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An unusual 5S rRNA, from Sulfolobus acidocaldarius, and its implications for a general 5S rRNA structure

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ABSTRACT

The nucleotide sequence of the 5S ribosomal RNA of the thermoacidophilic archaebacterium Sulfolobus acidocaldarius was determined. The high degree of evident secondary structure in the molecule has implications for the common higher order structure of other 5S rRNAs, both bacterial and eukaryotic.

INTRODUCTION

A sufficient diversity of 5S ribosomal RNAs now has been sequenced that the more obvious structural constraints on the molecule have been defined. Three main secondary structural elements, termed the molecular stalk, the tuned helix, and the common arm base, as illustrated for Escherichia coli 5S rRNA in Figure 1, are common to all 5S rRNAs so far sequenced (1). A fourth helix (the prokaryotic loop) is found only in the true bacteria; eukaryotic 5S rRNAs (1) and those of the archaebacteria (Luehrsen, G. Fox and Woese, in preparation) have a somewhat different helical arrangement in the corresponding region of the molecule.

The tertiary structural and the functional constraints in 5S rRNA will not be easily elucidated, if for no other reason because only 40 percent of the molecular sequence is in readily identifiable double stranded regions. In the course of examining

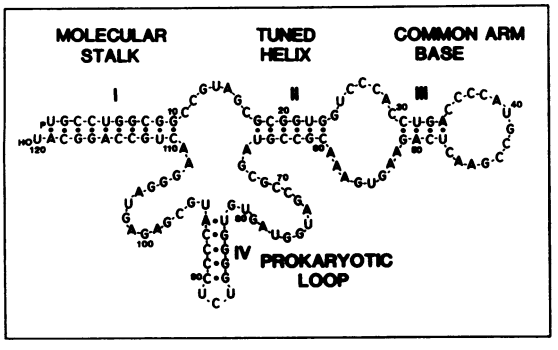

Figure 1. The nucleotide sequence of E. coli 5S rRNA, folded as suggested by Fox and Woese (1).

5S rRNA sequences from archaebacteria, a most unusual example, that of Sulfolobus acidocaldarius, has been encountered. In this case, approximately 60 percent of the residues appear to be involved in secondary structure. We feel that this molecule provides some insight into some of the more subtle aspects of a general 5S rRNA structure.

MATERIALS AND METHODS

S. acidocaldarius, obtained from Prof. T. Langworthy, University of South Dakota, was grown under conditions previously described (2) in the presence of [^{32}P] orthophosphate at ca. 200-300 μCi per ml. Cells were harvested by centrifugation, resuspended in a low ionic strength buffer, ruptured by adding Na dodecylsulfate, and total nucleic acids were purified by phenol extraction. 5S rRNA was purified by gel electrophoresis (3), and sequenced in two ways, by the standard, two-dimensional electrophoretic method developed by Sanger and coworkers (4,5), and by polyacrylamide gel sequencing techniques, using both enzymatic (6) and chemical degradative methods (7).

RESULTS AND DISCUSSION

Table I shows the RNases T_1 and A oligonucleotide catalogs for the molecule, and various partial digest fragments used to establish the order of the RNases T_1 and A fragments. This was sufficient information to establish the full sequence except in a small region, the vicinity of residue 25, where some uncertainty remained.

The methods of Donis-Keller et al (6) and Peattie (7) were then used to check this sequence and to resolve the remaining difficulty. The chemical protocol requires 3' end-labeled RNA and the molecule as isolated is heterogeneous, terminating with 2-4 U residues. Therefore, "half molecules" were generated by a preferred RNase T_2 cleavage near position 40, and the resultant fragments were isolated by gel electrophoresis. The isolated fragments were labeled at their 3' ends with [5'-^{32}P] pCp and run on polyacrylamide gels after appropriate chemical or enzymatic cleavage of the chains.

The two methods are in complete agreement except for residue 14, which seems to be a U and C mixture by the gel method (Fig. 2) but is homogeneously C by the Sanger method. We do not have an explanation for this, although in the three year interval that separated the two sequence determinations the original culture of S. acidocaldarius could have become populated by a variant. Residue 27 is homogeneously A by chemical or enzymatic digestion of end-labeled molecules, but two variant oligonucleotides, CAACACCC̆G and CACCC̆G, found in the original RNase T_1 catalog, suggest position 27 to be either A or G. The sequence shown is the one which we

TABLE I

A. RNase T_1 and RNase A Digestion Products

RNase T_1 Oligomers	Obs. Moles	Calc. Moles	RNase A Oligomers	Obs. Moles	Calc. Moles
G	—	14	U	—	11
CG	—	1	C	—	19
AG	2.5	2	AC	6	6
UG	4	4	AU	1	1
			GC	2	2
CCG	2	2	GU	3	3
CAG	1	>1<2			
AAG	1	1	AAC	1	0.5
CUG	1	1	AGC	1	>1<2
AUG	1	1	AGU	2	2
			GGU	1	1
UUAG	1	1			
			GAAC	1	1
AUCCG	1	1	AAGC	2	2
UUAAG	1	1	GAGC	1	1
			GGGC	1	1
C$\overset{*}{A}$CCCG	<1	<1	GGAU	1	1
AACCCG	1	1	GGGU	1	1
CUCACG	1	1			
UCACAG	1	1	C$\overset{*}{G}$GAC	1	1
AUACCG	1	1	GGGGC	1	1
			GGGAU	1	1
CCCACCCG	1	1			
			GAGGAU	1	1
CAACAC$\overset{*}{C}$CG	<1	<1	GGAAGU	1	1
CCCCACUAAG	1	1			
ACUCAUUUCG	1	1	pGC	—	1
pG	1	1			
$UUUU_{OH}$	<1	<1			

B. Partial Nuclease Digestion Products

RNase T_1 Fragments	RNase A Fragments	RNase U_2 Fragments
pGCCCACCCGGUCACAG	AGUGAGCGGGC	GUGGGGCCGUGGA
UUAAGCCGCUCACGUUAG		GCUGGGA
UUAGUGGGGCCGUGG	AC$\overset{*}{C}$CGGACUC	UCCGCA
AGGAUCCGCAGCCCCACUAAG	CCGGAAGUU	UUUCGAA
	ACGUU	CCGUGA
	AGUGGGGCCGU	
	CGUGGAU	
	GAGGAUCCGC	
	AAGCU	
	$GGGAUGGGUUUU_{OH}$	

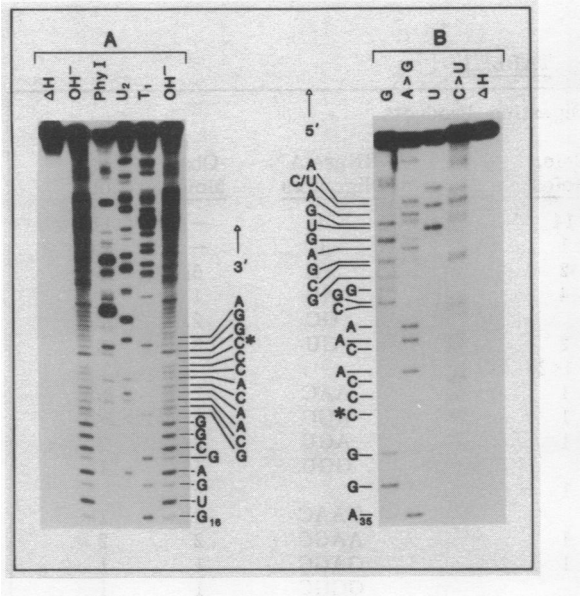

Figure 2. A sequencing gel of S. acidocaldarius 5S rRNA. A. 5'-^{32}P-labeled S. acidocaldarius 5S rRNA was digested partially with RNases U_2, T_1, or phy I, or by alkali, and resolved by gel electrophoresis as described (6). B. The 5' "half" (residues 1-ca. 40) of the 5S rRNA, isolated and labeled at its 3' end with [5'-^{32}P] cytidine bisphosphate as described in the text, was degraded with aniline, following treatment with sequence-specific reagents as detailed by Peattie (7).

consider the most reliable.

When folded into the phylogenetically common structure, the 5S rRNA from S. acidocaldarius appears to be more extensively base paired than either its typical bacterial counterpart or the 5S rRNAs found in other archaebacteria (1) (Fig. 3). The following points are evident:

1. The molecular stalk (helix I) contains a looped out, nonpaired A residue. This feature is not unique to S. acidocaldarius 5S rRNA, however, for although such a looped out base has not been encountered in eubacterial or eukaryotic cases, it is common in the molecular stalks of the archaebacterial 5S rRNAs now under study (Luehrsen, G. Fox, Woese, unpublished results).

2. The tuned helix (helix II) comprises eight base pairs. The majority of sequenced 5S rRNAs have a six base pair tuned helix, but occasional examples of seven and one of eight (Thermoplasma, 8) have been noted. The two base pair extension is toward the molecular stalk. It justifies the extension of helix II in other 5S rRNAs (below).

3. Although the common arm base, helix III, contains the normal number of pairs, one of the residues (C_{32}) is modified. Since the modified residue is not subject to alkaline hydrolysis (Fig. 2), it probably has a 2'-OH substitution.

4. The very strong, ten base pair helix V is unique among the bacteria to S. acidocaldarius, although a similar structure is a common feature of eukaryotic 5S rRNAs (9).

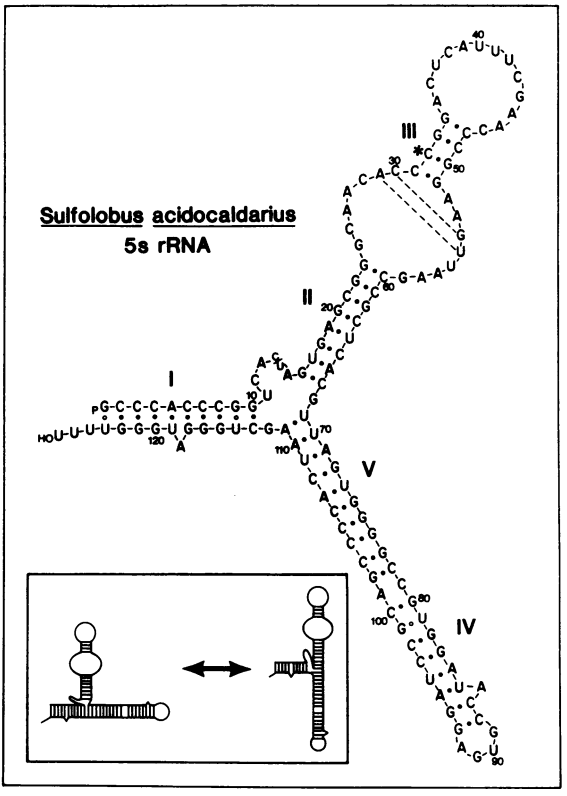

Figure 3. The S. acidocaldarius 5S rRNA is folded to maximize Watson-Crick complementarity, extending the Fox and Woese (1) pairing scheme. The inset diagrams two alternate stacking arrangements, as described in the text.

5. The Sulfolobus 5S rRNA contains some stretches of highly conserved sequence, for example CGAAC (positions 43-47), which is ubiquitous in prokaryotic 5S rRNAs at the 3' end of the loop defined by helix III.

6. The looped-out region separating helices II and III is probably highly structured (10,11), but no phylogenetically, completely consistent pairings are evident.

The pairing indicated by dotted lines in the S. acidocaldarius (Fig. 3) folding is a common theme among both the prokaryotes and the eukaryotes (12).

We suggest that the extreme helical content of the Sulfolobus 5S rRNA has implications for the structures of 5S rRNA from other organisms. Indeed, it suggests that all 5S rRNAs, both prokaryotic and eukaryotic, conform in more detail than had

previously been recognized to a common structure. Figure 4 shows the 5S rRNAs from E. coli and human (which are typical of their respective kingdoms) arranged to resemble the folding of the Sulfolobus counterpart as much as seems reasonable, with the following justifications.

The helix V extension of helix IV can be constructed in all cases if one accepts an occasional bulged base and some non-Watson-Crick pairings. Helix V in the E. coli case (Fig. 4) contains, with the exception of one G-G juxtaposition, only normal pairing and G-U or A-G pairs, for which latter two there are ample precedents. G-U pairs occur in many RNA duplexes (13), while A-G pairs are common features at the ends of tRNA anticodon stalks (14), and seem to occur within several helices in 16S rRNA (15). [A helix rich in these pairs probably would be quite irregular and presumably would easily undergo local denaturation in structure.]

The proposed helix V pairing in the E. coli case is supported by the observations of Noller and Garrett (10) that all of the G residues in this helix are resistant to modification by kethoxal. Additionally, the competitive pairing of the 3' sequence of

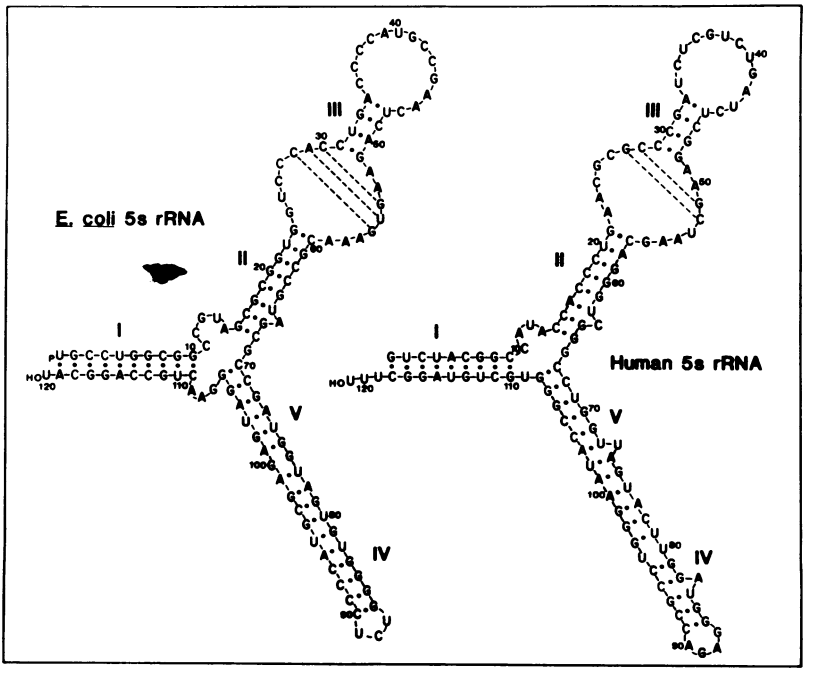

Figure 4. The 5S rRNAs of E. coli and human are drawn to conform as closely as possible to the S. acidocaldarius pairings (Figure 3).

helix V in Bacillus subtilis 5S rRNA with its Watson-Crick complement exposes the 5' sequence of the helix to RNase T_1 digestion (16). Analogous helices V are evident in the other eubacterial 5S rRNA sequences available. Limited pairing in this region has been put forth in many of the previously suggested eubacterial 5S rRNA foldings (11). The Sulfolobus 5S rRNA is the strongest evidence so far that such pairing exists, however.

Allowing the extension of helix IV into its loop (by bulging a base in the Sulfolobus and most eukaryotic examples) brings all examples, prokaryotic and eukaryotic, to a comparable loop size, 3-4 residues. The combined length of helices IV and V is 17 pairs in the eubacteria, 18 pairs in the eukaryotes and 19 pairs in Sulfolobus.

The E. coli helix II (the tuned helix), and that of other 5S rRNAs, can conform to the eight base-pair Sulfolobus helix II if the residue corresponding to E. coli residue A_{66} is bulged out and the established six base pair helix then extended by two additional Watson-Crick pairs in the direction of the molecular stalk (Fig. 4). The existence of this extension has already been suggested (12, 17, 18); it accomodates all 5S rRNAs. Although the figures do not show it as such, since there is no evidence, helix II probably also is extended in the direction of helix III by non Watson-Crick pairing or base stacking (generally an A-G, sometimes a G-G, seldom a G-U or A-A). Other irregular pairs in this region have been suggested by Studnicka et al. (12).

The extension of helix II to eight pairs and the construction of helix V tighten the structure sufficiently that two alternative coaxial stacking relationships among helices I, II and V seem plausible and may relate to 5S mechanism. Alternative stacking arrangements of these helices are diagramed in Figure 3, for the S. acidocaldarius structure. In one case, pairing between U_{11}-G_{112} brings helices I and V into coaxial stacking alignment. In an alternative case, pairing between A_{15}-G_{68} would coaxially align helices II and V. We have no basis on which to suggest either stacking arrangement; it is conceivable that both exist in the ribosome. The cyclic formation and disruption of these weak pairs at the ends of helices I and II could constitute a switch between the two conformational states. Analogous conformational alternatives seem possible with other 5S rRNAs. Other authors, as well, have addressed the possible involvement of conformational switches in 5S rRNA function (9, 12).

One difficulty in forming a common folding for the 5S rRNAs is that apparently homologous Watson-Crick pairings do not always involve homologous nucleotide sequences (19), particularly in helix I. One example of such inconsistency is illustrated in Figure 5, which aligns helices I of the E. coli and Bacillus subtilis 5S rRNAs. The significance of this is not clear, but it probably relates to our ignorance of higher order RNA structure. It may be that the A-C pairings shown for E. coli, because of stacking energies, extend helix I in analogy to that shown for B. subtilis.

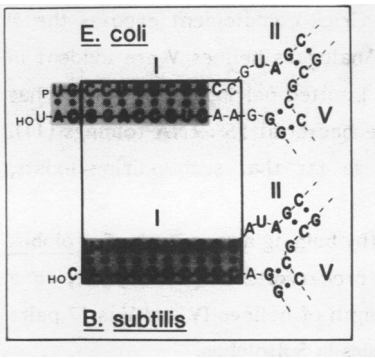

Figure 5. The aligned molecular stalks of E. coli and B. subtilis 5S rRNAs. The evident base pairings in helices I are shaded; identical residues are shown bold. The best alignment on the basis of nucleotide sequence homology is boxed.

The sites on the 5S rRNA molecule that are highly conserved in sequence are those in the loop closed by helix III and the bulged residues between helices II and III. Many of these residues appear not to be involved in internal structure; they are substantially reactive to chemical modifying reagents and to endonucleases. These residues, then, likely contain the sites whereby 5S rRNA interacts with other molecules.

Finally, it is not known why Sulfolobus should exhibit so much more obvious base pairing than do normal 5S rRNAs. Since the organism often grows at temperatures in excess of 80°C (in acid hot springs) (20), it is conceivable that stronger than normal structure is necessitated by this high temperature niche.

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