Hybrid 5 S ribosomal RNA encoded by a multicopy plasmid is incorporated into ribosomes of *Escherichia coli*

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Received 9 January 1984; revised version received 14 February 1984

The recombinant plasmid pJR3Δ contains a tandem pair of promoters from *rrnA* followed by a hybrid 5 S rRNA gene, derived from the two 5 S rRNA genes of the *rrnD* transcription unit, and a terminator. *Escherichia coli* cells transformed with this plasmid produce 2–3-times more 5 S rRNA compared to untransformed cells. The growth of cells containing this plasmid is not affected significantly. Although the sequence and the secondary structure of the plasmid-specific 5 S rRNA differ from those of its counterparts (e.g., from 5 S rRNA species encoded by chromosomal genes), it is processed properly and is incorporated into ribosomes.

Hybrid 5 S rRNA *Fingerprinting* *Ribosome* *Molecular stalk* *RNA processing* *Recombinant plasmid*

1. INTRODUCTION

The recombinant plasmid pJR3Δ contains a tandem promoter from the ribosomal transcription unit *rrnA* of *Escherichia coli*, a hybrid 5 S rRNA gene, that arose from a recombination event between the two 5 S rRNA genes of *rrnD*, and the transcription terminator of *rrnD* [1,2]. The *rrn* region of the plasmid is transcribed efficiently in *E. coli*, and under conditions in which RNase E, the enzyme responsible for the processing of 5 S rRNA, is active, the transcripts are processed to 5 S rRNA [2,3].

The 5 S rRNA gene of the plasmid consists of the 5′-end region of the first and of the 3′-end part of the second 5 S rRNA gene from the *rrnD* cluster. This arrangement results in the production of a hybrid 5 S rRNA, which is rather unusual. While all the 5 S rRNAs of *E. coli* can form a perfect molecular stalk [4] consisting of sequences from both ends of the molecule, this 5 S rRNA contains a CA pair in this stalk (nucleotides 6 and 120 in fig.1; this pair is either C-G or U-A in other 5 S rRNAs of *E. coli*). The weakening of the stem could affect significantly the conformation and, consequently, the maturation of the 5 S rRNA; i.e., the processing and/or the incorporation of this unusual 5 S rRNA into ribosomes.

Plasmid-carrying wild-type *E. coli* cells produce 2–3-times more 5 S rRNA than the parental cells and their growth rate is not significantly changed. Since cells carrying the pJR3Δ plasmid can be a source for the isolation of a homogeneous 5 S rRNA that could be very useful in solving a number of specific questions, it was necessary to find out whether or not the noncharacteristic 5 S rRNA generated from this plasmid is incorporated into ribosomes. We here demonstrate that this unusual 5 S rRNA is indeed incorporated into ribosomes.

2. EXPERIMENTAL

All methods followed established experimental procedures and are referred to in the table and figure legends.

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Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

3. RESULTS AND DISCUSSION

To determine whether or not the 5 S rRNA specified by the pJR3Δ plasmid is incorporated into ribosomes, ^{32}P-labeled 5 S rRNA was isolated from total cellular extracts and from ribosomal fractions of wild-type (*rne*$^+$) *E. coli* cells, strain N3433 [10], and from its plasmid-carrying transformant (strain N5712), after 10, 60 and 180 min of labeling. To estimate the amount of plasmid-encoded 5 S rRNA in strain N5712, its unusual 3′-end region was utilized. The hybrid 5 S rRNA contains an A at position 120 (fig.1), while all the other 5 S rRNAs, the sequence of which is known, have a G at this position. (The only exception is the 5 S rRNA encoded by the distal 5 S rRNA gene of *rrnD*. The 3′-end of the 5 S rRNA gene in the plasmid is derived from this distal 5 S

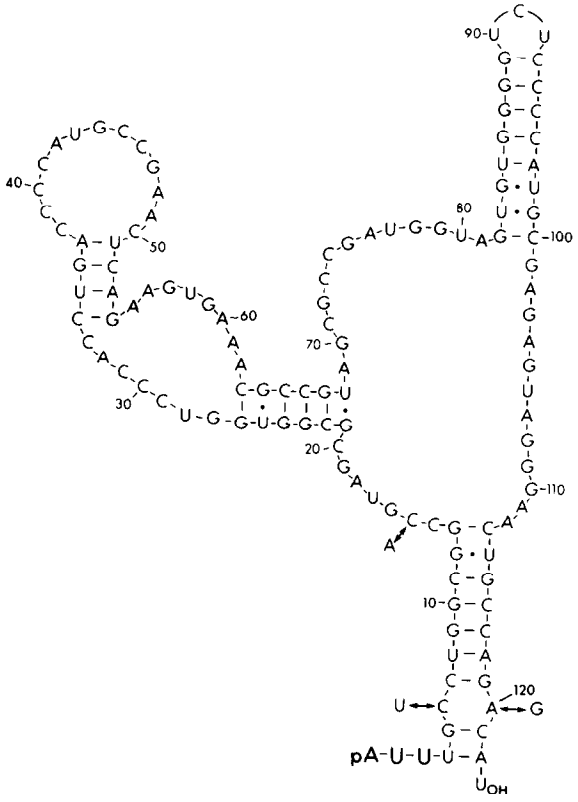

Fig.1. The sequence and a possible secondary structure of 5 S rRNA encoded by the plasmid pJR3Δ. The sequence of the hybrid 5 S rRNA is according to [2,5] and the secondary structure is as proposed in [6]. The heterogeneity among the 5 S rRNA sequences derived from the different ribosomal gene clusters at positions 6, 15 and 120 is indicated. At these positions 5 S rRNA from *rrnB* contains C, A and G [7]; from *rrnC* C, C and G [8]; from the first and second gene of *rrnD* C, C and G, and U, A and A, respectively [5]; from *rrnF* C, C and G [9]. (Sequences of 5 S rRNA genes from *rrnA*, *rrnE* and *rrnG* have not yet been published.) The 3 precursor nucleotides at the 5′-end are indicated by bold-face letters.

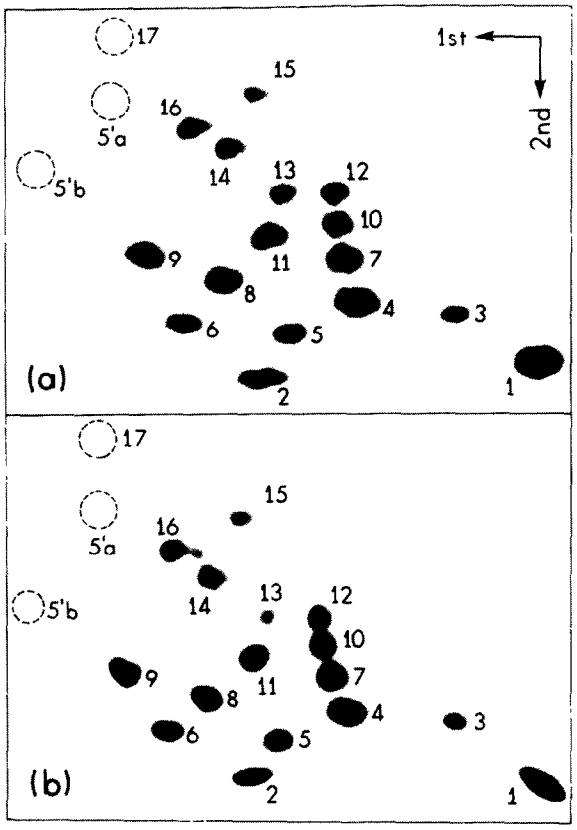

Fig.2. Pancreatic RNase fingerprint of 5 S rRNA from wild-type cells (a) and from their pJR3Δ-containing counterparts (b). Cells were labeled with $^{32}P_i$ (2.5 mCi/ml in 0.2 ml cultures) at A_{560} of 0.1. At 180 min after the addition of $^{32}P_i$, cells were harvested and extracts were prepared as in [11]. 5 S rRNA was isolated and purified in 4 successive polyacrylamide gels (5%/8%, containing 7 M urea; 15%; 12% with 7 M urea; 20%), eluted and precipitated [12]. RNA samples were digested with pancreatic RNase and the oligonucleotides were separated using the minifingerprinting method in [13]. Further characterization of the oligonucleotides was carried out by digesting the pancreatic oligonucleotides with RNase T_1 as in [14].

Table 1

Levels of plasmid-encoded 5 S rRNA in total cellular extracts and ribosomal fractions

Strain	Cellular fraction	Time of labeling (min)	p10b (AGACp)[a]		p13 (AGGCp)[b]		Percentage of plasmid-encoded 5 S rRNA[c]
			Molar yield	%	Molar yield	%	
N3433	Extracts	10	0.21	17	1.01	83	–
		60	0.08	6	1.17	94	–
		180	0.33	31	0.75	69	–
	Ribosomes	10	0.40	37	0.67	63	–
		60	0.18	15	1.00	85	–
		180	0.28	24	0.88	76	–
N5712	Extracts	10	0.71	66	0.36	34	59
		60	0.61	53	0.54	47	50
		180	0.70	65	0.38	35	50
	Ribosomes	10	0.78	71	0.32	29	54
		60	0.59	72	0.23	28	67
		180	0.63	66	0.32	34	56

[a] The quantitation of this oligonucleotide was performed as follows: Spot p10 (that consists of oligonucleotides GAACp, p10a and AGACp, p10b) was eluted from the polyethyleneimine–cellulose plate, digested with RNase T_1 and chromatographed in polyethyleneimine–cellulose plates as in [14]. The 4 spots (Gp, AACp, AGp, ACp) were excised and quantitated. Gp and AACp together were assumed to give one molar yield and used as reference to express the molar yield of AGp plus ACp

[b] Spots p11 (GGCp) and p13 (AGGCp) were excised from the polyethyleneimine–cellulose plates and quantitated. The molar yield of p13 was calculated using p11 as reference (p11 appears twice in the sequence of 5 S rRNA)

[c] The percentage of plasmid-specific 5 S rRNA was calculated as described in the text

Cells were grown as described in the legend to fig.2 and labeled with $^{32}P_i$ at A_{560} of 0.3 (for 10 and 60 min labeling) or 0.1 (for 180 min labeling). Total cellular extracts were prepared as in [11] and ribosomal fractions were isolated using the freeze-thaw–lysozyme method in [15]. 5 S rRNAs from whole extracts and ribosomal fractions were isolated, purified and fingerprinted after digestion with pancreatic RNase as described in the legend to fig.2

gene of *rrnD*, see above.) 5 S rRNAs containing A or G at this position can be distinguished from each other. When A appears in position 120 fingerprinting after digestion with pancreatic RNase leads to the oligonucleotide AGACp (fig.1, nucleotides 118–121; fig.2, oligonucleotide p10b; see legend to table 1). When the 5 S rRNA contains G at the same position the oligonucleotide AGGCp is found (p13 in fig.2).

These two oligonucleotides were quantitated in the 5 S rRNA preparations from strains N3433 and N5712 (table 1) and the relative amounts of plasmid-specific 5 S rRNA in the pJR3Δ-carrying strain were calculated (see legend to table 1). At all time points tested, 5 S rRNA containing G at position 120 is predominant in RNA isolated either from whole cell extracts or from ribosomes prepared from the cells without the plasmid (N3433); while in the plasmid-carrying cells the amount of 5 S rRNAs with A at position 120 is significantly increased.

Assuming that the transcription rate of chromosomal 5 S rRNA genes and the stability of host-specific 5 S rRNAs are not influenced by the

presence of the plasmid (see below), an equation can be used to estimate the relative amount of plasmid-specific 5 S rRNA:

$$5\ S_{pl} = 100 - \frac{100b}{a}$$

where 5 S_{pl} is the percentage of plasmid-encoded 5 S rRNA in strain N5712 as compared to the total amount of 5 S rRNA; a is the percentage of 5 S rRNA in strain N3433 containing G at position 120, and b is the percentage of 5 S rRNA in strain N5712 containing G at position 120. Using this equation we calculated that more than 50% of the 5 S rRNA in N5712 cells is produced from the plasmid (table 1). (In other strains carrying this plasmid up to 85% of the 5 S rRNA was coded by plasmid genes.) Since the relative amounts of the hybrid 5 S rRNA are similar in total cell extracts, and ribosomal fractions, regardless of the labeling time (table 1), we suggest that the plasmid-specific 5 S rRNA is stable and is efficiently utilized during ribosome assembly. Therefore, these experiments show that the plasmid-specific 5 S rRNA with the unusual primary structure that leads to a relatively weak molecular stalk is processed normally and is incorporated into mature ribosomes.

One could propose that the difference in the ratios of p13 to p10b in the two strains is not a manifestation of the expression of 5 S rRNA from the plasmid but rather that it is caused by the plasmid affecting differentially the expression of the chromosomal rRNA genes; i.e., another 5 S rRNA gene containing A in position 120 (fig.1) could be differentially expressed. This possibility is unlikely. We assume that a chromosomal 5 S rRNA gene that contains A in position 120 will contain T in position 6 (see fig.1). In this case the level of the T_1 oligonucleotide UCUGp (positions 6–9 in fig.1) should have increased proportionally to oligonucleotide p10b (positions 118–121), however, this did not happen. We carried out digestions with RNase T_1 and found that while UCUGp in strain N3433 was about 40%, in the plasmid containing strain (N5712) it became about 10% (100% being UCUGp plus CCUGp; nucleotides 6–9 in fig.1). This observation confirms our suggestion that the 5 S rRNA from the plasmid can be incorporated into ribosomes.

Authors in [16] showed that in extracts of *Bacillus stearothermophilus* a 5 S rRNA that contains an imperfect molecular stalk [4] can substitute, to a certain extent, a normal 5 S rRNA in reconstitution of 50 S ribosomal particles. Moreover, such ribosomes were shown to be comparable to ribosomes, reconstituted with normal 5 S rRNA, in a poly(U) directed polyphenylalanine synthesis [16]. The studies reported here indicate that all the in vivo functions of 5 S rRNA can be carried out by a 5 S rRNA with an imperfect molecular stalk.

ACKNOWLEDGEMENTS

Supported by a research grant from the Public Health Service National Institute of Health, GM19821. We are grateful to Dr W.M. Holmes for supplying us with the pJR3Δ plasmid.

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