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Escherichia coli 5S RNA binding proteins L18 and L25 interact with 5.8S RNA but not with 5S RNA from yeast ribosomes*

(structure and function of small rRNAs/ribosomal A-site/RNA–protein complexes/oligonucleotide binding/evolution)

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ABSTRACT **Reconstitution experiments showed that the two *Escherichia coli* 5S RNA binding proteins L18 and L25 form a specific complex with yeast 5.8S RNA and not with yeast 5S RNA. The yeast 5.8S RNA–*E. coli* protein complex was found to exhibit ATPase and GTPase activities that had previously been observed for the *E. coli* 5S RNA–protein complex. The tetranucleotide UpUpCpG, which is an analog of the tRNA fragment TpψpCpG, interacted strongly with 5S RNA–protein complexes from *E. coli* and *Bacillus stearothermophilus* and weakly with yeast 5.8S RNA. UpUpCpG did not bind to *E. coli*, *B. stearothermophilus*, or yeast 5S RNA or to the yeast 5.8S RNA–*E. coli* protein complex. It is suggested that 5.8S RNA evolved from prokaryotic 5S RNA and that the latter two RNAs are related and have similar functions in protein synthesis.**

Large ribosomal subunits of prokaryotic and eukaryotic organisms contain 5S RNA; eukaryotic ribosomes in addition contain 5.8S RNA (for recent review, see ref. 2). Because a number of different 5S RNAs have been sequenced, this molecule is ideally suited for evolutionary studies and investigations on protein–nucleic acid interaction (2–5).

Experimental evidence about the function of prokaryotic 5S RNA suggests that it participates directly in the binding of aminoacyl- (6, 7) and uncharged (8–11) tRNA to the ribosomal A-site. The biological functions of eukaryotic 5S and 5.8S RNA are less clear although it can be assumed that one of their functions is the binding of ribosomal proteins.

In previous comparative studies we have shown that prokaryotic 5S RNAs (from *Bacillus stearothermophilus*, *B. subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Micrococcus lysodeikticus*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Azotobacter vinelandii*, and *Halobacterium cutirubrum*) can be incorporated into biologically active 50S ribosomal subunits of *B. stearothermophilus*, whereas eukaryotic 5S RNAs [from yeast, beans, wheat germ, brine shrimp (*Artemia salina*), rat liver, and horse liver] cannot (12). In other reconstitution experiments it was possible to incorporate several prokaryotic but no eukaryotic 5S RNAs into biologically inactive 50S subunits of *E. coli* (13). On the basis of these results, prokaryotic and eukaryotic 5S RNAs may be divided into two distinct classes. In addition, it was possible to isolate and characterize specific homologous and heterologous 5S RNA–protein complexes (14). Therefore, it is likely that, during evolution, certain molecular aspects important for recognition, interaction, and function of prokaryotic 5S RNA and its specific binding to ribosomal proteins have been conserved.

This communication describes work that extends our previous comparative studies on 5S RNA. The data presented show that none of the 34 different *E. coli* 50S ribosomal proteins interacts with eukaryotic 5S RNA, whereas eukaryotic 5.8S RNA specifically binds to the proteins L18 and L25 which are the 5S RNA binding proteins in the *E. coli* ribosome (14, 15). The significance of this observation with respect to evolution, RNA–protein interaction, and conformational state as well as the possible functions of eukaryotic 5S and 5.8S RNA will be discussed.

Abbreviation: TR buffer, 30 mM Tris·HCl, pH 7.4/20 mM $MgCl_2$/320 mM KCl/6 mM 2-mercaptoethanol.

* This is paper no. 11 in a series on structure and function of 5S RNA. Paper no. 10 is ref 1.

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MATERIALS AND METHODS

Materials. ATP, GTP, CDP, GDP, UpU, and polynucleotide phosphorylase (polyribonucleotide:orthophosphate nucleotidyltransferase, EC 2.7.7.8; 30 units/mg) were purchased from Boehringer Mannheim (Germany). [5-^{3}H]Cytidine 5′-diphosphate (ammonium salt; 16 Ci/mmol), [8-^{3}H]guanosine 5′-diphosphate (ammonium salt; 11.5 Ci/mmol), and adenosine 5′-[γ-^{32}P]triphosphate (ammonium salt; 10 Ci/mmol) were obtained from Amersham Buchler, Braunschweig (Germany).

Preparation of Ribosomes, Ribosomal Proteins, and 5S and 5.8S RNA. *E. coli* 50S ribosomal subunits were isolated as previously described (16). Yeast (*Saccharomyces cerevisiae*) 80S ribosomes were prepared in collaboration with B. Schulz-Harder (Freie Universität, Berlin) according to the procedure in ref. 17. *E. coli* 5S RNA was prepared by phenol extraction of 50S ribosomal subunits and subsequent Sephadex G-100 gel filtration (18). To isolate yeast 5S and 5.8S RNA, 80S ribosomes were phenol extracted and the total RNA was precipitated with two volumes of ethanol at −20° for 12 hr (12). After low-speed centrifugation, the total RNA (1500 A_{260} units) was taken up in 6 M urea and heated at 60° for 5 min. Then, the RNA solution was rapidly chilled to 0° and applied to a Sephadex G-100 column (3.2 × 190 cm) that had been equilibrated with 0.05 M KCl/1% (vol/vol) methanol (18). The column was monitored at 260 nm and the peaks were further analyzed by polyacrylamide gel electrophoresis for RNA content (12). The 5S and 5.8S RNA fractions were concentrated by ethanol precipitation (two volumes of ethanol, −20°, overnight) and low-speed centrifugation. Total *E. coli* 50S ribosomal protein fraction was prepared as previously described (14).

Reconstitution and Isolation of 5S and 5.8S RNA–Protein Complexes. For the reconstitution of 5S and 5.8S RNA–protein complexes, a previous method (14) for 5S RNA–protein complexes was slightly modified; *E. coli* 5S RNA, yeast 5S RNA, or yeast 5.8S RNA was dissolved in 30 mM Tris·HCl, pH 7.4/20 mM $MgCl_2$ at 20 A_{260} units/ml and heated at 60° for 15 min. The RNA solutions were then slowly cooled to 0° and the buffer was adjusted to 30 mM Tris·HCl, pH 7.4/20 mM $MgCl_2$/320 mM KCl/6 mM 2-mercaptoethanol (TR buffer). Subsequently, 320 equivalent units of *E. coli* total 50S proteins (in TR buffer) was added to 10 A_{260} units of 5S RNA or 13 A_{260} units of 5.8S

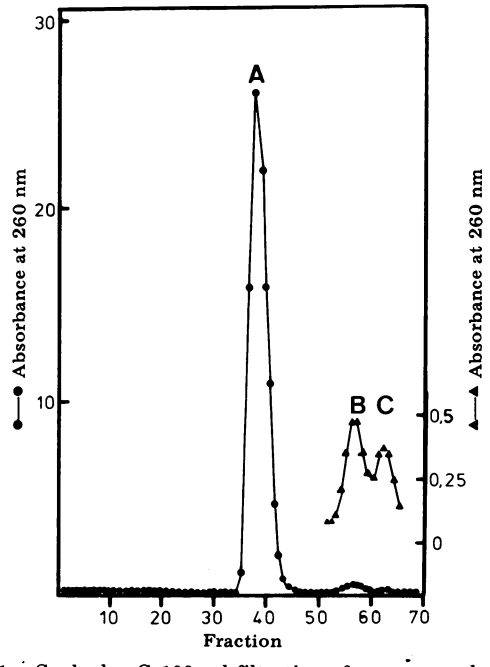

FIG. 1. Sephadex G-100 gel filtration of urea-treated yeast ribosomal RNA (1500 A_{260} units in 5 ml), isolated by phenol extraction of 80S ribosomes. Peak A corresponds to 28S and 18S RNA, peak B to 5.8S RNA, and peak C to 5S RNA. For experimental details see *Materials and Methods*.

RNA (1 equivalent unit of protein corresponds to the amount of protein obtained from 1 A_{260} unit of *E. coli* 50S ribosomes). The RNA–protein mixture was then incubated at 37° for 15 min and at 0° for 12 hr. The reconstituted 5S RNA- and 5.8S RNA–protein complexes were isolated by sucrose gradient centrifugation (Spinco SW 27 rotor). Each gradient consisted of 14 ml of 50% (wt/vol) sucrose overlayered by 2 ml of 20% sucrose and a 20-ml linear gradient of 5–15% sucrose. All sucrose solutions were made up in TR buffer. Centrifugation was carried out at 25,000 rpm (100,000 × *g*) at 4° for 60 hr. Harvesting of gradients was done as previously described (14). *B. stearothermophilus* 5S RNA protein complex was prepared as reported (14).

Two-Dimensional Gel Electrophoresis of Proteins. The fractions containing the 5S or 5.8S RNAs were first dialyzed against 15 mM Tris·HCl, pH 7.4/10 mM $MgCl_2$/30 mM NH_4Cl/6 mM 2-mercaptoethanol and then treated with 66% (vol/vol) acetic acid in 0.1 M $MgCl_2$ to extract the ribosomal proteins (19). Two-dimensional gel electrophoresis of the ribosomal proteins was carried out as described (20).

Enzymatic Activities. ATPase and GTPase hydrolysis was measured as described (21, 22), except that, for the yeast 5.8S RNA–*E. coli* protein complex, 0.091 A_{260} unit was used per assay.

Equilibrium Dialysis. This was performed in 30 mM Tris·HCl, pH 7.4/20 mM $MgCl_2$/320 mM KCl/6 mM 2-mercaptoethanol for 84 hr at 0°. Oligonucleotide synthesis and equilibrium dialysis were performed as described (23).

RESULTS

Pure *E. coli* 5S RNA was isolated by Sephadex G-100 gel filtration from an RNA mixture obtained by phenol extraction of 50S ribosomal subunits (18). As can be seen from Fig. 1, this procedure permits the separation of yeast 5S and 5.8S RNA in total RNA obtained from 80S ribosomes. The elution pattern revealed three peaks, of which peak A (void volume) consisted of 18S and 28S RNA. Peaks B and C were further analyzed by

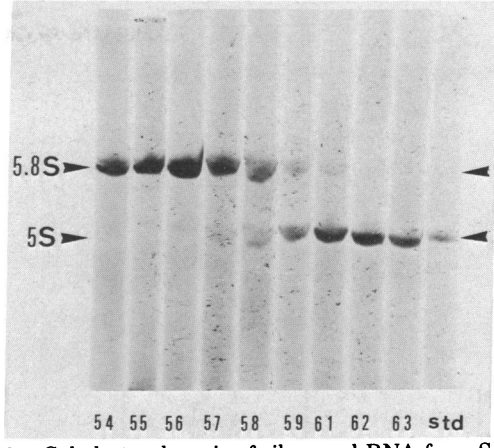

FIG. 2. Gel electrophoresis of ribosomal RNA from Sephadex G-100 fractions of peaks B and C of Fig. 1. The numbers below each gel correspond to the fraction numbers of the Sephadex G-100 run; "std." means *E. coli* 5S RNA was run as reference material. For other experimental details see *Materials and Methods*.

RNA gel electrophoresis. Fig. 2 shows that peak B corresponded to 5.8S RNA and peak C, to 5S RNA. The area under the A_{260} absorbance peak B was always 1.3 times that under peak C, suggesting a 1:1 molar ratio of 5.8S RNA/5S RNA in 80S ribosomes if a chain length of 158 nucleotides for 5.8S RNA (24) and 120 nucleotides for 5S RNA (25) is assumed.

To analyze possible interaction of *E. coli* 5S RNA, yeast 5S RNA, and yeast 5.8S RNA with *E. coli* proteins, the RNAs were incubated with *E. coli* 50S proteins as indicated under *Materials and Methods*. Subsequent sucrose gradient centrifugation yielded the A_{260} profiles shown in Fig. 3. A_{280} (not shown) was measured and the A_{260}/A_{280} ratio was determined. The gradients containing *E. coli* (Fig. 3*d*) and yeast (Fig. 3*c*) 5S RNAs and yeast 5.8S RNA (Fig. 3*a*) showed two distinct peaks (A and B) at 260 nm. The A_{260}/A_{280} ratios suggested that peak A contained mainly RNA and peak B, the ribosomal proteins.

Extraction of the material in the three A peaks (Fig. 3 *a*, *c*, and *d*) with acetic acid followed by two-dimensional gel electrophoresis showed that only *E. coli* 5S RNA and yeast 5.8S RNA interacted with ribosomal proteins; yeast 5S RNA did not. Fig. 4 shows the two-dimensional gel electrophoresis patterns for the RNA–protein complex containing *E. coli* 5S RNA (Fig. 4 *upper*) and yeast 5.8S RNA (Fig. 4 *lower*). The *E. coli* 5S RNA binding proteins were primarily E-L5, E-L18, and E-L25, in agreement with previous results (14). In addition, one can see small amounts of proteins E-L1, E-L10, E-L7/12, E-L27, and E-L30 (Fig. 4 *upper*). The proteins that interacted with yeast 5.8S RNA were E-L18 and E-L25.

Because *E. coli* and *B. stearothermophilus* homologous 5S RNA–protein complexes exhibit GTPase and ATPase activities (21, 22), we analyzed the yeast 5.8S RNA–*E. coli* protein complex for similar hydrolytic activities. The results (Table 1) show that the yeast 5.8S RNA–*E. coli* protein complex was active although less (approximately 50%) so than the *E. coli* 5S RNA–protein complex.

In prokaryotic 5S RNAs the conserved region around position 40 with the sequence CpGpApAp is able to bind the complementary tetranucleotide UpUpCpG only when the 5S RNA is complexed with its specific binding proteins (2). Therefore, we compared the binding of UpUpCpG to the different RNAs and RNA–protein complexes by equilibrium dialysis. As previously observed, the tetranucleotide UpUpCpG did not bind to free *E. coli* (1, 6) or *B. stearothermophilus* (1) 5S RNAs but only to their 5S RNA–protein complexes (Table 2). Similarly, free yeast 5S RNA did not bind UpUpCpG, but yeast 5.8S RNA interacted

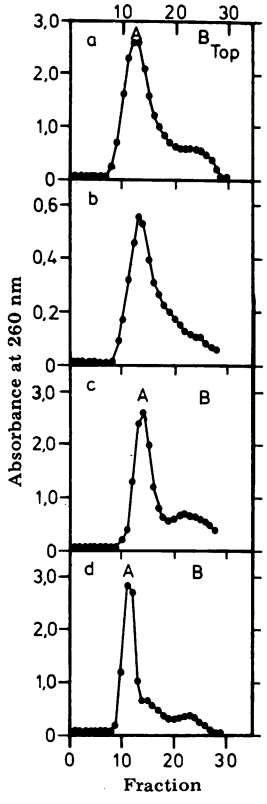

FIG. 3. Sucrose gradient centrifugation of RNA–protein complexes. (*a*) Yeast 5.8S RNA (33.5 A_{260} units in 1 ml) after incubation with *E. coli* total 50S proteins (815 equivalent units). (*b*) Yeast 5.8S RNA (5 A_{260} units in 0.25 ml). (*c*) Yeast 5S RNA (32 A_{260} units in 1 ml) after incubation with *E. coli* total 50S proteins (1000 equivalent units). (*d*) *E. coli* 5S RNA (32 A_{260} units in 1 ml) after incubation with *E. coli* total 50S proteins (1000 equivalent units). For other experimental details see *Materials and Methods*.

weakly with this oligonucleotide. On the other hand, interaction of *E. coli* proteins E-L18 and E-L25 to 5.8S RNA decreased the binding of the tetranucleotide UpUpCpG to the RNA.

DISCUSSION

Previous comparative studies have shown that prokaryotic 5S RNAs are significantly different from eukaryotic 5S RNAs: the latter cannot be incorporated into active 50S ribosomal subunits from *B. stearothermophilus*. The reconstitution experiments reported here support this earlier observation because we found that yeast 5S RNA does not interact with prokaryotic ribosomal proteins.

Eukaryotic 60S ribosomal subunits contain, besides 5S RNA, one additional small ribosomal RNA—namely, 5.8S RNA. Yeast 5.8S RNA consists of 158 nucleotides and is, therefore, nearly 30 nucleotides longer than prokaryotic and eukaryotic 5S RNAs. Because its sequence shows significant similarities to prokaryotic 5S RNAs we analyzed it for possible protein interaction with *E. coli* 50S ribosomal proteins. As shown in Fig. 4, the eukaryotic 5.8S RNA was found to interact with the prokaryotic ribosomal proteins L18 and L25 of *E. coli*, which have been identified as 5S RNA binding proteins. These results suggest that eukaryotic 5.8S RNA has evolved from prokaryotic 5S RNA. The observation that the 5.8S RNA–E-L18–E-L25 complex exhibits ATPase and GTPase activities (Table 1) indicates that the enzymatic activities of these prokaryotic proteins are not significantly altered when they bind to the eukaryotic RNA.

The tRNA fragment TpψpCpG or its synthetic analog UpUpCpG inhibits enzymatic aminoacyl-tRNA binding to the

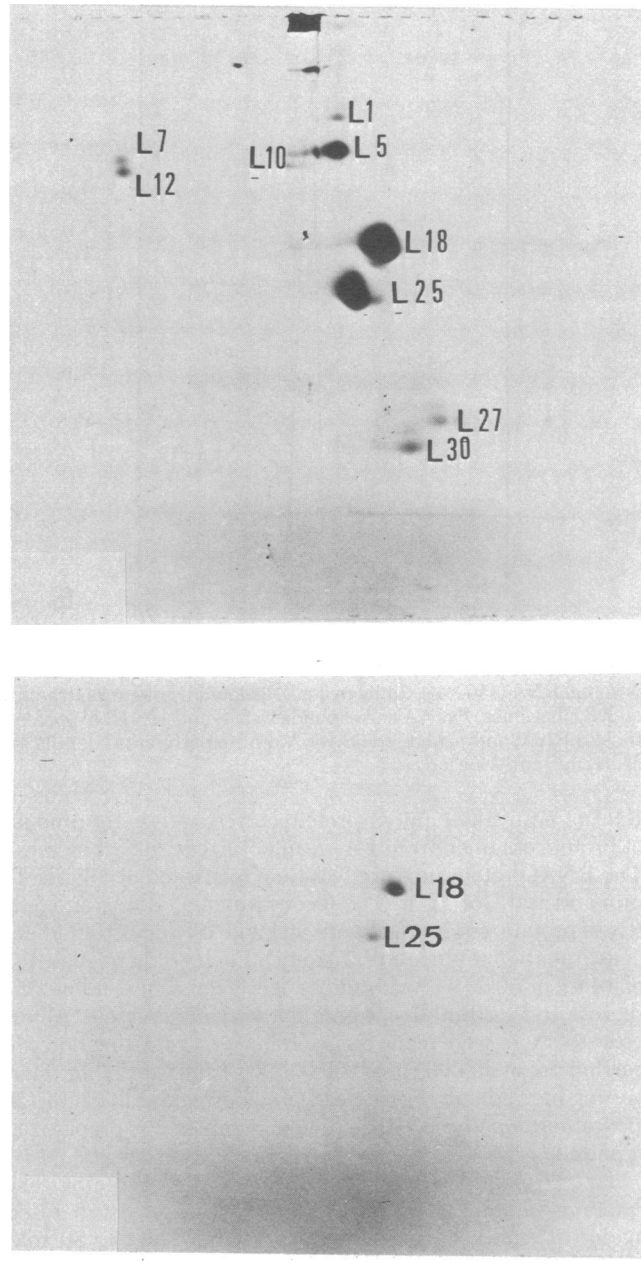

FIG. 4. Two-dimensional gel electrophoresis of proteins. (*Upper*) Extracted from the *E. coli* 5S RNA–*E. coli* protein complex (peak A of Fig. 3*d*). (*Lower*) Extracted from yeast 5.8S RNA–*E. coli* protein complex (peak A of Fig. 3*a*). There was no evidence of complex formation between yeast 5S RNA and any of the *E. coli* 50S proteins. For experimental details see *Materials and Methods* and ref. 14.

ribosomal A-site by interacting solely with the 50S ribosomal subunit (7). This interaction is possibly taking place with 5S RNA; it was shown that *E. coli* 5S RNA can only bind these oligonucleotides if it has first interacted with ribosomal proteins (6). Because of these functional implications we determined the binding of UpUpCpG to the different small ribosomal RNAs and their complexes. As summarized in Table 2, UpUpCpG did not interact with free 5S RNAs from *E. coli*, *B. stearothermophilus*, or yeast and showed intermediate binding affinity to yeast 5.8S RNA.

Of the RNA–protein complexes, only the ones from *E. coli* and *B. stearothermophilus* were found to bind the tetranucleotide. The observation that the apparent binding constant of UpUpCpG to the *B. stearothermophilus* 5S RNA–protein complex is significantly larger than that with the *E. coli* 5S

Table 1. ATPase and GTPase activities of *E. coli* and *B. stearothermophilus* 5S RNA–protein complexes and yeast 5.8S RNA–*E. coli* protein complex

RNA–protein complex		Hydrolysis, pmol	
RNA	Proteins*	ATP	GTP
Yeast 5.8S	E-L18, E-L25	38	10
E. coli 5S	E-L5, E-L18, E-L25	97	16
B. stearothermophilus 5S	B-L5, B-L22	90	60

ATP and GTP hydrolysis assays were performed under standard conditions (21, 22), except that 0.091 A_{260} unit of yeast 5.8S RNA–*E. coli* protein complex and 0.07 A_{260} unit of *E. coli* and *B. stearothermophilus* 5S RNA–protein complex was used. ATPase assays were carried out at 30° and GTPase assays, at 37°. In the absence of RNA, there is no detectable GTPase or ATPase activity (22).

* E indicates *E. coli* protein; B indicates *B. stearothermophilus* protein. It has previously been determined that *E. coli* proteins E-L5 and E-L18 correspond to *B. stearothermophilus* proteins B-L5 and B-L22, respectively (14).

RNA–protein complex has been made repeatedly and therefore suggests that the complementary *B. stearothermophilus* 5S RNA sequence (CpGpApA, positions 41–44) is more optimally oriented. The reason for this finding is not clear and could possibly be the fact that the *E. coli* 5S RNA–protein complex contains one additional protein, E-L25. The yeast 5.8S RNA–E-L18–E-L25 complex showed only weak binding of UpUpCpG (Table 2), and it is therefore clear that these two proteins cannot alter the RNA structure to stimulate the binding of the tetranucleotide. In this context it is worth pointing out that the latter complex did not contain protein E-L5, which is known to bind to those parts of *E. coli* and *B. stearothermophilus* 5S RNAs that contain the conserved sequence CpGpApA (V. Zimmermann and V. A. Erdmann, unpublished data).

On the basis of our results reported here—that yeast 5.8S RNA interacts with the *E. coli* 5S RNA binding proteins E-L18 and E-L25—and the fact that prokaryotic 5S RNAs (26) and eukaryotic 5.8S RNAs (27) are constituents of ribosomal RNA precursors which include the corresponding two large ribosomal RNAs, we propose that prokaryotic 5S RNA and eukaryotic 5.8S RNA are of the same evolutionary origin and that their functions in protein synthesis are similar. Previous experimental evidence supports the hypothesis that binding of tRNAs to the ribosomal A-site involves the conserved tRNA sequence TpψpCpGp and prokaryotic 5S RNA. The function of eukaryotic 5S RNA is less clear and it may well be involved in initiator tRNA binding to the 80S ribosomes (2).

Table 2. Binding constants of UpUpCpG to 5S RNAs, 5.8S RNA, and RNA–protein complexes

RNA or RNA–protein complex		Binding constant (K), M^{-1}
RNA	Proteins	
E. coli 5S	None	2,000
5S	E-L5, E-L18, E-L25	22,000
B. stearothermophilus 5S	None	3,000
5S	B-L5, B-L22	135,000
Yeast 5S	None	5,700
5.8S	None	12,100
5.8S	E-L18, E-L25	5,600

Equilibrium dialysis experiments were carried out as described under *Materials and Methods*. 5S RNA and 5S RNA–protein complexes were 11 μM; 5.8S RNA and 5.8S RNA–protein complex was 9 μM. The tetranucleotide UpUpCpG was at 10 nM with a specific activity of 11.5 mCi/mmol. The binding constants were determined as described (23).

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