Volume 16 Number 8 1988 Nucleic Acids Research

In vitro incorporation of eubacterial, archaebacterial and eukaryotic 5S rRNAs into large ribosomal subunits of *Bacillus stearothermophilus*

Roland K.Hartmann, Detlef W.Vogel, Richard T.Walker[1] and Volker A.Erdmann

Institut für Biochemie, FB Chemie, Freie Universität Berlin, Otto-Hahn-Bau, Thielallee 63, 1000 Berlin 33 (Dahlem), FRG and [1]Department of Chemistry, University of Birmingham, Birmingham B15 2TT, UK

Received January 7, 1988; Revised and Accepted March 8, 1988

ABSTRACT

Bacillus stearothermophilus large ribosomal subunits were reconstituted in the presence of 5S rRNAs from different origins and tested for their biological activities. The results obtained have shown that eubacterial and archaebacterial 5S rRNAs can easily substitute for *B. stearothermophilus* 5S rRNA in the reconstitution, while eukaryotic 5S rRNAs yield ribosomal subunits wih reduced biological activities. From our results we propose an interaction between nucleotides 42-47 of 5S rRNA and nucleotides 2603-2608 of 23S rRNA during the assembly of the 50S ribosomal subunit. Other experiments with eukaryotic 5.8S rRNAs reveal, if at all, a very low incorporation of these RNA species into the reconstituted ribosomes.

INTRODUCTION

The ribosomal 5S rRNAs are essentiel constituents of the large ribosomal subunits of prokaryotes and eukaryotes (1, 2). The large ribosomal subunits of eukaryotic ribosomes contain in addition a second small RNA designated as 5.8 S rRNA (2).

Eubacterial and eukaryotic 5S rRNAs represent two phylogenetically distinct classes of small ribosomal RNAs (3). All reported sequences of eubacterial 5S rRNAs conform to a secondary structure comprising four extended helices and a less defined fifth helix with a single stranded hairpin loop defined by helix C (3, 4). The hairpin loop c consists in most cases of 13 nucleotides and contains in eubacteria a highly conserved CGAAC-segment.

Eukaryotic 5S rRNAs display a secondary structure with five helical regions, thus suggesting a coaxial tertiary stacking of either helices A and D or helices B and D (3, 4, 5-8). In most published eukaryotic 5S rRNA sequences the loop defined by helix C consists of 12 bases. At the position of the CGAAC-sequence in prokaryotic 5S rRNAs eukaryotic 5S rRNAs manifest a semi-conserved PyNGNNPy-sequence (3, 9).

Despite these differences, eubacterial and eukaryotic 5S rRNAs display substantial structural similarities as the ubiquitous helices A, B and C

and the single stranded regions defined by these helices are almost identical.

Neither the eubacterial nor the eukaryotic secondary structure can be applied rigorously to archaebacterial 5S rRNA species (3). The 5S rRNAs from *H.cutirubrum* and *T.acidophilum* are provided with the typical eubacterial CGAAC-segment in the loop enclosed by helix C. Both species exhibit potential base pairing in the eukaryotic helix D region - with four base pairs for the *H.cutirubrum* and ten base pairs for the *T.acidophilum* molecule - and the eukaryotic feature of a looped out nucleotide in helix E (3). In the case of the *T.acidophilum* 5S rRNA, a coaxial stacking involving either helices A and D or B and D has been suggested, as proposed for the eukaryotic species (3, 4). The 5S rRNA from *T.acidophilum* displays the unique features of (i) a mispair in helix A, (ii) a very tenuous helical segment adjacent to loop c, (iii) an uninterrupted helix B and (iv) the extremely extended helix D.

In vitro reconstitution of prokaryotic ribosomal subunits (10-12) offers the possibility of incorporating altered ribosomal 5S rRNAs (13-17). Subsequent structural and functional analyses can refer to mechanistic aspects and molecular details of assembly and/or ribosomal function due to the altered component.

Comparative structural analysis (18) and ribosomal reconstitution experiments (19) with 5S rRNA from *Spinacea oleracea* chloroplast ribosomes established the eubacterial character of this 5S rRNA species.

In this study, we investigated the feasibility of incorporating 5S and 5.8S rRNAs from various phylogenetic groups into 50S ribosomal subunits of *B.stearothermophilus*. Reconstituted particles were analyzed by sucrose gradient centrifugation employing [^{32}P]-labelled 5S and 5.8S rRNAs and their biological activity was determined.

MATERIALS AND METHODS

Isolation of *B.stearothermophilus* 799 ribosomes and 23S rRNA

Ribosomes, ribosomal subunits and 23S rRNA were prepared according to published procedures (19). In this study the sucrose gradient buffer for the 23S rRNA preparation was 50 mM Na-acetate, 20 mM borate (pH 7.0).

Isolation of 5S and 5.8S rRNAs

B.stearothermophilus and *E.coli* 5S rRNAs were isolated as described (20) and rat liver 5S rRNA as published (21). 5S rRNA from *Caulobacter sp.* and 5.8S rRNA from rat liver were kindly provided by S. Leffler, New York, and P. Wrede, Berlin, respectively.

5S and 5.8S rRNAs from *Saccharomyces carlsbergensis* were isolated as published (22).

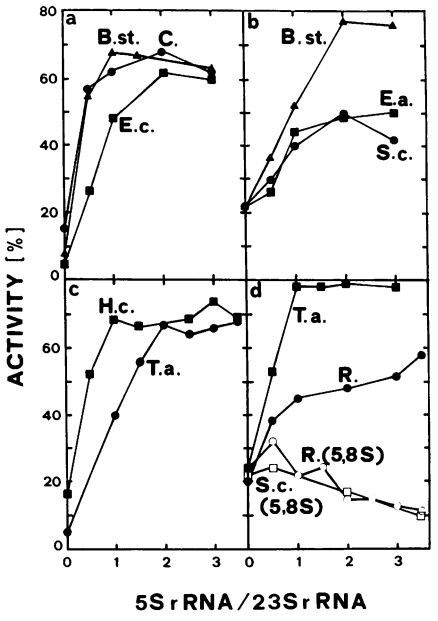


Figure 1: Activity of reconstituted *B.stearothermophilus* large ribosomal subunits in a poly(U)-dependent polyphenylalanine- sythesizing system as a function of the molar ratio of 5S rRNA or 5.8S rRNA to 23S rRNA. [a], 5S rRNA from *B.stearothermophilus* (B.st.), *E.coli* (E.c.) and *Caulobacter sp.* (C.); [b], 5S rRNA from *B.stearothermophilus* (B.st.), *Equisetum arvense* (E.a.) and Sacharomyces carlsbergensis (S.c.); [c], 5S rRNA from *Halobacterium cutirubrum* (H.c.) and *Thermoplasma acidophilum* (T.a.); [d], 5S rRNA from *Thermoplasma acidophilum* (T.a.) and *Rattus rattus* (R.) and 5.8S rRNAs from *Rattus rattus* [R.(5.8S)] and *Sacharomyces carlsbergensis* [S.c.(5.8S)]. 100% activity (corresponding to 55 phenylalanines polymerized per 50S subunit in 30 minutes) is defined as the activity of native 50S subunits from *B.stearothermophilus* in polyphenylalanine synthesis, and 0% as the activity of a reconstitution assay without 23S and 5S rRNAs.

5S rRNA from *Thermoplasma acidophilum* and *Halobacterium cutirubrum* were prepared according to references 23 and 24.

Preparation of *B.stearothermophilus* 50S ribosomal proteins

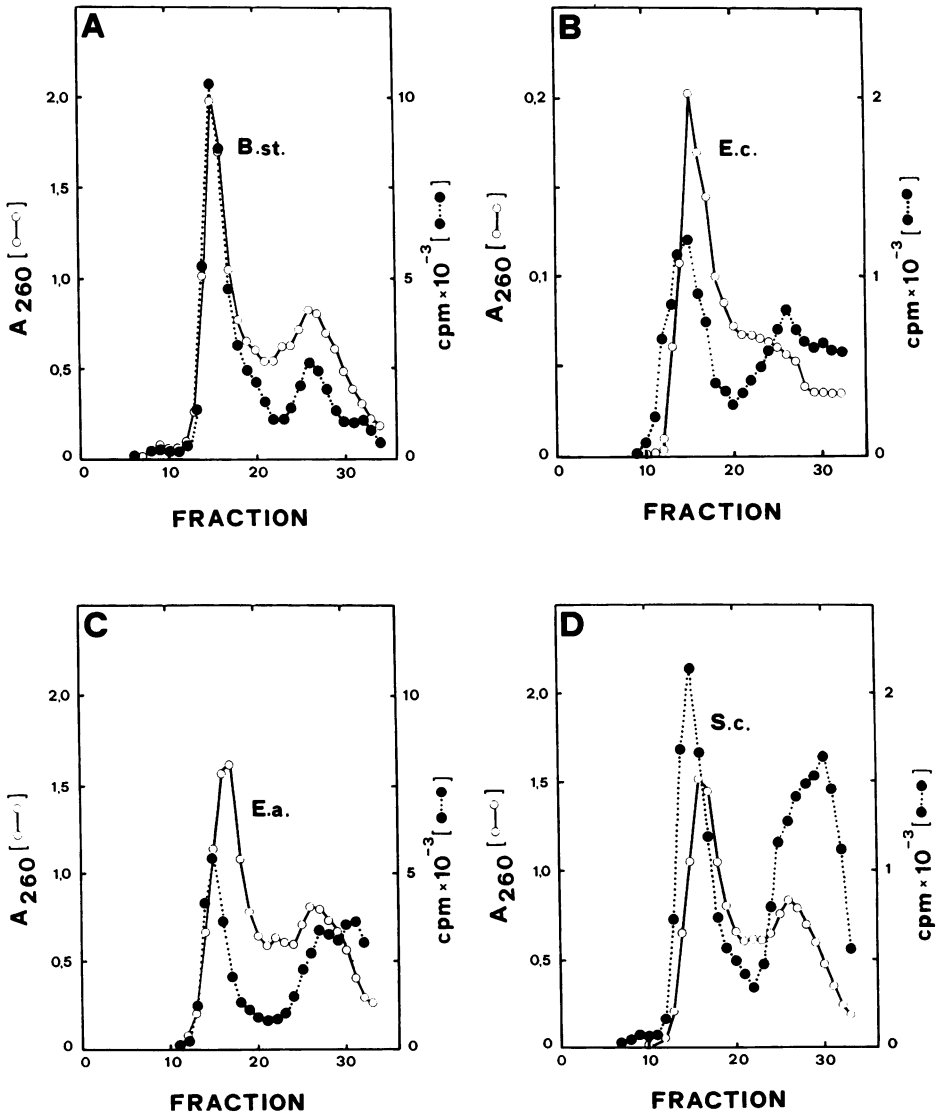
The isolation of total ribosomal proteins (TP 50) from 50S subunits of *B.stearothermophilus* has been reported (19, 25).

Reconstitution of 50S subunits, assay for biological activity and test for incorporation of 5S and 5.8S rRNAs

The two step reconstitution of *B.stearothermophilus* 50S ribosomal subunits has been described previously (19) and the biological activity of *in vitro*-assembled 50S particles was determined in a poly(U)-dependent polyphenylalanine-synthesizing system (19, 26).

Large ribosomal subunits were reconstituted with 5'-[^{32}P]-labelled (27) 5S and 5.8S rRNAs in order to investigate the incorporation of the mole-

cules. The reconstituted particles were analyzed by sucrose gradient centrifugation (19). Particles in Fig. 2 were reconstituted from 860 µg [A, C, D], 125 µg [B], 286 µg [E] or 362 µg [F, G] *B.stearothermophilus* 23S rRNA and 41 µg [A], 8,8 µg [B], 40 µg [C], 41 µg [D] or 13,2 µg [E] of the respective 5S rRNA or either 13,25 µg [F] or 13,5 µg [G] 5.8S rRNA. Amounts of added ribosomal proteins (TP 50) were optimized for each 23S rRNA preparation. Native *B.stearothermophilus* 50S subunits were co-centrifuged in each sucrose gradient run. Reconstituted particle fractions of comparable sedimentation velocity (to native 50S subunits) were analyzed in order to



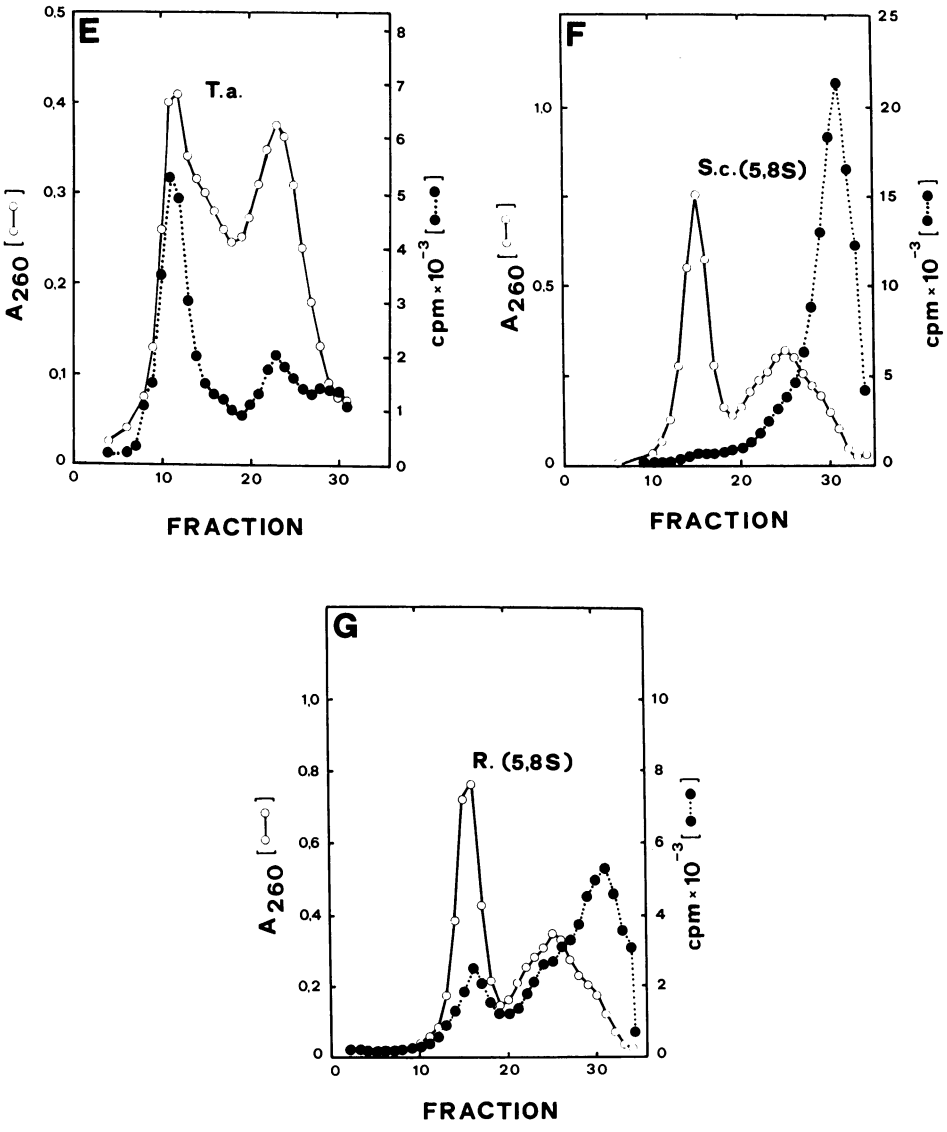


Figure 2: Sucrose gradients of *B.stearothermophilus* large ribosomal subunits reconstituted in the presence of 5'-[^{32}P]-labelled 5S rRNA from *B.stearothermophilus* (A), *E.coli* (B), *Equisetum arvense* (C), *Sacharomyces carlsbergensis* (D), *Thermoplasma acidophilum* (E) or in the absence of 5S rRNA and in the presence of 5.8S rRNA from *Sacharomyces carlsbergensis* (F) or *Rattus rattus* (G). For molar ratios of 23S to 5S or 5.8S rRNAs, respectively, see »MATERIALS and METHODS«.

quantify the amount of particle formation and to evaluate the molar ratio of 5S rRNA incorporation (Table I).

Particles reconstituted with 5S rRNA from *B.stearothermophilus* were mixed with [^{32}P]-labelled native 50S subunits from *E.coli* (a gift from R. Brimacombe) and analyzed by sucrose gradient analysis as described (19).

In addition, 5'-[^{32}P]-labelled 5S rRNA was re-extracted from those sucrose gradient fractions containing the reconstituted 50S subunits and sequenced according to published methods (28, 29) in order to validate the authenticity of the 5S rRNA incorporated.

RESULTS

50S ribosomal incorporation of eubacterial 5S rRNA species

Reconstitution mixtures of *B.stearothermophilus* large ribosomal subunits yielded comparable biological activities if reconstituted in the presence of eubacterial 5S rRNAs from *B.stearothermophilus*, *E.coli* or *Caulobacter sp.* (Fig. 1 a). Sucrose gradient analyses of reconstituted particles in the presence of [^{32}P]-labelled native 50S ribosomal subunits from *E.coli* revealed an identical migration behaviour (data not shown), thus proving that the reconstituted particles sediment with an S-value of 50. Furthermore no differences in biological activity were observed if eubacterial 5S rRNAs were not preincubated or were preincubated at 60°C - either followed by immmediate cooling on ice or renaturation in steps of 1°C/1,5 min. - (data not shown).

The sucrose gradient profiles for the incorporation of *B.stearothermophilus* or *E.coli* 5S rRNA (Fig. 2 A and B) showed no significant differences, thus supporting the exchangeability of eubacterial 5S rRNA species in the »in vitro« assembly of *B.stearothermophilus* 50S ribosomal subunits. Correlating the main absorption fractions (Fig. 2, fractions 12-17 in A and B) with the amount of incorporated 5S rRNA, we calculated a 1:1 molar ratio of 5S rRNA/50S particles (Fig. 2 A and B, Table I), i.e. each 50S particle contains one *B.stearothermophilus* or *E.coli* 5S rRNA molecule.

50S ribosomal incorporation of archaebacterial 5S rRNA species

Despite the conspicuous deviations from the eubacterial 5S rRNA structure - especially in the case of the *T.acidophilum* species - both archaebacterial 5S rRNA molecules clearly behaved in a eubacterial fashion in the reconstitution experiments (Fig. 1 c). Biological activities of the hybrid *B.stearothermophilus* ribosomal subunit preparations were indistinguishable from reconstitution assays performed with eubacterial 5S rRNA species. In addition, the sucrose gradient analysis, as exemplified for the *T.acidophilum* 5S rRNA (Fig. 2 E), shows the incorporation of the heterologous molecule. Again, as for the eubacterial 5S rRNAs from *B.stearothermophilus* and

small ribosomal RNA	small rRNA incorporation	% Particle Formation	Biological Activity
5S rRNA:			
B.stearothermophilus	100%	56%	60-80%
E.coli	100%	56%	60-80%
Caulobacter sp.	n.d.	n.d.	60-80%
H.cutirubrum	n.d.	n.d.	60-80%
T.acidophilum	100%	56%	60-80%
E.arvense	70%	56%	40-50%
S.carlsbergensis	70%	56%	40-50%
R.rattus	n.d.	n.d.	40-50%
5.8S rRNA:			
S.carlsbergensis	-	48%	< 20%
R.rattus	-	50%	< 20%
-	-	56%	< 20%

Table I: Analyses of *B.stearothermophilus* large ribosomal subunits reconstituted with different 5S or 5.8S rRNAs. 100% incorporation corresponds to one 5S rRNA molecule incorporated per one molecule 23S rRNA and 100% particle formation to 1 A_{260} unit of large ribosomal subunits reconstituted from 1 A_{260} unit of 23S rRNA. Values for particle formation were calculated from sucrose gradient profiles of reconstitution assays employing identical preparations of 23S rRNA and TP 50 proteins. Biological activity values (corresponding to plateau values in Fig. 1.) are based on multiple experimental series; n.d. = not determined.

E.coli, a 1:1 molar ratio of 5S rRNA to 50S particles could be calculated (Fig. 2 E, fractions 8-14).

50S ribosomal incorporation of eukaryotic 5S rRNA species

Reconstitution assays with eukaryotic 5S rRNAs from *S.carlsbergensis*, *E.arvense* and *R.rattus* displayed a significantly reduced biological activity (Fig. 1 b and d). Sucrose gradient analyses of ribosomal particles formed in the presence of [^{32}P]-labelled 5S rRNAs from *E.arvense* and *S.carlsbergensis* revealed a significant incorporation (Fig 2 C and D). However, the proportion of reconstituted particles containing a 5S rRNA molecule (Fig. 2, fractions 13-18 in C and D) was considerably reduced, i.e. in both cases only 70% of the particles incorporated a eukaryotic 5S rRNA molecule during assembly (Table I). The peak fractions of the reconstituted large ribosomal subunits (Fig. 2 C and D) displayed a reduced sedimentation velocity com-

pared to native 50S ribosomal subunits or particles reconstituted with eubacterial or archaebacterial 5S rRNA species (Fig. 2 A, B and E). This observation is illustrated by a significant displacement of the 260 nm absorption peaks relative to the radioactivity peaks of 5S rRNA incorporation (Fig. 2 C and D).

From our results, it is obvious that the decreased biological activity of particles, which were assembled in the presence of eukaryotic 5S rRNAs, is proportional to the formation of »minus 5S« particles (Table I). This implies that *B.stearothermophilus* large ribosomal subunits which contain a eukaryotic 5S rRNA molecule as an integral component, display activities similar to those of particles reconstituted with eubacterial or archaebacterial 5S rRNAs, at least in the polyphenylalanine-synthesizing system employed.

50S ribosomal assembly in the presence of eukaryotic 5.8S rRNA and in the absence of 5S rRNA

The large ribosomal subunit of eukaryotes contains a third 5.8S rRNA species which has been included in our studies.

Reconstitution assays performed in the presence of increasing amounts of 5.8S rRNA and in the absence of 5S rRNA showed a corresponding decrease in biological activity below the background activity of »minus 5S« particles (Fig. 1 d). This is in accordance with a 10-14% reduction of particle formation (Table I) compared to equivalent reconstitution assays performed in the absence (»minus 5S« assays [19], data not shown) or presence of any investigated 5S rRNA. Clearly, eukaryotic 5.8S rRNAs cause severe perturbations of prokaryotic assembly.

In the case of 5.8S rRNA from *R.rattus* small amounts of radioactively labelled 5.8S rRNA comigrated with the peak of assembled particles (Fig. 2 G). This latter finding may be due to the fact that 5.8S rRNA has a certain affinity to the *E.coli* binding proteins E-L18 and E-L25 (22).

DISCUSSION

In summary, our reconstitution experiments have given clear evidence that eubacterial and archaebacterial 5S rRNAs are more related to each other than to eukaryotic 5S rRNAs (Fig. 1 and 2). This result had not been anticipated since the 5S rRNA species from *T.acidophilum* displays, as outlined above, structural features which imply an even less overall similarity to eubacterial 5S rRNAs than for any of the eukaryotic 5S rRNAs

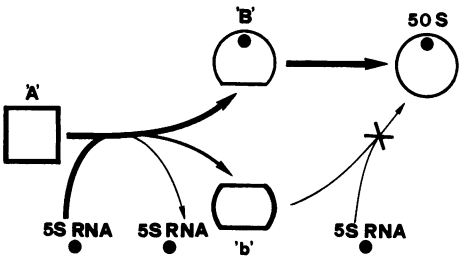


Figure 3: Directionality of 5S rRNA incorporation in the assembly of 50S subunits. As discussed under »DISCUSSION« the 5S rRNA interacts with 23S rRNA and ribosomal proteins in an early 50S precursor ('A'). If the interaction is perfect, it will lead to the formation of precursor 'B' to which the 5S rRNA is firmly bound. Alternatively, if this interaction is not optimal significant amounts of »dead end« particles 'b' are formed, which do not retain the 5S rRNA and which cannot revert to precursor 'A'. Only precursor 'B' undergoes a conformational change that leads to the formation of functional 50S subunits.

employed in these studies. The only exception is loop c of the *T.acidophilum* 5S rRNA which displays all eubacterial features such as the size of 13 nucleotides and the conserved bases including the two adjacent base pairs of helix C (3). Thus the data suggest that loop c is involved in crucial intermolecular contacts during early assembly, which can include RNA/protein and/or RNA/RNA interactions. This is consistent with the impaired incorporation of eukaryotic 5S rRNAs (Fig. 1 and 2) which manifest a different loop c structure (3).

Recent experiments employing deoxyhexanucleotide hybridization to complementary single stranded regions of 5S rRNAs and subsequent hydrolysis by RNase H (30) indicate that the CCGAAC-segment (or the corresponding sequence in eukaryotic 5S rRNAs) of loop c is the most pronounced region susceptible to RNase H hydrolysis in eubacterial and eukaryotic 5S rRNAs (S.Lorenz et al., manuscript in preparation), thus supporting its inferred importance as an exposed target site for intermolecular contacts.

A positive activation entropy has been reported for both rate-limiting steps of the »two step« reconstitution procedure of *E.coli* 50S ribosomal subunits (31). This high positive entropy of activation is especially pronounced for the first incubation, thus indicating a low degree of order in the initial phase of »in vitro« assembly. The initial course of particle formation is easily disturbed and suggests the existence of labile intermediates (31). The existence of a variety of crucial intermolecular contacts

			POSITIONS	REFERENCES
EUBACTERIA				
Anacystis nidulans			2580-2585	35
Bacillus stearothermophilus			2603-2608	36
Escherichia coli	23S	5'- G U U C G G	2603-2608	37
Pseudomonas aeruginosa	5S	C A A G C C - 5'	2593-2598	38
Thermus thermophilus			573-578	39
PLASTIDS				
Zea mays	23S	5'- G U U C G G	2698-2703	40
	5S	U A A G C C - 5'		
Nicotiana tabacum	23S	5'- A G U U C G G	2614-2620	41
	5S	U C A A G C C - 5'		
ARCHAEBACTERIA				
Methanococcus vannielii	23S	5'- A G G U U G G	2660-2666	42
	5S	U C U A G C C - 5'		
Halobacterium halobium	23S	5'- G G U C G G	2621-2626	43
Halobacterium maris mortui	5S	C A A G C C - 5'		44
EUKARYOTA				
Homo sapiens			4508-4514	45
Mus musculus	28S	5'- A G G U U A G	4199-4205	46
Rattus rattus	5S	U C U A G U C - 5'	4278-4284	47
Xenopus laevis			3675-3681	48
Saccharomyces cerevisiae	25S	5'- G G U U A G	2968-2973	49
Saccharomyces carlsbergensis	5S	C U A G C C - 5'	2969-2974	50
Physarum polycephalum	26S	5'- A G G U U A G	3291-3297	51
	5S	U C U A G C C - 5'		

Table II: Complementarities between 5S rRNA loop c nucleotides (corresponding to positions 42-47 of procaryotic 5S rRNAs) and nucleotides of the respective 23S equivalent rRNA located in the »peptidyltransferase« region (corresponding to positions 2603-2608 of *E.coli* 23S rRNA). Positions within 23S equivalent rRNAs are given according to the references indicated. The 5S rRNA sequences are taken from ref. 3.

during early assembly, provides a plausible explanation for the impaired incorporation of eukaryotic 5S rRNAs in the reconstitution procedure for *B.stearothermophilus* 50S ribosomal subunits. From the data presented in

rRNA species	Sequences involved in the interaction	Biological activity
23S B.stearo-thermophilus	3'-GUUCGG-5'	-
	||||||	
	******	
5S B.stearo-thermophilus	5'-CAAGCC-3'	60-80%
	******	
5S E.coli	5'-CAAGCC-3'	60-80%
	******	
5S H.cutirubrum	5'-CAAGCC-3'	60-80%
	******	
5S T.acidophilum	5'-CAAGCC-3'	60-80%
	**** *	
5S E.arvense	5'-CAAGAC-3'	40-50%
	* ****	
5S S.carlsber-gensis	5'-CUAGCC-3'	40-50%
	* ****	
5S R.rattus	5'-CUAGUC-3'	40-50%

Table III: Complementarity between nucleotides 2603-2608 of *B.stearothermophilus* 23S rRNA and nucleotides 42-47 of the 5S rRNAs used for reconstitution. Biological activity values are taken from Table I. Asterisks indicate nucleotides which are capable to basepair with the 23S rRNA sequence.

Fig. 1 b and d it is clear that even the presence of a 3-molar excess of eukaryotic 5S rRNA does not lead to the formation of particle populations with biological activities comparable to those obtained with eubacterial or archaebacterial species. We conclude that the deterioration of critical intermolecular contacts observed for eukaryotic 5S rRNAs results in a significant formation of »dead end« particles from which the 5S rRNA molecule has been irreversibly excluded (Fig. 3).

A search for conserved sequences in 5S rRNAs, which at the same time are complementary to conserved 23S rRNA sequences, revealed that nucleotides at position 42-47 in loop c (3) of eubacterial 5S rRNAs are complementary to nucleotides located in the 23S rRNA »peptidyltransfer« region, corresponding to position 2603-2608 (helix 81) of *E.coli* 23S rRNA (Table II). An extended search including large ribosomal subunit RNAs from plastids and eukaryotes (Table II) supports the proposed interaction which, if existent, might be stabilized by ribosomal proteins, especially in cases where the proposed base pairing would include a mismatch. In Table III the extents of complementarity between the *B.stearothermophilus* 23S rRNA hexanucleotide sequence (GUUCGG, position 2603-2608) and the different loop c sequences of

the 5S rRNAs used in these experiments are summarized. The eubacterial and archaebacterial 5S rRNA sequences permit the construction of six Watson-Crick base pairs, while the three eukaryotic 5S rRNAs only permit the formation of five base pairs, and then a G/U base pair for the *R.rattus* 5S rRNA has to be included. Since the prokaryotic 5S rRNAs yielded ribosomal particles displaying 60-80% activity compared to 40-50% activity for the eukaryotic 5S rRNAs (Fig. 1, Table I and III), the data would support the hypothetical interaction between 5S rRNA and 23S during early ribosomal assembly.

Other previously proposed interactions, i.e. between nucleotides 72-81 of *E.coli* 5S rRNA with nucleotides 143-154 of 23S rRNA (32) or nucleotides 69-78 of *E.coli* 5S rRNA with nucleotides 1759-1768 of 23S rRNA (33) and between nucleotides around position 95 of 5S rRNA with the 3'-end of 16S or 18S rRNAs (34) are not supported by our computer comparison studies (data not shown).

ACKNOWLEDGEMENTS

We are grateful to the »Deutsche Forschungsgemeinschaft« and the »Fonds der Chemischen Industrie« for financial support. We would like to thank H. Erdmann for drawing the figures. The 5S rRNA preparation from *H.cutirubrum* was a kind gift of Alan T. Matheson.

REFERENCES

1. Pieler, T., Digweed, M. and Erdmann, V.A. (1984) Alfred Benzon Symposium on Gene Expression 19, 353-376.
2. Erdmann, V.A. (1976) Progress in Nucleic Acids Research and Molecular Biology, Cohn, W.E. (Ed.) Vol. 18, pp. 45-90, Academic Press, New York.
3. Erdmann, V.A. and Wolters, J. (1986) Nucl. Acids Res. 4 (Supplement) r1-r59.
4. Fox, G.E., Luehrsen, K.R. and Woese, C.R. (1982) Zbl. Bakt. Hyg., I Abt. Orig. C 3, 330-345.
5. Studnicka, G.M., Eiserling, F.A. and Lake, J.A. (1981) Nucleic Acids Res. 9, 1885-1904.
6. Böhm, S., Fabian, H. and Welfe, H. (1982) Acta biol. med. germ. 41, 1-16.
7. Delihas, N. and Andersen, J. (1982) Nucleic Acids Res. 10, 7323-7344.
8. De Wachter, R., Chen, W.W. and Vandenberghe, A. (1982) Biochimie 64, 311-329.
9. Erdmann, V.A., Wolters, J., Digweed, M., Pieler, T., Lindschau, C., Lorenz, S. and Ulbrich, N. (1987) in Computer Handling and Dissemination of Data, Glaeser, P.S. (Ed.), pp. 373-380, Elsevier Science Publishers B.V. (North-Holland).
10. Nomura, M. and Erdmann, V.A. (1970) Nature 228, 744-748.
11. Nierhaus, K.H. and Dohme, F. (1974) Proc. Natl. Acad. Sci. USA 71, 4713-4717.

12. Londei, P., Teixido, J., Acca, M., Cammarano, P. and Amils, R. (1986) Nucleic Acids Res. 14, 2269-2285.
13. Raué, H.A., Lorenz, S. Erdmann, V.A. and Planta, R.J. (1981) Nucleic Acids Res. 9, 1263-1269.
14. Digweed, M., Erdmann, V.A., Odom, O.W. and Hardesty, B. (1981) Nucleic Acids Res. 9, 3187-3198.
15. Pace, B., Matthews, E.A., Johnson, K.D., Cantor, C.R. and Pace, N.R. (1982) Proc. Natl. Acad. Sci USA 79, 36-40.
16. Silberklang, M., RajBhandary, U.L., Lück, A. and Erdmann, V.A. (1983) Nucleic Acids Res. 11, 605-617.
17. Zagorski, L., Van Duin, J., Noller, H.F., Pace, B., Johnson, K.D. and Pace, N.R.(1984) J. Biol. Chem. 259, 2798-2802.
18. Pieler, T., Digweed, M. Bartsch, M. and Erdmann, V.A. (1983) Nucleic Acids Res. 11, 591-603.
19. Vogel, D.W., Hartmann, R.K., Bartsch, M., Subramanian, A.R., Kleinow, W., O'Brian, T.W., Pieler, T. and Erdmann, V.A. (1984) FEBS Lett. 169, 67-72.
20. Erdmann, V.A., Doberer, H.G. and Sprinzl, M. (1971) Molec. Gen. Genetics 114, 89-94.
21. Ulbrich, N. and Wool, I.G. (1978) J. Biol. Chem. 253, 9049-9052.
22. Wrede, P. and Erdmann, V.A. (1977) Proc. Natl. Acad. Sci. USA 74, 2706-2709.
23. Luehrsen, K.R., Fox, G.E., Kilpatrick, M.W., Walker, R.T., Domdey, H., Krupp, G. and Gross, H.J. (1981) Nucleic Acids Res. 9, 965-970.
24. Willick, G.E., Nazar, R.N. and Matheson, A.T. (1975) Biochemistry 18, 2855-2859.
25. Dohme, F. and Nierhaus, K.H. (1976) J. Mol. Biol. 107, 585-599.
26. Nirenberg, M.W. and Matthaei, J.H. (1961) Proc. Natl. Acad. Sci. USA 47, 409-423.
27. Silberklang, M., Gillum, A.M. and RajBhandary, U.L. (1979) Meth. Enzym. LIX, 58-109.
28. Donis-Keller, H., Maxam, A.M. and Gilbert, W. (1977) Nucleic Acids Res. 4, 2527-2538.
29. Peattie, D.A. (1979) Proc. Natl. Acad. Sci. USA 76, 1760-1764.
30. Lorenz, S., Hartmann, R.K., Piel, N., Ulbrich, N. and Erdmann, V.A. (1987) Eur. J. Biochem. 163, 239-246
31. Sieber, G. and Nierhaus K.H. (1978) Biochem. 17, 3505-3511
32. Herr, W. and Noller, H.F. (1975) FEBS Lett. 53, 248-252.
33. Glotz, C., Zwieb, C. and Brimacombe, R. (1981) Nucleic Acids Res. 9, 3287-3306.
34. Azad, A.A. and Deacon, N.J. (1980) Nucleic Acids Res. 8, 4365-4376.
35. Douglas, S.E. and Doolittle, W.F. (1984) Nucleic Acids Res. 12, 3373-3386.
36. Kop, J., Wheaton, V., Gupta, R., Woese, C.R. and Noller, H.F. (1984) DNA 3, 347-357.
37. Branlant, C., Krol, A., Machatt, M.A., Pouyet, J., Ebel, J.-P., Edwards, K. and Kössel, H. (1981) Nucleic Acids Res. 9, 4303-4324
38. Toschka, H.Y., Hartmann, R.K., Ulbrich, N. and Erdmann, V.A. (1987) Endocytobiosis and Cell Res. 4, 243-263
39. Hartmann, R.K., Vogel, D.W., Kröger, B., Ulbrich, N. and Erdmann, V.A. (1987) FEBS Lett. 218, 215-221.
40. Edwards, K. and Kössel, H. (1981) Nucleic Acids Res. 9, 2853-2869.
41. Takaiwa, F. and Sugiura, M. (1982) Eur. J. Biochem. 124, 13-19.
42. Jarsch, M. and Böck, A. (1985) Mol. Gen. Genet. 200, 305-312.
43. Mankin, A.S. and Kagramanova, V.K. (1986) Mol. Gen. Genet. 202, 152-161.

44. Specht, T., personal communication.
45. Gorski, J.L., Gonzalez, I.L. and Schmickel, R.D. (1987) J. Mol. Evol. 24, 236-251.
46. Hassouna, N., Michot, B. and Bachellerie, J.P. (1984) Nucleic Acids Res. 12, 3563-3583.
47. Hadjiolov, A.A., Georgiev, O.I., Nosikov, V.V. and Yavachev L.P. (1984) Nucleic Acids Res. 12, 3677-3693
48. Clark, G., Tague, B.W., Ware, V.C. and Gerbi, S.A. (1984) Nucleic Acids Res. 12, 6197-6220.
49. Georgiev, O.I., Nikolaev, N., Hadjiolov, A.A., Skryabin, K.G., Zakharyev, V.M. and Bayev, A.A. (1981) Nucleic Acids Res. 9, 6953-6958
50. Veldman, G.M., Klootwijk, J., deRegt, V.C.H.F., Planta, R.J., Branlant, C., Krol, A. and Ebel, J.P. (1981) Nucleic Acids Res. 9, 6935-6952.
51. Otsuka, T., Nomiyama, H., Yoshida, H. Kukita, T., Kuhara, S. and Sakaki, Y. (1983) Proc. Natl. Acad. Sci. USA 80, 3163-3167.