VITELLOGENIN SYNTHESIS IN ROOSTER LIVER: CHANGES IN LIVER POLYRIBOSOMES IN RELATION TO THE ACTIVATION OF NUCLEOLAR RNA POLYMERASE AND VITELLOGENIN SYNTHESIS

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Summary: Vitellogenin induction was correlated with changes in the activity of nucleolar RNA polymerase and the quantity and size distribution of liver polyribosomes at various times following an initial and subsequent administration of estrogen. The quantity of ribosomes per liver protein increased rapidly after stimulation to a maximum value which was approximately twofold when compared to the initial level. The concentration of plasma vitellogenin peaked slightly later. The increase in the quantity of ribosomes was accompanied by a rapid and dramatic increase in liver polyribosome size. With decreasing plasma levels of vitellogenin the amount of liver ribosomes also diminished and the polyribosome pattern returned to that characteristic of unstimulated roosters by the time vitellogenin had disappeared from the plasma. The magnitude of vitellogenin induction was 2.7-fold greater in response to the second estrogen injection. Of special interest was the slower activation of nucleolar RNA polymerase and the less extensive increase in the quantity of ribosomes when compared to the initial response.

Yolk protein formation in egg-laying vertebrates, such as hens, appears to involve synthesis of a yolk protein precursor by the liver, its secretion into the blood and uptake and processing by the developing oocyte. Synthesis of the avian yolk protein precursor, vitellogenin, can be induced in the livers of both immature and male birds by the administration of estradiol-17β and related steroids (see 1). Avian vitellogenin is a lipoglycophosphoprotein with a monomer molecular weight of 240,000 to 270,000 (1-3). The vitellogenin monomer is cleaved in the ovary to yield lipovitellin, with a molecular weight of about 180,000, and phosvitin, an extensively phosphorylated protein of molecular weight approximately 30,000, which contains 55 % serine (1-3). The determination of vitellogenin in plasma is relatively simple due to its unusually high content of serine phosphate (1,4). Estrogen treatment of roosters also causes a severalfold increase in the rate of hepatic synthesis of the protein component of very low density lipoprotein (5).

The quantity of ribosomes in tissues of adult animals is maintained at constant levels by mechanisms which are not yet sufficiently understood. In addition to changes in the rate of synthesis and degradation of ribosomal RNA

and protein, regulation at other steps such as maturation, modification, ribosome assembly and transport from the nucleus or a combination of these processes may be involved. Following estrogen induction of vitellogenin synthesis in roosters, total liver RNA increases considerably whereas DNA does not (6-8). This suggests that the amount of ribosomes relative to DNA is increased. It has also been shown that following estrogen treatment of immature chicks there is a parallel increase in ribosomal RNA with increased elongation factor content (9). Using a new magnesium precipitation technique it is possible to determine the total quantity of ribosomes in different types of tissues (10). I have used this method to study the quantitative aspects of ribosome synthesis and breakdown during the reversible stimulation of vitellogenin synthesis in roosters, which represents a large and selective activation of a quiescent protein structural gene in a well-differentiated tissue.

An interesting aspect of vitellogenin induction is also that, after rooster has responded to an injection of estrogen and plasma vitellogenin level has returned back to zero level, the animal responds to a subsequent injection of the hormone with a faster rate of accumulation of vitellogenin in plasma and an increased maximal level of plasma vitellogenin. Following the initial stimulation, vitellogenin first appears in the plasma after about four hours and a rapid rate of accumulation is reached after about 20 hours after the injection. Stimulation of the rooster with a second injection of estrogen two to three weeks after the first one results in the appearance of vitellogenin in plasma in about four hours but causes the level of vitellogenin to increase more rapidly, to reach a higher level, and to take longer to disappear (11-13). After the initial injection of estrogen de novo synthesis of RNA is required for induction of vitellogenin synthesis (1,4). However, the subsequent stimulation may be also qualitatively different since it has been recently demonstrated that after the initial stimulation when vitellogenin is no longer present in plasma or liver, the liver still contains appreciable amounts of vitellogenin mRNA, which is not associated with polyribosomes (14). The faster rate of vitellogenin synthesis during subsequent stimulations has also been suggested to be due to an increased ribosome content of the liver after the initial response (1,15). This paper reports the results of experiments designed to determine the quantitative and qualitative alterations in liver ribosomes during primary and secondary estrogen stimulation of yolk protein synthesis and their temporal relation to the activation of nucleolar RNA polymerase and levels of vitellogenin in plasma. While this study was made preliminary reports were also published from other laboratories (16-17).

MATERIALS AND METHODS

White Leghorn roosters weighing 1.0 to 1.2 kg were used. Estradiol-17β benzoate, 20 mg/kg in sesame oil, was injected intramuscularly as previously described (11). Blood samples were collected from the wing vein and vitellogenin in heparin plasma was determined on the basis of its high content of alkali-labile phosphorus (11). The isolation of liver nucleoli and the determination of RNA polymerase activity has been previously described (7).

To determine the quantity of ribosomes in the liver, the magnesium precipitation procedure was used with some modifications (10). A 10 % liver homogenate was prepared as follows. The rooster was anesthesized by injection of 60 mg/kg of sodium pentobarbital intraperitoneally. The liver was exposed and about half of each lobe was cut out and immediately put into ice-cold 0.9 % NaCl. About 5 g of tissue was weighed and minced in 9 volumes of a buffer containing 25 mM Tris/HCl, pH 7.5, 25 mM NaCl, 5 mM $MgCl_2$, 1 mg/ml of heparin and 2 % Triton X-100. The tissue was homogenized in a glass-teflon homogenizer with 15 strokes using a tight pestle. After centrifugation for 5 min at 28,000 g_{max}, the supernatant was decanted into another vessel and an equal volume of a second buffer (the previous buffer diluted with one fifth volume of 1 M $MgCl_2$ to yield 0.2 M $MgCl_2$) was added. The mixture was kept for 90 min in an ice-bath and 8-ml aliquots were layered over 4-ml pads of sucrose (0.2 M sucrose, 25 mM Tris/HCl, pH 7.5, 25 mM NaCl, 5 mM $MgCl_2$) in 15-ml polypropylene tubes and centrifuged for 10 min at 32,000 g_{max} with an angle rotor in a Sorvall RC-5 centrifuge. The supernatant was removed by aspiration partly into the sucrose pad and the upper portion of the tube was washed with distilled water. The wash was removed by aspiration and the tube was inverted to decant the remaining sucrose. After a few minutes, the walls were wiped dry with tissues and the pellet was dissolved in 20 mM Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), pH 7.5, rapidly frozen and stored in liquid nitrogen. For determination of total A_{254}-units, the pellets were dissolved in warm 0.5 % sodium dodecyl sulphate. All solutions were treated with diethyl pyrocarbonate as described (10).

Sucrose gradient centrifugation analysis of polyribosomes was carried out by layering samples containing 1-4 A_{254}-units in 0.025 to 0.050 ml over linear 3.7-ml sucrose gradients (0.7 to 1.5 M sucrose containing 0.1 mg/ml heparin). After centrifugation for 60 min at 337,000 g_{max} in a SW 60 Ti rotor, the gradients were pumped through a flow cell with a 0.5-cm light path and a continuous recording of absorbance at 254 nm was made using an ISCO UA-5 monitor. Samples frozen more than once were not used for studies of polyribosome profiles.

Protein was determined with bovine serum albumin as a standard (18). [$U^{14}C$]GTP (355 mCi/mmol) was obtained from New England Nuclear Corp. Estradiol benzoate was from Nutritional Biochemicals Corp. ATP, CTP and UTP were purchased as sodium salts from Boehringer, Mannheim.

RESULTS AND DISCUSSION

Figure 1 shows the results of the activation of nucleolar RNA polymerase and changes in the quantity of liver ribosomes and plasma vitellogenin during the primary and secondary estrogen response. Nucleolar RNA polymerase was rapidly activated in response to the estrogen injection reaching a maximal value after only 30 min. The high level of activity was maintained for at least three days. As previously reported (7), the activation did not occur as rapidly during the second estrogen response. The activity was not substantially different from the initial level four hours after the second

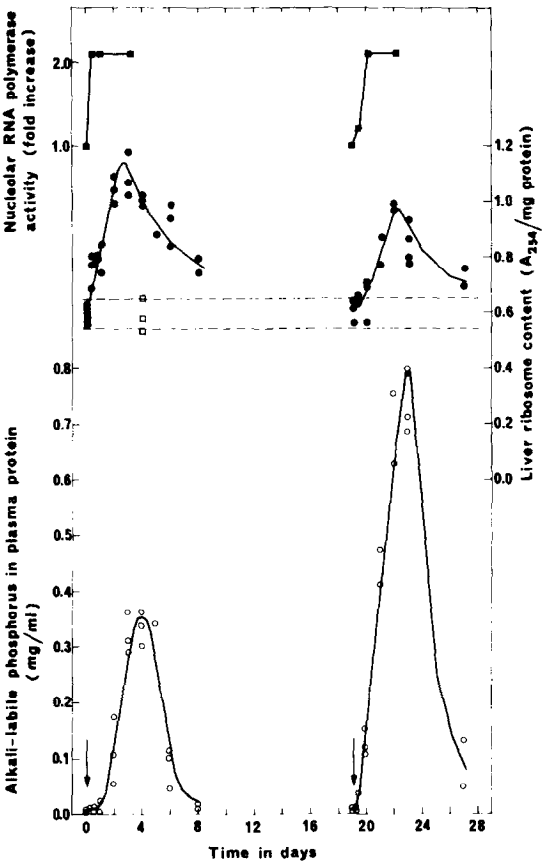

Figure 1. Activation of nucleolar RNA polymerase, changes in the levels of liver ribosomes and plasma alkali-labile protein-phosphate (representing vitellogenin) during the first and second estrogen response in roosters. Each symbol in ribosome and plasma phosphoprotein curves represents a value derived from one rooster. Values for RNA polymerase are calculated in part from previous data (7) and are means of 3 to 9 determinations each. The ribosome content is expressed relative to homogenate protein. The results are not different if expressed relative to liver DNA (not shown).

injection and only by 24 hours levels comparable to those found during the primary stimulation were found.

The effect of estrogen on the quantity of liver ribosomes is rapid and occurs prior to the increase in vitellogenin synthesis. Maximal levels which were approximately twofold as compared to the unstimulated level were found at day three, which was slightly prior to the peak of plasma vitellogenin. It has been demonstrated using antibodies against lipovitellin, that there is a progressive rise in the number of ribosomes synthesizing lipovitellin during the time when yolk protein synthesis occurs at an increasing rate. At the time

of maximal response 10 % of the ribosomes isolated from the post-mitochondrial supernatant are immunoprecipitated and almost all of these are derived from the membrane-bound population (19). The twofold increase in the total amount of liver ribosomes found in this study suggests that ribosomes involved in the synthesis of vitellogenin may only represent a small fraction of total ribosome population during the initial response. With decreasing levels of plasma vitellogenin the amount of liver ribosomes rapidly decreased almost to the control level by day eight. The decrease in plasma vitellogenin occurred slightly prior to the decline of tissue ribosomes.

As has been previously shown in several laboratories (11-13), the magnitude of vitellogenin synthesis upon the secondary stimulation was greater than during the first injection. The response was 2.7-fold greater based on the areas under the individual vitellogenin peaks. It was of interest that the increase of liver ribosomes was not as extensive as in response to the first injection, which is in accord with the slower activation of the nucleolar RNA polymerase.

The changes in size distribution of liver polyribosomes in response to the first (Figure 2) and second (Figure 3) estradiol injection were not substantially different. In both cases, the stimulation was followed during

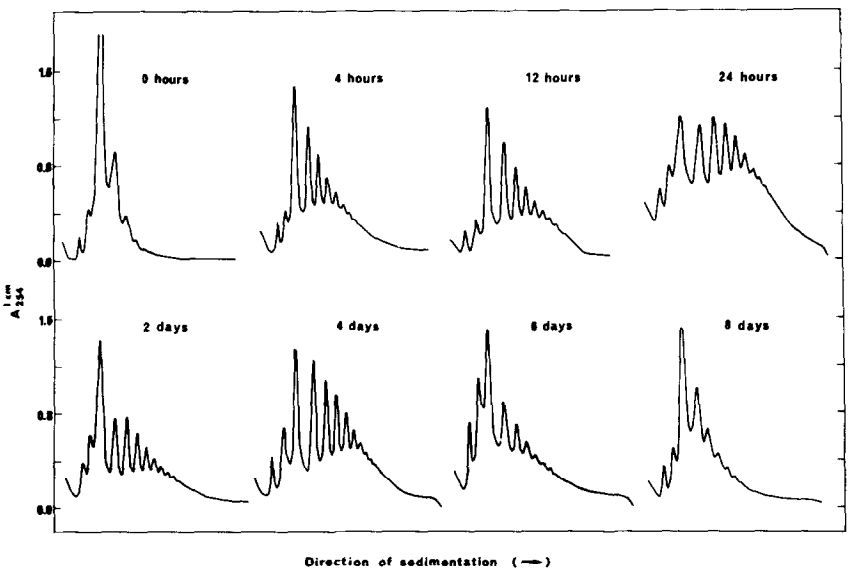

Figure 2. Changes in size distribution of liver polyribosomes during the first estrogen response. At every time-point a representative profile was chosen for presentation from 3 to 6 determinations. In each profile the highest peak corresponds to the monoribosome (80 S) peak.

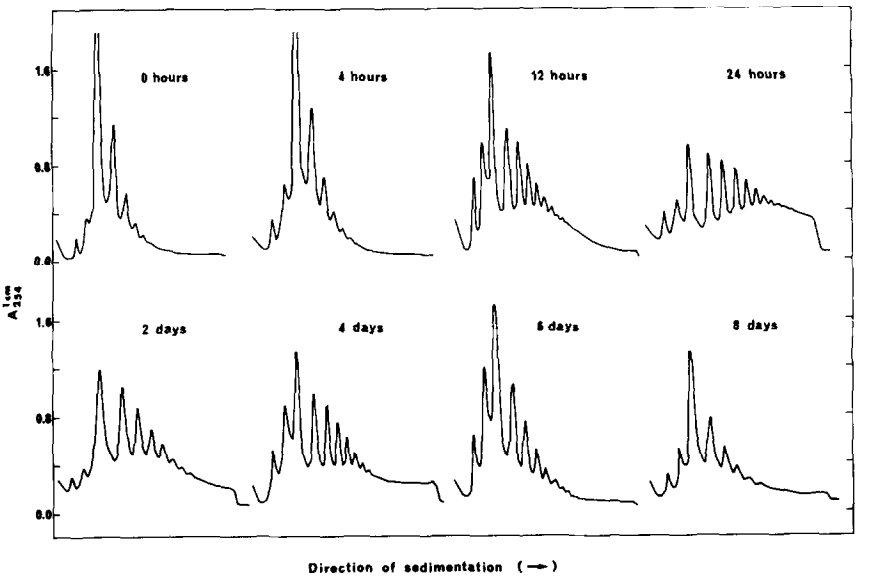

Figure 3. Changes in size distribution of polyribosomes during the second estrogen response. Details are as in Figure 2.

the first 24 hours by a dramatic and rapid shift of the gradient profiles to larger polyribosomes. Such a result implies that the rate of initiation of polypeptide synthesis has increased. The molecular weight of vitellogenin monomer is 240,000 to 270,000 (2,3), and the corresponding polyribosomes are reported to contain 25 to 40 ribosomes (20). In the gradient profiles shown here, these polyribosomes may be present near or at the bottom of the gradients especially in the one-, two-, and four-day samples. A clear qualitative shift to smaller polyribosomes was seen after day four when the quantity of ribosomes was also declining. By day eight the polyribosome profiles were almost indistinguishable from those derived from livers of unstimulated roosters. These results suggest that both the synthesis and degradation of liver ribosomes are under a sensitive control which at least in part is mediated through sex steroids. It is also interesting that the number of liver ribosomes diminished rapidly back to normal level as soon as the hormonal stimulation was over.

The more extensive synthesis of vitellogenin in response to a second injection of the hormone cannot be explained on the basis of a greater amount of ribosomes in the liver (1,15). In contrast, the quantity of ribosomes was normal at day 19 and the maximal stimulation of ribosome accumulation resulting from the second injection was less extensive. It is possible, however, that

a greater fraction of polyribosomes is functionally different and involved in the vitellogenin synthesis after the second injection. Specificity of function of newly synthesized ribosomes has been suggested in biosynthetic responses to hormones in many tissues (21). It has been recently shown that appreciable amounts of vitellogenin mRNA which is not associated with polyribosomes is present in the liver when the initial stimulation of vitellogenin synthesis is over and there is no vitellogenin present in plasma or liver (14). The importance of this mRNA for the more rapid and extensive secondary response remains to be established. A difference in the accumulation of ribosomes during subsequent hormonal stimulations suggests that the mechanisms in the first and second stimulation are not identical. The first injection probably involves many different steps in the redifferentiation of the liver to synthesize and secrete the yolk protein precursor, while subsequent injections may involve simpler mechanisms. The induction of vitellogenin synthesis by estrogen seems to provide possibilities for elucidating the mechanisms by which tissue levels of ribosomes are maintained and hormonally regulated.

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