
REVIEW

Investigation of Structure of the Ribosomal L12/P Stalk

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Received July 6, 2016

Abstract—This review contains recent data on the structure of the functionally important ribosomal domain, L12/P stalk, of the large ribosomal subunit. It is the most mobile site of the ribosome; it has been found in ribosomes of all living cells, and it is involved in the interaction between ribosomes and translation factors. The difference between the structures of the ribosomal proteins forming this protuberance (despite their general resemblance) determines the specificity of interaction between eukaryotic and prokaryotic ribosomes and the respective protein factors of translation. In this review, works on the structures of ribosomal proteins forming the L12/P-stalk in bacteria, archaea, and eukaryotes and data on structural aspects of interactions between these proteins and rRNA are described in detail.

DOI: 10.1134/S0006297916130022

Keywords: ribosome, stalk, ribosomal proteins

The cell organelles responsible for protein biosynthesis were discovered in the 1950s. These organelles were named ribosomes. The ribosome is a macromolecular ribonucleoprotein complex. Ribosomal RNA (rRNA) determines the main structural and functional properties of the ribosome, but the presence of both rRNA and ribosomal proteins is necessary for the normal functioning of the ribosome.

Ribosomal structure was originally studied by the methods of ultracentrifugation and electron microscopy. These studies showed that under certain conditions (e.g. the low concentration of magnesium ions) the ribosome dissociates into a small subunit and a large subunit. The large ribosomal subunit has three peripheral stalks: the lateral finger-shaped stalk (the L12 stalk in bacteria and the P stalk in archaea and eukaryotes) on one side of the subunit; the central protuberance that can be called the head of the large subunit in the middle; and the lateral L1 stalk on the other side [1, 2].

During protein biosynthesis, the ribosome interacts with messenger RNA (mRNA), transport RNA (tRNA), translation initiation, elongation, and termination factors, and other ligands [3]. The working cycle of the ribosome consists of three steps: initiation, elongation, and termination. Translation factors promote protein synthesis at each step of ribosomal working cycle. The lateral

L12/P stalk of the ribosome promotes interaction between the ribosome and the translation elongation and termination factors, while the bacterial L12 stalk is involved also in translation initiation, increasing the rate of association between the small and large ribosomal subunits [4]. Cryoelectron microscopy and the subsequent reconstruction of ribosomal structure have shown that the L12 stalk undergoes different conformational rearrangements during the ribosomal elongation cycle.

NOMENCLATURE OF RIBOSOMAL PROTEINS

The small and large ribosomal subunits contain many individual proteins. Nearly all are represented by a single copy on the ribosome. The first attempt to systematize ribosomal proteins was based on a standard experimental method: two-dimensional gel electrophoresis. It was the most convenient method for complete separation of ribosomal proteins by molecular size and charge (Fig. 1) [5].

Initially, ribosomal proteins from each type of organisms had their own designations in accordance with their electrophoretic separation. With that nomenclature, the same numbers could be given to nonhomologous proteins of different species. The comparison of amino acid sequences of ribosomal proteins revealed homology between the proteins of different species and demonstrated the evolutionary conservatism of most ribosomal proteins [6]. Hence, it was possible to create a new nomen-

Abbreviations: IF2, translation initiation factor 2; mRNA, messenger RNA; rRNA, ribosomal RNA; tRNA, transport RNA.

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Original and revised nomenclatures of ribosomal proteins (the table was taken with modifications from [7, 8])

Ribosomal proteins of the small subunit					Ribosomal proteins of the large subunit				
New name [#]	Domains of life*	Old name			New name [#]	Domains of life*	Old name		
		bacteria	archaea	eukaryotes			bacteria	archaea	eukaryotes
bS1	B	S1	–	–	uL1	BAE	L1	L1	L1/L10A [^]
eS1	AE	–	S3ae	S1/S3A [^]	uL2	BAE	L2	L2	L2/L8 [^]
uS2	BAE	S2	S2	S0/SA [^]	uL3	BAE	L3	L3	L3
uS3	BAE	S3	S3	S3	uL4	BAE	L4	L4	L4
uS4	BAE	S4	S4	S9	uL5	BAE	L5	L5	L11
eS4	AE	–	S4e	S4	uL6	BAE	L6	L6	L9
uS5	BAE	S5	S5	S2	eL6	E	–	–	L6
bS6	B	S6	–	–	eL8	AE	–	L8e	L8/L7A [^]
eS6	AE	–	S6e	S6	bL9	B	L9	–	–
uS7	BAE	S7	S7	S5	uL10	BAE	L10	P0	P0
eS7	E	–	–	S7	uL11	BAE	L11	L11	L12e
uS8	BAE	S8	S8	S22/S15A [^]	bL12	B	L12	–	–
aS8	A	–	L7ae	–	uL13	BAE	L13	L13	L16/L13A [^]
eS8	AE	–	S8e	S8	eL13	AE	–	L13e	L13
uS9	BAE	S9	S9	S16	uL14	BAE	L14	L14	L23
uS10	BAE	S10	S10	S20	eL14	AE	–	L14e	L14
eS10	E	–	–	S10	uL15	BAE	L15	L15	L28/L27A [^]
uS11	BAE	S11	S11	S14	eL15	AE	–	L15e	L15
uS12	BAE	S12	S12	S23	uL16	BAE	L16	L16	L10
eS12	AE	–	S12e	S12	bL17	B	L17	–	–
uS13	BAE	S13	S13	S18	uL18	BAE	L18	L18	L5
uS14	BAE	S14	S14	S29	eL18	AE	–	L18e	L18
uS15	BAE	S15	S15	S13	bL19	B	L19	–	–
bS16	B	S16	–	–	eL19	AE	–	L19e	L19
uS17	BAE	S17	S17	S11	bL20	B	L20	–	–
eS17	AE	–	S17e	S17	eL20	AE	–	LX	L20/L18A [^]
bS18	B	S18	–	–	bL21	B	L21	–	–
uS19	BAE	S19	S19	S15	eL21	AE	–	L21e	L21
eS19	AE	–	S19e	S19	uL22	BAE	L22	L22	L17
bS20	B	S20	–	–	eL22	E	–	–	L22
bS21	B	S21	–	–	uL23	BAE	L23	L23	L25/L23A [^]
eS21	E	–	–	S21	uL24	BAE	L24	L24	L26
eS24	AE	–	S24e	S24	eL24	AE	–	L24e	L24
eS25	AE	–	S25e	S25	bL25	B	L25	–	–
eS26	E	–	–	S26	bL27	B	L27	–	–
eS27	AE	–	S27e	S27	eL27	E	–	–	L27
eS28	AE	–	S28e	S28	bL28	B	L28	–	–
eS30	AE	–	S30e	S30	uL29	BAE	L29	L29	L35
eS31	AE	–	S27ae	S31/S27A [^]	eL29	E	–	–	L29
					uL30	BAE	L30	L30	L7
					eL30	AE	–	L30e	L30

Table (Contd.)

Ribosomal proteins of the large subunit				
New name [#]	Domains of life [*]	Old name		
		bacteria	archaea	eukaryotes
bL31	B	L31	–	–
eL31	AE	–	L31e	L31
bL32	B	L32	–	–
eL32	AE	–	L32e	L32
bL33	B	L33	–	–
eL33	AE	–	L33e	L33/L35 [^]
bL34	B	L34	–	–
eL34	AE	–	L34e	L34
bL35	B	L35	–	–
bL36	B	L36	–	–
eL36	E	–	–	L36
eL37	AE	–	L37e	L37
eL38	AE	–	L38e	L38
eL39	AE	–	L39e	L39
eL40	AE	–	L40e	L40
eL41	AE	–	L41e	L41
eL42	AE	–	L44e	L42/L36A [^]
eL43	AE	–	L43e	L43/L27A [^]
P1/P2	AE	–	P1	P1/P2

Note: ([#]) b, bacterial; e, eukaryotic; a, archaeal; u, universal. (^{*}) B, Bacteria; A, Archaea; E, Eukaryotes. Symbol ([^]) marks yeast/human eukaryotic ribosomal proteins.

clature for conservative homologous proteins of bacteria, archaea, and eukaryotes.

With the advent of the models of bacterial, archaeal, and eukaryotic ribosomes obtained by crystallography or cryoelectron microscopy, the absence of a universal nomenclature for ribosomal proteins began to impede the comparative analysis of structure. To solve this problem, a single nomenclature for ribosomal proteins of all domains of life was created in 2014 (table) [7]. The new nomenclature is based on the designation of ribosomal proteins of *E. coli* because the first ribosomal proteins were isolated from this organism, their amino acid sequences were identified prior to the sequences of other ribosomal proteins, and these proteins have been described most thoroughly in the literature. The prefix “u” (from “universal”) and the numbers of *E. coli* proteins were assigned to the proteins found in the ribosomes of all domains of life. The prefix “b” (from “bacterial”) was assigned to bacterial proteins that were shown to have no homologs in archaea and eukaryotes. The prefix “a” (from “archaeal”) was assigned to archaeal ribosomal proteins having no homologs in the bacterial and eukaryotic ribosomes. The prefix “e” (from “eukaryotic”) was assigned not only to the eukaryotic ribosomal proteins having no bacterial and archaeal homologs, but also to the homologous archaeal proteins.

In the proposed nomenclature, the ribosomal L12/P stalk proteins were given new designations. The bacterial protein L10 and its archaeal and eukaryotic homologs P0 were designated as uL10; the bacterial protein L12 was designated as bL12. Archaea and eukaryotes were shown to have no homologs of the bacterial protein L12 but con-

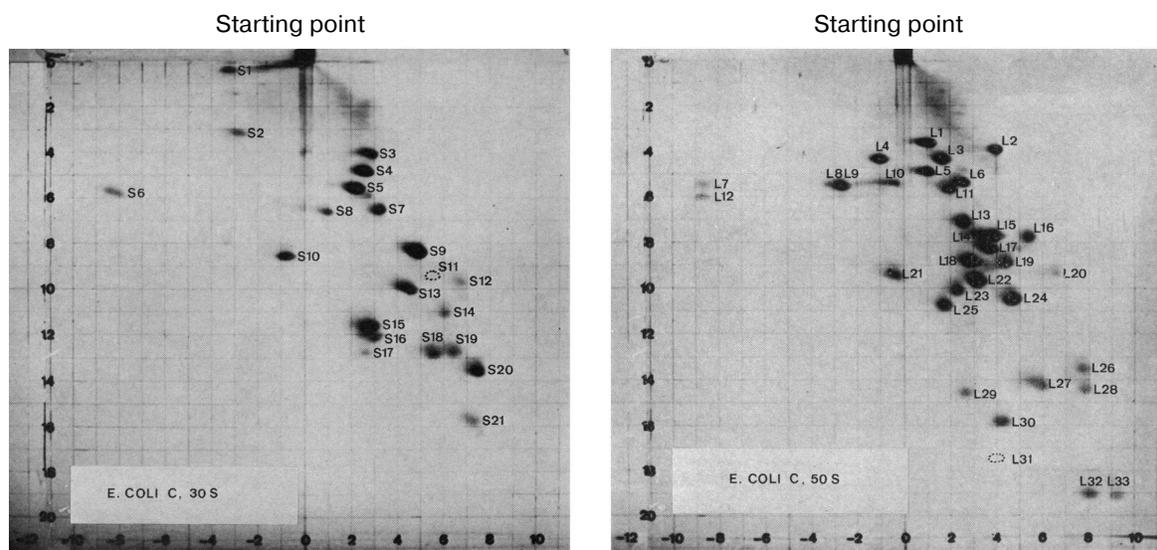


Fig. 1. Separation of the ribosomal proteins of the 30S (a) and the 50S (b) ribosomal subunits from *Escherichia coli* by 2D polyacrylamide gel electrophoresis under denaturing conditions, and the nomenclature of these proteins. First direction: horizontal; second direction: vertical (top-down). The proteins of the small ribosomal subunit are marked with S (English “small”) and the proteins of the large subunit are marked with L (English “large”). The figures with minor changes were taken from a work by Kaltschmidt and Wittmann [5].

tained its functional analogs named eP1/P2 in eukaryotes and aP1 in archaea. Accordingly, this lateral stalk in archaea and eukaryotes is now designated as a P stalk. The bacterial and archaeal proteins L11 and their eukaryotic analog L12e were designated as uL11.

The more detailed nomenclature will be used hereafter in this review. For example, the prefixes “b”, “a”, and “e” denoting bacterial, archaeal, and eukaryotic proteins, respectively, will be used for differentiating between the “universal” proteins uL10 and uL11 in addition to the prefix “u”.

COMPONENTS OF RIBOSOMAL L12/P STALK

Bacterial proteins bL10, bL11, and bL12. The ribosomal proteins bL10, bL11, and bL12, together with a fragment of domain II of 23S rRNA, form a characteristic morphological stalk of the bacterial ribosome called the L12 stalk. Two-step treatment of the large ribosomal subunit of *E. coli* with 1 M NH₄Cl and 50% ethanol at 0 and 37°C allows complete removal of these proteins from the ribosome [8].

Ribosomal protein bL12. The ribosomal protein bL12 was one of the first proteins isolated from ribosomes. It is the only protein of the large ribosomal subunit that is present in several copies [9]. The ribosomes of *E. coli* contain also an acetylated variant of this protein designated as ribosomal protein L7 according to the original nomenclature [5]. bL7 is an exact copy of ribosomal protein bL12; the only difference is that bL7 is acetylated at the N-terminal serine residue [10]. Because of their similarity, these proteins have been previously mentioned in the literature as protein bL7/L12. The bL7/bL12 protein ratio in a cell is not constant and depends on a cell growth phase. In the early logarithmic growth phase of *E. coli* cells, the level of protein bL7 in the ribosomes is minimal. An increasing amount is observed during log-to-stationary transition of the growth phase [11, 12].

During translation initiation, protein bL12 is needed for recognition of translation initiation factor 2 (IF2) in a complex with GTP as a component of the 30S preinitiation complex. This interaction results in a higher rate of association between the small and large ribosomal subunits [4].

The translation and error rates during protein biosynthesis on ribosomes depend on the presence of protein bL12 [13, 14]. The removal of this ribosomal protein from the ribosome impedes the binding of elongation factors EF1A and EF2 with the ribosome [15] and affects other factor-dependent functions, e.g. the binding of aminoacyl-tRNA with the A-site of the ribosome, translocation and, consequently, GTP hydrolysis [16, 17].

The protein bL12 has unique properties among bacterial ribosomal proteins. In addition to the fact that bL12 is a multicopy protein, its isoelectric point is in the acidic

region (pH 4.8) [18]. In aqueous solutions, isolated protein bL12 exists only as a dimer [19] or a tetramer [20].

The ribosomal protein bL12 consists of two domains and a long flexible linker [21–23]. The C-terminal domain of protein bL12 (bL12CTD) is responsible for interaction with translation factors [24, 25], while the N-terminal domain of bL12 (bL12NTD) is responsible for dimerization and binding with ribosomal protein bL10 [21]. The N- and C-terminal domains of bL12 are connected to each other with a flexible linker providing mobility of the protein molecule [26]. The length of this linker influences not only the mobility of the two domains relative to each other, but also the binding of elongation factors, GTP hydrolysis on the ribosome, and translation rate and accuracy [27, 28]. The removal of this region inactivates the bL12 protein [28].

In 1980, the C-terminal domain of the *E. coli* protein bL12 was crystallized and its structure was determined at 2.6 Å resolution [29]. It was the first crystal structure of a ribosomal protein. Much later, the spatial structure of the full-sized protein bL12 from *Thermotoga maritima* was obtained at 2.0 Å resolution [30]. The bL12NTD contains two short helices: α 1 and α 2. The long α 3 hinge helix is a linker that separates the N-terminal domain from the globular C-terminal domain. This helix is formed by 20 mainly hydrophobic amino acid residues. The C-terminal domain of protein bL12 has dense packing and consists of a three-stranded antiparallel β -sheet surrounded by three α -helices from one side [30].

In 2004, the spatial structure of protein bL7 dimer, the N-acetylated variant of the protein bL12 from *E. coli*, was determined using solution nuclear magnetic resonance (NMR) [26]. The protein bL7 has an elongated conformation. This structure of the protein bL7 differs greatly from the crystal structure of the protein bL12 from *T. maritima* in the linker region. The flexible linker in the structure of each bL7 monomer has no particular structural packing. The structure of bL7 dimer determined by NMR revealed how the protein dimerizes in solution (Fig. 2). Dimerization of protein bL7 occurs through the contact between two antiparallel V-shaped α - α -hairpins of the N-terminal domain, which form a symmetrical four-helix bundle.

The model of a molecular switch between the two states of the protein was proposed based on the determined protein structures of bL12 from *T. maritima* and bL7 dimer from *E. coli*. This model suggests that the region near the linker of protein bL12 plays the role of a molecular switch: the molecule adopts either a “closed” compact conformation, when the elongation factor is bound to the ribosome, or an “open” elongated conformation after GTP hydrolysis and dissociation of the elongation factor from the ribosome [26].

The bL12 dimers are bound to ribosomal protein bL10 with the formation of a strong ribosomal protein complex bL10–bL12 in solution. The thermal stability of

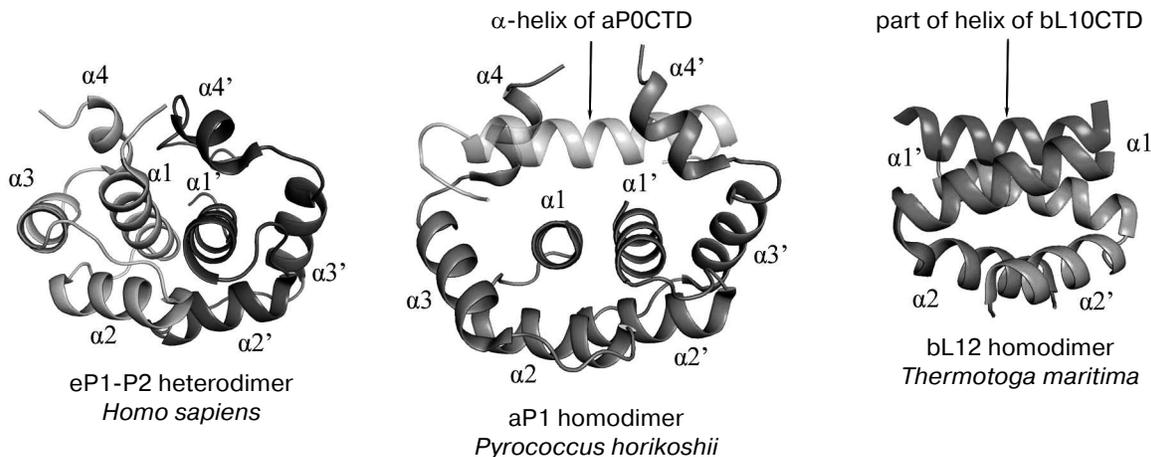


Fig. 2. Structural comparison of dimerization of eukaryotic proteins eP1/P2, archaeal protein aP1, and bacterial protein bL12 (the figure with minor modifications was taken from [34]).

bL10 and bL12 in the complex increases compared to the individual states [31]. The bL10–bL12 complex from *E. coli* remains stable in the presence of 6 M urea at pH 4.6. Hence, when the ribosomal proteins were systematized by two-dimensional electrophoresis, the protein complex bL10–bL12 was preserved under the denaturing conditions and was erroneously taken as an individual protein, which was named L8 [32].

For the complex of ribosomal proteins bL10–bL12 from *E. coli*, the ratio of proteins bL10 and bL12 was shown to be 1 : 4 by the methods of isotope dilution [33], equilibrium ultracentrifugation, and quantitative analysis of protein staining on the electrophoregram [31]. Therefore, it was believed for a long time that the bacterial complex of ribosomal proteins bL10–bL12 can exist only as a pentamer. The determination of the crystal structure of ribosomal complex bL10–bL12NTD from *T. maritima* changed the notion of the ratio of proteins bL10 and bL12. In this structure of the complex, six molecules of the N-terminal domain of protein bL12 formed a heptameric complex with one molecule of protein bL10 [34]. Hence, it was suggested that the difference between protein ratios in the bL10–bL12 complexes depended on the nature of the organism from which the proteins were isolated, and that the extra amino acid sequence in the C-terminal domain of protein bL10 of thermophilic bacteria could be a binding site for a third dimer of bL12 [35]. Results obtained by mass spectrometry confirmed this assumption. It was shown that the bL10–bL12 complexes from mesophilic bacteria are pentameric, while those from thermophilic bacteria are solely heptameric [35, 36].

Ribosomal protein bL10. Ribosomal protein bL10 acts as a bridge between bL12 dimers and the ribosome. The C-terminal part of protein bL10 from mesophilic bacteria contains two independent binding sites for bL12

dimers, whereas the N-terminal part of protein bL10 interacts with 23S rRNA [22].

The crystal structure of a complex of ribosomal protein bL10 with dimers of the N-terminal domain of protein bL12 from *T. maritima* was determined at 2.3 Å resolution [34]. Protein bL10 consists of two domains: the N-terminal RNA-binding domain, and the C-terminal domain to which protein bL12 is bound. The N-terminal domain is densely packed and contains an α/β motif. The C-terminal domain of bL10 is formed by a long and flexible C-terminal α -helix (helix $\alpha 8$). The $\alpha 8$ helix bends twice, forming three segments of 10 amino acid residues each. Each segment binds one bL12NTD dimer; therefore, three nearly identical elements can be distinguished in the region of contact between proteins bL10 and bL12 [34]. Between the N- and C-terminal domains of bL10 there is the so-called “rotation center”. This “rotation center” provides high mobility of helix $\alpha 8$ with the bL12NTD dimers relative to the RNA-binding domain, which is necessary for the functional activity of the lateral L12 stalk [34].

The site of bL10 binding to the ribosome is on the surface of the large ribosomal subunit. Chemical probing was used to determine the main binding region for protein bL10, which is in domain II of 23S rRNA and includes helices H42–44 [37]. The site of bL10 binding to 23S rRNA was localized by superimposing the known structure of the bacterial protein bL10 from *T. maritima* at the conservative RNA-binding domain on the structure of two N-terminal α -helices of the archaeal protein aL10, which was determined within the 50S ribosomal subunit from the archaeon *Haloarcula marismortui* [34, 38]. Based on these data, the maximum number of contacts were revealed between helices $\alpha 1$ and $\alpha 2$ of protein bL10 and helix H42 of 23S rRNA, which is in good agreement with the chemical probing data [34, 37]. It should be noted that most contacts between bL10 and 23S rRNA are in the sugar-

phosphate backbone of rRNA. Spatial packing of rRNA probably plays a key role in recognition of the uL10 binding site in the ribosomes of all organisms [34].

The genes of bacterial ribosomal proteins bL10 and bL12 are localized in the same operon. Ribosomal protein bL10 as a component of the bL10–bL12 complex is a translational repressor of its operon and is bound to mRNA upstream from the initiation codon of the bL10 gene [39, 40].

The analysis of rRNA showed that the crystal structure of the large ribosomal subunit of *Deinococcus radiodurans* contained a consensus motif in helix H42 of 23S rRNA around the GTPase center, the so-called “kink-turn motif”, which probably makes a major contribution to rRNA recognition by protein bL10 [41, 42]. The mRNA of the *rplJ* gene from *E. coli* might contain the same motif [43]. It is supposed that mRNA and rRNA interact with protein bL10 in a similar way.

Ribosomal protein bL11 is bound to 23S rRNA close to the bL10 binding site. The binding of proteins bL10 and bL11 is cooperative [44]. The cooperative effect can increase the affinity of bL10 to rRNA 100-fold [43]. Cooperative binding of bL10 and bL11 is probably determined by conformational changes in the rRNA structure [34]. Although mRNA and rRNA showed the same affinity for protein bL10 in experiments *in vitro*, the 100-fold cooperative effect of bL10 and bL11 binding to rRNA ensures almost complete occupation of ribosomes by the bL10–bL12 protein complex [43].

Ribosomal protein bL11. Ribosomal protein bL11 is a necessary component of the ribosomal 50S subunit, being located at the base of the L12 stalk. The bL11-lacking mutant strains of *Bacillus megaterium* are viable, but their growth is more than twice slower compared to cells of the wild type strain [45]. The function of bL11 on the ribosome is like that of bL12: it is involved in the interaction between the ribosome and elongation factors EF1A and EF2, termination factors RF1 and RF2, and it promotes the association of ribosomal subunits and stimulates GTP hydrolysis [46–48].

Spatial structures of the full-sized protein bL11 from *T. maritima* have been determined in the free state by the solution NMR technique [49] and in complex with the 23S rRNA fragment by X-ray structure analysis at 2.6 Å resolution [50]. Also, the structure of the 50S ribosomal subunit from *Deinococcus radiodurans* in complex with the antibiotic thiostrepton, where bL11 was visualized, was determined at 3.3 Å resolution [51].

Ribosomal protein bL11 consists of N- and C-terminal globular domains connected with a short linker. The $\alpha 1$ helix of the N-terminal domain of bL11 contains a conservative proline residue (Pro22, numbering for *T. maritima*), which interacts with thiazole antibiotics (e.g. thiostrepton and micrococcin). The linker between the bL11 domains is formed by a consensus motif of three amino acid residues (Thr72-Pro73-Pro74). The structure

of the C-terminal domain (bL11CTD) has a characteristic feature, namely, an elongated disordered loop (84–96 a.a.). This loop participates in RNA–protein interaction and is susceptible to conformational changes during rRNA binding [49, 50].

Protein bL11 interacts via the C-terminal domain with the large ribosomal RNA. Filter-binding assay has shown that the dissociation constant for the protein and rRNA is $1.2 \cdot 10^{-9}$ M [52]. The C-terminal domain of bL11 is bound with the minor groove of 23S rRNA formed by helices H43–44. The RNA-binding surface of bL11 is formed by the $\alpha 5$ helix, the N-terminal part of the $\alpha 3$ helix, as well as by the $\alpha 3$ – $\alpha 4$ and $\alpha 4$ – $\alpha 5$ loops located on each side of the $\alpha 5$ helix. The $\alpha 5$ helix is localized along the minor groove of 23S rRNA, forming the greatest number of contacts. The $\alpha 3$ – $\alpha 4$ and $\alpha 4$ – $\alpha 5$ loops, when binding to rRNA, adopt an ordered structure that repeats the surface of the minor groove of rRNA [50]. More than half of the hydrogen bonds of the complex are formed between the sugar-phosphate backbone of rRNA and the main chain of bL11CTD. This fact indicates that the spatial packing of 23S rRNA plays the key role in recognition of the bL11 binding site. Biophysical experiments have shown that bL11 stabilizes the tertiary structure of rRNA via the C-terminal domain [53]. The N-terminal domain of bL11 (bL11NTD) is involved in interaction with translation factors.

The ribosomal complex of protein bL11 with 23S rRNA is a target for thiazole antibiotics [54]. When binding with the ribosome, thiostrepton blocks and micrococcin stimulates GTP hydrolysis on EF2 [55]. The main thiostrepton/micrococcin binding site is localized in a slot between the $\alpha 1$ helix of bL11NTD and the 1067/1095 region of rRNA. Thus, thiostrepton blocks the functionally important structural rearrangements of bL11 through formation of a stable bL11–rRNA–thiostrepton complex.

Proteins of eukaryotic and archaeal P stalk. Eukaryotic and archaeal ribosomes contain a lateral P stalk with structural organization analogous to that of the bacterial L12 stalk. The eukaryotic ribosomal P stalk is formed by two types of P proteins and protein eL11. Protein eL10 is a P protein of the first type (previously denoted as P0) [56]. The proteins of the second type are small acidic proteins eP1/P2 of about 11 kDa [57]. The P proteins form a pentameric complex eL10–eP1/P2, where two heterodimers eP1/P2 are bound to protein eL10. The eukaryotic P stalk is a necessary component of the ribosome, and protein eL10 is vitally important for cell growth [58].

In archaea, the P stalk consists of ribosomal proteins aL11, aL10, and aP1. As concerns amino acid sequence, the archaeal proteins aL10 and aP1 are much more like eukaryotic proteins eL10 and eP1/P2 than to their bacterial analogs [59].

The archaeal P stalk is rather stable. Proteins aL10 and aP1 form a strong protein complex aL10–aP1,

which, like the bacterial complex bL10–bL12, is not destroyed in 6 M urea at pH 4.6. After the large ribosomal subunit has been treated with a high-concentration NH_4Cl and ethanol solution, protein aL10 is not completely removed from the ribosome as it interacts with rRNA with higher affinity than protein bL10 [60].

Ribosomal proteins eP1/P2 and aP1. The eukaryotic eP1/P2 proteins are very similar to each other both functionally and structurally. The number of groups and subgroups of eP1/P2 proteins varies between different species of organisms [61, 62]. In contrast to bacterial protein bL12 from *E. coli*, which is represented on the ribosome by one more N-acetylated copy, each type of eP1/P2 is encoded by a separate gene [61]. In solution, the eP1/P2 proteins are present as a stable heterodimer [63, 64].

The archaeal protein aP1 is encoded by a single gene in archaea and is not subject to modifications, although it is structurally similar to the eukaryotic proteins eP1/P2. In solution, as well as on the ribosome, protein aP1 is present as a dimer [60, 65].

Structurally, the archaeal aP1 and eukaryotic eP1/P2 can be divided into N- and C-terminal domains and a flexible linker connecting the domains (by analogy with bacterial protein bL12). Like in bL12, the N-terminal domain is responsible for dimerization of these proteins and for their binding with the ribosomal subunit via protein a/eL10 [66, 67].

During protein biosynthesis, archaeal protein aP1 delivers translation factors to the ribosome. The C-terminal part of the protein directly interacts with elongation factors aEF2 and aEF1 α , as well as with initiation factor aIF5B homologous to the bacterial initiation factor IF2 [68, 69]. As shown by surface plasmon resonance, protein aP1 binds elongation factor aEF2 irrespective of whether the factor is in a complex with GTP or GDP. Moreover, the protein complex aL10·(aP1)₆ can bind several aEF2 molecules, promoting an increase in the rate of aEF2 delivery to the GTPase-binding center and high rate of GTP hydrolysis [69].

The first 10 a.a. residues of eukaryotic protein eP1/P2 are important for heterodimerization and formation of a pentameric complex [70]. The C-terminal domain of eP1/P2 is responsible for interaction between the ribosome and translation factors [71].

The spatial structure of the full-sized heterodimer eP1/P2 from *Homo sapiens* has been determined by the solution NMR technique [72]. The N-terminal domains of proteins eP1 and eP2 consist of four α -helices and are rather compact (Fig. 2). The heterodimer of the eP1 and eP2 N-terminal domains is asymmetrical and is formed due to highly conservative hydrophobic residues of the α_1 , α_2 , and α_4 helices of eP1 and eP2. The primary point of contact is between the α_1 helices of eP1 and eP2. The C-terminal part has no specific structure. Thus, the C-terminal “tail” of the eP1–eP2 dimer can be at a distance up to 125 Å from the N-terminal domain. Due to the

elongated C-terminal “tail” of eP1/P2, translation elongation factors seem to be easily delivered to the GTPase-binding center [72].

The structure of the full-sized archaeal protein aP1 is unknown, but the crystal structures of dimers of the N-terminal domain of aP1 (in complex with aL10; 2.1 Å resolution) [67] and the C-terminal domain of aP1 (in complex with elongation factor aEF1 α ; 2.3 Å resolution) from *Pyrococcus horikoshii* have been determined [73]. The N-terminal domain of the archaeal protein aP1 is formed by four α -helices (Fig. 2). Dimerization of aP1 occurs due to hydrophobic interactions between the α_1 and α_2 helices of the two monomers. In contrast to the compact C-terminal domain of bacterial bL12, the C-terminal domain of archaeal aP1 (aP1CTD) is unstructured in the free state from the elongation factor [74]; however, during the interaction with translation factors, aP1CTD is structured with the formation of a long α -helix [73].

The structures of the archaeal dimer of the aP1 N-terminal domain and the eukaryotic heterodimer of the eP1 and eP2 N-terminal domains show similar packing of polypeptide chains, as opposed to the bacterial dimer of the bL12 N-terminal domain (Fig. 2). The basic structural difference between the aP1NTD dimer and the eP1/P2NTD heterodimer is the α_4 helix. The α_4 helix of aP1NTD in the structure of the archaeal complex aL10·(aP1)₆ adopts an “open” conformation, which allows it to bind with the short helix of aL10. The α_4 helix in the eukaryotic dimer eP1/P2NTD adopts a “closed” conformation but, quite probably, can adopt also the “open” conformation and, in this state, promote the binding of the eP1/P2 heterodimer with the C-terminal part of eukaryotic protein eL10 [66].

The α_3 helix of eukaryotic protein eP1 has a strongly hydrophobic surface and, on the contrary, the α_3 helix of eP2 has a hydrophilic surface. Neither the eP1 nor the eP2 α_3 helix is involved in the dimerization process. The asymmetry of eP1/P2 suggests that the eP1/P2 heterodimers in the pentameric complex are arranged in the following order: eP2–eP1 : eP1–eP2 [66].

In archaeal ribosomes, aL10 and aP1 are present as an aL10–aP1 complex in ratio 1 : 4 or 1 : 6, as determined by mass spectrometry [36, 59]. The ribosomes of hyperthermophilic archaea contain only the heptameric complex aL10·(aP1)₆, while mesophilic archaea were shown to have two ribosome populations with either pentameric or heptameric aL10–aP1 complex. The ratio of the ribosomes with pentameric and heptameric complexes varies through the cell life cycle. At the initial stage of cell growth, ribosomes contain mainly the pentameric complex aL10·(aP1)₄. When the cells pass into the stationary growth phase, the aL10 to aP1 ratio changes and the ribosomes with the heptameric complex aL10·(aP1)₆ become predominant [36].

The activity of GTP hydrolysis and polyphenylalanine synthesis in archaeal ribosomes containing the

trimeric complex aL10-(aP1)₂ have 55% of the activity of ribosomes with the heptameric complex. The activity of ribosomes with the pentameric complex is about 95%. Thus, the pentameric and heptameric complexes aL10-aP1 are not much different in availability for translation factors. The third dimer of the archaeal protein aP1 seems to be necessary for proper functioning of archaeal the ribosome at temperatures close to the optimal growth temperature (e.g. for *P. horikoshii*, it is 95°C) [59].

Ribosomal proteins a/eL10. Eukaryotic protein eL10 contains two structural elements for binding of two eP1-eP2 heterodimers [75]. By removing C-terminal amino acid residues, it has been shown that the first and second binding sites for the eP1-eP2 heterodimer are located in the region of 205-230 and 240-255 a.a., respectively (numbering for *Bombyx mori*) [75, 76].

The archaeal and eukaryotic ribosomal proteins a/eL10, like bacterial protein bL10, are intermediates between the archaeal aP1, the eukaryotic eP1/P2, and the ribosome. The archaeal and eukaryotic proteins a/eL10 consist of three domains (Fig. 3). The conservative (in all domains of life) N-terminal domain 1 of protein a/eL10 is an RNA-binding domain; it is responsible for the attachment of the ribosomal complex aL10-aP1 or eL10-eP1/P2 to the large ribosomal subunit. The second (specific domain) has been found only as a component of proteins a/eL10 of archaea and eukaryotes and is absent in the bacterial analog [77, 78]. The C-terminal helical domain is a place of attachment for the two eP1-eP2 heterodimers in the case of eukaryotic P stalk and two or three aP1 homodimers in the case of archaeal P stalk.

In 2010, the crystal structures of the archaeal ribosomal complex of aL10 with dimers of the N-terminal domain of aP1 from *P. horikoshii* (2.1 Å resolution) [67] and the two-domain N-terminal fragment of the archaeal ribosomal protein aL10 (aL10NTF) from *Methanococcus jannaschii* (1.6 Å resolution) [77] have been determined.

The N-terminal domain 1 of archaeal protein aL10 consists of two parts corresponding to amino acid residues

1-111 and 192-206 (numbering for *M. jannaschii*) [67, 77]. The second domain of aL10 is an insert in the first and contains amino acid residues 115-188. The two domains are connected with a linker consisting of two oppositely directed β-strands. Domain 2 can shift relative to domain 1 by 13 Å [77]. The C-terminal domain of protein aL10 is twofold longer (Fig. 3) than the bacterial bL10CTD and different in structure. It contains three independent α-helices connected with a short 6-a.a. linker. Each helix of the C-terminal domain of aL10 is bound to one dimer of protein aP1 [67].

The spatial structure of isolated eukaryotic protein eL10 has not been determined. A structural model of eukaryotic protein eL10 in complex with N-terminal domains of the eP1-eP2 heterodimer was suggested based on high homology between archaeal and eukaryotic proteins a/eL10 and the crystal structure of the archaeal protein aL10 in complex with dimers of the aP1 N-terminal domains [79]. This model predicted the structure of only the N-terminal domain 1 and the C-terminal domain of eL10 and, therefore, determination of the structure of its domain 2 is still relevant.

The region of archaeal and eukaryotic proteins a/eL10 corresponding to domain 2 ensures specific interaction between the ribosome and the eukaryotic or archaeal, but not bacterial, translation factors. The removal of this domain reduces to 40% the factor-dependent GTP hydrolysis and the level of polyphenylalanine synthesis by hybrid ribosomes of *E. coli* with eukaryotic and archaeal translation factors [67].

The N-terminal domain 1 of archaeal protein aL10 interacts with helix H42 of 23S rRNA domain II, similar to the binding of bacterial protein bL10 with 23S rRNA, and the major point of contact between the archaeal protein aL10 and rRNA is also located in the sugar-phosphate backbone of RNA ("kink-turn motif") [34].

The first 20 a.a. of eukaryotic protein eL10 are needed for binding with 26/28S rRNA [76]. Between amino acids 40 and 70, there is an arginine-rich region providing

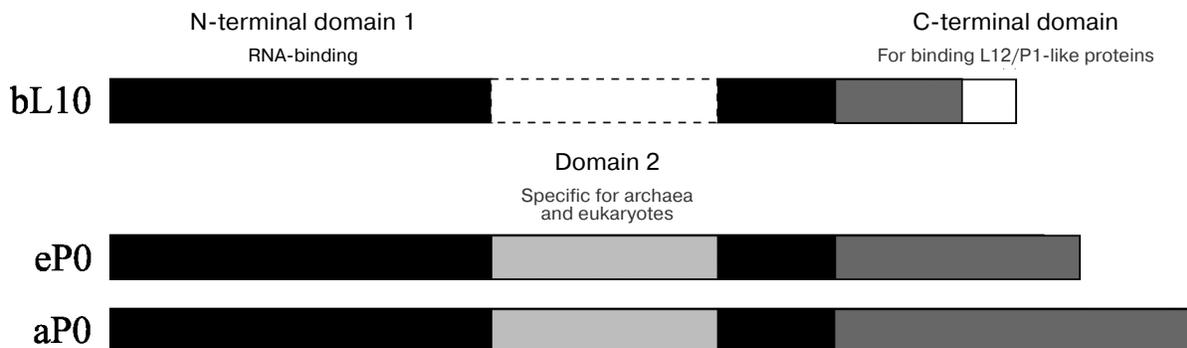


Fig. 3. Scheme of polypeptide chain sequences of ribosomal proteins uL10. The N-terminal domains 1 and 2 are rendered in black and light gray, respectively; the C-terminal domain is gray. The white rectangle with a solid line denotes extra amino acid sequences of the thermophilic bacterial proteins bL10.

an extra contact with rRNA [80]. In complex with proteins eP1/P2, the affinity of eL10 to rRNA increases [81]. It is most probable that eP1/P2 additionally functions as a modulator during the binding of eL10 with the ribosome.

Ribosomal proteins a/eL11. Eukaryotic ribosomal protein eL11 is a functional analog of the archaeal and bacterial proteins a/bL11. Chemical cross-linking of neighboring molecules has shown that eukaryotic protein eL11 interacts with eukaryotic elongation factors eEF1 α and eEF2 [82]. Protein eL11 binds with 26/28S rRNA in the region equivalent to the binding region for the archaeal/bacterial proteins a/bL11 on 23S rRNA.

It should be noted that the spatial structure of isolated eukaryotic protein eL11 has not been determined. It is only known about the model of eL11 as a component of the yeast ribosome as a polyalanine chain [83].

Archaeal protein aL11 is homologous to bacterial protein bL11 [65]. The spatial structure of archaeal protein aL11 has been partially visualized only within the 50S subunit of the ribosome from *H. marismortui* [84]. Protein aL11 consists of two domains connected with a short linker. Due to the low quality of electron density maps for the region of the N-terminal domain, the structural details of the latter are poorly discernible [84].

The archaeal aL11 is bound via the C-terminal domain to helices H43–44 of 23S rRNA domain II. The binding sites of archaeal aL10 and aL11 are located on rRNA side-by-side. It is important to note that archaeal protein aL11 stimulates binding of archaeal aL10 with RNA only at high temperatures (70°C); at low temperatures (37°C), stimulation is not observed [85]. The RNA-binding surface of protein aL11 is formed by the $\alpha 5$ helix and the $\alpha 3$ – $\alpha 4$ and $\alpha 4$ – $\alpha 5$ loops located on both sides of the $\alpha 5$ helix. The $\alpha 5$ helix of the C-terminal domain forms an extensive network of interactions with 23S rRNA, being localized along the minor groove of rRNA [84].

The N-terminal domain of archaeal aL11 promotes delivery of translation elongation and termination factors to the GTPase-binding center of the archaeal ribosome. It has been shown that the archaeal ribosome is sensitive to the peptide antibiotics thiostrepton and micrococccin. It is most probable that archaeal protein aL11 can be a target for this class of antibiotics [86].

INTERCHANGEABILITY OF LATERAL STALK IN BACTERIA, ARCHAEA, AND EUKARYOTES

After it had been shown that the L12/P stalk is a functionally important morphological element of ribosomes in bacteria, archaea, and eukaryotes, the first attempts were made to create a hybrid ribosome under *in vitro* conditions. In 1981, the first eukaryotic hybrid ribosome was reconstructed. After the removal of proteins

eL10, eL11, and eP1/P2 from the eukaryotic ribosome by extraction with NH₄Cl–ethanol mixture, the respective bacterial proteins were added to the “vacant” ribosome. However, the resulting hybrid ribosome was inactive [87]. In addition, the aL10–aP1 protein complex of the archaeal ribosome was replaced by the bacterial complex bL10–bL12 and, on the contrary, the bL10–bL12 complex of the bacterial ribosome was replaced by the archaeal complex aL10–aP1. Electron microscopy showed that the hybrid ribosomes had an L12/P stalk formed by the bL10–bL12 or aL10–aP1 complexes, but the functional activity of such ribosomes was not tested [88].

The first active hybrid bacterial ribosome from *E. coli* with eukaryotic P stalk of the rat ribosome was obtained in Hachimori laboratory [89]. This substitution changes the specific binding of bacterial EF2 into binding of eukaryotic eEF2 and stimulates GTPase activity in the latter. The level of GTP hydrolysis on eEF2 in the hybrid ribosome is comparable with the level of GTP hydrolysis on eEF2 in the eukaryotic 80S ribosome [68]. At the same time, the activity of the hybrid ribosome during polyphenylalanine synthesis in the presence of eukaryotic elongation factors eEF1 α and eEF2 is at the same level as in the eukaryotic ribosome [68, 76].

The archaeal P-stalk proteins, like their eukaryotic homologs, can substitute for bacterial L12-stalk proteins [68]. As a result of such substitution, the reconstructed hybrid ribosome becomes available both for the archaeal and eukaryotic elongation factors but not for the bacterial elongation factors. The activity of GTP hydrolysis and polyphenylalanine synthesis in the hybrid ribosome containing the archaeal lateral stalk proteins in the presence of eukaryotic elongation factors is comparable with those for the hybrid ribosome where the bacterial L12 stalk is replaced by its eukaryotic analog. It should be noted that polyphenylalanine synthesis and GTPase activity of the hybrid ribosome with the archaeal P stalk are the same as in eukaryotic and archaeal elongation factors [68].

The L12-stalk proteins of human mitochondria have a high degree of homology with the respective bacterial proteins. The mitochondrial L12 stalk can substitute for the analogous stalk on the ribosome of *E. coli* with the formation of a functionally active hybrid ribosome [90]. The resultant hybrid ribosome with the mitochondrial L12 stalk has a high activity of polyphenylalanine synthesis in the presence of bacterial or mitochondrial elongation factors.

Thus, these experiments have demonstrated the key role of the L12/P-stalk proteins in the specific recognition of translation factors. The uL10–L12-like complex (but not the uL11 proteins) is responsible for specific interaction between the ribosome and the translation factors. It is important to note that the change in specific recognition of the elongation factors caused by the replacement of lateral stalk proteins by their homologs (or

analogs) on the ribosome is accompanied by structural changes in 23S/28S rRNA regions around the sarcin–ricin loop and helices H43–44 [91].

CRYSTALLOGRAPHIC STUDIES OF THE L12/P STALK IN RIBOSOMES

The X-ray structure analysis made it possible to determine not only the significant morphological features of the ribosome, but also the internal structure of the ribosome, the tertiary structures of ribosomal RNA within the ribosome, and the localizations and structures of ribosomal proteins. The first ribosome crystals suitable for the X-ray structure analysis were obtained as early as in the late 1980s. However, the crystal structure of the 50S ribosomal subunit from the archaeon *H. marismortui* was determined for the first time at high resolution only in 2000 [38]. This structure contained 2711 out of 2923 nucleotide residues of 23S rRNA, the whole 5S rRNA, and the structures for 27 out of 31 ribosomal proteins. The electron density for the proteins aL1, aL10, aL11, and aP1 was absent, though previously they have been localized within this ribosomal subunit using electron microscopy and low-resolution diffraction data [92].

In 2009, the L12 stalk structure was determined within the bacterial 70S ribosome in complex with EF2 from *T. thermophilus* [93]. Elongation factor EF2 directly interacts with bL11 and the C-terminal domain of bL12. Due to low quality of electron density map for the region of the lateral stalk, the structure of protein complex bL10–bL12 was determined as a polyaniline chain. The $\alpha 8$ helix of bL10 is bent compared to its position in the structure of isolated complex bL10–bL12NTD, allowing the C-terminal domain bL12CTD to interact with both the N-terminal domain of bL11 and the G'-domain of EF2. In turn, the N-terminal domain of bL11, together with 23S rRNA nucleotide residues 1067 and 1095, interacts with domain V of elongation factor EF2 [93].

In the eukaryotic 80S ribosome from *S. cerevisiae*, the P stalk was visualized partially [83]. The proteins of this stalk could be placed into the electron density map only as polyaniline chains. The exception was the conservative N-terminal RNA-binding domain 1 of protein eL10, whose structure was determined fully. The overall packing of protein eL10NTD coincides with the packings of the N-terminal domains of bacterial bL10 and archaeal aL10.

The most complete structure of the archaeal P stalk could be determined after additional refinement of the structure of the 50S ribosomal subunit from the archaeon *H. marismortui* in 2013 [84]. As a result of revision, not only the P stalk but also some ribosomal components of previously unknown structure were visualized (e.g. the archaea-specific LX protein). Refinement of the large subunit structure made it possible to interpret the elec-

tron density map for approximately 2/3 of protein aL10 (the C-terminal domain as a polyaniline chain) and one dimer of the aP1 N-terminal domain (as a polyaniline chain), as well as to supplement the aL11 protein structure.

CONCLUSION

The lateral L12/P stalk plays a key role in the interaction between ribosome and translation factors. The structural organization of this stalk is similar in all domains of life, although the comprising ribosomal proteins are different: the proteins of the bacterial L12 stalk show a low homology, both in sequence and in structure, with the respective proteins that form the P stalk in archaea and eukaryotes.

In the last decade, there has been an enormous breakthrough in determination of spatial structures of the ribosome and the isolated proteins of this lateral stalk; however, there are problems thus far with understanding the interactions of these proteins between each other and with the high molecular weight rRNA. Works of recent years devoted to this subject have been considered in this review.

Acknowledgements

This work was supported by the Program of the Presidium of the Russian Academy of Sciences “Molecular and Cell Biology”.

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