IDENTIFICATION OF ALBUMIN-SYNTHESIZING POLYSOMES FROM MOUSE LIVER AND A MOUSE HEPATOMA CELL LINE

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Summary —Albumin-synthesizing polysomes from mouse liver and mouse hepatoma cells in in tissue culture have been localized on sucrose gradients with ^{125}I-labeled antimouse serum albumin used as a marker. Competition studies show that the ^{125}I-labeled antibody binds specifically to albumin-synthesizing polysomes from both tissues. The ^{125}I-labeled polysomes from liver and hepatoma cells have identical sedimentation properties on sucrose gradients, which indicates that the polysomes range in size from 9–14 ribosomes. This is comparable in size to polysomes from rat liver and Morris hepatoma. One significant difference between these albumin-synthesizing polysomes is that those extracted from hepatoma cells bind 70% less antibody than equivalent amounts of polysomes from liver cells. Since the level of albumin synthesis in the hepatoma cells is comparable to the level of albumin synthesis in vivo, this difference in antibody-binding capacity is not likely to be due to differences in polysomal content, but appears to be a characteristic difference between hepatoma and normal mouse liver cells.

Recent developments in immunochemical methodology have greatly facilitated the localization and isolation of polysomes engaged in tissue-specific protein synthesis. The binding of ^{125}I-labeled monospecific antibody to nascent polypeptide chains has been used to identify polysomes engaged in the synthesis of ovalbumin (1), rat serum albumin (2, 3), collagen (4), fibrinogen (5), immunoglobulin (6), and α- and β-globin (7). Binding of the antibody has been shown, in all cases, to involve the specific interaction of the antibody with nascent chains (1–3). Similar techniques have been used to identify specific polysomes in cells in culture. Polysomes specific for fatty acid synthetase (8) and immunoglobulin-synthesizing polysomes have been identified in cultured cells (9).

Abbreviations: MSA, mouse serum albumin; SDS, sodium dodecyl sulfate.

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Recently, it has been shown that the mouse hepatoma cell line derived from the solid tumor BW7756 continues to synthesize and secrete albumin (10–12), α-fetoprotein (13), and transferrin (14) after several years of continuous culture. Prior to isolating albumin-synthesizing polysomes by immunoprecipitation techniques and subsequent extraction of albumin mRNA, we thought it desirable to determine whether mouse liver and hepatoma cell albumin polysomes could be identified by the binding of ^{125}I-labeled antimouse serum albumin. In this study we show that iodinated antibody does specifically identify albumin-synthesizing polysomes from both tissues, and that mouse liver polysomes bind significantly more iodinated antibody than do equivalent amounts of hepatoma cell polysomes.

MATERIALS AND METHODS

Preparation of polysomes — Liver and brain polysomes were prepared from female DBA/2Bd mice by a modification of the method of Taylor and Schimke (2). Tissue was homogenized in 0.25 M sucrose in buffer A [50 mM Tris-HCl (pH 7.1) at 21°C, 25 mM NaCl, 5 mM $MgCl_2$] with 100 µg/ml sodium heparin. The postmitochondrial supernatant (14,500 X g for 15 min) was made 1% in Triton X-100 and sodium deoxycholate and gently mixed by repeated pipetting (15). After 10 min at 4°C, this mixture (6 ml) was layered over a discontinuous sucrose gradient consisting of 2.5 M sucrose (2 ml), 1.0 M sucrose (5 ml), and 0.5 M sucrose (0.5 ml), all in buffer A with 100 µg/ml heparin. Polysomes were withdrawn, by syringe, at the 2.5 M sucrose interface after centrifugation (SW-40 rotor, 40,000 rpm for 90 min at 2°C) and dialyzed at 0°C for 12 hr against buffer A with heparin.

Cells from mouse hepatoma cell line BW7756 were grown to confluency (approximately 1.5 X 10^7 cells) in 100-mm plastic Petri dishes (Falcon Plastics) in Waymouth's medium with insulin and 10% fetal calf serum. The cells were gently scraped with a rubber policeman in 0.5 ml buffer A with 100 µg/ml sodium heparin. The cell suspension was made 0.5% in Triton X-100, gently repipetted, and allowed to stand on ice for 10 min (15). No intact cells were visible after this treatment. After centrifugation (14,500 X g for 15 min), the supernatant was made 1% in Triton X-100 and sodium deoxycholate and polysomes were prepared as above. Approximately 2–3 A_{260} units of polysomes could be recovered from each dish ($A_{260}/A_{280} = 1.77$).

Preparation of antiserum — Mouse serum albumin (MSA) was extracted from serum by the method of Debro et al. (16). Further purification of serum albumin was achieved by a modification of the method of Taylor and Schimke (17). After an initial Sephadex G-100 step, the albumin peak was concentrated by $(NH_4)_2SO_4$ precipitation (66% saturation), chromatographed on DEAE cellulose (Bio-Rad DEAE-C), and finally rechromatographed on Sephadex G-100. The final preparation was analyzed by SDS-polyacrylamide gel electrophoresis and was found to be homogeneous and to have the expected molecular weight (68,000).

Rabbit antiserum to MSA was prepared and subsequently precipitated by $(NH_4)_2SO_4$ fractionation (17, 18). The precipitate was dissolved and equilibrated by dialysis in buffer C [10 mM Na_2HPO_4 (pH 7.2), 15 mM NaCl] and passed over DEAE cellulose, also in buffer C. Specific anti-MSA was prepared from the IgG fraction by column-affinity chromatography on MSA-derivatized Sepharose 4B (dimensions 1.0 X 7.0 cm) (19) by the method of Shapiro et al. (20).

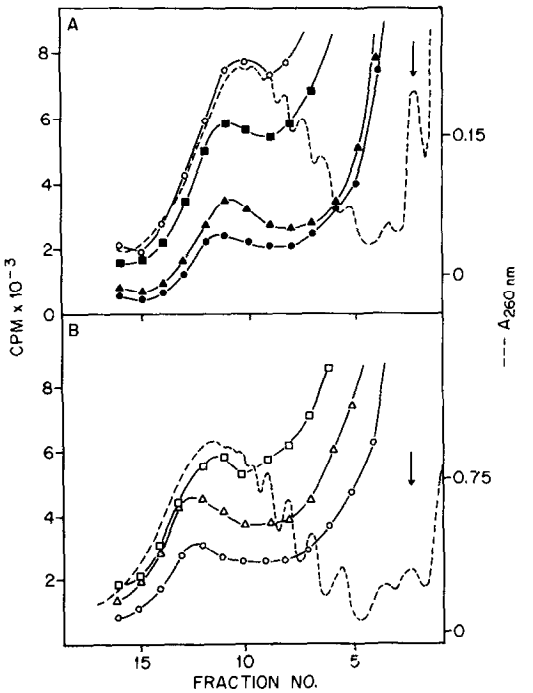

Figure 1. Binding of ^{125}I-labeled anti-MSA to cultured mouse hepatoma cell and mouse liver polysomes. (A) Sedimentation profile of hepatoma cell polysomes (2.9 A_{260} units) incubated with ^{125}I-labeled anti-MSA (1.94 X 10^5 cpm/µg). ●—● 3.2 µg; ▲—▲ 8 µg; ■—■ 16 µg; O—O 32 µg anti-MSA. Arrow indicates position of 80 S ribosomes. (B) Sedimentation profile of liver polysomes (12.66 A_{260} units) incubated with ^{125}I-labeled anti-MSA (2.94 X 10^4 cpm/µg). O—O 54 µg; △—△ 108 µg; □—□ 216 µg.

Iodination of anti-MSA was essentially as described (2) except that 2 mCi of ^{125}I was used, and labeled antibody was concentrated by $(NH_4)_2SO_4$ precipitation after Sephadex G-100 chromatography. ^{125}I-labeled anti-MSA, unlabeled anti-MSA, and IgG from a nonimmunized rabbit were rendered RNase free (20) and dialyzed against buffer A.

^{125}I-labeled antibody-polysome reaction — Labeled antibodies and dialyzed polysomes were mixed and incubated for 1 hour at 0°C in a final volume of 0.75 ml. Continuous sucrose gradients were prepared (11.5 ml, 0.5–1.5 M sucrose in buffer A with 40 µg/ml sodium heparin) over which was layered 1.0 ml of 0.5 M sucrose in buffer A containing 0.5% Triton X-100 and sodium deoxycholate. This detergent-containing layer was helpful in reducing contamination by unbound, labeled antibody in the upper regions of the underlying gradient. The antibody–polysome mixture was subsequently layered over the detergent-containing layer and centrifuged (Spinco SW-40 rotor, 40,000 rpm for 95 min at 2°C). Following centrifugation, the top two layers were removed. The gradient was displaced with 60% (w/v) sucrose and monitored at 260 nm with an ISCO absorbance monitor. Fractions (0.6 ml) were collected directly into glass scintillation vials to which Aquasol (New England Nuclear), diluted and acidified as suggested by the manufacturer, was added (15 ml). Radioactivity was determined by liquid scintillation spectrometry.

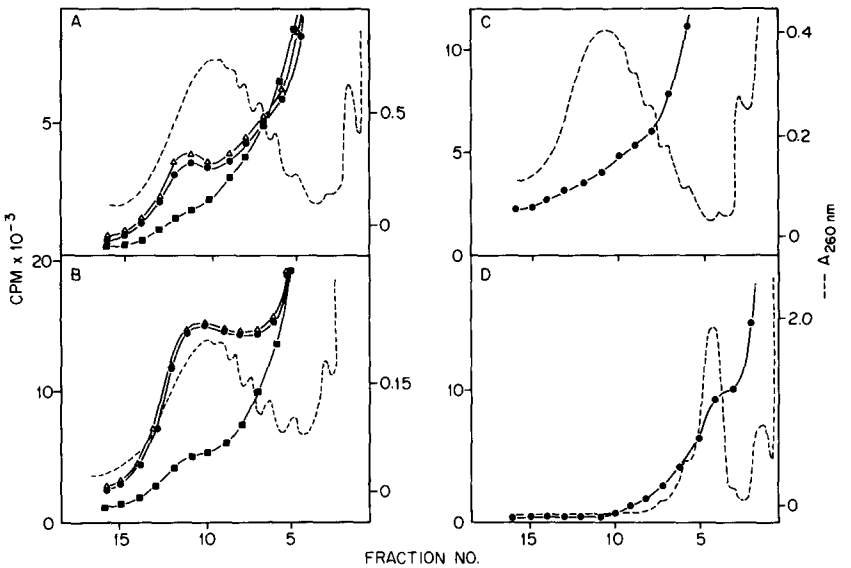

Figure 2. Specificity of ^{125}I-labeled anti-MSA binding to polysomes. (A) Competitive antibody binding assay. Hepatoma cell polysomes (2.6 A_{260} units) were preincubated for 45 min with nonimmunized rabbit IgG (15 µg, △—△), with unlabeled anti-MSA (15 µg, ■—■), or alone with no added protein (●—●). ^{125}I-labeled anti-MSA (8 µg, 1.94 X 10^5 cpm/µg) was added to each, incubated an additional hour, and centrifuged. (B) Competitive antibody binding assay. Liver polysomes (2.88 A_{260} units) were preincubated as above with nonimmunized rabbit IgG (54 µg, △—△), with unlabeled anti-MSA (59 µg, ■—■), or alone (●—●). ^{125}I-labeled anti-MSA (32 µg, 1.94 X 10^5 cpm/µg) was added to each and treated as above. (C) Brain polysomes (2.8 A_{260} units) were incubated with ^{125}I-labeled anti-MSA (32 µg, 1.94 X 10^5 cpm/µg) and centrifuged. (D) Liver polysomes (6.3 A_{260} units) were incubated with ^{125}I-labeled anti-MSA (54 µg, 1.94 X 10^5 cpm/µg) for 1 hour and then with ribonuclease A (8 µg, Worthington Biochemicals) for 30 minutes and centrifuged.

RESULTS

Interaction of ^{125}I-labeled antialbumin with hepatoma cell and liver polysomes — Polysomes from hepatoma cells in tissue culture were incubated with ^{125}I-labeled anti-MSA and centrifuged in a 0.5–1.5 M sucrose gradient. The sedimentation profile of the total polysome preparation and the binding of various amounts of anti-MSA are shown in Figure 1A. A similar experiment with mouse liver polysomes is shown in Figure 1B. In each of these cases, the peak of bound radioactivity occurs in fractions 10–12, which corresponds to polysomes composed of approximately 9–14 ribosomes. These observations agree with the sedimentation properties of ^{125}I-labeled antirat serum albumin bound to rat liver albumin polysomes (17).

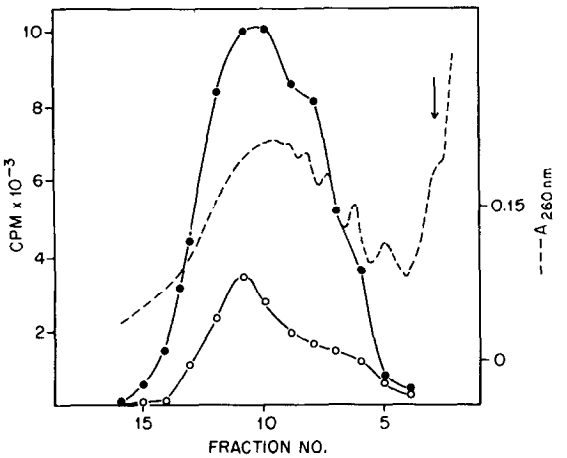

Figure 3. Comparison of ^{125}I-labeled anti-MSA binding to hepatoma cell and liver polysomes. ^{125}I-labeled radioactivity in fractions from Figure 2C (brain polysomes, 2.8 A_{260}, 32 μg ^{125}I-labeled anti-MSA) was subtracted from equivalent fractions from Figure 1A, open circles (hepatoma polysomes, 2.9 A_{260} units, 32 μg ^{125}I-labeled anti-MSA) and Figure 2B, solid circles (liver polysomes, 2.88 A_{260} units, 32 μg ^{125}I-labeled anti-MSA). The resulting radioactivity in each fraction represents anti-MSA binding corrected for non-specific adsorption. Total corrected counts in the liver polysome preparation (●—●) and the hepatoma polysome (O—O) are 5.52 X 10^4 and 1.61 X 10^4 cpm, respectively. A representative polysome profile is also included for reference.

Specificity of ^{125}I-labeled antialbumin binding to polysomes — The specificity of the antibody-binding reaction with hepatoma cell and liver polysomes is shown in Figures 2A and 2B. In these competitive binding assays, it may be seen that the binding of ^{125}I-labeled antialbumin in the peak regions is effectively eliminated by prior incubation with unlabeled specific antibody, and this binding is unaffected by prior incubation with non-immunized rabbit immunoglobulin.

Additional evidence for the specificity of ^{125}I-labeled anti-MSA binding to albumin-synthesizing polysomes is the inability of the ^{125}I-labeled antibody to bind to polysomes from brain tissue (Fig. 2C). Finally, the data in Figure 2D show that RNase treatment, after incubation with antibody, reduces the polysomes to monomers and dimers, and restricts bound radioactivity to the upper region of the gradient. These data indicate that the sedimentation of intact albumin-synthesizing polysomes is necessary in order to observe the radioactivity patterns seen in Figure 1.

Comparison of albumin-antibody binding capacities of hepatoma cell and liver polysomes — A direct comparison of the albumin-antibody binding capacities of hepatoma

cell and liver polysomes is complicated by the presence of contaminating or nonspecifically bound antibody seen in the upper regions (fractions 3–7) of the sucrose gradients. That this radioactivity is nonspecifically bound may be seen in the competitive binding assay (Figs. 2A and B), since the binding of labeled antibody is not preempted by prior incubation with unlabeled anti-MSA in fractions 3–7.

The data in Figures 1A (open circles) and 2B (solid circles) were corrected for nonspecific antibody adsorption and contamination from unbound antibody by subtraction of radioactivity in equivalent fractions from a gradient containing brain polysomes incubated with labeled albumin antibody (Fig. 3). Under identical conditions, hepatoma cell polysomes bind approximately 70% less ^{125}I-labeled anti-MSA than do an equivalent amount of liver polysomes. Examination of the competitive binding assays (Figs. 2A and B, solid squares) reveals that 15 μg of unlabeled anti-MSA essentially saturates the specific-antibody-binding sites, since little or no binding of labeled antibody subsequently occurs. In the comparative study, over twice the saturating amount of antibody was employed (32 μg), ensuring antibody binding to all available sites.

DISCUSSION

We have shown that ^{125}I-labeled antibodies to mouse serum albumin can be used to localize albumin-synthesizing polysomes from cultured mouse hepatoma cells and from normal adult liver. Maximal antibody binding occurred in the 9–14 ribosome region of the polysome profile. This is in excellent agreement with the results from similar experiments involving ^{125}I-labeled anti-rat serum albumin binding to rat liver polysomes (2). Our control experiments with polysomes from mouse brain tissue and RNase digestion indicate that antibody binding is dependent upon the presence of albumin nascent chains rather than the polysome structure alone. The specificity of the antibody-binding reaction in the peak region of the gradient was also indicated by the data from the competitive binding assay in which the binding of ^{125}I-labeled anti-MSA was only preempted by prior incubation with unlabeled anti-MSA. These observations indicate that unlabeled anti-MSA essentially saturates the available albumin antigenic determinants and prohibits subsequent binding by labeled antibody.

The level of albumin synthesis by the hepatoma cells has been shown in our laboratory to range from 8–10% of the total protein synthesized by the cells (13). This is comparable to the level of albumin synthesis observed to occur in vivo in rat (21) and in the cell-free translation of total mouse liver mRNA (22). Furthermore, the data show that there is no

difference in the size of the albumin polysomes from liver and hepatoma cells, thus inferring that the number of nascent chains per unit polysome is the same in each tissue. The observation that hepatoma cell polysomes bind 70% less antibody than do normal liver polysomes, while apparently synthesizing equivalent proportions of albumin, is perplexing and we currently have no explanation for these results. Possibly, the albumin mRNA in hepatoma cells is translated more rapidly than in liver, leading to approximately equal albumin synthesis from a smaller pool of cytoplasmic-albumin mRNA. Alternatively, processing of nascent albumin chains may be aberrant, resulting in diminished antigenicity and decreased antibody binding to polysomes engaged in the synthesis of albumin.

We have recently isolated albumin mRNA and have prepared a relatively complete complementary DNA (cDNA). Quantitative nucleic acid hybridization studies using this cDNA probe should reveal whether the differences in antibody-binding capacities of hepatoma cell and liver polysomes seen in these studies are a reflection of the cellular content of albumin mRNA and indirectly reveal whether some form of translational control is involved in the synthesis of albumin in these two systems.

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