RELATIVE ABUNDANCE OF SPECIFIC MESSENGER-RNA SPECIES IN THE FREE mRNP FRACTION OF RAT LIVER

J. ZÄHRINGER*, B. S. BALIGA+ and H. N. MUNRO

Laboratory of Physiological Chemistry, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Received 19 September 1979

1. Introduction

We demonstrated the presence in liver cell sap of a pool of untranslated ferritin mRNA [1] which plays a key role in the regulation of ferritin synthesis [2]. Liver cell sap normally contains a considerable amount of untranslated ferritin mRNA which can be rapidly recruited for ferritin polyribosome formation after iron administration. We have now extended this study to the distribution of albumin mRNA between the liver polyribosomes and the cell sap of normal and nephrotic rats, in order to determine whether there is a similar large pool of free mRNA in normal rats and whether such a pool contributes to the increase in polysomal albumin mRNA observed in nephrotic rats [3]. Unlike ferritin, there is little free albumin mRNA in the cell sap fraction and it remains unchanged by induction of albumin synthesis in nephrosis.

2. Material and methods

L-[4,5-^{3}H(N)]leucine (spec. act. 30–50 Ci/mmol) was obtained from New England Nuclear, Boston, MA, and oligo(dT)-cellulose from Collab. Res. Waltham, MA. Rat serum albumin was purchased from Sigma Chemical Co., St Louis, MO, and rat serum albumin antibody from ICN Pharm., which also provided puromycin aminonucleoside. Purified rat liver ferritin and ferritin antibody were kindly made available by Dr Maria Linder and wheat germ was donated by Dr Bryan Roberts, both of MIT. The concentration of RNA in solutions was determined by ultraviolet absorption, assuming $E^{1\%}_{260}$ nm to be 250 [3].

For studies of albumin synthesis by the liver, fasting male Sprague-Dawley rats (200–250 g body wt) obtained from Charles River Breeding Labs. (Wilmington, MA) were injected subcutaneously with 1.5 mg puromycin aminonucleoside/100 g body wt daily for 10 days, control rats being uninjected. On day 12, pooled livers from each group were homogenized in 2 vol. buffer containing 0.1 M Tris–HCl (pH 7.6). 0.1 M NaCl, 0.1 mM EDTA, 5 mM $MgCl_2$, 150 μg heparin/ml and 0.25 M sucrose, and centrifuged at 10 000 × g for 20 min. The resulting post-mitochondrial supernatant fraction (PMS) was used to obtain a microsome fraction and a post-ribosomal (cell sap) fraction. By analysis on sucrose gradients, it was shown that the post-ribosomal fraction contained only traces of 40 S and 60 S ribosomal subunits. The RNA from each fraction was extracted with phenol followed by separation of the poly(A)-containing mRNA by affinity chromatography on oligo(dT)-cellulose [4]. Each mRNA sample was then translated in a wheat germ S_{30} system [5] using [^{3}H]leucine in the incubation medium. The products of incubation were assayed for incorporation of [^{3}H]leucine into total trichloroacetic acid-precipitable material [6] and by immunoprecipitation with anti-albumin serum in the presence of carrier albumin [3]. The immuno-

* Present address: Medizinische Klinik I, Klinikum Großhadern, Marchioninistr. 15, 8000 München 70, FRG

+ Present address: Dept. Pediatrics, School Med., Univ. South Alabama, 2451 Fillingim St., Mobil, AL 36617, USA

precipitate was then dissociated with sodium dodecyl sulfate (SDS) and separated on SDS–polyacrylamide gradient gels (10–15%) followed by counting of 3 mm slices of the gel [2].

For studies of ferritin messenger RNA distribution, microsomal mRNA and cell sap mRNA prepared from fasting 100 g male rats were tested for their capacity to synthesize ferritin [7]. The mRNA samples were incubated with [^{3}H]leucine in the wheat germ system and newly synthesized ferritin peptide chains were precipitated with ferritin antibody in the presence of carrier rat liver ferritin. The precipitates were resolved by SDS–polyacrylamide gel electrophoresis and the incorporation into ferritin subunits was assayed as in [1,2].

3. Results and discussion

In each experiment, the total RNA from the microsomal (polyribosomal) and cell sap (post-ribosomal) fractions was extracted with phenol from ~100 g rat liver (10–20 rats, depending on size). The yields of total RNA were ~170–180 mg and 40–50 mg, respectively. In the case of the microsomal RNA, this is known to be an under-recovery because of the large numbers of membrane-bound polyribosomes which sediment with the nuclear-mitochondrial pellet during the preparation of the PMS [8]. Using oligo(dT)-cellulose, poly(A)-containing RNA was isolated from the total RNA of the microsomal and post-ribosomal fractions. Some 10–12 mg mRNA were recovered from the microsomal fraction and 1.5–1.7 mg mRNA from the post-ribosomal fraction. Thus 87% of the total poly(A)-containing RNA in the PMS occurs in the form of polyribosomes and 13% as free mRNA. Although this is an underestimation of the proportion of polyribosomal mRNA of the cytoplasm for the reason given above, the procedure appears to be reasonably reproducible. Rats made nephrotic by treatment with puromycin aminonucleoside showed similar relative amounts of total RNA and poly(A)-containing RNA in the microsomal and post-ribosomal fractions.

The albumin mRNA content of these two preparations of poly(A)-containing RNA was assayed by the procedure shown [3] to yield pure albumin after incubation of the mRNA in a wheat germ system followed by immunoprecipitation and recovery of the albumin on SDS–polyacrylamide gradient gels. As shown in fig.1, both microsomal and post-ribosomal mRNA preparations direct the synthesis of albumin in the wheat germ system. If ribosomes carrying nascent peptide chains are not spun down at the end of the wheat germ incubation, much of the material recovered from the albumin antibody precipitate consists of incomplete peptide chains (fractions 1–30 in fig.1). If the ribosomes are removed by centrifuging at the end of wheat germ incubation (45 000 rev./min in the Ti-50 rotor for 60–90 min), immunoprecipitation from the supernatant fraction yields only one peak of radioactivity co-migrating with carrier albumin. In order to obtain the maximum capacity for albumin synthesis, we have used the first

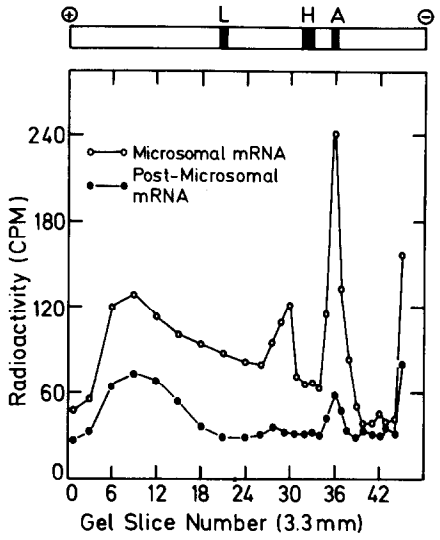

Fig.1. In vitro synthesis of nascent and released albumin chains by mRNA prepared from rat liver microsomal and post-ribosomal fractions. The mRNA samples were incubated with wheat germ S_{30} fraction in the presence of [^{3}H]leucine [2], carrier albumin was added and the albumin precipitated by antialbumin serum. Rat serum albumin (4 μg) was added to 50 μl incubation mixtures, which contained total trichloroacetic acid-precipitable material of 256 000 cpm for the microsomal mRNA incubation (o——o) and 217 000 cpm for the postribosomal mRNA incubation (•——•). Incubation for immunoprecipitation was for 60 min at 30°C and overnight at 4°C. Purification and SDS–gel analysis of the immunoprecipitates were performed as in [1]. *Abbreviations:* L, L-chain; H, H-chain of immunoglobin; A, albumin.

Table 1
Albumin and ferritin synthesis in a cell-free system containing wheat germ S_{30} and liver microsomal or postribosomal mRNA from normal or nephrotic rats

mRNA	Incorporation/Incubation		Albumin or ferritin / Total protein × 100
	Radioactivity in total protein (cpm)	Radioactivity in albumin (cpm)	
Albumin (normal)			
Microsomal	104 000	1660	1.6 ± 0.12
Post-ribosomal	45 000	137	0.3 ± 0.15
Albumin (nephrotic)			
Microsomal	108 500	2670	2.5 ± 0.19
Post-ribosomal	38 000	76	0.2 ± 0.10
Ferritin			
Microsomal	228 900	206	0.09 ± 0.01
Post-ribosomal	157 200	613	0.39 ± 0.05

Values refer to mean ∓ SEM of 12 cell-free incubations involving 6 different mRNA-preparations for each type of mRNA

Table 2
Distribution of total mRNA, albumin mRNA and ferritin mRNA in polysomal (microsomal) and free (post-ribosomal) forms in the post-mitochondrial supernatant PMS of rat liver

mRNA	mRNA content		
	Polysomal (%)	Free (%)	Total PMS (%)
Total mRNA			
Distribution in PMS[a]	87	13	100
Albumin mRNA			
% of total mRNA in fraction[b]	1.6	0.3	–
% of total mRNA in PMS[c]	1.4	0.04	1.44
Distribution in PMS[d]	97	3	100
Ferritin mRNA			
% of total mRNA in fraction[b]	0.09	0.39	–
% of total mRNA in PMS[c]	0.08	0.05	0.13
Distribution in PMS[d]	61	39	100

[a] See text
[b] From table 1
[c] Obtained by multiplying % of mRNA in fraction by total mRNA in fraction (e.g., albumin mRNA accounts for 0.3% of the free mRNA, which is 13% of total mRNA in PMS, so that free albumin mRNA is 0.04% of total mRNA in PMS)
[d] Relative proportions of albumin and of ferritin mRNA in PMS, based on preceding line in table

alternative and have counted the radioactivity recovered both under the albumin peak and in the nascent chains (table 1). In some studies not reported here, the second procedure was used and gave a similar relative distribution of albumin mRNA between microsomes and cell sap.

With the above system, uptake of radioactivity into albumin was 1.6% of the total protein radioactivity using the microsomal RNA preparation, and 0.3% for the post-ribosomal RNA preparation of normal rats (table 1). For nephrotic rats, the proportions were 2.5% and 0.2%, respectively. The greater relative incorporation into albumin by the microsomal mRNA of the nephrotic animals agrees with our findings [3]. Thus, in comparison with microsomal mRNA, the post-microsomal mRNA has a low albumin mRNA content which is not significantly affected by the increased albumin synthesis resulting from nephrosis (table 1). The distribution of ferritin mRNA was quite different from that of albumin mRNA (table 1). Ferritin accounted for only 0.09% of the total incorporation by microsomal mRNA in the wheat germ system, whereas it represented 0.39% of incorporation programmed by post-ribosomal mRNA. This agrees with our estimates [2] of distribution of ferritin mRNA.

From these data, and the distribution of total mRNA in the PMS, we can calculate the relative amounts of albumin mRNA and ferritin mRNA in the microsomal and post-microsomal fractions (table 2). Albumin mRNA accounts for 1.44% of the total mRNA in the PMS with 97% located in the microsomal (polyribosomal) fraction and 3% in the free mRNA of the cell sap. Since many of the membrane-bound polyribosomes are lost in the nuclear-mitochondrial pellet during preparation of the PMS, this is an underestimate of the disproportion in distribution. In contrast, similar calculations for ferritin mRNA indicate that the microsomes contribute only 61% of the total amount of this mRNA species in the PMS, while the cell sap provides 39%. Thus the distributions of albumin mRNA and of ferritin mRNA between microsomes and cell sap are quite different. We have shown [2] that iron administration increases the microsomal ferritin mRNA at the expense of the post-ribosomal free ferritin mRNA which suggests that this pool of ferritin mRNA functions as a reserve for emergency purposes. Albumin does not have a significant cytoplasmic reserve of free mRNA, and the increased microsomal albumin mRNA occurring in the livers of nephrotic rats must therefore be derived from the nucleus.

Acknowledgements

This investigation was supported by grant no. 15364–05 and by a research fellowship from the Deutsche Forschungsgemeinschaft to J. Zähringer.

References

[1] Zähringer, J., Baliga, B. S. and Munro, H. N. (1976) Biochem. Biophys. Res. Commun. 68, 1088–1093.
[2] Zähringer, J., Baliga, B. S. and Munro, H. N. (1976) Proc. Natl. Acad. Sci. USA 73, 857–861.
[3] Zähringer, J., Baliga, B. S. and Munro, H. N. (1976) FEBS Lett. 62, 322–325.
[4] Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408–1412.
[5] Roberts, B. E. and Paterson, B. M. (1973) Proc. Natl. Acad. Sci. USA 70, 2330–2334.
[6] Mans, R. J. and Novelli, G. D. (1961) Arch. Biochem. Biophys. 94, 48–53.
[7] Zähringer, J., Konijn, A., Baliga, B. S. and Munro, H. N. (1975) Biochem. Biophys. Res. Commun. 65, 583–590.
[8] Blobel, G. and Potter, V. R. (1967) J. Mol. Biol. 26, 279–292.