Proc. Natl. Acad. Sci. USA
Vol. 75, No. 2, pp. 686–690, February 1978
Biochemistry

Preferential transcription of the ovalbumin gene in isolated hen oviduct nuclei by RNA polymerase B

(RNA synthesis/mercurated polynucleotides/complementary DNA/affinity chromatography/α-amanitin)

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Communicated by Jacob Furth, November 14, 1977

ABSTRACT **The synthesis of ovalbumin mRNA sequences was studied in isolated nuclei from hen oviduct. Two different methods of analysis were used to distinguish *in vitro* synthesized from preexisting mRNA sequences: (*i*) Mercurated ribonucleotides were used for *in vitro* RNA synthesis, and the newly synthesized RNA was purified by chromatography on sulfhydryl-agarose and hybridized to radioactive ovalbumin cDNA. (*ii*) [^{3}H]UTP was used to label the *in vitro* synthesized RNA. Hybridization to unlabeled mercurated cDNA, RNase A digestion, and subsequent purification of the hybrids on SH-agarose allowed the quantitation of newly synthesized ovalbumin mRNA sequences. Approximately 0.1% of the newly synthesized RNA was identified as ovalbumin RNA by both methods. The synthesis of ovalbumin RNA progressed during the incubation of nuclei and was sensitive to actinomycin D and low concentrations of α-amanitin. The preferential *in vitro* transcription of the ovalbumin gene (1000-fold over random transcription of the chicken genome) by RNA polymerase B (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) suggests that the specificity of *in vivo* RNA synthesis is retained in isolated nuclei.**

The synthesis of the egg white proteins in the tubular gland cell of the chicken oviduct is primarily related to the cellular accumulation of the corresponding mRNAs (1–7). During stimulation with estradiol the amount of ovalbumin mRNA dramatically increases from a few copies to tens of thousands of copies per cell (2, 4, 5–7). Similar results have been found for the induction of ovomucoid and lysozyme mRNA (7). The steroid-mediated induction of specific mRNA molecules in the chicken oviduct may result from altered rates of synthesis and/or degradation. We therefore studied the synthesis rate of the egg white protein mRNAs. Because it was not possible to measure the rate of specific mRNA synthesis in the animals, we chose isolated oviduct nuclei as the cell-free system that might best reflect the transcriptional activity of the intact cell (8).

The analysis of specific mRNA synthesis in isolated nuclei and chromatin is difficult for two reasons. First, a specific mRNA is expected to represent only a small fraction of the total RNA synthesized. Thus, hybridization of *in vitro* synthesized, radiolabeled RNA to unlabeled cDNA and digestion of the nonhybridized RNA does not allow the detection of specific mRNA sequences over the RNase digestion background. Second, large amounts of the investigated sequences are usually present in the RNA endogenous to isolated nuclei and chromatin. Hybridization to radiolabeled cDNA would not be a suitable detection method because it does not distinguish *in vitro* synthesized from preexisting mRNA molecules. To overcome these difficulties, we used two different approaches, both exploiting the affinity of mercurated polynucleotides for sulfhydrylagarose (SH-agarose) (9, 10). Using either method we could show that ovalbumin mRNA sequences have been synthesized by RNA polymerase B in a highly selective manner in isolated hen oviduct nuclei.

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MATERIALS AND METHODS

Preparation of Mercurated Nucleotides and SH-Agarose. Hg-CTP and Hg-dUTP were prepared and characterized as described by Dale *et al.* (9). SH-agarose (2 μmol of sulfhydryl per ml) was prepared essentially as described by Cuatrecasas (11) from aminoethylagarose (Servachrom A-A1).

Isolation of Ovalbumin mRNA and Preparation of cDNAs. Ovalbumin mRNA ($mRNA_{oa}$) was purified and [^{3}H]cDNA was prepared as described (7, 12). Hg-cDNA was synthesized essentially under the same conditions with the following changes: the deoxynucleotides were used at 1 mM, dTTP was substituted by a mixture of 87.5% dTTP and 12.5% Hg-dUTP, 5 mM 2-mercaptoethanol replaced dithiothreitol, and pyrophosphate was omitted. [^{3}H]dCTP (22 Ci/mmol) or [^{32}P]dCTP (2 mCi/mmol) was used to label the Hg-cDNAs. Approximately 80% of [^{3}H]$cDNA_{oa}$ and Hg-[^{3}H]$cDNA_{oa}$ synthesized with a 12.5% substitution of dTTP by Hg-dUTP represented full-length transcripts. Hg-[^{3}H]cDNA and Hg-[^{32}P]cDNA were characterized by hybridization to $mRNA_{oa}$ and ^{125}I-labeled $mRNA_{oa}$ (^{125}I-$mRNA_{oa}$). Substitutions above 12.5% Hg-dUTP in the cDNA led to a lower rate of hybridization with $mRNA_{oa}$. Hg-$cDNA_{oa}$ synthesized with 100% Hg-dUTP substitution could not hybridize to $mRNA_{oa}$ (A. E. Sippel, unpublished observation). ^{125}I-$mRNA_{oa}$ was prepared according to Curtis and Weissmann (13).

***In Vitro* Synthesis and Purification of Hg-RNA and Hybridization to [^{3}H]cDNA.** Oviduct nuclei were prepared from HNL laying hens according to Ernest *et al.* (8) with the buffers used by Marshall and Burgoyne (14). The conditions for RNA synthesis were as reported previously (8) except that 14 mM 2-mercaptoethanol replaced 2.5 mM dithiothreitol, Tris·HCl at pH 8.0 replaced sodium *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Na Hepes) at pH 8.0, $MgCl_2$ replaced magnesium acetate, bovine serum albumin was omitted, and CTP or Hg-CTP was used at 0.5 mM. Nuclei were incubated at 25° at a DNA concentration of 1.5 mg/ml. [^{32}P]UTP (2 mCi/mmol) was used to label the RNA to low specific activity. The reaction mixture was then diluted 2-fold with a buffer containing 100 mM Tris·HCl at pH 7.5, 0.3 M NaCl, 15 mM

Abbreviations: $mRNA_{oa}$, ovalbumin mRNA; $cDNA_{oa}$, DNA complementary to ovalbumin mRNA; Hg-CTP, 5-mercuricytidine triphosphate; Hg-dUTP, 5-mercurideoxyuridine triphosphate; Hg-RNA, RNA containing Hg-CMP; Hg-cDNA, cDNA containing Hg-dUMP; SH-agarose, sulfhydrylagarose; R-SH eluate, the fraction eluted with 2-mercaptoethanol from the SH-agarose column; TN buffer, Tris/NaCl buffer; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; DNA_{fd}, phage fd DNA.

EDTA, and 1% sodium dodecyl sulfate. The RNA was extracted according to the hot phenol/dodecyl sulfate method (15) and chromatographed on Sephadex G-50 in TN buffer (10 mM Tris·HCl at pH 7.5/100 mM NaCl).

The eluted material was ethanol precipitated, then dissolved in 80% (wt/vol) formamide/10 mM Tris·HCl at pH 7.5/4 mM EDTA. Samples were heated at 67° for 3 min, adjusted to 40% formamide/2 mM EDTA in TN buffer and applied in a large volume (10 times bed volume) to the SH-agarose column (1.5 ml) with a flow rate of 15 ml/hr. RNA in the unbound fraction was collected and ethanol precipitated. The column was then washed at maximal rate with 50 ml of TN buffer, 10 ml of H_2O, 10 ml of 2 M NaCl in TN, and 30 ml of TN. The bound RNA was eluted with TN buffer containing 0.2 M 2-mercaptoethanol after the column was allowed to stand in this buffer for 3 min. To this R-SH eluate, yeast carrier RNA was added to 10 μg/ml, NaCl to 0.4 M, and 2-mercaptoethanol to 1.5 M. The RNA was left at room temperature for 3 hr to allow demercuration, and was then ethanol precipitated and dissolved in H_2O. Saturation hybridization of increasing amounts of the unbound and bound RNA to 0.1–0.15 ng [^{3}H]cDNA was carried out as described (7). [^{3}H]cDNA was ensured to be in 1- to 20-times excess over hybridizable RNA.

***In Vitro* Synthesis of [^{3}H]RNA and Hybridization to Hg-cDNA.** RNA was synthesized in isolated nuclei for 30 min at 25° as described above, using 20 μM [^{3}H]UTP (46 Ci/mmol), 1 mM of the other ribonucleotides, and 2.5 mM dithiothreitol. The RNA was extracted, chromatographed on Sephadex G-50, and ethanol precipitated as described above. Hybridizations to Hg-cDNA were carried out in 20–40 μl of 50% formamide/0.5 M NaCl/25 mM Hepes at pH 6.8/0.5 mM EDTA at 40° for 2 hr. Hg-cDNA was in 6- to 10-fold excess over endogenous ovalbumin RNA present in the input RNA. The hybridization mixture was diluted with 10 volumes of 10 mM Tris·HCl at pH 7.5/0.3 M NaCl/1 mM EDTA and yeast RNA at 2 μg/ml and incubated for 1 hr at 30° with RNase A at 40 μg/ml. The sample was applied to SH-agarose (2 ml) and incubated for 1 hr. The column was then washed with 12 ml of TN buffer and 8 ml of 2 M NaCl in TN. The bound material was eluted with 4 ml of TN buffer containing 0.2 M 2-mercaptoethanol. The acid-insoluble radioactivity in all fractions was determined.

RESULTS

Binding of Mercurated Nucleic Acids to SH-Agarose. In order to follow specific transcription in isolated nuclei, we distinguished *in vitro* synthesized from preexisting mRNA sequences either by incorporation of a mercurated nucleotide (9, 16–19) and subsequent isolation of the mercurated RNA by affinity chromatography on SH-agarose or by incorporation of a radioactive nucleotide and isolation of the specific hybrids after hybridization to mercurated cDNA. The basis of both procedures is the affinity of mercurated polynucleotides for SH-agarose. Therefore, it is necessary to find conditions under which the SH-agarose column exclusively binds mercurated polynucleotides with a high efficiency. The binding properties of the SH-agarose column under our conditions are summarized in Table 1. Routinely more than 80% of mercurated [^{3}H]RNA and Hg-[^{3}H]cDNA and 50% of hybrids containing Hg-cDNA bound to the column and could be eluted with 2-mercaptoethanol. Unmercurated [^{3}H]RNA, [^{3}H]cDNA, and $mRNA_{oa}$ did not bind to the column at a measurable level and could be recovered in the unbound fraction. The unspecific adsorption of unmercurated sequences is, if any, less than 10^{-4}% of the applied RNA.

Table 1. Binding of mercurated polynucleotides to sulfhydryl agarose

Sample	Amount, cpm (and μg or pg) Applied	Unbound	Bound
Hg-[^{3}H]RNA	102,000	1,785	97,000
[^{3}H]RNA	5,200,000	5,360,000	0
$mRNA_{oa}$	10.2 μg	10.4 μg	<10 pg
[^{3}H]$cDNA_{oa}$	20,882	21,020	0
Hg-[^{3}H]$cDNA_{oa}$	17,250	3,116	14,028
$mRNA_{oa}$·Hg-[^{3}H]$cDNA_{oa}$	14,493	7,601	6,817
^{125}I-$mRNA_{oa}$·Hg-$cDNA_{oa}$	12,563	6,253	6,424

The chromatography on SH-agarose of Hg-RNA or of Hg-cDNA and hybrids containing Hg-cDNA was performed as described in *Materials and Methods*. [^{3}H]Hg-RNA and [^{3}H]RNA were synthesized from calf thymus DNA by *Escherichia coli* RNA polymerase (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) under the conditions used by Dale *et al.* (9) with Hg-CTP or CTP and [^{3}H]UTP as label (specific activity 0.1 Ci/mmol). Twenty micrograms of polysomal poly(A)-containing RNA from hen oviduct, 50% of which is $mRNA_{oa}$, was prepared as described (12) and chromatographed on SH-agarose, and the $mRNA_{oa}$ content in the applied, unbound, and bound fractions was determined by cDNA excess hybridizations. $cDNA_{oa}$ and Hg-$cDNA_{oa}$ were prepared as described. $mRNA_{oa}$·[^{3}H]Hg-$cDNA_{oa}$ hybrids were obtained by hybridization in 10-fold excess of $mRNA_{oa}$ followed by S_1 nuclease digestion. ^{125}I-$mRNA_{oa}$·Hg-$cDNA_{oa}$ hybrids were obtained by hybridization in 10-fold excess of Hg-[^{32}P]$cDNA_{oa}$ followed by RNase A digestion.

Synthesis of Mercury-Labeled RNA and Analysis of Ovalbumin RNA Content. Fig. 1 shows the characteristics of RNA synthesis in isolated oviduct nuclei in the presence of a mercurated nucleotide. Products from both RNA polymerase A and RNA polymerase B were obtained with the mercury-substituted nucleotide as substrate, because 70% of Hg-RNA synthesis could be inhibited by α-amanitin at 2.5 μg/ml. For both enzymes, substitution of Hg-CTP for CTP reduced the rate of synthesis to 60% (Fig. 1A). Incubation of nuclei with actinomycin D, α-amanitin, or DNase or omission of Hg-CTP in the mixture suppressed the incorporation of radioactive UMP to 10%, 26%, 14%, and 8%, respectively (Fig. 1B). By means of the mercury label, the *in vitro* synthesized RNA was separated

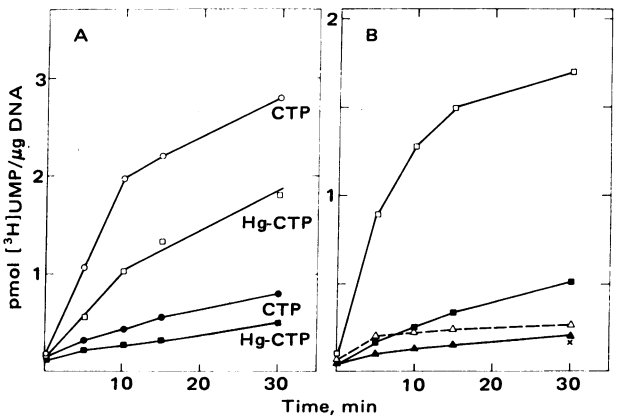

FIG. 1. RNA synthesis in isolated nuclei with mercurated CTP as substrate. (*A*) RNA was synthesized in isolated nuclei with Hg-CTP (□, ■) or CTP (○, ●) and using [^{3}H]UTP (0.5 Ci/mmol) as label in the absence (□, ○) or presence (■, ●) of 2.5 μg of α-amanitin per ml under the conditions described in *Materials and Methods*. Aliquots were taken at the indicated times and acid-insoluble radioactivity was determined as described (8). (*B*) RNA was synthesized with Hg-CTP in the absence of inhibitor (□) or in the presence of α-amanitin at 5 μg/ml (■), actinomycin D at 80 μg/ml (▲), or DNase at 80 μg/ml (△). Omission of Hg-CTP (×).

Table 2. Hybridization of purified newly synthesized Hg-RNA to labeled ovalbumin cDNA

Condition of RNA synthesis		*In vitro* synthesized Hg-[^{32}P]RNA		Ovalbumin mRNA		
Incubation time, min	Inhibitor	Total, ng	Bound to SH-agarose, ng	In unbound fraction, ng	In R-SH eluate, pg	In purified RNA synthesized *in vitro*, %
0		0	0	298	0	0
6		540	311	302	485	0.156
30		855	442	284	672	0.152
30	α-Amanitin (1 μg/ml)	328	149	293	48	0.032
30	Actinomycin D (40 μg/ml)	140	29	316	61	0.21

Each sample consisted of 8×10^8 nuclei. The conditions for synthesis, extraction, purification, and hybridization of Hg-[^{32}P]RNA (specific activity 2 mCi/mmol [^{32}P]UMP) are described in *Materials and Methods*.

from endogenous RNA on the SH-agarose column, which was shown to exclusively bind mercurated nucleic acids (Table 1). Routinely, 40–60% of the [^{3}H]UMP or [^{32}P]UMP incorporated into RNA that had been synthesized in the presence of Hg-CTP bound to the column. To totally eliminate contamination with endogenous RNA during the preparation procedure, we developed conditions that prevent aggregation of endogenous RNA to Hg-RNA and nonenzymatic mercuration of endogenous RNA. As pointed out by Dale *et al.* (20) and confirmed by us, nonenzymatic mercuration of RNA readily occurs in low salt buffer and at elevated temperature in the presence of acetate ions. Therefore, acetate ions were replaced by chloride ions in all steps of the RNA preparation and RNA samples were heat denatured before chromatography on SH-agarose.

$mRNA_{oa}$ sequences in the purified *in vitro* transcripts were titrated by cDNA excess hybridization. For hybridization analysis, Hg-[^{32}P]RNA of low specific activity was synthesized with the same number of nuclei under different incubation conditions (Table 2). The RNAs were then extracted and isolated on SH-agarose as described. The R-SH eluates of these samples, which are expected to contain the *in vitro* transcripts free of any endogenous contamination, were hybridized to [^{3}H]$cDNA_{oa}$. Table 2 summarizes these results. $mRNA_{oa}$ sequences were synthesized during the incubation of the nuclei, whereas no $mRNA_{oa}$ sequences were detectable in the R-SH eluate of the sample prepared from unincubated nuclei. Actinomycin D (80 μg/ml) or α-amanitin (5 μg/ml) reduced the *in vitro* synthesis of ovalbumin RNA to 10%. The observed extent of hybridization of the RNA in the R-SH eluates to [^{3}H]$cDNA_{oa}$ was strictly dependent on the *in vitro* incubation of nuclei and unrelated to the amount of endogenous $mRNA_{oa}$ chromatographed or to the total amount of Hg-RNA bound to the column. These results show that $mRNA_{oa}$ sequences were synthesized during the incubation of nuclei in a DNA template-dependent reaction by an α-amanitin-sensitive enzyme and represented 0.15% of the purified *in vitro* transcripts.

Synthesis of Radiolabeled RNA and Analysis of Ovalbumin RNA Content. Under these conditions, only elongation of *in vivo* initiated RNA chains occurs in isolated nuclei (8); therefore, Hg-RNA molecules should contain *in vivo* initiated sequences contiguous to *in vitro* elongated sequences. Hybridization to [^{3}H]cDNA thus would lead to an overestimate of *in vitro* synthesized $mRNA_{oa}$ sequences. Moreover, we found that the use of mercurated ribonucleotides can lead to artefacts in studies of *in vitro* transcription of chromatin (see *Discussion*). We therefore developed a more direct approach to study specific *in vitro* transcription (Fig. 2). Highly radiolabeled RNA was synthesized in isolated nuclei and hybridized to an excess of mercurated cDNA. The background problem caused by nonhybridized but RNase-resistant sequences was overcome by purification of Hg-cDNA-containing hybrids on SH-agarose.

As shown in Table 3, 0.03% of the input [^{3}H]RNA bound to SH-agarose and was eluted with 0.2 M 2-mercaptoethanol. When cDNA was omitted from the hybridization reaction, about 0.3% of the [^{3}H]RNA remained resistant to ribonuclease, but none of this radioactivity bound to SH-agarose. The retention of 0.03% of [^{3}H]RNA on SH-agarose is not due to unspecific aggregation to $Hg\text{-}cDNA_{oa}$, because this value is 30 times over the background value determined by control hybridization with [^{3}H]RNA synthesized in isolated rat liver nuclei. The amount of [^{3}H]RNA bound was reduced to 10% in a competition hybridization with $mRNA_{oa}$ added in 7-fold excess over Hg-cDNA (Table 3, experiment 1). The specificity of the purified [^{3}H]RNA·$Hg\text{-}cDNA_{oa}$ hybrids was furthermore tested with *E. coli* RNase H (hybrid nuclease). As shown in Table 4, 98% of the SH-agarose-bound radioactivity was sensitive to RNase H treatment. The effect of inhibitors is shown in experiment 2 of Table 3. Actinomycin D at 40 μg/ml reduced the synthesis of total and specific RNA to equal extents. α-Amanitin at 1 μg/ml completely abolished ovalbumin RNA synthesis, whereas reducing total RNA synthesis only to 35%. The inhibition of *in vitro* synthesis of $mRNA_{oa}$ sequences by actinomycin D and α-amanitin rules out the possibility of radioactive

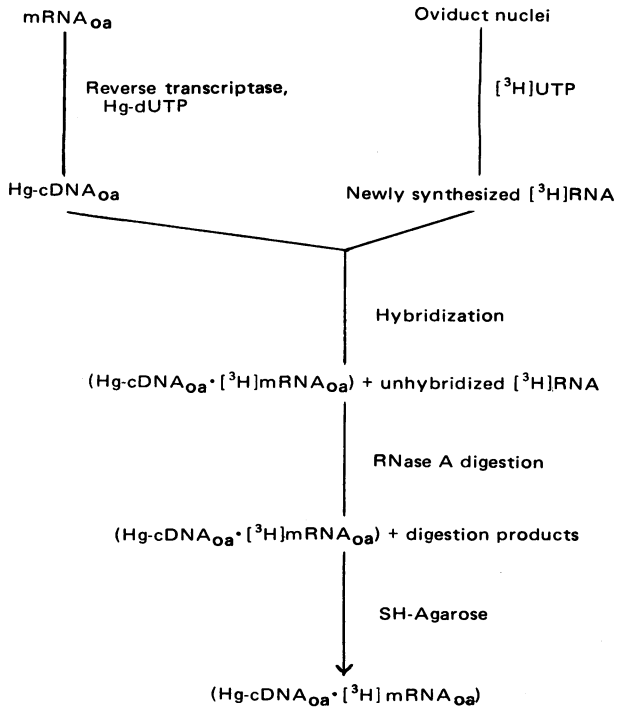

FIG. 2. Scheme for the detection of specific mRNA sequences in newly synthesized, radiolabeled RNA by a mercurated cDNA.

Table 3. Hybridization of [^{3}H]RNA synthesized in isolated nuclei to ovalbumin Hg-cDNA

Exp.	Transcription reaction	Hg-cDNA, ng	Input RNA cpm	Input RNA $mRNA_{oa}$, ng	$mRNA_{oa}$ added, ng	RNase A resistant, cpm	Bound to SH-agarose cpm	Bound to SH-agarose % of [^{3}H]RNA	Bound to SH-agarose % of [^{3}H]RNA, corrected
1	Complete	0	640,000	5.5	0	1,130	0	0	0
	Complete	72	640,000	5.5	0	1,432	201	0.0314	0.094
	Complete	72	640,000	5.5	500	1,030	18	0.0028	0.0084
	Rat liver nuclei	72	2,217,000	0	0	15,120	23	0.0010	—
2	Complete	84	798,040	15	0	1,512	204	0.026	0.078
	+ Actinomycin D (40 μg/ml)	84	110,720	15	0	266	28	0.025	0.075
	+ α-Amanitin (1 μg/ml)	84	299,760	15	0	596	0	0	0
	Complete, 5 min incubation	84	300,460	15	0	584	84	0.028	0.084

[^{3}H]RNA was synthesized in isolated oviduct nuclei for 30 min, using 20 μM [^{3}H]UTP (46 Ci/mmol) as described, except for the changes indicated. The content of endogenous $mRNA_{oa}$ in the samples was determined by [^{3}H]cDNA excess hybridization followed by a two-step digestion with S_1 nuclease and RNase A. In one hybridization 500 ng of $mRNA_{oa}$ was added as competitor, in another Hg-cDNA was omitted from the reaction. Hybridization, RNase A digestion, and SH-agarose chromatography were performed as described in *Materials and Methods*. The background value was determined by chromatography of [^{3}H]RNA that had been synthesized in isolated rat liver nuclei and hybridized to Hg-[^{32}P]$cDNA_{oa}$ under identical conditions. The efficiency of the purification procedure is 30%–35% as determined by chromatography of ^{125}I-$mRNA_{oa}$ hybridized to Hg-cDNA under the same conditions. The measured percentage of [^{3}H]RNA bound to SH-agarose was corrected for the purification efficiency determined with ^{125}I-$mRNA_{oa}$ after subtraction of the rat liver [^{3}H]RNA background.

tagging of endogenous $mRNA_{oa}$ sequences during the incubation of nuclei. The data show that $mRNA_{oa}$ sequences have been synthesized in a DNA-dependent reaction by RNA polymerase B and that 0.08–0.1% of the newly synthesized RNA was $mRNA_{oa}$ sequences.

DISCUSSION

To evaluate the manner in which steroid hormones effect accumulation of specific mRNAs, measurements of the rates of synthesis of hormone-dependent mRNAs are necessary. In this paper we investigated the rate of synthesis of $mRNA_{oa}$ sequences in isolated hen oviduct nuclei. The difficulties in detecting a low level of a newly synthesized specific sequence in the presence of large quantities of this sequence endogenous to nuclear RNA have been overcome by two different methods. Using these methods, both of which exploit the affinity of mercurated polynucleotides for SH-agarose, we have detected $mRNA_{oa}$ sequences synthesized in isolated nuclei. Multiple control experiments were performed to ensure the reliability of our identification procedure for newly synthesized specific RNA sequences. Thus in the case of mercury labeling of *in vitro* synthesized RNA we have developed conditions for RNA synthesis, extraction, and chromatography under which retention of endogenous sequences to SH-agarose through unspecific adsorption, through aggregation to Hg-RNA, and through chemical mercuration have not occurred at detectable levels. In the case of radiolabeling of *in vitro* synthesized RNA, the specificity of the hybrids has been carefully analyzed by competition with unlabeled $mRNA_{oa}$ and by digestion with RNase H. To give further support to the identification of ovalbumin RNA synthesized *in vitro*, we have studied the effect of inhibitors on total and specific RNA synthesis. Using both methods, we have shown that ovalbumin RNA synthesis in isolated nuclei is inhibited by actinomycin D and α-amanitin. On the basis of these controls we believe that the described experiments demonstrate the synthesis of ovalbumin mRNA sequences *in vitro*.

Table 4. Sensitivity of purified [^{3}H]RNA·Hg-$cDNA_{oa}$ hybrids to RNase H

Hybrids	Acid-insoluble products, cpm: No heat denaturation, No RNase H	No heat denaturation, RNase H	Heat denaturation, RNase H
[^{3}H]RNA·DNA_{fd}	9200	595	8438
[^{3}H]RNA·Hg-$cDNA_{oa}$	242	5	226

[^{3}H]RNA·Hg-$cDNA_{oa}$ hybrids were purified as described below Table 3. The material eluted with 0.2 M 2-mercaptoethanol was precipitated with 70% ethanol/0.3 M sodium acetate at pH 4.5/yeast RNA (10 μg/ml) at −20°. The pellet was dissolved in 600 μl of 40 mM Tris·HCl at pH 8.0/50 mM NaCl/5 mM $MgCl_2$/1 mM dithiothreitol/bovine serum albumin (30 μg/ml)/4% (vol/vol) glycerol and split into aliquots. Two of them were incubated in the absence or presence of *E. coli* RNase H at 10 units/ml for 30 min at 30° and one was heated to 100° for 3 min to denature the hybrids before treatment with RNase H. The acid-insoluble radioactivity was then determined. [^{3}H]RNA·DNA_{fd} hybrids were obtained by transcription of phage fd DNA by *E. coli* RNA polymerase, using [^{3}H]UTP (40 Ci/mmol).

The efficiency of $mRNA_{oa}$ synthesis is remarkable; 0.15% of the purified Hg-RNA and 0.08%–0.1% of the *in vitro* ^{3}H-labeled RNA are $mRNA_{oa}$ sequences. The discrepancy obtained with these two methods probably results from the fact that purified Hg-RNA chains are composed of *in vivo* initiated and *in vitro* elongated sequences, whereas only *in vitro* elongated sequences are detected when radiolabeled RNA is hybridized to unlabeled cDNA. From the total extent of incorporation into RNA it is obvious that the absolute rate of elongation *in vitro* is far below that observed *in vivo*. However, we believe that the high percentage of newly synthesized $mRNA_{oa}$ faithfully reflects the relative transcriptional rate of the ovalbumin gene *in vivo*, because it is likely that only elongation of *in vivo* initiated RNA chains has occurred *in vitro* and that the *in vitro* elongation rate is constant for all transcribed genes. Does the relative content of ovalbumin RNA found in the *in vitro* transcripts partly result from post-transcriptional events? Pulse–chase experiments would be required to rule out processing of RNA in isolated nuclei. Nevertheless, using both methods of analysis, we showed that nuclei incubated for a relatively short time (5 or 6 min) yield $mRNA_{oa}$ sequences with a specificity comparable to that found in nuclei incubated for 30 min (Tables 2 and 3). This indicates that degradative processing of RNA does not contribute in a significant way to the relative content of $mRNA_{oa}$ sequences in the *in vitro* transcripts. The transcripts from isolated hen oviduct nuclei contain ovalbumin RNA in a concentration comparable to that found in the total RNA extracted from oviduct nuclei (0.1%–0.15% of nuclear RNA is ovalbumin RNA; M. C. Nguyen-Huu, unpublished data). This

suggests that the nuclear steady-state concentration of ovalbumin RNA is controlled during transcription.

It has been demonstrated that transcription of chromatin with *E. coli* RNA polymerase gives rise to tissue-specific products (21, 22). Studying transcription of oviduct chromatin by *E. coli* RNA polymerase, Harris *et al.* (23), Tsai *et al.* (24), and Towle *et al.* (25) found that approximately 0.01% of the transcripts are $mRNA_{oa}$ sequences. A contamination of *in vitro* RNA with endogenous RNA sequences has, however, not been excluded. When we transcribed oviduct chromatin with *E. coli* RNA polymerase, using mercurated nucleotides, we observed that the apparent synthesis of ovalbumin mRNA sequences was not sensitive to actinomycin D. It has been shown that endogenous $mRNA_{oa}$ sequences were copurified with Hg-RNA. This copurification is mainly due to RNA-dependent synthesis of a complementary strand by the bacterial enzyme (26). Similar results have been published by Zasloff and Felsenfeld (27).

The direct engagement of RNA polymerase B in transcribing structural genes has been to date reported only for viral mRNAs (28–31) and for the fibroin mRNA (32). Our results demonstrate that RNA polymerase B transcribes the ovalbumin gene. We have shown that synthesis of ovalbumin mRNA sequences in isolated oviduct nuclei was completely inhibited by α-amanitin at 1 μg/ml, whereas total RNA synthesis was only reduced to 30% (Table 3). The incomplete inhibition found with the mercury labeling method (7%, Table 2) may result from side reactions that lead to a copurification of endogenous ovalbumin RNA. This could be due to the copurification of an Hg-RNA·$mRNA_{oa}$ hybrid produced by the action of RNA polymerase using $mRNA_{oa}$ as template (26, 27). Approximately 10,000 RNA polymerase B molecules are engaged in transcription in the oviduct nucleus (33). Because 0.12%–0.15% of polymerase B transcripts are $mRNA_{oa}$ sequences, we thus can calculate that 6–8 molecules of polymerase B are transcribing the ovalbumin gene, assuming that the elongation rate of polymerase B is identical on all genes, that all *in vitro* transcripts are conserved, and that both ovalbumin genes are being transcribed. This would represent a packing density of polymerases B of about one-half to one-third that found on rRNA cistrons (34).

The *in vivo* transcription rate of the ovalbumin gene is not known. Nevertheless, the preferential *in vitro* transcription of this gene (1000-fold over random transcription of the total chicken genome) suggests that the specificity of *in vivo* RNA synthesis is retained in isolated nuclei. The nuclear system described here should permit determination of the transcription rate of the egg white protein genes during hormone induction and withdrawal.

We would like to thank S. Jeep for expert technical assistance, I. Schallehn for preparation of the manuscript, Dr. H. Sternbach for providing *E. coli* RNA polymerase, and Dr. K. Moelling for the supply of radioactive RNA·DNA_{fd} hybrid.

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