Mouse Albumin mRNA in Liver and a Hepatoma Cell Line

PREPARATION OF COMPLEMENTARY DNA FROM PURIFIED mRNA AND QUANTITATION BY NUCLEIC ACID HYBRIDIZATION*

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Albumin, a major serum protein synthesized and secreted by the liver, is one of several serum proteins whose synthesis is regulated by hormonal and nutritional factors as well as during liver development. As part of our studies on the regulation of serum protein synthesis, we have isolated mouse albumin mRNA by direct immunoprecipitation of albumin-synthesizing polysomes and oligo(dT)-cellulose chromatography of albumin polysomal RNA. This albumin mRNA sedimented at about 17 S, which corresponds to a molecular weight of approximately 6.5 \times 10^5 or 2,000 nucleotides. Translation in vitro yielded a product which is immunoprecipitable with anti-mouse albumin and which showed a single radioactive peak having a molecular weight of 68,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. DNA, complementary to albumin mRNA, was synthesized with avian myeloblastosis virus RNA-dependent DNA polymerase. This complementary DNA was shown by alkaline sucrose density gradient sedimentation to have a molecular weight of 5.3 \times 10^5 which is equivalent to 1,740 nucleotides and represents approximately 87% of the total 17 S mRNA. Hybridization of the cDNA to its template mRNA gave a $R_t$ value of 2.3 \times 10^{-2} mol nucleotides \cdot s \cdot liter^{-1} (in 0.5 M NaCl). The resultant cDNA-mRNA hybrid displayed a melting temperature of 89°C when analyzed by thermal elution from a hydroxylapatite column, indicating a high degree of fidelity of the base pairings formed in this hybrid. Data from the hybridization analyses and cell-free translation studies indicate that the albumin mRNA is about 80 to 85% pure. Quantitation of albumin mRNA in total cytoplasmic RNA, by hybridization of cDNA under conditions of RNA excess, revealed that mouse liver contains about 10-fold more albumin mRNA sequences than Hepa-2 cells, a permanent mouse hepatoma cell line that has maintained the capacity to synthesize albumin.

A major function of the liver is to synthesize and secrete many of the proteins found in serum (Madden and Zeldis, 1968). These liver-specific serum proteins are of particular interest because many, if not all, are regulated in normal tissue by developmental (Gitlin and Gitlin, 1975; Koga and Tamaoki, 1974), hormonal (Belanger et al., 1975; Keller and Taylor, 1976; Roy, 1973; Rothschild et al., 1972a,b; Miller and John, 1970), and nutritional factors (Rothschild et al., 1972a,b; Miller and John, 1970; Munro, 1970). In addition, transcription and secretion of liver serum proteins are profoundly influenced by disease and cancer (Rothschild et al., 1972a,b; Peterman, 1960; Rotermund et al., 1970).

Another factor which makes these proteins attractive for study is their relative abundance. For example, albumin synthesis comprises about 10% of total liver protein synthesis (Peters and Peters, 1972), thus albumin mRNA is relatively abundant and comparatively easy to isolate and use to analyze the factors regulating the expression of the albumin gene. Additionally, large quantities of serum albumin are easily purified. Extensive data on physicochemical properties and complete amino acid sequences (Brown, J. R., 1976) are available for human and bovine serum albumin.

Finally, many hepatoma-derived tissue culture systems exist which continue to synthesize and secrete albumin as well as other liver-specific proteins (Richardson et al., 1968; Tashjian, Jr., et al., 1970; Ohanian et al., 1969; Szpirer and Szpirer, 1973; Peterson and Weiss, 1972; Bernhard et al., 1973).

The mouse hepatoma cell line Hepa, derived from RW7756, is one of these lines (Bernhard et al., 1973). It is well characterized and has been shown to maintain the synthesis of albumin and \( \alpha \)-fetoprotein (Bernhard et al., 1971, 1973; Papaconstantinou and Ledford, 1973), transferrin (Papaconstantinou et al., 1978), and ceruloplasmin (Darlington, et al., 1975) after many years in continuous culture.

In this communication, we report the purification and characterization of mouse albumin messenger RNA, the synthesis of cDNA, and the use of this probe to compare directly albumin mRNA levels in liver and in Hepa cells in tissue culture. This comparison is of interest to characterize the Hepa cells with respect to albumin mRNA and to extend previous studies involving the quantitation of albumin-synthesizing polysomes in liver and Hepa cells (Brown and Papaconstantinou, 1977), as well as to study the effects of hormonal and nutritional factors on albumin mRNA levels (Brown and Papaconstantinou, 1978).

EXPERIMENTAL PROCEDURES

The following procedures were used to prepare ribonucleic acid-free materials and solutions. All glassware was given a final rinse in 0.1% diethylpyrocarbonate and heated to 100°C for 5 h. Nonsterile nuclease-free materials were heated for at least 15 min in 0.1% diethylpyrocarbonate. Solutions were autoclaved prior to use.

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Extraction coefficients used were: (a) 20 A495/mg of RNA/ml, (b) mouse albumin: 0.69 A495/mg/ml, (c) rabbit and goat immunoglobulin: 1.4 A495/mg/ml.

Immunoprecipitation of Albumin-synthesizing Polysomes—The preparation of polysomes, antigen (albumin), and antialbumin IgG was done as previously described (Brown and Papaconstantinou, 1977). Dialyzed mouse liver polysomes (10 A260 units/ml) were incubated for 10 min with monospecific, affinity-purified rabbit anti-mouse albumin IgG in Buffer A (50 mm Tris (pH 7.3) at 21°C, 25 mm NaCl, 5 mm MgCl2, 200 μg/ml of sodium heparin (Sigma Chemical Co., St. Louis, Mo.), 0.4% Triton X-100, and sodium deoxycholate. Mouse serum albumin was added, stirred gently, and allowed to incubate an additional 90 min at 4°C. The resulting immunoprecipitate was collected by centrifugation at 2900 rpm, 10 min in the International centrifuge (JP-3), gently resuspended in one-fourth the original volume of Buffer A with 0.2% Triton X-100, 0.1 mM sucrose, and sodium heparin (500 μg/ml), and centrifuged as before. Analysed, small-scale immunoprecipitations were performed, in duplicate, in a volume of 0.5 ml, with one sample containing five A260 units of polysomes in addition to antibody and carrier albumin, while the companion sample contained only antibody and albumin. Immunoprecipitates were dissolved in 1 ml of 0.1 M NaOH and the absorbance of 260 nm was determined. Subtraction of the A495 reading of the immunoprecipitate without polysomes from the A495 reading of the immunoprecipitate with polysomes yields a net A495 reading of the polysomes immunoprecipitated by this technique. All precipitations were performed under conditions of antibody excess as determined independently from quantitative precipitin curves. Various aspects of the immunoprecipitation were done under conditions optimized by analytical immunoprecipitation. These conditions are 360 μg of antibody and 14 μg of albumin/ml of reaction mixture. Final immunoprecipitates were resuspended and phenol-extracted.

RNA Extraction, Oligo(dT)-Cellulose Chromatography, and Centrifugation—The immunoprecipitated polysomes were solubilized in SDS buffer (10 mm Tris (pH 7.4), 1 mm EDTA, 100 mm NaCl, 0.5% SDS) to give a solution equivalent to 15 to 20 A260 units of polysomes/ml and extracted in phenol-chloroform by the procedure of Perry et al. (1972). RNA was precipitated in ethanol and collected by centrifugation at 14,000 × g for 10 min. The precipitate was dried under a stream of N2 and dissolved in water. Poly(A)-containing RNA was prepared by two cycles of chromatography on oligo(dT)-cellulose type T3 (Collaborative Research, Waltham, Mass.) as described by Randle et al. (1976). Treatment of the first poly(A)-containing RNA fraction from the oligo(dT) column with MeSO and heat denaturation, prior to the second chromatography cycle, was essential for isolation of poly(A)-containing RNA from the immunoprecipitate with polysomes yields a net A495 reading of the immunoprecipitate without polysomes from the Ara, reading of the companion sample contained only antibody and albumin. Immuno-

Electrophoresis of Ribosomal RNA—The supernatant was collected by centrifugation at 2900 rpm, 10 min in the SW 50.1 rotor. The precipitated RNA was recovered by centrifugation, dried, dissolved in water, and frozen in 0.2 ml aliquots. The precipitation was collected by centrifugation (37°C for 1 h and at 4°C overnight, and the precipitate was centrifuged at 2500 rpm for 10 min) and washed twice in phosphate-buffered saline + 10 μg/ml leucine (once with Triton X-100 and sodium deoxycholate, and once without the detergents). The final immunoprecipitate was dissolved and processed for SDS-polyacrylamide gel electrophoresis and counting as described above for the CCI COOH-precipitated sample. Electrophoresis was performed in cylindrical gels (6 × 80 mm) of 10.5% polyacrylamide containing 0.1% SDS at 3 mA/gel for 90 min. After electrophoresis, gels were sliced, dissolved in H2O, and counted.

Cell-free Translation of RNA—An S-30 wheat germ lysate (100 to 115 A260/mg) was prepared as described by Marcu and Dudock (1974) and stored in 0.2 ml aliquots in liquid nitrogen. Wheat germ was purchased from General Mills (Vallejo, Calif.). Translation assays (50 to 200 μl) were made up as follows: 24 μl Henpes (pH 7.6), 100 mM KCl, 2.5 mM Mg(OAc)2, 1 mM diethioptol, 6 mM creatine phosphate, 200 μg/ml of creatine phosphokinase, 0.88 mM ATP (Sigma), neutralized, 0.018 mM GTP (Sigma). 20 μg 19 amino acids (minus leucine), 8 μg [3H]leucine (Amersham-Searle, Arlington Heights, Ill.; 324 mCi/mmol), 200 μl/ml of wheat germ S-30, 30 μg/ml of spermine (Sigma), 0.4% Triton X-100, 0.25% poly(A)-containing RNA. The reaction mixture was incubated for 90 min at 25°C, centrifuged at 40,000 rpm for 1 h in the type 65 rotor at 2°C, and the ribosomal pellets were either discarded or used as indicated. To analyze for incorporation of [3H]leucine into total protein, aliquots (25 to 75 μl) of the supernatant were diluted to 0.5 ml in phosphate-buffered saline (0.15 mM NaCl, 0.01 mM sodium phosphate buffer, pH 7.4) containing RNA was precipitated with an equal volume of 10% CCl4OOH, also containing 10 μg/ml heparin. After 30 min at 0°C, 1 ml of 5% CCl4COOH was added to the mixture and incubated in ice for an additional hour. The precipitate was collected by centrifugation (14,000 rpm for 10 min in Sorvall HB-4 rotor), resuspended in 5% CCl4COOH (10 ml mecumle), and pelleted. The final pellet was washed with ether, dried, and resuspended in 0.1% SDS, 0.1% sodium deoxycholate, sodium succinate buffer containing 10 μg/ml heparin. The mixture was collected by centrifugation (2000 rpm for 10 min) and washed twice in phosphate-buffered saline + 10 μg/ml leucine (once with Triton X-100 and sodium deoxycholate, and once without the detergents). The final immunoprecipitate was dissolved and processed for SDS-polyacrylamide gel electrophoresis and counting as described above for the CCI COOH-precipitated sample. Electrophoresis was performed in cylindrical gels (6 × 80 mm) of 10.5% polyacrylamide containing 0.1% SDS at 3 mA/gel for 90 min. After electrophoresis, gels were sliced, dissolved in H2O, and counted.
and counted. Molecular weight markers, run on separate gels, were stained and scanned: mouse serum albumin (molecular weight 68,000), ovalbumin (molecular weight 43,000), chymotrypsinogen (molecular weight 23,000), and bromophenol blue.

Synthesis of cDNA—Conditions for the synthesis of cDNA were as described by Kacian and Myers (1976). Avian myeloblastosis virus reverse transcriptase was a generous gift of Dr. R. W. Tennant (Oak Ridge National Laboratory). Following incubation (1 h, 37°C), aliquots (20 µl) of the reaction mixture (total volume 100 µl) were adjusted to 12.5 mM EDTA and 0.5 n NaOH in a final volume of 200 µl at room temperature. Samples were layered over 5 to 20% sucrose gradients (0.2 n NaOH, 0.5 n NaCl, 0.01 n EDTA) and centrifuged at 50,000 rpm for 4 h in SW 56 rotor at 20°C. Fractions were collected, and aliquots of each fraction were precipitated with CCl₃COOH in the presence of 5 µg of tRNA. The precipitates were collected on membrane filters and counted by liquid scintillation spectrophotometry.

Data from these gradients were processed by the "SLG" computer program (Buhl et al., 1972) to obtain sedimentation profiles and the average molecular weight of pooled fractions. These samples were neutralized and desalted by passage through a Sephadex G-50 (medium) column (30 x 1.4 cm) equilibrated with water. Aliquots of the breakthrough material were lyophilized and stored at -70°C. No significant degradation of cDNA was detectable when stored in this manner for up to 4 months.

cDNA-mRNA Hybridization—Hybridizations were carried out under conditions of RNA excess using the procedure of Dr. George Keller. The reaction was run in 6-ml polypropylene tubes (Falcon Plastics, Division of Becton Dickinson & Co., Oxnard, Calif.) using 10-µl aliquots of the reaction mixture which contained 0.025 M HEPES (pH 7.4), 0.5 M NaCl, 0.002 M EDTA, 0.1% SDS, CCl₃COOH (600 to 1000 cpm), and RNA as indicated. Samples were overlaid with mineral oil, incubated at 68°C for the appropriate times, removed, and frozen immediately in a dry ice-acetone slurry. Assayment of the extent of cDNA-RNA hybridization was done by digestion of all single strand species with S, nuclease (Miles, Elkhart, Ind.). The thawing sample was incubated at 37°C for 1 h, and the reaction was stopped by the addition of 2 ml of 12% CCl₃COOH followed by 100 µg of mouse DNA as carrier. The precipitates were collected after 1 h at 0°C on premoistened Whatman GF/C filters, washed five times with 5% CCl₃COOH, twice with ethanol (1:1), and dried. The precipitates were solubilized in scintillation vials in 1 ml of 0.1 n NaOH for 1 h at 50°C, neutralized with an equivalent of HCl, and counted in 10 ml of Aquasol (New England Nuclear, Boston, Mass.) by liquid scintillation spectrophotometry. Hybridization data are expressed as the percentage of total cDNA counts in each sample resistant to S, nuclease digestion. Rₒ = the initial concentration of RNA in mole of nucleotides per liter; t = time in seconds. A value of 321 g of RNA nucleotides per mol is used to calculate Rₒ. All counts were corrected for background radioactivity and intrinsic S, resistance of the cDNA probe (2 to 4%).

Melting Temperature of Albumin mRNA-cDNA Hybrids—To determine the Tₘₒ of albumin mRNA-cDNA hybrids, hybridization was performed in siliconized, 10-µl glass capillaries as described (Housman et al., 1974). The hybrid formed at Rₒ = 6 was expelled into 1.0 ml of 0.1 M phosphate buffer (pH 6.8), and adsorbed to hydroxylapatite (column dimension 1 x 2 cm) at 60°C. Thermal elution was performed as described by Niyogi and Thomas (1968) resulting in column fractions (1 ml each) representing the eluate from each 1°C increase in temperature. These samples were precipitated with CCl₃COOH, collected on filters, and counted.

RESULTS

Immunoprecipitation of Albumin-synthesizing Poly- some—Analytical small scale immunoprecipitations were performed to determine optimal conditions for the immunoprecipitation of albumin-synthesizing polysomes, using a modification of the method described by Schechter (1974). In Fig. 1 we summarize the results of this immunoprecipitation. For each concentration of antibody tested, carrier albumin was added in increasing amounts, always maintaining a condition of antibody excess. It may be seen that the highest concentration of antibody tested (310 µg/ml) precipitated no more polysomes than the intermediate concentration of antibody (360 µg/ml) or about 6 to 7% of the total polysome sample. The lowest concentration of antibody (180 µg/ml) was effective in precipitating no more than 2.5% of the polysomes. For the large scale immunoprecipitation of albumin polysomes, the intermediate concentration of antibody (360 µg of antibody/ml) was chosen with a corresponding albumin concentration of 14 µg/ml.

Size of Purified Albumin mRNA—Poly(A)-containing RNA from immunoprecipitated polysomes and total liver polysomes was purified by two cycles of oligo(dT)-cellulose chromatography and sedimented by SDS-sucrose density gradient centrifugation (Fig. 2A). The poly(A) RNA from immunoprecipitated polysomes, which sedimented as a single peak, had a sedimentation coefficient slightly smaller than the 18 S marker, i.e. approximately 17 S. This corresponds to a molecular weight of approximately 6.5 x 10⁵, or 2000 bases (Loening, 1968).

Total liver poly(A)-containing RNA was also prepared by two successive cycles of oligo(dT) cellulose chromatography. This RNA (Fig. 2B) sedimented as a broad peak with an average S value of 14 to 15 S. In both cases no 18 S or 28 S contaminant was observed after the second oligo(dT) step.

Translation of Albumin mRNA in a Cell-free System—Characterization of the products of the wheat germ cell free translation system programmed with total poly(A) RNA from mouse liver reveals that the synthesis of albumin is sensitive to KC1 concentration (Benveniste et al., 1976). Optimal synthesis of immunoprecipitable albumin occurs at 100 mM KC1, whereas total amino acid incorporation and incorporation into protein released from the wheat germ ribosomes were optimal at the lowest concentration tested (Fig. 3A). The data in Fig. 3B show that higher concentrations of KC1 favor both completion of albumin as well as release of radioactive protein from the ribosomes. However, since the translational efficiency at KC1 concentrations greater than 110 mM is low, all further translation assays were carried out at 100 mM KC1.

**Fig. 1 (left).** Immunoprecipitation of albumin-synthesizing polysomes with varying antibody concentrations. Conditions of immunoprecipitation were as described under "Experimental Procedures." Final antibody concentrations used were: 180 µg/ml (●—●); 360 µg/ml (■--■); 510 µg/ml (△--△). The inset shows the precipitin curve which characterizes the antibody used throughout this study. The final concentration of antibody was 180 µg/ml, and the A₅₀ value (ordinate) represents the absorbance at 280 nm of the washed immunoprecipitates dissolved in 1.0 ml of 0.1 n NaOH. MSA, mouse serum albumin.

**Fig. 2 (right).** Sedimentation of purified albumin mRNA and liver total poly(A)-containing RNA on SDS-sucrose density gradients. Direction of sedimentation is left to right, and arrows indicate sedimentation of 18 S and 28 S RNA markers run in separate gradients. A, poly(A)-containing RNA from immunoprecipitated polysomes; B, poly(A)-containing RNA from mouse liver.
Translation of RNA from the immunoprecipitated albumin polysomes is shown in Table I. Albumin synthesis in the wheat germ system increases from 4.5% of the total incorporation when programmed with total liver poly(A)-containing RNA to 49.5% when programmed with RNA from immunoprecipitated polysomes indicating an approximately 11-fold enrichment of albumin mRNA. This agrees with the results on the enrichment of rat albumin mRNA prepared by immunoprecipitation (Taylor and Tse, 1976).

Immunoprecipitated albumin synthesized in the wheat germ system programmed with total liver poly(A)-containing RNA was analyzed by electrophoresis in SDS-polyacrylamide gels. As may be seen in Fig. 4, albumin synthesized in vitro co-migrates with authentic serum albumin and is represented as a single peak of radioactivity. An identical pattern was observed when purified albumin mRNA was used as template (not shown). We could not detect preproalbumin as described by Strauss et al. (1977) probably because of the relatively low resolution afforded by external protein standards and because of the large (2 mm) slices taken for analysis.

Analysis of Albumin mRNA by Sucrose Density Gradient Sedimentation and Translation of Selected Fractions—Albumin mRNA extracted from immunoprecipitated polysomes was analyzed by SDS-sucrose density gradient sedimentation in order to determine whether any contaminating mRNA could be detected on the basis of size differences. Since no preliminary sizing of albumin mRNA was employed in purification and since the average size of liver poly(A)-containing RNA is considerably smaller than that of albumin mRNA (Fig. 2), any contaminating mRNA species in our preparation of albumin mRNA should be smaller than 17 S, the observed size of albumin mRNA (Fig. 2). Fractions from an SDS-sucrose gradient were collected and assayed in the wheat germ system for their translational capacity, i.e. both total protein synthesis (CCl₄COOH precipitation) and immunoprecipitation with antialbumin. The patterns in Fig. 5 show that both translational activities follow symmetrically the A₂₅₄ peak which represents the 17 to 18 S albumin mRNA. Since the profile for total amino acid incorporation follows the A₂₅₄ pattern and the profile of immunoprecipitable counts, we conclude that the albumin mRNA used in these studies is homogeneous in terms of size (17 to 18 S) and in terms of function (translatability). We conclude that significant copurification of heterologous mRNA species the same size as albumin mRNA, while not ruled out, seems highly unlikely.

Synthesis and Characterization of cDNA-cDNA to albumin mRNA was synthesized using the procedure of Kacian and Myers (1976) in which high concentrations of pyrophosphate are used to promote the synthesis of high molecular weight cDNA. Analysis of the product by alkaline sucrose gradients revealed a molecular weight of cDNA in excess of 5.7 x 10⁶ (data not shown). The number average molecular weight of pooled gradient fractions was about 5.3 x 10⁶; equivalent to 1740 bases or representative of about 87% of the length of template albumin mRNA.

Hybridization of the albumin cDNA to template mRNA (Fig. 6) indicates that a rapid pseudo-first order reaction occurs within two logs. The reaction plateaus at 81 to 82% hybridization and has a Rdₜ/₂ of 2.3 x 10⁻³ mol s⁻¹ liter⁻¹ in 0.5 M NaCl. These kinetic data are in good agreement with comparable analyses done with molecules of similar size and complexity (Shapiro and Schimke, 1975; Harris et al., 1975; Innis and Miller, 1977) when Rdₜ/₂ values are corrected to standard salt concentrations (Britten and Smith, 1968). Minor contaminants in albumin cDNA common to liver RNA but not found in Hepa-2 RNA probably account for the gradual transition to elevated plateau values in the hybridization driven by liver RNA. These contaminants could represent as much as 20% of albumin cDNA (and template albumin mRNA) and the Rdₜ/₂ of pure albumin mRNA to cDNA might be reduced accordingly from the apparent Rdₜ/₂ of 2.3 x 10⁻³ to 1.8 x 10⁻³ mol s⁻¹ liter⁻¹.

A comparison of total cytoplasmic RNA from mouse liver (Rdₜ/₂ = 1.3 x 10⁶) with the accompanying Rdₜ curve reveals that the albumin mRNA is enriched approximately 555-fold with respect to hybridizable albumin mRNA sequences. This is the approximate enrichment that one would predict based on albumin mRNA comprising 1.8% of albumin polysomal total RNA and albumin polysomes making up 10% of total liver polysomes.

Thermal denaturation analysis of albumin mRNA-cDNA hybrids was done to determine the stability of the duplexes formed. To do this, the hybrid formed at Rdₜ = 6.0 was adsorbed to hydroxylapatite and eluted at intervals of increas-

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**Table I**

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<thead>
<tr>
<th>RNA sample</th>
<th>Total Release</th>
<th>Mouse serum albumin</th>
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<tbody>
<tr>
<td></td>
<td>cpms/µg</td>
<td>% of total</td>
</tr>
<tr>
<td>Albumin mRNA</td>
<td>1,767</td>
<td>858</td>
</tr>
<tr>
<td>Total liver poly(A)-containing RNA</td>
<td>1,136</td>
<td>53</td>
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</table>
Fig. 4 (left). SDS-polyacrylamide gel electrophoresis of wheat germ translation of albumin mRNA. A, albumin immunoprecipitated from 100 µl of wheat germ reaction products programmed with total liver poly(A)-containing RNA; OV, ovalbumin; C, chymotrypsinogen; BPB, bromphenol blue.

Fig. 5 (right). Cell-free translation of albumin mRNA fractionated by SDS-sucrose density gradient centrifugation. Albumin mRNA was sedimented through 15 to 30% SDS containing sucrose gradients. Fractions were taken from the gradient and translated in the wheat germ system after which both total CCl₄COOH precipitable radioactivity (●—●) and albumin, immunoprecipitated, radioactivity (○—○) were determined.

Fig. 6. Hybridization of albumin cDNA to various RNA samples. Albumin cRNA (600 to 1000 cpm) was hybridized to various RNA samples and assayed for double strandedness by S, nuclease digestion. Albumin mRNA (4.4 µg/ml) with carrier E. coli tRNA (1 mg/ml, ●—●); mouse liver cytoplasmic RNA (1 mg/ml, ○—○); Hepa-2 cytoplasmic RNA (1 mg/ml, Δ—Δ).

Fig. 7. Thermal denaturation of albumin mRNA-cDNA hybrids on hydroxylapatite. Thermal chromatography of hybrid duplex was performed as described under “Experimental Procedures.”

Quantitation of Albumin mRNA in Mouse Liver and Hepa-2 Cells—One of the major purposes in isolating mouse albumin mRNA (and cDNA) was to study the regulation of albumin gene expression in cells in culture. Experiments were done, therefore, to determine the level of mouse serum albumin mRNA in young adult mouse liver and in Hepa-2 cells. Total cytoplasmic RNA from mouse liver and log phase Hepa-2 was prepared and hybridized to albumin cDNA under conditions of RNA excess.

Analysis of the Rₜ curves in Fig. 6 reveals that the reassociation kinetic data are approximately 6-fold more rapid in the reaction driven by liver cytoplasmic RNA (Rₐ/₂ = 1.3 × 10⁸ mol-s-liter⁻¹) as compared to the reaction driven by Hepa-2 cytoplasmic RNA (Rₐ/₂ = 8 × 10⁸ mol-s-liter⁻¹). Since the reaction rate is, under these conditions, inversely proportional to the relative concentration of albumin mRNA in each sample, liver cytoplasmic RNA contains 6-fold more albumin mRNA than an equivalent amount of Hepa-2 cytoplasmic RNA. Furthermore, mouse liver cells contain 1.5- to 2-fold more total cytoplasmic RNA than the log phase Hepa-2 cells (based on 1.7 × 10⁹ cells/g of liver, Bernhard et al., 1973). This raises the cellular concentration of liver albumin mRNA 10- to 12-fold above that in log phase Hepa-2 cells.

Size of Albumin mRNA Sequences from Liver under Denaturing Conditions—For an independent confirmation of the size of albumin mRNA, total liver RNA was isolated and centrifuged through 98% formamide sucrose density gradients. Pooled fractions from 18 gradients were hybridized in excess to albumin cDNA in order to determine the relative albumin mRNA content of each pooled fraction. These data are represented in Fig. 8. Hybridization data are expressed as the reciprocal of time in seconds required to achieve 50% hybridization. This reciprocal value is proportional to albumin mRNA concentration in each set of hybridizations and, therefore, is relative to albumin mRNA content in each fraction.

As may be seen in Fig. 8, the bulk of albumin mRNA sequences sediment at approximately 17 S under totally denaturing conditions, and this value is in agreement with the size of purified albumin mRNA previously determined under non-denaturing conditions.
of albumin mRNA we describe exceeds 80%. The hybridization of albumin cDNA to albumin mRNA occurs rapidly, within two logs, suggestive of a single component, pseudo-first order reaction. The hybridization plateaus over the $R_t$ values examined at 80 to 82% and implies a single species of nucleotide sequences greater than 80% in abundance. The possibility that there are two or more species of equal abundance in this mRNA preparation is highly unlikely because of the qualitatively identical kinetics seen in the hybridizations driven by purified albumin mRNA, total liver cytoplasmic RNA, and total Hepa-2 cytoplasmic RNA, all of which plateau at about 80% hybridization. It seems most improbable that these three RNA preparations could share major and identical abundances of nonalbumin mRNA sequences.

**Size of Albumin mRNA**—We have shown, using three independent methods of analysis, that albumin mRNA sediments in sucrose gradients at about 17 S (Figs. 2, 5, and 8). Other studies serve to confirm this finding (Taylor and Tse, 1976; Strair et al., 1977). Relative to 16 S and 18 S rRNA, 17 S RNA should have a molecular weight of approximately $6.5 \times 10^6$ or about 2000 bases (Loening, 1968). Mouse serum albumin, molecular weight 68,000, is composed of about 580 amino acids which would require a minimum of 1740 bases for translation. If preproalbumin is the primary translation product in mouse as in rat (Strair et al., 1977), then 54 more bases are needed, raising the total to 1794 bases. The remaining 200 or so bases could easily be accounted for by 3'-poly(A) stretches and nontranslated nucleotides at the 5'-end (Darnell, 1976; Brawerman, 1976). In summary, mouse albumin mRNA appears to be very close in size to the minimal size required for translational and structural considerations common to characterized messenger RNAs. Extensive noncoding regions are not apparent in contrast to other messenger RNA studied (Shapiro and Schimke, 1975; Innis and Miller, 1977; Lewin, 1975; Proudfoot et al., 1976). Recently, it has been reported that a 26 S form of rat albumin mRNA may exist in the nucleus, and it is presumably a precursor to the mature 17 S cytoplasmic form (Strair et al., 1978).

**Albunin mRNA in Normal Liver and Hepatoma Cells**—Relative albumin mRNA levels were determined, and it was shown that, per cell, liver has about 10-fold more albumin mRNA sequences than Hepa-2 cells. We have analyzed albumin synthesis in Hepa-2 cells and have determined that albumin synthesis makes up about 1 to 1.5% of total protein synthesis in log phase cultures. Thus, albumin synthesis, relative to total protein synthesis, is about 10-fold higher in liver than in Hepa-2 cells, and these differences are reflected in albumin mRNA levels in liver and Hepa-2 cells.

Previous studies in our laboratory showed that liver polysomes bound approximately 3.5-fold more iodinated albumin antibody than comparable amounts of Hepa-2 polysomes (Brown and Papaconstantinou, 1977). Accounting for yields of polysomes from each cell type, liver cells contain 5- to 10-fold more albumin-synthesizing polysomes than Hepa-2 cells. The difference between the two cell types in albumin mRNA levels (10-fold) and in albumin-synthesizing polysomes (5- to 6-fold), while it may reflect untranslated or poorly translated albumin mRNA in liver, probably reflects inaccuracies in the polysome-antibody binding technique.

At present we have no explanation for the apparent reduction in albumin synthesis and mRNA levels in Hepa-2 cells in tissue culture. Depressed albumin synthesis in transplantable hepatomas has been reported (Rotermund et al., 1970), and it is also possible that conditions of tissue culture, which alter tissue organization as well as a multitude of nutritional, hor-

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**DISCUSSION**

**Purity of Isolated Albumin mRNA**—Mouse albumin mRNA has been isolated by direct immunoprecipitation of albumin-synthesizing polysomes and chromatography on oligo(dT) cellulose. The theory underlying immunoprecipitation of polysomes engaged in the synthesis of specific proteins has been described in detail (Palmiter et al., 1972; Shapiro et al., 1974). These studies indicate that the main problem encountered in the immunological precipitation of polysomes as the major step in specific messenger RNA purification is nonspecific adsorption and trapping of heterologous polysomes, which is directly related to the size of the immunoprecipitate formed (Shapiro et al., 1974). This nonspecific precipitation is the major source of contaminating mRNA species in the final preparation. We have described conditions for minimal immunoprecipitate formation with acceptable yields of precipitated polysomes.

Assessment of the purity of albumin mRNA isolated by the procedure described is by analysis of cell-free translation and hybridization kinetics. Using the wheat germ cell-free system, we have shown that an 11-fold enrichment of translatable albumin mRNA was achieved in the purification process. On the basis of in vivo (Peters and Peters, 1972) and in vitro (Tse and Taylor, 1977) measurements in rats and in vitro measurements in mice (Faber et al., 1974), it has been shown that albumin synthesis makes up 8 to 10% of total liver protein synthesis. If all liver mRNA is translated with equal efficiency, then one would predict an enrichment of 10- to 12-fold in the isolation of pure albumin mRNA. Since we have achieved an 11-fold enrichment, we propose that our preparation of albumin is highly purified (>80%) with respect to total liver RNA.

Hybridization kinetic data further suggest that the purity of albumin mRNA we describe exceeds 80%. The hybridization of albumin cDNA to albumin mRNA occurs rapidly, within two logs, suggestive of a single component, pseudo-first order reaction. The hybridization plateaus over the $R_t$ values examined at 80 to 82% and implies a single species of nucleotide sequences greater than 80% in abundance. The possibility that there are two or more species of equal abundance in this mRNA preparation is highly unlikely because of the qualitatively identical kinetics seen in the hybridizations driven by purified albumin mRNA, total liver cytoplasmic RNA, and total Hepa-2 cytoplasmic RNA, all of which plateau at about 80% hybridization. It seems most improbable that these three RNA preparations could share major and identical abundances of nonalbumin mRNA sequences.

**Size of Albumin mRNA**—We have shown, using three independent methods of analysis, that albumin mRNA sediments in sucrose gradients at about 17 S (Figs. 2, 5, and 8). Other studies serve to confirm this finding (Taylor and Tse, 1976; Strair et al., 1977). Relative to 16 S and 18 S rRNA, 17 S RNA should have a molecular weight of approximately $6.5 \times 10^6$ or about 2000 bases (Loening, 1968). Mouse serum albumin, molecular weight 68,000, is composed of about 580 amino acids which would require a minimum of 1740 bases for translation. If preproalbumin is the primary translation product in mouse as in rat (Strair et al., 1977), then 54 more bases are needed, raising the total to 1794 bases. The remaining 200 or so bases could easily be accounted for by 3'-poly(A) stretches and nontranslated nucleotides at the 5'-end (Darnell, 1976; Brawerman, 1976). In summary, mouse albumin mRNA appears to be very close in size to the minimal size required for translational and structural considerations common to characterized messenger RNAs. Extensive noncoding regions are not apparent in contrast to other messenger RNA studied (Shapiro and Schimke, 1975; Innis and Miller, 1977; Lewin, 1975; Proudfoot et al., 1976). Recently, it has been reported that a 26 S form of rat albumin mRNA may exist in the nucleus, and it is presumably a precursor to the mature 17 S cytoplasmic form (Strair et al., 1978).

**Albunin mRNA in Normal Liver and Hepatoma Cells**—Relative albumin mRNA levels were determined, and it was shown that, per cell, liver has about 10-fold more albumin mRNA sequences than Hepa-2 cells. We have analyzed albumin synthesis in Hepa-2 cells and have determined that albumin synthesis makes up about 1 to 1.5% of total protein synthesis in log phase cultures. Thus, albumin synthesis, relative to total protein synthesis, is about 10-fold higher in liver than in Hepa-2 cells, and these differences are reflected in albumin mRNA levels in liver and Hepa-2 cells.

Previous studies in our laboratory showed that liver polysomes bound approximately 3.5-fold more iodinated albumin antibody than comparable amounts of Hepa-2 polysomes (Brown and Papaconstantinou, 1977). Accounting for yields of polysomes from each cell type, liver cells contain 5- to 10-fold more albumin-synthesizing polysomes than Hepa-2 cells. The difference between the two cell types in albumin mRNA levels (10-fold) and in albumin-synthesizing polysomes (5- to 6-fold), while it may reflect untranslated or poorly translated albumin mRNA in liver, probably reflects inaccuracies in the polysome-antibody binding technique.

At present we have no explanation for the apparent reduction in albumin synthesis and mRNA levels in Hepa-2 cells in tissue culture. Depressed albumin synthesis in transplantable hepatomas has been reported (Rotermund et al., 1970), and it is also possible that conditions of tissue culture, which alter tissue organization as well as a multitude of nutritional, hor-
monal, and growth factors, may have an effect on the steady state levels of albumin mRNA. Most recently, we have been able to show an increase in relative albumin synthesis and mRNA levels in stationary phase Hepa-2 cells to approximately in vitro levels by treatment with glucocorticoids and cyclic nucleotides (Brown and Papaconstantinou, 1978). These observations strongly indicate the importance of hormonal and nutritional factors in the maintenance of in vitro levels of albumin synthesis in cultured cells. Since our hybridization analyses are only capable of measuring steady state levels of albumin mRNA and provide no direct information as to its synthesis, processing, and degradation, studies on the mechanisms of this mRNA modulation must await the availability of relatively large quantities of albumin cDNA which will facilitate the direct analysis of pulse-labeled albumin mRNA by cDNA excess hybridization.

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Mouse Albumin mRNA
Mouse albumin mRNA in liver and a hepatoma cell line. Preparation of complementary DNA from purified mRNA and quantitation by nucleic acid hybridization.

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