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EFFECTS OF ESTROGEN ON GENE EXPRESSION IN ROOSTER LIVER

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Summary

The effects of estrogen on RNA sequence complexity and sequence frequency were studied in rooster liver. Both control and estrogen-treated liver contained total RNA sequence diversity of approximately 4.2×10^7 nucleotides. Two components were found in the reaction of chicken liver or brain RNA with unique DNA: RNA species present at high concentration and RNA species about 100-fold less abundant. Approximately 7×10^6 nucleotides of RNA sequence complexity were present at high concentration in estrogen-treated liver but not at high concentration in control liver.

Introduction

The effects of estrogen on vitellogenin induction (1-2) and ribosomal RNA processing (3) in chicken and Xenopus liver have been well characterized. The induction of vitellogenesis is associated with a great increase in total liver RNA (4) and an increase in the average size of hepatic polysomes (5-6). An 8-fold increase in the rate of nuclear RNA synthesis was observed 11 hr after estrogen administration in Xenopus liver, (7) with a large proportion of the hormone-induced RNA representing nonribosomal RNA, particularly during the early stages of induction. In spite of the activation of transcription of

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many diverse sequences with estrogen treatment, the only induced mRNA sequences which have been identified in liver are for low density lipoprotein and for vitellogenin mRNA (8-9) which is 2000-fold more abundant in treated animals.

The effects of estrogen on gene expression have been extensively studied particularly in the chicken oviduct (10-11). In oviduct, as in liver, estrogen causes profound transcriptional changes. Hybridization studies have shown that estrogen treatment leads to approximately a doubling of RNA sequence complexity present in estrogen-treated oviduct nuclear RNAs, (12) poly(A)-containing RNAs, (10) and poly(A)mRNAs (J. Monahan, personal communication) when compared to the RNAs of estrogen-withdrawn oviducts. Hahn found evidence that estrogen induced the transcription of new RNA species essential for yolk protein synthesis (13-14), but no quantitation of this induction has been done. The present study characterized the RNA sequence diversity and sequence frequency of total liver RNA from estrogen-treated or control roosters.

Methods and Materials

DNA Preparations: Radioactively-labeled and unlabeled chicken DNAs were prepared as described by Britten (15). The tritiated DNA (2.54×10^5 dpm/μg) was isolated (16) from primary cultures of chick fibroblasts and was sonicated (17) to 400 nucleotides in length as determined by $S_{20,w}$ values from alkaline sucrose gradients (18) according to the equation of Studier (19). Unique sequences were prepared by hydroxylapatite chromatography (20) of DNA incubated to a C_0t of 150. Sequences remaining single-stranded following this procedure were pooled and reincubated to C_0t = 180. Sequences again remaining single-stranded after hydroxylapatite chromatography were considered unique DNA.
RNA Preparations: Livers were obtained from White Leghorn hatching embryos, chicks, (males and females) or roosters. Estrogen administration was performed as described by Wetekam et al., (2). Total cellular RNA from liver was prepared by the method of Shearer and McCarthy (21), modified as described (20).
Hybridization Reactions: For measurements of unique DNA hybridization, chicken RNA (1,4,10,20,30 mg/ml) or DNA (1-20 mg/ml) was mixed with 0.018 μg of tritiated unique DNA in 5 μl glass capillary tubes in varying concentrations (0.18-1.2 M) of NaCl in Buffer A (10 mM EDTA, 0.5% SDS, 10 mM tris-HCl, pH 7.0), denatured at 110° for 3 min and then incubated at 70° (or 25° below T_m). The extent of unique DNA in hybrid was assayed by hydroxylapatite (15) or by S_1 nuclease (20). All samples were counted to <3% standard deviation. That contaminating DNA in RNA preparations did not account for the reaction with unique DNA was shown by alkaline degradation of samples of all RNAs used in these studies and elimination of all reaction with unique DNA by this means.

Results and Discussion

Unique DNA: Data from annealing reactions in which S_1 nuclease sensitivity of tritiated chicken unique DNA was measured during incubation with unlabeled

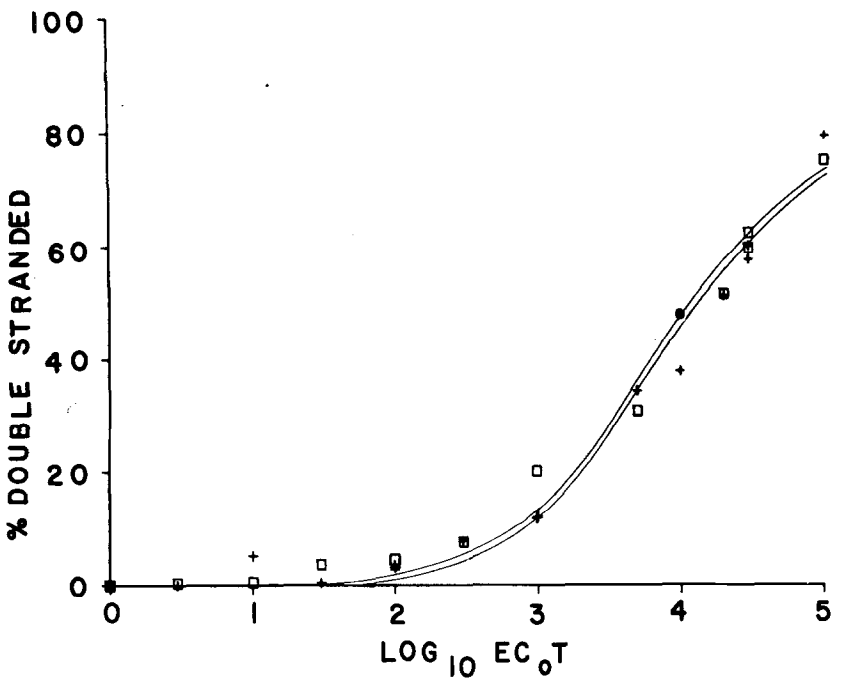

Fig. 1. Reaction of chick fibroblast unique DNA with chicken DNA. [^{3}]labeled unique DNA was incubated to the desired C_0t and the percent in well-matched hybrid was measured by S_1 nuclease at 45°, (squares) or S_1 nuclease at 25° (crosses).

total chicken DNA fragments are shown in Fig. 1. S_1 nuclease digestion at permissive (25°) and stringent (45°) temperatures (22) gave equivalent results. These data were computer-fit to a second order rate equation using the exponent value N = 0.44 (23). In order to show that hybrids were formed with single-copy DNA, RNA/DNA hybrid was purified by hydroxylapatite chromatography; the RNA components were degraded by treatment with alkali, and the remaining DNA was rehybridized with chicken total DNA. The DNA renatured with kinetics expected for unique DNA.

RNA sequence complexity and sequence frequency: In hybridization reactions of the unique [^{3}H] DNA with a vast molar excess of RNA, total liver RNA from a 13 week-old rooster (Fig. 2) hybridized to 4.86% of the unique DNA at saturation. The course of the hybridization reaction extended over at least four decades of ER_0t. The existence of at least two reasonably distinct

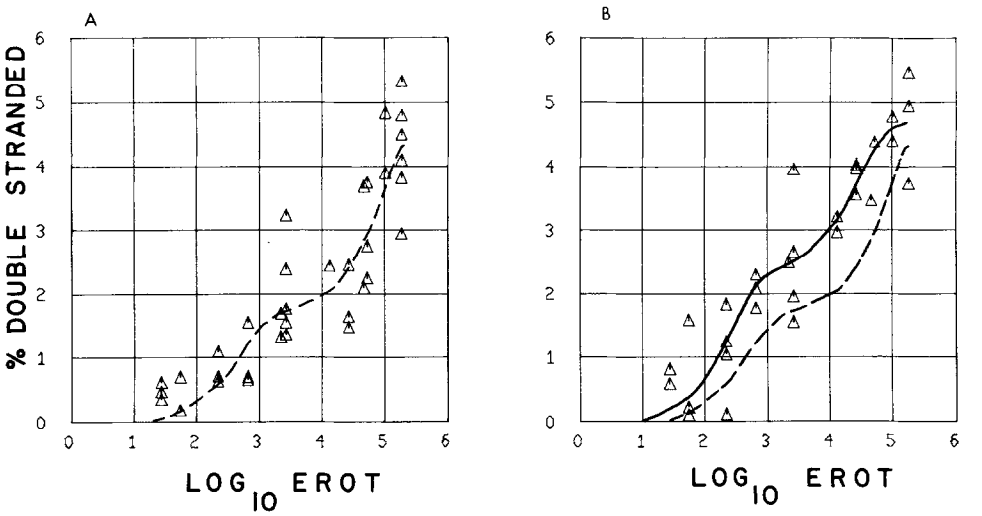

Fig. 2. A. RNA from control (triangles) or B. Estrogen-treated (triangles) rooster liver was incubated with [^{3}H]labeled unique DNA. The percent of unique DNA in hybrid was measured by S_1 nuclease as described in the Methods. The dashed line represents the best fit to the control liver RNA reaction; the solid line for the estrogen-treated liver RNA reaction.

reaction components could be distinguished, each component covering approximately 2 decades on the abscissa. The most rapidly renaturing component of control liver RNA, ($R_0t_{1/2}$ = 348), representing the vast majority of the mass of reactive RNA, contributed only 35% of the total RNA sequence complexity (1.7% of the unique DNA). The least abundant species ($R_0t_{1/2}$ = 67,300) contained the majority of the sequence diversity (3.2% of the unique DNA), but only a small fraction of the mass of the RNA. Total liver RNA from a 13 week old rooster which had received 17 β-estradiol treatments hybridized to 4.65% of the unique DNA at saturation (Fig. 2). Computer analysis of these data showed a rapidly renaturing component ($R_0t_{1/2}$ = 218), representing 2.3% of the unique DNA. Calculating the expected $R_0t_{1/2}$ for a pure component of RNA of complexity 4.6 x 10^7 nucleotides as previously described (24) predicts a $R_0t_{1/2}$ of 3.6. Thus, the rapidly-reacting component must represent about 1.5% of the RNA. The least abundant species ($R_0t_{1/2}$ = 21,650) had a sequence complexity of 2.4%. Thus, the total complexity of liver RNAs from

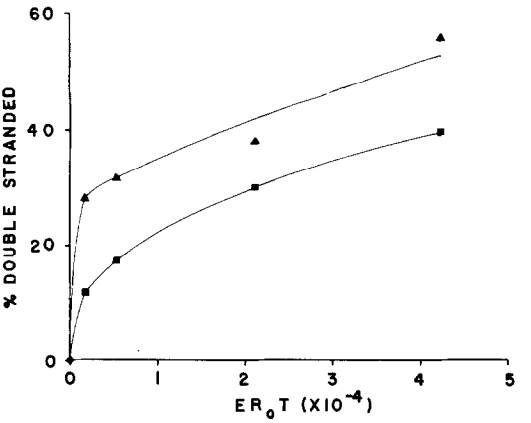

Fig. 3. RNA from control (squares) or estrogen-treated (triangles) rooster liver was incubated with a DNA probe complementary to rapidly-renaturing estrogen-treated liver RNA. The percent of the probe in hybrid was measured by S_1 nuclease as described in Methods.

control and estrogen treated animals was approximately the same. However, there were substantial differences in abundance of various RNA species.

Overlap of RNA sequences in RNA from normal and estrogen-treated roosters: To measure the fraction of those RNA sequences present in many copies in estrogen-treated rooster liver RNA, an initial purification of unique DNA complementary to the RNAs present at high concentration in estrogen-treated liver was made. The probe was prepared by hybridization of estrogen-treated liver RNA with chicken unique DNA to a R_0t of 10,000 and subsequent purification of the unique DNA in hybrid. RNA from control or estrogen-treated liver was then reacted with the enriched unique DNA probe (Fig. 3). Since the $R_0t_{1/2}$ of the fast component was 218, the first data point at R_0t = 1000 should already include the majority of the reaction of the rapid component, with further reaction representing uDNA of the slow component. The rapid reaction of estrogen-treated RNA with the probe was approximately 50% greater than control RNA, suggesting that RNA sequences equivalent to 7 x 10^6 nucleotides were present at high concentration in estrogen-treated liver but not in control liver.

In contrast to chick oviduct where estrogen induces the synthesis of 2×10^7 nucleotides of RNA not present in estrogen-withdrawn oviduct, few RNA sequences absent in control liver RNA appeared to be induced de novo in rooster liver by estrogen. Instead, estrogen caused a 3-4 fold increase in the concentration of all diverse, low-abundance liver RNA sequences. Both the concentration and the RNA sequence complexity of high-abundance liver RNA appeared to be increased by estrogen, with about 7×10^6 nucleotides of RNA present at high concentration in estrogen-treated RNA but not at high concentration in control RNA. Possibly, these sequences were present in unstimulated liver but at lower concentration. Since the high-abundance RNA sequences are present at 100-200 times the concentration of the low-abundance sequence a stimulation of this magnitude must have occurred in the synthesis of the abundant, estrogen-specific sequences as a result of estrogen treatment. Included in this class are sequences of vitellogenin mRNA. Wahli et al. (8) found that a species of poly(A)-containing RNA induced by estrogen (vitellogenin mRNA) is 2000 times more abundant in treated liver. However, many other sequences are also stimulated by estradiol since vitellogenin mRNA alone, would account for less than 0.1% of the RNA sequence complexity found at high concentration in estrogen-treated liver but not in control liver.

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