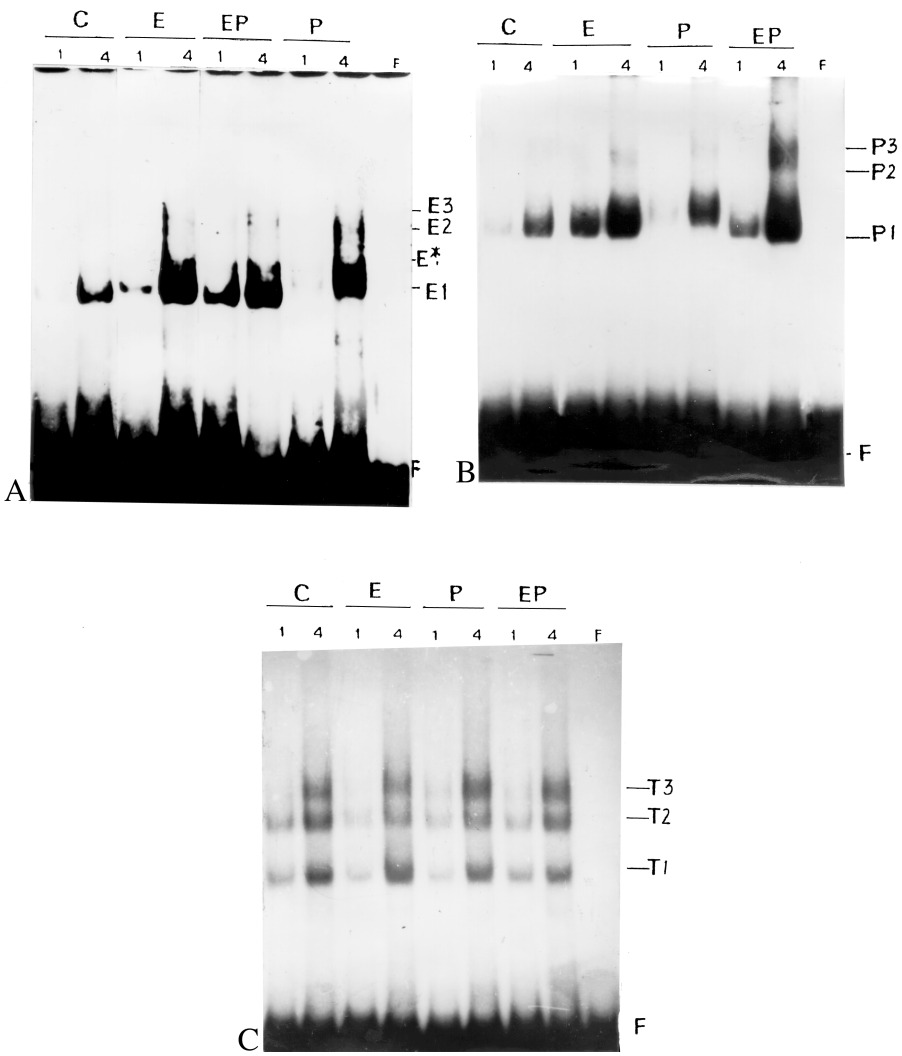

FIG. 4. Gel mobility shift assay of DNA fragments—One and 4 μg nuclear extract of the liver of the bird were incubated with 32P-labelled (A) 112 bp dsDNA containing ERE, (B) 149 bp dsDNA containing PRE and (C) 135 bp dsDNA containing TATA. C—vehicle, E—estradiol, P—progesterone, EP—E + P.

protein or post-translational modification of *trans*-acting factors. Such alterations in the DNA binding proteins may cause repression of transcription of VTG mRNA after administration of progesterone.

Fig. 4b shows that administration of either E alone or with P increases the level of PRE binding proteins. Estradiol induces ER and PR levels (26,27). Higher level of PRE binding proteins after E or E + P administration may be due to higher level of PR. Progesterone decreases the binding, and causes an upshift of DNA-protein complex. It is known to down-regulate its own receptor (28). On the other hand, the levels or affinity of TATA binding proteins do not change after administration of E or P (Fig. 4c).

Thus, the modulation of the VTG gene expression by estradiol and progesterone *in vivo* is due to the *trans*-acting factors that bind to its ERE and PRE.

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