Structure and function of intersubunit bridges in *procaryotic* ribosome

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Summary

The main function of ribosome is decoding of the genetic message and formation of peptide bonds. Protein synthesis is a dynamic process during which tRNA and mRNA are translocated through the ribosome. Ribosomal subunits, small and large, are joined together by a series of bridges, which make possible forming of an active ribosome. It this paper we present the functional importance of ribosomal bridges.

Key words:
ribosomal bridge, ribosome, ribosomal subunit, protein biosynthesis.

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1. Introduction

The ribosome is a ribonucleoprotein complex, composed of two subunits, called in *Procaryota* 30S – small subunit and 50S – large subunit (1) (Fig. 1). Both subunits are composed of ribosomal RNAs (rRNA) and proteins (2). The small subunit contains a single rRNA 16S (about 1500 nucleotides) and about twenty proteins; large subunit contains 23S and 5S rRNA (about 2900 nucleotides) and about 30 proteins (3). The function of the ribosome is decoding of the genetic message, which takes place in a small

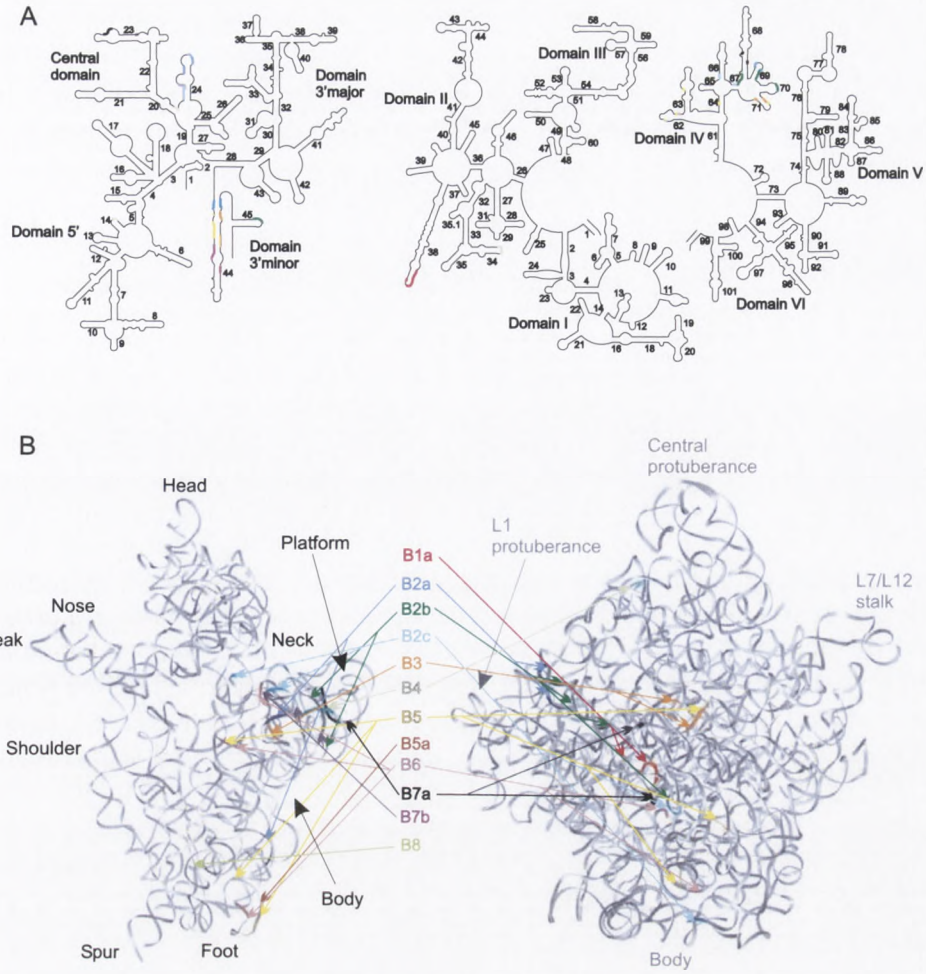


Fig. 1. Secondary (A) and tertiary (B) structure of 16S (on the left from *H. marismortui* – tertiary structure, *E. coli* – secondary structure) and 23S (on the right from *H. marismortui* – tertiary structure, *E. coli* – secondary structure) rRNA. The sequences that take part in forming of the ribosomal bridges have been shown in colors. The morphology of ribosomal subunits has been shown on the figure (4,7,9,12-14,25-27).

subunit and next catalyzing the formation of peptide bonds, which occurs in a large subunit (4). Protein synthesis is a dynamic process during which tRNAs and mRNA are translocated through the ribosome, this process is catalyzed by elongation factor G (EF-G) (5). The are three main sites, which can be occupied by tRNA molecules in the ribosome: A-, P- and E-site. The A-site, where aminoacyl-tRNA (aa-tRNA) binds is a place of recognition of the cognate aa-tRNA carrying amino acid encoded in the mRNA sequence. P-site is occupied with peptydyl-tRNA (6). The E-site is the exit

site, where deacylated-tRNA is released from the ribosome. It is known for 50 years that ribosomal subunits from all species can reversibly associate and dissociate. The key feature of translation systems is the existence of two ribosomal subunits that are joined together in the initiation phase of protein synthesis to form an active ribosome (7).

2. Ribosomal bridges – structure and function

Intersubunit contacts were first visualized as discrete bridges in low resolution cryo-electro microscopy studies (9). Analyses demonstrated the existence of the same contacts in yeast cytoplasmic ribosomes and the subset of these connections in mammalian mitochondrial ribosomes, what suggests that intersubunit bridges are conserved elements in ribosome structure (7). Ribosomal subunits, small and large, are joined by a series of bridges that are conserved among mitochondrial, bacterial and *eucaryal* ribosomes. Bridges play many roles, they are: (i) joining the subunits together in the initiation phase of protein biosynthesis, (ii) participating in the ribosome interactions with translational factors, (iii) transmitting the signals between the functional centers of the ribosome, (iv) mediating the relative movement of subunits during translation, and (v) modulating tRNA-ribosome interactions (7). The structure of 70S ribosomes (*prokaryotic*) has revealed 13 bridges; numbered: B1a, B1b, B2a, B2b, B2c, B3, B4, B5, B5a, B6, B7a, B7b, B8 (1,8). The majority of ribosomal bridges involve RNA-RNA interactions, but there are also interactions of RNA-protein type. Only one from 13 bridges, namely B1b, involves protein–protein interactions (7) (Fig. 1 and Table).

Table

Intersubunit bridges in the 70S ribosome

B	TYPE	30S		50S	
		16S rRNA or protein	rRNA or protein position	23S rRNA or protein	rRNA or protein position
1	2	3	4	5	6
B1a	P-R	S13 S19	92-94, 102-115 83-84	H38 H38	881-883, 886-888 884-885, 891-892
B1b	P-P	S13/S19		L5	134-153
B2a	R-R	H44	1406, 1408-1410, 1492-1496, 1517	H69	1912-1914, 1918-1919
B2b	R-R R-R	H24 H45	783-786, 791-794 1514-1516, 1516-1519	H67/H69 H71	1836-1838, 1912, 1918-1920, 1922-1923, 1928-1929 1932-1933
B2c	R-R	H24 H27	770-774 900-901	H66, H67 H67	1793-1794, 1830-1833 1832-1833

1	2	3	4	5	6
B3	R-R	H44	1483-1486	H71	1947-1949, 1960-1962
B4	R-R	H20	762-764	H34	715-718
	P-R	S15	40-44, 59-63, 86-87, C-end	H34	713- 717
B5	R-R	H44	1418-1419	H64	1718-1719, 1768-1769
	R-R	H44	1474-1476	H64	1689-1690
	R-R	H44	1420-1421	H71	1950-1951
	R-P	H44	1420-1423	L14	44-50
	R-R	H44	1473-1476	H62	1689-1690, 1702-1703, 1989
	R-R	H44	1413	H27	
B5a		H44	1418	–	–
B6	R-R	H44	1429-1431, 1474-1476	H62	1689-1690, 1704-1705
	R-P	H44	1431-1433	L19	1702-1705
	R-P	H44	1463-1465	L19	107-113
B7a	R-R	H23	698-699, 701-703	H68	1847-1849, 1896-1897
B7b	R-P	H23	712-713	L2	162-164, 172-174, 177-178
	R-P	H24	773-776	L2	177-178, 198-202
B8	R-P	H14	345-347	L14	116-119

Numbers refer to nucleotides position in *Escherichia coli* 16S and 23S rRNA, B – bridge, H – helix, S – small subunit protein, L – large subunit protein, P – P–protein–protein contact, P – R–protein–RNA contact, R – R–RNA–RNA contact (4,7,9,12-14).

Bridge B1a was observed clearly in the ribosome structure, especially in post-translocational complexes resolved by cryo-electron microscopy (cryo-EM) (9-11). This bridge connects the top of 50S with the head of the 30S and crosses the interface between the subunits above and parallel to the A- and P-site. This contact was observed between ribosomal protein S13 in small subunit (amino acids 83-84) and helix H38 in 50S (nucleotides in positions 884-885 and 891-892). Sequence of S13 in positions 92-94 and 102-115 makes also contacts with H38 in positions: 881-883 and 886-888 (7,9,12,14) (Fig. 2).

B1b bridge, as mentioned above, is the only bridge composed of protein-protein interactions. N-terminal end of protein S13 in small subunit contacts the 20-amino acid loop formed by residues 134-153 of protein L5 in large subunit (8). It was previously noticed that in post- and pretranslocational states protein L5 is located across the N-terminal end of S13 protein. S13 plays a crucial role in association of subunits and the fidelity of translocation causes its interactions with protein L5 in the central protuberance and with P-site tRNA (10,14). In pretranslocational state contact between proteins S13, S19, and L5 is weak, because S13 and the head of small subunit are rotated towards the E-site. In a hybrid state of the ribosome, one of the helices is extending from globular domain of S13 and running along the head of the small subunit to form a 'rail' that lies in a shallow groove of L5 (14).

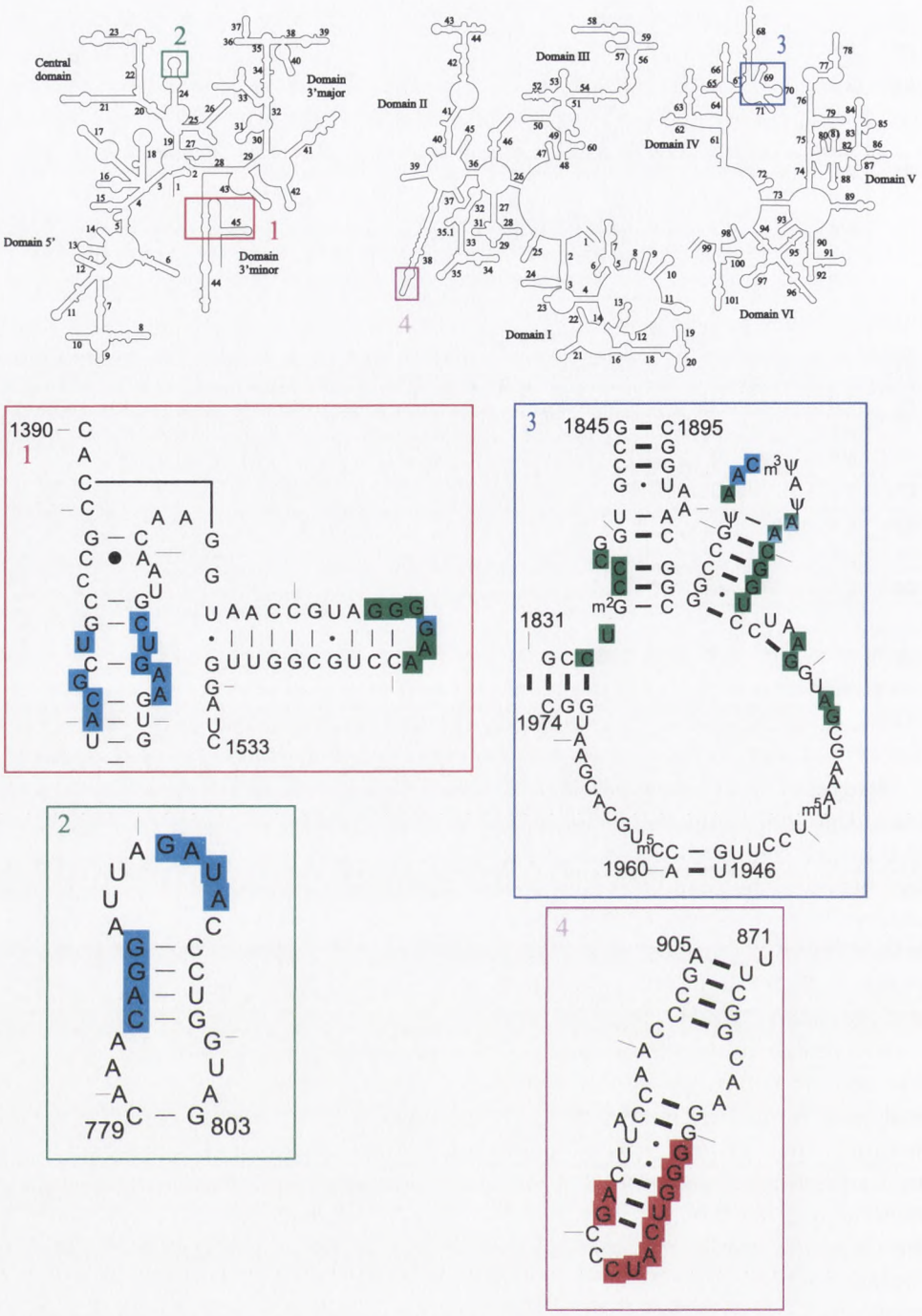


Fig. 2. Secondary structure of 16S (on the left from *E. coli*) and 23S (on the right from *E. coli*) rRNA. The sequences that take part in forming of the ribosomal bridges have been shown in colors: B1a – red, B2a – blue, B2b – green (4,7,9,12-14,27).

Bridge B2a is formed by 50S subunit and the penultimate stem of helix 44 of 16S rRNA, which is the dominant component of the interface of the small subunit. Moreover, B2a is adjacent to the mRNA decoding site between H44 of 16S rRNA and helix 69 of 23S rRNA and extends under the P-site towards H24 and H45 in small subunit. During the decoding process, the closing loop of H69 lies below the D-stem of A-site tRNA and the adjacent stem of H69 lies below the D-stem of P-site tRNA (9,15,16). This bridge involves interactions of 1914 loop of H69 of 23S rRNA (nucleotides in positions 1912-1914 and 1918-1919) with the decoding site of 16S rRNA (positions 1406, 1408-1410, 1492, 1496) and also with nucleotide in position 1517 (9). This bridge plays a key role in the subunit association and in the ribosome recycling process (13,17-19). At this step of protein biosynthesis, the ribosomal recycling factor (RRF) interacts with the ribosome and causes the tip of H69 to be released from interactions with the 30S in subunit dissociation process (13,18,19) (Fig. 2).

Interactions between small subunit helices H24 and H25, 45 and large subunit helices H67, H69 and H71 are building the bridge B2b (8,10,13,17). This contact lies in the central region of intersubunit space and has a role in forming an active ribosome (4). Helix H24 of 16S rRNA (nucleotides in positions 783-786, 791, 792, 794) interacts with H67 (positions 1836-1838) and H69 (position 1922). H45 of small subunit (nucleotides in positions 1514-1519 and 793) forms this contact by interacting with H67 (positions 1833, 1834), H69 (positions 1919, 1920, 1922, 1923, 1928, 1929) and H71 (positions 1932, 1933) (7,9,13) (Fig. 2).

The establishment of intersubunit bridge B2c is totally dependent on magnesium ions. This contact was observed between helices H24 and H27 of 16S rRNA, (sequences 770-774 and 900-901, respectively), and helices H66 and H67, (sequences 1793-1794 and 1830-1833, respectively) (20). The studies of these interactions demonstrated their importance for the stability and function of 70S ribosome (8) (Fig. 3).

Bridge B3, likewise B2a, involves interactions between the 50S and H44 of the 16S rRNA. Helix 71 in positions 1947-1449, 1960-1962 contacts the penultimate stem of H44 in positions 1483-1486 (7,9,13). B3 includes the largest RNA-RNA minor groove surface among the interface contacts, which causes small rearrangements in this bridge during translocation. Surfaces, composed of nucleotides, are complementary, what may help in stabilization of interactions during association (14,21,22) (Fig. 3). B4 has a crucial role in subunit association (13,14). This contact is likely to remain intact even during the translocation. The bridge involves stem-loop of helix 34 of 23S rRNA, which extends by 30Å from 50S subunit towards protein S15 in small subunit (14,23). Nucleotides in positions 713-717 in H34 interact with amino acids in positions 40-44, 59-63, 86-87 and C-end of S15 protein. Sequence in positions 715-718 of H43 interacts with H20 of 16S rRNA in positions 762-764 (7,9,14,17). In the ribosome structure, H34 is located close to the putative pivot point of the ratchet-like motion between subunits, what causes the need for its flexibility which allows to maintain intersubunit interactions (14,17) (Fig. 3).

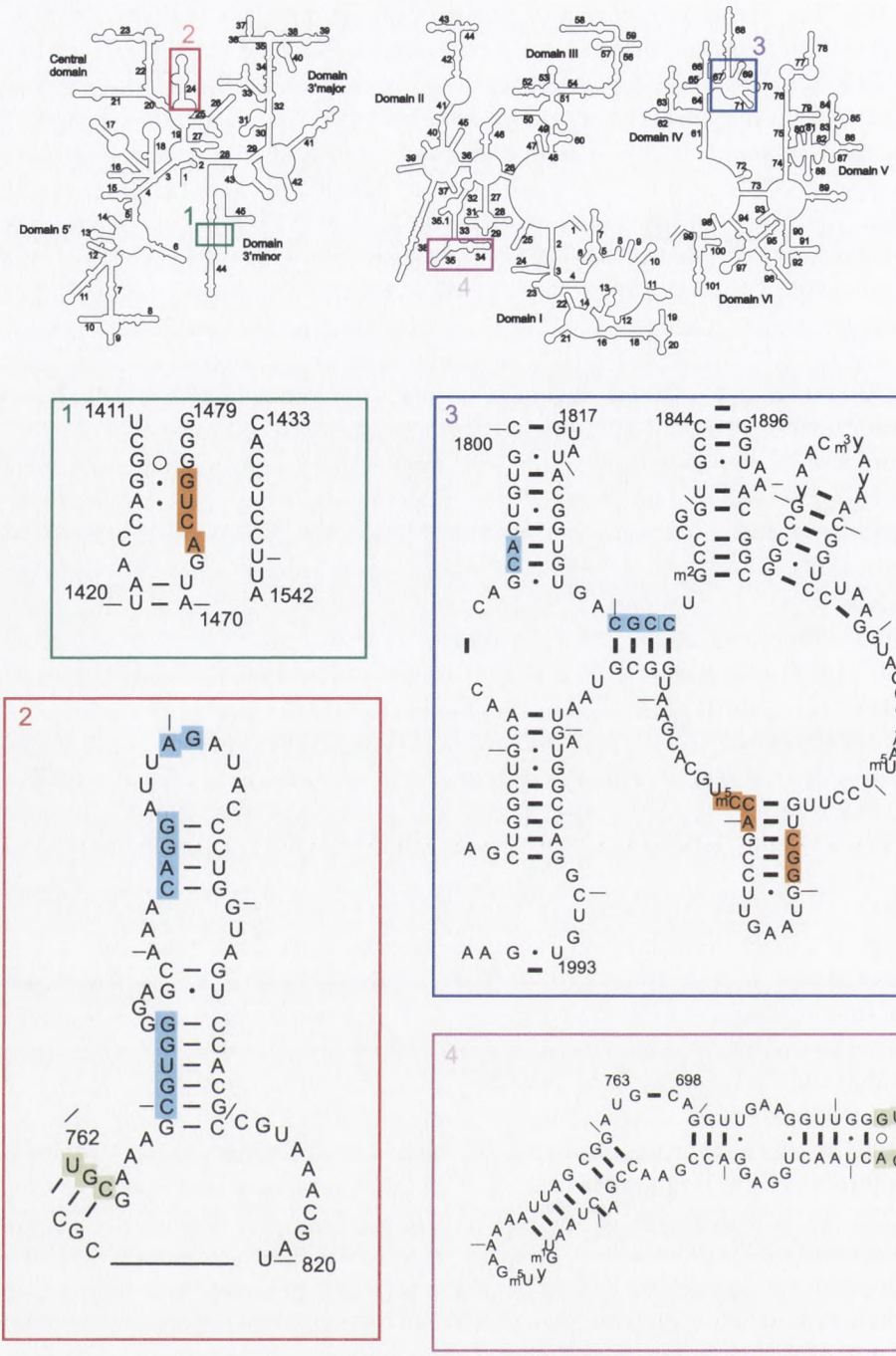


Fig. 3. Secondary structure of 16S (on the left) and 23S (on the right) rRNA. The sequences that take part in forming of the ribosomal bridges have been shown in colors: B2c – blue, B3 – orange, B4 – spring green (4,7,9,12-14,27).

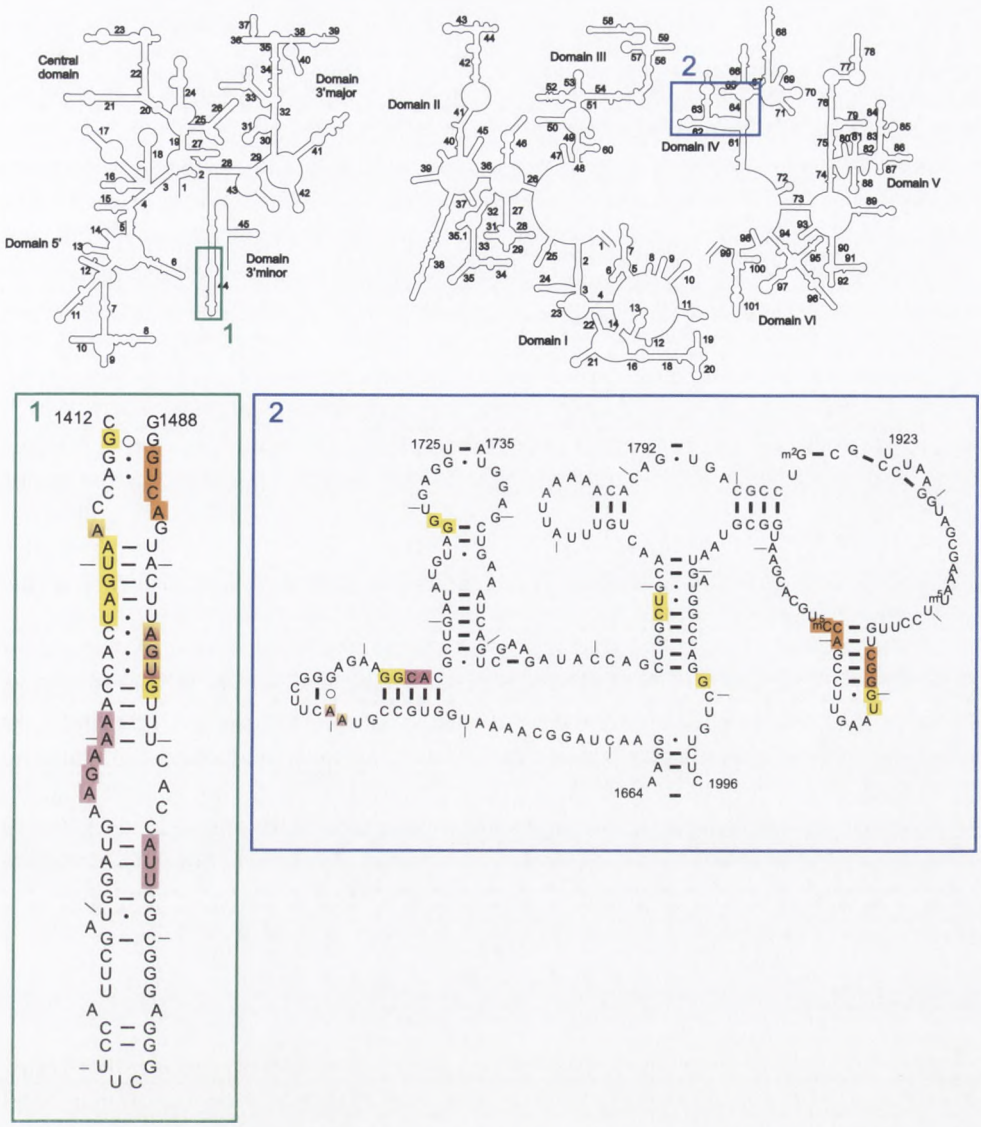


Fig. 4. Secondary structure of 16S (on the left) and 23S (on the right) rRNA. The sequences that take part in forming of the ribosomal bridges have been shown in colors: B5 – yellow, B5a – brown, B6 – dark pink (4,7,9,12-14,27).

Bridge B5 also plays a significant role in the association of subunits. Nucleotides forming H44 of the small subunit (positions 1418, 1419, 1474-1476) interact with minor groove of H64 (nucleotides in positions 1718, 1719, 1768, 1769, 1689-1890) in the large subunit (4). B5 is also built by contacts between: H44 (nucleotides 1420-1421) of 16S rRNA with H71 of 23S rRNA (positions 1950, 1951), H44 (positions 1473-1476) with H62 (nucleotides 1689, 1690, 1702, 1703) and H44 (nucleoti-

de in position 1413) with H27 (nucleotide in position 1989). Moreover, this bridge consists of one contact type, which is RNA-protein; H44 (nucleotides 1420-1423) of 16S rRNA interacts with protein L14 (amino acids 44-50) (8,9,13,20) (Fig. 4). Nucleotide 1418 of H44 is involved in building B5a bridge (7) (Fig. 4).

Bridge B6 is located in the close neighbourhood to B3 and involves interactions between H44 of 16S rRNA and H62 of 23S rRNA (14). The minor groove of H44 (sequences 1429-1431 and 1474-1476) and helix H62 (sequences 1689-1690 and 1704-1705) approach each other and leave 6Å gap, where a monolayer of water molecules could fit (20). There has been shown another contact in *Thermus* ribosome structure which states the part of B6; between H44 (sequences 1431-1433 and 1463-1465) and protein L9 in 50S subunit (amino acids 107-113) (7,9,13,14) (Fig. 4).

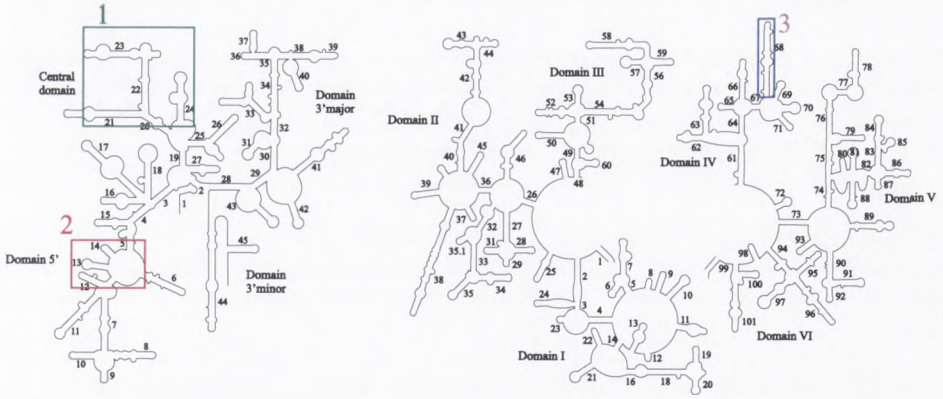
Helices H68 with H71 of 23S rRNA create long noncanonical coaxial arm, which lies horizontally along the top of the interface of large subunit, and forms bridges B2b and B7a. H23 of 16S rRNA (sequences 698-699 and 701-703) contacts the minor groove of H68 of 23S rRNA (sequences 1847-1849 and 1896-1897), and builds the bridge B7a (7,9,13,14). In the ratchet – like motion, the interface in this region shifts laterally whit respect to H68, what may suggest that during translocation this contact must break (14) (Fig. 5).

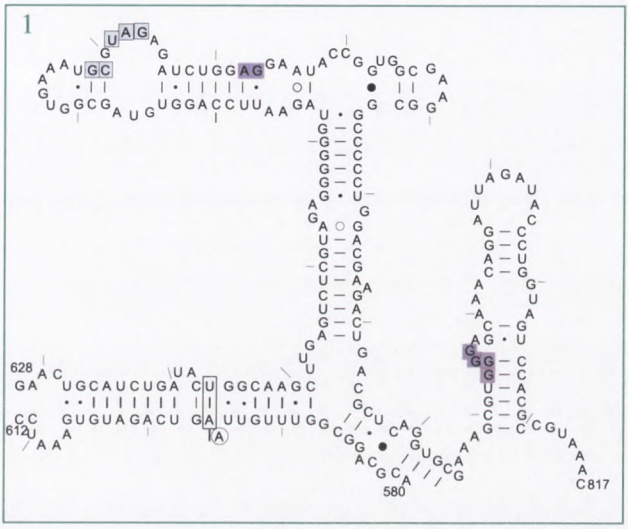
The bridge B7b is built by protein L2 that makes two contacts with helices 23 and H24 of 16S rRNA. This binding is located very close to protein S6 and therefore it could make a transient contact with this protein during translation. Protein L2, as a part of B7b, possibly plays a role in peptidyl transfer activity. Bridge B7b could be a relay between interactions tRNA in 30S and catalytic centre in 50S (9) (Fig. 5).

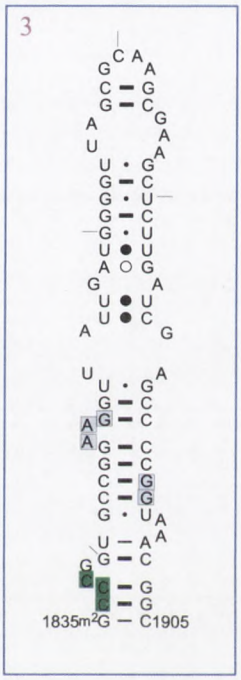
Helix 14, in positions 345-347, of 16S rRNA interacts with amino acids 116-119 of protein L14 from large subunit and builds bridge B8. Interactions with magnesium ions are crucial for stability of this contact (20) (Fig. 5).

3. Conclusions

Ribosomes are universally conserved complexes of rRNA and protein that translate genetic information from mRNA into proteins (24). Based on biochemical and structural evidences, the presence of the ribosomal bridges is crucial for the correct functioning of the active ribosome (25). Bridges hold the subunits together in a configuration appropriate for protein synthesis and may also transmit signals between the two subunits (9,13). Moreover, they can take part in relative displacement of 30S and 50S subunits during translocation, initiation factor IF3-ribosome and ribosome recycling factor – ribosome interactions. Presently scientists try to examine the contribution of each intersubunit bridge to its ribosomal function. Hopefully, advances in macromolecular crystallography and biochemical techniques (e.g. usage of rRNA mutants (7)) made during last decades will in short future help in elucidating all possible roles of intersubunit bridges.







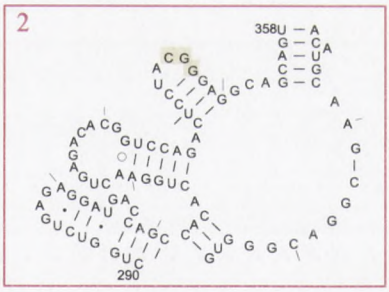


Fig. 5. Secondary structure of 16S (on the left) and 23S (on the right) rRNA. The sequences that take part in forming of the ribosomal bridges have been shown in colors: B7a – gray, B7b – purple, B8 – light green (4,7,9,12-14,27).

Acknowledgments

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