THE INTERACTION OF TRANSFER RIBONUCLEIC ACID WITH 50 S RIBOSOMAL SUBUNIT PROTEINS

M. USTAV, R. VILLEMS, M. SAARMA and A. LIND

Laboratory of Molecular Biology, Tartu University, Kingissepa Str 14/16, 202400 Tartu, Estonia, USSR

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1. Introduction

Viewing the nature of the tRNA–ribosome interaction from the tRNA side, a key role of codon–anticodon recognition [1] and the influence of various tRNA fragments [2] were studied. Except for the interaction of tRNA with 5 S RNA [3], to our knowledge no direct information on its binding to other ribosomal RNAs is available.

The affinity chromatography of ribosomal proteins on immobilised RNAs appeared to be a useful tool to study RNA–protein interaction [4–8]. In particular, this technique allowed the demonstration of the formation of a specific complex between tRNA and *Escherichia coli* ribosomal proteins [6–8]. Thus, Yukioka and Omori [6] reported that immobilised tRNA binds 50 S ribosomal subunit proteins L1, L17 and L22. Using a longer spacer group to immobilise tRNA, Burrell and Horowitz [7] found that the major proteins able to bind to tRNA are L3, L4, L5, L7 and L8/L9.

Here we describe a complex formation between 50 S ribosomal subunit proteins and tRNA. An extra long spacer group was used to immobilise tRNA. At moderate ionic strength and a low $MgCl_2$ concentration proteins L2, L15, L16, L17, L18, L22, L33 and L34 were found in the complex.

These results have been reported at the 3rd EMBO Ribosome Workshop, Bruxelles, 1976 and at 11th FEBS Meeting, Copenhagen, 1977

2. Experimental

50 S ribosomal subunits from *Escherichia coli* MRE600 were prepared according to Hardy et al. [9] or purchased from IREA (Olaine, USSR). Proteins were extracted from the subunit by acetic acid treatment [10] and dialysed against 10 mM Tris–HCl buffer pH 7.4, containing 6 mM 2-mercaptoethanol and various concentrations of $MgCl_2$ and KCl. Proteins were identified by two-dimensional gel electrophoresis [11].

Transfer ribonucleic acid was from *E. coli* MRE600 (Boehringer, Mannheim), purified on Sephadex G-75 chromatography, or extracted from rat liver [12]. 3′-End oxidised tRNA was covalently linked to commercial Epoxy-activated Sepharose 6B (Pharmacia) and affinity chromatography of the *E. coli* ribosomal subunit proteins was performed as described earlier [8].

Statistical tetranucleotide mixture was prepared from high-molecular-weight rRNA digest with the cobra venom unspecific endonuclease as described [13]. Tetranucleotide fraction containing 5′-end phosphate groups and without the latter were linked to the Sepharose essentially as tRNA. Concentration of tetranucleotides in the gel was approx. 1 mM.

3. Results and discussion

Three different types of control experiments were needed. Firstly, the adipic-acid–hydrazide–epoxy arm contains a positively charged amino group and

may therefore interact with ribosomal proteins. Secondly, an unspecific electrostatic interaction may also occur between ribosomal proteins and negatively-charged phosphate groups of nucleotides. To test the latter possibility we linked the tetranucleotides to Sepharose. Neither the arm alone, nor the tetranucleotides linked to Sepharose via this arm were able to bind ribosomal proteins. A more specific control experiment was provided with immobilised native ribonucleic acid. It was shown that 5 S RNA from rat liver does not bind the *E. coli* 50 S ribosomal subunit proteins. We also wish to emphasise this result for another reason. Namely, as will be shown below, partial 'overlapping' between proteins able to bind to tRNA and *E. coli* 5 S RNA was found. It may be proposed that the *E. coli* tRNA contains a small amount of 5 S RNA and that what one actually sees is the binding of ribosomal proteins to this contaminant. However, tRNA–Sepharose gel prepared either from *E. coli* or from rat liver tRNA binds these proteins equally well. Since rat liver tRNA obviously does not contain *E. coli* 5 S RNA, it is indeed tRNA which binds, among other proteins some '5 S RNA proteins'.

Does tRNA interact with ribosomal proteins within the 50 S ribosomal subunit? Besides the TψCG sequence interaction with 5 S RNA [4], there is, to our knowledge, no experimental basis to assume RNA–RNA type complementary basepairing between tRNA and ribosomal RNA. Although a common CCA sequence among all tRNAs could be a suitable candidate for an RNA–RNA interaction, the highly specific ability of CA-Phe to inhibit the binding of aminoacyl-tRNA to the ribosome [14] indicates an RNA–protein interaction because of the evident weakness of the alternative interaction of the dinucleotide CA with the ribosomal RNA. Furthermore, it was recently shown [15] that tRNA forms a stable complex with the 50 S ribosomal subunit even without the presence of the 30 S subunit and mRNA. Since this binding was not inhibited by TψCG, the possibility of the 5 S RNA–tRNA interaction is unlikely in their experiment. Thus, there are a number of indirect pieces of evidence for tRNA interaction with the 50 S ribosomal subunit proteins.

To study this question experimentally we performed affinity chromatography of free 50 S subunit proteins on the immobilised tRNA column. The influence of ionic strength and $MgCl_2$ concentration was studied. As in experiments with 30 S subunit proteins [8] a strong dependence of the amount of protein bound upon ionic strength was observed. At a constant $MgCl_2$ concentration (20 mM) the set of proteins bound to the immobilised tRNA in 0.1 M and 0.2 M KCl was identical, containing proteins L2, L16, L17, L22 and L33 as the main ones and L1, L6, L15, L20, L21 and L26 at lower amounts.

Affinity chromatography experiments in this study were performed with a large excess of tRNA over an individual protein. Therefore, the amount of protein bound does not necessarily reflect only their binding ability but also their proportion in the initial protein mixture. This is particularly true for the protein L26. Since it is identical with the small subunit protein S20 [16] and exists in the 70 S ribosome as a single copy [17], its content in our 50 S subunit was very low. When the total 70 S ribosome protein was used in the affinity chromatography experiment, S20 (L26) gave a strong spot on two-dimensional (data not shown).

It is well established that by varying the $MgCl_2$ concentration it is possible to regulate orientation of tRNA within the peptidyl transferase center (e.g., see [18]). Thus, though we do not know to what extent the immobilised tRNA mimics the situation within the ribosome, it was interesting to study the effect of $MgCl_2$ concentration on the complex.

The data obtained at two widely separated $MgCl_2$ concentrations are summarised in table 1. Some obvious differences can be seen – at a low $MgCl_2$ concentration

Table 1

Escherichia coli 50 S ribosomal subunit proteins identified in the complex with immobilised tRNA at various $MgCl_2$ concentrations

$MgCl_2$ concn.[a]	Main proteins	Minor and variable proteins
1 mM	L2, L15, L16, L17, L18, L22, L33, L34	L1, L4, L20, L26, L21
30 mM	L2, L16, L17, L33	L1, L6, L15, L20, L21, L22

[a] To keep the overall ionic strength equal at both $MgCl_2$ concentrations, 200 mM and 100 mM KCl was used in the binding buffer, respectively. Other components as indicated in Experimental, and legend to fig.1

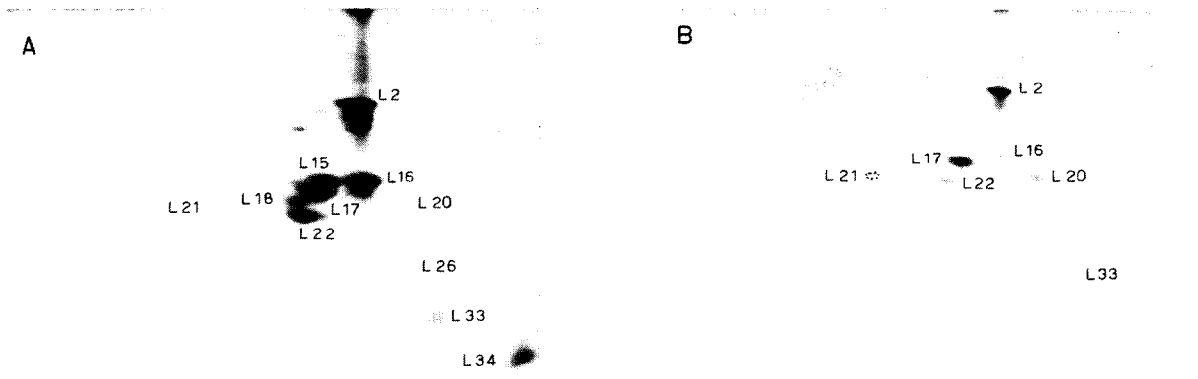

Fig.1. Two dimensional gel electrophoresis of *E. coli* 50 S ribosomal subunit proteins bound to the immobilised tRNA at 4°C. 50 S subunit proteins, 2.3 mg, were passed through the affinity column containing 1.6 mg of the immobilised tRNA in vol. 0.4 ml. Buffers: 10 mM Tris–HCl, pH 7.5, 6 mM 2-mercaptoethanol. (A) 50 S subunit proteins bound in 1 mM $MgCl_2$, 200 mM KCl, other components as indicated above. (B) 50 S subunit proteins bound in 30 mM $MgCl_2$, 100 mM KCl, other components as indicated above. 0.24 mg and 0.11 mg of bound proteins were used in the analysis, respectively. Two dimensional polyacrylamide gel electrophoresis system as described by Howard and Traut [11].

two strong new spots appeared, corresponding to proteins L18 and L34 (fig.1A). On the other hand, at a high $MgCl_2$ concentration (fig.1B) relative amounts of proteins L1 and L6 are higher than at a low $MgCl_2$ concentration. Since the overall ionic strength in both experiments was maintained the same, any difference must be due to the bivalent cation concentration.

It seems reasonable to assume that 50 S subunit proteins interacting with tRNA are located in the neighbourhood of the peptidyl transferase centre. Indeed, proteins L2, L6, L13–L15, L26/L27, L18 and L32/L33 have been shown to be near to this centre by various affinity labelled tRNAs as well as by analogues of chloramphenicol and puromycin (for review see [19]). Moreover, proteins L1, L15, L20 and L33 that we found in the complex with tRNA have been shown to crosslink with EFTu [20].

There is a reasonably good agreement between our data and those by Burrell and Horowitz [7]. A much smaller number of proteins able to bind to tRNA, as reported by Yukioka and Omori [6], most likely reflects the important role of the length of the spacer group connecting RNA with the Sepharose granule.

Do all proteins found in the complex interact directly with tRNA? It seems possible that protein–protein interactions may also have a stabilising role with the complex. A strong argument supporting this idea becomes evident from the results on 5 S RNA–ribosomal protein interaction [21]. That is, besides proteins L2, L5, L17, L21 and L25 which have been identified in this complex, very low amounts of other proteins, among them L6, L15, L16, L22, L26 and L33 were also found. Similar results, obtained by a different method were recently reported [22]. In that case proteins L2, L5, L18 and L25, and to a lesser extent proteins L7, L12, L13, L15, L16, L17, L19, L21 and L28 were found in the 5 S RNA–protein complex. Thus, we propose that weak protein-protein interactions between certain 50 S ribosomal subunit proteins are amplified in the presence of RNA and become strong enough to keep them in the complex. More specifically, the presence of either tRNA or 5 S RNA in the mixture of 50 S subunit proteins allows the formation of a considerable part of the peptidyl transferase centre.

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