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IDENTIFICATION OF ALBUMIN mRNPs IN THE CYTOSOL OF FASTING RAT LIVER AND INFLUENCE OF TRYPTOPHAN OR A MIXTURE OF AMINO ACIDS

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SUMMARY

In rats fasted for 24-30 hours, albumin mRNA sequences are released from membrane-bound polysomes to enter the free cytosol fraction. A significant portion of these sequences are present in albumin mRNPs, distinguished from free albumin mRNA and 40S subunit complexes by Cs_2SO_4 equilibrium density centrifugation. Refeeding a mixture of 20 amino acids restores albumin mRNA to membrane-bound polysomes, demonstrating the importance of amino acid supply in the mRNP-polysome equilibrium and in regulation of albumin synthesis.

Recently, using molecular hybridization technology with purified albumin [^{3}H] cDNA, we have observed that 97-98% of albumin mRNA sequences in normal rat liver are located in membrane-bound polyribosomes (1). When rats are fasted for 24-30 hours, there is disaggregation of membrane-bound polyribosomes and a shift of albumin mRNA sequences to free polyribosomes and to the post-ribosomal supernatant fraction (2). Albumin mRNA sequences in the post-ribosomal supernatant fraction are present as intact 17S albumin mRNA molecules and do not represent degradation products (2). In this communication we report evidence that albumin mRNA in the liver post-ribosomal supernatant fraction of fasted rats is present at least in part as mRNA-protein complexes (albumin mRNPs) and that albumin mRNA can reenter membrane-bound polyribosomes upon refeeding a mixture of 20 amino acids. When rats are refed with tryptophan alone for 1 hour, albumin mRNA shifts from the supernatant fraction to free monosomes but little material enters membrane-bound polyribosomes. These studies suggest that albumin mRNA sequences shifted from membrane-bound polyribosomes to free polyribosomes and mRNP complexes during acute starvation are functionally reutilized upon restoration of amino acid supply.

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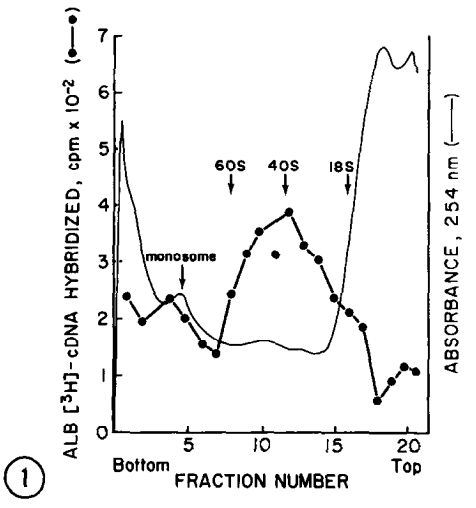
MATERIALS AND METHODS

Male Sprague-Dawley rats (220-250 gm) were used for all studies. Animals were maintained on Purina chow diet. On the day prior to sacrifice, food was withdrawn at 9:00 A.M. and the animals were sacrificed at 9:00 A.M. the following morning or given tryptophan or a mixture of 20 amino acids by stomach tube 1 hour prior to sacrifice (3,8). All subsequent procedures for preparation of liver membrane-bound polysomes, free-polysomes, a post-ribosomal supernatant fraction and various RNAs were as reported previously (1,2). Preparation of purified albumin [^{3}H] cDNA and cDNA-mRNA hybridization assays were also as reported previously (1,2,4). Sucrose gradient centrifugation, using simultaneously prepared exponential gradients, was performed by the method of Noll (5). Cesium sulfate density gradient centrifugation of RNA-protein complexes was performed by the method of Greenberg (6).

RESULTS

Previously, we reported that fasting of rats for 24-30 hrs markedly increases the content of albumin mRNA in the post-ribosomal supernatant fraction (2). As shown in Fig. 1, when a post membrane (free polysome plus supernatant) fraction of fasted rat liver was centrifuged in a sucrose gradient under conditions to pellet polysomes, the bulk of albumin mRNA sequences sedimented as a broad peak between 30-60S. Small amounts of albumin mRNA were identified with 80S monomers and free polysomes (pelleted material) and very few sequences were found in the 17-18S region, the position expected for free albumin mRNA (4). This suggested that albumin mRNA in the post-ribosomal supernatant fraction was present in mRNA-protein complexes. Material from the peak region of this gradient was placed over a preformed, linear 1.25 - 1.75 gm/cm^3 Cs_2SO_4 density gradient in buffered 0.5 M NaCl, centrifuged to equilibrium, and assayed for albumin mRNA distribution in comparison to deproteinized mRNA and 40S ribosomal subunits centrifuged in parallel gradients. As shown in Fig. 2, distinct peaks of albumin mRNA were identified at densities of 1.63 gm/cm^3 (the position of deproteinized albumin mRNA) and 1.33 gm/cm^3 (the position expected for RNPs on Cs_2SO_4). No distinct peak was found at a density of 1.52 gm/cm^3 (the position of 40S subunit marker). Since glutaraldehyde fixation of RNPs could not be performed prior to centrifugation (this would render the RNA incapable of hybridization), the percentage of albumin mRNPs



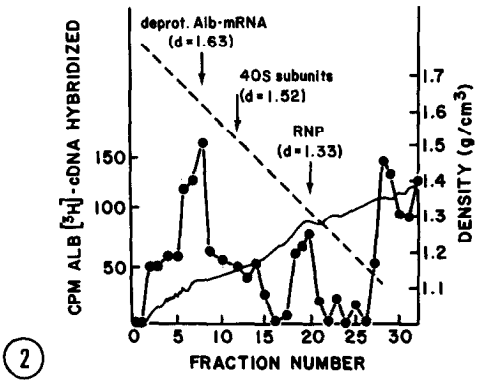


Figure 1. Sucrose gradient analysis of albumin mRNA sequences in the subribosomal fraction of liver cytoplasm from fasted rats. A 400 µl aliquot of the post-membrane fraction of fasted liver cytoplasm (supernatant fraction prepared by centrifugation of liver homogenate at 131,000 x g for 12 min) was layered over a 12 ml, 10-40% ($^{W}/_{V}$) exponential sucrose gradient in 10 mM Hepes pH 7.4, 75 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA. Centrifugation was at 38,000 rpm for 5 hr in a Beckman #SW41 rotor at 2°C and 0.5 ml fraction were collected. RNA was prepared from each fraction by digestion with proteinase K (1 mg/ml), followed by ethanol precipitation. Each fraction of RNA was pelleted, resuspended in deionized distilled water, and assayed for albumin mRNA sequence content by hybridization to albumin [^{3}H]-cDNA as described previously (1). The solid line represents the absorbance profile of the gradient monitored with an Altex UV monitor #153 and arrows indicate the positions of monosomes, 60S subunits, 40S subunits and 18S RNA centrifuged in parallel gradients.

Figure 2. Cesium sulfate density gradient analysis of albumin mRNA sequences in enriched particles from the postribosomal supernatant fraction of fasted rats. 400 µl of pooled fractions (the peak region from Fig. 1) was layered on top of a preformed linear Cs_2SO_4 gradient (initial density range: 1.25 to 1.75 g/cm^3) in buffer containing 10 mM Hepes, pH 7.4, 75 mM KCl, 5 mM $MgCl_2$ and 500 mM NaCl. Centrifugation was at 50,000 rpm in a Beckman #SW60 rotor for 45 hours at 20°C. After centrifugation, gradients were withdrawn from the bottom of each tube and absorbance monitored at 254 nM. Samples from each fraction were diluted with deionized distilled water and RNA was prepared and analyzed for albumin mRNA sequence content as described in Fig. 1. The arrows indicate the position of deproteinized albumin mRNA (determined by hybridization analysis) and liver 40S subunits (determined by absorbance profile) centrifuged in parallel gradients.

vs free albumin mRNA could not be determined. However, from Fig. 1, it would appear that a high proportion of albumin mRNA sequences in the supernatant fraction are present in mRNP complexes.

TABLE I

Albumin mRNA Sequence Content in Subcellular Fractions of Rat Liver

	Polysomal		Post-ribosomal
	Membrane-bound	free	Supernatant
	(albumin mRNA, ng/liver)		
Fasted	1782	73	376
Tryptophan refed	1719	199	168
Amino acid refed	2150	35	154

Membrane-bound polysomes, free polysomes and a post-ribosomal supernatant fraction were isolated from liver homogenates of rats fasted for 24 hrs or fasted and refed tryptophan or a mixture of 20 amino acids 1 hr prior to sacrifice (see Materials and Methods). RNA was prepared from these subcellular fractions and assayed quantitatively for albumin mRNA sequences by hybridization to albumin [^{3}H] cDNA (1).

The content of albumin mRNA in membrane-bound polysomes, free polysomes, and the post-ribosomal supernatant fraction of fasted rats and rats refed with tryptophan or a mixture of 20 amino acids for 1 hr was determined (Table 1). Total cytoplasmic albumin mRNA did not change during this period. Tryptophan refeeding tripled albumin mRNA sequences in free polysomes with a proportionate reduction of sequences in the post-ribosomal supernatant fraction. However, albumin mRNA sequences in free polysomes were located almost exclusively in monosomes (Fig. 3). There was no change in albumin mRNA content in membrane-bound polysomes (Table 1). With a mixture of 20 amino acids, albumin mRNA content in the post-ribosomal supernatant fraction was again reduced (Table 1). However, albumin mRNA content in free polysomes was also reduced and there was a net increase in albumin mRNA sequences in membrane-bound polysomes, the normal subcellular location of albumin synthesis.

DISCUSSION

For many years it has been known that protein synthesis is depressed under adverse cellular growth conditions. Qualitatively, it has been shown in tissue culture cells that cytoplasmic mRNA can be shifted reversibly from

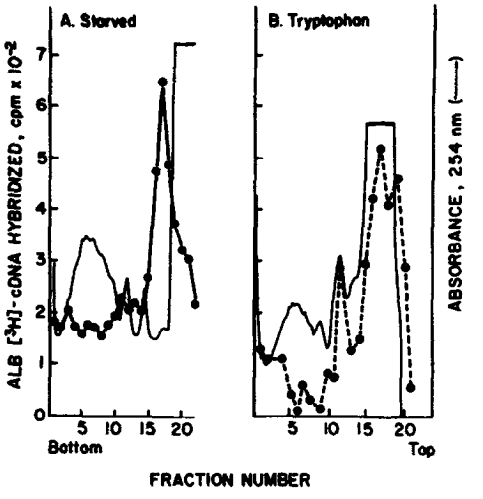


Figure 3. Sucrose gradient analysis of albumin mRNA sequences in the post-membrane (free polysome plus supernatant) fraction of liver cytoplasm from fasted and tryptophan refed rats. 400 µl (3.3 A_{200} units RNA) of the supernatant fraction of liver homogenate (after centrifugation at 131,000 x g for 12 min) of rats fasted for 24 hrs (A) or fasted for 24 hrs and given tryptophan 1 hr prior to sacrifice (B) was layered over a 12 ml 10-40% ($^{w}/_{v}$) exponential sucrose gradient. Centrifugation in the Beckman #SW41 rotor was for 2 hr. Gradients were fractionated, and RNA was isolated from each fraction and assayed for albumin mRNA sequences as noted in Fig. 1. The absorbance profile was determined by continuous recording with an Altex UV monitor #153.

polysomes to mRNPs during starvation or amino acid deprivation (7,8), temperature shock (9,10), or inhibition of protein synthesis initiation (11,12). Other studies have identified cytoplasmic mRNPs containing specific mRNAs as normal constituents in cellular development or differentiation, e.g. maternal histone mRNA in Xenopus oocytes (13,14) and myosin mRNA in undifferentiated myoblasts (15,16). Uninduced (Fe^{+++}depleted) rat liver cells have also been reported to contain non-translated ferritin mRNA (17), and it has been proposed that mRNPs may serve as a storage depot for specific mRNAs when they are not engaged in protein synthesis (13-19). These studies are limited, however, in that mRNA has been identified only qualitatively either by pulse labeling of poly A^{+} RNA or by translation in a heterologous system.

To deal with quantitative aspects of the subcellular distribution and function of a specific eukaryotic mRNA, we have recently purified rat liver

albumin mRNA and have prepared a complementary [^{3}H] DNA probe of high specific activity (4). Using this probe we have determined that 97-98% of albumin mRNA sequences in normal rat liver are present in membrane-bound polysomes (1). The present study shows that when rats are fasted for 24-30 hours, a significant portion of albumin mRNA sequences is shifted from membrane-bound to free polysomes and to mRNPs in the post-ribosomal supernatant fraction. When starved rats are refed a mixture of 20 amino acids for 1 hour, there is no change in total cytoplasmic albumin mRNA, but albumin mRNA sequences shift back to membrane-bound polysomes. This appears to represent functional reutilization of albumin mRNA from mRNPs, but this point will require additional documentation.

In the present study, the shift in albumin mRNA sequences to the cytosol fraction was not as great as we had observed previously (2). However, we feel that this may be due to a seasonal variation in the effect of fasting. It is interesting that an increase in albumin mRNA in free polysomes was not observed during amino acid refeeding. This is consistent with predictions from the "signal sequence" hypothesis for synthesis of secretory proteins proposed by Blobel and Dobberstein (20). According to this model, ribosomes synthesizing albumin would be removed from the free polysome pool as soon as they reach the oligosome stage and the N-terminal hydrophobic precursor region of the growing polypeptide chain (ie. the "signal sequence") is exposed (assuming hydrophobic sites in the membrane are also available for interaction). Therefore, it would be difficult to trap albumin mRNA in free polysomes especially during specific reactivation of protein synthesis. However, during brief tryptophan refeeding, albumin mRNA was found in the monomer fraction of free polysomes, and little material reentered membrane-bound polysomes. This is consistent with an intermediate or lag phase in the recovery process. The present studies, therefore, suggest that amino acid supply is intimately involved in modulation of hepatic protein synthesis and that events related to the basic protein synthesis mechanism play a vital role in the equilibrium and subcellular distribution of albumin mRNA.

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