NEW ASPECTS OF THE EUKARYOTIC RIBOSOMAL SUBUNIT INTERACTION

R. VILLEMS*, M. SAARMA*, A. METSPALU and I. TOOTS

**Laboratory of Molecular Genetics, Institute of Physics* and *Laboratory of Molecular Biology, Tartu State University, 14/16 Kingissepa Str., 202400 Tartu, Estonia, USSR*

Received 30 July 1979

1. Introduction

The molecular mechanism of ribosomal subunit interaction is perhaps one of the less understood aspects of current ribosomology. For prokaryotes, we have indirect data on the involvement of these or other ribosomal components in this interaction, as suggested from chemical modification and Fab inhibition studies (eg. [1,2]). Even less is known about the eukaryotic ribosomes. Therefore recent reviews of eukaryotic ribosome research scarcely deal with this problem [3,4].

Experiments with immobilized 5.8 S RNA reveal the ability of 5.8 S RNA to bind 40 S subunit proteins [5–7].

Here we show that both 40 S subunit proteins and the subunits interact with the immobilized 5.8 S RNA. It is suggested that the 5 S RNA–protein complex may be involved in 40 S subunit binding.

2. Experimental

Rat liver ribosomes and ribosomal subunits were prepared as in [8]. The functional activity of 40 S subunits was checked by codon-directed Met-tRNA$_f^{Met}$ binding assay as in [9,10].

Extraction of ribosomal proteins, isolation of 5 S RNA and 5.8 S RNA was essentially as in [8]. Preparation of RNA–Sepharose gels was as in [11].

Affinity chromatography of 40 S ribosomal subunits on rat liver 5.8 S RNA and 5 S RNA immobilized to epoxy-activated Sepharose was performed in 10 mM Tris–HCl (pH 7.6) buffer containing 10–30 mM $MgCl_2$, 100 mM KCl and 6 mM 2-mercaptoethanol. 40 S subunits (0.1–0.2 mg) in 50–100 μl buffer were applied to the column at 7 ml/h flowrate. The column was washed with 20 vol. buffer and the bound subunits were eluted with buffer containing 1 M KCl and 5 mM EDTA. The chromatography was monitored by LKB Uvicord II UV-absorptiometer.

The pre-formed 5 S RNA–60 S subunit protein complex was prepared as follows: 2 ml 60 S subunit total proteins (0.4 mg/ml) was applied to the 5 S RNA column and washed with 5 vol. binding buffer. After that, the sample of 40 S subunits was passed through the column as above.

All experiments were carried out at 1–3°C. Other details are given in figure legends.

3. Results and discussion

The interaction of ribosomal subunits is evidently a very complex molecular event. So far, very little is known about its mechanism in eukaryotes.

Earlier we showed that a component of the rat liver 60 S ribosomal subunit, 5.8 S RNA, forms a complex not only with the proteins of the homologous subunit but also with proteins of the small subunit [5,6]. This result was confirmed in [7]. Obviously, it was intriguing to see whether this phenomenon has any functional significance. Figure 1a clearly demonstrates that the immobilized 5.8 S RNA gives a stable complex with rat liver 40 S ribosomal subunit. No complex was found between 60 S subunit and the immobilized 5.8 S RNA.

No complex was found between 40 S subunit proteins and the immobilized 5 S RNA [8,12]. Also in these studies no complex of 40 S particle with the

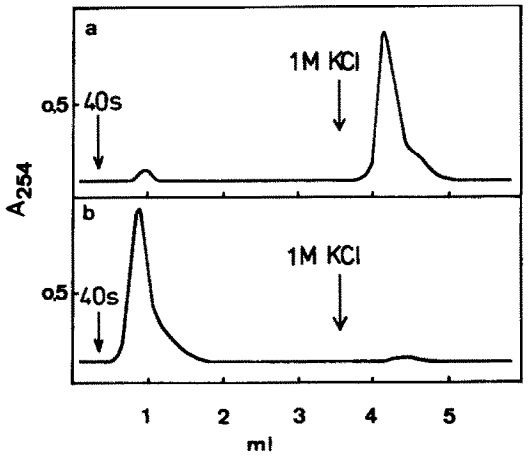

Fig.1. Affinity chromatography of rat liver 40 S subunits on the immobilized rat liver 5.8 S RNA (0.1 mg 5.8 S RNA in 0.4 ml gel). (a) 40 S subunits (0.1 mg) in 10 mM Tris–HCl buffer (pH 7.6) containing 100 mM KCl, 10 mM $MgCl_2$ and 6 mM mercaptoethanol (BB_{10}) were loaded onto 5.8 S RNA–gel, washed with 20 column vol. BB_{10} and eluted with 1 M KCl, 5 mM EDTA in 10 mM Tris–HCl buffer (pH 7.6) (EB). (b) Affinity chromatography of 40 S subunits (0.1 mg) on Sepharose column (0.5 ml) without RNA linked to the spacer group.

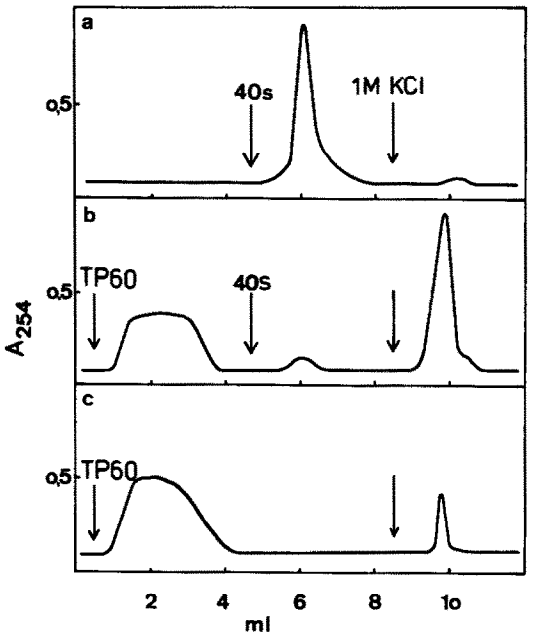

Fig.2. Affinity chromatography of rat liver 40 S ribosomal subunits on the immobilized rat liver 5 S RNA (0.12 mg in 0.5 ml gel). (a) 40 S subunits (0.1 mg) loaded to 5 S RNA–gel and eluted with EB, as in fig.1. (b) 40 S subunits (0.1 mg) loaded to the preformed 5 S RNA–60 S subunit protein complex. Total protein of 60 S subunits (TP60) (2 ml at 0.4 mg/ml) was passed through the column. After washing the column with 5 column vol. BB_{10}, a sample of 40 S subunits was applied to the column. The bound 40 S subunits and proteins were eluted with EB. (c) Optical profile of affinity chromatography of TP60 in BB_{10} on the immobilized 5 S RNA. Total protein (2 ml at 0.8 mg/ml) was passed through the column and eluted with BB_{10}.

immobilized 5 S RNA was observed (fig. 2a). Since Sepharose alone also did not bind 40 S subunits (fig. 1b) we conclude that the 5.8 S RNA–40 S subunit interaction is specific.

At given 40 S subunit and 5.8 S RNA concentrations, the plateau level of the subunit binding to the 5.8 S RNA column was reached when only ~12% of the bound RNA was coupled with the subunit (assuming 1:1 stoichiometry of the complex). Since no change of plateau was observed at higher subunit concentrations, this value probably reflects the accessibility of 5.8 S RNA and/or the conformationaly active fraction in the gel.

Freshly prepared 40 S subunits were fully active in binding to the 5.8 S RNA-affinity column. However, if stored at 2–4°C for a longer period, the bound fraction of 40 S subunits gradually decreased up to almost total loss of the binding capacity after a week. Although not analyzed yet, this loss is probably due to the enzymatic degradation of subunits.

As shown in [8], the complexes between 60 S subunit proteins with 5.8 S RNA, 5 S RNA and tRNA overlap. In particular, proteins L6, L7 and L18 were found in both 5.8 S RNA and 5 S RNA–protein complexes [8]. Here, from the interaction between 5.8 S RNA and 40 S subunit an indication of the proximity of these proteins to the subunit interface became evident. Hence, an experiment with preformed 5 S RNA–60 S subunit proteins was performed, to see whether this complex interacts with the 40 S subunit. The answer was yes (fig. 2b). Although it seems likely that proteins L6, L7, L8, L18 and L35, which are bound under these conditions to 5 S RNA [8], are responsible for the subunits sticking to the affinity column, the possibility of a direct contribution of 5 S RNA should also be considered.

The data presented above is to our best knowledge the first direct evidence of the involvement of certain eukaryotic ribosomal components in subunit interaction. It remains to be seen what might be the functional role of this interaction. However, what we want to stress here is the correlation of results obtained at the level of immobilized RNA–ribosomal protein interaction with more complex systems. This statement can be further illustrated by reference to *Escherichia coli* 5 S RNA. This RNA was found to interact not only with *E.coli* 50 S ribosomal subunit proteins, but also with a set of 30 S subunit proteins [13,14]. With a technique similar to that described above we found a stable tRNA-independent, binding of 30 S subunit to the immobilized 5 S RNA (in preparation). Also, these results indirectly support the hypothesis of the functional similarity between the prokaryotic 5 S RNA and the eukaryotic 5.8 S RNA. Besides their assumed role in tRNA binding to the ribosomal A-site [15,16], they both seem to participate in the ribosomal subunit interaction.

Acknowledgement

We are grateful to Dr I. Wool for sending us his manuscripts prior publication.

References

[1] Litman, D. J., Beekman, A. and Cantor, C. R. (1976) Arch. Biochem. Biophys. 174, 523–531.
[2] Morrison, C. A., Garrett, R. A., Zeichhardt, H. and Stöffler, G. (1973) Mol. Gen. Genet. 127, 359–368.
[3] Bielka, H. and Stahl, J. (1978) Int. Rev. Biochem., Amino Acid and Protein Biosynthesis II (Arnstein, H. R. V. ed) vol. 18, pp. 79–168, University Park Press, Baltimore.
[4] Wool, I. G. (1979) Ann. Rev. Biochem. in press.
[5] Saarma, M., Villems, R., Metspalu, A., Toots, I., Ustav, M. and Lind, A. (1978) 12th FEBS Meet. Dresden, 1978, abst. no. 1629.
[6] Toots, I., Metspalu, A., Lind, A., Saarma, M. and Villems, R. (1979) FEBS Lett. 104, 193–196.
[7] Ulbrich, N., Lin, A. and Wool, I. G. (1979) J. Biol. Chem. in press.
[8] Metspalu, A., Saarma, M., Villems, R., Ustav, M. and Lind, A. (1978) Eur. J. Biochem. 91, 73–81.
[9] Gupta, N. K., Chatterjee, B. and Majumdar, A. (1975) Biochem. Biophys. Res. Commun. 65, 797–805.
[10] Gupta, N. K., Chatterjee, B., Chen, Y. C. and Majumdar, A. (1975) J. Biol. Chem. 250, 853–862.
[11] Ustav, M., Remme, J., Lind, A. and Villems, R. (1979) Bioorg. Khim. 5, 365–369.
[12] Ulbrich, N. and Wool, I. G. (1978) J. Biol. Chem. 253, 9049–9052.
[13] Ustav, M., Lind, A., Saarma, M. and Villems, R. (1977) Bioorg. Khim. 3, 1570–1572.
[14] Burrell, H. R. and Horowitz, J. (1977) Eur. J. Biochem. 75, 533–544.
[15] Nishikawa, K. and Takemura, S. (1974) FEBS Lett. 40, 106–109.
[16] Wrede, P. and Erdmann, V. A. (1977) Proc. Natl. Acad. Sci. USA 74, 2706–2709.