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RNA–RNA and RNA–protein interactions in 30 S ribosomal subunits

Association of 16 S rRNA fragments in the presence of ribosomal proteins

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The *E. coli* 16 S rRNA with single-site breaks centered at position 777 or 785 was obtained by RNase H site-specific cleavage of rRNA. Spontaneous dissociation of the cleaved 16 S rRNA into fragments occurred under 'native' conditions. The reassociation of the 16 S rRNA fragments was possible only in the presence of ribosomal proteins. The combination of S4 and S16(S17) ribosomal proteins interacting mainly with the 5'-end domain of 16 S rRNA was sufficient for reassociation of the fragments. The 30 S subunits with fragmented RNA at ca. 777 region retained some poly(U)-directed protein synthetic activity.

Ribosome structure; Fragmented 16 S rRNA; RNase H hydrolysis; RNA–RNA interaction; RNA–protein interaction

1. INTRODUCTION

The assembly of ribosomal subunits from rRNA and proteins is accompanied with very compact folding of RNA within RNP complexes. It was proposed that protein-induced alterations in rRNA tertiary structure play an important role in fine-tuning of conformation and dynamic of rRNA in ribosomal subunits [1]. In this work we have investigated 16 S rRNA from *E. coli* ribosomes fragmented at region 770–800. This region contains several universally conserved bases and some of them directly participate in protein synthesis [2]. We have found that dissociation of the 16 S rRNA cleaved by RNase H at regions 773–782 or 781–800 into two halves occurs under non-denaturing conditions. The reassociation of these fragments was investigated. The presence of ribosomal proteins was found to be indispensable for reassociation. A minimal set of ribosomal proteins which are capable of stimulating the association of fragments consists of S4 and S16(S17). Here we also report functional characteristics of the 30 S subunits with fragmented 16 S rRNA.

2. MATERIALS AND METHODS

2.1. *Materials*

E. coli MRE 600 was the source of 30 S and 50 S ribosomal subunits prepared according to Makhno et al. [3]. 16 S rRNA was prepared as described previously [4]. *E. coli* RNase H was purchased from Biolar (Olaine, Latvia). Oligonucleotides were gifts from Drs. V. Zarytova and T. Oretskaja. Ribosomal proteins were a generous gift from Mrs. T. Bystrova.

The hydrolysis of the 16 S rRNA with RNase H and analysis of cleaved RNA by PAG electrophoresis were performed as described by Bogdanov et al. [5]. The molar 16 S rRNA/oligonucleotide ratio was 1:2 for oligonucleotide I and 1:5 for oligonucleotide II. The concentration of RNase H was 2.6×10^3 units/ml and 1.3×10^3 units/ml, respectively. To denature, the samples of cleaved RNA were incubated at 60°C for 3 min in TM buffer (20 mM Tris-HCl, pH 7.6, 20 mM $MgCl_2$, 6 mM β-mercaptoethanol), then cooled. Fragments of RNA were assayed by sucrose gradient centrifugation [4].

2.2. *Formation of the RNA-protein complexes*

A mixture of RNA fragments and proteins (4 molar excess of the protein) was incubated for 40 min at 40°C in TMK buffer (TM buffer containing 330 nM KCl). Final concentration of RNA was 1 mg/ml. All samples were then cooled and were layered on top 5–20% sucrose gradients in TMK buffer. Centrifugation was in Beckman SW 50.1 rotor at 4°C for 2.5 h at 45000 rpm. The fractions corresponding to RNP complexes were combined and precipitated with ethanol. Proteins were analyzed by SDS-PAG electrophoresis [6] using 7–25% acrylamide.

Reconstitution of 30 S subunits, protein synthetic assays in the presence of poly(U) were performed as described previously [4].

Association of 30 S subunits to 50 S subunits was assessed by analytical ultracentrifugation: 25 pmol of 30 S subunits and 25 pmol of 50 S subunits were incubated for 30 min at 37°C in 120 μl of reassociation buffer (20 mM Tris-acetic acid, pH 7.3, 12 mM $Mg(OAc)_2$, 110 mM NH_4OAc, 6 mM β-mercaptoethanol) and centrifuged in Spinco E ultracentrifuge at 42000 rpm using ultraviolet absorption method.

3. RESULTS AND DISCUSSION

Previously we have shown that *E. coli* 16 S rRNA could be cleaved with RNase H at specific sites in the presence of complementary oligodeoxyribonucleotides [5]. In this work the oligonucleotides T_3GCTC_4 (I) and

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Abbreviations: rRNA, ribosomal RNA; RNase, ribonuclease; RNP, ribonucleoprotein; TP30, total proteins of 30 S subunits

$C_2AG_3TATCA_2TC_2TGT_2$ (II) complementary to the 16 S rRNA regions 773–782 (16 S RNAI) and 781–800 (16 S RNAII), respectively, were used that allowed us to cut the RNA chain roughly into two halves (Fig. 1). One can see from Fig. 2 that from 40% to 50% of 16 S rRNA, cleaved at these sites, spontaneously dissociates into corresponding fragments under 'native' conditions. The dissociation can be completed by brief heating at 60°C (Fig. 2C). The recent version of the model of the 16 S rRNA secondary structure suggests that two fragments studied in this work are fastened together with 17 base pairs [7]. They are organized into four short helices separated by rather long single-stranded regions. We have found that at least in free 16 S rRNA these helices are easily melted at room temperature even at high concentrations of Mg^{2+}. It is also clear that two halves of the 16 S rRNA chain in free state are not intertwined or involved into any strong interdomain tertiary interactions.

The dissociation of the fragmented 16 S rRNA into two halves was irreversible. However, in the presence of TP30 the fragments of both types were effectively reconstituted into 30 S particles which comigrated with native subunits in sucrose gradient. From 15% to 20% of particles, sedimented as a 'light shoulder', are likely to represent RNP containing separate halves of 16 S rRNA (Fig. 3). The capacity of reconstituted 30 S particles to associate with 50 S subunits was very low even at high concentration of Mg^{2+}. Their translational activity was also low but it was rather different for 30 S subunits reconstituted from 16 S rRNAI and 16 S rRNAII. In contrast, 30 S subunits containing the fragmented 16 S rRNAII were almost completely inactive in poly(U)-directed cell-free system, and the small ribosomal subunits reconstituted from 16 S rRNAI showed up to 35% activity of 30 S subunits assembled with native 16 S rRNA (Fig. 4). This demonstrates that an essential part of the 30 S subunits containing the intact 783–799 stem-loop region is able to restore their functional activity in the presence of poly(U) and Phe-$tRNA^{Phe}$. These findings correlate with the data obtained with other methods [2,8,9].

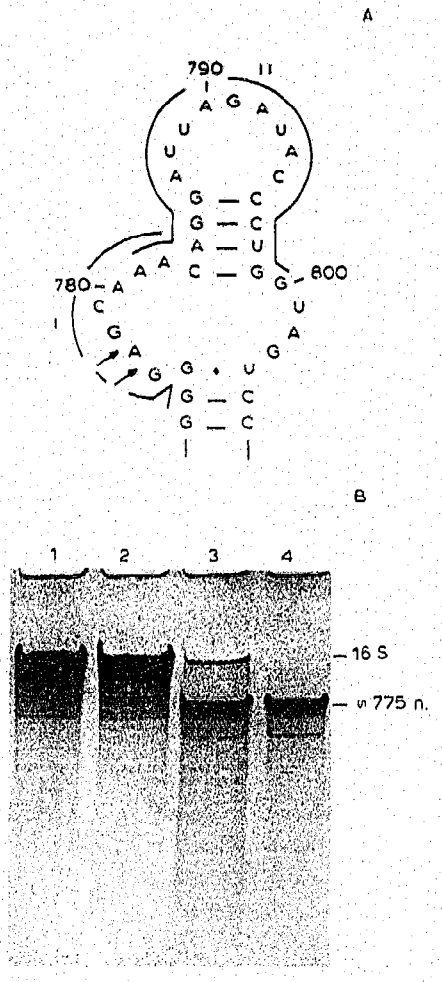

Fig. 1. (A) Diagram of secondary structure of the central part of *E. coli* 16 S rRNA, showing the position of complementary oligonucleotides. (B) Electrophoretic analysis of 16 S rRNA fragmented in central region. 1 = Native 16 S rRNA; 2 = native 16 S rRNA incubated with RNase H; 3 = 16 S rRNAII; 4 = 16 S rRNAI.

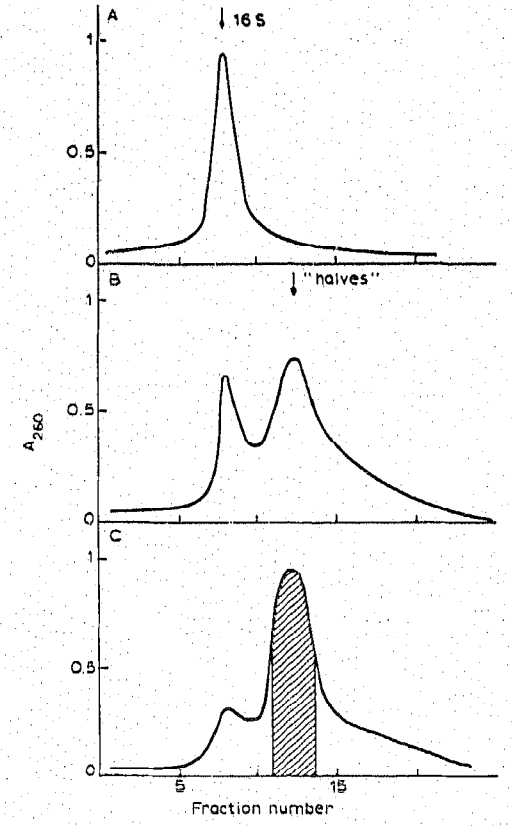

Fig. 2. Analysis of 16 S rRNA by sucrose gradient centrifugation. (A) Native 16 S rRNA. (B) 16 S rRNAI. (C) 16 S rRNAI heated for 3 min at 60°C.

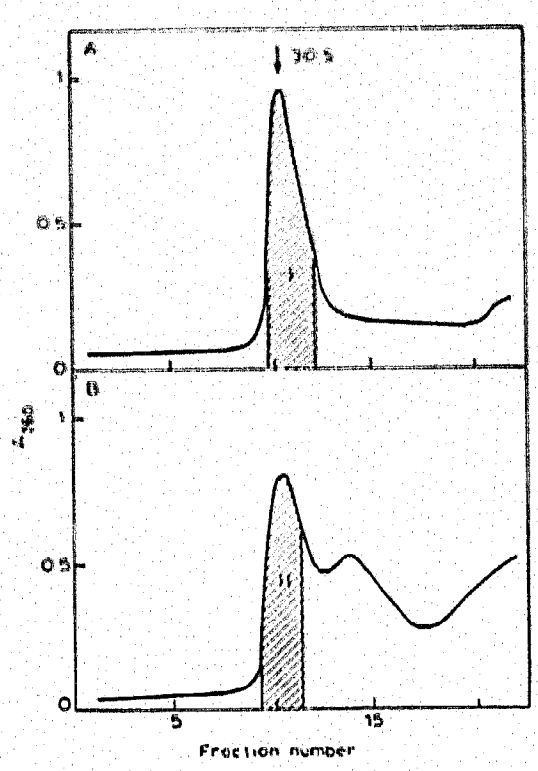

Fig. 3. Analysis of reconstituted 30 S subunits by sucrose gradient centrifugation. 30 S subunits reconstituted from: (A) native 16 S rRNA; (B) fragments of 16 S rRNAI recovered from appropriate fractions of sucrose gradients (see hatched area in Fig. 2C). 30 S subunits recovered from appropriate fractions (hatched areas I and II) were used in protein synthetic assays and for association with 50 S subunits.

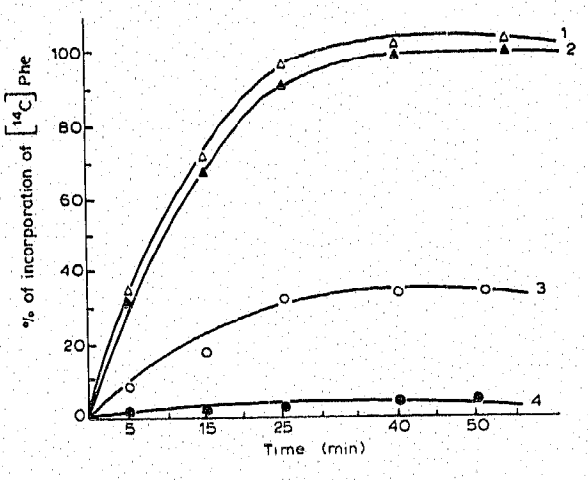

Fig. 4. In vitro poly(U)-directed protein synthesis. Polyphenylalanine incorporation by ribosomes containing: 1 = native 30 S subunits; 30 S subunits with: 2 = native 16 S RNA; 3 = 16 S RNAI; 4 = 16 S RNAII. The 240 pmol of [^{14}C]phenylalanine incorporated into TCA-insoluble material/pmol of 70 S ribosomes were defined as 100% (the mean of background incorporation in blank probes without mRNA was 24 pmol/pmol of 70 S ribosomes).

The ability of 16 S rRNA halves to associate only in the presence of 30 S proteins deserved special attention. It was suggested long ago that ribosomal proteins promote the formation of the compact structure of RNA within ribosomes due to their capacity to change the initial RNA conformation [1]. In particular, it was demonstrated that proteins S4 and S8 strongly affect the 16 S rRNA structure outside of their binding sites [10-13]. We have found, however, that neither S4 nor S8 stimulated the association of the 16 S rRNA halves although both proteins bound to these rRNA fragments with the same efficiency as to the intact 16 S rRNA (data not shown).

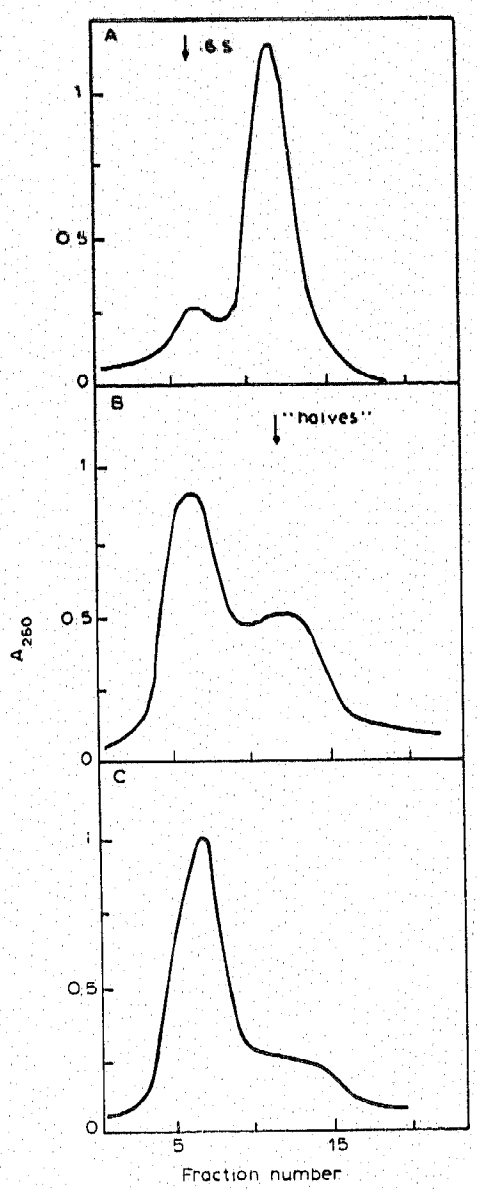

Fig. 5. Analysis of RNP-complexes by sucrose gradient centrifugation. Complexes of 16 S rRNA halves (see hatched area in Fig. 2C) with: (A) protein S4; (B) mixture S4, S16(S17); (C) mixture S4, S8, S16(S17).

The minimal set of 30 S subunit proteins which was capable of inducing reassociation of the 16 S rRNA halves consisted of S4 and S16(S17) (Fig. 5). The ratio between S16 and S17 in our protein preparation was 2:1. It was previously shown that protein S17 binds independently to 16 S rRNA and protects several nucleotides in the immediate neighborhood of its binding site. In contrast, binding of S16 depends on the presence of S4. It changes the pattern of chemical modification - about 45 nucleotides scattered in the 5'-half of 16 S rRNA whereas no S4 and S16 binding sites were found in the 3'-half of 16 S rRNA [14]. One can suggest that proteins S4 and S16 change the conformation of the 5'-half of the 16 S rRNA and make it competent for association with corresponding fragments. As can also be seen from Fig. 5C, S8 stimulates the transition of the 16 S rRNA halves into associated form. This is not surprising since some of S8 footprinting sites are located in the segments belonging to both 16 S rRNA halves [13].

Finally, we conclude that the results presented here demonstrate unambiguously that protein induced RNA-RNA interactions initiate the rRNA compact folding within ribosomal subunits.

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