Effect of Estrogen on Ovalbumin Gene Expression in Differentiated Nontarget Tissues†

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ABSTRACT: By use of cloned DNA fragments as probes, low levels of ovalbumin RNA sequences (structural and intervening sequences) were detected in nuclear RNA extracts of nontarget tissues, such as liver, spleen, brain, and heart of chicks. The expression of the ovalbumin gene sequences was hormone dependent. In estrogen-stimulated chicks, a low level of ovalbumin RNA sequences, ranging from 0.2 to 0.7 molecule per cell, was present in nontarget tissues while less than 0.01 molecule per cell could be found in the same tissues of unstimulated chicks. A significant amount of the ovalbumin mRNA sequences was also found in polysomes of liver and brain. The ovalbumin mRNA sequences could be translated into proteins which were only localized in a few cells among the entire population of liver cells as determined by an immunocytochemical assay. These results suggest that there are some cells in liver, spleen, heart, and brain which can respond to hormone stimulation and produce ovalbumin mRNA and its translational product.

Although the molecular events which govern the development of a multicellular organism from a single cell (fertilized egg) are poorly understood, it is generally believed that as differentiation of an organism proceeds, the developmental potential of each cell becomes more and more restricted. Transcriptional control mechanisms have been implicated in the restriction of the developmental potential of a cell since a portion of the RNA sequences transcribed is tissue specific. Previous studies of RNA synthesis have focused on mRNAs which code for proteins characteristic of a differentiated tissue such as ovalbumin in the chick oviducts (Harris et al., 1975; McKnight et al., 1975), hemoglobin in erythroid tissues (Ross et al., 1974), and fibroin in the silk moth (Suzuki & Suzuki, 1974). However, very little is known concerning the synthesis of such phenotypic mRNAs in other differentiated tissues. Recently, low concentrations of globin mRNA sequences have been found in adult liver and other nonerythroid tissues (Gilmour et al., 1974; Humphries et al., 1976; Ono & Cutler, 1978). The origin and the role of such sequences in the nonerythroid tissues are not well-defined. We have, therefore, chosen to study the expression of ovalbumin gene sequences in tissues of the chick other than oviduct. Using a highly radioactive and pure hybridization probe prepared from cloned fragments of the ovalbumin gene, we have detected a small but significant number of ovalbumin gene transcripts in liver, spleen, heart, and brain tissues which normally are not responsible for the synthesis of ovalbumin. Surprisingly, the concentration of such transcripts in the above tissues is hormone dependent. Finally, the distribution of the ovalbumin messenger RNA sequences in the cellular population and the expression of those sequences into protein are also reported.

Materials and Methods

Materials

All unstimulated tissues (liver, heart, brain, and spleen) were obtained from 4-week-old white Leghorn chicks. In the case of stimulated tissues, chicks were weekly implanted subcutaneously with a 20-mg pellet of diethylstilbestrol (DES) (Sigma Chemical Co.) which provided continuous release of DES for 8–9 days. To prepare hormone-withdrawn oviducts, we subcutaneously injected chicks daily with 2.5 mg of diethylstilbestrol for 14 days and then withdrew them from all hormones for 14 days. Laying hens and roosters were obtained from Research Farms. Restriction endonuclease *Hha*I was purchased from Bethesda Research Laboratories. S_1 nuclease was obtained from Miles Laboratories. DNA polymerase I was a product of Boehringer. [^{3}H]dCTP and [^{3}H]TTP were purchased from New England Nuclear Corp. Rabbit anti-chicken ovalbumin was obtained from Cappel Laboratories and goat IgG–horseradish peroxidase conjugate against rabbit IgG was purchased from Miles Biochemicals. All other chemicals were reagent grade and were purchased from Fisher Scientific Co.

Methods

Preparation of Specific Hybridization Probes to Detect Ovalbumin Sequences. Structural sequence probes (OV, OV_L, and OV_R) were prepared from pOV_{230}, a chimeric plasmid previously constructed in our laboratory (McReynolds et al., 1977). A DNA fragment containing essentially the entire ovalbumin structural gene (OV) was obtained by digestion of pOV_{230} with endonuclease *Hha*I, followed by purification by gel electrophoresis. It was labeled with ^{3}H to high specific activity by nick translation (Mackey et al., 1977). A single-stranded probe was then prepared as previously described (Roop et al., 1978). The specific activity of the OV probe was 7.7×10^6 cpm/μg. Fragments containing DNA sequences corresponding to the 5′ terminus (OV_L) and the 3′ terminus (OV_R) of ovalbumin mRNA ($mRNA_{ov}$) were obtained by simultaneous digestion of pOV_{230} with endonuclease *Hin*dII and *Hae*III, and single-stranded probes were then prepared from the purified fragments (Roop et al., 1978). The specific activities of the OV_L and OV_R probes were 5×10^6 and 9×10^6 cpm/μg, respectively. Intervening sequence probes $OV_{2.4}$ and $OV_{1.8}$ were prepared from cloned *Eco*RI fragments of the natural ovalbumin gene and labeled by nick translation. The specific activities of the double-stranded probes $OV_{2.4}$ and $OV_{1.8}$ were 6×10^7 and 4×10^7 cpm/μg, respectively.

Isolation of Nuclei from Various Chick Tissues. Oviduct, liver, spleen, brain, and heart were freshly removed from chicks, hens, or roosters and were used for preparation of nuclei. Nuclei from oviduct, spleen, and heart tissues were

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isolated according to the procedure of Tsai et al. (1978). Preparations of chick liver and brain nuclei were slightly modified. The tissues were homogenized in 5% citric acid with a tissuemizer at 4 °C for only 20 s. Other steps were essentially as previously described (Tsai et al., 1978). DNA and RNA content was determined by diphenylamine and orcinol assays as described elsewhere (Tsai et al., 1975).

Isolation of Nuclear and Polysomal RNA. Nuclear RNA was isolated from purified nuclei of various tissues according to the method of Tsai et al. (1978). Polysomes were isolated according to the procedure of Palacios et al., and RNA was isolated as described above. The purified RNA was lyophilized, stored at −20 °C, and used for hybridization.

RNA–DNA Hybridization. RNA excess hybridization experiments were performed in a final volume of 100 μL in tapered reaction vials containing 0.6 M NaCl, 10 mM Hepes (pH 7.0), and 2 mM EDTA. RNA (5 mg/mL) was boiled together with the [^{3}H]DNA probe [0.1 ng for structural sequence probes and 0.016 and 0.02 ng (800–1000 cpm) for intervening sequence probes $OV_{2.4}$ and $OV_{1.8}$, respectively] for 5 min at 100 °C at a RNA/DNA ratio of (5–32) × 10^6. Samples were then incubated at 68 °C for time intervals ranging from 10 min to 70 h. Following hybridization, the samples were treated with S_1 nuclease (1600 units), and the S_1 nuclease resistant hybrids were determined as previously described (Harris et al., 1976). Equivalent R_0t values, obtained at 0.18 M NaCl, were plotted (Britten et al., 1974), and $R_0t_{1/2}$ was determined at half-maximum of hybridization.

Immunocytochemical Method. Oviducts and livers freshly removed from both DES-stimulated and unstimulated chicks were fixed immediately in a 4% paraformaldehyde and 0.1% glutaraldehyde mixture in 0.15 M NaCl and 0.07 M sodium phosphate buffer, pH 7.4, overnight. After washing with the above buffer for 1 h, we dehydrated the tissue fragments with ethanol and embedded them in paraffin. The paraffin blocks were cut by a microtome to a thickness of 5 μm. The sections were mounted on glass slides, incubated at 57 °C overnight, and then deparaffinized and rehydrated by washing with decreasing concentrations of ethanol to 50%. For inhibition of the endogenous peroxidase activities, sections were incubated with 1% H_2O_2 in absolute methanol at room temperature for 10 min, washed with buffer, and further incubated with 0.05% Pronase in H_2O at room temperature for 10 min to reduce background staining (Finley et al., 1978). The sections were first incubated with rabbit antiserum against ovalbumin (0.1 mg/mL) at room temperature for 1 h and then reacted with a goat IgG–peroxidase conjugate against rabbit IgG at a dilution of 1:60. Finally, the sections were washed with buffer and then incubated with 0.05% 3,3′-diaminobenzidine tetrahydrochloride in 0.05 M Tris-HCl, pH 7.6, and 0.01% H_2O_2 for 10 min at room temperature (Karnovsky, 1965). Sections thus obtained were fixed with 0.05% OsO_4 in 0.1 M sodium phosphate, pH 7.4, for 2 min. Some sections were stained with hematoxylin for 2 min; others were unstained. The final preparations were dehydrated, mounted with piccolytexylene, and viewed under a light microscope. In some control experiments, rabbit antibody against ovalbumin was either omitted or preincubated with excess ovalbumin (500 μg) prior to reacting with the sections.

Results

Expression of Ovalbumin Structural Sequence in Nontarget Tissues. A specific probe for ovalbumin messenger RNA sequences was prepared from a cDNA clone (pOV_{230}), a chimeric plasmid constructed in our laboratory (McReynolds et al., 1977). This plasmid contains a DNA complement to

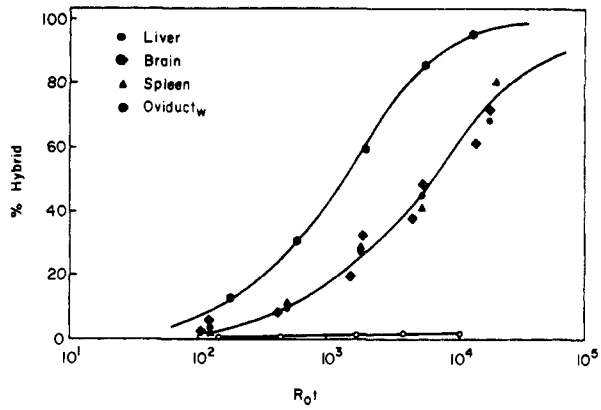

FIGURE 1: Hybridization of nuclear RNA to ^{3}H-labeled probes (OV) corresponding to structural sequences in the ovalbumin gene. The RNAs used were extracted from liver (●), brain (♦), and spleen (▲) of estrogen-stimulated chicks and from oviduct (●) of withdrawn chicks. RNA isolated from liver of stimulated chicks was hydrolyzed with 0.2 N NaOH at 70 °C for 20 min prior to hybridization (○). Hybridization was performed as described under Methods.

all but 12 nucleotides at the 5′ end of the ovalbumin mRNA. pOV_{230} DNA was digested with restriction endonuclease *Hha*I. The *Hha*I fragment containing the ovalbumin-specific sequences was then purified and labeled with [^{3}H]dCTP by nick translation to a specific activity of 8 × 10^6 cpm/μg. A single-stranded ovalbumin probe was then prepared as reported by Roop et al. (1978). The ovalbumin mRNA probe (OV) was neither contaminated by plasmid sequence nor contaminated by anticoding DNA sequence (Roop et al., 1978). This single-stranded probe was then hybridized to an excess of total nuclear RNA isolated from the liver, spleen, and brain of estrogen-stimulated chicks. As shown in Figure 1, nuclear RNA from estrogen-stimulated liver, spleen, and brain all reacted similarly with the probe with an apparent $R_0t_{1/2}$ of at least 4.3 × 10^3 M s. Nuclear RNA of oviducts prepared from chicks first stimulated and then withdrawn from estrogen reacted only slightly faster at a $R_0t_{1/2}$ of 1.6 × 10^3 M s. The rate of hybridization to nuclear RNA from nontarget tissues which do not contain high concentrations of ovalbumin when subjected to hormone stimulation is much slower in comparison with that of nuclear RNA from estrogen-stimulated chick oviducts (7.5 × 10^{-1} M s). Figure 1 shows that the hybridization reactions using nontarget cell RNA were almost complete at a R_0t value of 10^4 M s. When the nuclear RNA preparation from liver was subjected to alkaline hydrolysis prior to hybridization reaction, no significant level of hybridization was observed which indicated that the reaction was indeed due to RNA–DNA hybrids and not due to reannealing because of chick DNA contamination. To further substantiate that the hybrids resulted from true $mRNA_{ov}$ sequences, we determined the thermal stability of the RNA–DNA duplexes. The melting profile of the hybrids formed between total liver nuclear RNA and DNA_{ov} was identical with that of pure $mRNA_{ov}$ and DNA_{ov} (Figure 2). The melting curve had a sharp transition with a T_m of 84 °C in 2 × SSC. The results indicated that authentic $mRNA_{ov}$ sequences are indeed present in nontarget tissues.

Expression of Intervening Sequences of the Ovalbumin Gene in Nontarget Tissues. Recently, it has been demonstrated that the structural gene sequence of the natural ovalbumin gene is interrupted 7 times by intervening sequences (Breathnach et al., 1977; Dugaiczyk et al., 1978). As reported earlier, these intervening sequences are fully transcribed but at a 10-fold lower level in estrogen-stimulated chick oviduct tissue (Roop et al., 1978). It was of interest to determine whether inter-

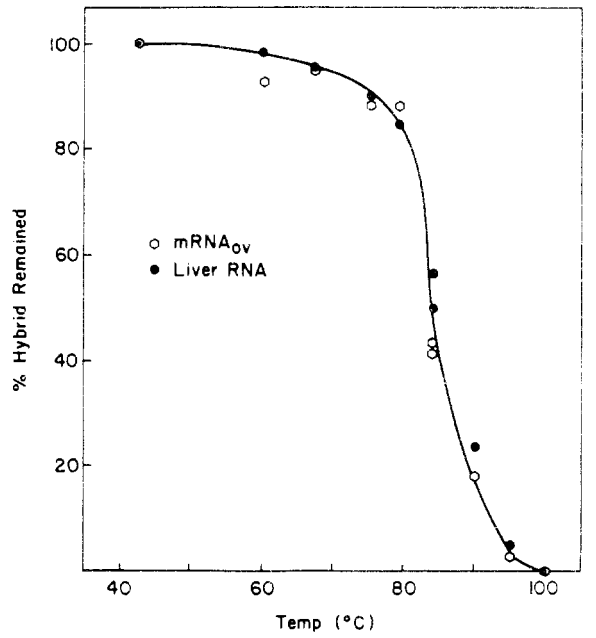

FIGURE 2: Melting profile of RNA-[^{3}H]DNA$_{ov}$ hybrids. Hybridization of [^{3}H]DNA$_{ov}$ to nuclear RNA from liver was carried out to a R_0t of 2×10^4 M s, and samples were diluted to 0.3 M NaCl. After incubation at the indicated temperatures for 5 min, the samples were treated with S_1 nuclease as described under Methods (●). Hybridization of [^{3}H]DNA$_{ov}$ to pure mRNA$_{ov}$ was carried out to a R_0t of 2.5×10^{-1} M s, and the melting temperature was determined as described above (○).

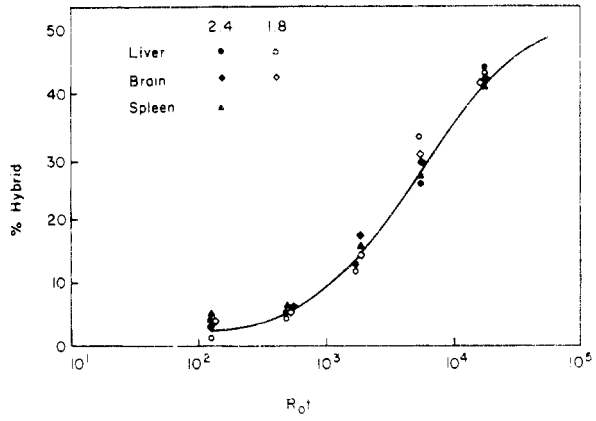

FIGURE 3: Hybridization of nuclear RNA to ^{3}H-labeled probes corresponding to intervening sequences in the ovalbumin gene. The probes used were OV$_{2.4}$ (closed symbols) and OV$_{1.8}$ (open symbols). The RNAs used were extracted from liver (●, ○), brain (♦, ◇), and spleen (▲) of stimulated chicks. Hybridization was performed as described under Methods.

vening sequences were also expressed in nontarget tissues. To this end, 2.4- and 1.8-kbp *Eco*RI fragments of the natural ovalbumin gene were purified from pOV$_{2.4}$ and pOV$_{1.8}$ clones (Roop et al., 1978) and labeled to 2.4×10^7 cpm/μg with [^{3}H]TTP and [^{3}H]dCTP by nick translation. These labeled fragments were used as probes for intervening sequences since 93% of the 2.4-kbp fragment and 95% of the 1.8-kbp fragment correspond to intervening sequence regions of the ovalbumin gene. As revealed in Figure 3, transcripts complementary to intervening sequences were also detected in all nuclear RNA preparations from liver, spleen, and brain of estrogen-stimulated chicks. The concentration of the intervening sequences was similar to that of the structural sequences of ovalbumin gene ($R_0t_{1/2} = 4.4 \times 10^3$ M s). These results were different from data obtained by using a nuclear RNA preparation of

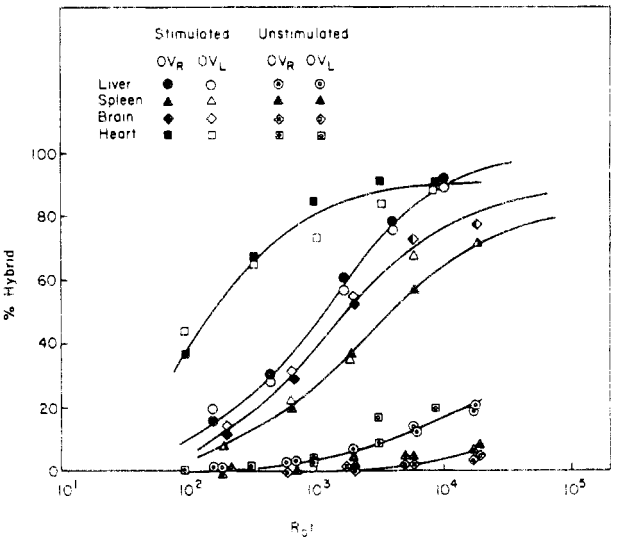

FIGURE 4: Hormone induction of ovalbumin gene expressions in nontarget tissues. The probes used were OV$_R$ (●, ♦, ▲, ■, ⊙, ◇, △, and □) and OV$_L$ (○, ◇, △, □, ⊙, ◇, △, and ⊡). The RNAs used were isolated from liver (●, ○), brain (♦, ◇), spleen (▲, △), and heart (■, □) of stimulated chicks and from liver (⊙, ⊙), brain (◇, ◇), spleen (△, △), and heart (⊡, ⊡) of unstimulated chicks.

estrogen-stimulated oviduct cells where the level of intervening sequences was 10 times less than that of the structural sequences (Roop et al., 1978). Therefore, the rate of RNA processing or RNA degradation must be slower in nontarget tissues.

It should be noted that the maximum extent of hybridization of the 2.4- and 1.8-kbp fragments to RNA reached approximately 45% (Figure 3). Assuming that both strands of the 2.4- and 1.8-kbp DNA fragments were labeled to the same extent by nick translation and that only the coding strands are transcribed into RNA in vivo, the 50% level was equivalent to 100% hybridization of the theoretical maximum. These data are consistent with our previous reports in oviduct cells that all intervening sequences are transcribed in vivo.

Hormone-Dependent Expression of the Ovalbumin Gene Sequences. As reported earlier, the expression of the ovalbumin gene in oviduct tissue is clearly hormone dependent (Roop et al., 1978). Withdrawal of hormone from chronically stimulated chickens leads to a dramatic decrease in detectable ovalbumin sequences from 3000 molecules per tubular gland cell nucleus to two molecules per cell nucleus (Roop et al., 1978). It was of interest to examine whether the expression of ovalbumin structural sequences in nontarget tissues is under hormonal control. Total nuclear RNA was isolated from estrogen-stimulated and unstimulated liver, spleen, brain, and heart and hybridized to ovalbumin gene probes. In this particular experiment we used the probes corresponding to the 5′ end of the ovalbumin gene (OV$_L$) and the 3′ end of the ovalbumin gene (OV$_R$) prepared by *Hae*III digestion. As illustrated in Figure 4, nuclear RNA from each estrogen-stimulated nontarget tissue hybridized well with the left and the right probes of the ovalbumin structural sequences. At a R_0t of 10^4 M s, the hybridization reaction was essentially complete. The similarity of hybridization kinetics of the left and the right probe indicates that the low level of ovalbumin gene expression in these nontarget tissues does not favor any region of the gene. In the case of RNA preparations isolated from unstimulated chick tissues, even at a R_0t value of 2×10^4 M s, only 20% hybridization was attained in heart and liver tissues and less than 6% hybridization was observed in spleen and brain tissues. This indicated the presence of less than 0.01

Table I: Concentration of $mRNA_{ov}$ Sequences in Total Nuclear RNA of Various Tissue

tissues	$R_0t_{1/2}$[a] × 10^{-3} (M s)	fraction of $mRNA_{ov}$[b]	RNA/DNA	molecules of $mRNA_{ov}$[c] per cell nucleus
$oviduct_W$	1.6	1.9×10^{-6}	0.07	0.3
$liver_{DES}$	3.8	7.8×10^{-7}	0.16	0.32
$heart_{DES}$	2.1	1.4×10^{-6}	0.22	0.7
$spleen_{DES}$	4.0	7.5×10^{-7}	0.09	0.16
$brain_{DES}$	3.5	8.6×10^{-7}	0.26	0.5

[a] The $R_0t_{1/2}$ was an average value calculated from three sets of experiments in the case of heart tissues and five sets of experiments in the case of the other tissues. [b] Fraction of $mRNA_{ov}$ = [$R_0t_{1/2}$ for pure $mRNA_{ov}$ hybridized to $cDNA_{ov}$ (3×10^{-3})]/($R_0t_{1/2}$ obtained for RNA from a given tissue). [c] Molecules of $mRNA_{ov}$ = (fraction of $mRNA_{ov}$)(RNA/DNA)(2.6×10^{-12} g of DNA)[(6.02×10^{23} molecules)/(6.23×10^5 g of $mRNA_{ov}$)].

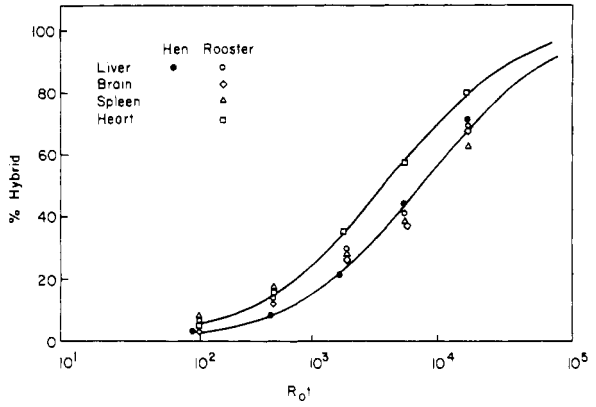

FIGURE 5: Hybridization of nuclear RNA to [^{3}H]DNA_{ov} probe corresponding to structural sequences in the ovalbumin gene. Hybridization was performed as described under Methods. The RNAs were extracted from liver of hens (●) and liver (○), brain (◇), spleen (△), and heart (□) of estrogen-treated roosters.

molecule of ovalbumin structural sequences per cell nucleus. Similarly, very low levels of hybridization (<5%) were observed when intervening sequence probes were used (data not shown). Thus, a low level of expression of the entire ovalbumin gene sequences in nontarget tissue can be mediated also by steroid hormones.

It should be noted that the concentration of ovalbumin sequences in these differentiated tissues is highest in heart and progressively decreases in liver, brain, and spleen. As tabulated in Table I, the number of molecules of ovalbumin structural sequences in various stimulated tissues is very low, with the majority of the nontarget tissues having an average of only 0.4 molecule per cell nucleus. The slight variation in reaction kinetics among RNA preparations (Figures 1 and 4) might be a function of the state of the tissue and/or its relative responsiveness to hormone stimulation.

Expression of Ovalbumin Structural Sequences in Nontarget Tissues of the Hen and Estrogen-Stimulated Rooster. In the chronically stimulated chicks, a high dose of estrogen (2.5 mg/chick) was administered daily. We considered that this high level of estrogen might artificially induce the expression of ovalbumin gene sequences in nontarget tissues. Thus, it was of interest to examine whether nontarget tissues from laying hens contained ovalbumin structural sequences where hormone was present at a physiological level. Total nuclear RNA was isolated from the liver of hens and hybridized to a single-stranded OV probe. The hybridization kinetics as demonstrated in Figure 5 were very similar to the

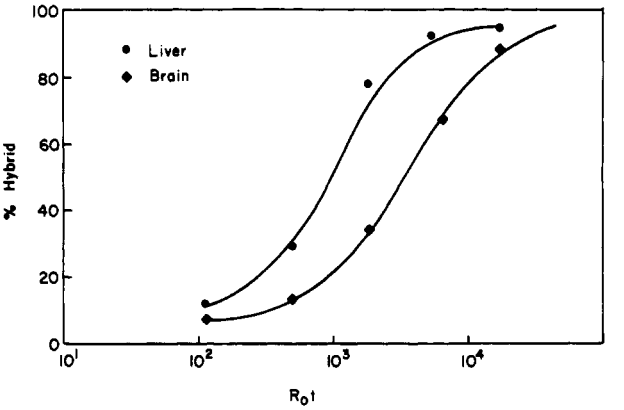

FIGURE 6: Hybridization of polysomal RNA to structural sequence probe (OV_{230}). Polysomal RNAs were isolated from liver (●) and brain (♦) of stimulated chicks. Hybridization was performed as described under Methods.

kinetics presented for nontarget tissues from estrogen-stimulated chicks in Figure 1. The extent of the hybridization at a R_0t value of 2×10^4 M s was roughly 70% and the $R_0t_{1/2}$ was about 4×10^3 M s. This result indicates that physiological levels of estrogen are enough to mediate transcription of the ovalbumin gene sequences.

The hormonal control of ovalbumin gene expression in nontarget tissues was not limited to female chicks. When nontarget tissues from diethylstilbestrol-stimulated male chickens were examined, a significant level of ovalbumin sequences was again observed (Figure 5). Similarly, transcripts of intervening DNA sequences were also detected in these tissues (data not shown). Therefore, a detectable level of sequences from the entire ovalbumin gene is transcribed in hormone-stimulated male chicks.

Presence of Ovalbumin Structural Sequences in the Cytoplasm of Estrogen-Stimulated Liver and Brain Tissues. We searched next for ovalbumin mRNA sequences in polysomes of nontarget tissues. Polysomal RNAs were prepared from estrogen-stimulated chick liver and brain and hybridized to the ovalbumin DNA probe. As plotted in Figure 6, polysomal RNA from stimulated liver or brain protected more than 90% of the ovalbumin probe from digestion by S_1 nuclease. Polysomal RNA from liver reacted slightly faster with the probe having a $R_0t_{1/2}$ of 9×10^2 M s, while RNA from brain had an apparent $R_0t_{1/2}$ of 3.6×10^3 M s. Those $R_0t_{1/2}$ values correspond to more than 10 molecules of $mRNA_{ov}$ sequences per cell, a level much greater than the nuclear concentration. Therefore, this result suggested that in nontarget tissue, mature ovalbumin mRNA sequence is transported to the cytoplasm following synthesis.

Identification of Ovalbumin in Tissue Sections of Estrogen-Stimulated Liver Slices Using Immunocytochemical Techniques. The presence of ovalbumin structural sequences in the cytoplasm of nontarget tissues prompted us to investigate whether these sequences could be translated into functional protein. An immunocytochemical method, instead of a translation assay, was employed for these studies since it enabled us to detect the presence of ovalbumin as well as to reveal the relative concentrations of this protein among individual cells. To ascertain the effectiveness of the assays, we first localized ovalbumin in oviduct tissues from estrogen-stimulated chicks. Sections of oviduct tissue were incubated with pure ovalbumin antiserum, reacted with goat IgG–peroxidase conjugated against rabbit IgG, and then reacted with diaminobenzidine as described under Methods. Ovalbumin is identified as a dark-stained reaction product. As illustrated

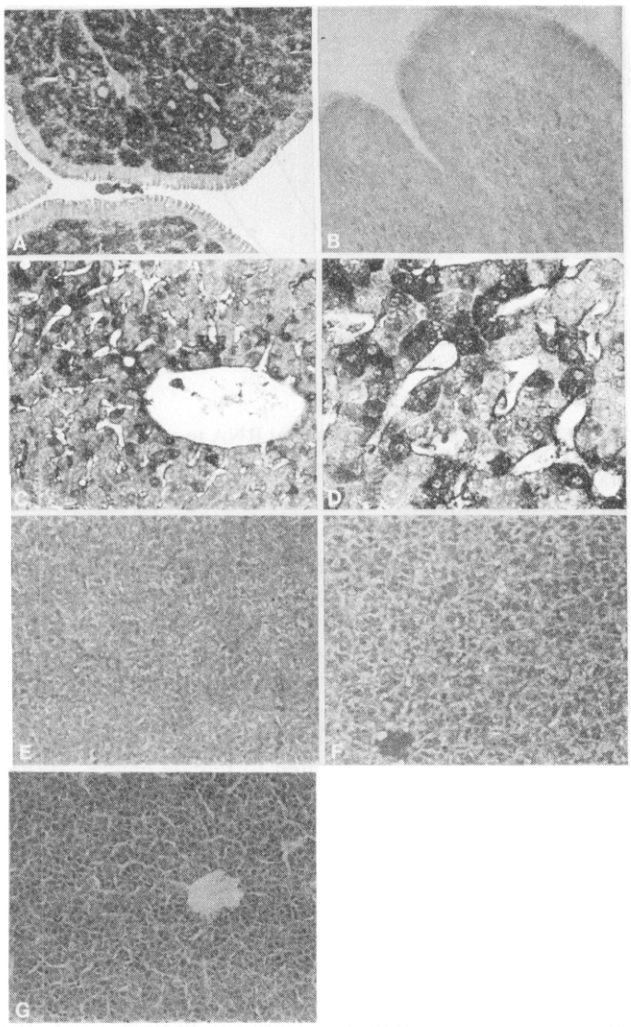

FIGURE 7: Localization of ovalbumin in liver by immunocytochemical techniques. (A) Cross section of oviduct from estrogen-stimulated chicks. The secretory glandular structure in the lamina propria are all stained with dark brown reaction product (250×). (B) Cross section of oviduct from unstimulated chicks, showing no reaction product in the mucosa and lamina propria (500×). (C) Liver section from estrogen-stimulated chicks, showing reaction product in sporatic hepatocytes (225×). (D) Liver section from estrogen-stimulated chicks at high magnification (600×). (E) Liver section from unstimulated chicks, showing no reaction product in the hepatocytes. Section was counterstained with hematoxylin (150×). (F) Liver section from estrogen-stimulated chicks was reacted with antibody against ovalbumin which had previously been incubated with excess ovalbumin. No reaction product is noted. Section was counterstained with hematoxylin (150×). (G) Liver section from estrogen-stimulated chicks reacting only with second antibody peroxidase conjugate against rabbit IgG but not antibody against ovalbumin. No reaction product is evident. Section was counterstained with hematoxylin (125×).

in the micrograph of Figure 7A, ovalbumin is located in the glandular structure of the lamina propria of the oviduct mucosa, and no reaction was observed in the superficial epithelial layer. By contrast, no reaction product could be detected when sections of unstimulated chick oviducts were analyzed under identical conditions (Figure 7B). These results indicate that the reaction was specific for ovalbumin. When liver sections from estrogen-stimulated chicks were reacted with antibody, the dark-stained reaction product was observed in a few of the hepatocytes (Figure 7C,D). The distribution of the positive cells was random. Some were scattered throughout the entire hepatic lobule while others were localized in a small area. Only 8 out of 44 sections of liver slices from 22 different estrogen-stimulated chicks revealed the presence of ovalbumin-containing cells. It should be noted that only a few cells (<100) out of each positive liver slice (~200000 cells) showed intense reaction products. In contrast, not a single positive ovalbumin-containing cell could be found in 30 liver sections of 15 unstimulated chicks (Figure 7E).

To ensure that the positive products indeed resulted from specific interaction between ovalbumin antiserum and ovalbumin, we performed the following control experiments. First, rabbit antibody specific for ovalbumin was reacted with an excess of ovalbumin prior to incubation with the liver sections from estrogen-stimulated chicks. The excess amount of ovalbumin completely blocked the specific reaction so that no positive cell could be found in the micrograph of these control sections (Figure 7F). Second, when antibody against ovalbumin was omitted, no positive cells were observed in the estrogen-stimulated liver sections (Figure 7G). The sections used in these control experiments were obtained from the same block which has previously been shown to contain ovalbumin-bearing cells. Therefore, the absence of positive reaction products in the controls substantiates the specific nature of the immunocytochemical reactions and suggests that ovalbumin indeed is localized in only a few cells of the entire population of hepatocytes.

Discussion

The existence of cloned DNA fragments of the ovalbumin gene has enabled us to prepare highly labeled, single-stranded sequences, specific probes which permit the detection of less than one molecule of ovalbumin mRNA per cell nucleus (Roop et al., 1978). Using these specific probes, we have attempted to determine whether ovalbumin DNA sequences are expressed only in oviduct or if they can be expressed at any level in other differentiated chick tissues such as liver, brain, and spleen. These latter tissues are generally thought to be nontarget tissues in regard to the ovalbumin response to steroid hormones. It was of interest that a low level of structural ovalbumin sequences, ranging from 0.2 to 0.7 molecule per cell nucleus, was found in all tissues examined. Surprisingly, the expression of low levels of ovalbumin mRNA sequences in these nontarget tissues was hormone dependent since significant levels of $mRNA_{ov}$ sequences were only detected in tissues isolated from estrogen-stimulated chicks but not from unstimulated chicks. The low concentration of ovalbumin mRNA sequences in the nontarget tissues of unstimulated chicks indicates that ovalbumin gene expression in nontarget tissue could be induced more than 20-fold upon administration of hormone. Similar levels of $mRNA_{ov}$ sequences were also found in hen liver which argue against artifactual induction of ovalbumin mRNA synthesis by an overdosage of hormone.

Recently, precursor RNA molecules of ovalbumin have been demonstrated by denaturing gel electrophoresis and by pulse–label and chase experiments (Tsai et al., unpublished experiments). The size of the largest precursor molecules suggests that the entire natural gene, structural and intervening sequences, is transcribed into a continuous transcript. Thus, it was of interest to examine whether intervening sequences are also expressed in nontarget tissues of hormone-stimulated chicks. The detection of similar low levels of intervening RNA sequences in liver, brain, and spleen of chicks indicated that the entire ovalbumin natural gene was transcribed in these differentiated tissues.

We have reported previously that the concentration of structural sequences is much higher than that of intervening sequences in total nuclear RNA extracts of estrogen-stimulated chick oviduct (Roop et al., 1978). Our current results revealed similar levels in the nuclei of the nontarget tissues that we examined. For determination of whether intervening sequences

are processed in nontarget tissues, polysomal RNA from such tissues was isolated and analyzed for the presence of $mRNA_{ov}$ sequences. Low levels of $mRNA_{ov}$, but no intervening sequences (data not shown), were detected in polysomal RNA. Thus, it appears likely that ovalbumin gene transcripts were processed to mature ovalbumin mRNA which accumulated in the cytoplasm of liver and other nontarget tissues.

Recent studies from Chambon's laboratory have demonstrated that ovalbumin-related genes, termed X and Y genes, are present in chick DNA (Royal et al., 1979). Both genes contain sequences which are homologous to certain regions of ovalbumin structural sequence. The low levels of ovalbumin sequences detected in our studies do not appear to result from transcription of the X and Y genes for the following reasons. First, the extent of hybridization of structural sequences observed in our studies was greater than 80% which indicates a large degree of homology. Second, the hybridization kinetics were identical when either the 5′ or 3′ end of the structural probe was used, suggesting that homologous sequences are present throughout the entire RNA. Third, the intervening sequences detected were completely homologous to ovalbumin intervening sequences. Finally, the melting temperature indicated no mismatching in the hybrids formed. Therefore, the low levels of ovalbumin mRNA sequences detected in the various chick tissues examined in the present study do not arise from expression of the X and Y genes.

The work of Gurdon (1974) and Bernardino & King (1967) has demonstrated that nuclei from highly differentiated tissues can interact with enucleated fertilized eggs and support the development of a whole organism; however, the success rate is much lower than that when nuclei from early embryos are used. Caplan & Ordahl (1978) have proposed that within any tissue of a developing organism, there is a fraction of cells which remain uncommitted. These cells have unrestricted developmental potential and their number decreases as the organism becomes more highly developed. Our immunocytochemical studies demonstrated that among the entire population of liver cells examined, only a few contain high concentrations of ovalbumin and are capable of reacting with the ovalbumin-specific antibody. This observation is consistent with the above view that a small proportion of cells in the liver are undifferentiated or uncommitted and thus have the potential to develop into other cell types.

Recently, a low level of globin RNA sequences was detected in nonerythroid tissues, such as adult brain and liver (Humphries et al., 1976; Ono & Cutler, 1978), in cultured fibroblasts (Groudine & Weintraub, 1975), and in *Xenopus* liver oocytes (Perlman et al., 1977). Also, RNA complexity analyses have showed that the majority of the RNA sequences are common in various stages of development (Kleene & Humphries, 1977; Affara et al., 1977) as well as between different cells and tissues (Axel et al., 1976; Galau et al., 1976; Young et al., 1976; Hastie & Bishop, 1976; Ryffel & McCarthy, 1975) although the RNA sequences may differ widely in concentration. In light of our current results, it would be of interest to determine whether the synthesis of these low levels of RNA sequences could arise also from a few uncommitted cells.

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