REVIEW ARTICLE

5 S rRNA: structure and interactions

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5 S rRNA is an integral component of the large ribosomal subunit in all known organisms. Despite many years of intensive study, the function of 5 S rRNA in the ribosome remains unknown. Advances in the analysis of ribosome structure that have revealed the crystal structures of large ribosomal subunits and of the complete ribosome from various organisms put the results of studies on 5 S rRNA in a new perspective. This paper summarizes recently published data on the structure and function of 5 S rRNA and its interactions in complexes with proteins, within and outside the ribosome.

Key words: ribosomal proteins, ribosome, 5 S rRNA, transcription factor IIIA (TFIIIA).

INTRODUCTION

Ribosomes are large ribonucleoprotein (RNP) particles consisting of two unequally sized subunits that associate upon the initiation of translation. Their role is to provide an appropriate environment for the correct positioning of mRNA, tRNAs and translation factors during the decoding process, as well as catalytic activity for peptide bond formation. In bacteria, the large ribosomal subunit (50 S) is composed of two rRNA molecules (5 S and 23 S rRNAs) and 34 proteins. The eukaryotic 60 S subunit contains three rRNAs (5 S, 28 S and 5.8 S) and 50 proteins. The small subunit in bacteria (30 S) and eukaryotes (40 S) consists of one rRNA, of 16 S and 18 S respectively. The number of proteins in the small subunit varies from 21 in bacteria to over 30 in eukaryotes. The small ribosomal subunit is responsible for decoding, whereas the large subunit performs catalytic functions. Because of their key role in protein biosynthesis, ribosomes have been the subject of intensive studies over several decades. The final goal is to elucidate the mechanisms of peptide bond formation and the specificity of translation. In the last few years, several crystal structures of large and small ribosomal subunits, as well of as a whole ribosome from various organisms, have been solved [1–4]. They show fine detail of the spatial organization of rRNAs and their interactions with proteins.

Ribosome research is also important from the medical point of view, since a number of antibiotics target specific sites on both ribosomal subunits, interfering with various aspects of their function. The crystal structures of ribosomal subunits, their components and whole ribosomes with bound antibiotics are therefore crucial for inferring the mechanisms and selectivity of drug action [5–8].

For a long time it was believed that rRNAs provide only a structural scaffold for ribosomal proteins, which were regarded as the 'true' catalysts responsible for protein biosynthesis. It was demonstrated, however, that the peptidyltransferase activity is preserved after removal of a significant number of large-subunit proteins from *Thermus aquaticus* ribosomes [9]. Strong evidence for the involvement of 23/28 S rRNA in the catalytic activity of peptidyltransferase emerged from the crystal structure of the large ribosomal subunit from *Haloarcula marismortui* [1,10,11]. rRNA domains are also important for the activities of other functional sites of the ribosome, which suggests that the first ribosomes were all-RNA particles, and that proteins were added later in the course of evolution [8].

The smallest RNA component of the large subunit, present in almost all types of ribosome, is 5 S rRNA. For many years 5 S rRNA was used as a model molecule for studies on RNA structure and RNA–protein interactions, and as a phylogenetic marker. This led to the accumulation of a large amount of nucleotide sequence data [12]. 5 S rRNA was found to be absent from the mitochondrial ribosomes of some fungi, vertebrates and most protists [13]. In human cells, the cytoplasmic 5 S rRNA is imported efficiently into mitochondria, where its role is unclear, but the estimated number of 5 S rRNA molecules corresponds roughly with the number of mitochondrial ribosomes [14]. The precise role of 5 S rRNA in ribosome function is not fully understood. Based on the results of cross-linking experiments, it was suggested that it may serve as a signal transducer between the peptidyltransferase centre and domain II responsible for translocation [15], or as a determinant of large-subunit stability [16]. Its importance for the protein biosynthesis machinery was demonstrated in *Escherichia coli*, in which deletion of more than one 5 S rRNA gene greatly impairs the growth rate [17].

SECONDARY STRUCTURE OF 5 S rRNA

The 5 S rRNA molecule is approx. 120 nucleotides long, with a molecular mass of approx. 40 kDa. Regardless of its origin, 5 S rRNA can be folded into a common secondary structure. It consists of five helices (I–V), two hairpin loops (C and E), two internal loops (B and D) and a hinge region (A), organized in a

Abbreviations used: CKII, 'casein kinase II'; NLS, nuclear localization sequence; RNP, ribonucleoprotein; TFIIIA, transcription factor IIIA.

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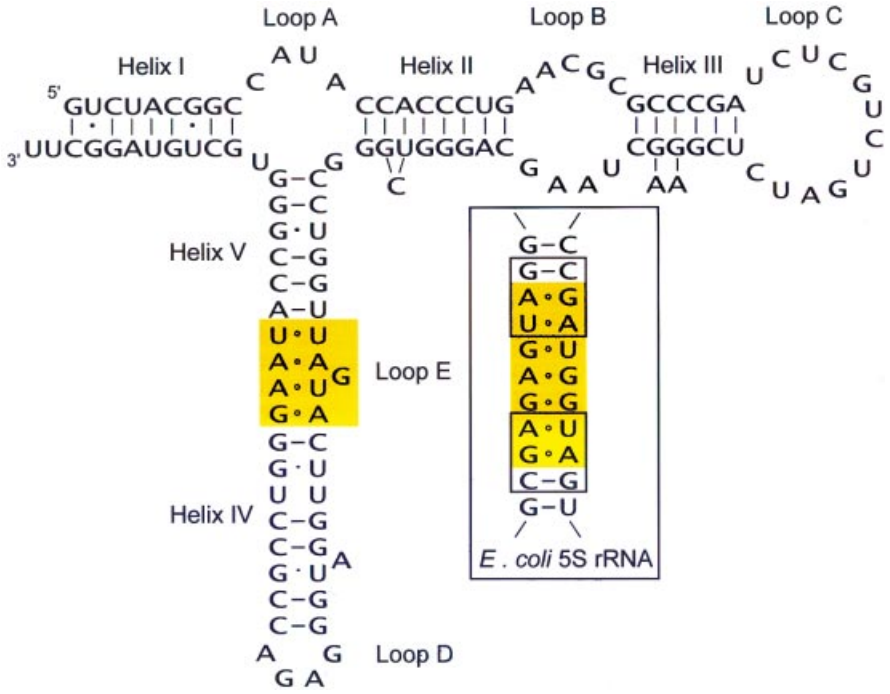

Figure 1 Secondary structure of human 5 S rRNA

In all organisms, the structure consists of five double-stranded regions (I–V) and five loops (A–E). Loop E, which differs between eukaryotic/archaeal and eubacterial 5 S rRNAs, is highlighted in yellow. The palindromic motifs within eubacterial loop E are boxed.

three-helix junction (Figure 1). Its general features have been confirmed in a number of structural studies and comparative sequence analyses. In Eukaryota and Archaea, the structure of 5 S rRNA is better preserved than in Eubacteria, where much more nucleotide sequence variability is observed.

The most notable difference between the eubacterial and archaeal/eukaryotic 5 S rRNAs is in the structure of internal loop E (Figure 1). In both cases the nominally single-stranded regions on the 5′ and 3′ sides of the loop pair with each other, forming a well defined tertiary structure composed of non-Watson–Crick base pairs. In Eubacteria, both sides of the loop have equal length. In Archaea and Eukaryota, however, the 5′ portion is one nucleotide longer than its 3′ counterpart. This asymmetry results in a single-nucleotide bulge. A detailed description of the loop E structures will be given in the following section on the tertiary structure of 5 S rRNA.

The crystal structure of a large ribosomal subunit from *H. marismortui* allowed verification of its secondary structure that had been inferred from phylogenetic analysis and structural studies in solution. Most of the base pairs predicted by comparative sequence analysis were detected in the crystal structure. A different pattern of base pairs was proposed for the region of loop B and helix III, where the three Watson–Crick base pairs flanking a dinucleotide bulge were not observed. Furthermore, a complicated network of tertiary interactions was also observed within the loop A/helix II and hairpin loop C region (Figure 2) [1].

TERTIARY STRUCTURE OF 5 S rRNA

No crystal data for the entire, uncomplexed 5 S rRNA are yet available. Crystallization of *Thermus flavus* 5 S rRNA under microgravity conditions yielded crystals that diffracted at 7.8 Å [18]. A low-resolution structure in solution was obtained using synchrotron X-ray scattering [19]. There are, however, some high-resolution crystal and NMR data for isolated 5 S rRNA domains, which are discussed below.

Helix I

The crystal structure of a synthetic helix I of *Thermus flavus* 5 S rRNA was solved at a resolution of 2.4 Å. The helical parameters of the RNA are similar to those found in the tRNA structure, and deviate slightly from a canonical A-RNA. Water molecules form an intricate network of inter- and intra-strand hydrogen bonds, maintaining the tertiary structure of the helix. An interesting feature found in this structure is the occurrence of tandem G · U pairs stabilized by three water molecules that mediate interactions between the nucleotides, compensating the loss of direct base-to-base hydrogen bonds [20].

Helix II

Helix II forms a binding site for ribosomal proteins and transcription factor IIIA (TFIIIA). A conserved feature of this region is a single-nucleotide bulge. The crystal structure of helix II of *Xenopus laevis* 5 S rRNA with a cytosine bulge revealed two distinct conformations at 2.2 Å and 1.7 Å resolution [21]. In both cases, the RNA retains the A-RNA conformation; the extrahelical cytosine is flipped out and points upwards in form I and downwards in form II. These differences have little influence on the conformation of the double-stranded region. In form I, a widening of the major groove suggests the possibility of substantial distortion of the sugar–phosphate backbone, which can be responsible for specific protein binding. The extrahelical base itself can also be a part of the protein-binding site [22].

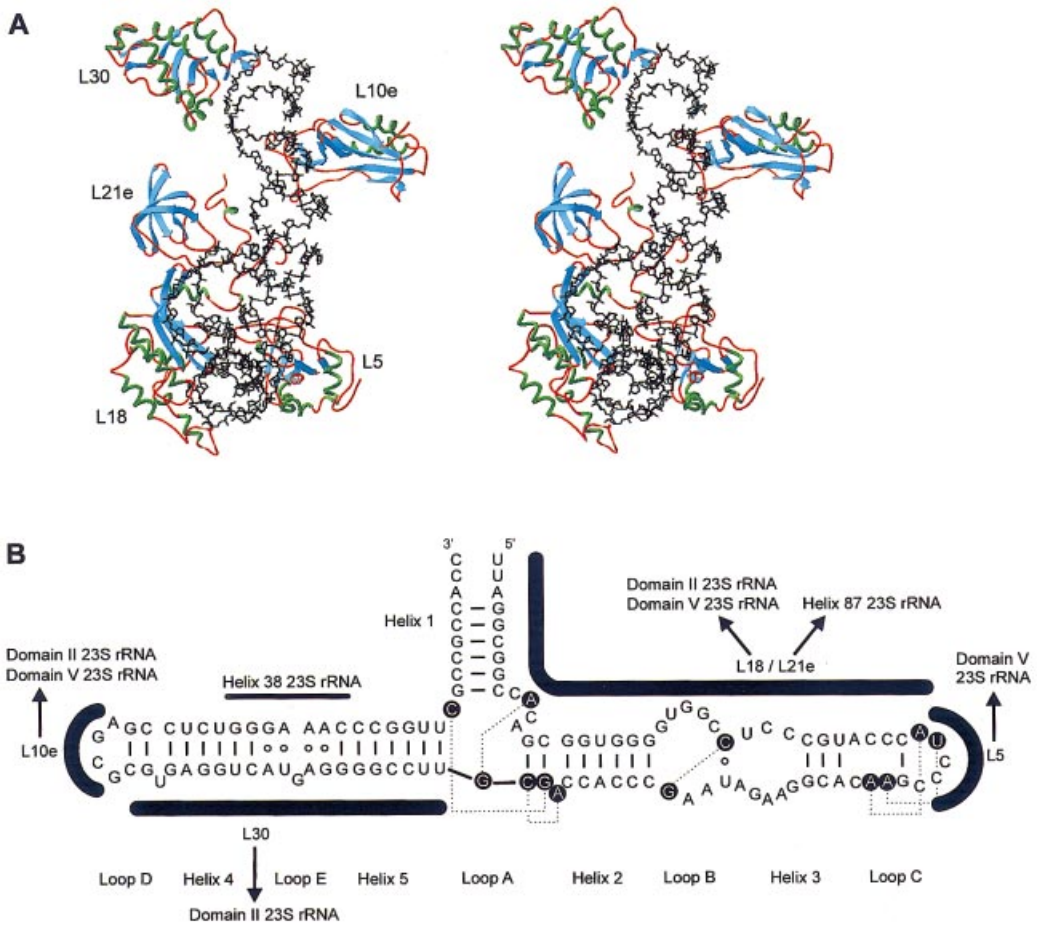

Figure 2 Interactions of 5 S rRNA in the ribosome

(**A**) Stereo view of 5 S rRNA with interacting proteins, as seen in the crystal structure of the 50 S ribosomal subunit from *H. marismortui*. (**B**) Base pairs observed within 5 S rRNA from *H. marismortui* in the large ribosomal subunit (compare with Figure 1) showing regions of interactions with proteins. The nucleotides involved in tertiary interactions are shown as black circles.

Helix III

Helix III contains a two- or three-nucleotide bulge in its 3′ portion. This feature is well conserved in both prokaryotes and eukaryotes, although the nature of bulge nucleotides and flanking base pairs varies. In *X. laevis*, the two single-stranded adenosines were shown to be crucial for high-affinity binding of the L5 ribosomal protein [23]. An NMR structure of an RNA stem with an AA bulge, corresponding to helix III of *X. laevis* oocyte 5 S rRNA, showed that the adenosines are located in the minor groove of the RNA helix and that they are stacked upon the 3′ flanking guanosine (Figure 3B). This structure confirmed the results of chemical modification studies of uncomplexed 5 S rRNAs in solution [24,25].

Helix IV

A crystal structure of helix IV of 5 S rRNA from *T. flavus* has been determined at 1.6 Å resolution. An interesting feature of this structure is an unusual G · C base pair in wobble-like conformation as a result of protonation of the cytosine at position N-3. This base pair is stabilized by water molecules and RNA–RNA interactions. Its formation is influenced by the crystal packing, and is unlikely to exist in solution [26]. Another structural motif found in helix IV is a tandem of G · U pairs. This motif belongs to the most frequent type (type 1) of adjacent G · U pairs, where the guanosines are stacked but located on opposite strands [27,28]. This motif was also found in structures of helix IV from *E. coli* [29,30] and helix I from *T. flavus* [20].

Loop D

Tertiary structures of loop D have been solved for an *E. coli* 5 S rRNA fragment with a UCU loop [30] and for a *T. flavus* GCGA tetraloop [31]. The NMR structure of loop D from *E. coli* shows that U^{87} stacks upon C^{88} and that the bases are oriented towards the major grove, while U^{89} sticks out into solution [30]. The *T. flavus* loop D tetraloop belongs to a conserved class of 5′-GNRA-3′ tetraloops. Its conformation is, however, different from those found in the hammerhead ribozyme (GAAA) [32] and other GNRA tetraloops in solution [33]. In loop D, the 5′ guanine (G^{87}) interacts with the 3′ adenine ($A^{89.1}$) via two hydrogen bonds: between N-2 of G^{87} and N-3 of $A^{89.1}$, and between N-3 of G^{87} and N-6 of $A^{89.1}$. C^{88} and G^{89} point towards the solvent and do not form any hydrogen bonds with other nucleotides [31].

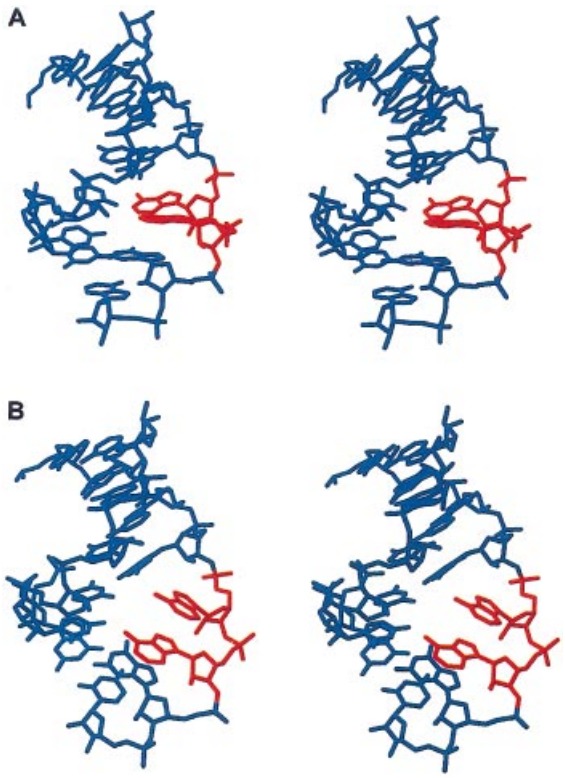

Figure 3 Differences in conformation of helix III with a dinucleotide bulge between a crystal structure found in a large ribosomal subunit from *H. marismortui* (A) and the NMR structure of an uncomplexed RNA fragment with a sequence corresponding to helix III of 5 S rRNA from *X. laevis* (B)

The structures depicted in (**A**) and (**B**) are described in [1] and [23] respectively.

Loop E

In bacteria, loop E is a part of the binding site for protein L25 [34]. The solution and crystal structures of RNA fragments encompassing loop E reveal that it possesses a highly ordered structure that involves a number of non-canonical base pairs (Figure 4) [29,30]. In Eubacteria, the sequence of loop E is partially palindromic, which is reflected in its tertiary structure. It consists of three portions, each of which includes three base pairs (with flanking Watson–Crick pairs). Each of the palindromic motifs consist of a G · C pair, followed by a sheared A · G pair and a reversed Hoogsteen U · A pair. A characteristic feature of this region is a cross-strand stack between the two adenine bases. The backbone is kinked at adenosines that form U · A pairs. This results in narrowing of the major groove and larger cross-strand distances in the central portion of loop E. The palindromic motifs are separated by three non-canonical base pairs with unusual geometries that involve bifurcated hydrogen bonds (G · U and G · G) or a single hydrogen bond (A · G) and are stabilized by five magnesium ions bound in the major groove [29]. Sequence analysis of eubacterial 5 S rRNAs reveals that the majority of substitutions of base pairs within loop E are isosteric with base pairs observed in the crystal structure [35].

In eukaryotic and archaeal 5 S rRNAs, loop E contains a single-nucleotide bulge. The structure of *X. laevis* loop E has been solved by NMR on a synthetic 27 nt duplex [36]. Its structure is different from that of eubacterial loop E, but shows striking similarity to a structural motif found in the sarcin/ricin loop of 23 S rRNA [37–39]. A sarcin/ricin loop motif has also been found to occur frequently in other rRNA structures [40]. It consists of a sheared A · G pair, followed by a *trans*-Hoogsteen A · U pair which forms a base triple with the bulged guanine in the major groove and a *trans*-parallel-Hoogsteen–Hoogsteen A · A pair (Figure 5). Regions of RNA structures where a sarcin loop motif has been identified show that the base pair substitutions are compatible, in terms of stereochemistry, with observed tertiary structures [40].

Structure and interactions of 5 S rRNA within the ribosome

In bacteria, 5 S rRNA together with proteins L5, L18 and L25 form the central protuberance of the large ribosomal subunit. Cross-linking experiments on *E. coli* ribosomes showed that helices II and III of 5 S rRNA are located close to domains II (helix 35) and V (helices 81–86) of 23 S rRNA [41]. The cross-links of U^{89} with nucleotides in helices 39, 41 and 89 of 23 S rRNA locate loop D close to the peptidyltransferase centre and GTPase region [42]. Interactions between helix 89 and 5 S rRNA were also demonstrated by *in vitro* selection of 5 S rRNA-binding aptamers with sequences identical with fragments of helices 53 and 89 of 23 S rRNA. The complex-formation between synthetic loop 89 and 5 S rRNA protects several nucleotides in helices II and III and loop B of 5 S rRNA and two guanosines in helix 89 against hydrolysis by nucleases S1 and V1 [43].

The interactions of loop D of 5 S rRNA and helix 39 of 23 S rRNA were studied by site-directed mutagenesis of A^{960} of 23 S rRNA, the substitution of which results in increased reactivity of N-3 of C^{90} in 5 S rRNA [44]. Based on an NMR structure in which C^{90} forms a non-standard base pair with G^{86} [30] and weak conservation of loop D in bacteria, it was suggested that A^{960} contacts the backbone and not the bases, and that its substitution has an indirect effect on the loop structure by changing the conformation of the backbone [44].

It has also been suggested that 5 S rRNA forms a link between the peptidyltransferase centre and domain II [15]. Within the ribosome, 5 S rRNA can be functionally substituted by antibiotics that interact simultaneously with domains II and V of 23 S rRNA [45].

The most detailed picture of the tertiary structure of 5 S rRNA is available from X-ray analyses of large ribosomal subunits [1,3]. *Haloarcula marismortui* 5 S rRNA forms a standard 5 S rRNA structure that consists of three stems projecting out from loop A (Figure 2). In the crystal, the stem containing helices II and III stacks on the stem containing helices IV and V, and helix I is folded in the central part of the molecule. The nucleotides in the multibranch loop A form unusual tertiary interactions with the nucleotides in helix II [1]. A similar structure emerged from X-ray analysis of the *Deinococcus radiodurans* large ribosomal subunit [3]. One has to note that the relative orientation of helices I and IV/V is different from that observed in the structure of fragment I of *E. coli* 5 S rRNA, in which helix I is stacked on and almost co-axial with helices V and IV [34]. A similar structure was obtained in a structural model derived from synchrotron X-ray scattering in solution for *T. flavus* 5 S rRNA [19]. Both of these structures resemble theoretical models for spinach chloroplast and *Xenopus* oocyte 5 S rRNAs [46]. An extended structure of wheat 5 S rRNA has been confirmed by X-ray footprinting, which showed that over 50 % of guanosine residues in 5 S RNA react with hydroxyl radicals [47].

The crystal structure of a complete ribosome-bound 5 S rRNA gives an opportunity to compare it with the structures of the separated domains. An interesting difference was observed in

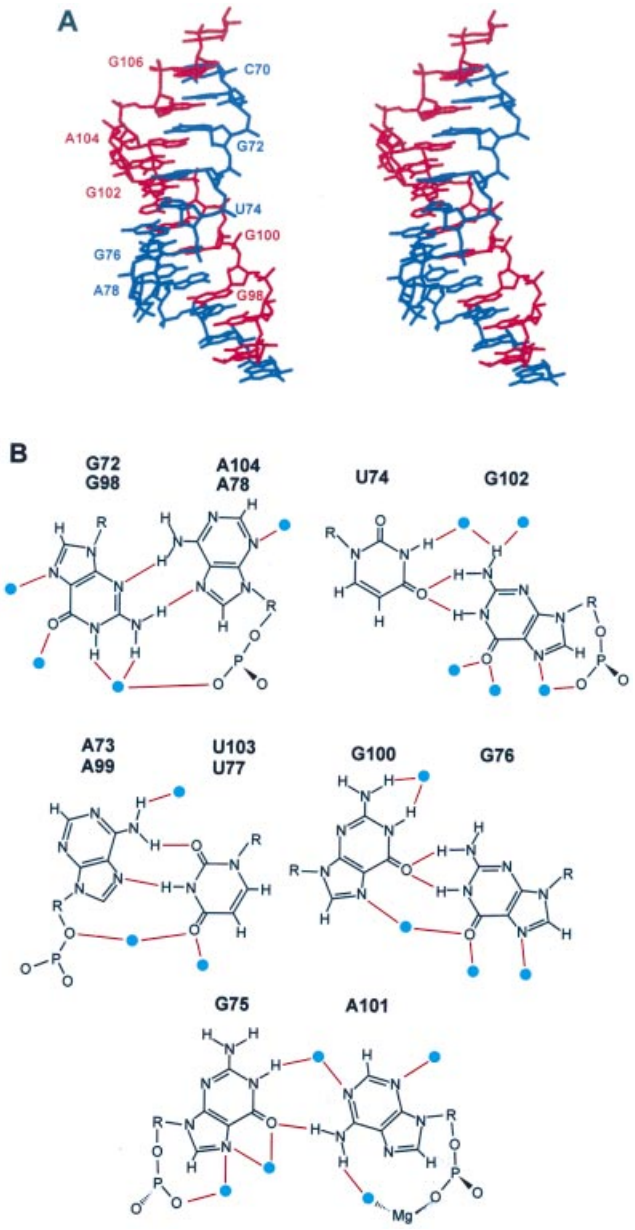

Figure 4 Loop E of *E. coli* 5 S rRNA

(**A**) Stereoview of the crystal structure of loop E of 5 S rRNA from *E. coli* [29]. The two RNA strands are shown in different colours. (**B**) Non-canonical base pairs found in the structure of bacterial loop E. Hydrogen bonds are shown in red. Water molecules are represented by blue circles.

Table 1 Direct and indirect (protein-mediated) contacts between 5 S rRNA and 23 S rRNA within the crystal structure of the *Halorcula marismortui* large ribosomal subunit

* Interaction inferred for *E. coli* ribosome from UV cross-linking experiments.

5 S rRNA domains	← Interactions →	23 S rRNA domains
	RNA–RNA	
Helices IV/V	Backbones	Helix 38 (domain II)
Loop E (77, 104, 105)	A-minor motif	A-rich loop (955, 1013, 1014)
Loop D (U^{89})*	Cross-link*	C^{2475} (domain V)*
	RNA–protein–RNA	
Helices IV/V	L30	Domain II
Helices I/II/III	L18	Helix 87 (domain V)
Helices I/II/III	L21e	Domains II and V
Loop C	L5	Helix 86 (domain V)
Loop D	L10e	Domains II and V

helix III. In the NMR structure, the two bulged adenosines were shown to be inserted into the helix and stacked upon the 3′ flanking base [23] (Figure 3B). Within the ribosome, they form an adenosine platform (Figure 3A) similar to that found in the P4–P6 domain of the group I intron [48]. Formation of adenosine platforms is usually induced by RNA–RNA interactions. However, since this region of 5 S rRNA is not involved such interactions, it seems that the transition may result from protein binding [23,25].

From the ribosome structure, it is evident that most partners in the interactions of 5 S rRNA are proteins (Table 1). There is only one significant RNA–RNA contact, between the loop E region of 5 S rRNA and helix 38 of 23 S rRNA. This interaction involves the reciprocal interactions of adenine residues 77, 104, 105 of 5 S rRNA and adenines 955, 1013 and 1014 of 23 S rRNA, forming so-called A-minor motifs, in which adenines form intra- or inter-molecular interactions using their N-1/C-2/N-3 edges [49].

INTERACTION OF 5 S rRNA WITH RIBOSOMAL PROTEINS

RNA–protein interactions play an important role in biological systems. Recognition of rRNA by proteins has been the focus of ribosome research for many years. Due to its relatively small size and the fact that it can form specific complexes with ribosomal proteins outside the ribosome, 5 S rRNA is used as a model molecule for studying RNA–protein interactions. The most obvious protein targets of 5 S rRNA are ribosomal proteins, but it has also been found to interact with several non-ribosomal

Figure 5 NMR structure of a fragment of a sarcin/ricin loop with identity with loop E of eukaryotic/archaeal 5 S rRNA

The opposite strands are shown in red and blue, with a bulged G residue [37] in green.

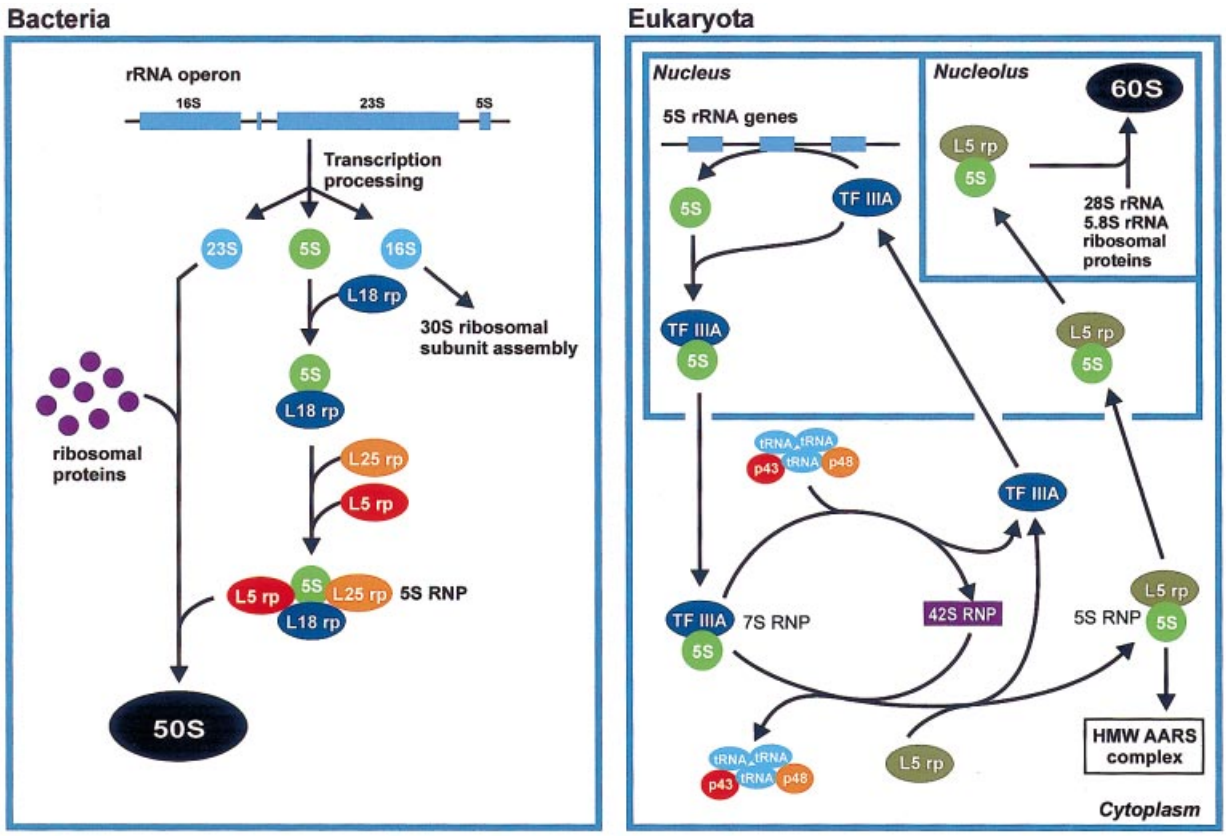

Figure 6 Interactions of 5 S rRNA with proteins in prokaryotic and eukaryotic cells

In bacteria, 5 S rRNA is synthesized as a part of the primary transcript from the rRNA operon. Before incorporation into a large ribosomal subunit, 5 S rRNA forms a complex with three ribosomal proteins (rps): L18, L25 and L5. In eukaryotes, 5 S rRNA genes constitute independent units that are transcribed by RNA polymerase III. TFIIIA is involved in the initiation of transcription. It can bind 5 S rRNA, and as a 7 S RNP is exported from the nucleus to the cytoplasm. 5 S rRNA can be exchanged from 7 S RNP to form another storage particle, 42 S RNP, or a 5 S RNP complex with ribosomal protein L5 that is exported to the nucleus and then to the nucleolus, where it is incorporated into the large ribosomal subunit. In the cytoplasm, 5 S RNP was also found to associate with high-molecular-mass complexes of aminoacyl-tRNA synthetases (HMW AARS).

proteins. A summary of the protein interactions of 5 S rRNA is given in Figure 6.

Eukaryotic ribosomal protein L5

In eukaryotes, 5 S rRNA is incorporated into the ribosome in a complex with ribosomal protein L5 (5 S RNP). After transcription, 5 S rRNA is exported from the nucleus to the cytoplasm in a complex with TFIIIA as a 7 S RNP particle (see section on TFIIIA below), and then re-enters the nucleus in the complex with protein L5. The interactions of 5 S rRNA with TFIIIA and a ribosomal protein are crucial for the regulation of 5 S rRNA biosynthesis. Overexpression of protein L5 in *Xenopus* embryos results in increased synthesis of 5 S rRNA from otherwise inactive genes. The exchange of 5 S rRNA between 7 S RNP and L5 releases TFIIIA, which can return to the nucleus and activate the synthesis of more 5 S rRNA. Thus the biosynthesis of 5 S rRNA is tightly linked to the synthesis of L5 [50,51].

The analysis of the human L5–5 S rRNA complex in an *in vitro* translation system shows that specific RNA–protein recognition is a co-translational event, and that 5 S rRNA is required at the beginning of the translation process. 5 S rRNA binds specifically to a 16-amino-acid region at positions 35–50 of the nascent L5 chain that is required, but not sufficient, to form a 5 S RNP complex. This initial interaction is specific: human L5 protein does not bind *E. coli* 5 S rRNA or U5 small nuclear RNA (snRNA) [52].

5 S rRNA can be imported into the nucleus only in a complex with L5, which ensures correct stoichiometry between RNA and protein [53]. In *Xenopus* L5, three nuclear localization sequences (NLSs) have been identified. The import of 5 S RNP to the nucleus requires the N-terminal NLS-1 (positions 1–25) and the C-terminal NLS-3 (positions 261–285) [54]. NLS-2, located in the central part of the molecule, is not required, and is probably masked by bound 5 S rRNA [55]. In human L5 there are two sequence elements responsible for nuclear export and nucleolar localization, at positions 21–37 and 255–265. An additional nuclear export signal has been identified at positions 101–111 [56].

There is no clearly identifiable RNA-binding domain in L5 protein from *Xenopus laevis*; almost all of the protein is required for high-affinity binding to 5 S rRNA [22,54,57]. The RNA target for L5 protein binding is helix III and loop C of 5 S rRNA. 5 S rRNA–L5 complex-formation is insensitive to ionic strength, which suggests a significant contribution of non-polar interactions [22]. It was proposed that a key recognition event involves intermolecular stacking. Analysis of *X. laevis* L5 mutants has revealed three tyrosine residues (Tyr^{86}, Tyr^{99} and Tyr^{226}) that are essential for binding of 5 S rRNA. Mutations of two other tyrosines (Tyr^{95} and Tyr^{207}) result in a 6–10-fold decrease in 5 S

rRNA binding. These tyrosine residues are well conserved in all eukaryotic L5 and bacterial L18 (the bacterial counterpart of eukaryotic L5) proteins. 5 S rRNA binding is not affected by substitutions of Tyr^{86} and Tyr^{226} with phenylalanine. The tyrosine at position 99 is absolutely required, which suggests it may make a direct contact with RNA via its hydroxy group [57].

In human cells, L5 protein associates with the β subunit of the protein kinase CKII ('casein kinase II') [58] and forms a complex with CKII holoenzyme and p53 protein [59]. CKII can phosphorylate serine residues in L5 protein, causing an approx. 5-fold decrease in 5 S rRNA-binding activity [60]. It was also noted that L5 can interact with protein phosphatase-1, which, together with CKII, could provide a mechanism for the modulation of 5 S rRNA–L5 interactions [61].

In yeast, mutations in L5 were shown to increase the efficiency of programmed ribosomal frameshifting in the 5′ and 3′ directions. In these mutants, peptidyl-tRNA shows a lower affinity for the ribosome. Although L5 does not make any direct contacts with peptidyl-tRNA, 5 S rRNA plays an indirect role as a bridge between L5, L11 and peptidyl-tRNA [62]. These interactions became evident from analysis of the crystal structure of the *Thermus thermophilus* ribosome with bound mRNA and tRNAs [2].

Bacterial ribosomal protein L5

In bacteria, the 5 S RNP complex, which is incorporated into ribosomes as a single unit, consists of three ribosomal proteins, i.e. L5, L18 (the bacterial equivalent of eukaryotic L5) and L25, as well as 5 S rRNA [34].

The crystal structure of L5 protein from *Bacillus stearothermophilus* was determined at 1.8 Å resolution. The central part of the molecule forms an antiparallel, five-stranded β-sheet similar to an RNA recognition motif. Mutagenesis of amino acids in β-strands and loops identified four residues in strands β1 (Asn^{37}) and β3 (Thr^{90}) and loops α2–β2 (Gln^{63}) and β2–β3 (Phe^{77}) whose replacement with alanines resulted in 5–25-fold decreases in binding of 5 S rRNA. Comparison of *Bacillus* L5 with the structure of L5 from *H. marismorturi* revealed differences not only in weakly conserved N- and C-terminal regions but also in putative 5 S rRNA-binding domains. Interestingly, a conserved phenylalanine (Phe^{77}) in the β2–β3 loop, which is essential for RNA binding in *Bacillus* L5, is located far away from 5 S rRNA in the *H. marismortui* ribosome and cannot make direct contact with RNA. It was suggested that Phe^{77} is involved in stabilization of the correct RNA-binding structure, but is not in direct contact with 5 S rRNA [63,64].

The crystal structure of *T. thermophilus* L5 in complex with a fragment of *E. coli* 5 S rRNA determined at 2.3 Å resolution shows that the structure of the N-terminal domain is close to the structure of L25 and binds 5 S rRNA, but the C-terminal domain represents a new fold composed of seven β-strands connected by large loops. The sequence of that protein shows identity with that of the general stress protein CTC [65].

Bacterial ribosomal protein L25

Bacterial protein L25 binds to the loop E/helix IV region of 5 S rRNA. Despite obvious differences between prokaryotic and eukaryotic 5 S rRNAs, the RNA–protein interactions in this region are of particular interest, because of the unusual RNA structure formed by an array of non-canonical base pairs [29,30,39]. NMR analysis of free L25 protein from *E. coli* showed a structure consisting of a six-stranded β-barrel and two α-helices. The β-strands show topology identical with that found in the anticodon-binding domain of glutaminyl-tRNA synthetase (GlnRS) from *E. coli*. However, there are differences in the lengths and orientation of the loops, and there is very low sequence similarity [66]. The interactions with RNAs are also different; GlnRS binds single-stranded RNA, whereas L25 interacts with a helical region.

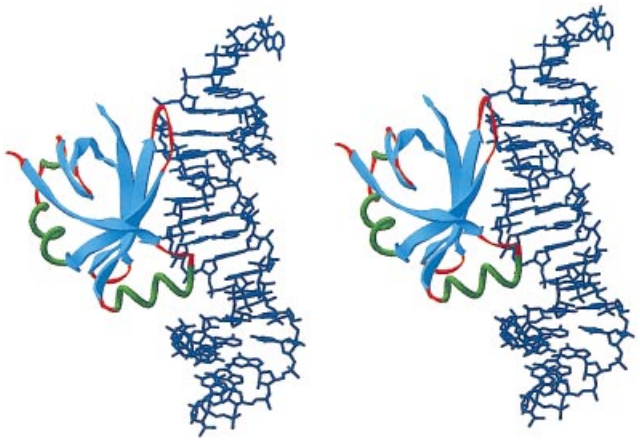

Figure 7 Crystal structure of *E. coli* 5 S rRNA fragment encompassing helix V/loop E/helix IV/loop D with ribosomal protein L25

The details of the interactions of 5 S rRNA with ribosomal protein L25 were studied using complexes of 5 S rRNA fragments containing loop E with L25. The NMR structure was determined for a complex of L25 with a 37 nt fragment of 5 S rRNA [67], and the crystal structure was solved for a complex of L25 with an 18 bp duplex at 1.8 Å resolution (Figure 7) [34]. Both structures demonstrated that the L25–RNA interactions are mediated by two distinct protein motifs. The first is a surface of the β-barrel that contacts the minor groove of loop E of 5 S rRNA. There are several amino acids in four of the six β-strands (β-2, β-3, β-5 and β-6) whose side chains contact one strand of RNA in the 5′ portion of the loop (positions 73–77). Most of the contacts involve hydrophobic amino acids located in strands β-2 and β-3. On the other hand, the amino acids that contribute to binding in strands β-5 and β-6 are mostly hydrophilic, and include at least three base-specific contacts. A comparison of the RNA–protein complex with available structures of the free protein and the loop E region of 5 S rRNA shows that complex-formation does not induce any major changes in the conformation of either the β-sheet surface or a matching RNA region. The second RNA-binding element of L25 is an α-helix (α-1) whose N-terminal part interacts with the major groove of RNA on the border between helix IV and loop E. Interestingly, the formation of helix α-1 is induced by RNA binding, since this fragment is not structured in the uncomplexed protein [66]. The amino acids in helix α-1 and the flanking N-terminal region bind RNA exclusively through hydrophilic side chains. Lysine at the N-terminus of the helix is inserted into the widened major grove and interacts with bases and phosphate groups of the RNA backbone [67]. The structures show the importance of non-canonical base pairs within loop E that induce considerable distortion of the A-RNA structure. On the other hand, non-standard base pairs provide a complex hydrogen-binding surface and shape that is a major determinant of the specificity of the interaction. The crystal structure also demonstrated an important role for bivalent metal ions in stabilization of the RNA structure that is necessary for protein binding [34,68].

Bacterial ribosomal protein L18

In bacteria, ribosomal protein L18 is the equivalent of eukaryotic protein L5. It forms a stable complex with 5 S rRNA that further stimulates binding of L5. In *E. coli* and *Pyrococcus furiosus* it has been shown that this stimulation depends on the presence of 17 and 15 N-terminal amino acids respectively [69,70].

NMR analysis has shown that *T. thermophilus* L18 protein consists of two α-helices, a β-sheet composed of three antiparallel and a short parallel β-strand, and a helical turn [71]. The tertiary structure is very similar to that of ribosomal protein S11 from *T. thermophilus*, and these proteins share 21.6 % identical amino acids [71]. Comparison of the free L18 structure with its structure in ribosomes [1–3] revealed that there are no substantial differences in the β-sheet that makes numerous contacts with RNA. On the other hand, the N-terminal portion, which is unstructured in solution, forms a helical turn that contacts helix II and loop A of 5 S rRNA and the neighbouring 23 S rRNA domain as well as L5 protein. This situation is similar to the induced formation of the α-1 helix observed in the interaction of protein L25 with the loop E/helix IV region [34].

Other bacterial proteins

Mobility shift assays of total protein extracts of *E. coli* showed that, in addition to the three ribosomal proteins mentioned above (L5, L18 and L25), 5 S rRNA can also bind ribosomal protein L2 and the heat-shock protein HSP70. It has been proposed that L2 protein plays a role by providing the bridge joining 23 S rRNA and 5 S rRNA, thus constituting the peptidyltransferase centre. Binding of HSP70 to 5 S rRNA suggests that this protein may bind transiently to the ribosomes and promote the correct folding of the nascent polypeptide chain [72].

Interactions of 5 S rRNA with proteins within the ribosome

The full picture of the interactions of 5 S rRNA within the ribosome will emerge from analysis of the structures of the ribosomes. A high-resolution structure of the 50 S subunit of the *H. marismortui* ribosome, which has become a standard reference for ribosomal studies, shows precise topology of the ribosomal components [1]. 5 S rRNA makes only one direct contact with 23 S rRNA through the A-minor motifs [49]. There are, however, a number of contacts mediated by ribosomal proteins that are bound to both RNAs (Table 1). Three ribosomal proteins – L5, L18 and L30 – have substantial interactions with 5 S rRNA, while the interactions of L21e and L10e are somewhat weaker (Figure 2) [1]. Ribosomal protein L18 binds 5 S rRNA helices I, II and III, as well as helix 87 of 23 S rRNA. The same region of 5 S rRNA is bound by protein L21e, which mediates its interaction with domains II and V of 23 S rRNA. Domain V of 23 S rRNA also makes two other indirect contacts with 5 S rRNA through proteins L5 (that binds to loop C) and L10e (bound to loop D). It seems that one of the major roles played by the ribosomal proteins is maintenance and stabilization of the correct relative orientation of the RNA domains, and in some instances also of their tertiary structures.

INTERACTIONS WITH NON-RIBOSOMAL PROTEINS

TFIIIA

In eukaryotes, the biosynthesis of 5 S rRNA depends upon binding of TFIIIA to the internal control region of the 5 S rRNA gene. In *Xenopus* oocytes, TFIIIA is found in the complex with 5 S rRNA (7 S RNP) that serves as a storage particle for 5 S rRNA. The exchange of 5 S rRNA from 7 S RNP with ribosomal protein L5 (see above) results in the formation of 5 S RNP, which can enter the nucleus and then the nucleolus, where it is used in ribosome biosynthesis (Figure 6).

A unique feature of TFIIIA is its ability not only to specifically recognize the 5 S rDNA promoter sequence, but also to bind the transcription product or 5 S rRNA. The RNA- and DNA-binding activities of TFIIIA depend on the presence of nine zinc fingers – specific elements found in many DNA- and/or RNA-binding proteins.

The mechanism and specificity of TFIIIA binding to DNA have been extensively studied. A crystal structure of the first six zinc fingers bound to a 31 bp 5 S rRNA promoter at 3.1 Å resolution showed details of the positioning of the fingers along the DNA [73]. The first three fingers wrap the DNA in the major groove and seem to play the role of prime determinants of sequence specificity by making extensive direct contacts with bases. The next three fingers form a spacer element and lie along one side of the DNA molecule. Only finger 5 interacts with the bases in the major groove. Although they are missing from the crystal structure, biochemical studies suggest that the remaining three fingers, 7–9, are also responsible for specific sequence recognition, similar to fingers 1–3 [73].

Much less is known about the interactions of TFIIIA with 5 S rRNA. Currently no crystal structure of this RNA–protein complex is available. Systematic analysis of the 5 S rRNA-binding properties of TFIIIA mutants showed that the interaction depends primarily on the three middle fingers 4–6, with the first three fingers making very little contribution to binding [74]. Experiments in a heterologous system with *X. laevis* TFIIIA and a plant 5 S rRNA from *Lupinus luteus* demonstrated that a peptide composed of the first three fingers can in fact bind 5 S rRNA, protecting helices IV and V and part of helix II from hydrolysis by V1 nuclease [75]. Successive addition of zinc finger domains showed that finger 4 interacts with helix II, fingers 5 and 6 interact with the central part of 5 S rRNA including helices I and II, and fingers 7–9 cover part of helix II, loop B and helix III [75]. Binding of TFIIIA to mutant 5 S rRNAs showed that this interaction is structure-specific. The minimal 5 S rRNA fragment that is recognized and bound by TFIIIA consists of helices I, III and V and loops A, B and E [76,77]. The most important structural element of 5 S rRNA required for TFIIIA binding is loop A; only one of the four nucleotides (C^{12}) can be changed without affecting protein binding [78]. Cleavage within this region also leads to a significant loss of TFIIIA binding activity [79]. Loop A was proposed to form a binding site for finger 6, which contains a conserved Thr-Trp-Thr sequence with an essential tryptophan residue at position 177 (in *X. laevis* TFIIIA) [77]. It was suggested that a stacking interaction exists between Trp^{177} and nucleotides within loop A, since mutation of this residue to alanine totally abolishes RNA binding, while its substitution with phenylalanine does not affect binding [78]. Another amino acid critical for high-affinity binding of truncated (fingers 4–7) TFIIIA to 5 S rRNA is a lysine at position 118 in finger 4 [80]. The same mutation in a full-length TFIIIA, however, has little effect on complex-formation, which suggests that the contribution of finger 4 to binding is small [81].

Detailed analysis of binding to 5 S rRNA using protein fragments encompassing zinc fingers 4–6 or 4–7 of TFIIIA revealed that fingers 4–6 are sufficient for high-affinity binding, and that the interaction of finger 7 with the RNA is much weaker. This is consistent with the observation that the loss of loop B of 5 S rRNA, which is a target for finger 7, does not influence binding of the truncated molecule by a protein fragment encompassing zinc fingers 4–7 of TFIIIA [82].

p43 protein

In addition to 7 S RNP complexes, in amphibian oocytes 5 S rRNA is also stored in larger, 42 S RNP particles (thesaurisomes). In *Xenopus*, approximately one-half of oocyte 5 S rRNA is associated with TFIIIA and the other half is present in the form of 42 S RNPs (Figure 6). In *Pleurodeles waltl*, the 42 S RNP is a major storage form of 5 S rRNA [83]. Thesaurisomes consist of two proteins: thesaurin a (p50), a tRNA-binding protein that shows identity with elongation factor 1α, and thesaurin b (p43), a nine-zinc-finger protein responsible for 5 S rRNA binding [84]. Although p43 and TFIIIA both bind 5 S rRNA with nine zinc-finger motifs, they show little sequence identity. Unlike TFIIIA, p43 binds only to 5 S rRNA and not to its gene [85], and recognizes stems II and V and loop D of 5 S rRNA [86].

Although the overall similarity of the sequences of TFIIIA and p43 is low, in both cases zinc finger 6 contains the conserved sequence Thr-Trp-Thr that has been found to be critical for TFIIIA binding to 5 S rRNA. Interestingly, in p43, substitution of this motif does not influence RNA binding [79]. In p43 the first fingers are responsible for high-affinity RNA binding, while isolated fingers 5–9 do not bind 5 S rRNA at all [81].

Comparative analyses of the sequence requirements in p43 and TFIIIA for binding of 5 S rRNA, as well as the results of studies on mutations in truncated and full-length proteins, have suggested an important role of the interactions between the individual fingers within the structure of the protein. This can explain the results of finger-swapping experiments showing that, even though they alone do not possess RNA-binding activity, fingers 4–7 of p43 can functionally substitute for fingers 4–7 of TFIIIA [81].

Other proteins

In *Trypanosoma brucei* two novel proteins have been identified that may play a role in 5 S rRNA transport and the biogenesis of ribosomes. The proteins, p34 and p37, are highly similar, and are localized in the nucleus. Both form specific complexes with 5 S rRNA, which suggests that they may be involved in a pathway of ribosome assembly [87].

In rat liver, a complex of 5 S rRNA and L5 ribosomal protein was found to be associated with a high-molecular-mass complex of aminoacyl-tRNA synthetases [88] and complexes of threonyl- and histidyl-tRNA synthetases [89]. The presence of 5 S RNP in the macromolecular aminoacyl-tRNA synthetase complex enhanced the activities of methionyl- and isoleucyl-tRNA synthetases [90]. It was further shown that 5 S RNP associates specifically with methionyl-tRNA synthetase, but the details of their interactions are not fully understood [91].

PERSPECTIVE

The crystal structures of ribosomal subunits and of whole ribosomes open a new perspective for all RNA studies. They contribute much to the understanding of 5 S rRNA structure and its possible function as a ribosomal component. There are, however, some open questions remaining. Some details of 5 S rRNA structure within the ribosome differ from those obtained for uncomplexed fragments in solution [21]. One may ask whether the structure of free 5 S rRNA is different from that observed in the ribosome, and to what extent the interaction with ribosomal proteins and the environment of the ribosome influence its structure. The definitive answer to this question may be obtained by analysis of the crystal structure of the whole free 5 S rRNA or of its complexes with other proteins outside the ribosome.

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