5 S RNA AND 5.8 S RNA BUILD UP EUKARYOTIC SUBRIBOSOMAL DOMAINS ACTIVE IN tRNA BINDING

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Received 5 October 1981

1. Introduction

The codon–anticodon interaction is obviously not a sole direct contact between tRNA and ribosome. Various tRNA affinity and photo-affinity analogues show its proximity to a number of ribosomal proteins [1–3], while nitrocellulose membrane filtration [4] and affinity chromatography [5,6] revealed direct interaction between tRNA and ribosomal proteins outside of ribosome. It has been also postulated that tRNA, both elongator and initiator, may interact with 5 S RNA and 5.8 S RNA via complementary base-pairing between certain conserved sequences [7]. This hypothesis, however, lacks experimental evidence.

We suggested earlier that ribosomal proteins which form a complex with the immobilized tRNA are located close to the peptidyl transferase center [5,8]. Thereafter, partial overlap of the proteins of 5 S RNA–, 5.8 S RNA– and tRNA–protein complexes allowed us to suggest that 5 S RNA– and 5.8 S RNA–protein complexes are ribosomal domains active in tRNA binding [5].

Here, we show that both 5 S RNA– and 5.8 S RNA–60 S ribosomal subunit protein complexes form a binding site for one molecule of deacylated tRNA. Also, the tRNA–TP60 complex is able to bind a second molecule of tRNA.

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Part of these results were presented at the symposium 'Biological Implications of Protein–Nucleic Acid Interactions', Poznan, 1980 [19]

2. Experimental

Rat liver ribosomal 5 S RNA, 5.8 S RNA and tRNA were prepared essentially as in [5] and their purity was controlled by urea–polyacrylamide gel electrophoresis [9].

All tRNA binding experiments were performed with 5′-^{32}P-labelled rat liver tRNA. After dephosphorylation [10] and ethanol precipitation, 1–2 nmol tRNA was dissolved in 10–20 μl of 50 mM Tris–HCl buffer (pH 8.0) containing 10 mM $MgCl_2$, 5 mM DTT and incubated with 2 μl T4 polynucleotide kinase and 100–300 pmol [γ-^{32}P]ATP (1000–2000 Ci/mmol, Isotop, USSR) at 37°C for 30 min. Labelled tRNA was purified by polyacrylamide gel electrophoresis, eluted as in [11], and reprecipitated with cold tRNA as a carrier. All binding experiments were carried out in 10 mM Tris–HCl buffer (pH 7.5) containing 20 mM $MgCl_2$, 300 mM KCl and 6 mM 2-mercaptoethanol (BB). Before use, tRNA sample was kept at 60°C for 10 min in BB and cooled slowly to room temperature.

5′-End-labelled 5 S RNA and 5.8 S RNA were prepared and purified identically to tRNA.

Rat liver 60 S ribosomal subunit proteins (TP60) were isolated as in [5] and analyzed according to [12,13]. All protein samples were centrifuged at 20 000 rev./min for 10 min before each experiment.

About 1–3 mg RNA was immobilized per 1 ml wet epoxy-activated Sepharose 6B (Pharmacia) gel essentially as in [5]. Preformation of 5 S RNA–, 5.8 S RNA– and tRNA–TP60 complexes is described in [5] and was carried out in BB as follows: TP60 (0.15–0.5 mg/ml) was applied to affinity columns at a flow rate 5 ml/h and the columns were washed with 20 vol. BB. For TP60, 1 mg/ml was taken as 1×10^{-6} M

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.75

[14] and total M_r as 1.06×10^6 [15]. These preformed columns were further used for tRNA binding experiments as well as for control experiments with free 5 S RNA and 5.8 S RNA binding.

5'-^{32}P-Labelled deacylated tRNA, 5 S RNA or 5.8 S RNA were applied to affinity columns at 5×10^{-5} M. The unbound RNA was washed out with BB (~10 column vol.) and the bound RNA with proteins were eluted by 8 M urea and 4 M LiCl containing solution [16].

The background value for tRNA binding to 0.3 ml RNA-gel column without TP60 present was ~1–1.5% of the loaded tRNA. Other experimental details are given in legends to figures.

Crude *E. coli* tRNA synthetase, known to aminoacylate eukaryotic $tRNA^{Met}$, including initiator tRNA [17], was isolated as in [18] by Dr J. Remme in this laboratory. Aminoacylation of the tRNA bound to the preformed 5 S RNA–TP60 complex was done as in [18].

3. Results

5 S RNA, 5.8 S RNA and tRNA used for immobilization, as well as their ^{32}P-labelled counterparts, were at least 99% pure (fig.1).

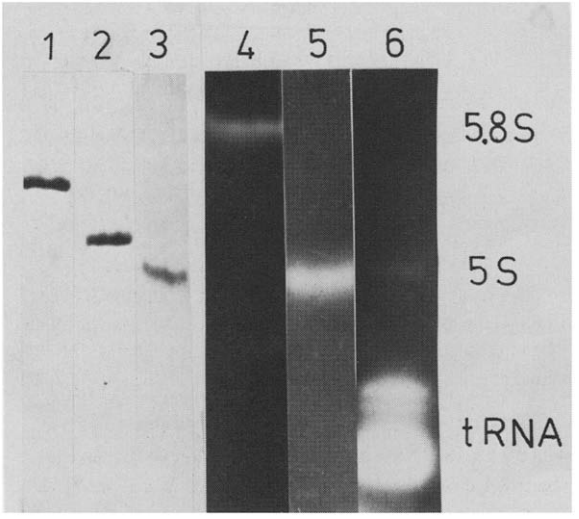

Fig.1. Electrophoresis of rat liver 5 S RNA, 5.8 S RNA and tRNA in urea–10% polyacrylamide gel: (1) 5.8 S RNA; (2) 5 S RNA; (3) tRNA; (4) 5.8 S [5'-^{32}P]RNA; (5) 5 S [5'-^{32}P]-RNA; (6) [5'-^{32}P]tRNA. Cold RNAs were stained with methylene blue and radioactive RNAs autoradiographed.

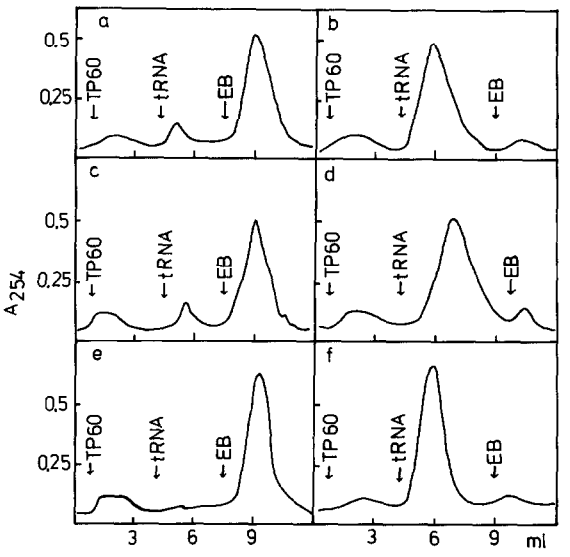

Fig.2. Affinity chromatography of [5'-^{32}P]tRNA on 5 S RNA, 5.8 S RNA and tRNA columns. (a) Total protein from 60 S subunit (TP60) (2 ml, 0.26 mg/ml), was applied to the 5 S RNA-column at flow rate of 5 ml/h; the column was then washed with 10 column vol. BB and a 30 μl sample of tRNA (20 A_{260} units/ml) mixed with [5'-^{32}P]tRNA (~20 000 cpm) was applied to the column. The bound tRNA and proteins were eluted with 8 M urea, 4 M LiCl. Optical profile was monitored by Uvicord II. (b) 5 S RNA column was washed with 10 column vol. BB and a tRNA sample (as in (a)) was applied to the column. The column was washed with 8 M urea, 4 M LiCl and the optical profile was measured by Uvicord II. (c) As (a), but 5.8 S RNA column was used; (d) as (b), but 5.8 S RNA column; (e) as (a), but tRNA column; (f) as (b), but tRNA column.

The complexes of 5 S RNA, 5.8 S RNA and tRNA with TP60, formed in 20 mM $MgCl_2$ and 150 mM KCl containing buffers, are described in [5,8] and the complexes formed in 20 mM $MgCl_2$ and 300 mM KCl, in [6,19–21]. These complexes have several proteins in common [5,19]. Therefore, and for reasons given in section 1, we undertook a series of experiments to reveal possible interaction of tRNA with these RNA–protein complexes.

Fig.2 shows that none of these 3 immobilized RNAs is able to bind free tRNA, while all preformed RNA–TP60 complexes do. Next, a more detailed analysis of this phenomenon was done:

(i) The dependence of the amount of the bound tRNA upon the amount of TP60 used for complex formation was monitored (fig.3a);
(ii) The constant amounts of different preformed

RNA–TP60 complexes were titrated with an increasing quantity of tRNA (fig.3b).

The first series of experiments show that 5 S RNA–, 5.8 S RNA– and tRNA–TP60 complexes all bind nearly 1 mol deacylated tRNA/1 mol RNA–TP60 complex. Approximate molar concentration of the preformed complexes were calculated from the known molar amount of TP60 used (see section 2), and by assuming that under conditions of these experiments, all proteins of the complex, present in TP60, were bound to the immobilized RNAs. The latter situation was achieved by performing experiments in conditions where the molar amount of an immobilized RNA was much higher (~50–times) than that of the applied TP60. Electrophoretic analysis of the affinity column flowthroughs confirmed this prediction (not shown). Hence, the molar amount of the RNA–TP60 complexes can be taken equal to the molar amount of TP60, applied to the column. Titration experiments, shown in fig.3b agree with the results of the first series, and, in addition, reveal that tRNA binding reaches plateau in all complexes studied. Calculations show that 1 mol 5 S RNA–, 5.8 S RNA– and tRNA–TP60 complexes bind at saturation 0.9, 0.8 and 0.95 mol deacylated tRNA, respectively.

The possibility of forming a 5 S RNA–protein L5–5.8 S RNA ternary complex was demonstrated earlier [16]. Here, as an important control experiment, we examined the binding of free 5 S [5′-^{32}P]-RNA and 5.8 S [5′-^{32}P]RNA to homologous preformed complexes. Results, shown in fig. 3a, clearly reveal that the preformed 5 S RNA–TP60 complex does not bind free 5 S RNA and the 5.8 S RNA–TP60 complex does not bind free 5.8 S RNA. These results, together with quantitative data given above, strongly support the idea that the binding of tRNA to these complexes is specific.

Next, a more specific question was asked. Namely, according to the hypothesis [7], eukaryotic initiator tRNA and 5 S RNA interact via complementary base pairing between sequences GAUC and GAUC, respec-

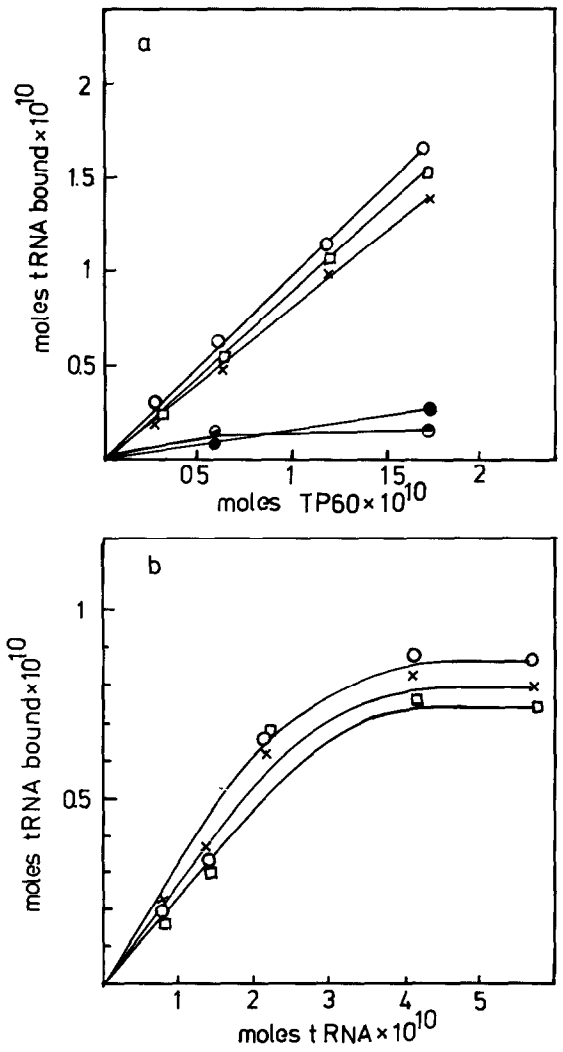

Fig.3. (a) The binding of deacylated [^{32}P]tRNA to the 5 S RNA–, 5.8 S RNA– and tRNA–TP60 complexes as the function of the amount of TP60 applied to the immobilized 5 S RNA, 5.8 S RNA and tRNA–Sepharose gel columns in BB: 1 A_{260} unit of tRNA was taken as 1.54×10^{-9} mol; 1 A_{260} of 5 S RNA, 1×10^{-9} mol; 1 A_{260} unit of 5.8 S RNA, 0.75×10^{-9} mol. To 0.5 ml 5 S RNA (20 A_{260} units/ml), 5.8 S RNA (21 A_{260} units/ml) and tRNA (38 A_{260} units/ml) columns in BB indicated amounts of TP60 were applied and the columns were washed with 15 column vol. of BB. After that saturating amounts of tRNA (15 μl, 0.3 A_{260} units, ~60 000 cpm Čerenkoff counts) were applied to the columns, washed again with 20 column vol. BB and the bound tRNA together with proteins were eluted with 8 M urea–4 M LiCl. (–○–) tRNA column; (–×–) 5 S RNA column; (–□–) 5.8 S RNA column. In identical conditions 15 μl 5 S [5′-^{32}P]RNA (~0.4 A_{260} units, ~ 50 000 cpm by Čerenkoff) was applied to the 5 S RNA–TP60 column (–⊖–) and 5.8 S [5′-^{32}P]RNA (15 μl, 0.6 A_{260} units, ~45 000 cpm by Čerenkoff) was applied to the 5.8 S RNA–TP60 column (–●–). (b) The titration of 5 S RNA–, 5.8 S RNA– and tRNA–TP60 complexes with increasing amounts of [^{32}P]tRNA. About 95 pmol 5 S RNA–, 5.8 S RNA– and tRNA–TP60 complexes were titrated with increasing amounts of [5′-^{32}P]tRNA in BB as fig.2a. Other details are given in section 2.

tively. Therefore, we compared the quantity of $tRNA^{Met}$ in the initial tRNA sample and in the tRNA fractions bound and unbound to the preformed 5 S RNA–TP60 complex. No specific enrichment of the unbound tRNA with $tRNA^{Met}$ was found. A similar result was obtained with the 5.8 S RNA–TP60 complex.

4. Discussion

Although several alternative speculations are possible (too low K_a, codon-induced conformational change of tRNA etc. [22,23]), our results do not support the idea of a direct interaction between 5 S RNA or 5.8 S RNA with tRNA, as it was postulated in [7]. Conformational analysis of 5 S RNA and 5.8 S RNA led us to an identical conclusion [24]. Therefore, we rather suggest that 5 S RNA and 5.8 RNA together with a definite limited set of ribosomal proteins form binding sites for tRNA in the eukaryotic ribosome. Supportive evidence for the interaction of tRNA with rat liver 5 S RNA–protein complex, although more indirect, comes from the observation that 5 S RNA–L5 complex, in the presence of EF2 and tRNA, exhibits ATPase and GTPase activities [25].

In sum, the results presented in this paper lend further credence to the suggestion [5,8] according to which the eukaryotic ribosomal peptidyl transferase centre is, at least partly, organized by these 2 low-M_r ribosomal RNAs and interacting ribosomal proteins.

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