Specific Transcription in Chicken Liver Chromatin by Endogenous RNA Polymerase II

COMPARISON OF AN ESTROGEN-INDUCIBLE GENE WITH A CONSTITUTIVELY EXPRESSED GENE*

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We have developed a system for the in vitro transcription of specific genes in rooster liver chromatin by endogenous RNA polymerase II that maintains the specificity of transcription in vivo. Radioactive transcripts synthesized in vitro were identified and quantitated by hybridization to a vast excess of cloned cDNA. The cDNA preparations employed corresponded to vitellogenin mRNA, the synthesis of which is responsive to estrogen stimulation in vivo, and chicken serum albumin mRNA, the synthesis of which is not significantly affected by estrogen stimulation in vivo. Comparing the pattern of transcription of the albumin and vitellogenin genes in chromatin from the liver of the normal rooster with the pattern in chromatin from the liver of the estrogen-stimulated rooster, we found that prior estrogen treatment of the rooster is attended by a slight decrease in the differential rate of transcription of the albumin gene and approximately a 10-fold increase in the differential rate of transcription of the vitellogenin gene. Because this pattern of transcription reflects the estrogen-induced changes in transcription observed in vivo, chromatin preparations from the livers of normal and estrogen-stimulated roosters can be used to investigate regulation of specific gene transcription at the molecular level in vitro.

Knowledge of the ways in which RNA synthesis is regulated is necessary for understanding the mechanisms involved in control of gene expression in eukaryotic organisms. In eukaryotes, there are at least three forms of DNA-dependent RNA polymerase: RNA polymerase I is responsible for ribosomal RNA synthesis; RNA polymerase II, for heterogeneous nuclear RNA and mRNA synthesis; and RNA polymerase III, for the synthesis of 5 S RNA, tRNA, and other small RNA molecules (1). To date, accurate in vitro transcription systems that utilize chromatin plus exogenous DNA-dependent RNA polymerase have been limited to studies of transcription by RNA polymerase II (2). Some correct in vitro syntheses of 5 S RNA utilizing endogenous RNA polymerase III in chromatin isolated from nuclei of mouse myeloma cells has been reported by Marzluff and Huang (3). Accurate in vitro transcription by RNA polymerase II has heretofore been limited to transcription by whole isolated nuclei, probably because essential components necessary for transcription by this enzyme are labile or are lost during isolation of RNA polymerase II. Recently, several laboratories have demonstrated the synthesis, in vitro, of various species of RNA by RNA polymerase II in isolated nuclei (4–7).

We have sought to develop an in vitro system that achieves faithful transcription by RNA polymerase II and that potentially can be manipulated so that its transcription pattern can be altered in a controlled way. As a first approach, rather than attempt to utilize isolated RNA polymerase II, we have used chromatin from the nuclei of rooster liver as the transcriptional material. This chromatin, isolated by a modification of the procedure of Marzluff and Huang (3), has high levels of endogenous RNA polymerase II activity and all transcription has been carried out by the endogenous, chromatin-associated RNA polymerase. Since it will be advantageous, eventually, to study ways in which transcription of certain genes can be altered, we have chosen to study transcription of a gene whose expression can be regulated in a controlled manner. The vitellogenin gene, whose expression is regulated by estrogen (8), fulfills this requirement. Administration of 17β-estradiol to males of oviparous species results in a several thousandfold increase in the concentration of vitellogenin mRNA in the liver (9–11). In vivo, there is only about 1 molecule of vitellogenin mRNA/cell in liver from normal roosters whereas vitellogenin mRNA constitutes 20% of polyadenylated RNA in livers of estrogen-treated roosters (10). Since it is known that estrogen treatment results in elevated levels of RNA polymerase I and II activities in rooster liver (12) and in rat uterus (13), it is necessary to ascertain that any increases in the transcription of an estrogen-inducible gene in vivo are, in fact, specific for estrogen-inducible genes, and do not simply reflect the elevated levels of RNA polymerase associated with estrogen stimulation. We have addressed this problem by also studying the transcription of a gene in the same tissue whose expression is constitutive and relatively unaffected by estrogen, the gene that specifies chicken serum albumin (14).

In the experiments described here, we show that transcription of the vitellogenin and albumin genes in chromatin preparations from the livers of normal and estrogen-treated roosters does mirror the pattern found in vivo. In addition, the transcription of these two genes that we have measured is carried out by the endogenous RNA polymerase II, as judged by its sensitivity to low levels of a-amanitin (15).

EXPERIMENTAL PROCEDURES

Materials—Six-week-old cockerels were obtained from Truslow Farms, Inc. Dithiothreitol, ultrapure ammonium sulfate, sucrose, and Tria base were from Bethesda Research Labs. Nucleoside triphos-
specific transcription in chicken liver chromatin


the abbreviations used are: SDS, sodium dodecyl sulfate; SSC, 0.15 M sodium chloride, 0.015 M sodium citrate; TUNES Buffer, 0.1 M Tris-HCl, pH 8.0; and TdT, terminal deoxyribonucleotidyl transferase.

conditions for RNA synthesis—In experiments in which the transcriptional activity of chromatin was measured, the assay was as follows: Individual in vitro transcriptions were initiated by the addition of 0.5 nCi of [α-32P]UTP or [α-32P]CTP, specific activity 2000 Ci/mmol, at a concentration of 2 x 105 M, and the other three nucleotide triphosphates at concentrations of 4 x 106 M. After incubation, yeast tRNA was added (final concentration 0.1 mg/ml) and MnCl2 and α-amanitin as indicated. The reaction was terminated by the addition of SDS to a final concentration of 0.16% and of trichloroacetic acid to a concentration of 10%. The chromatin was mechanically dispersed and the entire mixture was collected onto Whatman GF/C filters. The filters were washed with 30 ml of 10% trichloroacetic acid containing sodium pyrophosphate (20 mm) and then washed with 1 ml of 0.5 M KCl, followed by a 10% trichloroacetic acid wash. The filters were then air-dried and immersed in Protosol (New England Nuclear) for 18 h at 55°C, neutralized with 0.5 ml of 0.7 n acetic acid, and counted in Dimilume (Packard Instruments). All reactions were done in duplicate. For each reaction described, the incorporation of [3H]UMP into RNA was determined by subtracting the radioactivity of the incubation mixture from the radioactivity of the reaction mixture containing yeast tRNA, DNA, and the enzyme. The radioactivity of the reaction mixture containing yeast tRNA, DNA, and the enzyme was determined by the addition of the DNA filters (4 mm diameter, each containing 4 pg of DNA) to the RNA solution (50 μl). Incubation was for 18 h at 37°C. The solution was then filtered, washed with 1 ml of 0.5 M NaCl (0.1% SDS), 2% TEF, 0.1% SDS, and 0.1% Triton X-100, and the filters were air-dried and counted as described above. The percentage of the radioactive label incorporated into the DNA was determined as described previously (24).

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quantitation of specific transcripts by DNA-excess hybridization—Nitrocellulose filters (Schleicher and Schuell, B6) containing 32P-labeled DNA from the chimeric plasmid pFR322:albumin (26) or pBR322:vitellogenin were prepared according to the method of Jacquet et al. (27). Labeled RNA was denatured by heating at 80°C for 10 min in a solution containing formamide (50%), NaCl (0.3 M), sodium citrate (0.03 M), yeast tRNA (100 μg/ml), and poly(A) (100 μg/ml). The hybridization reactions were started by the addition of the DNA filters (4 mm diameter, each containing 4 μg of DNA) to the RNA solution (50 μl). Incubation was for 18 h at 42°C. At the end of the reaction, the filters were treated as follows: washed twice with 5 ml of 1.4 X SSC at 30°C for 1 h; washed twice with 5 ml each of 0.1 X SSC at 30°C for 20 min; incubated for 1 h at room temperature in 2 X SSC with boiled pancreatic RNase (20 μg/μl) and 5 μg/μl of tRNA; washed with 5 ml each of 2 X SSC at 30°C for 10 min; finally, washed successively with 5 ml each of 20% ethanol containing 0.1 M NaCl for 20 min until no more radioactivity washed off the filters. The filters were then dried and counted in Econofluor (New England Nuclear).

thermal denaturation of RNA-DNA hybrids—After the radioactive activity on the filters was determined, the filters were removed from scintillation fluid, dried, and placed in 1 ml of 2 X SSC. The tubes containing the filters were placed in a circulating water bath and the temperature was slowly raised. At 3-min intervals, the buffer was removed and another 1 ml of 2 X SSC was added to each tube. The radioactivity in each eluted fraction was determined by liquid scintillation counting in Aquasol. The isolation and characterization of chimeric plasmids containing albumin DNA has been described previously. The plasmid used in this study contained an albumin DNA that was 520 nucleotides long (26).

specific transcription in chicken liver chromatin

The amino-terminal portion of Vitellogenin—Clones containing recombinant DNA sequences that hybridized strongly with both 32P-labeled vitellogenin mRNA and vitellogenin cDNA were selected from the large number of clones isolated from the repeated screenings of the plasmid DNA by agarose gel electrophoresis of plasmid DNA that had been digested with the restriction endonucleases Eco RI or Pst I. Two clones were selected as sources of vitellogenin DNA for monitoring transcription of the vitellogenin gene in vitro. Both contained vitellogenin DNA fragments 700 to 750 nucleotides long.

The qualification of specific transcripts by DNA-excess hybridization—Nitrocellulose filters (Schleicher and Schuell, B6) containing 32P-labeled DNA from the chimeric plasmid pFR322:albumin (26) or pBR322:vitellogenin were prepared according to the method of Jacquet et al. (27). Labeled RNA was denatured by heating at 80°C for 10 min in a solution containing formamide (50%), NaCl (0.3 M), sodium citrate (0.03 M), yeast tRNA (100 μg/ml), and poly(A) (100 μg/ml). The hybridization reactions were started by the addition of the DNA filters (4 mm diameter, each containing 4 μg of DNA) to the RNA solution (50 μl). Incubation was for 18 h at 42°C. At the end of the reaction, the filters were treated as follows: washed twice with 5 ml of 1.4 X SSC at 30°C for 1 h; washed twice with 5 ml each of 0.1 X SSC at 30°C for 20 min; incubated for 1 h at room temperature in 2 X SSC with boiled pancreatic RNase (20 μg/μl) and 5 μg/μl of tRNA; washed with 5 ml each of 2 X SSC at 30°C for 10 min; finally, washed successively with 5 ml each of 20% ethanol containing 0.1 M NaCl for 20 min until no more radioactivity washed off the filters. The filters were then dried and counted in Econofluor (New England Nuclear).

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Specific Transcription in Chicken Liver Chromatin

RNA synthesized in vitro was performed on horizontal slab gels of chromatin from control animals, 87% of the RNA synthesis that of chromatin from normal roosters. As is the case with chromatin from the liver of estrogen-treated roosters was almost twice as high as that of normal roosters, and of estrogen-treated roosters was subjected to electrophoresis in 1.5% agarose containing methylmercuric hydroxide (5 mM). The RNA was then transferred, by blotting, to diazobenzoxymethyl paper and hybridized with °P-labeled V28 plasmid DNA (7.3 × 10⁹ cpm/μg of DNA), as described by Alwine et al. (30). The chicken liver RNA samples analyzed in the autoradiogram were: Track 1, total RNA from an estrogen-treated rooster (5 μg); Track 2, poly(A⁺)-RNA from a laying hen (5 μg); and Track 3, total RNA from a normal rooster (25 μg).

**FIG. 1. Hybridization of vitellogenin plasmid DNA (V28) to RNA from chicken livers.** Samples of RNA from livers of hens, of normal roosters, and of estrogen-treated roosters were subjected to electrophoresis in 1.5% agarose containing methylmercuric hydroxide (5 mM). The RNA was then transferred, by blotting, to diazobenzoxymethyl paper and hybridized with °P-labeled V28 plasmid DNA (7.3 × 10⁹ cpm/μg of DNA), as described by Alwine et al. (30). The chicken liver RNA samples analyzed in the autoradiogram were: Track 1, total RNA from an estrogen-treated rooster (5 μg); Track 2, poly(A⁺)-RNA from a laying hen (5 μg); and Track 3, total RNA from a normal rooster (25 μg).

**RESULTS**

Chromatin prepared from rooster liver, as described under “Experimental Procedures,” was transcriptionally active without the addition of exogenous RNA polymerase. In this procedure, the chromatin was used as an insoluble aggregate since we have found that over 80% of the transcriptional activity was lost by shearing. Studies on the kinetics of incorporation of °H]UMP into RNA showed that RNA synthesis proceeds linearly for 45 min providing that phosphocreatine and phosphocreatine kinase were present. We have determined the divalent cation requirements of the reaction and have found that RNA synthesis was optimal at 1 mM magnesium ion and 5 mM magnesium ion. In order to favor transcription by RNA polymerase II over RNA polymerases I and III, the reaction mixture was adjusted to 0.05 M ammonium sulfate. All reactions were carried out at 25°C.

Having optimized the transcription reaction, we determined the activity of RNA polymerases I, II, and III in chromatin from the livers of normal and estrogen-treated roosters after a 20-min incubation. The results are shown in Table I. Under the reaction conditions that we have used, chromatin from normal rooster livers incorporated 0.41 pmol of UMP into RNA per μg of DNA in 20 min; 87% of this RNA synthesis was carried out by RNA polymerase II, as judged by its inhibition by low levels (1 μg/ml) of α-amanitin (15). There was almost no detectable RNA polymerase III activity under these conditions. The transcriptional activity of chromatin from the liver of estrogen-treated roosters was almost twice that of chromatin from normal roosters. As is the case with chromatin from control animals, 87% of the RNA synthesis was inhibited by α-amanitin at 1 μg/ml.

The level of RNA synthesis by chromatin was approximately 30% of the level of RNA synthesis by isolated intact rooster liver nuclei. We have determined the size distribution of the newly synthesized RNA molecules by subjecting the RNA to agarose gel electrophoresis under denaturing conditions (29). The RNA synthesized in vitro had an average size of between 150 and 250 nucleotides, which is smaller than the RNA synthesized by isolated rooster liver nuclei.

In order to study transcription of the vitellogenin and albumin genes in chromatin, we have analyzed the RNA synthesized in vitro by filter hybridization of the transcripts to filter-bound cloned albumin DNA (26) and cloned vitellogenin DNA, under conditions of vast DNA excess. The sizes of the cloned chicken DNA fragments are 520 nucleotides for the albumin sequence (20% of the length of albumin mRNA (26)), and 750 nucleotides for the vitellogenin sequence (11% of the length of vitellogenin mRNA (10)).

As described under “Experimental Procedures,” the chimeric plasmids that were used as sources of vitellogenin DNA, were constructed from cDNA prepared by reverse transcription of highly purified vitellogenin mRNA. Before DNA from these plasmids was used to monitor transcription of the vitellogenin gene in vitro, however, the identity of the inserted DNA fragments was confirmed by two different procedures.

Plasmid DNA that had been labeled with °P by nick-translation was hybridized to a diazotized cellulose blot (30) of various preparations of liver RNA that had been subjected to electrophoresis on agarose gels containing methylmercuric hydroxide (23). The plasmid DNA hybridized specifically to an RNA species that was the size of vitellogenin mRNA, and that was present in RNA isolated from the livers of both hens and estrogen-treated roosters but not in RNA from the livers of normal roosters (Fig. 1). As an additional check on the identity of the cloned vitellogenin DNA fragments, we have compared the genomic sequences that hybridize with °P-labeled vitellogenin mRNA, with those that hybridize with nick-translated plasmid DNA. Chicken DNA was digested with the restriction endonuclease Eco RI and subjected to
chromatography on RPC-5. Four of the fractions from this column contained DNA fragments that hybridized with vitellogenin mRNA. The aggregate size of these fragments was approximately 20,000 base pairs. We have determined that each of the vitellogenin clones used for monitoring transcription hybridized with one of these four fragments. Fig. 2 shows the results obtained when one of the RPC-5 fractions that hybridized to vitellogenin mRNA was purified further by preparative agarose gel electrophoresis. Samples of each of the fractions from the preparative gel were electrophoresed on a second agarose gel, transferred to a nitrocellulose filter (31), and hybridized with either [32P]vitellogenin mRNA or nick-translated plasmid DNA. Hybridization with either vitellogenin mRNA or the plasmid DNA yielded identical results. Both hybridized to a single 6000-nucleotide-long restriction fragment.

Prior to using the filters for monitoring transcription in vitro, the specificity and quantitative characteristics of the hybridization assay were studied. The overall efficiency of hybridization under our conditions was 30 to 35%, as determined by hybridization of pBR322 [32P]cRNA to nitrocellulose filters containing pBR322 DNA. Purified vitellogenin mRNA fragmented to a size comparable with that of the in vitro transcripts and labeled with [32P] (28), was hybridized to nitrocellulose filters containing pBR322:vitellogenin. As was expected from the efficiency of hybridization and the size of the cloned DNA fragment, 3% of the labeled vitellogenin RNA hybridized to the filter. Hybridization to the albumin sequence was determined by hybridizing [32P]-labeled (28) poly(A) RNA from normal rooster liver to filters containing pBR322:albumin. The level of albumin mRNA in poly(A) RNA from normal rooster liver is 15% by weight. In the hybridization reaction, 1% of the poly(A) RNA hybridized to the pBR322:albumin filter, as expected, since the inserted sequence was 20% of the length of albumin mRNA and the hybridization efficiency was 30%. As a final characterization, we have used the filters containing the cloned albumin and vitellogenin DNA to measure the levels of vitellogenin and albumin mRNA in total RNA isolated from rooster liver during the vitellogenic response and have obtained results comparable to those obtained with the same RNA preparations by liquid hybridization utilizing vitellogenin and albumin cDNA. We have determined the levels of albumin and vitellogenin RNA in rooster liver nuclei. In our preparation of chromatin, as was found by Marzluff and Huang (3), we found that 90% of the nuclear RNA was removed. However, to determine what amount of plasmid DNA we should bind to the filters for hybridization, we have assumed that all of the vitellogenin and albumin RNA sequences present in nuclei are present in chromatin and have used an amount of plasmid DNA that is in 150-fold excess over that in nuclear RNA.

Table II shows the results of two experiments in which we have measured the transcription of the vitellogenin and albumin genes in chromatin isolated from livers of normal and estrogen-treated roosters. These represent the highest (23.5) and lowest (3.14) ratio of stimulation of vitellogenin RNA synthesis that we have observed in five different experiments. In Experiment A, synthesis of vitellogenin RNA in chromatin from the liver of estrogen-stimulated roosters comprised 0.47% of the total RNA synthesized during a 15-min reaction; albumin RNA, synthesized in the same reaction, comprised 0.12% of the RNA. In contrast, there was very little vitellogenin RNA synthesized in chromatin from normal roosters (0.02%).

1 These experiments were performed in collaboration with Drs. S. Tilghman and P. Leder.
3 K. P. Mullinix and R. G. Deeley, unpublished observations.

![Fig. 2. Analysis of Eco RI restriction fragments of chicken genomic DNA.](http://www.jbc.org/)

**Specific Transcription in Chicken Liver Chromatin**

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![2a](http://www.jbc.org/)

![2b](http://www.jbc.org/)
0.0005% of the input counts per min. In Table III, we show of the very small amount of transcription of the vitellogenin amount of radioactivity hybridized to the filters was in some gene in chromatin from the livers of normal roosters. The absolute level of vitellogenin RNA synthesis by chromatin from estrogen-treated animals. The synthesis of vitellogenin RNA in chromatin from the liver of normal animal, however, appeared to be elevated compared to Experiment A. The synthesis of vitellogenin RNA in chromatin from the liver of estrogen-treated roosters increased 1.7-fold over that found in Experiment A, it can be seen that the absolute amount of albumin RNA synthesis was inhibited by o-amanitin at this concentration. These results indicate that the endogenous RNA polymerase II in our chromatin was responsible for the specific RNA synthesis that was cpm hybridized x 33, and for albumin, the correction factor is cpm x 15. The quality of the hybrids formed under our hybridization conditions was determined by thermally denaturing the RNA-DNA hybrids. Fig. 3 shows that the T_m of the hybrids was the average values of five experiments in which we have studied the synthesis of vitellogenin and albumin mRNAs, and for the efficiency of hybridization. For vitellogenin, the correction factor is cpm hybridized x 33, and for albumin, the correction factor is cpm x 15.

### Table II

Summary of composite results of experiments measuring the synthesis of vitellogenin and albumin RNA in chromatin

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Source of chromatin</th>
<th>DNA on filter*</th>
<th>Δ cpm*</th>
<th>RNA Input</th>
<th>Transcripts hybridized*</th>
<th>Estrogen-stimulated/normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Normal rooster liver</td>
<td>Vitellogenin</td>
<td>14</td>
<td>2.6 x 10^6</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>252</td>
<td>2.6 x 10^6</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen-treated rooster liver</td>
<td>Vitellogenin</td>
<td>855</td>
<td>6.0 x 10^6</td>
<td>0.47</td>
<td>23.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>438</td>
<td>5.6 x 10^6</td>
<td>0.12</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>B Normal rooster liver</td>
<td>Vitellogenin</td>
<td>54</td>
<td>1.3 x 10^6</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>80</td>
<td>0.8 x 10^6</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen-treated rooster liver</td>
<td>Vitellogenin</td>
<td>759</td>
<td>5.7 x 10^6</td>
<td>0.44</td>
<td>3.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>234</td>
<td>2.1 x 10^6</td>
<td>0.13</td>
<td>0.87</td>
<td></td>
</tr>
</tbody>
</table>

* Each filter (4 mm diameter) contained 4 µg of plasmid DNA

* Δ counts per min were computed by subtracting from the counts per min bound to the filters containing vitellogenin or albumin DNA the numbers of counts per min bound to filters containing pBR322 DNA (between 50 and 55 cpm).

* Calculated by correcting the numbers of counts per min actually hybridized to the filters for the lengths of the inserted chicken sequences compared to the lengths of the vitellogenin and albumin mRNAs, and for the efficiency of hybridization. For vitellogenin, the correction factor is cpm hybridized x 33, and for albumin, the correction factor is cpm x 15.

### Table III

Summary of composite results of experiments measuring the synthesis of vitellogenin and albumin RNA in chromatin

<table>
<thead>
<tr>
<th>Source of chromatin</th>
<th>Specific RNA</th>
<th>Transcripts*</th>
<th>Stimulated/normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rooster liver</td>
<td>Vitellogenin</td>
<td>0.053 ± 0.024</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>Vitellogenin</td>
<td>0.483 ± 0.046</td>
<td></td>
</tr>
<tr>
<td>Normal rooster liver</td>
<td>Albumin</td>
<td>0.166 ± 0.020</td>
<td>0.71</td>
</tr>
<tr>
<td>Estrogen-treated rooster liver</td>
<td>Vitellogenin</td>
<td>0.119 ± 0.011</td>
<td></td>
</tr>
</tbody>
</table>

* Calculated as described in Table II, Footnote c.
molecules and we do not know whether all of the RNA existing RNA molecules.

This laboratory has shown that chicken liver nuclei contain advantage in the use of such tissue culture systems is that not done experiments to measure initiation of new RNA transcripts synthesized by nuclei from Bl cells. One major premature termination of transcription. In addition, we have of the RNA isolated from the chromatin-directed transcrip-

we cannot rule out the possibility that it may result from high levels of ribonuclease and we suspect that the small size endogenous RNA polymerase in chromatin from myeloma cells. In addition, Bitter and Roeder (4) have isolated large preparation of chromatin and have found that the RNA is quite small (average size of 206 nucleotides). Marzluff and Huang (3) isolated large transcripts synthesized in vitro by E. coli RNA polymerase to chromatin causes increased transcription, there is very little preferential enhancement of transcription of the globin gene in chromatin isolated from chicken erythroid cells (35), and no preferential synthesis of the 5 S RNA in chromatin from Xenopus laevis (2). In our experiments, we have avoided the use of mercurated nucleotides to measure specific transcription (34). In fact, it has been reported that although the addition of E. coli RNA polymerase to chromatin causes increased transcription, there is very little preferential enhancement of transcription of the globin gene in chromatin isolated from chicken erythroid cells (35), and no preferential synthesis of the 5 S RNA in chromatin from Xenopus laevis (2). In our experiments, we have avoided the use of mercurated nucleotide precursors and have chosen, instead, to hybridize 32P-labeled RNA synthesized in vitro to nitrocellulose filters containing specific cloned chicken DNA in sufficiently vast excess such that endogenous RNA does not compete with hybridization of the newly synthesized RNA.

In order to establish conditions under which the transcriptional activity of chromatin reflects the activity of the liver cell in vivo, we have monitored the specific transcription of both a hormonally regulated gene and a gene that is expressed constitutively in the liver. We have measured the size distribution of the RNA that was synthesized in vitro in our preparation of chromat in and have found that the RNA is quite small (average size of 200 nucleotides). Marzluff and Huang (3) isolated large transcripts synthesized in vitro by endogenous RNA polymerase in chromatin from myeloma cells. In addition, Bitter and Roeder (4) have isolated large transcripts synthesized by nuclei from B1 cells. One major advantage in the use of such tissue culture systems is that these cells contain very low levels of ribonuclease. Work in this laboratory has shown that chicken liver nuclei contain high levels of ribonuclease and we suspect that the small size of the RNA isolated from the chromatin-directed transcription reactions is a result of digestion by ribonuclease although we cannot rule out the possibility that it may result from premature termination of transcription. In addition, we have not done experiments to measure initiation of new RNA molecules and we do not know whether all of the RNA synthesis that we measure results from elongation of pre-existing RNA molecules.

By measuring the levels of transcription of the vitellogenin gene by endogenous RNA polymerase II in chromatin isolated from the livers of normal and estrogen-treated rooster, we have determined that while there is little, if any, synthesis of vitellogenin RNA by endogenous RNA polymerase II in chromatin from normal rooster liver, vitellogenin RNA comprises approximately 0.5% of the transcription products in chromatin from the liver of the estrogen-treated rooster (Table II). The significance of the low levels of transcription of the vitellogenin gene in chromatin from livers of normal roosters is difficult to assess. From what is known of the situation in vivo (7), it would be predicted that the vitellogenin gene would be almost completely dormant in chromatin from normal rooster liver. The actual numbers of counts per min bound to the vitellogenin filters in transcripts from chromatin from unstimulated rooster liver are very low (between 14 and 54) after a hybridization reaction to which approximately 2 x 106 cpm were added. It is possible that this material is adventitiously bound "noise" and cannot be removed even after our stringent washing conditions. In recent experiments, in which transcription of the ovalbumin gene in isolated nuclei from chickens after withdrawal from diethylstilbestrol was studied, Swaneck et al. (7) have found similarly low amounts of radioactivity bound to filters containing cloned ovalbumin DNA. Because this radioactivity could not be removed when ovalbumin mRNA was used as a competitor, these authors concluded that there was no transcription of the ovalbumin gene in nuclei from withdrawn chickens.

It is also possible that in the preparation of the chromatin, there is some slippage of RNA polymerase or of other mole-
cules, allowing the vitellogenin gene to be inappropriately transcribed at a very low level. Such a problem could have been responsible for the results obtained in a study of vitel-
genin RNA synthesis involving the addition of purified chicken liver RNA polymerase II to chromatin from normal and estrogen-treated rooster liver by Dierks-Ventling (36). She found no difference in levels of vitellogenin RNA synthe-
sis between chromatin from normal and estrogen-treated roosters. Possibly, experimental conditions used in those experiments, including shearing of the chromatin, the use of heparin in the reactions, the hybridization conditions used, and the addition of exogenous purified RNA polymerase caused a loss of specific transcription. Finally, there may always be, in vivo and in vitro, a low level of transcription of the vitellogenin gene resulting in a species of RNA that is rapidly degraded so that it never accumulates in the cyto-

In the two experiments reported in Table II, the differential rate of synthesis of vitellogenin RNA in chromatin from livers of estrogen-treated roosters compared to that from normal roosters varies from 23.5 to 3.1. From the magnitude of the variance in the results of five experiments (Table III), it can be seen that the major source of variation is the value of the level of the transcription of the vitellogenin gene in chromat in from livers of normal roosters, whereas there is very little variability in the values obtained for the transcription of the vitellogenin gene in chromatin from livers of estrogen-treated animals or of albumin RNA synthesis in chromatin from livers of both normal and estrogen-treated roosters. Whereas there was a 2-fold increase of total transcription in chromatin from estrogen-treated rooster liver, the differential rate of transcription of the albumin gene in chromatin from estrogen-treated rooster liver was decreased by 30% compared to chromatin from normal rooster liver (Table III). Williams et al. (37) have shown by translation assays in the wheat germ cell-free system that there is a 50% decrease in the percentage of functional albumin mRNA during the vitellogenic response. The de-
increased differential rate of synthesis of albumin RNA in chromatin from estrogen-treated rooster liver compared to normal liver is in accordance with those results. However, Gordon et al.\(^1\) have studied the relative concentration of albumin mRNA isolated from rooster liver during the vitelligenic response by hybridization to cloned chicken albumin DNA. They found that when a correction was made for the increased RNA content of livers from estrogen-treated roosters, an increase commensurate with the increased transcriptional activity of nuclei and chromatin, the actual numbers of albumin mRNA molecules/cell were found to remain relatively constant. Since there is a 1.7-fold increase in the absolute rate of transcription of the albumin gene in vitro in chromatin from estrogen-treated rooster liver compared to normal rooster liver, it is possible that estrogen treatment results in a decreased half-life of albumin RNA in vivo in order to maintain a constant number of albumin mRNA molecules/cell.

It should also be noted that the molar transcription rates of the albumin gene (estrogenized and normal rooster) and the vitellogenin gene (estrogenized rooster) are remarkably similar. In view of recent observations on the homologies among regions of genomic DNA preceding the 5’ termini of several species of eukaryotic messenger RNA molecules (38), it is tempting to speculate that genes coding for abundant messengers might be transcribed at similar rates.

We conclude that the chromatin used in these studies retains the ability to specifically transcribe both the vitellogenin and albumin genes by endogenous RNA polymerase II. This system offers many advantages in the study of specific transcription. Since the components of specific transcription, including active RNA polymerase, are present in our chromatin preparation, it will be possible to determine what treatments will cause the loss of the specific transcription of these genes. In addition, it is of great interest to try to learn how to affect specific transcription of the vitellogenin gene in chromatin from livers of normal animals. We have recently developed procedures for preparing crude nuclear lysates from rooster liver in which the RNA polymerase is dependent on exogenous DNA for activity (17) and we intend to use such preparations to manipulate the transcriptional patterns of chromatin prepared from rooster liver.

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K P Mullinix, M B Meyers, J L Christmann, R G Deeley, J I Gordon and R F Goldberger