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Three-dimensional structural model of eubacterial 5S RNA that has functional implications

(*Escherichia coli* 5S RNA/nuclease S1/nucleic acid–protein interaction/looped-out adenines/tertiary structure)

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ABSTRACT ***Escherichia coli* 5S RNA and its specific protein complexes were hydrolyzed with the single-strand-specific nuclease S1. Based on the results, a tertiary structural model for *E. coli* 5S RNA is proposed in which ribosomal proteins E-L5, E-L18, and E-L25 influence the conformation of the RNA. This may be of significance for ribosomal function. Comparison of the proposed *E. coli* 5S RNA structure with those of 18 other prokaryotic 5S RNAs led to a generalized eubacterial 5S RNA tertiary structure in which the majority of the conserved nucleotides are in non-base-paired regions and several conserved "looped-out" adenines (in *E. coli*, adenines -52, -53, -57, -58, and -66) are implied to be important for protein recognition or interaction or both.**

The structure of ribosomal 5S RNA has been studied extensively for more than a decade (1), and these studies have led to the proposal of a large number of structural models (some of them are summarized in refs. 1–3 and others are described in refs. 4–12). Comparison of the primary structures of eukaryotic and prokaryotic 5S RNAs led to a universal four-helix model (4) that is generally accepted to be a minimal secondary structure model. For eukaryotic 5S RNA, the model can be extended to a five-helix model (10–13).

Although they have a common ancestry, prokaryotic and eukaryotic 5S RNAs are structurally distinct. Moreover, 50S ribosomal reconstitution experiments showed that different prokaryotic 5S RNAs are functionally interchangeable whereas eukaryotic 5S RNAs cannot replace the prokaryotic molecule in bacterial ribosomes (14). The question of whether the small ribosomal RNA is just a static structural component of the ribosome or whether it takes part in the dynamic process of protein biosynthesis, such as interacting with tRNA (for review, see refs. 1, 15, and 16) or the two large ribosomal RNAs (17, 18), can not yet be answered satisfactorily.

The precise function of the 5S RNA can be determined only after its structure is known. Therefore, we analyzed *Escherichia coli* 5S RNA and its specific protein complexes by nuclease S1 digestion, previous studies with tRNA having established that this nuclease is single-strand specific (19). Based on similar nuclease S1 digestion studies, we here present a structural model for *E. coli* 5S RNA including tertiary interactions that leads to an extended four-helix model for eubacterial 5S RNA. Furthermore, we report experimental evidence for a conformational switch in *E. coli* 5S RNA mediated by its ribosomal binding proteins E-L5, E-L18, and E-L25. This could facilitate interactions between 5S RNA and tRNA, 16S RNA, and 23S RNA.

MATERIAL AND METHODS

Material. Uniformly ^{32}P-labeled *E. coli* MRE600 50S ribosomal subunits were a gift from R. Brimacombe. Nuclease S1 was from Boehringer Mannheim, RNases T1 and A were from Sankyo (Tokyo), and polyethyleneimine-cellulose plates were from Macherey and Nagel (Düren, Federal Republic of Germany). All other chemicals were purchased from Merck (Darmstadt, Federal Republic of Germany).

Isolation of 5S RNA; Proteins E-L5, E-L18, and E-L25; and 5S RNA–Protein Complexes. Unlabeled (20) and labeled (21) *E. coli* MRE600 5S RNAs were isolated from 50S ribosomal subunits as published. *E. coli* 5S RNA binding proteins E-L5, E-L18, and E-L25 were isolated by 5S RNA affinity chromatography (unpublished data) and the complexes were reconstituted as described (21, 22).

Nuclease S1 Digestions. Hydrolysis of 10 μg of free 5S RNA was carried out in 90 μl of buffer F (0.1 M NaCl/5 mM $ZnCl_2$/30 mM NaOAc/5% glycerin, pH 5), and 5S RNA–r-protein complexes containing 10 μg of RNA were digested in 90 μl of buffer C (0.3 M KCl/20 mM $MgCl_2$/5 mM $ZnCl_2$/30 mM NaOAc, pH 6.0). The reaction was started by adding 200 units of nuclease S1 in buffer F or 2,000 units of nuclease S1 in buffer C; after incubation at 37°C for various time intervals, digestion was stopped by phenol extraction of the proteins and ethanol precipitation of the RNA. The digest was characterized on a 20% polyacrylamide/7 M urea gel. To estimate quantities of the individual fragments, gels stained with toluidine blue were scanned at 650 nm.

Identification of ^{32}P-Labeled Nuclease S1 Fragments. Sequence analysis of the fragments was carried out as published (23).

RESULTS

To distinguish between primary and secondary nuclease S1 cleavage sites in *E. coli* 5S RNA, digestion experiments were carried out under various conditions. The results show that limited nuclease S1 digestion of *E. coli* 5S RNA yields five distinct groups of fragments (Fig. 1). All fragments were isolated and their sequences were determined by polyethyleneimine-cellulose mapping (Fig. 2) and secondary analysis (Tables 1 and 2). The entire sequence was as follows:

$$\begin{array}{l}
\overset{1}{\text{p}}\overset{2}{\text{U}}\text{G C}\overset{3}{\text{C}}\overset{4}{\text{U}}\text{G G C}\overset{3}{\text{G}}\text{ G}_{10}\overset{5}{\text{C}}\text{CG U}\overset{6}{\text{A}}\text{G C}\overset{4}{\text{G}}\text{ CG}_{20}\\
\overset{3}{\text{G}}\overset{7}{\text{U}}\text{G}\overset{3}{\text{G}}\overset{8}{\text{U}}\text{CCCAC}_{30}\text{CUG A}\overset{9}{\text{C}}\text{CCCAU}_{40}\text{G}\overset{5}{\text{C}}\text{CG}\\
\overset{10}{\text{A}}\text{ACUCA}_{50}\text{G}\overset{5}{\text{A}}\text{AG}\overset{7}{\text{U}}\text{G}\overset{11}{\text{A}}\text{AAC}_{60}\text{G}\overset{5}{\text{C}}\text{CG}\overset{6}{\text{U}}\text{AG}\overset{4}{\text{C}}\text{G}\\
\overset{5}{\text{C}}_{70}\text{CG}\overset{6}{\text{A}}\text{UG}\overset{3}{\text{G}}\overset{6}{\text{U}}\text{AG U}_{80}\text{G}\overset{7}{\text{U}}\text{G}\overset{7}{\text{G}}\overset{3}{\text{G}}\overset{3}{\text{G}}\overset{3}{\text{G}}\\
\overset{12}{\text{U}}\text{CUC}_{90}\text{CCCAUG CG}\overset{4}{\text{A}}\overset{4}{\text{G}}_{100}\text{ A}\overset{4}{\text{G}}\text{ U}\overset{6}{\text{A}}\text{G}\overset{3}{\text{G}}\\
\overset{3}{\text{G}}\overset{13}{\text{A}}\text{AC}_{110}\text{UG C}\overset{14}{\text{C}}\text{AG}\overset{3}{\text{G}}\overset{15}{\text{C}}\text{AU}_{120}\text{-OH.}
\end{array}$$

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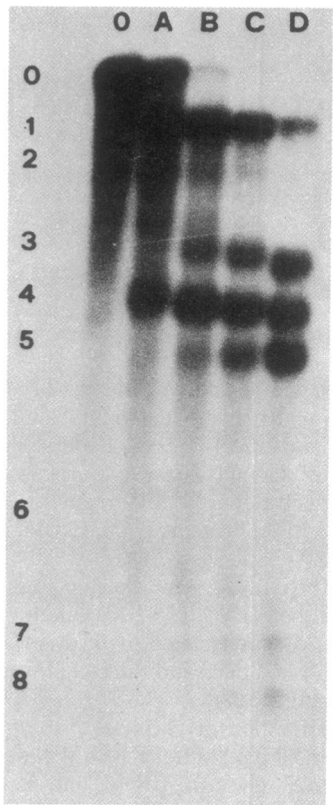

FIG. 1. Nuclease S1 digestion of ^{32}P-labeled *E. coli* 5S RNA under standard conditions in buffer F for 10 (lane A), 60 (lane B), 180 (lane C), and 300 (lane D) min. Numbering is according to I and Table 1.

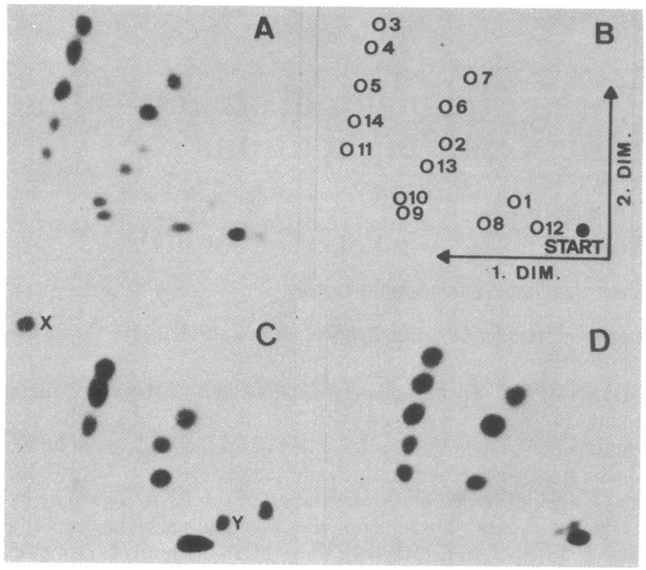

FIG. 2. RNase T1 maps of *E. coli* 5S RNA (*A* and *B*) and nuclease S1 fragments C1 (*C*) and C4 (*D*) on polyethyleneimine-cellulose (23). Numbering is according to I and Table 1.

Although the primary nuclease S1 cleavage site is behind uridine-40, other cleavages are observed behind cytidines -12, -36, -37, -38, and -88; adenosines -39, -59, and -104; guanosines -51, -69, -86, -96, and -100; and uridines -87, -89, and -95 (Fig. 3), suggesting that these nucleotides are in single-stranded or weakly base-paired regions of the 5S RNA molecule.

It is interesting that fragments C3, C4, and C6 (Fig. 2*B* and Tables 1 and 2) contain interrupted sequences, implying that these fragments are held together by base-paired regions that remain stable during electrophoresis in 7 M urea. Similar stabilities toward high concentrations of urea have previously been observed in the case of *Bacillus stearothermophilus* 5S RNA (22).

Since the primary nuclease S1 cleavage occurs around uridine-40, we followed the rate of hydrolysis in this region of the RNA in the presence of all possible combinations of the 5S RNA binding proteins E-L5, E-L18, and E-L25. Before these studies were carried out, the possible influence of the binding buffer (see *Materials and Methods*) on the specificity of nuclease S1 was analyzed. Although higher amounts of the enzyme were required, no differences in the cleavage pattern of the free 5S RNA were observed (data not shown). Addition of the individual proteins to the 5S RNA showed that E-L5 and E-L18 inhibited nuclease S1 cleavage, while E-L25 was without any effect (Table 3). E-L25, which had no influence on the binding of E-L5 (25), had a surprising effect when analyzed in its presence; namely, it counteracted the inhibition of nuclease S1 observed with E-L5 alone (Table 3). As can also be seen in Table 3, the three

Table 1. Relative molar yields of ribonuclease T1 oligonucleotides from individual nuclease S1 fragments shown in Fig. 1

RNase T1 spot*	Sequence	Theoretical,† mol	5S RNA	Nuclease S1 fragment, no. A1	B1	C1	C2	C3	C4	C5	D6	D7	D8	RNase A fragment(s)
1	pUG	1	1, 0	—	—	—	—	—	0, 9	—	—	—	1, 0	pUp, Gp
2	CCUG	1	0, 7	—	—	—	—	—	0, 8	—	—	—	1, 0	Cp, UpGp
3	G	11	11, 7	9, 9	7, 7	6, 6	8, 3	4, 7	4, 8	4, 8	6, 6	2, 6	3, 1	Gp
4	CG, AG	7	6, 5	5, 0	4, 0	3, 7	4, 8	2, 0	3, 2	3, 9	2, 0	—	0, 8	Cp, Gp, ApGp
5	CCG, AAG	5	4, 3	4, 5	2, 4	2, 8	1, 6	2, 8	0, 9	—	—	—	—	Cp, Gp, ApApGp
6	UAG, AUG	5	4, 0	4, 5	2, 4	2, 8	3, 8	3, 4	0, 8	1, 3	2, 2	—	—	Gp, Up, ApGp, ApUp
7	UG	4	4, 4	3, 5	3, 2	2, 6	2, 1	2, 6	1, 2	—	1, 8	—	—	Up, Gp
8	UCCCACCUG	1	0, 8	—	—	—	—	—	1, 1	—	—	—	—	Cp, ApCp, Gp, Up
9	ACCCCAUG	1	0, 8	—	—	—	—	—	—	—	—	—	—	Cp, ApCp, Gp, ApUp
10	AACUCAG	1	0, 9	1, 0	0, 3	—	—	—	—	—	—	—	—	Cp, ApApCp, ApGp, Up
11	AAACG	1	1, 1	1, 1	1, 2	1, 2	—	1, 0	—	—	—	—	—	ApApApCp, Gp
12	UCUCCCCAUG	1	1, 3	0, 8	1, 0	1, 0	1. 0	0, 2	—	—	—	—	—	Cp, Gp, Up, ApUp
13	AACUG	1	1, 1	0, 9	0, 8	0, 9	1, 0	—	—	1, 0	—	1, 0	—	ApApCp, Gp, Up
14	CCAG	1	1, 5	1, 5	1, 0	1, 0	1, 0	—	—	1, 0	—	1, 0	—	Cp, ApGp
15‡	CAU-OH	1	1, 1											Cp, ApU-OH

* As indicated in I.
† Expected for intact 5S RNA.
‡ Spot 15 migrates with the solvent front and is therefore usually not detected.

Table 2. Extra spots in the RNase T1 maps of nuclease S1 fragments from *E. coli* 5S RNA and their sequences as identified by maps on polyethyleneimine-cellulose after digestion with RNase A

Fragment	Extra spot	Sequence
A1	—	Guanosine-41 to uridine-120
B1	—	Guanosine-41 to uridine-12/ adenosine-52 to uridine-120
C1	—	Adenosine-52 to uridine-120
C2	pCGp	Cytidine-60 to uridine-120
C3	pUCUCCCCAU-OH	Adenosine-52 to guanosine-96
	pCUCCCCAU-OH	Adenosine-52 to guanosine-86/ uridine-87 to uridine-95
		Adenosine-52 to guanosine-86/ cytosine-88 to uridine-95
C5	pUCCCCAUGp	Uridine-89 to uridine-120
	pCCCCAUGp	Cytosine-90 to uridine-120
D6	pUCCCCAUGp	Cytosine-70 to guanosine-86/
	pCCGp	uridine-89 to guanosine-100
C4	ACC-OH	Uridine-1 to cytosine-37/ uridine-48 to guanosine-51
	ACCC-OH	Uridine-1 to cytosine-38
	UCAGp	
D7	pGp	Guanosine-105 to uridine-120
D8	CpC-OH	Uridine-1 to cytosine-12

proteins together showed the highest observed inhibition (58%) of the hydrolysis at uridine-40.

DISCUSSION

Comparison of the nuclease S1 cleavage patterns with previously proposed *E. coli* 5S RNA structural models showed that the data fit best to a base-pairing scheme that combines elements of previously proposed 5S RNA models (4, 7, 26, 27) if one assumes an additional tertiary base pairing between GCCG-44 and UGGU-77 (Figs. 3 and 4*A*). Based on this structure, we built a molecular model (Nicholson molecular model, Labquip, Reading, England) that is shown schematically in Fig. 4. Helices I and IV are coaxial and helices II and III are antiparallel to each other in this model (Figs. 3 and 4). The three-dimensional model, consisting of 26 G·C, 7 G·U, and 6 A·U base pairs, is in general agreement with IR spectroscopic results (2) and hydrogen exchange studies (29), as well as with previous chemical (30) and nuclease (31) modification studies (see also ref. 1). Results obtained by complementary oligonucleotide binding studies (32, 33) are also in agreement with the model under the assumption that double-stranded regions containing G·U base pairs or "looped-out adenines" are weakened and may therefore be accessible for oligonucleotide binding. Recently reported internal crosslinks in *E. coli* 5S RNA between guanines-2 and -112 and between guanines-41 and -72 (34, 35) are in excellent agreement with the three-dimensional structure proposed. To our knowledge, the latter crosslink is satisfied only in the model proposed here; structural models proposing interactions between CCAU-40 and AUGG-76 (16, 36) are not consistent with our experimental findings.

Nuclease hydrolysis studies with the double-strand-specific ribonuclease from the venom of the cobra *Naja naja oxiana* produced cleavage sites (24, 37) that are all within double-stranded regions of the proposed model (Fig. 3).

The overall shape of the Nicholson molecular model was measured to be 80 × 140 Å (Fig. 4*B*), which is in agreement with previous physical measurements (1, 38) as well as with our low-angle neutron scattering (25, 39) and electron microscopy (28) data.

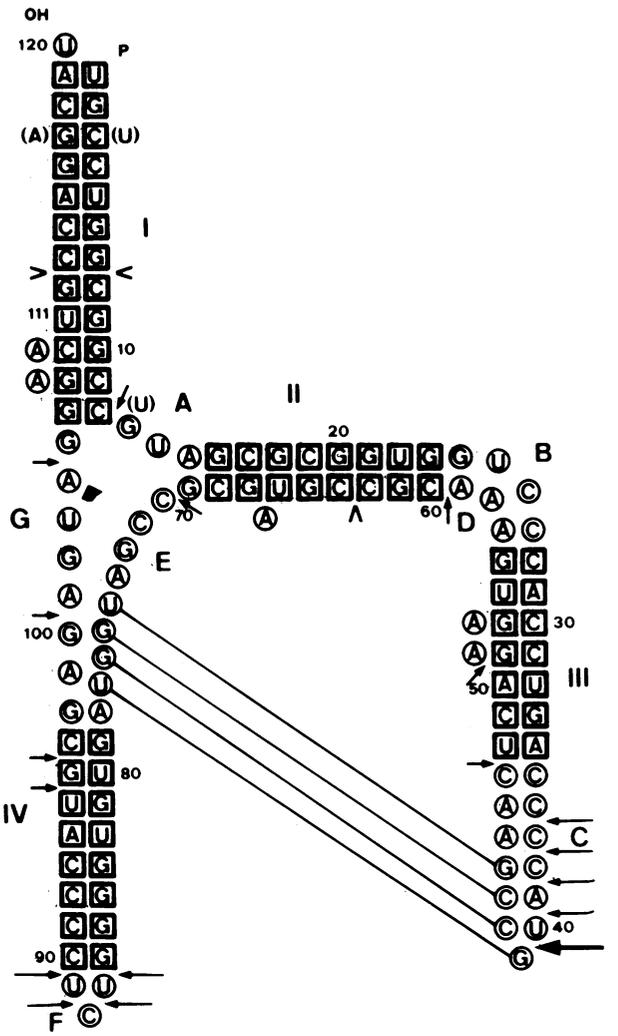

FIG. 3. Proposed secondary structure of *E. coli* 5S RNA having an additional tertiary interaction between GCCG-44 and UGGU-77; ⟶ and →, sites of strong and weak nuclease S1 cleavage, respectively; >, site of cleavage by double-strand-specific RNase (*Naja naja oxiana*; ref. 24).

The primary binding sites of proteins E-L5, E-L18, and E-L25, as determined by partial ribonuclease digestion studies of the corresponding 5S RNA–protein complexes (21), are indicated in Fig. 4*B*. As it turns out, the 5S RNA structure is made up of three surfaces, each of which serves as the primary binding site for the one ribosomal protein. From Fig. 4, it is also possible to envision that the structure of the central part of the molecule might be regulated by the three 5S RNA binding proteins during protein synthesis to facilitate possible interactions between 5S RNA and tRNA (1), 16S ribosomal RNA (17), or 23S ribosomal RNA (18), as illustrated in Fig. 4*C*. The observation that

Table 3. Influence of binding of proteins E-L5, E-L18, and E-L25 to *E. coli* 5S RNA on the accessibility of uridine-40 to nuclease S1

Protein	% inhibition
E-L5	27
E-L18	20
E-L25	0
E-L5/L18	41
E-L5/L25	0
E-L18/L25	18
E-L5/18/25	58

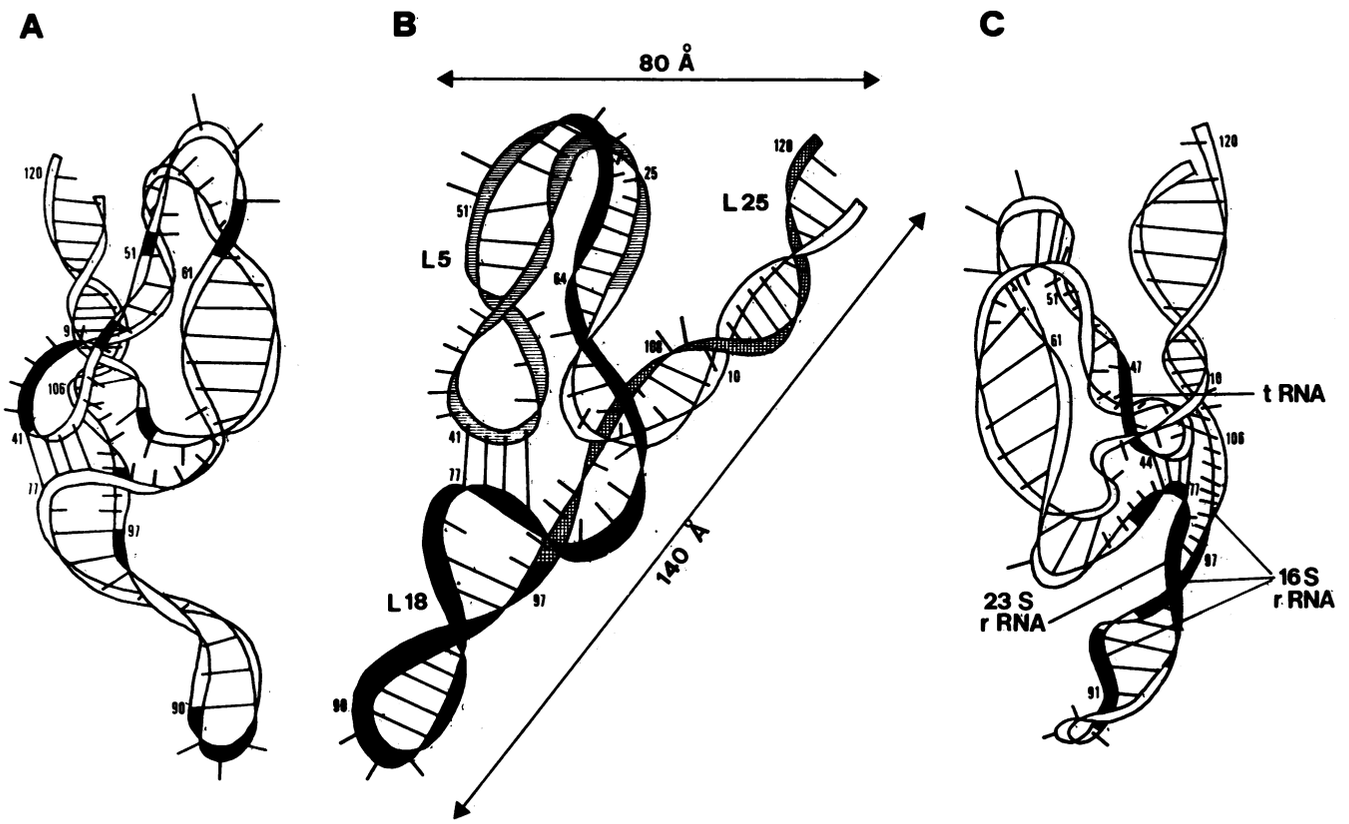

FIG. 4. Schematic representation of a molecular tertiary structural model of *E. coli* 5S RNA from three perspectives based on nuclease S1 digestion studies. (*A*) Shaded areas indicate sites of nuclease S1 cleavage (see also Fig. 3). (*B*) Binding regions of the *E. coli* 5S RNA binding proteins E-L5, E-L18, and E-L25 (21) and overall dimensions of the molecule as estimated by model building. Note that similar dimensions for *E. coli* 5S RNA have been measured by electron microscopy (28). (*C*) Hypothetical regions of interaction between *E. coli* 5S RNA and 16S RNA (17), 23S RNA (18), and tRNA (1).

the three 5S RNA binding proteins may alter the RNA structure in such a way that the rate of nuclease S1-mediated cleavage at uridine-40 is influenced by these proteins becomes plausible by the structural proposal shown in Fig. 4. Conformational changes of the 5S RNA due to protein interaction have previously been reported (24, 40, 41) and agree with proposals that the 5S RNA structure is altered during protein synthesis (8, 36). The results also agree with the proposal that ribosomal proteins bind preferentially to double-stranded RNA regions (41).

In addition, the distance determination of 75–85 Å between the mass centers of proteins E-L18 and E-L25 in the 5S RNA–protein complex (24, 39) is also satisfied by the model shown in Fig. 4.

By superimposing the structure presented here on the eubacterial 5S RNA sequences so far published (42), we derived the general secondary structure for eubacterial 5S RNAs shown in Fig. 5. In this structure, at least three base pairs within the region of tertiary interaction are always possible. It is also of interest that the conserved nucleotides are found primarily in single-stranded regions and in helix III. We propose that the two conserved adenines in the single-stranded section D, which are the most readily accessible adenines for modification by chloracetaldehyde (30) and the ones looped out from the double-stranded section III, as well as the conserved single looped-out adenine of helix II are important for protein recognition and interaction. The possible function of single-base bulge loops in 16S RNA as protein recognition signals has been proposed recently (44). In addition, we conclude, from model building, that these adenines are important for obtaining the tertiary structure proposed, so that the base pairs of the tertiary interaction are most likely not the driving force behind the three-dimensional structure. We also point out that the single-stranded section G is very rich in purines, so that the nucleotides should stack well on top of one another and thereby favor the coaxial arrangement of helices I and IV.

Comparison of the generalized eubacterial 5S RNA secondary structure with the recent proposal for eukaryotic 5S RNAs (13) reveals similarities in that the conserved nucleotides are also primarily found in the single-stranded regions of the RNA. It is possible that the eukaryotic helix V (13) serves the same role as the single-stranded section G in prokaryotes as discussed above.

We congratulate Dr. W. Hoppe on the occasion of his 65th birthday. We thank Prof. H. G. Wittmann for continuous support and helpful discussions and Miss H. Mentzel for drawing the figures. This work was supported by the Deutsche Forschungsgemeinschaft (Sfb 9/B 5).

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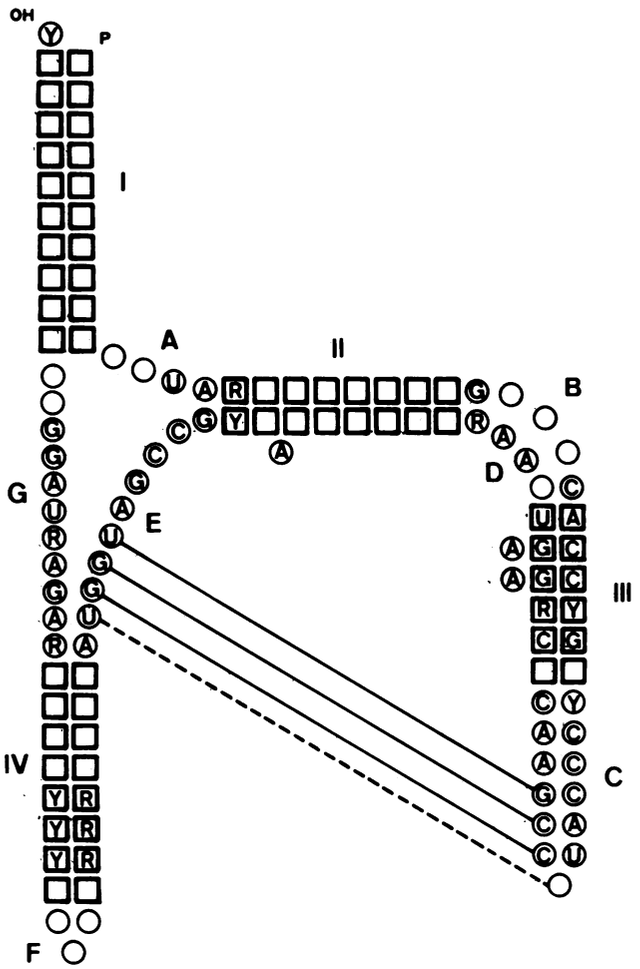

FIG. 5. General secondary structural model for eubacterial 5S RNA, deduced from the alignment of 20 eubacterial 5S RNA sequences (42) and strictly following the secondary structure shown in Fig. 3. Conserved and semiconserved nucleotides (R, purine; Y, pyrimidine) are indicated; conserved means present at an identical structural position in at least 90% of the sequences. For detailed discussion of the application of this model to various eubacterial 5S RNA sequences, see ref. 43.

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