ACTIVITIES OF *B. STEAROTHERMOPHILUS* 50 S RIBOSOMES RECONSTITUTED WITH PROKARYOTIC AND EUKARYOTIC 5 S RNA*

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1. Introduction

Reconstitution experiments have clearly demonstrated the importance of 5 S RNA as a structural component in the 50 S ribosomal subunit [1, 2]. 50 S ribosomes reconstituted without 5 S RNA are void of several proteins and show greatly reduced biological activities. From these experimental findings it became clear that for studying the biological function of 5 S RNA, modification of the RNA followed by reconstitution and protein synthesizing assays, would be helpful. Two principle types of modifications were at our disposal: i) chemical modification and ii) natural modification as has been undergone in different pro- and eukaryotic organisms during evolution.

Using chemical modification it has been shown that the 3′ terminal base in 5 S RNA does not play an essential role in the structure and function of the 50 S subunit [3, 4]. More recently, we could show that selective chemical modification of two adenines per 5 S RNA molecule has significant effects on its function, but not on its structure (Erdmann, Sprinzl and Pongs, in preparation).

The other type of 5 S RNA modification mentioned above, i.e., the natural one, will be analysed here. Earlier experiments have shown that it is possible to use 16 S RNA of different bacterial strains and *E. coli* 30 S proteins to reconstitute active hybrid 30 S subunits [5]. Similar experiments were carried out with 23 S RNA from *Staphylococcus aureus* and proteins from *Bacillus stearothermophilus* yielding active 50 S ribosomal subunits [6].

In this report we specifically asked the question whether or not different 5 S RNA species could be incorporated in *B. stearothermophilus* 50 S subunits to yield active ribosomes. Such study would possibly cast some light on whether 5 S RNA could be used for determining to what extent different bacterial ribosomes are related to each other. Furthermore, since the 5 S RNA sequences from two bacterial and from several higher developed organisms are known [7–10], we hope that this study will give the basis from which one could start a correlation study between 5 S RNA structural elements and their possible function in protein synthesis.

The results presented here show clearly that all prokaryotic 5 S RNA's tested were active in reconstituted *B. stearothermophilus* 50 S subunits, whilst eukaryotic 5 S RNA'S were not.

2. Experimental

2.1. Bacterial strains

The following bacterial strains were grown in media, as cited by the references, to mid log phase before harvesting: *E. coli* A19 [2], *B. stearothermophilus* strain 799 [2], *Proteus vulgaris* [11], *Bacillus subtilis* [11], *Micrococcus lysodeicticus* [12], *Azotobacter vinelandii* [13], *Pseudomonas fluorescens* [11], *S. aureus* [12].

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2.2. Preparation of ribosomes and poly-U assay

Bacterial ribosomes were isolated as previously described [1]. Ribosomes from yeast (*Saccharomyces cerevisiae*) and beans (*Vicia faba*) were a gift from Dr. H.G. Janda. Rat and horse liver ribosomes were prepared as described by H. Bielka [14]. Ribosomes from wheat germ were prepared as previously described [15], whilst *Artemia salina* ribosomes were a gift from J. Zimmermann. Poly-U directed polyphenylalanine synthesis was measured as previously published [2]. Yeast $tRNA^{Phe}$ was a generous gift from Dr. O. Pongs.

2.3. Preparation of 5 S RNA

Ribosomal RNA was either prepared by phenol extraction of ribosomes [16] or phenol extraction of crude bacterial extracts. When the crude extract was used as the RNA source, the bacterial cells (30 g) were mixed with 60 g of alumina (Alcoa, A 305) in an ice cold mortar. The cells and alumina powder were mixed until an even paste was obtained and grinding was then continued for a total of 4 min. Broken bacteria and alumina were then taken up in 150 ml 0.01 M Tris-HCl (pH 7.8), 0.01 M $MgCl_2$, 0.03 M NH_4Cl, 0.006 M mercaptoethanol (TMA I) contaning 2 μg DNAase (RNAase-free, Boehringer, Germany) per ml. The suspension was allowed to stand for 15 min on ice before an equal volume of phenol saturated with 0.25 M phosphate buffer (pH 7.0) was added. After 10 min of vigorous shaking the sample was centrifuged at low speed (10 000 *g*; 15 min; 4°C). The aqueous phase was re-extracted with buffer saturated phenol until a clear interphase between buffer and phenol was obtained. Usually four to five phenol extractions were required. RNA was precipitated from the aqueous phase with 2 vol. of −20°C ethanol over night. Pure 5 S RNA was then isolated by Sephadex G-100 chromatography as described previously by Erdmann et al. [3].

2.4. Gel electrophoresis of RNA

Polyacrylamide gel electrophoresis was essentially carried out according to Loening [17] and Peacock and Dingman [18] with the following modifications: Composite gels were prepared consisting of a 10% gel on the bottom (4.5 cm) and 3.1% acrylamide gel (4.5 cm) on the top. The combination of these two gels had the advantage of allowing the separation of 23 S, 16 S, 5 S rRNA and tRNA. Generally 0.1 A_{260} units, in 25 μl electrophoresis buffer, of 5 S RNA were applied per gel. In order to obtain sharper bands the RNA samples were applied to the gels in 5% sucrose. Electrophoresis was carried out at 2 mA per gel until the bromophenol blue marker had migrated to the end of the 10% gel. To stain the RNA bands 0.1% toluidine blue in 1% acetic acid (45 min) was used. The gels could rapidly be destained in 3 hr using 5% acetic acid.

3. Results

The different species of 5 S RNA were checked for their purity by polyacrylamide disc electrophoresis (fig. 1). All samples employed for reconstitution experiments were at least 95% pure, as judged from densitometer tracings of the RNA gels. Fig. 1 shows polyacrylamide gels of some prokaryotic and eukaryotic 5 S RNA preparations. Since all 5 S RNA's so far sequenced contain approx. 120 nucleotides per molecule [7–10], it should be expected that they have identical mobility in the RNA disc electrophoresis. *B. stearothermophilus* 23 S RNA and 50 S proteins were reconstituted for 1 hr at 60°C [2–4] with the following different bacterial 5 S RNA's: *B. stearothermophilus, E. coli, P. vulgaris, S. aureus, M. lysodeicticus, A. venelandii, B. subtilis* and *Ps. fluorescens.* After reconstitution the ribosomes were tested for their ability to participate in a poly-U directed polyphenylalanine synthesis. The results are summarized in table 1 and show clearly that all bacterial 5 S RNA's tested exhibit biological activity when incorporated in *B. stearothermophilus* 50 S subunits. The findings that *E. coli* and *Ps. fluorescens* 5 S RNA are active in the *B. stearothermophilus* system is in agreement with previously published results [2, 3, 19].

B. stearothermophilus and *E. coli* 5 S RNA's were the most active when tested (table 1), but since such factors as growth conditions and age of cell culture when harvested could possibly influence the 5 S RNA preparations we feel that the differences in activities observed may not be significant. *Ps. fluorescens,* for example, showed only 75% of the activity when compared with *B. stearothermophilus* 5 S RNA (table 1) whilst Fahnestock and Nomura (personal communication and [20]) have found no difference in activity.

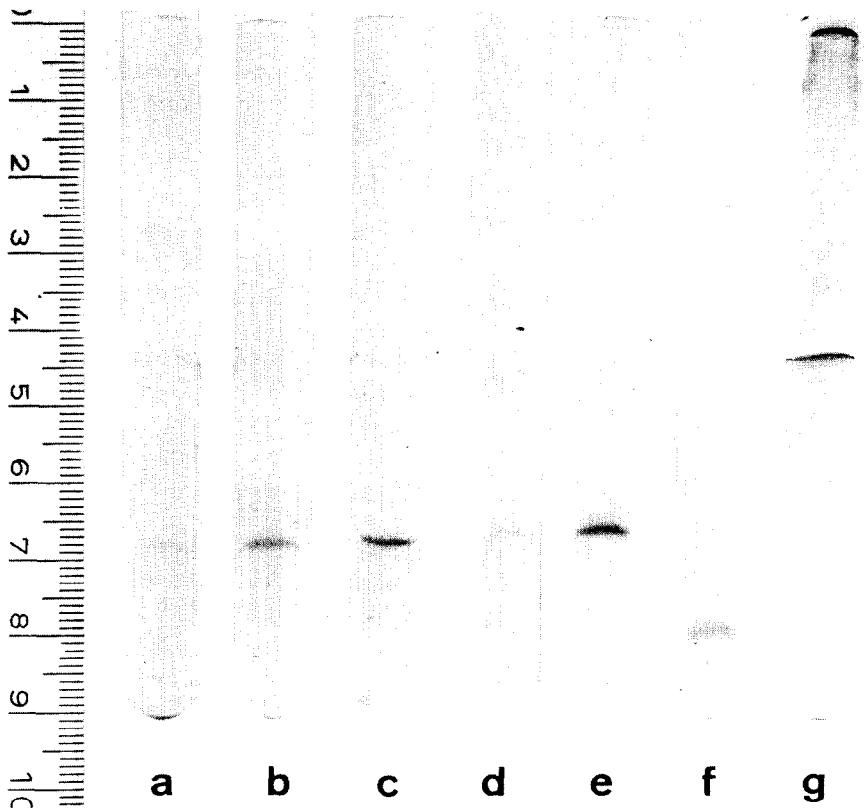

Fig. 1. Disc gel electrophoresis of different 5 S RNA's: a) *B. stearothermophilus*, 0.095 A_{260} unit; b) *Micrococcus lysodeicticus*, 0.1 A_{260} unit; c) *Azotobacter vinelandii*, 0.13 A_{260} unit; d) *Saccharomyces cerevisiae*, 0.05 A_{260} unit; e) Rat liver, 0.1 A_{260} unit; f) $tRNA^{Phe}$ (yeast) 0.11 A_{260} unit; g) 23 S RNA (*E. coli*), 0.5 A_{260} unit. Upper 4.5 cm: 3.1% acrylamide gel. Bottom 4.5 cm: 10% acrylamide gel.

The selection of eukaryotic 5 S RNA's tested included yeast, wheat germ, beans, *Artemia salina*, rat liver and horse liver. The results obtained showed clearly that eukaryotic 5 S RNA's are not active in bacterial ribosomes (table 1). Polyacrylamide gel electrophoresis analysis of such reconstituted ribosomes revealed that none of the eukaryotic 5 S RNA's were incorporated. It remains to be seen whether these eukaryotic 5 S RNA's formed a specific 5 S RNA–protein complex as found for *B. stearothermophilus* and *E. coli* [19] but did not get incorporated into the reconstituted 50 S subunit or whether they could not even form the 5 S RNA–protein complex. Nevertheless this question seems to be worth further analysis, since the answer could yield valuable information in respect to what structural elements of 5 S RNA are important for the interaction with the 5 S RNA binding proteins.

Recently, we have tested 5 S rRNA from *Halobacterium cutirubrum*, which was also active in our *Bacillus* system (P. Wrede, A.T. Matheson and V.A. Erdman, unpublished results). Although the *Halobacterium* ribosomes are only stable in 3–4 M KCl [21–23] the incorporation of *Halobacterium* 5 S RNA into *B. stearothermophilus* 50 S subunits was possible at the relatively low salt concentration of the *Bacillus* reconstitution system. Since we obtained activities with 5 S RNA's from distantly related bacteria as well as from bacteria in extreme growth conditions (high salt and temperature) we conclude that specific structural and functional elements of these RNA's must have been strongly conserved during evolution.

Table 1
Activities of reconstituted *B. stearothermophilus* 50 S ribosomes with different 5 S RNA's.*

Species	Order	Poly-U activities♦
E. coli	Enterobacteriaceae	+++ ♦
Proteus vulgaris	Enterobacteriaceae	+++
B. stearothermophilus	Bacillaceae	+++
B. subtilis	Bacillaceae	++(+)
M. lysodeicticus	Micrococcaceae	+++
S. aureus	Micrococcaceae	+++
Ps. fluorescens	Pseudomonadaceae	++(+)
A. vinelandii	Azotobacteriaceae	+++
S. cerevisiae		---•
Bean		---
Wheat germ		---
Artemia salina		---
Rat liver		---
Horse liver		---

* The data summarized in this table represents the average of at least three reconstitutions with each 5 S RNA preparation.
♦ 1.0 A_{260} unit of 50 S subunits was tested with 1.0 A_{260} unit of 30 S [2]. Each assay was carried out in duplicates.
♦ Bacterial 5 S RNA's yielded activities between 7000 cpm [++(+)] and 10 000 cpm [+++] above the −5 S RNA particle blank.
• All eukaryotic 5 S RNA's showed activities between 0 and 100 cpm above background [- - -]. −5 S RNA particles + 30 S = 700 cpm. 50 S + 30 S = 20 000 cpm.

4. Discussion

The results presented indicate that the possible differences in the sequences of the various bacterial 5 S RNA's [24] do not influence their functions. Since all bacterial 5 S RNA's exhibited approximately similar activities, the differences found between them are too small to correlate biological activity of the different 5 S RNA's with the taxonomic relationship among the tested bacteria (see Bergey's Manual of Determinative Bacteriology, 1957, for taxonomy of bacteria).

It is evident from table 1 that there are two groups of 5 S RNA's: i) Those from bacteria, even if they belong to a wide taxonomic spectrum, are fully replaceable and active in the *Bacillus* system. ii) On the other hand the 5 S RNA's from all tested eukaryotes, regardless whether they are lower or higher plants as well as lower or higher animals, are completely inactive in this system. Apparently the structure of the eukaryotic 5 S RNA's is too different from those of bacterial 5 S RNA's for a structural and functional replacement. There seems to be no 5 S RNA with an intermediary behaviour which is more evidence for the two separate classes of pro- and eukaryotic ribosomes.

Recently Bellemare et al. [25] tested three prokaryotic and six eukaryotic 5 S RNA's in a partial reconstitution system with *E. coli* 50 S ribosomal subunits. The eukaryotic 5 S RNA's did not bind to the reconstituted subunits. This result is in agreement with our findings. From the three prokaryotic 5 S RNA's tested, *E. coli* and *Ps. fluorescens* showed significant binding, whilst *B. stearothermophilus* 5 S RNA bound only 1/10 as well as *E. coli* 5 S RNA to the reconstituted subunits. The latter finding is surprising, since we could not find any significant differences in binding and activities of *B. stearothermophilus* 50 S subunits reconstituted with eight different bacterial 5 S RNA's. A possible explanation for this discrepancy may lie in the fact that the *E. coli* partial reconstitution does not yield biologically active 50 S subunits or that the higher temperature in the *B. stearothermophilus* reconstitution system helps to overcome some minor structural differences between the different bacterial 5 S RNA's.

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