Regulation of Gene Transcription by Estrogen and Progesterone

LACK OF HORMONAL EFFECTS ON TRANSCRIPTION BY ESCHERICHIA COLI RNA POLYMERASE*

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Estrogen and progesterone markedly stimulate transcription of ovalbumin and conalbumin (transferrin) genes in chick oviduct as measured by hybridization of labeled RNA synthesized in isolated nuclei to immobilized plasmid DNA containing these gene sequences. Using this direct assay for specific gene transcription, we explored the basis of previous reports indicating that steroid hormones also cause changes in oviduct chromatin structure that can be detected by Escherichia coli RNA polymerase. We observed no effect of these hormones on the ability of E. coli RNA polymerase to transcribe specifically the conalbumin and ovalbumin genes 8½ h after hormone administration when transcription of these genes by endogenous RNA polymerase was elevated 5- and 30-fold, respectively. Furthermore, we were unable to detect any significant effect of either of these hormones on the total number of E. coli RNA polymerase binding sites in oviduct nuclei or chromatin. In contrast, after several days of hormone administration, we detected an apparent preferential ovalbumin RNA synthesis by E. coli RNA polymerase and this effect could be transferred to unstimulated nuclei by a 0.35 m salt extract of active nuclei. However, further experiments revealed that this preferential ovalbumin RNA synthesis is an artifact produced by transcription from contaminating ovalbumin mRNA. We conclude that E. coli RNA polymerase does not recognize steroid hormone-induced changes in oviduct chromatin.

The mechanisms by which genes respond to regulatory signals are not yet understood in molecular terms. However, activation of transcription can be subdivided into: (a) control of gene commitment, which involves changes in chromatin that allow a gene to be transcribed if an appropriate signal is presented; and (b) actual stimulation of gene transcription by the cellular signal. For example, in most chicken cells, the ovalbumin genes are not committed to respond to any known environmental signal, but in the oviduct tubular gland cells and their immediate progenitors, the ovalbumin gene is responsive to estrogen receptors (1-3). However, the presence of steroid receptors is not sufficient to allow transcription since the liver also contains estrogen receptors that stimulate vitellogenin and transferrin RNA transcription but have no effect on ovalbumin mRNA transcription (4, 5).

Several methods have been devised to probe the change in chromatin structure accompanying gene commitment. One of these involves measuring the sensitivity of genes to nucleases. Most, if not all, active genes are digested preferentially by DNase I, and in some cases these genes are unusually sensitive to staphylococcal nuclease as well (6, 7). The ovalbumin gene is degraded selectively in hen oviduct nuclei (or chromatin), but not in liver or erythrocyte nuclei, and this sensitivity persists even when estrogen is withdrawn leaving the gene transcriptionally inactive (8, 9). These results suggest that DNase I sensitivity reflects either prior transcription or gene commitment rather than active transcription, per se.

Escherichia coli RNA polymerase has also been used to probe chromatin structure of specific genes. By titrating chromatin with increasing amounts of this enzyme, the number of binding sites can be determined and the transcripts can be assayed for the abundance of specific gene sequences. This technique was first used to explore globin gene structure. The conclusions from several laboratories were that the globin genes are transcribed preferentially by E. coli RNA polymerase and this property could be transferred by a non-histone fraction in chromatin dissociation-reconstitution experiments (10-14). Similar conclusions were reached by O'Malley and coworkers, who showed that preferential transcription of the ovalbumin gene by E. coli RNA polymerase is a consequence of hormone action (15-18). According to their view, exposure to estrogen produces a large increase in the total number of E. coli RNA polymerase binding sites in chromatin, and preferential transcription of ovalbumin mRNA (18, 19). Their results suggest that E. coli RNA polymerase and DNase I recognize different features of chromatin structure since the preferential transcription changes with hormone withdrawal and restimulation, whereas DNase sensitivity does not. In all of these experiments, specific gene transcription was monitored by hybridizing total nuclear RNA with labeled cDNA. In some cases mercurated nucleotides were used so that newly synthesized RNA could be separated from pre-existing RNA (20). Despite many precautions and controls, these experiments have been plagued with technical difficulties, stemming largely from the presence of mRNA sequences in the nuclei and chromatin fractions and an unsuspected ability of E. coli RNA polymerase to use RNA as a template (21-24).

Until recently, it was not possible to measure transcription of specific genes directly; now the availability of recombinant DNA plasmids containing specific gene sequences has allowed a relatively straightforward assay of transcription by isolated nuclei with either endogenous or exogenous RNA polymerase (25-27). In these experiments, labeled RNA transcripts are hybridized to immobilized plasmids. If an internal standard is included to monitor hybridization efficiency, then the percent of sequences complementary to the specific gene sequences can be determined accurately. When this assay is used with
nuclei and endogenous RNA polymerase, it essentially measures the relative number of RNA polymerases on the gene in question since there is very little initiation in these systems and chain elongation is usually limited to a few hundred nucleotides.

In this study, we have examined the relationship between hormonal activation of ovalbumin gene transcription by endogenous RNA polymerase and the susceptibility of the ovalbumin gene to be transcribed by E. coli RNA polymerase. We observed no significant change in either the total number of exogenous RNA polymerase binding sites or in specific transcription of the ovalbumin or conalbumin genes by E. coli RNA polymerase during the first few hours of secondary stimulation with either estrogen or progesterone. Under these conditions nuclear receptor levels have increased 5-fold and transcription of the ovalbumin and conalbumin genes by endogenous RNA polymerase has increased 30- and 5-fold, respectively.

MATERIALS AND METHODS

Animals and Injections—White Leghorn chicks, 3 to 4 days old, were given 10 days of primary estrogen stimulation with 15-mg diethylstilbestrol pellets and then withdrawn for 2 days by removing the pellets. Chicks were then given secondary stimulation by subcutaneous administration of 17 beta-estradiol benzoate or progesterone; both steroids were dissolved in 90% corn oil, 10% ethanol at a concentration of 5 mg/ml.

Preparation of Templates—Nuclei were isolated essentially as described by Mulvihill and Palmer (28), except that 0.5 mM phenylmethylsulfonyl fluoride was included in the homogenization buffer. Briefly, the magnonic portions of the oviducts were homogenized in a low ionic strength buffer containing Triton X-100, filtered through glass wool, diluted 1:1 with 2.4 M sucrose, and centrifuged through 2.4 M sucrose using the SW 27 rotor (Beckman). The nuclear pellets were suspended in 50 mM Tris, pH 8; 5 mM MgCl₂; 5 mM dithiothreitol; 0.5 mg/ml of bovine serum albumin, and collected on GF/C filters (Whatman) by filtration activity associated with blank filters from the radioactivity hybridized to filters containing specific gene sequences. This value was then corrected for the hybridization efficiency, divided by the input radioactivity, and expressed as a relative rate in parts per million (ppm). The parts per million values have not been corrected for the length of the cDNA sequences in the ovalbumin and conalbumin plasmids which are 1800 and 2300 nucleotides, respectively.

RESULTS

To investigate the effects of steroid hormones on the in vitro transcription of oviduct chromatin by E. coli RNA polymerase, we initially carried out titration experiments similar to those of Tsi et al. (30) and Johnson and Baxter (29). Fig. 1 shows the results of an experiment in which nuclei and chromatin isolated from oviducts of hormone-withdrawn chicks as well as chicks stimulated for 8 hr with either estrogen or progesterone were assayed with varying amounts of E. coli RNA polymerase. With both nuclei (Fig. 1A) and chromatin (Fig. 1B), the quantity of RNA synthesized increased with higher concentrations of polymerase until a plateau level was obtained. In the case of chromatin, the plateau value of 35 to 45 pmol of UMP/μg of DNA with approximately 10 μg of polymerase is similar to that reported by Johnson and Baxter (29) but less than that reported by Schwartz et al. (31). With nuclei, a lower plateau level (20 to 25 pmol of UMP/μg of DNA) was observed consistently. This difference suggests that structural changes occur during chromatin isolation that increase its capacity to act as a template for E. coli RNA polymerase. The lower value obtained with nuclei is probably not explained by nonspecific binding of the enzyme to sites other than chromatin since the level of incorporation has clearly plateaued.

In contrast to previous reports (31–34), we did not observe a significant and reproducible steroid-induced change in total template activity for E. coli RNA polymerase; both nuclei and chromatin templates isolated from hormone-withdrawn and estrogen- or progesterone-stimulated birds yielded similar levels of incorporation. In addition to measuring total incorporation, we have carried out rifampicin-challenge assays similar to those used before (30) in order to determine whether the steroid-induced change of chromatin can be observed only by measuring the initial round of transcription. In this assay, E. coli RNA polymerase is allowed to bind to chromatin in the absence of nucleotide triphosphates; then the nucleotide triphosphates are added to start polymerase elongation, and at the same time, heparin and rifampicin are added to prevent

![Fig. 1. Template activity of oviduct nuclei and chromatin.](image-url)
further polymerase initiation. These experiments (Fig. 1B, inset) also failed to demonstrate a significant change in template capacity. Experiments of both types have been repeated several times using chromatin from oviducts stimulated for shorter and longer periods, as well as from liver, another estrogen-responsive tissue. In all cases, we have failed to observe a significant change in template capacity.

To examine the effects of hormonal treatment on expression of specific genes, we isolated oviduct nuclei and either allowed endogenous RNA polymerases to elongate RNA chains in the presence of \( \alpha[^{32}P]UTP \) or added a-amanitin to inhibit endogenous RNA polymerase, form B (25, 27), and then added \( E. coli \) RNA polymerase and \( \alpha[^{32}P]UTP \). The radioactive RNA molecules synthesized in each case were hybridized to immobilized plasmid DNAs containing ovalbumin or conalbumin gene sequences. We did not observe a significant change in template capacity.

Table I shows that nuclei from birds treated with estrogen or progesterone for 8 h gave substantially increased rates of ovalbumin and conalbumin mRNA transcription by endogenous polymerases compared to nuclei from hormone-withdrawn chicks. With estrogen treatment, the rate of ovalbumin mRNA transcription increased about 35-fold, while with progesterone, transcription increased 25-fold. There were smaller increases in the rate of transcription from the conalbumin gene with estrogen and progesterone (Table I); however, this smaller response primarily reflects the higher constitutive rate of conalbumin mRNA synthesis (88 ppm in this experiment) compared to ovalbumin mRNA transcription (18 ppm). The significant rate of transcription from the conalbumin gene accounts for the higher concentration of conalbumin mRNA in oviduct tissue of hormone-withdrawn birds (about 100 molecules/cell) compared to ovalbumin mRNA (about 10 molecules/cell). Addition of a-amanitin to these nuclei (1 to 10 \( \mu \)g/ml) suppressed both ovalbumin and conalbumin mRNA transcription (25–27 and Table I). Quantitation of the number of nuclear estrogen receptors by exchange assay (28) showed that after 8 h of estrogen treatment \( \textit{in vivo} \), the number of nuclear estrogen receptors had increased 6-fold to 2000 molecules/cell (data not shown); nuclear progesterone receptors were not measured in this experiment, but in similar experiments a 5-fold increase is generally observed (35). These observations show that hormone treatment had significant effects on both the number of nuclear receptors and the endogenous transcriptional activity of two oviduct genes. This set of assays was performed on the same nuclei as were used for the titration of the number of \( E. coli \) RNA polymerase binding sites described in Fig. 1. When \( E. coli \) RNA polymerase was added to these nuclei in the presence of a-amanitin, there was no significant stimulation of either ovalbumin or conalbumin RNA transcription in response to hormonal treatment (Table I). We did note, however, that when estrogen was given for 4 days, the rate of ovalbumin RNA transcription by endogenous RNA polymerase increased to about 2800 ppm and conalbumin RNA synthesis reached 1300 ppm. The synthesis of both gene products was almost completely suppressed by a-amanitin while total RNA synthesis was inhibited only 50%. When these nuclei were tested with \( E. coli \) RNA polymerase in the presence of a-amanitin, we observed a 4-fold increase in total RNA synthesis and a 34-fold increase in the relative rate of ovalbumin RNA synthesis; conalbumin RNA synthesis showed the same trend with prolonged estrogen treatment (Table II).

To explore the basis by which prolonged hormonal treatment increases the ability of ovalbumin gene sequences to be transcribed by \( E. coli \) RNA polymerase, we prepared a crude non-histone fraction by washing nuclei with 0.35 \( M \) KCl and added this fraction to nuclei from withdrawn oviduct tissue.

**Table I**

<table>
<thead>
<tr>
<th>Source of nuclei or chromatin*</th>
<th>Ovalbumin gene transcription</th>
<th>Conalbumin gene transcription</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endogenous RNA polymerase (nuclei)*</td>
<td>( E. coli ) RNA polymerase (nuclei)*</td>
</tr>
<tr>
<td>Oviduct, withdrawn</td>
<td>18 (2.8)†</td>
<td>68 (9.6)</td>
</tr>
<tr>
<td>Oviduct, 8% h of estrogen</td>
<td>746 (3.4)</td>
<td>135 (13.0)</td>
</tr>
<tr>
<td>Oviduct, 8% h of progesterone</td>
<td>544 (3.6)</td>
<td>77 (13.2)</td>
</tr>
</tbody>
</table>

* Nuclei were prepared from chicks withdrawn from prior diethylstilbestrol treatment or from withdrawn chicks given 1 \( mg \) of progesterone or estradiol-benzoate for 8 h. Nuclei were isolated and chromatin was prepared as described under "Materials and Methods." Where exogenous RNA polymerase is indicated, 35 \( \mu \)g of \( E. coli \) RNA polymerase and 200 ng of a-amanitin were included in the reaction. Exogenous RNA polymerase stimulated total incorporation 17- to 24-fold.

† Chromatin (11 to 15 \( \mu \)g) was incubated in a 100-\( \mu \)l reaction mixture (see "Materials and Methods") with 8.8 \( \mu \)g of \( E. coli \) RNA polymerase and 200 ng of a-amanitin. There was no endogenous activity in the chromatin samples.

§ Values in parentheses represent total input radioactivity added to each hybridization reaction (\( \times 10^{-6} \)).
Table III shows that a salt extract of 4-day estrogen-treated, oviduct nuclei stimulated ovalbumin RNA synthesis in withdrawn nuclei about 3-fold, whereas a similar salt extract from withdrawn oviduct nuclei had no effect. In a similar experiment, a salt extract from oviduct nuclei of birds given 10 days of primary stimulation increased ovalbumin RNA synthesis in withdrawn oviduct nuclei over 10-fold. This extract also had the remarkable effect of increasing ovalbumin RNA synthesis in chick liver nuclei, where ovalbumin mRNA is not synthesized normally, and in mouse liver nuclei, where ovalbumin mRNA is never transcribed (Table IV). Further experiments revealed that actinomycin D did not inhibit the synthesis of ovalbumin RNA sequences; in fact, the relative rate of ovalbumin RNA synthesis increased from 730 to 2900 ppm (Table III). If the salt extract was deproteinized with sodium dodecyl sulfate and proteinase K, followed by phenol-chloroform extraction and the resulting nucleic acids were added to withdrawn nuclei, at the same concentration as in the original extract, then it was clear that the stimulatory activity resided in the nucleic acid fraction (Table III). We were unable to detect DNA in this fraction and DNase I treatment had no effect. Table III also shows that the salt extract alone served as a template for synthesis of ovalbumin RNA with a relative rate of ovalbumin RNA synthesis of 8000 ppm. The RNA from this fraction was a better overall template, but the relative rate of ovalbumin RNA synthesis decreased to 1550 ppm. In another experiment in which the relative rate of ovalbumin synthesis in the salt extract alone was 3700 ppm, we measured the concentration of ovalbumin mRNA by hybridization with CDNA to be 0.39%, or 3900 ppm by weight. These observations suggested that E. coli RNA polymerase might be copying ovalbumin mRNA that was contaminating the nuclei and was extracted subse-

<table>
<thead>
<tr>
<th>TABLE III</th>
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<tbody>
<tr>
<td><strong>Stimulation of E. coli RNA polymerase-mediated ovalbumin RNA synthesis by salt extract and RNA from estrogen-stimulated oviduct nuclei</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nuclei</th>
<th>Additions</th>
<th>Ovalbumin RNA synthesis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oviduct (WD)*</td>
<td>None</td>
<td>268 (1.60)</td>
</tr>
<tr>
<td>Oviduct (WD)</td>
<td>Salt extract (WD)*</td>
<td>247 (1.44)</td>
</tr>
<tr>
<td>Oviduct (WD)</td>
<td>Salt extract (ES)*</td>
<td>736 (1.35)</td>
</tr>
<tr>
<td>Oviduct (WD)</td>
<td>RNA from salt extract (ES)</td>
<td>792 (1.74)</td>
</tr>
<tr>
<td>Oviduct (WD)</td>
<td>Salt extract (ES) + AcD*</td>
<td>2907 (0.40)</td>
</tr>
<tr>
<td>Oviduct (WD)</td>
<td>Ovalbumin mRNA*</td>
<td>4454 (1.51)</td>
</tr>
<tr>
<td>None</td>
<td>Salt extract (ES)</td>
<td>8779 (0.21)</td>
</tr>
<tr>
<td>None</td>
<td>RNA from salt extract (ES)</td>
<td>1551 (1.26)</td>
</tr>
</tbody>
</table>

* All assays (50 μl) contained: 25 μl of nuclei (or buffer), 10 μl of salt solution (or salt extract or RNA, see Footnote d), and 15 μl of other additions to give the final composition indicated under "Materials and Methods." E. coli RNA polymerase was added to 175 pg/ml and α-amanitin was present at 3 μg/ml.

* Nuclei (600 μg of DNA/ml) were from chicks withdrawn (WD) for 2 days from diethylstilbestrol stimulation. When no nuclei were added, the nuclei storage buffer was added instead.

* Numbers in parentheses refer to total input radioactivity (×10^4) in each hybridization.

* Nuclei (900 μg of DNA/ml) from withdrawn (WD) or 7-day estrogen-stimulated (ES) chicks were pelleted at 80,000 × g and resuspended in 40% of the original volume of 350 mM KCl, 5 mM MgCl₂, 5 mM KH₂PO₄, pH 7.2. After 15 min, the nuclei were centrifuged for 3 min at 15,000 × g and the supernatant was saved as the salt extract. RNA was isolated from 75 ml of extract by sodium dodecyl sulfate-proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation. The RNA was dissolved in 75 μl of the salt solution.

* Actinomycin D (AcD) was added at a final concentration of 100 μg/ml.

![TABLE IV](attachment:image)

<table>
<thead>
<tr>
<th>TABLE IV</th>
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<tbody>
<tr>
<td><strong>Transfer of ovalbumin stimulatory activity to liver nuclei by salt extracts of estrogen-stimulated oviduct nuclei</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nuclei</th>
<th>Ovalbumin RNA transcription by E. coli RNA polymerase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minus salt extract</td>
</tr>
<tr>
<td></td>
<td>ppm</td>
</tr>
<tr>
<td>Oviduct, withdrawn</td>
<td>37 (12.4)*</td>
</tr>
<tr>
<td>Liver, chicken</td>
<td>22 (11.1)</td>
</tr>
<tr>
<td>Liver, mouse</td>
<td>53 (15.1)</td>
</tr>
</tbody>
</table>

* The salt extract was prepared as described in Table III from chicks given 10 days of primary stimulation with diethylstilbestrol pellets. The assays were performed as detailed under "Materials and Methods.*

* Numbers in parentheses represent total input radioactivity (×10^4) in each hybridization.

DISCUSSION

Our results are consistent with previous studies indicating that administration of either estrogen or progesterone to withdrawn chicks increases the number of nuclear receptors 5-fold within 30 min, and that these increases are associated with increases in the number of endogenous RNA polymerases (Form B) transcribing the ovalbumin and conalbumin genes (25, 27). However, we question the significance of the hormonal increase in total exogenous E. coli RNA polymerase binding sites previously reported (31-34), as well as the effects of estrogen and progesterone on specific gene transcription by E. coli RNA polymerase (16-19).

We have been unable to detect a significant effect of hormone stimulation on the total number of E. coli RNA polymerase binding sites in oviduct nuclei or chromatin even when transcription was limited to a single round using the rifampicin assay. We used the chromatin isolation procedure of Johnson and Baxter (29), who showed that glucocorticoids have rapid effects on the number of E. coli RNA polymerase binding sites, and this procedure is basically the same as that used by Tsai et al. (30). Since chromatin is a very complex structure it is possible that subtle differences in isolation procedures have dramatic effects on subsequent template activity. For this reason, we have also examined the binding of E. coli RNA polymerase to nuclei assuming that they represent a more physiological state than chromatin. Nuclei can be isolated in the presence of nonionic detergents, such as Triton X-100, in which case the nuclear membrane is lost, thereby allowing free access to large molecules such as RNA polymerase. However, using nuclei we also failed to note a significant effect of hormone stimulation on the total number of E. coli RNA polymerase binding sites. The 2-fold difference in plateau values attained when titrating nuclei and chromatin with...
E. coli RNA polymerase probably reflects the removal or rearrangement of chromatin constituents during isolation of the latter.

The effect of steroid hormones on E. coli RNA polymerase binding sites reported by others (25-32) must persist for a short time in the absence of the steroid receptors, since the number of RNA polymerase binding sites generated is much greater than the number of nuclear receptors but the nature of the imprint remains elusive. Furthermore, the physiological significance of the large hormonal-induced increase in E. coli RNA polymerase binding sites is unclear since there is very little effect of these hormones on the number of endogenous RNA polymerases actually involved in transcription during the first few hours of secondary stimulation (36); indeed, there is only about a 2-fold change between withdrawn and fully stimulated oviduct when cell proliferation is not involved. O'Malley and co-workers have argued that the increase in E. coli RNA polymerase binding sites reflects a 2½-fold increase in the number of active genes (measured as mRNA complexity) after hormonal stimulation (37). However, two other groups working on the same system with similar methods have detected essentially no change in the number of active genes (38, 39).

Perhaps a more serious conflict with previous reports involves transcription of specific gene sequences by E. coli RNA polymerase. Our studies indicate that E. coli RNA polymerase does not recognize the hormone-induced increase in nuclear receptors or specific transcription by endogenous RNA polymerases using either nuclei or chromatin as a template (Table I). The explanation for this discrepancy is not entirely clear but it may result from technical difficulties associated with the indirect assays used to measure specific transcription in all former experiments of this type. It is perhaps significant that in experiments in which specific ovalbumin gene transcription was assayed, the oviduct chromatin template was always taken from chicks that had been treated with estrogen for a long time (15-17). Considering the potential for artifacts associated with contaminating RNA sequences, these experiments would be much more convincing if chromatin templates were prepared at very early times after hormone stimulation, i.e. at times prior to the accumulation of massive amounts of ovalbumin mRNA. In our experiments, we inhibited endogenous RNA polymerase with α-amanitin (Table II) in order to examine the effects of exogenous RNA polymerase. It is possible that these "frozen" polymerases might block E. coli RNA polymerase initiation or elongation. However, a rate of ovalbumin mRNA synthesis of 750 pnpm (the maximum rate shown in Table I) is equal to only 3.7 RNA polymerase molecules/ovalbumin gene (0.075% x 10,000 polymerases/cell (36) + 2 genes/cell). Given that the ovalbumin gene spans 7.8 kilobases (40, 41) and assuming random spacing, the endogenous RNA polymerases are about 2000 bases apart. This distance is nearly 3 times larger than the average E. coli transcript (700 bp) measured by Tsai et al. (30, 31). Endogenous RNA polymerase activity is totally lost during the isolation of chromatin, but we still observed no effect of hormones on general or specific E. coli RNA polymerase transcription. Thus, it seems unlikely that endogenous polymerases significantly impede E. coli RNA polymerase initiation or elongation.

Although we did not detect any significant increase in ovalbumin or conalbumin gene transcription by E. coli RNA polymerase upon hormonal stimulation, we did notice a much higher background level of hybridization when exogenous RNA polymerase was used. This high background might reflect the fact that these genes are committed to respond to steroid hormone receptors. If so, it may be related to the phenomenon of increased nuclear sensitivity that tends to be associated with go- conversion (9).

The experiments presented in Tables II to IV indicate that there is a real problem associated with E. coli RNA polymerase copying contaminating mRNA. Our observations on this activity of E. coli RNA polymerase are by no means unique (21-24). In a recent paper, Pays et al. (24) suggest that the problem stems largely from the use of Mn²⁺ in the transcription reaction. However, in our experiments 5 mm Mg²⁺ was the only divalent cation added and ovalbumin mRNA still served as a template for E. coli RNA polymerase. Non-histone fractions prepared by simple salt extraction of nuclei are rich sources of contaminating RNA sequences. For example, the RNA that we extracted with 0.35 m salt was about 0.4% ovalbumin mRNA, a value close to the abundance of this mRNA in total cellular RNA after prolonged estrogen treatment.

This contamination makes meaningful chromatin reconstitution experiments very difficult (21-24, 42). With the advent of cell-free transcription systems that show correct initiation with Form B polymerase (43, 44), it is going to be very tempting to look at regulation of transcription by various enzyme fractions. These experiences with E. coli RNA polymerase should serve as a warning for the possibility of similar artifacts with eukaryotic RNA polymerases.

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Steroid Hormones and Transcription by E. coli RNA Polymerase