A COMPARATIVE STUDY OF THE PROTEIN COMPONENTS OF NUCLEAR AND POLYSOMAL MESSENGER RIBONUCLEOPROTEIN

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1. Introduction

It was shown previously that newly synthesized DNA-like RNA ("D-RNA") is liberated from chromatin associated with globular protein particles designated informofers i.e. in the form of specific ribonucleoprotein (RNP) complexes [1, 2]. In the cytoplasm, m-RNA occurs in two forms: as free m-RNA designated informosomes [3, 4] or as m-RNP associated with polysomes [5, 6].

It was shown that a portion of the D-RNA complexed with protein in nuclear ribonucleoprotein particles is transferred into cytoplasm and corresponds to true mRNA [7–9]. However, it is not known whether the informofers as a protein unit are engaged in the transport of mRNA through the nuclear membrane. The direct answer to this question may be obtained by comparing the proteins of nuclear and cytoplasmic RNP particles. It was found [2, 10] that the nuclear RNP particles contain only one main protein component with a molecular weight of approximately 40,000. Each informofer contains a number of such apparently identical subunits; they have been shown to be an integral part of the nuclear 30 S RNP particle, rather than a fortuitously bound contaminant [11]. In the absence of mercaptoethanol two very characteristic additional components are formed from the main component by means of S–S bridges. Recently it was shown that polysomes contain proteins which are very similar to nuclear proteins in their mobility in polyacrylamide gels [12–14]. However, in these experiments the authors could not exclude the contamination of polysomes by nuclear material arising from nuclear leakage during homogenization and centrifugation. For this reason, in the present study, the proteins of polysomal mRNP from rabbit reticulocytes were compared with those of nuclear RNP. Reticulocytes do not contain nuclei and consequently polysomes cannot be contaminated with nuclear material. Another advantage of this system is that reticulocyte polysomes are very homogeneous and after their dissociation with EDTA mRNA is released in the form of a homogeneous ribonucleoprotein of sedimentation constant approx. 14 S [15]. This mRNP can be isolated and studied free from ribosomal subunits, and contains two protein components [16]. Here the protein components associated with mRNA in nuclear RNP and in polysomes were isolated and compared. The electrophoretic mobility of one of

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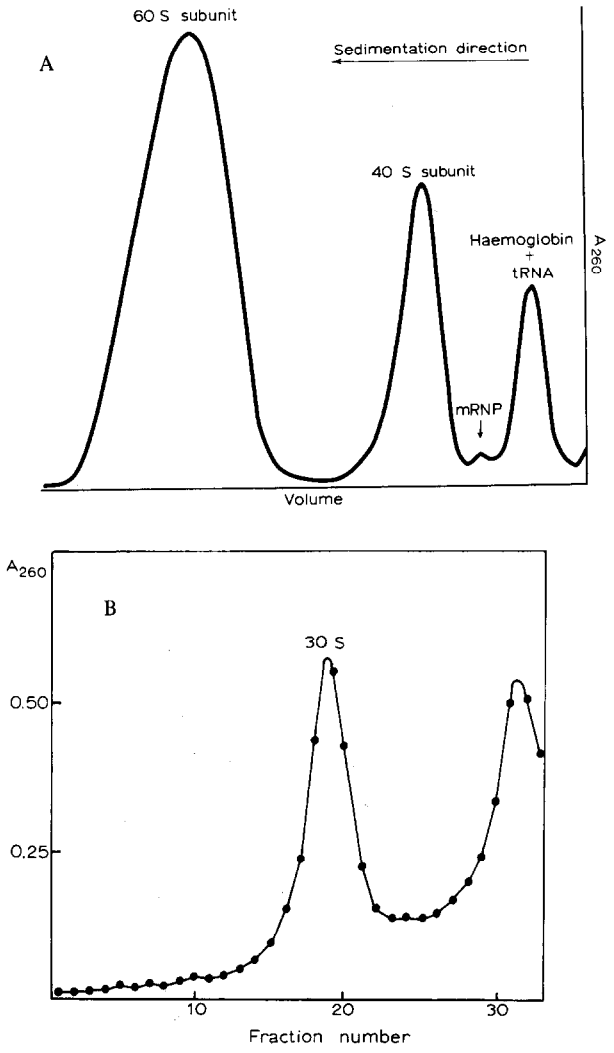

Fig. 1. (A) Sedimentation profile of EDTA-treated reticulocyte polysomes. 14 S RNP fractions were collected for protein analysis. 10–30% sucrose gradient, 42,000 rpm, 18 hr, 4°. (B) Sedimentation profile of nuclear extracts obtained according to Samarina et al. [1]. The 30 S D-RNP fractions were collected for protein analysis.

the cytoplasmic components was similar but not identical to the single nuclear component.

2. Materials and methods

The cytoplasmic ribonucleoprotein was prepared by the method of Chantrenne et al. [15] as modified by Williamson et al. [17]. Approximately 50 ml packed reticulocytes, obtained from phenylhydrazine-treated rabbits, were lysed in 0.001 M $MgCl_2$, and polysomes were isolated. These were suspended in 0.05 M KCl, 0.001 M Tris pH 7.5 at a concentration of approximately 5 mg/ml, and a half-volume of 0.1 M EDTA, pH 7.0 was added. The EDTA-treated polysomes were then centrifuged on a 10–30% sucrose gradient in either the B-XIV or B-XV Zonal rotor (M.S.E. Ltd., Crawley, Sussex, England) for a sufficient time to separate the 14 S messenger ribonucleoprotein peak clearly from the 40 S ribosomal subunits and also from the mixture of tRNA and haemoglobin remaining at the top of the gradient (fig. 1A). 14 S mRNP fractions from three runs were pooled and concentrated by dialysis against 0.05 M KCl, 0.001 M Tris, pH 7.5. Approximately 2.5 mg 14 S mRNP were obtained from 200 mg polysomes starting material. The 14 S mRNP is slightly contaminated by haemoglobin but uncontaminated by 40 S ribosomal subunit material, as demonstrated by the absence of 18 S RNA from material analysed on polyacrylamide gels. All buffers and sucrose solutions were pre-treated with diethylpyrocarbonate ("Baycovin", a gift from Bayer Chemicals, Ltd., London) before use to inactivate ribonucleases; this was essential for reproducible results.

The nuclear D-RNP in the form of 30 S particles was obtained from rat and rabbit liver and mouse Ehrlich ascites carcinoma cells as described previously [1] (fig. 1B). For the isolation of protein from nuclear [18] and cytoplasmic mRNP particles two techniques were used: (1) Solutions containing the particles were pre-treated with pancreatic RNase (20–50 μg/ml at 30° for 15–30 min). The proteins were precipitated by 10% trichloroacetic acid in the cold and collected by centrifugation, washed with 70% ethanol and dried. The precipitate was dissolved in 0.1–0.2 ml of 10% sucrose, 6 M urea, 0.06 N KOH neutralized with acetic acid to pH 6.8, and treated with 0.14 M mercaptoethanol at 30° for 2 hr. (2) Solutions containing the particles were dialysed against 6 M urea, 0.01 M Tris, pH 7.2, and applied to a DEAE-cellulose (Chromedia DE-32) column. In these conditions the RNA of nuclear particles is adsorbed and 95% of the proteins is not retained. With cytoplasmic particles only about half of the

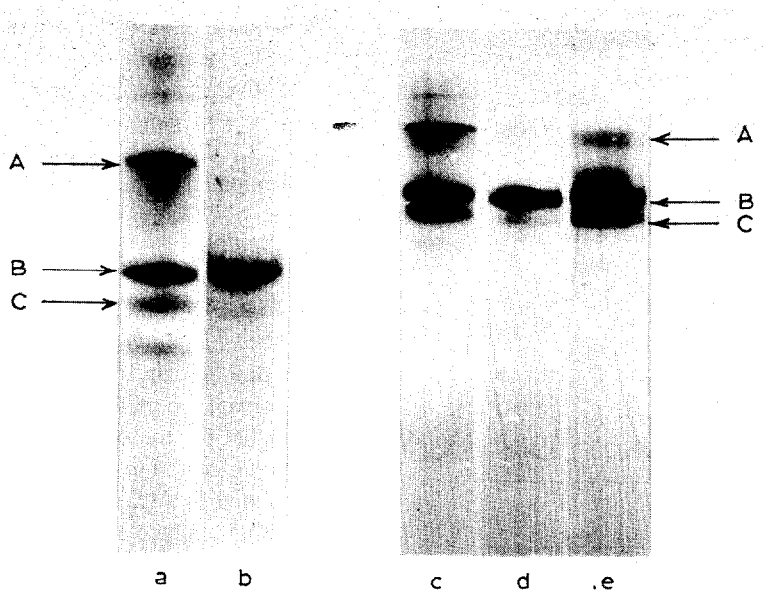

Fig. 2. Electrophoresis of nuclear and cytoplasmic proteins in polyacrylamide gel. a,b: Proteins of nuclear 30 S RNP particles from rat liver before (a) and after (b) mercaptoethanol treatment. c, d, e: Proteins of nuclear 30 S RNP particles from rabbit liver (c), rat liver (d), and mouse Ehrlich ascites carcinoma cells (e) without mercaptoethanol treatment.

protein passed through the column. Another part was retained on the column and was then eluted by urea containing 2 M NaCl. All protein solutions were dialysed against 1 M acetic acid and lyophilised. Analyses of proteins were performed by electrophoresis in polyacrylamide gel in 6 M urea, pH 4.5 [19, 20].

3. Results and discussion

As the reticulocytes do not contain nuclei, the comparison of proteins from cytoplasmic mRNP was carried out with the proteins from nuclear RNP of other tissues. Therefore, it was necessary to confirm that nuclear proteins from different tissues were similar. The proteins of nuclear D-RNP particles obtained from rat and rabbit liver and from mouse Ehrlich ascites carcinoma cells were analysed. One can see from fig. 2c, d, e that the nuclear proteins isolated from these sources have exactly the same characteristic distribution: three main bands before (fig. 2a), and one main band after (fig. 2b), mercaptoethanol treatment. The exact coincidence of the bands is clearly visible using a split gel (fig. 3a). Thus neither tissue nor species specificity was found and one can conclude that in the nuclei of different tissues D-RNA is combined with protein components of identical mobility. Therefore a comparison of proteins isolated from different organs seems to be valid.

In contrast to nuclear proteins, proteins isolated from 14 S mRNP show a heterogeneous distribution in polyacrylamide gel electrophoresis. Among a number of minor components two prominent bands can be recognized (fig. 3d). Their positions are very similar to those found for proteins extracted with DOC from rat polysomes [12] and for rabbit reticulocytes [16]. The mobility of one of these bands is similar but not identical to that of the main protein component of informofers. This is clearly visible on split gels (fig. 3b). The nuclear and rapidly migrating polysomal components may also be resolved when mixed together at low concentration (fig. 3c). In contrast to nuclear proteins the patterns of polysomal proteins do not differ after mercaptoethanol treatment. A prominent diffuse band with the mobility of haemoglobin was also found (fig. 3d, h) in amounts depending on the quality of the preparation.

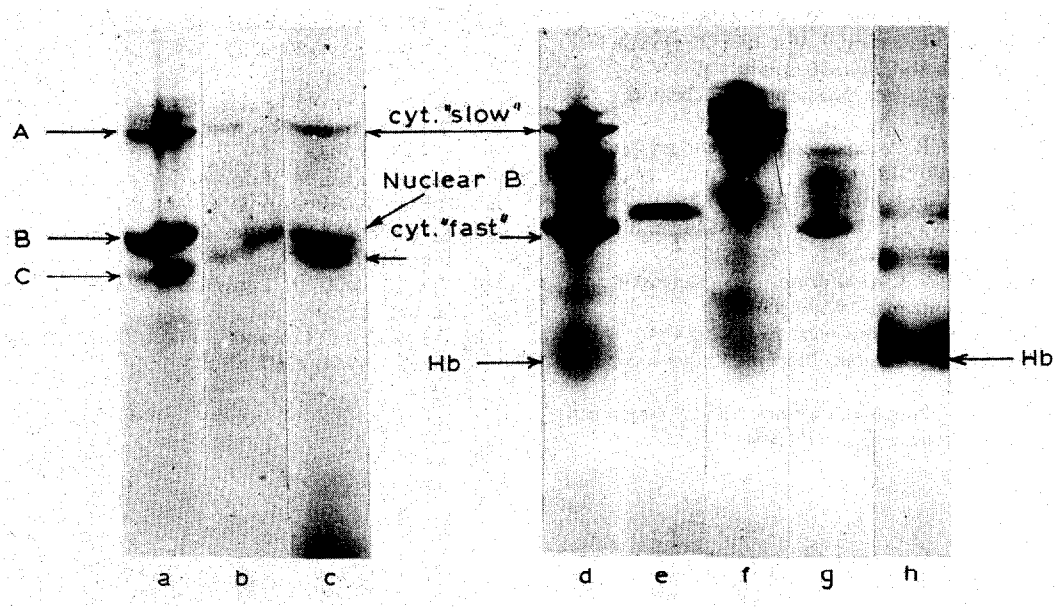

Fig. 3. (a) Split gel of proteins of nuclear 30 S RNP particles from rat and rabbit liver. (b) Split gel of proteins from nuclear 30 S RNP and polysomal mRNP particles. (c) Mixture of proteins of nuclear 30 S RNP particles with proteins of polysomal mRNP. (d) Proteins of cytoplasmic 14 S mRNP particles. (e) Proteins of nuclear 30 S RNP particles from rabbit liver. (f) Fraction of cytoplasmic proteins eluted from DEAE-cellulose, same conditions as nuclear proteins. (g) Fraction of cytoplasmic proteins eluted from DEAE-cellulose by urea 2 M NaCl. (h) Haemoglobin treated in the same way as mRNP particles. All samples except (a) were treated with mercaptoethanol.

In experiments with DEAE-column fractions the two main polysomal proteins were separated. The slowly migrating component is not retained by the column (fig. 3f), while the rapidly migrating component is eluted only with high salt concentration (fig. 3g). The difference may depend upon a higher stability of the complexes between the proteins of the fast component and mRNA. Thus in this respect the fast component again differs from the nuclear proteins.

One can conclude that the proteins combined with D-RNA (which includes mRNA) in the nucleus differ from those in polysomal mRNP, although the electrophoretic properties of some of the messenger-associated polysomal proteins are rather similar to those of nuclear proteins. This similarity may explain why other authors who have found this protein in polysomal mRNP described it as identical to the nuclear one.

If 14 S mRNP is a true native form of polysomal mRNP, one can conclude that the protein component of nuclear RNP is not incorporated with the mRNA into polysomes, and that in all probability the informofers participate only in nuclear mRNA transport and do not pass from nucleus to cytoplasm.

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