Purification of Ovalbumin Messenger Ribonucleic Acid by Specific Immunoabsorption of Ovalbumin-synthesizing Polysomes and Millipore Partition of Ribonucleic Acid*

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SUMMARY

Ovalbumin-synthesizing polysomes were isolated from total oviduct polysomes by reaction with anti-ovalbumin antibody and adsorption to a matrix of glutaraldehyde-linked ovalbumin. The RNA from immunoabsorbed ovalbumin polysomes was then deproteinized and selectively adsorbed to Millipore filters. Several types of experiments indicated the resulting RNA fraction is highly enriched for ovalbumin mRNA.

As an approach to understanding the molecular events involved in the regulation of specific protein synthesis in higher organisms, several laboratories have focused on the isolation of mRNA specific for a given protein. The mRNAs for hemoglobin (1-5), myosin (6, 7), the light chain of an immunoglobulin (8, 9), ovalbumin (10), lens a-crystallines (11, 12), histones (13-16), and silk fibroin (17) have been identified and in some cases purified and partially characterized. The methodology followed for the isolation of specific mRNA in most cases has been based on the fact that the protein comprises a large percentage of the total protein synthesized in the system, in addition to a special characteristic of the protein such as size or a unique amino acid composition. This type of methodology restricts the possibility of isolation of specific mRNAs to a very few systems. An immunological approach based on the specificity of an antibody prepared against the native protein and reactive against nascent polypeptide chains associated with mRNA in polysomes has been used in our laboratory to purify ovalbumin mRNA from other messengers. This approach has the possible advantage of being generally applicable to the isolation of specific mRNAs.

Several studies have shown that antibodies against complete proteins react with specific polysomes (18-29). We have demonstrated that [3H]anti-ovalbumin binds specifically to ovalbumin-synthesizing polysomes (30) and that such polysomes can be precipitated by sequential addition of ovalbumin and anti-ovalbumin (30, 31). Ovalbumin nascent chains and ovalbumin mRNA activity have been shown in the immunoprecipitate (31). When attempting to isolate large amounts of polysomes, this precipitation technique has some disadvantages: a large excess of antibody must be used, and the polysomal components must be extracted from the precipitate that contains a large amount of protein. This excess protein increases the possibility of RNA degradation. In the present study, as a first step in the purification of ovalbumin mRNA, ovalbumin-synthesizing polysomes were isolated by a method based on immunoadsorption. This technique avoids the disadvantages of immunoprecipitation.

The second phase in the purification of ovalbumin mRNA was based on a difference in physicochemical properties between mRNA and rRNA. Several workers have purified presumptive mRNA on the basis of an adenine-rich sequence attached to the message. Poly(dT) cellulose (32) and poly(U) cellulose (33, 34) have been used to isolate presumptive messengers containing an adenine-rich region from heterogeneous RNA populations. Selective adsorption to Millipore has been reported by Brawerman et al. (35) to enrich for mRNA in two different types of cells and by Phillipson et al. (33) to isolate RNA fractions containing an adenine run from virus-specific RNA. During the course of this work Means et al. (36) showed that hen oviduct rapidly labeled polysomal RNA isolated from a sucrose gradient region that contains ovalbumin mRNA activity (8 to 17 S) binds to Millipore filters. This rapidly labeled RNA contains a fraction resistant to RNase suggesting the presence of a poly(A) sequence. We have used the Millipore-binding technique of Brawerman et al. (35) to enrich the mRNA in the RNA extracted from immunoabsorbed ovalbumin polysomes.

MATERIALS AND METHODS

The sterile techniques used in the preparation of reagents and equipment which came in contact with polysomes or RNA have been described (30).

Chemicals—Ovalbumin, five times recrystallized, was from Nutritional Biochemical Corp., bovine serum albumin from Miles Labs., Inc., ribonuclease and deoxyribonuclease from Worthington, and Pronase from Calbiochem, glutaraldehyde...
Oviduct, sodium deoxycholate; BSA, bovine serum albumin; anti-temperature. The mixture was centrifuged for 10 min on a magnetic stirrer at room temperature with 2 ml of the glycine buffer. The mixture was then centrifuged 10 min at 6000 rpm and the supernatant discarded.

Isolation of Ovalbumin-synthesizing Polyosomes—Polysome preparations at a concentration of 10 A260 units per ml in polysome buffer (see above) containing 100 µg per ml of heparin were incubated with pure anti OV (1 mg of antibody per 20 A260 units of polyosomes) at 4° for 45 min. The reaction mixture was transferred to a Corex centrifuge tube containing ovalbumin matrix (400 mg/20 A260 units of polyosomes) and incubated with constant stirring at 4° for 45 min. The preparation was centrifuged in a Sorval HB-4 swinging rotor for 10 min at 6000 rpm and the supernatant saved.

Ethanol Precipitation of RNA—RNA samples were made 0.1 M in NaCl and adjusted, if necessary, to neutral pH by addition of 0.2 volume of 1 M Tris-HCl, pH 7.0. Two volumes of ethanol were added and the RNA precipitated a minimum of 6 hours at -20°. The precipitated RNA was pelleted out of the ethanol at 14,000 × g for 20 min at 0°.
Deproteinization of RNA—Two methods of deproteinizing RNA from polysomes were used alternately or in combination as indicated. In one method polysomes were made 0.05 M in EDTA, 1% in SDS, and 0.1 M in NaCl, and precipitated with 2 volumes of ethanol as described above for RNA. The precipitate was dissolved in 1 ml of 0.5% SDS-acetate EDTA buffer (0.02 M sodium acetate, 0.005 M EDTA, pH 5.0). The sample was then layered on a 1.15-Ml 5 to 20% sucrose gradient in 0.5% SDS-acetate EDTA buffer and centrifuged at 40,000 rpm for 6 hours at 20° in a Spincor SW 41 rotor. The gradient was pumped through a flow cell in a Gilford recording spectrophotometer and the material in the lower part of the gradient (including all the 18 S RNA peak) was collected and precipitated with ethanol as described above. The material in the upper part of the gradient, consisting of the tRNA, SDS-treated proteins, and heparin (added as a nuclelease inhibitor during polysome isolation) was discarded.

Alternatively polysomes were made 1% with Sarkosyl, salt and ethanol added as indicated above, precipitated at least 6 hours at -20°, and the precipitate dissolved in 1% Sarkosyl, 0.01 M EDTA, Tris-acetate, pH 6.5. Dry heat-sterilized CsCl was then added to this solution to a density of 1.723 to 1.739 g per cc (about 105 g of CsCl per 80 ml of solution). Aliquots of the sample (8.8 ml) were dispensed into Beckman pollycollen tubes, overlaid with mineral oil, and centrifuged 60 hours at 21°, 33,000 rpm, in a Beckman type 40 rotor. Under these conditions the larger RNA species pelleted while the detergent-treated proteins, any DNA present, and tRNA, which does not come to equilibrium because of its small size, remained in the CsCl solution. At the end of the centrifugation, oil and water were drawn off and the RNA pellet redissolved in 0.01 M Tris-Cl, pH 7.5, and ethanol-precipitated several times. Up to 10 mg of RNA can be loaded on one such gradient.

We find the SDS-sucrose gradient technique results in RNA with a higher specific protein-synthesizing activity, while the CsCl technique is more suited to bulk deproteinization of RNA.

Reticulocyte Lysate Assay—Ovalbumin synthesis was measured by immunological precipitation of ovalbumin from a rabbit reticulocyte lysate protein-synthesizing system. This procedure was as described by Rhoads et al. (10), except that the antibody precipitates were washed by pelleting through sucrose as described by Rhoads' and that in some reactions, where indicated, 3H-labeled leucine was replaced with [3H]isoleucine, specific activity 6 Ci per mm, at 24 μCi per ml of reaction mixture.

Polyacrylamide Gel Electrophoresis—For RNA this procedure was performed as described by Maizel (40). The “Neutral SDS-EDTA” system was used, and gels were pre-electrophoretically treated 1 hour at 5 ma per gel. Gels were scanned at 200 nm in a Gilford recording spectrophotometer with a linear transport device. For protein the procedure was as described by Palmeter et al. (38).

Millipore Adsorption Procedure—This procedure was essentially after Brawerman et al. (35). A 47-mm Millipore HAWP filter was soaked overnight in Buffer A (0.5 M KCl, 0.01 M Tris-Cl, 0.001 M MgCl₂, pH 7.6, at the temperature of RNA passage, either 0 or 20° as indicated). The filter was placed in a Millipore fritted glass collection apparatus and washed 10 times under vacuum with 10 ml of Buffer A. The RNA sample, in Buffer A, was allowed to flow through the filter by gravity. Up to 10 mg of RNA in 100 ml of buffer have been passed through one filter. After the sample had passed through, the filter was washed 10 times with 10-ml aliquots of Buffer A under vacuum. This procedure was done at 0 and 20° with no apparent differences in the RNA fractions obtained. The filter was then removed and placed in a sterile, covered Petri dish. Three milliliters of Buffer A were added, the dish agitated, briefly, and the buffer removed and discarded. This step was to remove any RNA trapped on the filter under the edges of the glass chimney. Three milliliters of low salt buffer (0.01 M EDTA, 0.5% SDS, pH 5.0) were added, the filter agitated for 15 min at 20°, and the solution removed and saved. This procedure was repeated four more times, and the combined fractions were ethanol-precipitated three times. Low pH buffer was used to diminish the possibility of RNA degradation.

Results

Isolation of Ovalbumin-synthesizing Polysomes—The method developed for isolating ovalbumin-synthesizing polysomes involves: (a) incubation of oviduct polysomes with anti-ovalbumin which binds specifically to ovalbumin-synthesizing polysomes (30); (b) reaction of the antibody-polysome complex with immobilized ovalbumin prepared by cross-linking with glutaraldehyde; (c) washing of the matrix to remove nonspecifically trapped material; and (d) elution of the bound polysomes with EDTA which liberates the ribosomal subunits and mRNA from the nascent polypeptide chains that remain attached to the γ-globulin-ovalbumin gel complex. As seen in Fig. 1, when hen oviduct polysomes were treated in this way, some A₂₆₀ absorbing material was bound to the gel and released with EDTA. That the adsorption of polysomes to the OV-matrix depends on its previous reaction with anti-OV is shown in Fig. 2. When poly-

*Robert E. Rhoads, manuscript in preparation.

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somes were not incubated with antibody, the amount of 
A260 absorbing material eluted from the ovalbumin matrix was very small (less than 2%). Concentrations higher than 0.1 mg of anti-OV used, and that appropriate control experiments must be done if these techniques are to be applied to other systems. In the rest of the experiments presented the immunoadsorbents were washed with sucrose, detergents, and salt in addition to the polysome buffer.

To prove directly the specificity of the immunoadsorption method ovalbumin mRNA was measured by its capacity to direct ovalbumin synthesis in a heterologous cell-free system. Under RNase-free conditions, the specific ovalbumin-synthesizing activity (ovalbumin synthesized per amount of RNA) of the RNA extracted from immunoadsorbed polysomes should be higher than that from total polysomes. Such an experiment is presented in Fig. 5. Hen oviduct polysomes were treated as described for the isolation of ovalbumin-synthesizing polysomes and that nonspecific trapping is random, the polysome preparation after immunoadsorption is estimated to be more than 95% pure.

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ovalbumin was measured by the technique of Palmiter et al. (38) in explants from the oviduct magnum. The relative rate of ovalbumin synthesis was determined after stimulation of young chicks with estrogen and 18 hours later polysomes were prepared from the tissue in culture and found to be 177. Therefore, the highest purification expected would now be about 6-fold. Ovalbumin polysomes was on the order of the expected increase.

In the hen oviduct, the mRNA activities of adsorbed and nonadsorbed polysomes were examined in a t.oluene-based scintillation-fluid after solubilization with NCS Open bars, A260; shaded bars, 3H counts per min. Inset, the A260 and radioactivity recovered from the fractions in A (washings), B (elution), or Total (washing and elution) are presented.

The presence of ovalbumin mRNA activity in the nonadsorbed polysomes can be explained by the fact that the concentration of anti-OV used was less than saturating. The increase in specific synthesizing activity after purification of the ovalbumin polysomes was on the order of the expected increase. Ovalbumin represents 60 to 65% of the total protein synthesized in the hen oviduct. Thus assuming the starting polysomes are 60 to 65% specific for ovalbumin, the highest purification expected would be only about 1.5- to 1.6-fold.

Additional proof of the specificity of the isolation of ovalbumin polysomes was obtained by comparison of the nascent polypeptides associated with the immunoadsorbed polysomes with those of the total polysomes (Fig. 7). Nascent chains from adult hen oviduct were labeled with a 5 min pulse of [3H]labeled amino acids in vitro. We have previously shown that with a pulse of this duration nascent polypeptides and not ribosomal proteins are labeled (31). Polysomes isolated from the tissue were treated with anti-OV and OV-matrix and eluted with 1% SDS. The pattern of nascent chains was determined by acrylamide-SDS electrophoresis. Immunoadsorbed nascent polypeptides showed a pattern in accordance with that expected for ovalbumin nascent chains, i.e. there was a sharp increase in radioactivity in the region where the ovalbumin standard appeared and then a gradual decrease. This pattern is significantly different from that seen in Fig. 7 for nascent chains from total polysomes. This result is similar to that found when direct precipitation of ovalbumin polysomes was used (31). However, immunoadsorbed ovalbumin-synthesizing polysomes were purified by the immunoadsorbent method and the specific ovalbumin-synthesizing activity of the RNA determined. Approximately 9.5% of the A260 absorbing material was adsorbed, then eluted from the matrix. This material showed a 7-fold increase in specific ovalbumin-synthesizing activity compared to the original polysomes, while the nonadsorbed polysomes exhibited a 4-fold decrease in specific ovalbumin-synthesizing activity.

The relative rate of ovalbumin synthesis was measured by the technique of Palmiter et al. (38) in explants of the tissue in culture and found to be 177%. Therefore, the highest purification expected would now be about 6-fold. Ovalbumin polysomes from a mixture of labeled liver and nonlabeled oviduct polysomes. A 2-week-old chick received an intraperitoneal injection of [3H]uridine (2 mCi). After 20 hours it was killed, the liver was removed, and a polysome preparation was made as described previously (30). The final preparation had a specific activity of 15,000 cpm per A260 of polysomes. Hen oviduct polysomes (20 A260 units) and labeled liver polysomes (4 A260 units, 6 x 10⁶ cpm) were mixed and treated for isolation of ovalbumin polysomes as described under "Materials and Methods." Fractions collected from the different steps were precipitated with ethanol and A260 measured as described in Fig. 1. To measure radioactivity, an aliquot of the sample was precipitated with 5% trichloroacetic acid, collected on a glass fiber filter, and counted in a t.oluene-based scintillation-fluid after solubilization with NCS

![Graph](https://via.placeholder.com/150)

**Fig. 4.** Immunoadsorption of ovalbumin-synthesizing polysomes from a mixture of labeled liver and nonlabeled oviduct polysomes. A 2-week-old chick received an intraperitoneal injection of [3H]uridine (2 mCi). After 20 hours it was killed, the liver was removed, and a polysome preparation was made as described previously (30). The final preparation had a specific activity of 15,000 cpm per A260 of polysomes. Hen oviduct polysomes (20 A260 units) and labeled liver polysomes (4 A260 units, 6 x 10⁶ cpm) were mixed and treated for isolation of ovalbumin polysomes as described under "Materials and Methods." Fractions collected from the different steps were precipitated with ethanol and A260 measured as described in Fig. 1. To measure radioactivity, an aliquot of the sample was precipitated with 5% trichloroacetic acid, collected on a glass fiber filter, and counted in a t.oluene-based scintillation-fluid after solubilization with NCS Open bars, A260; shaded bars, 3H counts per min. Inset, the As0 and radioactivity recovered from the fractions in A (washings), B (elution), or Total (washing and elution) are presented.

![Graph](https://via.placeholder.com/150)

**Fig. 5.** In vitro synthesis of ovalbumin by RNA extracted from immunoadsorbed hen oviduct polysomes. Hen oviduct polysomes (100 A260 units) were treated for isolation of ovalbumin polysomes by immunoadsorption as described under "Materials and Methods." Aliquots from nontreated polysomes (O-O), polysomes incubated with anti-OV only (A-A), polysomes that were not adsorbed after incubation with anti-OV and ovalbumin matrix (D---D), and polysomes eluted with EDTA from the ovalbumin matrix (●-●) were made 0.05 M in EDTA, 1% in SDS, and 0.15 M in NaCl and precipitated overnight at -20° with 2 volumes of ethanol. After centrifugation at 14,000 x g the precipitate was dissolved in 0.5% SDS-acetate EDTA buffer, centrifuged through a 5 to 20% continuous sucrose gradient, and the material in the lower part of the gradient collected as described under "Materials and Methods." The fractions were made 0.1 M in NaCl and precipitated with ethanol. The RNA was reprecipitated three times more with ethanol, dissolved in water, and assayed at different concentrations in the reticulocyte lysate system for the synthesis of ovalbumin as described under "Materials and Methods," using [3H]leucine as the labeled amino acid.
FIG. 6. In vitro synthesis of ovalbumin by RNA extracted from immunoadsorbed chick oviduct polysomes. Chicks received a secondary stimulation with estrogen for 18 hours as previously described (38). Oviduct magnum was isolated and the relative rate of ovalbumin synthesis was measured by incubating explants of the oviduct in Hanks’ medium with [3H]-amino acids and then determining the percentage of the total protein synthesized that was precipitated by anti-OV (38). Ovalbumin was 17% of the total protein synthesized. Polysomes prepared from the same oviduct (20 A260 units) were treated for immunoadsorption as described under “Materials and Methods.” The A260 of the different fractions during the treatment was measured as in Fig. 1 and is presented in the inset. Shaded fractions represent the eluted polysomes. RNA was extracted from total (O—O), nonadsorbed (Δ—Δ), and adsorbed polysomes (●—●), treated as in Fig. 5, and assayed at different concentrations in the reticulocyte lysate system for the synthesis of ovalbumin as described under “Materials and Methods.” Isoleucine was used as the labeled amino acid.

FIG. 7. SDS-acrylamide gel electrophoresis of nascent polypeptide chains from total and immunoadsorbed polysomes. Explants of hen oviduct were incubated in Hanks’ medium with [3H]-amino acids for 5 min as previously described (31). Polysomes were prepared and treated for immunoadsorption as described under “Materials and Methods” except the elution was made with 1% SDS at 20°. Aliquots from total and eluted polysomes were mixed with [3H]ovalbumin and precipitated with 5% trichloroacetic acid. The precipitates were dissolved in boiling SDS buffer (38) and applied to 10% acrylamide gels (6 × 90 mm). Electrophoresis was as described before (38). Gel slices were shaken overnight at 37° in 0.7 ml of NCS and counted in a toluene-based scintillator fluid.
TABLE I

Percentage of total ovalbumin messenger activity and of [3H]poly(A) bound and not bound to Millipore filters. Oviduct polysomes were prepared, and the RNA deproteinized by the CsCl method. Of this RNA, 214 A260 units, and 0.6 µg (0.1 µCi) of [3H]poly(A) were mixed in 200 ml of Buffer A and adsorbed, then eluted from a Millipore filter at 4°C as described under “Materials and Methods.” That fraction which did not bind was immediately repassed over another Millipore filter. That fraction which bound was ethanol-precipitated three times, dissolved in Buffer A, then repassed over a Millipore filter. Aliquots from each fraction were trichloroacetic acid-precipitated and counted as described under “Materials and Methods” to determine the amount of [3H]poly(A) present. Other aliquots were assayed at three RNA concentrations, with [3H]leucine as the labeled amino acid, to determine the total ovalbumin messenger activity present in each fraction (see “Materials and Methods”).

| Material passed over Millipore filter | [3H] cpm (poly(A)) bound | mRNA activity bound | [3H] cpm (poly(A)) not bound | mRNA activity unbound |
|--------------------------------------|--------------------------|---------------------|-----------------------------|----------------------|
| Total oviduct polysomal RNA + [3H]poly(A) | 39 | 30 | 36 | 59 |
| Repass of bound material | 72 | 96 | 0.9 | <2 |
| Repass of unbound material | 5 | <1 | 77 | 91 |

Fig. 8. Ovalbumin synthesizing activity of Millipore-treated RNA. Oviduct polysomes were isolated and the RNA deproteinized by pelleting in a CsCl gradient, then centrifuging in a 5 to 20% sucrose gradient with 0.5% SDS-acetate-EDTA buffer as described under “Materials and Methods.” The RNA was then fractionated by Millipore filters. Aliquots of the RNA were assayed at different concentrations in the reticulocyte system with [3H]leucine as the labeled amino acid. Initial polysomal RNA (●); fraction not bound by Millipore (■); fraction bound by Millipore (○).

To test whether the sedimentation profile of ovalbumin-synthesizing activity had been changed by Millipore binding of the RNA, the following experiment was performed. The RNA fraction which bound to Millipore was centrifuged in a 5 to 20% sucrose gradient, and the fractions assayed for ovalbumin messenger activity. The results shown in Fig. 10, indicate that ovalbumin messenger activity sediments as a sharp and symmetrical peak slightly more slowly than 18 S rRNA. This result agrees with that described by Rhoads et al. (10) for ovalbumin messenger activity in total oviduct polysomal RNA.

FIG. 9. Acrylamide gel electrophoresis of Millipore-adsorbed RNA. RNA was prepared by the CsCl method and 25 µg of either total oviduct polysomal RNA or Millipore-adsorbed RNA were run on polyacrylamide gels and scanned at 260 nm as described under “Materials and Methods.” The background represents the scanning of a separate gel run without RNA. A, total RNA; B, Millipore-adsorbed RNA.

FIG. 10. Concentration of ovalbumin messenger RNA, determined by the trichloroacetic acid precipitation technique, in the Millipore adsorbed and nonadsorbed RNA fractions. (A) Absorbance at 260 nm as a function of RNA concentration. (B) Amount of ovalbumin messenger RNA as a function of RNA concentration.

DISCUSSION

In order to purify a specific mRNA two different problems must be faced: (a) the purification of the specific mRNA from...
By comparing the capacity to synthesize ovalbumin in adsorbed when oviduct-synthesizing 17%, ovalbumin was used. Ovalbumin-synthesizing polysomes were specifically immuno-
synthesized in the assay of the 25-ml aliquot. For all fractions the sedimentation characteristics of the RNA (1, 2, 7, 8, 11, 12, 17) increased the adsorption of the polysomes (Fig. 2); (c) hen oviduct polysomes do not react when the immunoadsorbent system is formed by a protein (BSA) not found in the oviduct and its corresponding antibody (Fig. 3); (d) polysomes from a tissue that does not synthesize ovalbumin (liver) do not react with the anti-OV and ovalbumin system (Fig. 4); (e) the capacity of the RNA to synthesize ovalbumin in vitro is increased in immunoadsorbed polysomes. This suggests that the initial step, the binding of the y-globulin to the nascent chains of polysomes, requires that the reaction take place in solution, at least for this immunological system.

After purifying ovalbumin mRNA from other messengers, the small amount of pure protein cannot be obtained, similar results should be obtained if the polysomes are first reacted with the specific antibody and then with a matrix of anti-y-globulin prepared in a different species than the original antibody. Preliminary results indicate that this is indeed the case for ovalbumin polysomes. Another possibility is to incubate the polysomes directly with a matrix of antibody against the protein. However, this latter technique results, in our hands, in very low yields when applied to ovalbumin-synthesizing polysomes. The immunoadsorption technique, as presented here, requires a small amount of purified antibody and a large amount of protein to prepare the matrix. This is suitable only for proteins such as ovalbumin which can be purified in large amounts. When applying this technique to systems in which a large amount of pure protein cannot be obtained, similar results should be obtained if the polysomes are first reacted with the specific antibody and then with a matrix of anti-y-globulin prepared in a different species than the original antibody. Preliminary results indicate that this is indeed the case for ovalbumin polysomes.

Other methods for isolating specific mRNA have been based on the sedimentation characteristics of the RNA (1, 2, 7, 8, 11, 12, 17). Immunological techniques have the potential of being generally applicable and are not restricted to systems in which a protein comprises a very large percentage of the total protein being synthesized. As illustrated by the experiment in Fig. 6, ovalbumin-synthesizing polysomes were specifically immunoadsorbed when oviduct-synthesizing 17% ovalbumin was used. By comparing the capacity to synthesize ovalbumin in vitro of the RNA extracted from immunoadsorbed and total polysomes it can be concluded that a high degree of purification was achieved. Moreover, ovalbumin polysomes have been specifically precipitated and intact mRNA has been recovered from an in vitro protein synthesizing system, where ovalbumin is only about 3% of the total protein being synthesized.

The purification of ovalbumin mRNA from other messengers and (b) the purification of the mRNA from other types of RNA, namely rRNA. Different techniques must be applied to each of these problems.

The purification of ovalbumin mRNA from other messengers has been based on the specific immunoadsorption of ovalbumin-synthesizing polysomes. The specificity of the immunoadsorption technique is evidenced by the following: (a) polysomes that have not been reacted with anti-OV do not bind to the ovalbumin matrix; (b) increasing the amount of the antibody increases the adsorption of the polysomes (Fig. 2); (c) hen oviduct polysomes do not react when the immunoadsorbent system is formed by a protein (BSA) not found in the oviduct and its corresponding antibody (Fig. 3); (d) polysomes from a tissue that does not synthesize ovalbumin (liver) do not react with the anti-OV and ovalbumin system (Fig. 4); (e) the capacity of the RNA to synthesize ovalbumin in vitro is increased in immunoadsorbed polysomes (Figs. 5 and 6); (f) nascent polypeptide chains isolated from immunoadsorbed polysomes show a pattern that is in accordance with that expected for ovalbumin nascent chains (Fig. 7).

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The immunoadsorption technique, as presented here, requires a small amount of purified antibody and a large amount of protein to prepare the matrix. This is suitable only for proteins such as ovalbumin which can be purified in large amounts. When applying this technique to systems in which a large amount of pure protein cannot be obtained, similar results should be obtained if the polysomes are first reacted with the specific antibody and then with a matrix of anti-y-globulin prepared in a different species than the original antibody. Preliminary results indicate that this is indeed the case for ovalbumin polysomes. Another possibility is to incubate the polysomes directly with a matrix of antibody against the protein. However, this latter technique results, in our hands, in very low yields when applied to ovalbumin-synthesizing polysomes. This suggests that the initial step, the binding of the y-globulin to the nascent chains of polysomes, requires that the reaction take place in solution, at least for this immunological system.

After purifying ovalbumin mRNA from other messengers, the main contaminants in the RNA preparation should be the rRNA species. To enrich for mRNA, the preparation was partitioned by the Millipore filter technique. This step resulted in an increase of 20-fold in the capacity of the RNA to synthesize oval-
bumin in vitro (Fig. 8). The electrophoresis pattern of RNA indicated that the relative concentration of rRNA had decreased (Fig. 9). By rough calculation mRNA should comprise approximately 1% by mass of polysomal RNA. Therefore, an increase of 26-fold in specific protein-synthesizing activity suggests that the fraction after Millipore treatment contains about 25% mRNA.

We have also been able to isolate an RNA fraction with increased specific ovalbumin-synthesizing activity by the phenol partition method of Smith et al. (43). We prefer the Millipore method because of the difficulty in quantitative recovery of messenger activity from the phenol phase, and because exposure to phenol induces an aggregation of ovalbumin mRNA molecules which becomes apparent upon rate zonal centrifugation in aqueous solution. Such aggregation after exposure to phenol has been reported for other RNA species (44).

Our results indicate (Table I) that some ovalbumin mRNA binds to Millipore filters and some does not. We feel one explanation for this behavior could be a difference in the length, or in the presence, of the adenine-rich sequence presumed to be on ovalbumin mRNA. Whether this heterogeneity exists normally in the cell or is an artifact of RNA isolation remains to be seen.

We have evidence that our polysome preparations contain little or no nonpolysomal RNA (30, 31). Both deproteinizing procedures used in our laboratory remove the 4 S and 5 S RNAs, so the major RNA species remaining after immunoadsorption of polysomes and Millipore treatment of RNA should be ovalbumin mRNA, 18 S rRNA, and 28 S rRNA. Since ovalbumin mRNA activity sediments in a sucrose gradient near the 18 S region before (10) or after Millipore treatment (Fig. 10), rate zonal sedimentation of the Millipore-treated RNA should separate ovalbumin messenger from the 28 S rRNA contaminant. This added step should result in an RNA preparation purer for ovalbumin messenger. Our immediate interest in immunologically purified mRNA is to synthesize complementary DNA sequences with Rous Sarcoma Virus DNA polymerase (45). Under our conditions for DNA synthesis, rRNA is not significantly transcribed. Therefore, the amount of RNA present in our purified messenger preparations should not interfere with the synthesis of a homogenous hybridization probe complementary only to ovalbumin mRNA.

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