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Translational active mRNPs from rabbit reticulocytes are qualitatively different from free mRNA in their translatability in cell-free system

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The translatability of polyribosomal and free mRNPs from rabbit reticulocytes and their mRNA was compared. Both classes of mRNPs turned out to be active in rabbit reticulocyte lysates. Considerable differences between mRNPs and mRNA have been revealed. The most striking feature of mRNPs was that high concentrations of mRNPs do not inhibit protein biosynthesis, whereas high concentrations of mRNA strongly inhibit this process. This inhibition is specific for mRNA and does not occur at the addition of the same amount of rRNA from *E. coli*. The features of mRNP translation are not the result of addition of the supplementary translation factors within particles. The specific function of mRNP proteins in the process of translation is under discussion.

Ribonuclear protein; mRNA; Reticulocyte; Translation; (Rabbit)

1. INTRODUCTION

It is well known that mRNA exists in eukaryotic cell in the form of ribonucleoprotein particles (mRNPs or informosomes) [1]. Cytoplasmic mRNPs can exist in a free form (free mRNPs) and as a part of polyribosomes (polyribosomal mRNPs) [1–3]. Free cytoplasmic mRNPs can contain both mRNA, in equilibrium with that of polyribosomes [4], and masked mRNA [5–9]. Some investigators found translation factors within polyribosomal mRNPs [3,5,10,11]. However, these factors can represent only minor components among mRNP proteins [12]. The comparison of translatability of polyribosomal mRNPs with their mRNA practically did not show any differences [6,13–16].

In this work we compared the translation of polyribosomal mRNPs from rabbit reticulocytes with the free mRNA from these particles in a rabbit reticulocyte cell-free system in a wide range of template concentrations and revealed the qualitative differences between mRNA and mRNPs. The translational activity of free mRNPs from rabbit reticulocytes was similar to that of polyribosomal mRNPs. This means that free mRNPs from rabbit reticulocytes can be considered as translationally active mRNPs in equilibrium with polyribosomal mRNPs.

2. MATERIALS AND METHODS

Reticulocytosis was induced in rabbits by injection of phenylhydrazine [17]. Lysis of the reticulocytes and separation of the lysate was performed as in [18]. Polyribosomal and free mRNPs were isolated by chromatography on oligo(dT)-cellulose as in [10] except that binding of mRNPs with resin was done at 150 mM NaCl. Eluted mRNPs were pelleted by centrifugation at 45000 rpm for 12 h at 4°C (Beckman rotor Ti 60) and dissolved in buffer 10 mM TEA-HCl, pH 7.8. The mRNA was isolated from mRNPs and the rRNA from *E. coli* ribosomes by extraction with a SDS/phenol/chloroform mixture [20], precipitated with 2.5 vols of ethanol, washed 5-fold with ethanol and dissolved in buffer 10 mM TEA-HCl, pH 7.8. The concentration of free RNA and RNA within the mRNPs was determined spectrophotometrically taking 1 unit A_{260} RNA = 1 unit A_{260} mRNPs = 44 μg of RNA. Centrifugation of formaldehyde-fixed mRNPs in CsCl gradients was done as in [21]. The amount of protein was determined by staining with amido black on nitrocellulose filters [18,22]. SDS gel electrophoresis of proteins was done according to the method in [23]. Preparation of the cell-free translation system from lysates of rabbit reticulocytes was performed as described in [24].

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3. RESULTS AND DISCUSSION

Fig.1 shows the distribution of the preparations of polyribosomal and free mRNPs from rabbit reticulocytes in the CsCl gradient. Polyribosomal mRNPs had a buoyant density in CsCl of about 1.45 g/cm^3 corresponding to the protein/RNA weight ratio of approximately 2:1 [1,2]. Free mRNPs were mainly distributed in the CsCl zone with a density of 1.39 g/cm^3 corresponding to the protein/RNA weight ratio of approximately 3:1 [1,2]. The preparations of particles did not contain any free proteins with a buoyant density in CsCl of about 1.35 g/cm^3. Mainly globin mRNA was found in the preparations of RNA from both classes of mRNPs (data not shown).

The data in fig.2 show that polyribosomal mRNPs contain two major proteins with molecular masses of 78 and 50 kDa as well as some minor polypeptides. Several major proteins with molecular masses from 150

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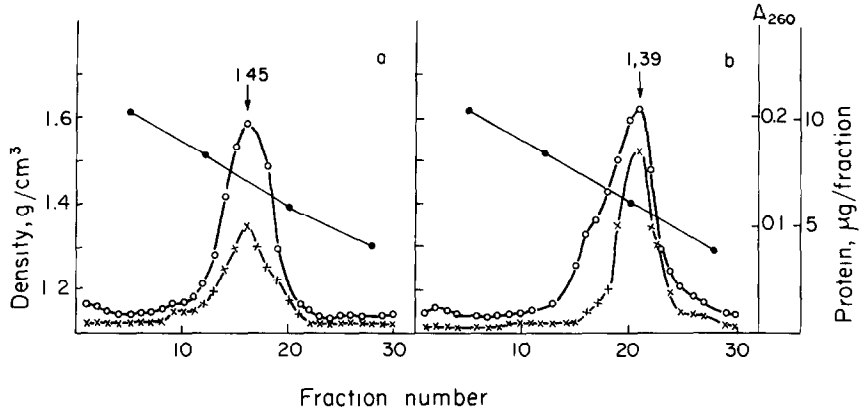

Fig.1. Density distribution in the CsCl gradient of preparations of polyribosomal mRNPs (a) and free mRNPs (b) from rabbit reticulocytes. Formaldehyde-fixed mRNPs were centrifuged in the preformed CsCl gradient in the presence of formaldehyde in a SW-55 rotor, Spinco L3-50 at 4°C, 36000 rpm for 36 h. (○—○) Absorbance at 260 nm; (●—●) CsCl density; (×—×) protein.

to 30 kDa as well as numerous minor components are present in free mRNPs. These results are in agreement with the published data [2,19].

Fig.3 shows that the nuclease-treated rabbit reticulocyte lysate becomes active in protein biosynthesis after the addition of cytoplasmic mRNPs of both classes. With the increase of the mRNPs concentration, the protein synthesizing activity reaches its maximal level and retains this level up to a mRNP concentration of 200 μg/ml. The activity of a cell-free system, depending on free mRNA concentration, has quite a different character. In low concentrations, free mRNA is translated more actively than mRNA within mRNPs. However, with the increase of the free mRNA concentration, the activity of the system is only 50–80% of the level of the maximal activity of the system in the presence of mRNPs; at a further increase of the free mRNA concentration the activity of the system sharply decreases and becomes practically inactive at an mRNA concentration of 200 μg/ml.

Thus, it follows: (i) both classes of cytoplasmic mRNPs from rabbit reticulocytes, the polyribosomal and free mRNPs, are active in protein biosynthesis in a cell-free system from rabbit reticulocytes; (ii) high concentrations of mRNA within mRNPs do not inhibit the translation, whereas the same concentrations of deproteinized mRNA strongly inhibit protein biosynthesis.

Inhibition of protein biosynthesis by an excess of mRNA [25,26] as well as high concentrations of rRNA [27] and polynucleotides [28,29] has already been described. It has been shown that RNA and polynucleotides inhibit the process of translation at the step of initiation [27,28]. It has been suggested that these compounds inhibit protein biosynthesis by interacting with some initiation factors [27–29], in particular with eIF-2 [26]. Our results show that mRNA inhibits protein biosynthesis much more effectively than rRNA from *E. coli* (fig.4). This testifies to a specific binding between the mRNA and the translation

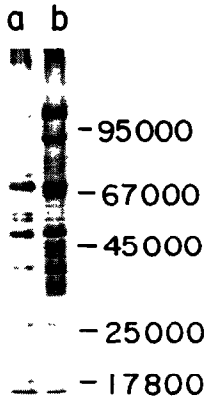

Fig.2. SDS gel electrophoresis of polyribosomal (a) and free mRNPs (b) proteins from rabbit reticulocytes.

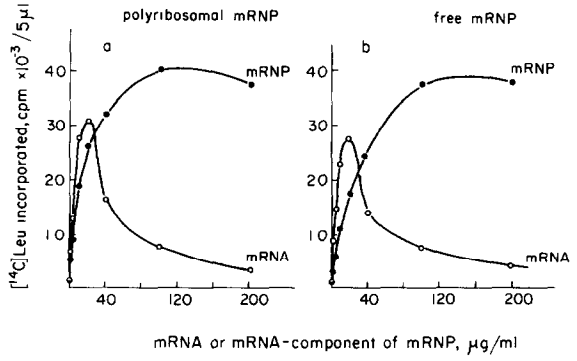

Fig.3. Translation of polyribosomal mRNPs (a) and free mRNPs (b) from rabbit reticulocytes in a cell-free system of protein synthesis from rabbit reticulocytes. (●—●) mRNPs; (○—○) mRNA, isolated from the corresponding class of mRNPs. Cell-free translation systems were incubated for 1 h at 37°C. 5 μl of the incubation mixture were taken for analysis.

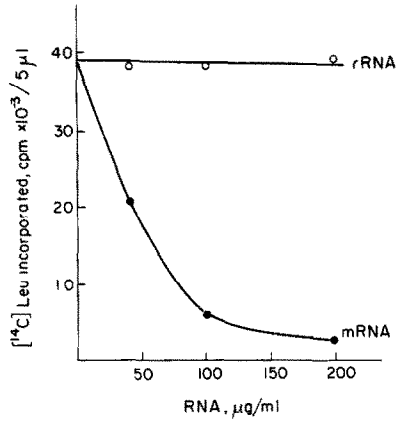

Fig.4. Effect of mRNA from polyribosomal mRNPs (●—●) and rRNA from *E. coli* (○—○) on protein biosynthesis in the cell-free system from rabbit reticulocytes. Various amounts of mRNA and rRNA were added to the cell-free system containing 20 µg/ml mRNA. The cell-free translation systems were incubated for 1 h at 37°C. 5 µl of the incubation mixture were taken for analysis.

factors which limit protein biosynthesis under an excess of templates. mRNPs proved to be unable to overcome the inhibition of the cell-free system induced by an excess of free mRNA (table 1). This result indicates that mRNPs did not contain any detectable amount of the translation factors limiting protein biosynthesis under an excess of mRNA.

On the grounds of these results we believe that cytoplasmic mRNP proteins play a specific role in the process of translation. These proteins organize the mRNA structure, shielding it from an occasional interaction with the translation factors. This may result in a more effective usage of the translation factors in protein biosynthesis. This can explain the revealed differences in translation of mRNPs and mRNA, in particular the fact that high concentrations of mRNPs, unlike those of mRNA, do not inhibit protein biosynthesis.

Table 1

Effect of mRNA excess on mRNPs translation in a cell-free system of protein biosynthesis

RNA concentration (µg/ml)			[14C]Leucine incorporated (cpm/5 µl)
mRNA	Polyribosomal mRNPs[a]	Free mRNPs[a]	
100	–	–	7844
–	100	–	51234
–	–	100	47328
100	100	–	8698
100	–	100	9212
20	–	–	33156
200	–	–	3422
–	200	–	47050
–	–	200	45296
–	–	–	1364

[a] Concentration of the mRNA-component of mRNP is given (see section 2)

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REFERENCES

[1] Spirin, A.S. (1969) Eur. J. Biochem. 10, 20–35.
[2] Preobrazhensky, A.A. and Spirin, A.S. (1978) Prog. Nucleic Acid Res. Mol. Biol. 21, 1–37.
[3] Larson, D.E. and Sells, B.N. (1987) Mol. Cell. Biochem. 74, 5–15.
[4] Bag, J. and Sells, B.H. (1979) Eur. J. Biochem. 99, 507–516.
[5] De Herdt, E., Piot, E., Wahba, A. and Slegers, H. (1982) Eur. J. Biochem. 151, 455–460.
[6] Civelli, O., Vincent, A., Buri, J.F. and Scherrer, K. (1976) FEBS Lett. 72, 71–76.
[7] Jenkins, N.A., Kaumeyer, J.F., Young, E.M. and Raff, R.A. (1978) Dev. Biol. 63, 279–298.
[8] Geohegan, T., Cereghini, S. and Brawerman, G. (1979) Proc. Natl. Acad. Sci. USA 76, 5587–5591.
[9] Sinclair, G.D. and Dixon, G.H. (1982) Biochemistry 21, 1869–1877.
[10] Schmidt, H.P., Kohler, K. and Setyono, B. (1982) J. Cell. Biol. 98, 893–898.
[11] Gorlach, M. and Hilse, K. (1986) EMBO J. 5, 2629–2635.
[12] Minich, W.B., Klimjuk, V.I. and Ovchinnikov, L.P. (1985) Biokhim. (USSR) 50, 465–474.
[13] Sampson, J. and Borghett, A.F. (1972) Nature New Biol. 238, 200–202.
[14] Frienstein, G. and Blobel, G. (1974) Proc. Natl. Acad. Sci. USA 71, 3435–3439.
[15] Ernst, V. and Arnstein, H.R.V. (1975) Biochim. Biophys. Acta 378, 251–259.
[16] Chen, J.H., Lavers, G.C. and Spector, A. (1976) Biochim. Biophys. Acta 418, 39–51.
[17] Adamson, S.D., Herbert, E. and Godchaux, W. (1968) Arch. Biochem. Biophys. 125, 671–683.
[18] Ovchinnikov, L.P., Seriakova, T.A., Avanesov, A.T., Alzhanova, A.T. and Radzhabov, H.M. (1978) Eur. J. Biochem. 90, 517–525.
[19] Jain, S.K., Pluskal, M.G. and Sarkar, S. (1979) FEBS Lett. 97, 84–90.
[20] Belitsina, N.V., Ajtkhozhin, M.A., Gavrilova, L.P. and Spirin, A.S. (1964) Biokhim. (USSR) 29, 363–374.
[21] Ovchinnikov, L.P., Avanesov, A.T., Seriakova, T.A., Alzhanova, A.T. and Radzhabov, H.M. (1978) Eur. J. Biochem. 90, 527–535.
[22] Schaffner, W. and Weissmann, C. (1973) Anal. Biochem. 56, 502–514.
[23] Laemmli, U.K. (1970) Nature 227, 680–685.
[24] Pelham, H.R.B. and Jackson, R.J. (1976) Eur. J. Biochem. 67, 247–256.
[25] Gozes, I., Schmitt, H. and Littauer, U.Z. (1975) Proc. Natl. Acad. Sci. USA 72, 701–705.
[26] Barrieux, A. and Rosenfeld, M.G. (1978) J. Biol. Chem. 253, 6311–6314.
[27] Wettenhall, R.E.H. and Slobbe, A. (1976) Biochim. Biophys. Acta 432, 323–328.
[28] Lodish, H.F. and Nathan, D.G. (1972) J. Biol. Chem. 247, 7822–7829.
[29] Jacobson, A. and Favreau, M. (1983) Nucleic Acids Res. 11, 6353–6368.