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Apolipoprotein (apo) B and apoII gene expression are both estrogen-responsive in chick embryo liver but only apoII is estrogen-responsive in kidney

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Abstract

Estrogen regulates the hepatic synthesis of a variety of proteins required for egg yolk production in oviparous vertebrates. In chickens, two of these proteins, apolipoprotein (apo) B and apoII, comprise the major protein components of specialized very low density lipoprotein particles that transport triacylglycerols and cholesterol to the developing egg yolk. In the adult, apoB is synthesized constitutively in liver, small intestine, and kidney but is estrogen-responsive only in the liver. In this work we have examined the embryonic expression of the apoB and apoII genes in yolk sac, liver, kidney, and small intestine. The 14 kb apoB mRNA was first detected at day 3 of development in vascular yolk sac, a tissue involved in the transfer of yolk lipids into the embryonic circulation. Constitutive apoB mRNA expression was detectable in liver at day 6.5 and in kidney at day 7.5, but in intestine was barely detectable before hatching. The hepatic apoB gene acquired estrogen-responsiveness at day 6.5 and its hormone-dependent expression increased throughout development in concert with the estrogen-responsive expression of the apoII gene. In contrast, the constitutively expressed apoB gene in kidney remained unresponsive to estrogen. Surprisingly, the apoII gene was found to be responsive to estrogen in both the embryonic kidney and small intestine. ApoII mRNA induction by estrogen in kidney at day 11 was at 10% of the level in the liver but estrogen-responsiveness decreased later in development and was low in the adult. The lack of estrogen-responsiveness of the constitutively expressed apoB gene in embryonic kidney suggests that tissue and gene-specific factors in addition to the estrogen receptor are required for estrogen-dependent expression of apoB and apoII genes.

Keywords: Apolipoprotein B mRNA; Apolipoprotein II mRNA; Estrogen receptor; Chicken embryo; Kidney; Liver

1. Introduction

Estrogen regulation of gene expression in vitellogenesis has been a valuable model for dissecting the molecular mechanisms of steroid hormone action (Wahli, 1988). In laying hens estrogens promote the hepatic biosynthesis of the vitellogenins which are transported to the oocyte where they are deposited in egg yolk granules as nutrient sources for embryonic development (Bergink et al., 1974). Estrogens also stimulate the hepatic production of specialized very low density lipoprotein (VLDL) particles which contain equal mass amounts of apolipoprotein (apo) B and apoII and serve to transport cholesterol and triacylglycerol to the developing egg yolk (Capony and Williams, 1980). Although each of these genes is believed to be transcriptionally regulated by the same estrogen receptor, the regulation of each gene shows distinctive features with regard to tissue specificity and the development of estrogen-responsiveness during embryogenesis. For example, in the adult chicken apoB exhibits high constitutive expression in liver, intestine, and kidney, but is estrogen-responsive only in liver (Blue et al., 1980; Capony and Williams, 1980; Nadin-Davis et al., 1980; Kirchgessner et al., 1987). In contrast, the apoII and vitellogenin genes are expressed only in the liver and only in response to estrogen. In chick embryo liver, the apoII gene becomes sensitive to estrogen stimulation between the 7th and 9th days of development in parallel with the appearance of the estrogen receptor whereas the vitellogenin gene does not acquire estrogen-responsiveness until day 11 despite the presence of receptor

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(Elbrecht et al., 1981; Elbrecht et al., 1984, Evans et al., 1988).

In the present study, we have investigated the tissue specificity of basal and estrogen-regulated apoB and apoII gene expression in kidney, intestine and liver of the developing chick embryo. These are both single-copy genes (Meijlink et al., 1981; Wiskocil et al., 1981). Our experiments reveal several surprising findings. First, in the embryo the apoII gene is estrogen-responsive not only in liver but also in kidney and intestine. Estrogen-inducible apoII expression in kidney and intestine is detectable at low levels as early as day 7.5 of development, increases dramatically at day 11 and subsequently declines at day 15 to the low level of expression seen in the adult. Thus, the apoII gene, although not normally expressed in kidney or intestine during development, is rendered estrogen-responsive during a brief developmental window. Second, despite constitutive expression of apoB in embryonic kidney and relatively high expression of the estrogen receptor, the apoB gene is not estrogen-responsive in this tissue. Third, the apoB gene, although constitutively expressed in embryonic kidney and liver, is not expressed in the intestine until after hatching. These results reveal that each gene exhibits a developmental program that is tissue specific, embryonic-stage specific, and hormone-specific. These complex patterns of gene expression suggest a model in which each gene is regulated by the interplay of multiple gene-specific and tissue-specific transcription factors.

2. Materials and methods

2.1. Eggs and injections

Fertile White Leghorn eggs were purchased from Cook's Hatchery (Truro, Nova Scotia) and grown in a humidified incubator at 38°C. Eggs were injected with 1.25 mg of 17-β-estradiol in 50 μl of propylene glycol, or propylene glycol only as controls. Injections were carried out 48 h prior to dissection. Dissection of yolk sac tissue was carried out from day 3 onwards. The vascular yolk sac or area vasculosa was identified as a red ring of primitive capillaries around the embryo bounded by the sinus terminalis. The distal side of the sinus terminalis is the non-vascular yolk sac (area vitellina) (Freeman and Vince, 1974). Tissues were frozen in liquid N_2.

2.2. Isolation of RNA

Total cellular RNA was prepared by homogenization of frozen tissue in 7.3 M guanidine hydrochloride followed by ethanol precipitation and phenol/chloroform extraction (Protter et al., 1982). Formaldehyde-denatured cytosol RNA used in the dot blot analysis was isolated according to the method of White and Bancroft (1982).

2.3. Northern blot hybridization

Total RNA was denatured with 50% (v/v) formamide, 2 M formaldehyde at 65°C and subjected to electrophoresis, transfer to nitrocellulose or Gene Screen™, prehybridization, hybridization at 42°C for 48 h with ^{32}P-labeled probes and washing according to the method of Thomas (1980). The filters were then subjected to autoradiography at −70°C using Kodak XAR-5 film and photographed or scanned with a Macintosh Color OneScanner and analysed with the NIH Image program version 1.54. Dot blot hybridizations and washings were carried out in the same manner as for Northern blots. ApoB and apoII single-stranded probes were prepared as outlined below for the solution hybridization assay. Double stranded cDNA probes (β-actin and chick estrogen receptor pCEO) were labeled by the random primer method using the oligo labeling kit from Pharmacia. Specific activities of 10^9 dpm/μg of DNA were obtained. pCEO was a gift from P. Chambon.

2.4. Solution hybridization assays for apoB and apoII mRNA

The chicken apoB cDNA clone CB12 was subcloned into M13mp18 and a unique Pvu II site located 233 nucleotides from the 3′ end of the clone CB12 was selected for preparation of an apoB probe (Kirchgessner et al., 1987). ApoII cDNA was subcloned into M13mp9, and a 240 nucleotide fragment was used as a probe as previously described (Williams et al., 1986). High specific activity ^{32}P-labeled single stranded apoB or apoII cDNA were synthesized and hybridized in excess with total RNA or with template DNA (to generate standard curves). The S1 nuclease resistant hybrids were acid-precipitated, collected on glass fiber filters, and counted by scintillation spectrometry. ApoB and apoII mRNA values were determined based on the specific activity of the probe hybridized to a given quantity of RNA. Hybridization data were normalized to molecules/ hepatocyte given that the average chicken liver cell contains 10.9 pg of total cellular RNA and that liver tissue is 70% hepatocytes (Williams et al., 1978).

3. Results

3.1. Tissue specificity of basal apoB expression in embryonic tissues

Figs. 1 and 2 show Northern and dot blot analysis of apoB mRNA in a variety of tissues in the developing chicken embryo. The 14 kb mRNA is expressed in liver and kidney as previously reported in roosters (Kirchgessner et al., 1987). Embryonic intestine contains little or no apoB mRNA, unlike rooster intestine (Fig. 1).

Fig. 2 shows that apoB is expressed to a relatively high degree in the embryonic yolk sac, more in the vascularized than in non-vascular tissue. The Northern blot reveals that the 14 kb apoB mRNA shows a tendency to break down in a recognizable pattern, giving products which run just ahead of the 18S and 28S ribosomal RNA positions on the gel. This is particularly noticeable in Fig. 2 for the day 8 and 14 samples. These are not non-specific bands since

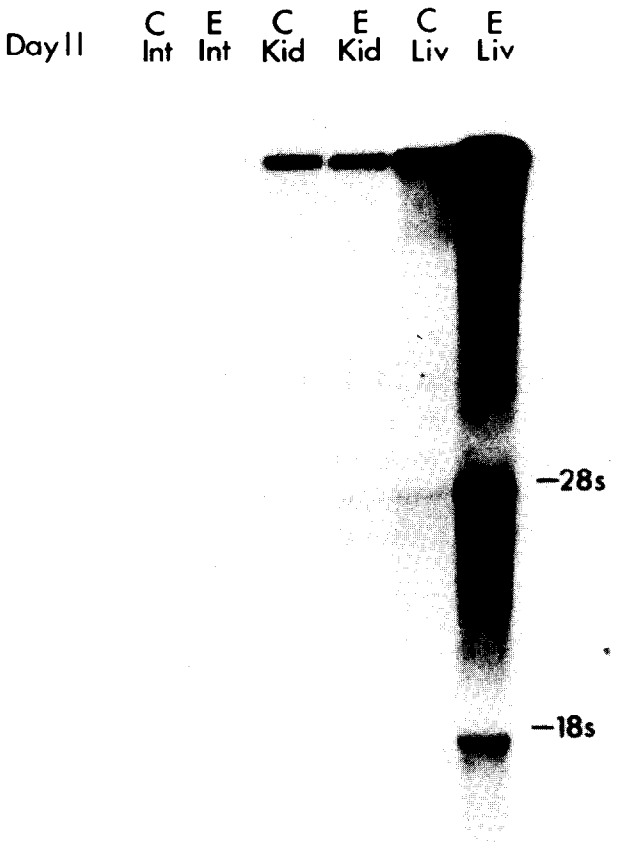

Fig. 1. ApoB mRNA in chicken embryo liver and kidney. Northern blot analysis of total liver RNA (30 μg/lane) from day 11 embryos. C Int, control intestine; E Int, intestine from estrogen-treated embryos; C Kid, control kidney; E Kid, kidney from estrogen-treated embryos; C Liv, control liver, E Liv, liver from estrogen-treated embryos. The upper band represents the 14 kb apoB mRNA. The overexposed E liv sample reveals some apoB mRNA breakdown products running just ahead of the 18S and 28S ribosomal RNA markers.

they are absent in the heart RNA lane. The dot blots in Fig. 2 show that apoB mRNA can be detected in 3-day vascular yolk sac and that at day 6 it constitutes about the same proportion of total RNA in vascular yolk sac as in adult liver.

Quantitation of apoB mRNA levels at different stages of development was carried out with embryonic intestine, kidney and liver tissue using a sensitive solution hybridization assay described by Williams et al. (1986) (Table 1). The results show that apoB mRNA can be detected in embryonic liver and kidney from the 7.5th day of development. Expression of apoB mRNA in the embryonic intestine is considerably lower, which is consistent with the observations that intestinal apoB protein is not synthesized in embryonic tissue (Nadin-Davis et al., 1980) and that nutrient absorption is directly from yolk sac into the embryonic circulation, not involving the intestine (Noble and Cocchi, 1990).

3.2. Development of responsiveness to estrogen

In order to assess estrogen responsiveness at the mRNA level, embryonated eggs at various stages were injected with estradiol and after 48 h of incubation, the embryos were dissected and total RNA was prepared from pooled frozen tissues. Tissues from 2 dozen embryos were pooled for each RNA preparation at day 15, 6 dozen at day 11 and 8–12 dozen at the earlier stages. cDNA excess liquid hybridization analysis using apoB and apoII single-stranded cDNA probes and Northern blot analysis were carried out. The results in Figs. 3 and 4 show that estrogen responsiveness of both hepatic apolipoprotein genes develops over days 6.5–9, but with somewhat different characteristics. ApoB estrogen responsiveness increases fivefold from day 6.5 to 9 (from 42 ± 11 to 210 ± 5 molecules per hepatocyte), while apoII responsiveness increases 60-fold (from 12 ± 1 to 748 ± 84 molecules per hepatocyte) over the same period. Basal apoB expression increases approximately fivefold over day 6.5 to day 15.

In contrast to the liver, apoB expression in the embryonic kidney does not exhibit estrogen responsiveness. Values of 13 ± 6, 3 ± 1, 17 ± 2, 29 and 63 ± 20 apoB mRNA molecules per hepatocyte were obtained for kidney RNA preparations from estrogen-treated 7.5-, 9-, 11- and 15-day embryos and estrogen-treated roosters, respectively. These concentrations are very close to those for RNA from control preparations at the same stages of development (Table 1). For apoII mRNA, on the other hand, we consistently find estrogen inducibility during development in both kidney and intestine. ApoII mRNA levels are somewhat variable and considerably lower than in liver but are reproducibly found in RNA from estrogen-treated embryos but, with one exception as noted in Table 2, not from untreated or vehicle-treated controls. To ascertain that the solution hybridization assay was measuring authentic apoII mRNA

Table 1

Development of basal apoB mRNA expression apoB mRNA (molecules/cell)

Day in development	ApoB mRNA (molecules/cell)		
	Intestine	Kidney	Liver
7.5	0	11 ± 1	31 ± 6
9.0	0	2 ± 1	39 ± 6
11.0	2 ± 1	16 ± 3	75 ± 28
15.0	1	32	94 ± 24
Adult	16	60 ± 12	110 ± 15

ApoB mRNA levels were measured by the DNA-excess liquid hybridization as given in Section 2. The results are the mean and range for two separate RNA preparations from pooled tissues except for day 15 and adult intestine and day 15 kidney where single preparations from pooled tissues were used.

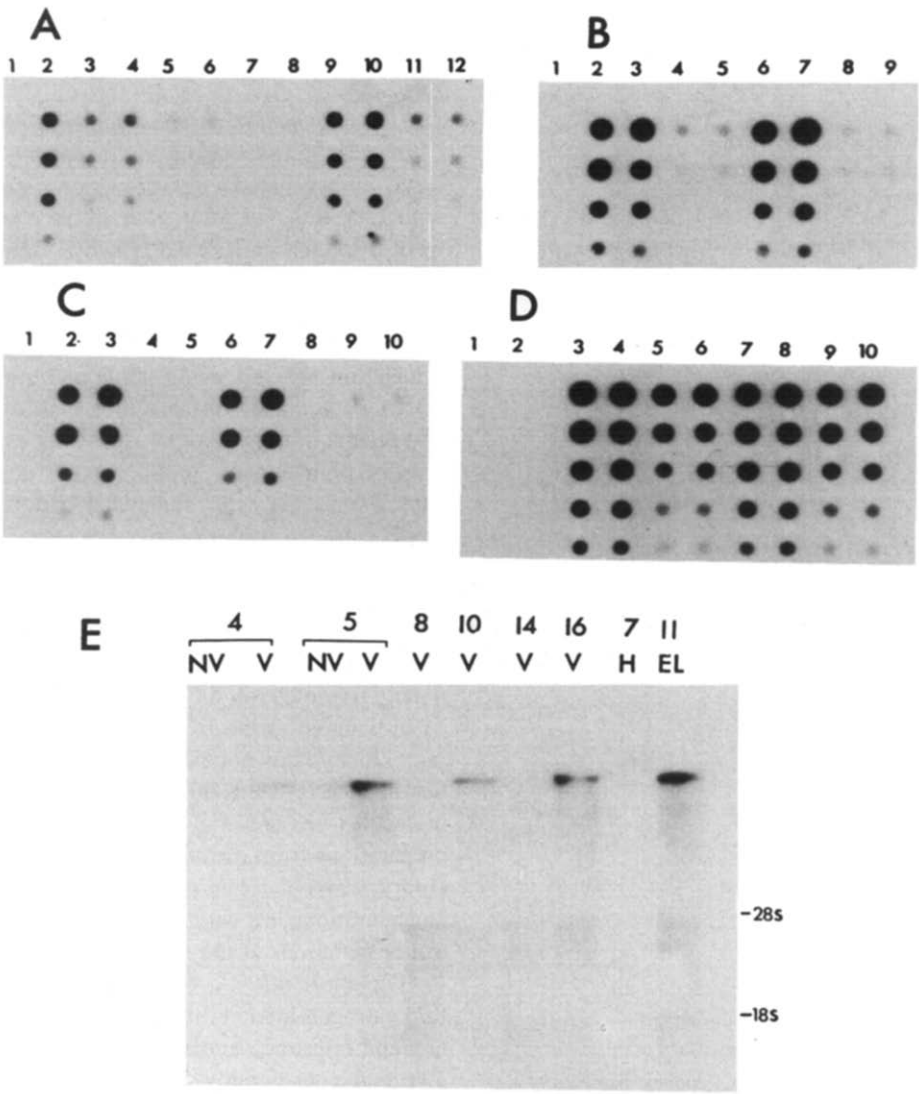

Fig. 2. ApoB mRNA in the embryonic yolk sac. This composite dot blot and Northern blot shows apoB expression in serial dilutions from total RNA or cytosol RNA preparations from vascular and non-vascular yolk sac compared to adult chicken liver. (A) Lane 1, day 19 brain RNA; lane 2; adult chicken liver RNA; lanes 3 and 4, 3-day vascular yolk sac plus embryo RNA; lanes 5 and 6, 3-day non-vascular yolk sac RNA; lanes 7 and 8, 4-day embryo thoracic region RNA; lanes 9 and 10, 4-day vascular yolk sac RNA; lanes 11 and 12, 4-day non-vascular yolk sac RNA. Serial dilutions of RNA contained from 5.0 to 0.625 μg of total RNA. (B) Lane 1, 19-day brain RNA; lanes 2 and 3, adult chicken liver RNA; lanes 4 and 5, 6-day embryo thoracic region RNA; lanes 6 and 7, 6-day vascular yolk sac RNA; lanes 8 and 9, 6-day non-vascular yolk sac RNA. Serial dilutions of RNA contained from 5.0 to 0.625 μg of total RNA. (C) Lane 1, 19-day brain RNA; lanes 2 and 3, adult chicken liver RNA; lanes 4 and 5, 7-day embryo thoracic region cytosol; lanes 6 and 7, 7-day vascular yolk sac cytosol; lane 8, blank; lanes 9 and 10, 7-day non-vascular cytosol yolk sac cytosol. Serial dilutions of RNA were from 5.0 to 0.625 μg; serial dilutions of cytosol contained the equivalent of 50 μl, 0.5 μ1, 0.25 μl and 0.125 μ1 of cytosol prepared as outlined in Section 2. (D) Lanes 1 and 2, 19-day brain RNA; lanes 3 and 4, 11-day liver cytosol; lanes 5 and 6, 11-day vascular yolk sac cytosol; lanes 7 and 8, 13-day liver cytosol; lanes 9 and 10, 13-day vascular yolk sac cytosol. Serial dilutions of RNA and cytosol were as in (C). (E) Northern blot, showing the 14 kb apoB mRNA in yolk sac and embryonic liver tissues. The numerals refer to the days of embryonic development. Thirty micrograms of RNA was used per lane. NV, non-vascular yolk sac; V, vascular yolk sac; H, heart; EL, liver from 11-day embryos which had been estrogen-treated for 48 h.

and not an unprocessed nuclear precursor or a related gene product, kidney and intestine RNA were examined by Northern blot analysis. As shown in Fig. 5, the apoII probe detected a single 800 nt mRNA band in kidney and intestine RNA that co-migrated with the estrogen-induced apoII mRNA in liver RNA. We have also examined the transcription start sites for kidney and intestine apoII transcripts by primer extension analysis (Binder et al., 1990). ApoII mRNAs from kidney and intestine showed the same pattern of three clustered transcription start sites that we previously reported in liver (data not shown). Since any tissue-specific difference in the splicing pattern of the primary transcript would be detected as a size difference of the apoII mRNA, these analyses confirm that estrogen induces authentic apoII mRNA transcripts in kidney and intestine.

Interestingly, the estrogen-responsiveness of kidney and intestine apoII genes is very low between days 7 and 9 of

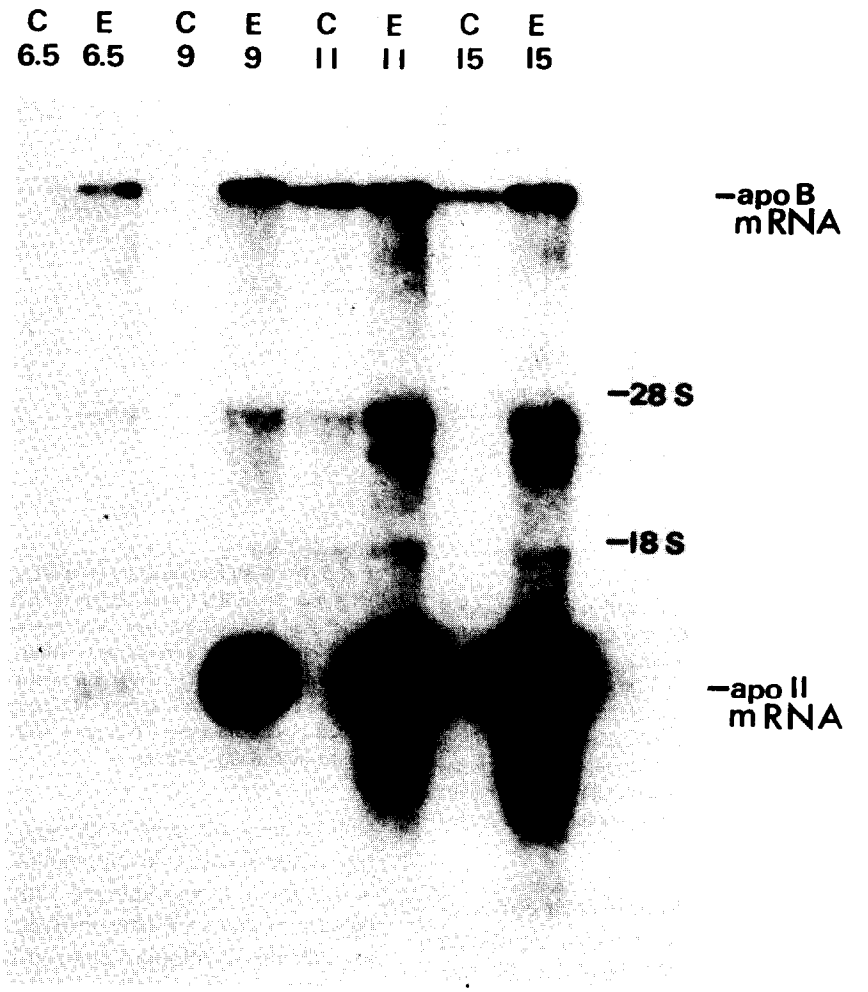

Fig. 3. Development of estrogen responsiveness of apoB and apoII genes. Northern blot analysis of total RNA prepared from liver from control or estrogen-treated embryos. 30 μg of RNA was used per lane. (C) Control E, estrogen-treated embryos The numerals refer to the days of development at the time of liver dissection. Estradiol or propylene glycol vehicle injections were given 48 h previously.

development, increases markedly at day 11, and declines at day 15 (Table 2). At the peak of responsiveness at day 11, the level of apoII mRNA induction in kidney and intestine is about 10% of that in the liver (Fig. 4), but by day 15 the levels of expression are 1% or less than in liver. We were able to detect apoII mRNA transcripts in kidney and intestine RNA from adult estrogen-treated roosters by primer extension analysis, but the mRNA content was too low to quantitate reliably (data not shown). This result suggests that the estrogen-responsiveness of the apoII gene is essentially extinguished in adult kidney and intestine.

3.3. Estrogen receptor expression in liver and kidney

Estrogen receptor characterized in chicken liver exhibits a K_d for estradiol of 1–2 nM (Lazier and Jordan, 1982). Salt extracts of nuclei of chicken kidney similarly show specific estradiol binding activity, with a K_d of 1–2 nM (data not shown). The concentration of the renal nuclear estrogen receptor from birds which had been estrogen-treated was 47 ± 6 fmol/mg protein in three independent preparations. This is about half that found in liver nuclear salt extracts prepared by the same method from similarly treated birds. As expected, nuclear extracts from untreated birds contained little or no receptor activity.

Estrogen receptor mRNA expression in embryonic kidney and liver from both estrogen-treated and untreated birds was examined using the Northern hybridization technique. β-Actin mRNA was also measured as a loading control. Fig. 6 shows that estrogen receptor mRNA (7.5 kb) can be detected in hepatic total RNA from embryos and birds. Only traces were detectable at early stages of development and a major increase appeared to take place around day 15. Fig. 6 also shows that estrogen receptor mRNA is detectable in embryonic and adult kidney, but in contrast to liver, it is at least as abundant in early stages as later. The level in adult kidney appears to be less than in day 15 liver. No consistent effect of estrogen treatment on estrogen receptor mRNA levels was seen. A separate experiment using

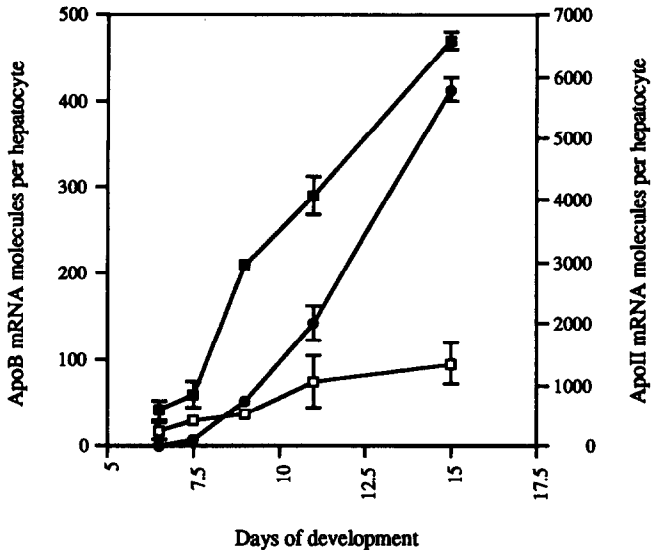

Fig. 4. Solution hybridization assay of hepatic apoB mRNA and apoII mRNA in development. Single-stranded apoB and apoII cDNA probes were used for cDNA excess solution hybridization. RNA was prepared from embryos which had been estrogen-treated or vehicle-treated 48 h previously. The abcissa refers to the stage of development at the time of death. ApoB mRNA: □, control embryos, ■, estrogen-treated embryos. ApoII mRNA: estrogen-treated embryos, ●. Note that apoII mRNA is not detectable without prior estrogen treatment of the embryos. The results are the mean and range for two independent preparations from pooled tissues.

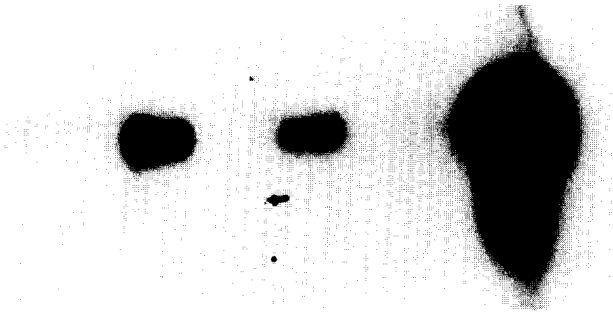

Fig. 5. Northern blot analysis of apoII mRNA in embryonic kidney and intestine. Total RNA was prepared from day 11 and day 15 embryos which had been estradiol or vehicle-treated for 48 h (30 μg RNA/lane). The Northern blots were probed with a ^{32}P labeled apoII cDNA probe. CI, control intestine (11 days); EI, intestine from estrogen-treated 11-day embryos; CK, control kidney (11 days); EK, kidney from estrogen-treated 11-day embryos; CL control liver (15 days); EL, liver from estrogen-treated 15 day embryos.

four independent RNA preparations from liver of estrogen-treated and control 15 day embryos showed no significant differences in estrogen receptor mRNA levels in the two groups (data not shown).

4. Discussion

It is clear from the results reported here that the expression of the apoB gene in chicken embryos is under distinctive tissue specific developmental programs. ApoB mRNA is first found in vascularized yolk sac RNA, at a level comparable to that seen later in liver. Assimilation of yolk lipids into the developing embryo is a complex process and involves uptake of yolk lipid into the yolk sac endoderm, where hydrolysis and re-esterification of the major lipids and reassembly into new lipoprotein particles takes place (Noble and Cocchi, 1990). Yolk nutrients taken up by the yolk sac endoderm are transferred to mesodermal blood vessels of the vascular yolk sac and transported to the embryo. Obviously apoB could be a key constituent of the yolk sac lipoprotein particles. The cues for yolk sac apoB expression are not clear, but our findings suggest that they could be mesodermal or blood-borne component(s) since apoB expression is associated mainly with the vascularized membrane.

The relative paucity of apoB mRNA in embryonic intestine is consistent with previous observations on the lack of apoB protein synthesis in that tissue (Nadin-Davis et al., 1980). Since the yolk sac and not the embryonic intestine has the primary role in embryonic nutrient absorption, it is reasonable that intestinal apoB expression develops only after hatch (Kirchgessner et al., 1987).

The role of the apoB formed by the avian kidney is not understood. It is not estrogen-sensitive and thus is probably not related to egg laying. There are no reports that lipoprotein particles are synthesized by the avian or mammalian kidney although lipoprotein particle production by the proximal segment of lamprey kidney has been observed (Hatae and Fujita, 1987). The kidney apoB is very similar

Table 2

Ontogeny of apoII mRNA responsiveness to estrogen in kidney and intestine

Day in development	ApoII mRNA (molecules/cell)			
	Kidney		Intestine	
	Control	Estrogen-treated	Control	Estrogen-treated
7.5	0	3 ± 1	0	6 ± 4
9.0	0	1 ± 0.5	0	1 ± 8
11.0	0	159 ± 33	0	230 ± 130
15.0	5 ± 4	36 ± 25	0	7 ± 2

ApoII mRNA levels were measured by the DNA excess liquid hybridization method as described in Section 2. The results are the mean and range for two independent preparations from pooled tissues for each stage and treatment group except for day 11 kidney from estrogen-treated embryos where five independent preparations were used. One preparation from the 15-day control kidney exhibited a low level of apoII mRNA but no other control preparations gave any hybridization above background.

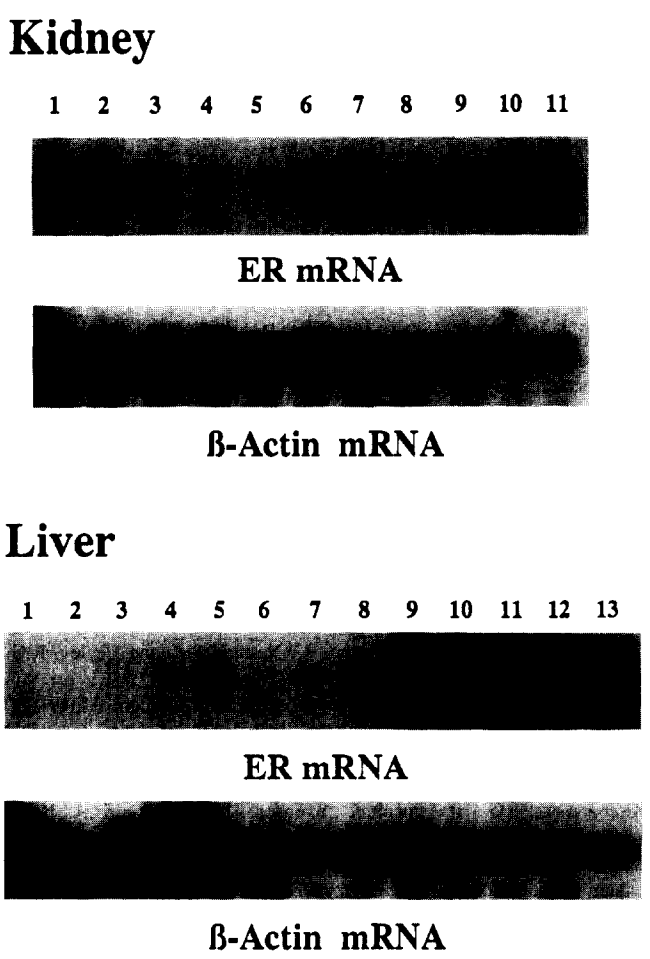

Fig. 6. Northern blot analysis of estrogen receptor mRNA in embryonic liver and kidney. Northern blots (40 μg total RNA per lane) were probed with ^{32}P-labeled CEO cDNA for chicken estrogen receptor mRNA (7.5 kb) and with ^{32}P-labeled β-actin cDNA for β-actin mRNA (2.3 kb). The numbers on the upper two panels (kidney) refer to RNA prepared from embryonic kidney at the following stages: 1, day 6.5 control; 2, day 6.5 estrogen-treated; 3, day 7.5 control; 4, day 7.5 estrogen-treated; 5, day 9 control; 6, day 9 estrogen-treated; 7, day 11 control; 8, day 11 estrogen-treated; 9, adult estrogen-treated; 10, day 15 liver control; 11, day 15 liver estrogen-treated. The numbers on the lower two panels (liver) refer to RNA prepared from embryonic liver: 1, day 6.5 control; 2, day 6.5 estrogen-treated; 3, day 7.5 control; 4, day 7.5 estrogen-treated; 5, day 9 control; 6, day 9 estrogen-treated, 7, day 11 control; 8, day 11 estrogen-treated; 9, day 15 control; 10, day 15 estrogen-treated; 11, day 19 control; 12, day 19 estrogen-treated, 13, rooster control.

to hepatic apoB; both proteins show the same reactivity to antibody against plasma apoB and both show identical partial protease maps with V8 protease and elastase using [^{35}S]methionine or [^{3}H]glucosamine labeling (Blue et al., 1980). However some differences in mRNA processing leading to minor differences in the expressed proteins cannot be ruled out at this stage.

Our finding of estrogen-stimulated apoII mRNA induction in embryonic kidney was unexpected and raises a number of interesting questions. First, what, if any, are the functional consequences of apoII mRNA induction? Earlier literature suggests that estrogen has a role in calcium homeostasis through modulation of PTH and vitamin D_3 action in avian kidney (Castillo et al., 1977; Forte et al., 1983), but a possible role for apoII in these processes is not at all obvious. Rat kidney contains estrogen receptor (Hagenfeldt and Eriksson, 1988) and several estrogen-regulated proteins have been identified (Muekler et al., 1984; Whitman et al., 1990) but since apoII is strictly found in birds it is likely any function would be linked to some unique aspect of avian renal physiology.

A second question is, why does estrogen induce apoII mRNA expression in kidney and small intestine but have no effect on apoB? It is possible that the apoB gene in embryonic intestine is not estrogen responsive because it is in an inactive chromatin configuration. However, this cannot explain the lack of estrogen responsiveness in kidney since apoB is expressed constitutively in embryonic and adult kidney. One possibility is that estrogen-mediated transcription of the apoB gene requires a liver-specific transacting factor other than the estrogen receptor and in addition to factors required for estrogen-dependent expression of the apoII gene (Brooks and Levy-Wilson, 1993). This possibility is illustrated in Fig. 7 which shows hypothetical regulatory factors associated with the apoB promoter. In this scheme, factor(s) A is required for constitutive apoB expression and is present in embryonic liver and kidney but does not appear in intestine until after hatching. Factor B. which is present in embryonic liver but is absent in kidney and intestine, is required in conjunction with the estrogen receptor (ER) for estrogen-induced apoB expression. In a similar vein, factor C is specific for the apoII gene and is required in conjunction with ER for estrogen-induced apoII expression. In this model the transient window of estrogen-

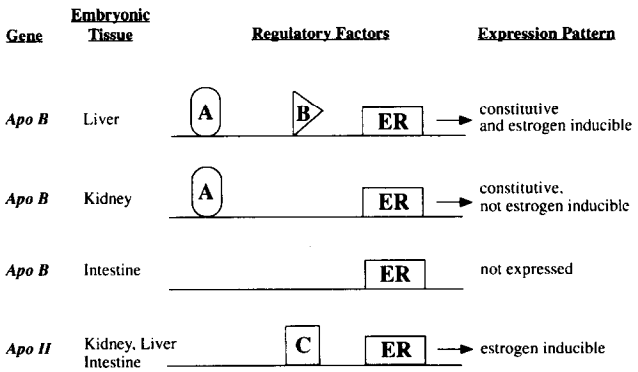

Fig. 7. Interplay of putative regulatory factors to explain the expression pattern of apoB and apoII genes during embryonic development. Factor A is required for apoB constitutive expression in the embryonic liver and kidney and is missing in embryonic intestine. Factor B is unique to embryonic and post-hatch liver and is required for estrogenic induction of apoB but not apoII. Factor C is specific for estrogen inducibility of the apoII gene in all three tissues and may be expressed transiently in embryonic kidney and intestine. Estrogen receptor (ER) is present in all three tissues. In post-hatch tissues the simplest model would be that factor A persists in liver and kidney and is acquired in intestine. Factor B would remain liver-specific and factor C would be lost in kidney and intestine but not liver.

responsiveness of the apoII gene in kidney and intestine could reflect the transient expression of factor C and the loss of apoII estrogen responsiveness in the adult despite the presence of the receptor. This model is speculative but serves to illustrate that the complex tissue-specific pattern of apoB and apoII expression in the embryo may reflect the interplay of several tissue-specific and gene-specific regulatory factors.

This is the first demonstration of estrogen receptor binding activity in chicken kidney and of estrogen receptor mRNA in embryonic kidney. Traces of estrogen receptor mRNA have been reported earlier in adult chicken kidney using a reverse-transcriptase PCR method (Ninomiya et al., 1992). It is possible that estrogen receptor expression is limited to a portion of the kidney, and immunocytochemical analysis or in situ hybridization will be necessary to further investigate this issue. It is also important to determine the physiological target genes of the renal receptor. The estrogen receptor concentration in kidney of hatched birds is somewhat less than in liver but is high enough so that physiological functions are feasible.

The analysis of differential estrogen regulation of particular genes and of tissue specificity in hormonal responsiveness promises to give basic insights into gene regulation. Of the estrogen-sensitive genes involved in vitellogenesis, sequence and functional analysis of the 5′ regulatory regions of the apoII and vitellogenin genes point to the key role of estrogen response elements as well as liver-specific factors. The response elements are present in different arrangements depending upon the species and the particular gene (Wijnholds et al., 1991; Seal et al., 1991). It is also probable that local chromatin structure and the presence of trans-acting positive and negative transcription factors in addition to the estrogen receptor could be of central importance in the development of estrogen responsiveness (Haché et al., 1987; Hoodless et al., 1990; Beato, 1991). Future studies focused on the avian apoB promoter and regulatory elements will serve to define those elements necessary for tissue-specific expression and to test whether estrogen-responsiveness of apoII and apoB genes requires distinct gene-specific factors acting in concert with the estrogen receptor.

Acknowledgements

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