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DEXAMETHASONE AND ESTROGEN REGULATE XENOPUS LAEVIS ALBUMIN mRNA LEVELS*

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The regulation of albumin mRNA levels by steroid hormones was examined in a primary Xenopus liver culture system. In the absence of exogenous steroid hormone, albumin mRNA levels declined rapidly in culture. Dexamethasone is required for preservation of albumin mRNA levels in culture and effects a >10 fold induction of albumin mRNA in cultures maintained in steroid hormone-free medium. Estrogen can override the effect of dexamethasone and elicits a decline of greater than 80% in albumin mRNA levels. Our cDNA clones of the mRNAs encoding the two 74,000 dalton and one 72,000 dalton cellular albumins allowed us to show that all three albumin mRNAs were down regulated by estrogen. © 1986 Academic Press, Inc.

The synthesis and secretion of serum albumin represents a major differentiated function of hepatocytes. Albumin mRNA levels in mammalian systems are relatively unresponsive to regulation (1). In contrast, Xenopus laevis albumin mRNA levels exhibit a complex pattern of regulation in response to estrogen (2-6). Albumin mRNA levels and albumin synthesis decline and then recover following estrogen administration. Down regulation of albumin mRNA levels by estrogen has been reported to be mediated by a combination of a decreased rate of albumin gene transcription and an increased rate of albumin mRNA degradation (6) and by an increased rate of albumin mRNA degradation with no change in albumin transcription rate (7). The investigation of albumin synthesis using cell culture systems is complicated by the rapid decline in albumin production in most primary cultures and by the low level of albumin synthesis in most established hepatocyte lines (8).

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In this report we describe the hormonal regulation of albumin synthesis in a primary Xenopus culture system we have shown exhibits an estrogen-mediated induction of vitellogenin mRNA which is comparable to the in vivo process (9). We demonstrate that dexamethasone is required for the maintenance of albumin mRNA levels in this system, and that it can efficiently induce albumin mRNA when added back to steroid hormone free culture medium. In contrast to its complex in vivo pattern of regulation, estrogen represses albumin mRNA levels in a simple linear fashion. These studies were made possible by the isolation and characterization of a set of Xenopus albumin cDNA clones which contains clones of the mRNAs coding for both the 74,000 dalton and the 72,000 dalton serum albumins.

EXPERIMENTAL PROCEDURES

Preparation and Characterization of Albumin cDNA Clones. A small cDNA library was prepared from size fractionated (10) 18S poly(A) RNA (11) from male Xenopus liver. Double stranded cDNA (13) was tailed with dCTP (14), annealed to pBR322 tailed with dGTP, and transformed into E. coli RR1 (15,16).

The cDNA clones were screened by differential colony hybridization (16) with [^{32}P]cDNA prepared from estrogen stimulated (minus probe) and control mRNA (plus probe). Albumin cDNA clones were identified as clones of mRNAs whose levels decline following estrogen administration and their identities confirmed by hybridization-selection-translation (17). The homology between albumin clones was determined by restriction mapping, cross hybridization as a function of temperature (18) and salt concentration (19).

Xenopus Liver Culture and Quantitation of Albumin mRNA Levels. Adult male Xenopus were either untreated or injected with 2 mg of estradiol-17β 60-65 days previously. Primary liver cube cultures were prepared and maintained as described by Brock and Shapiro (9) in medium containing insulin and triiodothyronine supplemented as indicated with dexamethasone and estradiol-17β.

Quantitation of Albumin mRNA Levels. Albumin mRNA levels were determined by quantitative RNA dot hybridization essentially as described for

vitellogenin mRNA (9). A control with known albumin mRNA content was used as a standard in each hybridization in order to allow calculation of the number of molecules of albumin mRNA per cell (9,10). RNA blots were carried out after fractionation on glyoxal and transfer to GeneScreen (New England Nuclear). Hybridization was according to the manufacturer's directions. The membranes were washed twice in 2X SSC (1X SSC = 0.15 M NaCl, 15 mM Na•Citrate pH 7.0) for 1 hr at 25° and for 30 min at 65° in 2X SSC, 0.5% SDS; 1X SSC, 0.1% SDS; 0.5X SSC, 0.1% SDS.

RESULTS

Production and Characterization of Albumin cDNA clones. *Xenopus* albumin cDNA clones were identified in a small cDNA library by differential colony hybridization using cDNA probes prepared from control *Xenopus* liver (in which albumin mRNA is abundant) and from estrogen stimulated liver (in which albumin mRNA levels are low. After hybridization with the cDNA probes and with one of the albumin cDNA clones, pXA1a, approximately 60 of our 500 clones were shown to be albumin clones. This suggests that albumin comprises approximately 5% of total male *Xenopus* liver RNA and 12% of the size fractionated RNA used to prepare the cDNA library.

The proteins encoded by the cDNA clones were identified by hybridization-selection-translation (Fig. 1). Two *Xenopus* albumin proteins, which we

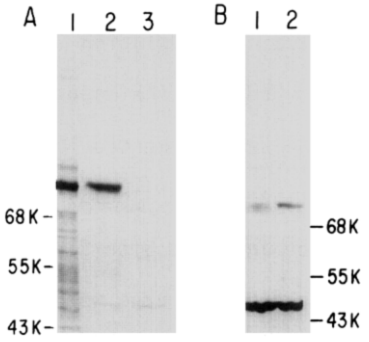

Fig. 1. Identification of Xenopus albumin cDNA clones by hybridization-selection-translation. *Xenopus* albumin cDNA clones corresponding to both the 74 Kd and the less abundant 72 Kd albumin were identified by hybridization-selection-translation (17) with approximately 200,000 cpm of [^{35}S] translation product synthesized from the hybrid selected RNA. Panel A: lane 1, translation products obtained with total *Xenopus* RNA; lane 2, hybrid selection of a *Xenopus* albumin 74 Kd clone pXA2b; lane 3, hybrid selection of a clone not coding for albumin. Panel B: lane 1, hybrid selection of clone pXA1a which codes for a 72 Kd albumin and of a 74 Kd albumin clone pXA3e (lane 2).

designate as the abundant 74 Kd albumin and the less abundant 72 Kd albumin are found in Xenopus. These proteins are encoded by related genes (18). Our hybrid selections (Fig. 1B, lane 1) and other data (see below) indicated that clone pXA1a is a 72 Kd albumin clone.

A set of clones were characterized in extensive cross hybridization studies (data not shown [19]) and by restriction mapping. These studies are consistent with the existence of three classes of albumin cDNA clones (Fig. 2). This work also demonstrated that under ordinary hybridization

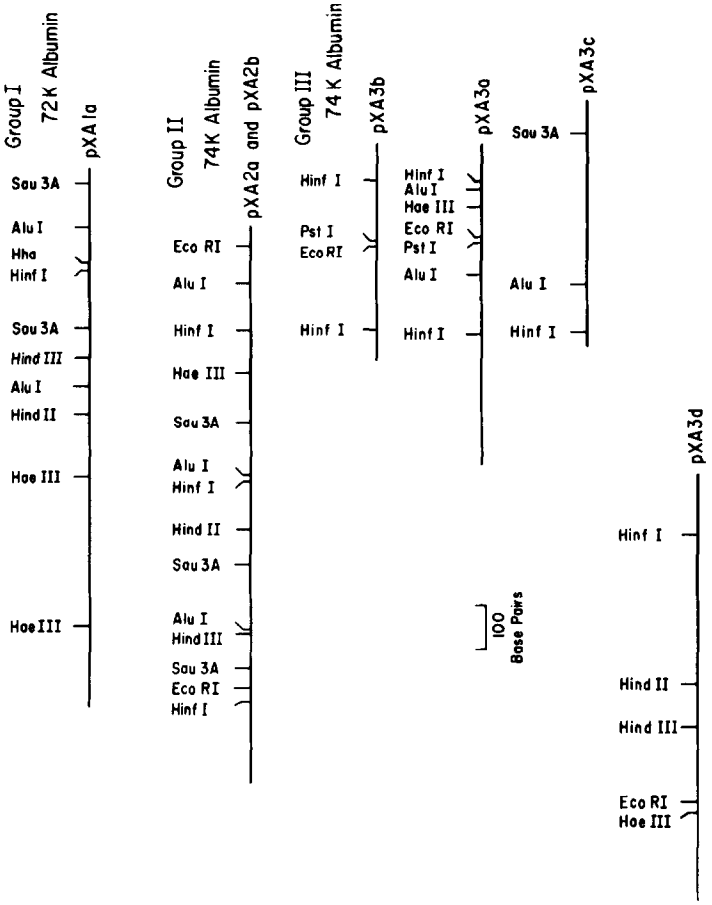

Fig. 2. Restriction maps of Xenopus albumin cDNA clones. The restriction endonuclease cleavage sites located in 7 albumin cDNA clones are presented. No cleavage sites were identified for Taq I, Acc I, Bgl I, Xho I and Xba I. The orientation of the clones was determined by end labelling with polynucleotide kinase followed by cleavage with restriction enzymes to obtain fragments labelled on one strand. The labelled fragments were isolated after agarose gel electrophoresis and hybridized to immobilized Xenopus RNA. The clones are drawn 5' to 3' and are ordered in groups based on restriction mapping and cross-hybridization studies.

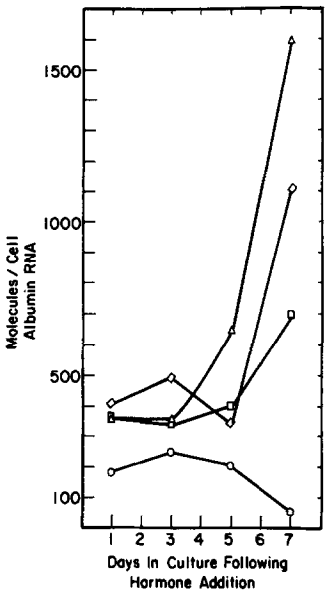

Fig. 3. Induction of albumin mRNA in Xenopus liver cubes by dexamethasone and T_3. Xenopus liver cubes were depleted of albumin mRNA by maintenance in steroid hormone-free medium for 7 days. On day 7 they were divided into groups which received: (o) no additional hormone; $1 \cdot 10^{-8}$ M T_3, and (□) $1 \cdot 10^{-8}$ M dexamethasone; (◇) $1 \cdot 10^{-7}$ M dexamethasone; (Δ) $5 \cdot 10^{-6}$ M dexamethasone. At the indicated times, albumin mRNA levels were determined as described in "Experimental Procedures".

conditions a single albumin cDNA clone of the mRNA encoding the 74 Kd albumin will effectively cross-hybridize with both the 74 Kd and 72 Kd albumin mRNAs.

Hormone Regulation of Albumin mRNA Levels in Primary Cultures. The effects of dexamethasone and estradiol-17β on albumin mRNA levels in primary cultures were examined. Although this culture system is capable of efficient induction of vitellogenin mRNA in chemically defined medium lacking other steroid hormones (9), levels of albumin mRNA decline rapidly in the absence of exogenous glucocorticoids (data not shown). The ability of dexamethasone to restore albumin mRNA levels after 8 days in primary culture is shown in Fig. 3. All three concentrations of dexamethasone dramatically increased albumin mRNA levels relative to a control culture. At the highest level of dexamethasone ($5 \cdot 10^{-6}$M), albumin mRNA levels were induced 30 fold and were still increasing when the experiment was terminated.

This culture system was used to examine the effect of secondary estrogen stimulation on albumin mRNA levels. Primary cultures were prepared from

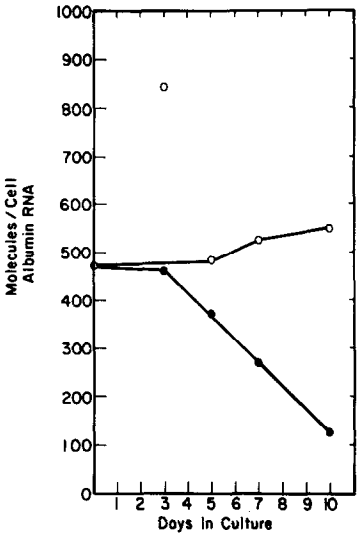

Fig. 4. Estrogen repression of albumin mRNA. Male Xenopus were injected with 2 mg of estradiol-17β. After 60 days at which time the cells contain no detectable vitellogenin mRNA, the livers were excised and placed in culture. After 2 days to adapt to the culture medium (16) the cultures were maintained in either $1 \cdot 10^{-6}$ M dexamethasone (o) or in the above hormone plus $1 \cdot 10^{-6}$ M estradiol-17β (•). Albumin mRNA levels were determined as described in "Experimental Procedures".

livers of withdrawn male Xenopus laevis and maintained in medium containing $2 \cdot 10^{-6}$M dexamethasone. After 2 days estradiol-17β ($1 \cdot 10^{-6}$ M) was added to half the cells and albumin mRNA levels were determined at various times. Albumin mRNA levels remained essentially constant in liver cubes maintained in dexamethasone (Fig. 4). Estradiol-17β produces a linear decline in albumin mRNA levels which are 18% of control levels after 10 days.

These studies demonstrated that dexamethasone can restore or maintain albumin mRNA levels in culture while estrogen represses albumin mRNA. Because our standard hybridization conditions detect both the quantitatively major mRNA coding for the 74 Kd albumin and the much less abundant mRNA coding for the 72 Kd albumin, these experiments did not exclude the possibility that the 72 Kd albumin mRNA was regulated independently of the major 74 Kd albumin mRNA. We therefore examined the relative ratios of these mRNAs by blot hybridization of RNAs from the experiment shown in Fig. 4, under stringent conditions. The ratios of the two albumin mRNAs did not change during steroid hormone treatment (Fig. 5).

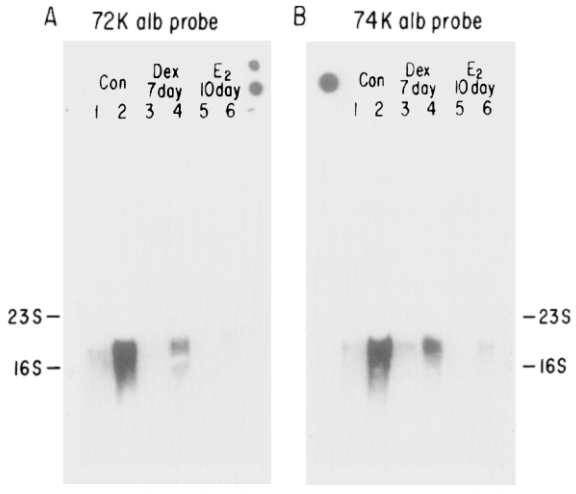

Figure 5. Blot hybridization of albumin probes to Xenopus RNAs. The RNA samples from Fig. 4 and from a control male Xenopus were fractionated on denaturing glyoxal gels transferred to GeneScreen and hybridized (see "Experimental Procedures"). Panel A shows hybridization under stringent conditions to nick-translated clone pXA1a (72 K alb probe) while panel B shows hybridization to pXA2b (74 K alb probe). For each sample the left lane contained 2.5 µg of RNA while the right lane contained 10 µg of RNA.

DISCUSSION

Despite its high abundance in mammals and Xenopus, the maintenance of high levels of albumin secretion in primary hepatocyte cultures has often proven difficult (8). Our data demonstrating the importance of dexamethasone in maintenance of albumin mRNA (Fig. 4) and in reinducing albumin mRNA to >30% of in vivo levels (Fig. 3) is consistent with its reported role in mammals (21-23).

The ability of estrogen to repress albumin mRNA levels (Fig. 4) in culture and override the inductive effect of glucocorticoids resembles its in vivo effects. The striking differences in the reported effects of estrogen in two recent studies (6,7) may be due to the absence of dexamethasone from the culture medium and to the carryover of low but variable amounts of dexamethasone in the different primary culture systems employed. Regulation of Xenopus albumin mRNA levels by dexamethasone and estrogen in primary Xenopus liver cultures represents a useful model for the study of the interrelationships between a maintenance hormone (dexamethasone) and a regulatory hormone (estradiol-17β).

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