IMMOBILIZED EUKARYOTIC 5.8 S RNA BINDS *ESCHERICHIA COLI* AND RAT LIVER RIBOSOMAL PROTEINS

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1. Introduction

The eukaryotic ribosome contains two low-molecular weight RNAs, 5 S RNA and 5.8 S RNA (for reviews [1,2]). The larger species is specific only for eukaryotic ribosome and, like 5 S RNA, is a component of the large subunit.

Earlier we found that the immobilized rat liver 5.8 S RNA forms a complex with 60 S subunit proteins L5, L6, L7 and L18 [3]. The yeast 5.8 S RNA has been shown to interact also with *E. coli* ribosomal proteins L18 and L25 [4], specific for the prokaryotic 5 S RNA.

Here we demonstrate that the immobilized rat liver and bovine 5.8 S RNAs bind rat liver 40 S ribosomal subunit proteins S6, S14 and S23/S24. 5.8 S RNAs bind also a set of *E. coli* 50 S subunit proteins consisting of L2, L17, L19 for the yeast 5.8 S RNA and L2, L17, L19, L20 and L21 for bovine liver 5.8 S RNA. L18 was visible as a minor protein of the yeast 5.8 S RNA–protein complex.

2. Experimental

Rat liver ribosomal subunits were prepared according to the slightly modified method by Sherton et al. [5] as described elsewhere [3]. Ribosomes from *E. coli* MRE600 strain were isolated according to Held et al. [6]. 30 S and 50 S ribosomal subunits were prepared according to Hardy et al. [7] and proteins from ribosomal subunits were extracted by acetic acid treatment. Ribosomal proteins were identified by two-dimensional gel electrophoresis. The system by Howard and Traut [8] was used for the *E. coli* proteins and that by Sherton and Wool [9] for eukaryotic proteins. The latter was modified by the use of 15% gel in the second dimension. For 40 S subunit proteins the nomenclature by Wool's laboratory [10] as interpreted earlier [3] was followed.

Mammalian liver ribosomal 5.8 S RNA was isolated as described in [3]. Yeast (*Saccharomyces cerevisiae*) ribosomal RNA was prepared according to Rubin [11]. Yeast and bovine RNAs were fractionated on 10% acrylamide preparative gel slabs (0.3 × 20 × 15 cm) according to the method of Donis-Keller et al. [12]. The bands of 5.8 S RNA were visualized by ultraviolet light, and eluted from the gel [13]. Finally 5.8 S RNA was precipitated with ethanol, dissolved in 0.1 M NaCl and repurified on Sephadex G-100 column.

Immobilization of RNAs was as in [14]. Epoxy-activated Sepharose 6B (Pharmacia) was allowed to react with adipic acid dihydrazide at pH 9.5 for 12 h at a room temperature. RNA oxidized with $NaIO_4$ was coupled with the gel at pH 5.8. The affinity chromatography experiments with immobilized 5.8 S RNA and 5 S RNA were performed essentially as described earlier [3]. Various control experiments were performed to obtain the conditions where the unspecific binding of ribosomal proteins to the gel would be ruled out. To exclude unspecific electrostatic interactions we tested the ability of the immobilized UMP and the statistical tetranucleotides to bind ribosomal proteins. Under ionic conditions of the present study neither these gels nor the Sepharose with the spacer group alone were able to interact with

ribosomal proteins [3,14–16]. Furthermore, neither mammalian nor yeast 5 S RNA gels interacted with the rat liver 40 S subunit proteins, and were equally unable to bind *E. coli* 30 S subunit proteins as well. These experiments strongly suggest that within the range of ionic conditions used throughout this study the complexes described below are specific.

3. Results and discussion

The purity of the final 5.8 S RNA preparation was checked on 10% polyacrylamide gel (fig.1).

Applying affinity chromatography technique we demonstrated recently that the rat liver 60 S subunit proteins L5, L6, L7 and L18 form complex with the immobilized homologous 5.8 S RNA [3]. Here we examined further the interaction of 5.8 S RNA with the eukaryotic ribosomal proteins. Unlike rat liver 5 S RNA [3], the immobilized mammalian 5.8 S

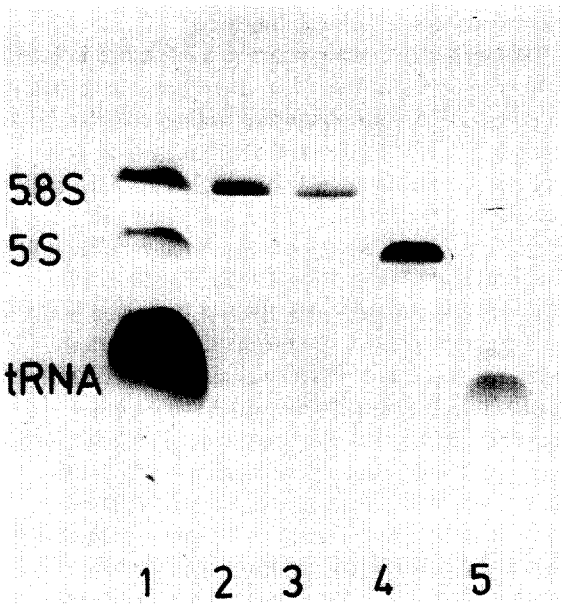

Fig.1. Polyacrylamide gel electrophoresis of bovine and yeast 5.8 S RNA preparations. Separation gel contains 9.7% acrylamide and 7 M urea (pH 8.3). Tank solution is 50 mM Tris–borate buffer (pH 8.3) containing 1 mM EDTA. The sizes of the gel plates were 1.5 × 13 × 200 mm. Samples of 5.8 S RNA consisted of 0.5 A_{260} units of RNA; yeast sRNA as a marker contained 1 A_{260} unit. (1) Yeast sRNA; (2) Yeast 5.8 S RNA; (3) Bovine 5.8 S RNA; (4) Rat liver 5 S RNA; (5) Rat liver tRNA.

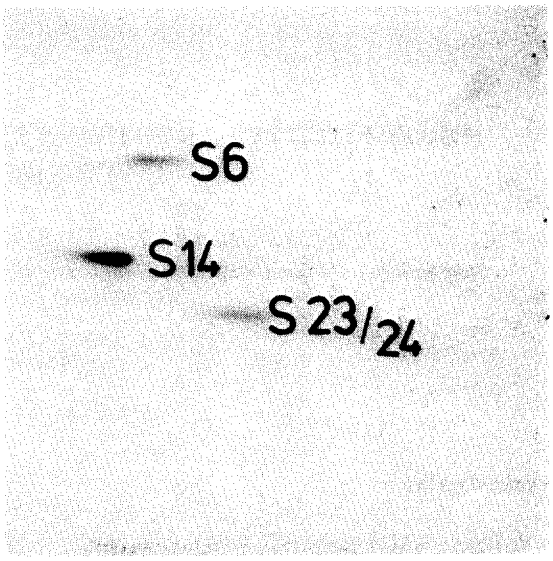

Fig.2. Two-dimensional gel electrophoresis of rat liver 40 S ribosomal subunit proteins bound to the immobilized bovine liver 5.8 S rRNA. 10 ml of 40 S subunit proteins (0.3 mg/ml) in 10 mM Tris–HCl buffer (pH 7.5), containing 6 mM 2-mercaptoethanol, 150 mM KCl and 20 mM $MgCl_2$ at 4°C were applied to the column. Affinity column consisted of 0.5 ml of Sepharose with 0.5 mg of bound 5.8 S RNA. 0.12 mg of bound proteins were analysed on two-dimensional polyacrylamide gel electrophoresis system as in [9].

RNA, either rat or bovine, forms a complex with 40 S subunit proteins S6, S14 and S23/S24 (fig.2). The latter pair is unseparable on two-dimensional gel slabs in the conditions used (see also [17]).

This observation indicates the location of 5.8 S RNA on the subunits interface. Supporting evidence for the suggestion comes from the positioning of protein S6, as well as S23, on the interface [18,19].

Thus, although strongly bound to the large subunit via base-pairing with 28 S RNA [20,21], this low-molecular weight RNA may participate in the subunits interaction. However, we note that 40 S subunit proteins found in the complex were also present in a similarly obtained tRNA–protein complex [3]. Since the contamination of 5.8 S RNA with any significant quantity of tRNA is excluded (fig.1) the phenomenon should have a different explanation. The tRNA molecule, too, interacts with both ribosomal subunits. Does 5.8 S RNA share common proteins with tRNA, or does it mimic, while immobilized, a certain conformational feature of tRNA needed for the ribosomal

protein binding, is uncertain yet. The binding of radioactive tRNA to the preformed 5.8 S RNA–protein complex seems to favour the former explanation (Toots, unpublished observation).

The yeast 5.8 S RNA, as shown by sucrose density gradient centrifugation, forms a complex with *E. coli* ribosomal proteins L18 and L25 [4]. These two proteins, as well as L5, bind independently and specifically to *E. coli* 5 S RNA [22].

Various indirect evidences suggest the interaction between the prokaryotic 5 S RNA and tRNA in the ribosomal A-site mediated by complementary base-pairing between the universal GTψC sequence of tRNA and GAAC of 5 S RNA (review [1]). The eukaryotic 5 S RNA, however, lacks this sequence (for primary structures of 5 S RNA and 5.8 S RNA [23]). In contrary to that, the molecule of 5.8 S RNA contains GAAC [23]. All these facts led Wrede and Erdmann to the hypothesis, according to which in the eukaryotic ribosome 5.8 S RNA instead of 5 S RNA interacts with tRNA in the A-site [4], as proposed earlier by Nishikawa and Takemura [24].

At ionic conditions (0.32 M KCl, 20 mM $MgCl_2$) used by Wrede and Erdmann [4] the immobilized 5.8 S RNA did not bind 50 S subunit proteins. On the other hand, to avoid protein aggregation we used in our experiments roughly a hundred times lower protein concentration than these authors, which could be the reason of our negative result. The third difference, compared with [4] is the modification of the 3′-end ribose due to the technique of immobilization. That, however, does not influence the ability of the immobilized *E. coli* 5 S RNA to bind L25 [25,26]. At lower potassium chloride concentration (0.2 M and 0.1 M), in 10 mM $MgCl_2$, we were able to identify a group of 50 S subunit proteins in the complex with the immobilized 5.8 S RNA. The bovine liver 5.8 S RNA-bound proteins are depicted on fig.3. Although similar, the yeast 5.8 S RNA–protein complex contained smaller number of proteins (table 1).

In agreement with Wrede and Erdmann [4] we did not detect the binding of *E. coli* ribosomal proteins to the eukaryotic 5 S RNA, demonstrating that neither yeast [4] nor mammalian 5 S RNA (present study) forms a complex with prokaryotic ribosomal proteins.

Nevertheless, disagreement between the present data with the results by Wrede and Erdmann is evident. What we found, is a relatively wide set of *E. coli*

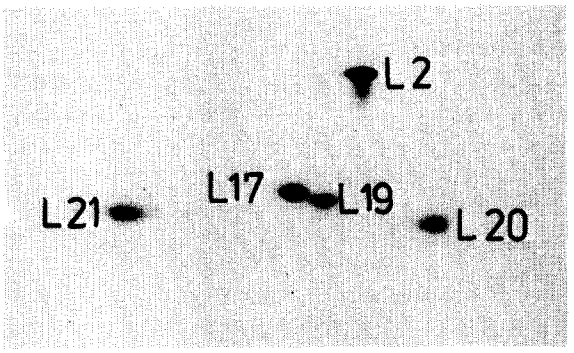

Fig.3. Bovine 5.8 S RNA bound proteins from *E. coli* 50 S ribosomal subunit. About 3.5 mg of 50 S subunit proteins in 10 ml 10 mM Tris–HCl buffer (pH 7.5), containing 6 mM 2-mercaptoethanol, 100 mM KCl and 10 mM $MgCl_2$ were allowed to interact with 0.35 mg of immobilized bovine 5.8 S RNA. Bound proteins, 0.25 mg, were analysed on two-dimensional polyacrylamide gel electrophoresis system as in [8].

50 S subunit proteins bound to 5.8 S RNA. L18, the main protein found in [4] was indeed present in the immobilized yeast 5.8 S RNA–protein complex, but as a marginal component (table 1). However, also marginally, this protein was found in the immobilized tRNA–protein complex [16]. Moreover, we observed, in identical conditions, the binding of L18 to the immobilized poly(A) and poly(C) [27]. In addition to these results at high concentrations L18 was shown to bind even to the *E. coli* 5 S RNA in multiple copies [28]. Overgoing puts the specifity of L18 interaction with 5.8 S RNA into question.

We never detected L25 in our 5.8 S RNA–protein complexes. Notably the third *E. coli* 5 S RNA protein,

Table 1
Eukaryotic 5.8 S RNA bound proteins from the 50 S ribosomal subunit of *E. coli*[a]

5.8 S RNA source	Concentration of KCl[b]	
	0.1 M	0.2 M
Yeast	L2, L17, L19 (L18)	L2 (L17, L19)
Bovine liver	L2, L17, L19, L20, L21 (L1, L3, L4, L5)	L21 (L1, L2, L3, L4, L17, L19, L20)

[a] Marginal proteins are given in brackets
[b] In 10 mM Tris–HCl (pH 7.5) 10 mM $MgCl_2$, 6 mM 2-mercaptoethanol

L5, was present at very low amount in the bovine liver 5.8 S RNA set (table 1). Again, although this fact seems to favour the idea of similarity between 5.8 S RNA and the *E. coli* 5 S RNA, it must be also noted that Burrell and Horowitz found this protein bound to their immobilized tRNA [24].

Further experiments are in progress to reveal the possible meaning of 5.8 S RNA–*E. coli* proteins complex.

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