CHARACTERIZATION OF A FREE CYTOPLASMIC RIBONUCLEOPROTEIN PARTICLE CARRYING MESSENGER-LIKE RIBONUCLEIC ACID

N. NIKOLAEV, H. WELFLE* and A.A. HADJIOLOV

*Section of Molecular Genetics, Institute of Biochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria and *Department of Cell Physiology, Central Institute of Molecular Biology, Academy of Science, Berlin-Buch, GDR*

Received 1 September 1972

1. Introduction

The mechanism of transport of messenger RNA from the nucleus into the cytoplasm is still controversial. Studies with liver [1–4] and other cells [5, 6] have shown that rapidly labelled RNA is associated with free ribonucleoprotein particles sedimenting mainly in the 45 S zone of density gradients (referred further as "45 S RNP"). These particles contain nascent 18 S rRNA and heterogeneous, messenger-like RNA (mlRNA). Two possibilities have been envisaged: (i) the "45 S RNP" represents the nascent smaller ribosomal subparticle carrying mlRNA [1, 5] and (ii) "45 S RNP" is a mixture of smaller ribosomal subparticles and protein-bound mlRNA, informosomes [7, 8]. All studies on the characterization of the "45 S RNP" were carried out by analyses of the rapidly labelled RNA species in this fraction. Here, we report results on the characteristics of both the RNA and the proteins of rat liver "45 S RNP". The obtained evidence shows that this RNP particle contains both 18 S rRNA and mlRNA. However, all the proteins are identified by 2D polyacrylamide gel electrophoresis as typical for the smaller ribosomal subparticle.

2. Methods and materials

The experiments were carried out with albino rats weighing 150 to 170 g. For the preparative isolation of RNP particles 30 to 60 rats were used. *In vivo* labelling of RNA was with 100 μCi per rat of [6-^{14}C] orotic acid for 90 min.

The animals were sacrificed, the livers removed, rinsed in saline and homogenized in 0.25 M sucrose, 0.05 M Tris-HCl (pH 7.5), 0.025 M KCl, 0.005 M $MgCl_2$ and 0.005 M β-mercaptoethanol. The homogenate was centrifuged at 17 000 *g* for 10 min. Free and membrane-bound polysomes were sedimented at 100 000 *g* for 60 min. The supernatant was centrifuged at 100 000 *g* for 4 hr. The sediment contains all postmicrosomal RNP (only 4 S RNA is found in the supernatant). The RNP sediment was suspended in the same buffer and:

(a) 150 ml were layered on 800 ml of 7.4 to 38% sucrose density gradient in the same buffer and centrifuged for 3 hr at 35 000 rpm (20°) in the B XV Ti rotor. The shape of the gradient was according to [9];
(b) fractionated in a 50 ml 10 to 30% linear sucrose gradient run for 17 hr at 18 000 rpm (20°) in the Spinco SW 25.2 rotor;
(c) fractionated in 14 ml 10 to 30% linear sucrose gradient run for 3 hr at 40 000 rpm (20°) in the Spinco SW 40 rotor.

Lipoprotein contaminants were removed from the postmicrosomal RNP fraction or from the 45 S zone of sucrose gradients by treatment with 1% Na deoxycholate plus 2% Brij 35, followed by recentrifugation in one of the above gradients.

The reference smaller ribosomal subparticle was obtained from polysomes by KCl-puromycin dissociation as described previously [10].

The proteins were extracted from the RNP fractions by 0.25 N HCl plus 0.005 M β-mercaptoethanol for 30 to 60 min and precipitated with 5 vol acetone at

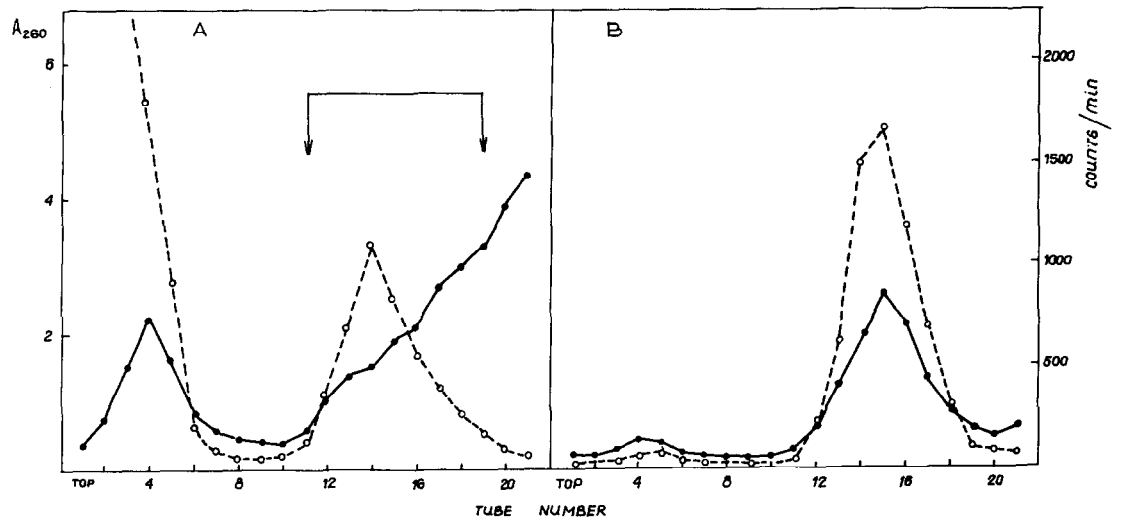


Fig. 1. Sucrose density gradient fractionation of postmicrosomal ribonucleoproteins. Left – total postmicrosomal RNP from the liver of rats labelled for 90 min *in vivo* with [^{14}C]orotate. Right – Ribonucleoproteins from the 45 S region (pooled fractions designated by arrows) after detergent treatment (1% Na deoxycholate plus 2% Brij 35) and recentrifugation. (●–●–●), A_{260}; (○—○—○), radioactivity. For details see text.

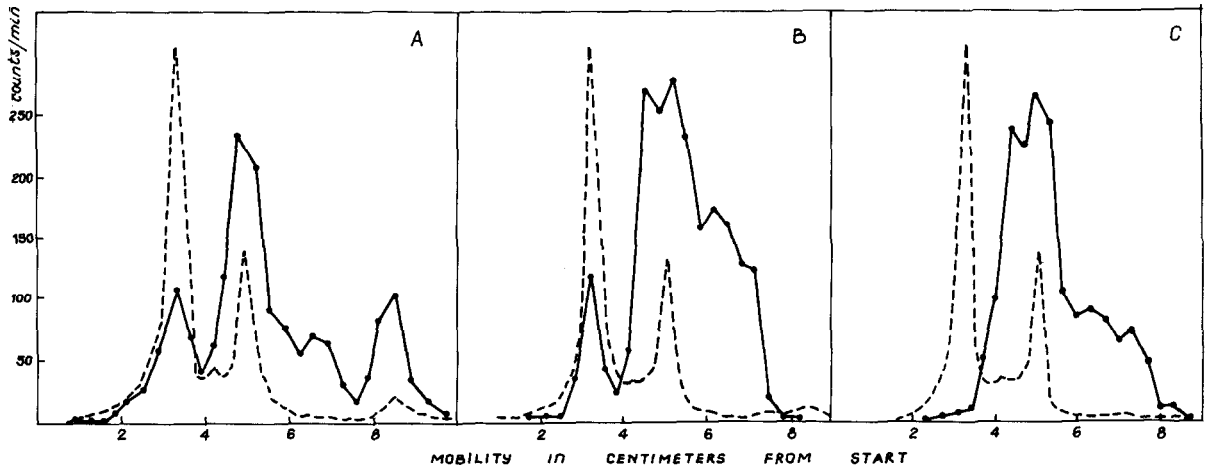


Fig. 2. Agar gel electrophoresis pattern of rat liver rapidly labelled RNA from: (A) polysomes; (B) postmicrosomal ribonucleoproteins and (C) 45 S RNP isolated from total postmicrosomal ribonucleoproteins by detergent treatment and subsequent sucrose density gradient centrifugation. The dotted line represents the A_{260} pattern of marker 28 S plus 18 S rRNA.

−20° for 3 to 4 hr. The 2D polyacrylamide gel electrophoresis was carried out according to the technique of Welfle [11, 12] with the ionic strength of the buffer doubled in the first dimension gel.

Appropriate dilutions of gradient fractions were counted with the use of a toluene–Triton X-100 (2:1)-PPO-DimethylPOPOP phosphor in a Packard Tricarb 3000 spectrometer. The extraction and the agar gel electrophoretic analysis of total and labelled RNA from the RNP fractions is described elsewhere [4].

3. Results

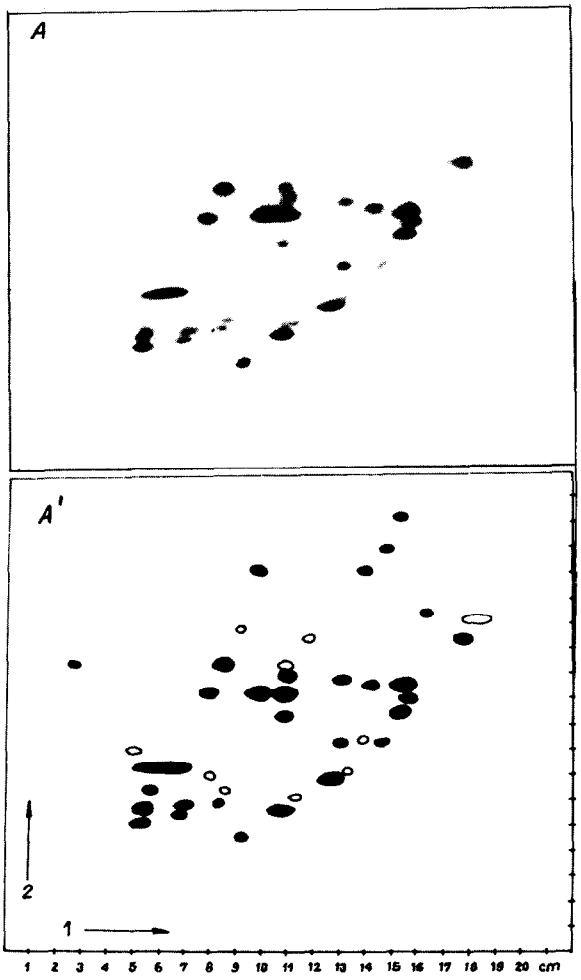
The sucrose density gradient analysis of the postmicrosomal RNP fraction (fig. 1.A) shows a very shallow A_{260} peak in the 45 S zone. However, the [^{14}C] orotate labelled material is more strictly localized in this region [2, 3]. Similar results were obtained upon fractionation of the postmicrosomal RNP material by zonal centrifugation. After detergent treatment of either the postmicrosomal RNP or the material from the 45 S region and recentrifugation, a homogeneous peak by both A_{260} and radioactivity is observed in the 45 S zone (fig. 1.B). These results show that removal of lipoproteins and other contaminants from the material in the 45 S region unmasks a homogeneous RNP peak without changing its sedimentation characteristics. The specific activity of the RNA extracted from the "45 S RNP" material is identical both before (2850 cpm per A_{260} unit) and after (2720 cpm per A_{260} unit) detergent treatment, thus showing that the latter does not remove any substantial amount of total or labelled RNA from the "45 S RNP".

Analysis of the RNA extracted from polysomes, total postmicrosomal RNP and "45 S RNP" (fig. 2) shows that at the labelling time studied, the label is located in both 28 S and 18 S rRNA (the labelling of 18 S rRNA prevailing markedly) and a heterogeneous, mlRNA in the zone between 6 S and 26 S. The RNA from the "45 S RNP" isolated prior or after detergent treatment yields identical agar gel electrophoresis patterns, the total amount of label being approximately equally distributed between 18 S rRNA and mlRNA.

The 2D polyacrylamide gel electrophoresis pattern (fig. 3) of the "45 S RNP" proteins reveals all the main spots typical of the smaller ribosomal subparticle. Two proteins with high mobility in both directions, not visible in the figure, were found in other runs at reduced running times [12]. The additional protein spots observed in the "45 S RNP" protein pattern are also typical modification products of the smaller ribosomal subparticle proteins, which can be seen in some preparations, especially upon extended periods of RNP isolation and extraction. Most likely they reflect alterations in the proteins of the smaller ribosomal subparticle occurring during the isolation procedure. All the proteins of the smaller ribosomal subparticle and the "45 S RNP" are basic with a relatively low molecular weight. No acidic proteins could be detected in either RNP particle.

4. Discussion

Our results clearly show that the free "45 S RNP" of rat liver cytoplasm contains both nascent 18 S rRNA and mlRNA. However, only the proteins typical of the smaller ribosomal subparticle could be detected. Considering the sensitivity of the technique (less than 5 μg of protein per spot), any single protein constituting more than about 1% of the "45 S RNP" proteins should have been detected. Therefore, if the presumed messenger RNP does exist in the "45 S RNP" its amount should be less than about 2% of this fraction. Our experiments do not rule out also the possibility that the presumed messenger RNP proteins are not extracted by our technique or that they coincide in position with the proteins of the smaller ribosomal subparticle. These possibilities remain open and require further studies. It has been shown recently [13, 14] that globin mRNA, released upon dissociation of reticulocyte ribosomes, is bound to distinct proteins which were isolated by SDS treatment of the mRNP complex. Yet, it remains to be seen whether these proteins are required for the binding of mRNA to the smaller ribosomal subparticle or they are structural components of free mRNP particles. The fact that the mild detergent treatment used in the present work does not remove total or labelled RNA from the "45 S RNP" indicates that the lipoproteins and other contaminants removed by this treatment are not required for the binding of liver mlRNA to the "45 S RNP". Several authors [15, 16] have demonstrated the artifactual formation of RNP particles with lower



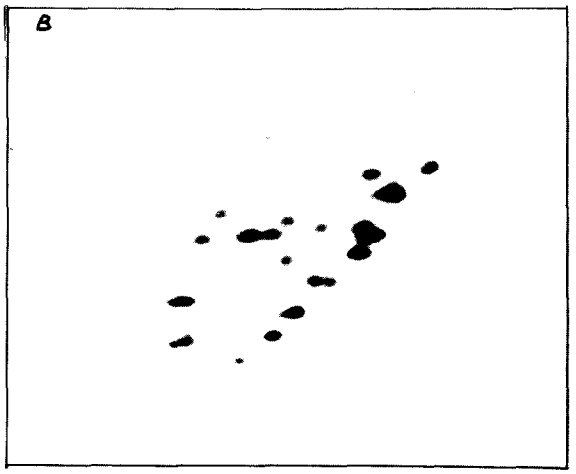


Fig. 3. Two-dimensional (2D) pattern of the proteins from: (A) and (A') – the 45 S RNP and (B) the smaller ribosomal subparticle derived from polysomes by KCl–puromycin dissociation. The scheme in (A') indicates the position of the main protein spots observed in (A). The open circle spots are modification products typical for the smaller ribosomal subparticle. The arrows in (A') indicate the direction of the first (1) and second (2) polyacrylamide gel electrophoresis runs.

buoyant density in CsCl gradients upon incubation or mixing of ribosomal subparticles or rapidly labelled RNA with cytoplasmic proteins. Therefore, the observation of particles with lower buoyant density [6–8] does not prove unequivocally the existence of free messenger RNP. Our present results are more in line with the earlier suggestion that the nascent smaller ribosomal subparticle carries mlRNA [1, 5] and support the concept of a more direct involvement of the ribosome in the nucleo-cytoplasmic transfer of messenger RNA [17, 18].

Acknowledgement

The authors are greatly indebted to Prof. Dr. H. Bielka for his continuous interest and helpful suggestions.

References

[1] E.C. Henshaw, M. Revel and H.H. Hiatt, J. Mol. Biol. 14 (1965) 241.
[2] E.C. Henshaw and J. Loebenstein, Biochim. Biophys. Acta 199 (1970) 405.
[3] B.M. Mullock, R.H. Hinton, M. Dobrota, D. Froomberg and E. Reid, European J. Biochem. 18 (1971) 485.
[4] A.A. Hadjiolov, N. Nikolaev and A. Shulga, Intern. J. Biochem., in press.

[5] E.H. McConkey and J.W. Hopkins, J. Mol. Biol. 14 (1965) 257.
[6] P. Tiollais, F. Galibert and M. Boiron, European J. Biochem. 18 (1971) 35.
[7] A.S. Spirin, European J. Biochem. 10 (1969) 20.
[8] G. Spohr, N. Garnboulan, C. Morel and K. Scherrer, European J. Biochem. 17 (1970) 296.
[9] E.F. Eikenberry, T.A. Bickle, R.R. Traut and C.A. Price, European J. Biochem. 12 (1970) 113.
[10] J. Stahl, H. Welfle, K. Dressler and H. Bielka, Acta Biol. Med. Germ. 28 (1972) 451.
[11] H. Welfle, Acta Biol. Med. Germ. 27 (1971) 211.
[12] H. Welfle, J. Stahl and H. Bielka, FEBS Letters 26 (1972) 228.
[13] B. Lebleu, G. Marbaix, G. Huez, J. Temmerman, A. Burny and H. Chantrenne, European J. Biochem. 19 (1971) 264.
[14] G. Blobel, Biochem. Biophys. Res. Commun. 47 (1972) 88.
[15] J. Kempf, D. Popovic and P. Mandel, FEBS Letters 9 (1970) 41.
[16] D.M. Woodcock and J.N. Mansbridge, Biochim. Biophys. Acta 240 (1971) 218.
[17] A.A. Hadjiolov, Progr. Nucleic Acid Res. Mol. Biol. 7 (1967) 195.
[18] H. Harris, Nucleus and Cytoplasm, Oxford Univ. press (1968).