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Genes for 7S RNAs Can Replace the Gene for 4.5S RNA in Growth of *Escherichia coli*

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4.5S RNAs of eubacteria and 7S RNAs of archaebacteria and eukaryotes exist in a hairpin conformation. The apex of this hairpin displays structural and sequence similarities among both 4.5S and 7S RNAs. Furthermore, a hyphenated sequence of 16 nucleotides is conserved in all eubacterial 4.5S RNAs examined. In this article I report that 7S RNAs that contain this 16-nucleotide sequence are able to replace 4.5S RNA and permit growth of *Escherichia coli*.

4.5S RNA is a metabolically stable, 114-nucleotide RNA (10) essential for the growth of the bacterium *Escherichia coli* (6). Genetic analyses indicate that the essential role of 4.5S RNA is performed on translating ribosomes (4, 5). Suppressors reducing the requirement for 4.5S RNA reside in the genes for protein synthesis elongation factor G, in the elongation factor G-binding site of 23S rRNA, in aminoacyl-tRNA synthetases, and in tRNAs. The binding of 4.5S RNA to ribosomes is enhanced in the suppressor mutant extracts examined, compared with wild-type extracts (4, 5). Consistent with the conclusion that the role of 4.5S RNA is in translation are the observations that extracts prepared from 4.5S RNA-depleted cultures are defective in translation (2) and that depletion of 4.5S RNA induces a heat-shock response (3), a response inducible by perturbations of ribosome function (24).

Interestingly, a consensus structure for 4.5S RNAs of eubacteria (7) is very similar to that for 7S RNAs of eukaryotes and archaebacteria (12, 18, 23). The eukaryotic 7S RNA is an essential component of the signal recognition particle, a ribonucleoprotein that participates in protein secretion (25). The portion of 7S RNAs bearing similarity to 4.5S RNA is shown in Fig. 1.

I cloned genes for various 7S RNAs into the expression vector pGFIB-1 (15) and examined their ability to complement the deletion of *ffs*, the structural gene for 4.5S RNA. This vector allows the expression of eubacterial 4.5S RNAs at a level sufficient for complementation of *ffs* (7). An approximately 400-bp *Ssp*I fragment containing the gene for 7S RNA from the archaebacterium *Methanococcus voltae* was cloned from pMv7S (13) into the *Sma*I site of pGFIB-1. This *Ssp*I fragment starts 2 nucleotides upstream from the structural gene for 7S RNA and ends approximately 90 nucleotides beyond its 3′ end. The resulting clone, pSB1548, directs the synthesis of an RNA species not directed by the vector alone (Fig. 2). The ability of pSB1548 to complement the deletion of *ffs* was tested in the following manner.

The tester strain for complementation, S1610 F$^-$ *araD139* Δ(*ara-leu*)*7697* Δ*lacX74 galU galK hsdR rpsL ffs*::*kan-591* λ*vir*^{r} [λ *c*I857 *nin5 Xho*I::Φ(P*tac-ffs*)], and its use have been described previously (7). In this strain, the sole copy of the gene for 4.5S RNA is carried within a λ *c*I857 prophage. Lysogens of λ *c*I857 can be cured of the prophage with high efficiency (26) (Table 1). However, loss of the prophage results in loss of the gene for 4.5S RNA. Thus, the cured bacterium can only survive if provided with another gene that expresses 4.5S RNA function. Such cured bacteria are detected by their ability to form colonies at 42°C; the prophage in the uncured lysogens are induced and kill the cell at 42°C.

After the curing regimen, a culture of S1610 harboring a complementing plasmid can form similar numbers of colonies when measured at 42 and 30°C. Examples of this (Table 1) are the *ffs*$^+$-bearing plasmids pSB832 (5) and pSB432 (6). In both cases, the efficiency of plating at 42 relative to 30°C is close to unity. In comparison, the respective vectors lacking *ffs*, pBR327 (21) and pACYC184 (8), form colonies at less than 10^{-5} times the efficiency at 42°C relative to the efficiency at 30°C. Since the *M. voltae* clone, pSB1548, permits an efficiency of curing similar to that obtained with

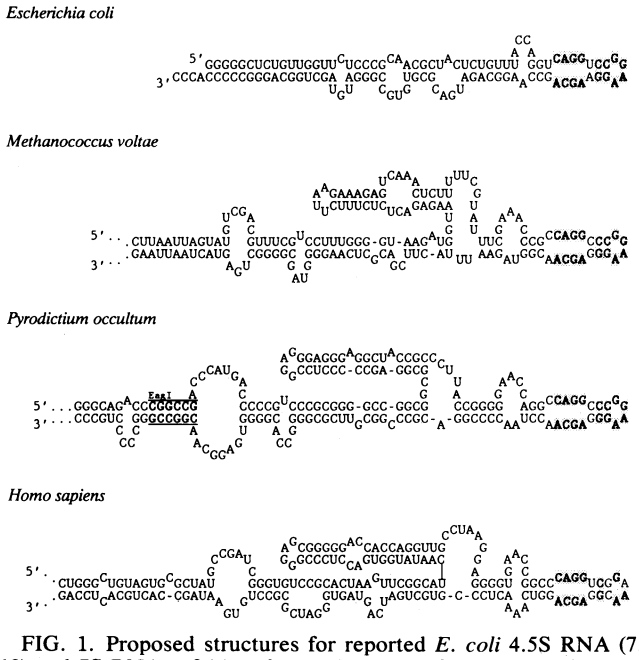

FIG. 1. Proposed structures for reported *E. coli* 4.5S RNA (7, 18) and 7S RNAs of *M. voltae* (13), *P. occultum* (14), and *Homo sapiens* (18). Shaded nucleotides are those found to be conserved in all eubacterial 4.5S RNAs examined (7, 22). Only the apical portions of the 7S RNAs are displayed. The locations of the *Eag*I sites used to clone an internal portion of *P. occultum* 7S RNA are indicated.

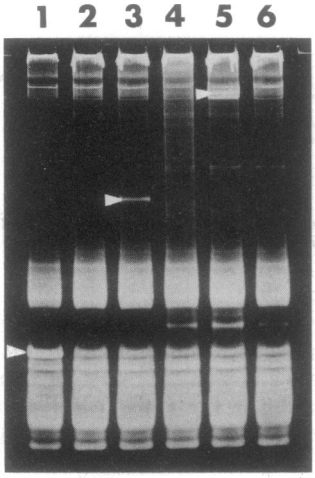

FIG. 2. Ethidium bromide-stained, native 12% polyacrylamide gel of total small RNA from various transformants of S971 (HfrH *lacI*^{q} [4]). RNA was extracted as described previously (4), except samples were not purified by DEAE chromatography. All lanes contain 1.5 A_{260} units of RNA, except lane 1, which contains 1 A_{260} unit. Arrowheads adjacent to bands in lanes 1, 3, and 5 indicate RNA species whose synthesis is directed by plasmids encoding genes from *E. coli*, *P. occultum*, and *M. voltae*, respectively. Lanes contain RNA from cells harboring the following plasmids: lane 1, pSB432; lane 2, pACYC184; lane 3, pSB1600; lane 4, pGFIB-1; lane 5, pSB1548; and lane 6, pSB1636.

ffs$^+$-bearing plasmids (Table 1), I conclude that it provides 4.5S RNA function. The vector pGFIB-1 alone fails to permit efficient curing.

A second genetic test for 4.5S RNA function was conducted in strain S1533 HfrH *lacI*^{q} *relA1 spoT1 lacY*::Tn*10 mini-tet ffs*::*kan-591* [λ *imm*^{434} *c*$^+$ *nin5 Xho*I::Φ(P*tac-ffs*)]

TABLE 1. Complementation tests[a]

Plasmid (description)	EOP	
	S1610 (42°C/30°C)	S1533 (−IPTG/+IPTG)
ColE1 derivatives		
pBR327 (vector)	<0.00001	ND
pSB832 (*ffs*$^+$ in pBR327)	0.8	ND
pSP65 (*lacO*-bearing vector)	<0.00001	0.54
pGFIB-1 (vector)	0.000004	<0.001
pSB1548 (*M. voltae* 7S)	1.6	0.97
pSB1636 (human 7SL)	0.0000011	<0.001
P15A derivatives		
pACYC184 (vector)	<0.00001	<0.001
pSB1600 (*P. occultum* 7S)	1.6	1.2
pSB432 (*ffs*$^+$ in pACYC184)	0.96	0.8

[a] Plasmids listed above were transformed (9) by using modifications described previously (7). Transformants of ColE1 derivatives were selected on YT agar with 200 μg of ampicillin per ml, and transformants of P15A derivatives were selected on YT agar with 25 μg of chloramphenicol per ml. Transformants of S1610 were selected at 30°C, purified at least once, and pulse-cured as described previously (7). Transformants of S1533 were selected at 37°C on medium supplemented with 0.1 mM IPTG and purified at least once prior to growth in YT broth supplemented with antibiotic and IPTG. Dilutions of log-phase cultures were spread on YT agar supplemented with antibiotic and with and without IPTG. Values shown represent a ratio of efficiency of plating (EOP) of cells grown at 42 and 32°C or with and without IPTG, as indicated. ND, Not done.

(5). In this strain, the sole copy of the gene for 4.5S RNA is regulated by the *lac* operator. This strain requires inducers of *lac* such as isopropyl-β-D-thiogalactopyranoside (IPTG) for growth. Complementing plasmids such as pSB432 (Table 1) permit growth in the absence of an inducer, whereas the vector pACYC184 alone does not. pSB1548, like pSB432, permits growth of S1533 in the absence of IPTG (Table 1). This test confirms the results obtained with S1610. Note that *lacO* carried on multicopy plasmids can titrate *lac* repressor (11) and relieve the IPTG requirement for induction of 4.5S RNA (7, 22; pSP65 [16], Table 1).

The gene for 7S RNA of the archaebacterium *Pyrodictium occultum* was initially recloned as a 2.6-kb *Bam*HI fragment into the *Bam*HI site of pGFIB-1 from its original clone in λ (14). The plasmid clone is able to complement *ffs*. Since the insert is so large compared with the size of 7S RNA, I recloned the 150-bp *Eag*I fragment internal to the *P. occultum* gene that contains the region of homology with eubacterial 4.5S RNAs (Fig. 1) into the *Eag*I site within the gene for tetracycline resistance of pACYC184 (8). The resulting plasmid, pSB1600, directs the synthesis of an RNA species not directed by the vector alone (Fig. 2) and is also able to complement *ffs* (Table 1).

Like the 7S RNA genes from *M. voltae* and *P. occultum*, the gene for 7S RNA of the archaebacterium *Sulfolobus sulfataricus* contains the conserved 16 nucleotides of eubacterial 4.5S RNAs (data not shown). The clone containing this RNA gene in pGEM4 isolated by Kaine (12) is able to complement the deletion of *ffs* when tested in strain S1610 (data not shown). This gene was not characterized further.

The sequence of human 7SL RNA matches 13 of the 16 nucleotides in the sequence shared by the eubacterial 4.5S RNAs (Fig. 1). The ability of the gene for human 7SL RNA to complement *ffs* was examined in the following manner. The human gene from the *Alu*I site at +34 to just past the end of the structural gene was recloned from phR-wt (gift of C. Zwieb) into the *Sma*I site of the polylinker of pGFIB-1. The resulting clone, pSB1636, behaved unlike clones of other 7S RNAs tested. It neither directed the synthesis of any new RNA species (Fig. 2), nor was it able to complement the deletion of *ffs* (Table 1). Clearly, the inability of pSB1636 to complement *ffs* could simply be due to a low steady-state level of 7SL RNA encoded by the plasmid. However, by recycling the selection, I was unable to isolate spontaneous, plasmid-associated mutations from the clone of human 7SL RNA able to complement the *ffs* deletion by using strain S1610. Such mutations should have been detected had they occurred at a frequency of at least 1 in 10^{10} plasmid-bearing cells.

The complementation behavior of the 7S RNA genes examined here indicates that the 16 conserved nucleotides and a secondary structure similar to that of the apex of the 4.5S RNA hairpin may be sufficient for 4.5S RNA function. Five nucleotides of this 16-nucleotide sequence lie within the decamer of sequence identity between *E. coli* 4.5S RNA and 23S rRNA. Mutations of the nucleotide adjoining this decamer in 23S RNA reduce the 4.5S RNA requirement of *E. coli* (5), implying that this sequence identity between 4.5S and 23S RNAs is relevant to 4.5S RNA function. Results reported here and those of an earlier comparison of 4.5S RNAs (7) indicate that it is a subset of the nucleotides common between 4.5S and 23S RNAs contained in a 4.5S RNA-like structure that is necessary for 4.5S RNA function rather than the entire decamer of sequence identity.

It is seductive to conclude that since 7S RNAs can replace 4.5S RNA, the function of 4.5S RNA is the same as 7S RNA.

Although the function of 7S RNA in archaebacteria remains unknown (12), the known function of 7S RNA in eukaryotes is in protein secretion (reviewed in reference 18). Supporting this hypothesis is the observation that homologs of proteins associated with eukaryotic 7S RNA in its role in protein secretion have been found in *E. coli* (1, 19). If this is indeed the function of 4.5S RNA, it is surprising that a direct involvement of 4.5S RNA in protein secretion has not yet been detected (1, 17, 20). On the other hand, all genetic and physiological analyses of 4.5S RNA function have found that its role is in translation (2, 4, 5). This suggests that 4.5S RNA has a role in *E. coli* translation more general than functioning solely in the synthesis of secreted proteins, and perhaps functions in addition to a role in protein secretion will be found for 7S RNAs.

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