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Review

Structure and functions of 5S rRNA✪

Mirosława Z. Barciszewska[1], Maciej Szymański[1], Volker A. Erdmann[2] and Jan Barciszewski[1]✉

[1]*Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland, and* [2]*Institut für Biochemie, Freie Universität Berlin, Berlin, Germany*

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The ribosome is a macromolecular assembly that is responsible for protein biosynthesis in all organisms. It is composed of two-subunit, ribonucleoprotein particles that translate the genetic material into an encoded polypeptides. The small subunit is the site of codon-anticodon interaction between the messenger RNA (mRNA) and transfer RNA (tRNA) substrates, and the large subunit catalyses peptide bond formation. The peptidyl-transferase activity is fulfilled by 23S rRNA, which means that ribosome is a ribozyme. 5S rRNA is a conserved component of the large ribosomal subunit that is thought to enhance protein synthesis by stabilizing ribosome structure. This paper shortly summarises new results obtained on the structure and function of 5S rRNA.

In all organisms, messenger-directed protein synthesis is catalysed by ribosomes. The ribosome is a large (about 2.6 MDa), very complex molecular machine composed of rRNAs and proteins [1–4]. In the past few years a combination of X-ray crystallography, NMR spectroscopy and cryoelectron microscopy has provided new data on the structure of ribosomes [5]. A bacterial ribosome (70S) consists of the two unequal subunits. The small one, 30S, is composed of 16S rRNA (1542 nucleotides) and 21 ribosomal proteins. The 50S (large) subunit containing 34 different

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✉Corresponding author: Prof. dr hab. Jan Barciszewski, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Z. Noskowskiego 12, 61-704 Poznań, Poland; tel. (48 61) 852 8503, fax. (48 61) 852 0532; e-mail: jbarcisz@ibch.poznan.pl

Abbreviations: mRNA, messenger ribonucleic acid; tRNA, transfer ribonucleic acid; NMR, nuclear magnetic resonance; EM, electron microscopy; NLS, nuclear localisation signal; NES, nuclear export signal; CKII, casein kinase II; GlnRS, glutaminyl-tRNA synthetase; TF IIIA, TF IIIB, TF IIIC, transcription factor IIIA, IIIB, IIIC; ZIF, zinc finger protein; CSB, Cockayne syndrome group B; CPD, cyclobutane pyrimidine dimer; NER, nucleotide excision repair; NTP, nucleotide triphosphate; L5, ribosomal protein L5; RNP, ribonucleic acid particle.

proteins (L1–L34), 23S rRNA of 2904 nucleotides and 5S rRNA, has been recently crystallized and its structure solved with a high resolution [6–11]. Ribosomal 5S RNA, a 120 nucleotide long RNA of 40 000 Da, is found in virtually all ribosomes with the exception of mitochondria of some fungi, higher animals and most protists [12]. In bacteria it binds three proteins: L5, L18 and L25, but in Eukaryota 5S rRNA molecule binds only ribosomal protein L5. Recently the complete atomic structure of the large subunit of the halophile archaea *Haloarcula marismortui* has been solved at 2.4 Å resolution [13, 14]. In this paper we describe recent results of structural studies concerning 5S rRNA. The primary structures of 5S rRNAs are available at URL: http://biobases.ibch.poznan.pl/5SData/. The database is a new version of the last year edition [15]. Other results have been discussed in a recent minireview on 5S rRNA [16].

CURRENT STRUCTURE OF 5S rRNA

The model of atomic structure of *H. marismortui* 50S ribosomal subunit includes 2833 of its total 3045 nucleotides (212 nucleotides are missing) and 27 out of 31 proteins [13, 14]. 5S rRNA of *H. marismortui* contains 122 nucleotides but no modified ones, as studied by MALDI mass spectrometry [17]. *H. marismortui* has two rDNA operons coding for 5S rRNA, rrnA and rrnB. The last one differs in cytidine to guanosine substitution at position 107 [17]. All nucleotides of 5S rRNA are well ordered in the crystal structure of the 50S ribosomal subunit. The 5S rRNA structure consists of three stems projecting out from loop A. The helix 2 and helix 3 (domain β) of the 5S rRNA molecule stack on its helix 4 and 5 (domain γ). Domain α is somehow folded in the central part of the molecule. A similar shape for *Thermus flavus* 5S rRNA in solution has been established from synchrotron X-ray scattering data using an *ab initio* simulated annealing algorithm [18]. 5S rRNA forms a long bent elongated molecule consisting of two domains: A-D-E and B-C. In that model no interactions between the domains E and C can be observed [18].

In the crystal structure of the subunit, 5S rRNA and 23S rRNA do not interact extensively with each other. There are some RNA–RNA interactions of the backbones of helices 4 and 5 of 5S rRNA and of helix 38 of 23S rRNA [13, 14]. A cryo-electron microscopic (EM) reconstruction at 7.5 Å resolution of *Escherichia coli* 50S ribosomal subunit combined with known cross-links of 5S rRNA to 23S rRNA have suggested close contacts of the residue U89 of 5S rRNA to several sites within the helix 38 and uridines of helices 2 and 3 to sites in the region of helices 83–85 of 23S rRNA. A total of 24 cross-link sites defining tertiary contacts with or between the 23S and 5S rRNA have been suggested from EM reconstruction data [19]. These observations were furthermore supported by mutations at position A960 (domain II), which caused structural rearrangements in loop D of 5S rRNA and in the peptidyltransferase region of domain V [20]. As 5S rRNA is an integral component of ribosomes, its omission during subunit reconstruction results in a dramatic decrease of the peptidyltransferase activity. However, in the presence of some antibiotics, the *Thermus aquaticus* 50S subunit activity can be partially restored [21]. Those results indicate functional role of 5S rRNA in the specified interdomain interactions, rather than only spatial proximity [20, 21]. Recently crystals of *T. flavus* 5S rRNA were obtained under micro gravity conditions and data were collected at 7.8 Å resolution [22].

INTERACTION OF 5S rRNA WITH PROTEINS

5S rRNA is the only known RNA species that binds ribosomal proteins before it is incorporated into the ribosomes both in prokaryotes and eukaryotes (Fig. 1). In eukaryotes, the 5S rRNA molecule binds ribosomal protein L5, whereas in bacteria it interacts with the three ribosomal proteins L5, L18 and L25. The 5S rRNA assembly to 23S rRNA requires proteins L18 and L5, but not L25, which earlier binds to 5S rRNA [1–5].

Of those three proteins, L18 binds the most tightly and that binding stimulates the interaction of L5 with 5S rRNA. In the crystal structure of the 50S subunit, protein L18 joins helix 1 (domain α) and helices 2 and 3 (domain β) of 5S rRNA to helix 87 of 23S rRNA [13]. L18 from *E. coli* retains its 5S rRNA binding activity after removal of its first 17 amino acids, but the cleaved protein no longer stimulates the binding of L5. It has been shown that phosphorylation of *Bacillus stearothermophillus* L18 protein is critical for binding of 5S rRNA at physiological pH. A good candidate for the phosphorylation is Ser 57 of L18 [23].

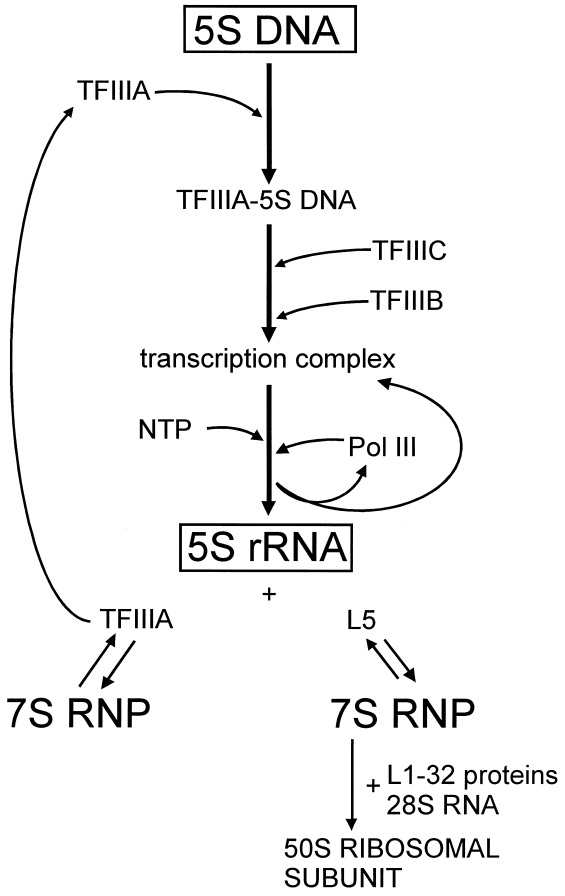

Figure 1. The biosynthesis pathway, activities and functions of 5S rRNA.

In the crystal structure of the 50S subunit of *H. marismortui,* protein L21e mediates an interaction between domains α and β of 5S rRNA and domains II and V of 23S rRNA. Protein L30 binds helices 4 and 5 to domain II. On the other hand, loop C and D of 5S rRNA are linked to domain V by protein L5, and to domains II and V by protein L10e, respectively [13, 14].

Ribosomal protein L5 is localized in both the cytoplasm and the nucleus of eukaryotic cells, accumulating mostly in the nucleoli. L5 binds specifically to 5S rRNA and is involved in nucleocytoplasmic transport of this RNA. Mapping of elements in ribosomal protein L5 that mediate nuclear protein import, defines three separate such activities, NLS 1–3, which are functional in both oocytes and somatic cells. They consist of amino acid residues: 21–37 (NLS), 101–111 (NES) and 255–265 (NLS). On the other hand, the RNA binding activity involves N-terminal as well as C-terminal parts of L5 [24, 25]. Human protein L5 is phosphorylated *in vitro* by protein casein kinase (CKII), resulting in a decrease in 5S rRNA binding activity. Phosphorylation occurs on serine residues within two fragments, 142–200 and 272–297, of L5. It seems that phosphorylation of L5 by CKII is one of the mechanisms that regulate nucleolar targeting of 5S rRNA and/or ribosome assembly in the cell [26].

The tertiary structure of L25 solved by NMR showed a high similarity to tRNA anticodon-binding domain of glutaminyl-tRNA synthetase. L25 shows a new topology for binding of RNA. Many of the residues of L25 are distributed throughout the amino-acid sequence and are located on a continuous surface of the protein structure. Heteronuclear NMR spectroscopy of the complex of L25 and a 37 nucleotide RNA molecule containing the E-loop and helix IV of *E. coli* 5S rRNA, shows that one side of the six-stranded β-barrel of L25 recognizes the minor groove of the E-loop with very little change in the conformation of either the protein or the RNA [27]. The minor groove recognition module includes two parallel β-strands of L25. Binding of the RNA also induces changes of a flexible loop to an α-helix in L25. The N-terminal tip interacts with the widened major groove at the E-loop/helix IV junction of the RNA [27].

The crystal structure of *E. coli* ribosomal protein L25 bound to an 18-base pair portion of 5S rRNA which contains loop E has been determined at 1.8 Å resolution. The structure of loop E is almost the same as that of uncomplexed RNA. The most conserved amino-acid residues among the known

L25 protein sequences interact with 5S rRNA backbone. In contrast, those side chains which interact with bases are mostly not conserved but in many cases, covary with the 5S rRNA sequence with which they interact [28]. Although the structure of L25 protein shows a striking similarity to the anticodon binding domain of *E. coli* glutaminyl-tRNA synthetase (GluRS), these two proteins interact with their RNA substrates in completely unrelated ways by using different parts of their structures. GluRS recognizes primarily unpaired bases of the anticodon loop, but L25 recognizes the RNA duplex [28]. Although its NMR structure is similar to the crystal structure, some differences are observed probably due to differences in divalent metal ion contents in the measured samples [27, 28].

5S rRNA as well 5S DNA bind transcription factor III A (TF IIIA) with high affinity (K_d about 1 nM). The central four zinc fingers (ZIF 4–7) of the nine finger TF IIIA have been shown to bind 5S rRNA with comparable affinity as the entire TF IIIA, but ZIF 4–6 protein binds 5S rRNA with an about sevenfold lower binding activity. It has been suggested that ZIF 7 contributes to affinity and specifity in that interaction. High affinity binding activity of ZIF 4–6 is encoded in 5S rRNA fragment of 55 nucleotides comprising loop A, helix V, loop E and helix IV, but not helix II which is involved in binding of ZIF 7 [29].

Eukaryotic 5S rRNAs of cytoplasmic ribosomes are usually encoded by separate genes arranged in tandem arrays of repeating units. Their number varies significantly up to several thousands in vertebrates and plants. In prokaryotes and organelles, 5S rRNAs are synthesised as part of a single long transcript, together with 16 and 23S rRNAs. The 5S ribosomal genes in higher eukaryotes are located independently from the 45S rDNA repeats containing 18S, 5.8S and 26S ribosomal RNA genes. The 5S rRNA genes in *Macaca fascicularis* are organized in tandem repeats, which are unusually large and complex. They consist each of a 7.3 kb DNA fragment containing two 5S rRNA genes, linked to a 4.3 kb fragment with one gene. The total number of genes in the repeats is about 50–100 per haploid genome of *M. fascicularis* compared with 100–150 5S rRNA genes in the repeats in human genome [30].

Transcription of the eukaryotic 5S rRNA genes by polymerase III is strongly inhibited by p53 [31]. It has also been shown that loss of the Cockayne syndrome group B repair protein (CSB) or over expression of p53 causes fragility of the genes transcribed by polymerase II (U1 and U2 snRNA) and polymerase III (5S rRNA). In the absence of functional CSB, RNA polymerase would stall on the U1, U2 and 5S RNA genes, locally blocking metaphase chromatin condensation and thereby causing metaphase fragility. It seems that CSB protein functions as an elongation factor. Transcription of highly structured RNAs might be unusually dependent on that elongator factor to restart or abort the RNA polymerases stalled by the secondary structure in the nascent transcript [32].

The presence of TF IIIA alters formation of the UV-induced photoproducts (cyclobutane pyrimidine dimers, CPDs), primarily in the transcribed strand of the 50 bp internal control region (ICR) of DNA. During nucleotide excision repair *in vitro*, CPD removal is reduced at most sites in both strands of the ICR when TF IIIA is bound. Efficient repair occurs just outside of the TF IIIA-binding site [33]. Surprisingly, ICR containing three CPD sites (56, 75 on the transcribed strand and 73 on non-transcribed strand) are repaired rapidly when TF IIIA is bound. This suggests that CPDs partially displace TF IIIA [33]. Mutual effects of CPDs formation and nucleosome folding in chromatin have been analyzed on the *Xenopus borealis* somatic 5S rRNA gene [34]. Nucleosome folding modulates CPD formation in the dyad axis region of the nontranscribed strand of 5S rDNA where DNA helix is tightly bound to the H3-H4 tetramer [34] and strongly inhibits nucleotide excision repair (NER) at many CPD sites. However, inhibition of NER by mononucleosomes is not significantly influenced by the rotational orientation of CPDs on the histone surface [35].

Reconstruction of a DNA fragment containing *X. borealis* 5S rRNA gene into a nucleosome restricts the binding of TF IIIA to the ICR of the gene. Removal of all core histone allows TF IIIA

to bind to DNA. It has been shown that the tail domains of H3 and H4 modulate the ability of the TF IIIA to bind to DNA and they are the primary arbiters of the transcription factor access to intranucleosomal DNA [36].

SECONDARY STRUCTURE OF 5S rRNA

The nucleotide sequence of 5S rRNA is highly conserved throughout nature and phylogenetic analysis alone provided an initial model for its secondary structure. The secondary structure of all analysed 5S rRNAs consists of five helices (I–V), two hairpin loops (C and E), two internal loops (B and D) and a hinge region (A), organised into three helix junction. The general secondary structure models of eukaryotic and prokaryotic 5S rRNAs are shown on Fig. 2. Most of the 5S rRNA sequences can be folded according to one of these models, although Eubacteria and Archaea show much higher variability than the Eukaryota. The ability of the sequence to adopt a correct consensus secondary structure can be used to discriminate between genes and pseudogenes, that are often found in eukaryotic genomes. Improved programming algorithms for 5S rRNA secondary structure prediction were recently published [37–39]. The calculated structures for *E. coli* and *X. laevis* 5S rRNAs are different from the model shown in Fig. 2. The secondary structure elements of these 5S rRNAs, which include five helical regions, three internal loops, and two hairpin loops, form an unknown three dimensional structure, can be studied in solution by chemical modification, site directed mutagenesis, physical methods and computer modelling. Many three dimensional structure models of 5S rRNA have been proposed (see for review [16]) but they all differ from that found within the 50S ribosomal subunit (Fig. 3) [13].

5S rRNA DATABASE

To get a consistent picture of the structure–function relationships of 5S rRNA, detailed knowledge concerning the primary structure of

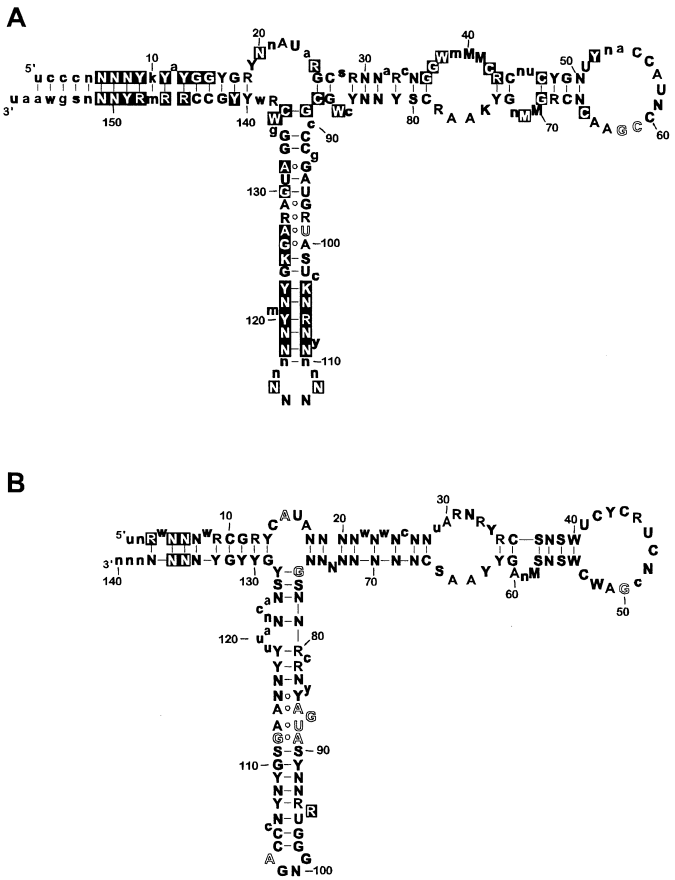

Figure 2. The general secondary structure model of eubacterial (A) and eukaryotic (B) 5S rRNAs.

The totally conserved positions (nucleotides) are identified and marked. Lower case letters show the insertions, and letters within the boxes deletions.

this RNA species from different sources is required.

The 5S rRNA database contains the 5S rRNA coding sequence as well as the information on the length of the original clone and location of the structural gene. The data base contains 2193 nucleotide sequences of 5S rRNAs and 5S rDNAs published till the end of 2000. In Table 1 we show distribution of the sequence entries for the main taxonomic groups. Files with the primary structure data and the nucleotide sequence alignments are available *via* the WWW at http://rose.man.poznan.pl/5SData/ or http://biobases.ibch.poznan.pl/5SData/. Any nucleotide sequence can be retrieved using the taxonomy browser or alphabetical list of organisms.

Figure 3. The tertiary structure of 5S rRNA found within the crystals of 50S ribosomal subunit of *H. marismortui*.

Table 1. The nucleotide sequence entries in the 5S rRNA database for major taxonomic groups

Taxonomic group	Number of entries
Eubacteria	491
Archaea	60
Organelles	75
Mitochondria	10
Chloroplasts	65
Eukaryota	1567
Protista	76
Fungi	234
Animals	396
Plants	861
Total:	2193

CONCLUSIONS

Ribosomes were visualized in electron micrographs as early as in 1943 and 5S rRNA was discovered 20 years later. The next four decades witnessed big advances in our understanding of the ribosome with the use of biochemical, genetic and low resolution structural approaches. At that time many experimental data accumulated also on 5S rRNA, but its precise function remains unknown. Now, 60 years later we can see almost all components of the large ribosomal subunit at 2.4 Å resolution. This big discovery will stimulate further studies aimed at understanding that structure in terms of function. Thus 5S rRNA become again an attractive model system for exploring fundamental issues of RNA conformation and RNA protein interaction, due to its relatively small size, ease of preparation and rich array of non-canonical base pairs [1–5].

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