Physical and Chemical Characterization of Purified Ovalbumin Messenger RNA* 

(Received for publication, March 10, 1975)

SAVIO L. C. WOO, JEFFREY M. ROSEN, CHARLES D. LIARAKOS, YONG C. CHOI, HARRIS BUSCH, ANTHONY R. MEANS,† AND BERT W. O’MALLEY

From the Departments of Cell Biology and Pharmacology, Baylor College of Medicine, Houston, Texas 77025

DONALD L. ROBBERSON

From the Department of Biology, M.D. Anderson Hospital and Tumor Institute, University of Texas System Cancer Center, Houston Texas 77025

Preparative agarose gel electrophoresis under denaturing conditions has been successfully employed to purify large quantities of ovalbumin mRNA from hen oviducts. The mRNA thus prepared is physically homogeneous based on its migration as a single component on electrophoresis in both analytical acid-urea agarose gels and formamide-containing, neutral polyacrylamide gels; it also sediments as a single peak in sucrose gradients containing 70% formamide. The mRNA is chemically free of ribosomal RNA contamination since its oligonucleotide fingerprint map after complete T1 ribonuclease digestion contains no detectable specific large oligonucleotide markers of ribosomal RNAs. It is also not contaminated by other biologically active messenger RNAs because, when it is added to the cell-free wheat germ translation system, the only protein product synthesized is ovalbumin as analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and specific immunoprecipitation.

Ovalbumin mRNA has a nucleotide composition of 32.3% A, 21.0% G, 25.7% U, and 20.7% C [(A+U)/(G+C) = 1.41]. The mRNA contains a heterogeneous poly(A) tract ranging from 20 to 140 residues with a number average chain length of 62 adenylate residues. The molecular weight of the sodium salt of the purified mRNA is approximately 650,000 ± 63,000, corresponding to a chain length of 1890 ± 180 nucleotides, as determined by electron microscopy under completely denaturing conditions. This value is in close agreement with the values obtained from: (a) sucrose gradient centrifugation in the presence of 70% formamide, (b) evaluation of poly(A) content in the mRNA and the number average chain length of its poly(A) tract; and (c) sedimentation velocity studies in the presence of 3% formaldehyde.

When 125I-labeled ovalbumin mRNA is allowed to hybridize with a large excess of chick DNA, the observed kinetics of hybridization reveal no appreciable reaction between the mRNA and the repeated sequences of the chick DNA, although the mRNA appears to be approximately 600 nucleotides longer than necessary to code for ovalbumin. It thus appears that the entire ovalbumin mRNA is primarily transcribed from a unique sequence in the chick genome.

Our previous work has shown that the estrogen-inducible ovalbumin mRNA can be substantially purified from total nucleic acid extracts of hen oviducts (1, 2). Further purification of ovalbumin mRNA can be achieved by employing Sepharose 4B column chromatography to effect a partial fractionation of total cellular mRNAs from DNA and stable RNAs in total tissue nucleic acid extracts (3). Preparative agarose gel electrophoresis under conditions of acidic pH and in the presence of 6 M urea is a precise sizing technique for fractionation of high molecular weight nucleic acids and is capable of removing quantitatively the similarly sized 18 S rRNA from ovalbumin mRNA. Combining these techniques, it is possible to purify large quantities of ovalbumin mRNA to apparent physical homogeneity as judged by gel electrophoresis under two different types of denaturing conditions. Furthermore, careful analysis of the total protein products synthesized in a cell-free wheat germ translation system indicates that
ovalbumin mRNA thus prepared is free of other biologically active oviduct mRNAs. The absence of ribosomal RNAs and their fragments in the ovalbumin mRNA preparations, as indicated by the oligonucleotide fingerprint maps of the 14-1L-RNAs after complete T1 ribonuclease digestion, has allowed further characterization of this mRNA with respect to its chemical composition, physical size, and sequence complexity.

**EXPERIMENTAL PROCEDURE**

**Materials**

White Leghorn laying hens were purchased from Rich-Glo Farm, LaGrange, Texas; their ovicells were rinsed with cold 0.9% NaCl solution (saline) and frozen in liquid nitrogen immediately after dissection. Liquefied phenol was purchased from Fisher Scientific Co., New Jersey, and redistilled before use. Sepharose 4B and Sephadex G-25 were purchased from Pharmacia Fine Chemicals, Sweden. Creatine phosphate, creatine phosphokinase, and sodium dodecyl sulfate were from Sigma Chemical Co. Wheat germ was kindly supplied as a gift by General Mills, Inc., Minneapolis, Minn. [*P*]-Poly(U) (23 mCi/mmol, 1°Cyanine (260 mCi/mmol), ultrapure urea, and sucrose (ribonuclease-free) were purchased from Schwarz/Mann, Orangeburg, N. Y. Nitrocellulose filters (HAWP 0.45 µ) were purchased from Millipore Corp., Bedford, Mass. Ribonuclease T1 was obtained from Sankyo, Ltd., Japan, and pancreatic ribonuclease A was from Worthington. Na141 (carrier-free, >500 mCi/ml) was from Nalz51 (carrier-free, >500 mCi/ml) was from New Jersey, and redistilled before use. Sepharose 4B and Sephadex G-25 were purchased from Pharmacia Fine Chemicals, Sweden.

**Preparation of Wheat Germ S-30**

A 30,000 x g supernatant fraction was prepared from wheat germ by a modification of the procedure of Roberts and Paterson (4) as previously described (5). Briefly, wheat germ was ground with glass beads in a buffer and the homogenate was centrifuged for 10 min at 30,000 x g. The supernatant was applied to a Sephadex G-25 column (2.6 x 70 cm) without preincubation, and the turbid fractions excluded from the column were pooled and dispensed into liquid nitrogen. The frozen spheres were stored in a liquid nitrogen tank; the activity was stable for at least 6 months.

**In Vitro Protein Synthesis Assay**

The in vitro translation assay using wheat germ S-30 was carried out essentially as previously described (5). RNA was incubated with the wheat germ S-30 fraction in the presence of 1°Cyanine and total mRNA activity was quantitated by measuring total incorporation of radioactive amino acid into trichloroacetic acid-precipitable material. Ovalbumin mRNA activity was assessed by using a monospecific antibody against ovalbumin as described (6). Specific activity is defined as number of picomoles of 1°Cyanine incorporated into ovalbumin per mg of exogenous RNA used under these conditions.

**Analysis of In Vitro Synthesized Protein Products by Gel Electrophoresis**

The in vitro synthesized protein products by gel electrophoresis was carried out as previously described (5). The wheat germ ribosomes were removed by ultracentrifugation and the post-ribosomal supernatant fractions were treated with EDTA and pancreatic ribonuclease A prior to analysis by the sodium dodecyl sulfate polyacrylamide gel electrophoresis procedure of Weber and Osborn (7).

**Purification of Ovalbumin mRNA**

Throughout the purification procedure described below, all glassware was washed in 1 N NaOH and then extensively rinsed with deionized water. All stock reagents, except those containing phenol, were filtered through Millipore filters (0.45 µ). Gloves were worn at all times. RNA solutions were adjusted to 0.25 M KCl when precipitated with 2 volumes of alcohol at -20°C overnight. When not specified, centrifugation was at 12,000 x g for 10 min at 2°C.

**Extraction of Total Nucleic Acid**

Frozen hen oviducts were chopped into small pieces with a hammer and immediately extracted twice at room temperature with 10 volumes of phenol-saturated sodium dodecyl sulfate buffer, pH 8.0, as described previously (5). The total nucleic acid was precipitated with alcohol, centrifuged, and redissolved in water. The yield of total nucleic acid was generally 4 to 5 mg/g of frozen oviduct. About 1 to 1.5 g of pooled total nucleic acid extract was usually employed for further purification.

**Nitrocellulose Filtration—Ovalbumin mRNA was partially purified from the total nuclear acid extract by nitrocellulose filters essentially as described previously (1). Total extract was filtered through nitrocellulose filters and the adsorbed RNA was eluted from the filters with 0.1 M Tris-HCI, pH 7.6, containing 0.5% sodium dodecyl sulfate. The eluate was chilled and the precipitated sodium dodecyl sulfate was removed by centrifugation. Potassium chloride was added to 0.25 M and the insoluble potassium dodecyl sulfate was quantitatively removed by centrifugation. The supernatant was transferred to fresh tubes with Pasteur pipettes, and the RNA was precipitated with alcohol. Precipitates were collected by centrifugation and redissolved in water.

**Sepharose 4B Column Chromatography—RNA extracted from the nitrocellulose filters was subjected to gel filtration on a Sepharose 4B column (2.5 x 100 cm) as described (3). About 20 mg of filtered RNA, in a total volume of 10 to 15 ml, was heated for 1 min at 70°C and quickly cooled in an ice-water bath before being applied to the column which was developed at 4°C with 0.1 M sodium acetate, pH 5.0, containing 1 mM EDTA, at a flow rate of approximately 10 ml/hour. Fractions of 8 ml were collected, and RNA in each fraction was precipitated with alcohol, centrifuged, and redissolved in water. Ovalbumin mRNA activity as well as total mRNA activity in each fraction was assayed by the wheat germ translation system. Fractions enriched in ovalbumin mRNA activity were pooled.

**Second Nitrocellulose Filtration—RNA pooled from the Sepharose column was filtered through nitrocellulose paper for a second time. Filtration was carried out as before, except that only 100 µg of RNA was applied to each filter. Adsorbed RNA was subsequently eluted from the filters, precipitated with alcohol, centrifuged, and redissolved in small amounts of water.

**Preparative Agarose Gel Electrophoresis—Electrophoresis on acid-urea agarose gels yielded high resolution for RNA transcribed in vivo (high molecular weights) and was, therefore, used on a preparative scale for the purification of ovalbumin mRNA. Agarose gels have extremely low viscosity, however, and cannot remain in the gel electrophoresis column without support. To overcome this difficulty, an adapter to the Buchler Poly-Prep electrophoresis apparatus was designed for support of the gel. The adapter was made of a 6.0 cm tall lucite column (outside diameter, 5.0 cm; inside diameter, 4.2 cm), which fits precisely into the gel electrophoresis chamber. It was held tightly in the gel column by two vition O-rings fitted over two grooves engraved on both ends of the adapter. A piece of Nytex screen was secured at the bottom of the adapter with the aid of a snap ring. The adapter was fitted into the gel column, and the gel was cast inside the adapter.

A 2% agarose gel was made by gently refluxing 1.0 g of agarose in 50 ml of 0.025 M sodium citrate, pH 3.5, containing 6 m urea, for 10 min with stirring. The slightly opaque solution was cooled in a 62°C water bath, and 40 ml of it were transferred, using a separatory funnel, into the adapter in the gel electrophoresis chamber. The gel solution in the adapter was slowly cooled to 4°C by regulating the temperature of coolant which circulated continuously in the column jackets. Overlaying the gel surface with water was avoided. An opaque agarose gel formed in the adapter within 2 hours. The gel-forming insert was replaced by the glass membrane holder. An elution chamber of 1 mm in depth was established by retracting the glass membrane holder one-half turn. The lower and upper chambers were filled with 0.025 and 0.05 m sodium citrate, pH 3.5, respectively. The elution chamber was filled with 0.125 M sodium citrate, pH 3.5, containing 6 m urea and 350 µl/sucrose. It is imperative not to raise the elution buffer level higher than 6 m urea in the elution buffer chamber or the agarose gel will float in the gel column by increased hydraulic pressure from the elution buffer. The gel then was subjected to pre-electrophoresis at 4°C for 30 min at a constant current of 5 mA. RNA recovered from the second nitrocellulose filtration step was mixed with 3 volumes of layering buffer (0.020 M sodium citrate, pH 3.5, containing 8 M urea, 0.05% g/liter of sucrose and 0.05% g/liter of bromophenol blue), and the solution was applied to the top of the agarose gel using a peristaltic pump at low speed. Electrophoresis was continued immediately at 35 mA, and voltage was generally 50 to 80 volts throughout electrophoresis. RNA was eluted from the elution chamber by drawing elution buffer through the chamber at a rate of 0.5 ml/min using a peristaltic pump. It is necessary to draw the elution buffer through the elution chamber rather than having it through because forcing buffer into the elution chamber would cause the agarose gel to

Downloaded from http://www.jbc.org/ by guest on March 24, 2020
Electron Microscopy of Purified Ovalbumin mRNA

RNA samples in distilled water were prepared for electron microscopy by the formamide-urea modification of the Kleinschmidt technique (11). RNA samples were centrifuged at 50 to 60 μg/ml at 100,000 x g for 1 hr, and each pellet was re-dissolved in 0.5 ml of 0.5 M NaCl. Three units of pancreatic ribonuclease A and 1 unit of ribonuclease T1 were added, and the solution was incubated at 37° for 1 hr. The digest was subjected to electrophoresis on 12% polyacrylamide gels as described (13). The gels were sliced into 1 mm slices and transferred to DEAE-cellulose. Each gel slice was soaked in 0.1 ml of 0.5 M NaCl and homogenized with a Teflon pestle. The homogenate was centrifuged at 12,000 x g for 15 min, and the supernatant fractions were used for hybridization with [3H]poly(U) (14). The extent of hybridization radioactivity along the gel reflected the migration of poly(A) chains. The weight average and number average chain lengths in purified ovalbumin mRNA were then estimated by comparing to the migration of standard poly(A)s of known chain lengths.

Determination of Average Length of Poly(A) in Purified Ovalbumin mRNA

The average length of poly(A) in purified ovalbumin mRNA was determined by a modification of the procedure of Morrison et al. (12). Twenty micrograms of purified ovalbumin mRNA were dissolved in 50 μl of 0.02 M Tris-HCl, pH 7.6, containing 0.002 M EDTA and 5 mM MgCl2. The temperature of the pancreatic ribonuclease T1 was added, and the solution was incubated at 37° for 1 hr. The digest was subjected to electrophoresis on 12% polyacrylamide gels as described (13). The gels were sliced into 1-mm slices after electrophoresis. Each gel slice was soaked in 0.1 ml of 0.5 M NaCl and homogenized with a Teflon pestle. The homogenate was centrifuged at 12,000 x g for 15 min, and the supernatant fractions were used for hybridization with [3H]poly(U) (14). The extent of hybridization radioactivity along the gel reflected the migration of poly(A) chains. The weight average and number average chain lengths in purified ovalbumin mRNA were then estimated by comparing to the migration of standard poly(A)s of known chain lengths.

Oligonucleotide Fingerprint Analysis

13H labeled ovalbumin mRNA, chick S ribosomal RNA or chick 28 S ribosomal RNA was digested separately at 37° for 45 min in 0.02 M Tris-HCl (pH 7.4)/0.002 M EDTA with T1 RNase or RNAse A at an enzyme to substrate ratio of 1:20 (w/w). Each digest was fractionated by electrophoresis on Cellogel or cellulose acetate strips followed by a thin layer of homomixtures (15). Each homomixture is a 3% solution of Torula yeast RNA (Sigma, grade VI) in 0.01 M Tris-Cl, 0.01 M NaCl, 10 mM EDTA, pH 7.4. The yeast RNA was hydrolyzed in 1 M KOH at room temperature for 5 (mixture C1) or 15 min (mixture C2) and neutralized with glacial acetic acid and ethanol. For "C homomixtures, the yeast RNA was hydrolyzed in 1 M KOH at room temperature for 20 min. The RNA was precipitated at room temperature before dissolving in the urea solution. A homomixture consisting of 25% mixture B and 75% mixture C2 (v/v) was used to fractionate T1 RNase digests, a homomixture of 20% mixture B and 80% mixture C2 (v/v) was used for pancreatic RNase digests. Following homochromatography in the second dimension, the thin layer plates were air dried and autoradiographed using Dupont Cronex 4 x-ray film.
The resulting nucleosides were oxidized to 2',3'-nucleoside dialdehydes. Analysis of Nucleotide Composition of Ovalbumin mRNA used to determine the extent of DNA-DNA reassociation by hydroxyl-denatured. The remaining 0.5 ml portions of the assay mixture were subjected to preparative agarose gel electrophoresis, the nitrocellulose filtration step. Finally, when 3.6 mg of this RNA additional slight purification was affected by the second ribonuclease resistant radioactivity to the total trichloroacetic acid-precipitable 125I-ovalbumin mRNA radioactivity was observed throughout the hybridization experiment, and the percent hybridization was determined by calculating the ratio of the ribonuclease resistant radioactivity to the total trichloroacetic acid-precipitable radioactivity. The values obtained were corrected for a control value obtained by determining the ribonuclease resistance of the 125I-mRNA in the reaction mixture in which the DNA was not denatured. The remaining 0.5 ml portions of the assay mixture were used to determine the extent of DNA-DNA reassociation by hydroxylapatite chromatography as described previously (2).

**Analysis of Nucleotide Composition of Ovalbumin mRNA**

Nucleotide composition analysis of ovalbumin mRNA was accomplished using the tritium derivative method (19). Ovalbumin mRNA was digested for 18 to 24 hours at 37°C with a mixture of pancreatic RNase A, alkaline phosphatase, and snake venom phosphodiesterase. The resulting nucleosides were oxidized to 2',3'-nucleoside dialdehydes with NaIO₄ and subsequently reduced to 4H-nucleoside trialcohols with KBH₄ (10 Ci/mmol). In each determination, an enzyme blank (no RNA) and a buffer blank (no RNA and no enzymes) were carried out simultaneously with the RNA sample. Individual nucleoside trialcohols were resolved by two dimensional chromatography on 0.10 mm cellulose thin layers (20 x 20 cm, EM Laboratories, Inc., Germany). The 4H-nucleoside trialcohols were visualized by autoradiography as described (20) and eluted with 2 N NH₄OH for counting in an Omnifluor (New England Nuclear) scintillator. The mean of five 2-min cycles (3 to 8 x 10⁵ cpm) was used to calculate the mole percentage of each 4H-nucleoside trialcohol.

**RESULTS**

The purification of ovalbumin mRNA from total hen oviduct nucleic acid extract is shown in Table I. The total nucleic acid extract can direct the incorporation of 0.63 pmol of valine into ovalbumin per μg of RNA used in the wheat germ translation assay. When 1300 mg of total nucleic acid extract were filtered through nitrocellulose filters, 22.2 mg of RNA were recovered with an increase in specific activity of 18-fold. This step can be replaced by oligo(dT)-cellulose column chromatography which yields slightly better purification and recovery of the mRNA (10). Gel filtration of this RNA on a Sepharose 4B column chromatography which yields slightly better purification and recovery of the mRNA. The 4H-nucleoside trialcohols were visualized by autoradiography as described (20) and eluted with 2 N NH₄OH for counting in an Omnifluor (New England Nuclear) scintillator. The mean of five 2-min cycles (3 to 8 x 10⁵ cpm) was used to calculate the mole percentage of each 4H-nucleoside trialcohol.

**TABLE I**

| Procedure                  | Amount | Specific Activity | Ovalbumin/total protein |
|----------------------------|--------|-------------------|-------------------------|
| 1300.0 mg                  | 22.2   | 11.6              | 65                      |
| Sepharose peak RNA         | 7.2    | 22.9              | 72                      |
| Sepharose reabsorbed RNA   | 0.6    | 36.7              | 79                      |
| Preparative gel RNA        | 0.6    | 92.5              | 92                      |

* RNA samples were prepared as described in the text. Several concentrations of each sample were assayed in the wheat germ translation system, and the values obtained above represent initial activities. Each picomole of valine is equivalent to 457 cpm.

* Per cent of total protein synthesized in the wheat germ translation system represented by ovalbumin. Since only 85 to 90% of a 13C-labeled ovalbumin is precipitable with the antibody in this assay, a correction factor of 10% had been added to these values.

Thus, the purification of ovalbumin mRNA by this procedure was 146-fold, with an over-all activity yield of approximately 6.7%. From 1300 mg of total extract, 0.6 mg of purified ovalbumin mRNA was obtained. The yield generally ranged from 0.5 to 1.0 mg of ovalbumin mRNA for different preparations.

Preparative agarose gel electrophoresis is capable of separating RNA species that are very similar in molecular size. This technique was employed at the last step in the purification procedure to remove quantitatively the residual contaminating 18 S rRNA from the ovalbumin mRNA. It consistently resolved RNA preparations from the second nitrocellulose filtration step into two major RNA peaks (Fig. 1). The faster migrating peak was subsequently identified as the 18 S rRNA, and when assayed in the wheat germ translation system, all ovalbumin mRNA activity was associated with the slower migrating RNA peak. Total mRNA activity in the fractions also was assayed in the wheat germ translation system by measuring the incorporation of labeled amino acid into trichloroacetic acid-precipitable material. The major peak of activity was associated with the peak of ovalbumin synthesizing activity. The presence of two additional small peaks of activity in fraction eluted prior to and coincident with the 18 S rRNA indicates the separation of some other mRNA species from ovalbumin mRNA.

The content of nucleic acids at each stage of purification was analyzed by analytical agarose gel electrophoresis (Fig. 2). Total nucleic acid extract contains many minor RNA bands in addition to the major stable cellular RNA species and DNA (Fig. 2, Gel A). Consequent to nitrocellulose filtration, there appears a preferential enrichment in a single RNA species migrating at 21 S (Fig. 2, Gel B). After Sepharose 4B column chromatography and a second nitrocellulose filtration step, the only remaining major species of nucleic acid are the 21 S RNA and the 18 S rRNA (Fig. 2, Gel C). DNA 28 S RNA, and most of the minor RNA species have been quantitatively removed. Finally, the only RNA species present after preparative agarose gel electrophoresis is the 21 S RNA (Fig. 2, Gel D). No contamination of ovalbumin mRNA was detected in these analytical gels. Furthermore, when examined by electrophoresis in polyacrylamide gels containing 98% formamide, ovalbumin mRNA also migrates as a 21 S RNA species with no
The wheat germ translation system, which has an intrinsically low endogenous protein synthesizing activity, was next employed to assess the biological purity of ovalbumin mRNA. The ratio of the ovalbumin antibody-precipitable radioactivity to total trichloroacetic acid-precipitable radioactivity in the postribosomal supernatant is a measure of the fraction of total biologically active messenger RNAs represented by ovalbumin mRNA. When this analysis was carried out for the RNA samples at each stage of purification, the percent ovalbumin mRNA increased from 45% in the total nucleic acid extract to over 90% in the purified ovalbumin mRNA preparations (Table I). This is at best a minimum estimation because incomplete ovalbumin molecules due to premature release from the ribosomes are not recognizable by the antibody (6) but will be precipitated by trichloroacetic acid.

The total protein products synthesized in the wheat germ translation system in response to purified ovalbumin mRNA were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The entire postribosomal supernatant fraction was applied to the gel and the radioactivity distribution in the gel is shown in Fig. 3. Only one peak of radioactivity is present and its electrophoretic mobility is identical with the ovalbumin marker. The broadness of the peak is probably not due to the presence of different peptide products, but rather to a distribution of various lengths of ovalbumin synthesized in vitro. This interpretation is supported by the observation that an identical radioactivity profile was obtained when immunoprecipitated product was analyzed. The absence of radioactivity peaks in other areas of the gel suggests that proteins of other sizes are not being synthesized. These experiments thus indicate that purified ovalbumin mRNA is not demonstrably contaminated by other biologically active oviduct messenger RNAs.
schematic) represents a characteristic pattern from 28 S rRNA (Fig. 6c, schematic). The absence of ribosomal RNA-specific patterns of large oligonucleotides from the T1 Rnase fingerprint map of ovalbumin mRNA (Fig. 5a or 6a) indicates that the messenger RNA contains no detectable contamination with 18 S or 28 S ribosomal RNAs or with their degradation products which contain the large oligonucleotide marker fragments.

All of these data provide strong evidence that the ovalbumin mRNA has been purified to apparent homogeneity. Therefore, it seems suitable to further characterize this molecule with respect to various physical and chemical parameters. The first such experiment undertaken was to determine the nucleotide composition of ovalbumin mRNA. This analysis was performed using the tritium derivative method (19) and the nucleotide composition of ovalbumin messenger RNA is presented in Fig. 7 (inset table). These data represent the average of four determinations on two different RNA preparations: A+U = 58.0% and G+C = 41.4%. An autoradiograph of the chromatographic separation of the four nucleoside triphosphates (A,U,G,C) and the tritiated background spots (B1, B2, B3) produced in both the buffer and enzyme blank reactions is also shown in Fig. 7. The spots labeled X1, X2, and X3 appear in all of the nucleotide composition determinations and represent less than 1% of the total radioactivity (A+U+G+C+X) eluted from the plate. These X spots have not been identified as yet but could represent modified nucleotides occurring in the ovalbumin mRNA molecule.

The molecular weight of ovalbumin mRNA was estimated by several independent methods. Initially, we utilized gel electrophoresis, assuming that the molecular weights of Escherichia coli 16 S and 23 S rRNAs are 560,000 and 1,080,000, and hen oviduct 18 S and 28 S rRNAs are 700,000 and 1,580,000, respectively (13). The molecular weight of ovalbumin mRNA was estimated to be 900,000 ± 90,000 from both acid-urea agarose gel electrophoresis (Fig. 8, Panel A) and neutral formamide polyacrylamide gel electrophoresis (Fig. 8, Panel B). Since the average residue molecular weight (sodium salt) is 343.5 in ovalbumin mRNA according to its nucleotide composition (Fig. 7), such a molecular weight estimate would correspond to a chain length of 2620 ± 262 nucleotides. The molecular weight of ovalbumin mRNA was also estimated from its sedimentation velocity in sucrose gradients. Partially purified ovalbumin mRNA sediments at 16 S to 18 S in the presence of 0.1 M NaCl at pH 5.0 (2). Under these conditions, RNAs sedimenting faster than 18 S rRNA also contain various amounts of ovalbumin mRNA activity, indicating that the mRNA is forming aggregates with itself or other RNA species. When sucrose gradient centrifugation is carried out in the presence of 70% formamide, however, partially purified ovalbumin mRNA sediments as a sharp peak at approximately 15.5 S to 16.5 S (10). Furthermore, the fact that there is no ovalbumin mRNA activity in the faster sedimenting RNA species indicates that aggregation does not occur under the denaturing conditions employed. When purified ovalbumin mRNA was analyzed in the same manner, a sharp RNA peak sedimenting at approximately 16 S was noted, which was essentially superimposable with the peak of ovalbumin mRNA activity when assayed in the wheat germ translation system (Fig. 9, inset). Compared to the stable cellular RNAs, the molecular weight of ovalbumin mRNA can be estimated by this technique to be 550,000 ± 30,000 (Fig. 9), which corresponds to a polyribonucleotide chain length of 1600 ± 90 residues. It is apparent that this molecular weight estimation is considerably different from the value obtained by the gel electrophoretic techniques. Consequently, other procedures were required to determine the true figure.

Since 4.2 ± 0.2% of the purified hen oviduct ovalbumin mRNA is composed of polyadenylic acid (10), the approximate molecular weight of ovalbumin mRNA can also be estimated from the length of its polyadenylic acid tract. Purified ovalbumin mRNA, therefore, was exhaustively digested with pancreatic ribonuclease A and ribonuclease T1 in the presence of 0.3 M NaCl, and the lengths of the poly(A) tracts in the digest were determined by electrophoresis in polyacrylamide gels. Poly(A) in the purified ovalbumin mRNA digest migrates
FIG. 5. Nucleotide fingerprint maps of the complete T1 RNase digest of [3H]-labeled (a) ovalbumin mRNA (1.47 × 10^6 cpm), (b) ovalbumin mRNA (1.57 × 10^6 cpm) plus 18 S rRNA (1.18 × 10^6 cpm), and (c) 18 S rRNA (1.29 × 10^6 cpm). A schematic tracing of each autoradiograph is also shown. The large T1-resistant oligonucleotides as a broad band between standard polyadenylic acids composed of 90 residues and 45 residues (Fig. 10, Panel A). A plot of the log of molecular weight of standard poly(A)s versus distance of migration yields a linear relationship (Fig. 10, Panel B); therefore, the chain length of poly(A) in each slice can be estimated. Calculation of the weight average and number average chain length of poly(A) in the ribonuclease digest of purified hen oviduct ovalbumin mRNA yields values of 68 and 62, respectively. Since 62 nucleotides constitute 4.2% of the entire molecule, purified ovalbumin mRNA should roughly be 1467 ± 75 nucleotides long, corresponding to a molecular weight of approximately 520,000 ± 25,000. Although this procedure is not the most accurate method available for determining the molecular weight of ovalbumin mRNA, the
estimate obtained is in agreement with the value obtained from sucrose gradient centrifugation.

The molecular weight of ovalbumin mRNA also can be estimated by determining its sedimentation coefficient after reaction with formaldehyde. Polynucleotides are converted into random coils by reacting with formaldehyde, so that the sedimentation coefficient in 3% formaldehyde is related to molecular weight by the equation $s_{20, w} = 0.65 \times M^0.4$ (21). Purified ovalbumin mRNA was denatured with formaldehyde and sedimented as described (21). A linear relationship between the log of migration distance versus time in minutes was obtained with a standard deviation of the regression coefficient.
of less than 2%. The \( s_{20, w} \) value obtained from three independent experiments was 9.12 \( \pm \) 0.10, which converts to a molecular weight of 450,000 \( \pm \) 12,000. This value, however, must be corrected by a factor of 1.2, since there appears to be one chain breakage per 5 molecules (see Fig. 12). Hence the corrected value should be 540,000 \( \pm \) 14,000, corresponding to a chain length of 1572 \( \pm \) 42 nucleotide residues.

The final approach to determine accurately the molecular weight of purified ovalbumin mRNA is to measure its length directly by electron microscopy under denaturing conditions. When spread in 4 M urea dissolved in formamide as previously described (11), ovalbumin mRNA molecules appear to be completely denatured as evidenced by the uniform contour throughout their length (Fig. 11). Length measurements on two independent ovalbumin mRNA preparations provide length distributions that are representative of a homogenous species of RNA (Fig. 12). Both the number average length and the weight average length for the two preparations are 0.5 \( \pm \) 0.05 \( \mu \)m (Fig. 12). Since RNA molecules have an average residue spacing of 2.65 \( \AA \) per nucleotide in this spreading procedure, ovalbumin mRNA would be composed of 1890 \( \pm \) 180 nucleotide residues. The molecular weight of ovalbumin mRNA, therefore, is estimated to be 650,000 \( \pm \) 63,000. This determination of molecular weight for ovalbumin mRNA is compatible with the values obtained by sucrose gradient centrifugation, poly(A)
analysis, and sedimentation velocity in formaldehyde, but is considerably lower than the value obtained by gel electrophoresis.

It is apparent that ovalbumin mRNA is considerably larger than is required to code for a protein composed of 387 amino acid residues. Using the complementary [3H]DNA probe, it has been previously reported that ovalbumin mRNA is transcribed from unique DNA sequences of the chick genome (22, 23). The [3H]cDNA probe used in these studies, however, was not a complete copy of the mRNA and could only reveal the sequence complexity of the 3'-terminal portion of the mRNA molecule. We have extended this study by performing hybridization experiments using 32P-labeled ovalbumin mRNA and a large excess of chick DNA to examine whether the entire ovalbumin mRNA molecule is transcribed from the unique sequence portion of the oviduct genome. A typical DNA reassociation curve is shown in Fig. 13. As previously reported (18), approximately 30% of the chick genome is composed of repeated sequences, while the remaining 70% is primarily unique sequences. Total chick DNA reassociates at a characteristic C0.1 value of 170 under the rather stringent conditions employed. Thus, the expected C0.1 value for unique sequence DNA reassociation under these conditions would be $170 \times 0.70 = 243$. Observation of the DNA/32P-ovalbumin mRNA hybridization curve reveals only one major transition with a C0.1 value of 550 (Fig. 13). These data suggest that the entire ovalbumin mRNA molecule is primarily transcribed from a unique DNA sequence in the chick genome.

**Fig. 10.** Determination of the length of poly(A) tract in ovalbumin mRNA by gel electrophoresis. The ribonuclease digest of 20 µg of purified ovalbumin mRNA was electrophoresed on a 12% polyacrylamide gel (13). The poly(A) contents in various slices of the gel were determined by [3H]poly(U) hybridization (14), as described under "Methods." Migration of standard poly(A)s of known chain length was similarly determined in separate gels; and migration of 5 S and 4 S RNAs was determined by staining. Panel A, extent of [3H]poly(U) hybridization of ribonuclease digest of purified ovalbumin mRNA through the gel was plotted versus gel slices; migration of standards employed are indicated by the arrows. Panel B, log of molecular weight was plotted versus distance of migration in the gel in the same experiment.

**Fig. 11.** Formamide-urea spreading of ovalbumin mRNA from two independent preparations. The magnification was the same in the two representative fields as indicated by the bar length of 1 µm.

**Fig. 12.** Frequency distributions of lengths observed in two separate ovalbumin mRNA samples spread by the formamide-urea procedure. The bar above the histograms represents the interval over which the number average length ($L_n$) and weight average length ($L_w$) were determined. The average lengths and standard deviations for the two RNA samples are also shown.

**Fig. 13.** Hybridization of 32P-ovalbumin mRNA with large excess of total chick liver DNA. DNA was sheared to 400 base pairs long and allowed to react with 32P-ovalbumin mRNA at a ratio of 3 x 10^3. The percentages of DNA/DNA reassociation and DNA/32P-mRNA hybridization were determined as described under "Methods." The per cent DNA/32P-mRNA hybridization values obtained were corrected by a 7% background ribonuclease resistance as determined by treating an aliquot of the reaction mixture in identical manner except that the DNA was not denatured. ○—○, per cent denatured DNA; ■—■, per cent RNA hybridized.
**DISCUSSION**

Ovalbumin mRNA has been purified from hen oviducts on a preparative scale by the combined use of nitrocellulose adsorption, Sepharose 4B column chromatography and preparative agarose gel electrophoresis. The purified mRNA migrates as a single component in both acid-urea agarose gels and formamide-containing neutral polyacrylamide gels (Fig. 2, Gel D, and Ref. 10). It also sediments as a single symmetrical peak in formamide-containing neutral sucrose gradients (Fig. 9, inset).

When examined by electron microscopy under completely denaturing conditions, length distributions for two ovalbumin mRNA preparations appear homogeneous (Fig. 12). Furthermore, prolonged radioautography of agarose gels of \(^{125}\)I-ovalbumin mRNA preparations failed to develop other radioactive RNA bands. These combined results indicate a high degree of size homogeneity of the purified ovalbumin mRNA.

When purified ovalbumin mRNA was assayed in the wheat germ translation system, greater than 90% of the protein synthesized was immunoprecipitable with a monospecific antibody against ovalbumin. Furthermore, when analyzed by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels, the \(in vitro\) synthesized proteins present in the post-ribosomal supernatant fraction migrate as ovalbumin. These data suggest that the ovalbumin mRNA preparations are essentially free of other functionally active oviduct RNAs.

Comparison of the \(T_1\) RNase resistant oligonucleotides from different RNA species by routine nucleotide composition analysis is not possible since \(^{125}\)I is incorporated largely into cytidine, and to a much lesser extent into uracil (16). Therefore, the \(T_1\) resistant oligonucleotides of \(^{125}\)I-ovalbumin mRNA were compared with those from \(^{125}\)I-labeled 18 S or 28 S rRNA by mapping a mixture of equal amounts of mRNA and 18 S or 28 S rRNA. \(T_1\)-resistant oligonucleotides that are identical with respect to nucleotide composition and chain length (although not necessarily nucleotide sequence) will co-chromatograph. Since additive maps were obtained, these mixture experiments demonstrate that the large, \(T_1\)-resistant oligonucleotides of ovalbumin mRNA and 18 S or 28 S rRNA chromatograph independently of each other in a specific pattern characteristic of the parent RNA species. The absence of patterns of large, \(T_1\)-resistant oligonucleotides specific for 18 S or 28 S rRNA from the \(T_1\) RNase map of ovalbumin mRNA indicates that the mRNA is not contaminated with either of these RNAs or with any of their degradation products which contain the RNA-specific, large oligonucleotides. This, however, does not rule out the remote possibility of contamination of mRNA with an RNA degradation product longer than 1500 nucleotides and completely devoid of the RNA-specific, large oligonucleotides. Furthermore, since the mixture experiments were carried out with approximately equal amounts of mRNA and rRNA (both in terms of mass and radioactivity), it is also possible that small amounts of rRNA might exist in purified ovalbumin mRNA preparations that were undetected by autoradiography for 1 day. However, this appears unlikely since prolonged (4 to 7 days) autoradiography of the mRNA thin layer plates failed to develop any additional rRNA-specific, large oligonucleotide spots. Thus, the contamination of ovalbumin mRNA with either 18 S or 28 S chick rRNA or their degradation products appears to be highly unlikely.

The preparative nature of the purification method described (greater than 0.5 mg of purified material/preparation) has permitted detailed physical and chemical characterization of the mRNA. In this regard, the molecular weight of ovalbumin mRNA has previously been determined in our laboratory as well as others (10, 24). However, the results were inconclusive since a large discrepancy existed between the values obtained by gel electrophoresis and by sucrose gradient centrifugation. The mRNA migrates as a 21 S species during electrophoresis in both acid-urea agarose gels and formamide-containing polyacrylamide gels, corresponding to a molecular weight of about 900,000 \(\pm\) 90,000 or a chain length of 2620 \(\pm\) 262 nucleotides; however, it sediments at approximately 16 S in sucrose gradients containing 70% formamide, corresponding to a molecular weight of about 550,000 \(\pm\) 30,000 or a chain length of about 1,600 \(\pm\) 90 nucleotides. Therefore, we employed three additional independent methods to estimate the molecular weight of ovalbumin mRNA, and the results are summarized in Table II. The molecular weight estimates from poly(A) analysis, sedimentation velocity in 3% formaldehyde and electron microscopy are in reasonable agreement with the value obtained from sucrose gradient centrifugation, but considerably less than the value obtained from the gel electrophoresis methods. Among the methods employed, only electron microscopy has the capability of measuring the linear lengths of RNA molecules independent of secondary structure and nucleotide composition (11). The absence of secondary structure within ovalbumin mRNA molecules under conditions employed is demonstrated by the uniform contour (Fig. 11). The only remaining variable in this method of molecular weight determination for RNAs is the residue spacing which ranges only between 2.6 to 2.7 A/residue for a variety of RNAs tested (11). Therefore, the value of 650,000 \(\pm\) 63,000 obtained by electron microscopy should be a more reliable estimate of the molecular weight of ovalbumin mRNA.

The discrepancy in RNA molecular weight determination between the methods of gel electrophoresis and sucrose gradient sedimentation is by no means unique to ovalbumin mRNA. Molecular weights of the two HeLa cell mitochondrial ribosomal RNAs were estimated to be 720,000 and 420,000 based on their electrophoretic mobilities (25, 26) and 540,000 and 350,000 by sucrose gradient sedimentation analysis (27), respectively. More recently, a similar observation has also been made in several messenger RNAs. Hemoglobin and myosin mRNAs sedimented at 5 S and 26 S in sucrose gradients, while migrated at 12 S and greater than 28 S in gels, respectively (28, 29). Gel electrophoresis at two different temperatures yielded a

| Method of determination | Conditions employed | Estimated molecular weight | Estimated chain length (nucleotide residues) |
|-------------------------|---------------------|----------------------------|--------------------------------------------|
| Agarose gel electrophoresis | 6 M \(\text{urea, pH 3.5}\) | 900,000 \(\pm\) 90,000 | 2,620 \(\pm\) 262 |
| Polyacrylamide gel electrophoresis | 98% formamide | 550,000 \(\pm\) 30,000 | 1,600 \(\pm\) 90 |
| Sucrose gradient centrifugation | 70% formamide | 510,000 \(\pm\) 20,000 | 1,476 \(\pm\) 75 |
| Poly(A) analysis | Nondenaturing | 540,000 \(\pm\) 14,400 | 1,572 \(\pm\) 42 |
| Sedimentation velocity | 3% Formaldehyde | 650,000 \(\pm\) 63,000 | 1,890 \(\pm\) 180 |

TABLE II: Molecular weight estimation of ovalbumin mRNA
molecular weight estimation of 400,000 for the immunoglobulin κ chain mRNA (30, 31), but was only estimated to be approximately 300,000 by sucrose gradient centrifugation (32, 33). A possible cause of molecular weight over-estimation for messenger RNAs by gel electrophoresis is that the electrophoretic mobility of RNA is not only a function of chain length, but also a function of nucleotide composition and secondary structure. Cytoplasmic ribosomal RNAs have higher G+C content and it is conceivable that the presence of some residual double strandedness in the molecules under the denaturing conditions employed will enhance their mobility in the gels. In fact, secondary structure is still evident in 28 S rRNA even after the molecule is denatured. In this experiment, a background of 7% was subtracted from the hybridization curve. Although ovalbumin mRNA is 1,890 nucleotides long, its coding portion consists of only 1161 nucleotides since ovalbumin is composed of 387 amino acids. With the consideration that there are, on the average, 62 polyadenylc acid residues at the 3’ terminus of the molecule, there are still more than 600 nucleotides in the molecule with unknown function. Based on the analysis employing the complementary [3H]DNA probe, it has previously been reported by us and others that ovalbumin mRNA is transcribed from the unique sequence DNA in the chick genome (22, 23). This analysis, however, was not capable of revealing the sequence complexity of the mRNA’s 5’ end because the cDNA employed at that time was not a complete copy of the mRNA and represented only approximately 400 nucleotides of the mRNA’s 3’ end. The sequence complexity of the entire ovalbumin mRNA molecule was, therefore, analyzed using 125I-mRNA to hybridize with total chick DNA at a DNA/RNA ratio of 3 × 10⁶. Under the hybridization conditions employed, unique sequence DNA will reassociate at a Cₛₚₛ value of 243 as previously reported (2, 18). There was only one major transition in the DNA/ovalbumin mRNA hybridization curve with a characteristic Cₛₚₛ value of 550 (Fig. 13). In this experiment, a background of 7% was subtracted from all the experimental points. This background value was determined by processing a reaction mixture in which the DNA was not heat-denatured. This extent of background ribonuclease resistance in the mRNA molecule is probably due to intramolecular snap-back of the mRNA molecule when reaction mixtures were cooled to hybridization temperature after heating. Thermal denaturation studies have shown that the heat-denatured ovalbumin mRNA molecule rapidly regains extensive secondary structure when quick cooled, independently of RNA concentration. The fact that a maximum of only 23% of the input 125I-mRNA has hybridized is not surprising when the following factors are taken into consideration. Since there are 2 × 10⁶ base pairs of DNA per haploid chick genome and ovalbumin mRNA is composed of 1890 nucleotides, the fraction of the DNA in the chick genome that is complementary to ovalbumin mRNA is only 1890 nucleotides/2 × 10⁶ nucleotides, or approximately 10⁻⁴. Hence, at a total DNA to RNA ratio of 3 × 10⁸, the ratio of complementary DNA to ovalbumin mRNA is only 3 × 10⁴ × 10⁻⁴, or 3, if the entire ovalbumin mRNA is transcribed from unique DNA sequences. At this complementary sequence ratio, the theoretically maximal per cent of hybridization possible should be 75% (35). The second factor to consider is that under DNA excess conditions, the rate of DNA/RNA hybridization is only 40 to 70% of the rate of DNA/DNA reassociation (36, 37). Under the conditions employed, the iodine-labeled mRNA also seems to hybridize with its DNA complement at a slower rate than the competing DNA. The Cₛₚₛ value for DNA/125I-mRNA hybridization is 550, whereas the expected Cₛₚₛ value for unique sequence DNA/DNA reassociation is only 243. Since true DNA excess condition was not established, the maximum hybridization possible in this experiment should be corrected by the factor 243/550 × 75%, or 33%. The experimentally obtained value for maximum hybridization of only 23% is not unexpected, since ribonuclease treatment under the stringent digestion conditions employed could have resulted in the loss of some partially mismatched hybrids. Nevertheless, only one major transition at a Cₛₚₛ value of greater than 100 was observed in the hybridization curve. Although short tracts of repetitive sequences (20 to 30 nucleotides) may not be detected by this technique, it is reasonable to conclude that the majority of the ovalbumin mRNA molecule is transcribed from a unique sequence in the chick genome. This interpretation is further supported by our recent ability to generate a [3H]DNA complementary to the entire ovalbumin mRNA molecule. Hybridization of this cDNA to total chick DNA has revealed no repeated sequence within this molecule.²

Acknowledgments—The authors wish to express their gratitude to Dr. N. T. Van for his help with the sedimentation velocity experiments, to Mr. Bill Weill for his excellent technical assistance and Drs. J. Wood, R. Peterson, and J. Sinkovics for the use of the electron microscopy facilities. Dr. Sinkovics’ Electron Microscopy Laboratory was the gift of Mr. and Mrs. L. Lippman.

REFERENCES
1. Rosenfeld, G. C., Comstock, J. P., Means, A. R., and O’Malley, B. W. (1972) Biochem. Biophys. Res. Commun. 47, 387-392
2. Rosen, J. M., Harris, S. E., Rosenfeld, G. C., Liarakos, C. D., and O’Malley, B. W. (1974) Cell Differentiation 3, 103-116
3. Woo, S. L. C., Harris, S. E., Rosen, J. M., Chan, L., Sperry, P., Means, A. R., and O’Malley, B. W. (1974) Prep. Biochem. 4, 555-572

² N. T. Van, J. W. Holder, S. L. C. Woo, A. R. Means, and B. W. O’Malley, manuscript in preparation.
³ J. M. Monahan, S. E. Harris, S. L. C. Woo, and B. W. O’Malley, manuscript in preparation.
4. Roberts, B. E., and Paterson, B. M. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 2330-2334
5. Means, A. R., Woo, S. L. C., Harris, S. E. and O'Malley, B. W. (1975) Cell. Mol. Biochem. 7, 33-42
6. Means, A. R., Comstock, J. P., Rosenfeld, G. C., and O'Malley, B. W. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 1146-1150
7. Weber, K., and Osborn, M. (1969) J. Biol. Chem. 244, 4406-4412
8. Rosenfeld, G. C., Comstock, J. P., Means, A. R., and O'Malley, B. W. (1972) Biochem. Biophys. Res. Commun. 48, 1685-1703
9. Weil, P. A., and Hampel, A. (1973) Biochemistry 12, 4361-4367
10. Rosen, J. M., Woo, S. L. C., Holder, J. W., Means, A. R., and O'Malley, B. W. (1975) Biochemistry 14, 69-78
11. Robberson, D., Aloni, Y., Attardi, G., and Dividson, N. (1971) J. Mol. Biol. 60, 473-484
12. Morrison, M. R., Merkel, C. G., and Lingrel, J. B. (1973) Mol. Biol. Rep. 1, 55-60
13. Loening, U. E. (1969) J. Mol. Biol. 38, 355-365
14. Gillespie, D., Marshall, S., and Gallo, R. C. (1972) Nature New Biol. 236, 227-231
15. Brownlee, G. G., and Sanger, F. (1969) Eur. J. Biochem. 11, 295-399
16. Commerford, S. L. (1971) Biochemistry 10, 1993-2000
17. Marmur, J. (1961) J. Mol. Biol. 3, 208-218
18. Rosen, J. M., Liarakos, C. D., and O'Malley, B. W. (1973) Biochemistry 12, 9803-9809
19. Randerath, E., Yu, C.-T., and Randerath, K. (1972) Anal. Biochem. 48, 172-198
20. Randerath, K. (1970) Anal. Biochem. 34, 188-206
21. Boedtker, H. (1968) J. Mol. Biol. 25, 61-70
22. Harris, S. E., Means, A. R., Mitchell, W. M., and O'Malley, B. W. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 3776-3780
23. Sullivan, D., Palacios, R., Stavnezer, J., Taylor, J. M., Faras, A., J., Kiely, M. L., Summers, N. M., Bishop, J. M., and Schmide, R. T. (1973) J. Biol. Chem. 248, 7530-7539
24. Hainco, M. E., Carey, N. H., and Palmiter, R. D. (1974) Eur. J. Biochem. 43, 549-560
25. Vesco, C., and Pennman, S. (1969) Proc. Natl. Acad. Sci. U. S. A. 62, 218-220
26. Attardi, G., Aloni, Y., Attardi, B., Ojala, D., Picamattoccia, L., Robberson, D. L., and Storrie, B. (1970) Cold Spring Harbor Symp. Quant. Biol. 35, 599-619
27. Attardi, B., Canvioto, B., and Attardi, G. (1969) J. Mol. Biol. 44, 47-70
28. Williamson, R., Morrison, M., Lanyon, G., Eason, R., and Paul, J. (1971) Biochemistry 10, 3014-3021
29. Morrie, G. E., Busch, E. A., Rourke, A. W., Tepperman, K., Thompson, W. C., and Heywood, S. M. (1972) Cold Spring Harbor Symp. Quant. Biol. 37, 555-541
30. Brownlee, G. G., Cartwright, E. M., Cowan, N. J., Jarvis, J. M., and Milstein, C. (1973) Nature New Biol. 244, 236-240
31. Stavnezer, J., Huang, R. C. C., Stavnezer, E., and Bishop, J. M. (1974) J. Mol. Biol. 88, 43-63
32. Swan, D., Avis, H., and Leder, P. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 1967-1971
33. Delovitch, T. L., and Bagioni, C. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 173-178
34. Wellauer, P. K., Dawid, I. R., Kelley, D. F., and Perry, R. P. (1974) J. Mol. Biol. 89, 397-407
35. Ross, J., Gielen, J., Packman, S., Ikawa, Y., and Leder, P. (1974) J. Mol. Biol. 87, 697-714
36. Melli, M., Whitfield, C., Rao, K. V., Richardson, M., and Bishop, J. O. (1971) Nature New Biol. 231, 8-12
37. Bishop, J. O. (1972) Biochem. J. 126, 171-155
Physical and chemical characterization of purified ovalbumin messenger RNA.
S L Woo, J M Rosen, C D Liarakos, Y C Choi, H Busch, A R Means and O'Malley
J. Biol. Chem. 1975, 250:7027-7039.

Access the most updated version of this article at http://www.jbc.org/content/250/17/7027

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/250/17/7027.full.html#ref-list-1