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REVIEW

Ribosomal Proteins: Structure, Function, and Evolution

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Abstract—The question concerning reasons for the variety of ribosomal proteins that arose for more than 40 years ago is still open. Ribosomes of modern organisms contain 50-80 individual proteins. Some are characteristic for all domains of life (universal ribosomal proteins), whereas others are specific for bacteria, archaea, or eucaryotes. Extensive information about ribosomal proteins has been obtained since that time. However, the role of the majority of ribosomal proteins in the formation and functioning of the ribosome is still not so clear. Based on recent data of experiments and bioinformatics, this review presents a comprehensive evaluation of structural conservatism of ribosomal proteins from evolutionarily distant organisms. Considering the current knowledge about features of the structural organization of the universal proteins and their intermolecular contacts, a possible role of individual proteins and their structural elements in the formation and functioning of ribosomes is discussed. The structural and functional conservatism of the majority of proteins of this group suggests that they should be present in the ribosome already in the early stages of its evolution.

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Key words: ribosomal proteins, RNA–protein interactions, ribosome, evolution

RIBOSOMAL PROTEINS OF THREE DOMAINS OF LIFE: DIVERSITY, LIKENESS, AND DIFFERENCES IN THEIR STRUCTURES

The variety of *Escherichia coli* ribosomal proteins was first demonstrated 40 years ago [1], when the bacterial ribosome was found to contain at least two dozens of different polypeptides. During some following years the multiplicity of ribosomal proteins was shown to be characteristic for many other bacteria [2]. The separation of ribosomal proteins by two-dimensional electrophoresis in polyacrylamide gel elaborated during the same period [3] allowed researchers to perform a rather rapid analysis of ribosomal proteins from members of all domains of life [4-6]. The resulting data showed that ribosomes from bacteria and archaea contained 50-60 different polypeptides, and eukaryotic ribosomes contained about 70. However, these results did not answer a fundamental question of whether some dozens of polypeptides found in the ribosome were individual proteins. To answer this question, at least for *E. coli* ribosomes, still 10 years were required [7, 8]. Works whose purpose was to determine primary structures of all ribosomal proteins of *E. coli* and sequencing their genes resulted in affirmative: ribosomes of *E. coli* contain 53 individual proteins. Most of these proteins were small (50-150 amino acid residues), and the comparative analysis of their primary structures failed to detect among them elongated homologous segments (no more than six identical residues) [7, 9]. Thus, each ribosomal protein was found to have a unique amino acid sequence. The modern analysis of primary structures of ribosomal proteins from other bacteria, archaea, and eucaryotes results in the same conclusion [10, 11].

Concurrently with determination of primary structures of ribosomal proteins from *E. coli*, similar studies were performed on proteins from other organisms [7, 12, 13]. This promoted the elucidation of the problem of relationship between proteins from different domains of life. Such an attempt was realized in 1995: the known by that time primary structures of ribosomal proteins of rat, human, yeast, some archaea, and the bacterium *E. coli* were compared [14]. This analysis revealed 31 families of ribosomal proteins that are present in ribosomes from members of all domains of life. By the end of the XX century, a technical and methodical success occurred in sequencing whole genomes. Therefore, already in 2002 a more large-scale comparative study on genes of ribosomal proteins was performed [15]. Sixty-six genomes from different species were analyzed (45 bacteria, 14 archaea, and

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seven eucaryotes). Fifty-seven different proteins were found in bacteria, 68 in archaea, and 78 in eucaryotes (Fig. 1). Among 102 families of ribosomal proteins, only 34 proteins (universal ribosomal proteins) were found in members of all domains of life (15 and 19 in the small and large ribosomal subparticles, respectively). This finding confirmed the earlier conclusion [14].

Now, based on analysis of primary structures and some other features of known ribosomal proteins, some fundamental conclusions can be formulated about their diversity, conservativeness, and evolution of structure. In accordance with the above-described grouping of all known ribosomal proteins, a group of universal ribosomal proteins consists of 34 members, which can be found in all domains of life [15] (Fig. 1). This group includes 15 proteins of the small (S2-S5, S7-S15, S17, and S19) and 19 proteins of the large (L1-L6, L10-L15, L18, L22-L24, L29, L30, and L7ae) ribosomal subparticles. The universality of this group of proteins is also confirmed by results of other studies. Thus, the proteins of this group are detected in organelle ribosomes of the majority of organisms studied, and genes of nearly all these proteins are present in the genome of the smallest bacterium *Mycoplasma genitalium* [16-19]. Nevertheless, now, considering new experimental data, it would be reasonable to somewhat correct the list of universal ribosomal proteins. Thus, the bacterial ribosomal protein L16 was not included into this group [15]. However, still in 2001 the central region of the primary structures of the L16 bacterial protein and of the L10e protein of the archaean *Haloarcula marismortui* were shown to be very alike [20]. Moreover, these proteins were shown to be structural and functional homologs with a similar spatial package and an identical location inside the ribosome [21, 22]. Another example is presented by the ribosomal protein L7ae, which was characterized as a possible representative of universal ribosomal proteins [15]. On one hand, this protein, found in eucaryotic and archaean ribosome [23-25], was not found in bacterial ribosomes [26-29]. On the other hand, now the gene encoding the L7ae protein is detected in genomes not of all bacteria, but only in genomes of some bacteria from the classes of Bacilli and Clostridia [11, 15]. Therefore, this protein more is probably not a universal ribosomal protein. Thus, the list of universal ribosomal proteins is suggested to be not final and will undergo further changes.

The first sufficiently complete analysis of the ribosomal protein gene distribution inside the chromosome was performed 25 years ago for *E. coli* [8]. Dozens of genomes of different organisms sequenced from that time [11] has allowed researchers to comparatively analyze the distribution of genes encoding different ribosomal proteins inside chromosomes of archaea and bacteria [30]. The genes of the majority of universal ribosomal proteins in both bacteria and archaea were found to be accumulated in some large operons (thus, in *E. coli* the genes encoding 30 of these 34 proteins are accumulated in five operons: *spc*, *S10*, *Alpha*, *L11*, and *str*). However, genes of the majority of non-conservative ribosomal proteins are dispersed inside the chromosome combining into operons with genes that often are not related with translation. On one hand, accumulation of the ribosomal genes into large operons is favorable for easy and effective control of the regulation of their synthesis [8]. On the other hand, this grouping of universal ribosomal proteins on the gene level can indicate the proximity of the time of their appearance in the ribosome.

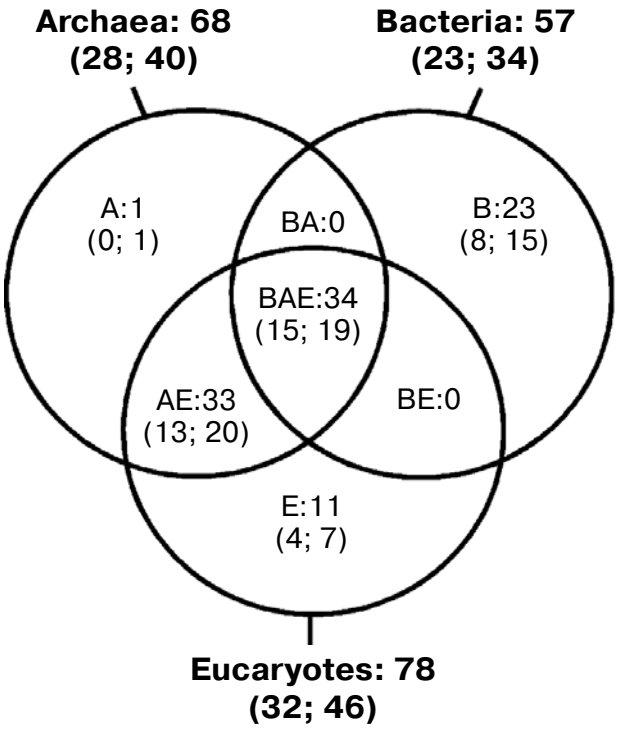

Fig. 1. Venn diagram presenting the distribution of ribosomal proteins among three domains of life: bacteria (B), archaea (A), and eucaryotes (E). The total number of ribosomal protein families is shown for each domain. Numerals in parentheses indicate the number of proteins in the small and large ribosomal subparticles, respectively. The scheme is taken from work [15] and modified.

The detailed comparative analysis of primary structures of universal ribosomal proteins has revealed that they consist of blocks [31]. These blocks are segments of an amino acid sequence of 8-70 amino acid residues that are unique for each protein family (Fig. 2). Just the existence of these conservative blocks within proteins of all domains of life clearly indicates that these proteins could be present in the proto-ribosome still before the divergence of the three branches: archaea, bacteria, and eucaryotes. Moreover, some universal proteins (S9-S13, L1, L5, L6, L11, L14-L16, and L29) were found to essentially retain their size and nearly lack additional non-conservative blocks in their structure [31] (Fig. 2). Note that the majority of the above-mentioned proteins are

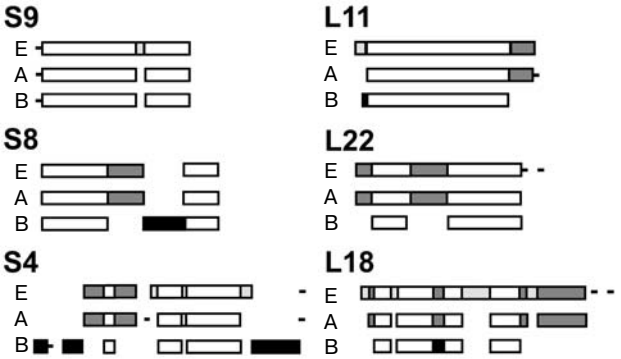

Fig. 2. Schematic picture of alignments the primary structures of some universal ribosomal proteins from bacteria (B), archaea (A), and eucaryotes (E). Proteins with minimal (S9 and L11), some (S8 and L22), and maximal (S4 and L18) changes in the structure are presented as examples. Regions that are alignable, that is, homologous, in proteins from all domains of life are shown in white. Regions detected only in bacteria are shown in black, dark-gray indicates regions in archaea and eucaryotes, and light-gray indicate regions detected only in eucaryotes. The interrupted line shows unalignable regions. The scheme is compiled based on data from work [31].

involved in formation of functional sites of the ribosome. It seems that the retention of the structure of these segments (blocks) in the universal proteins may be necessary for maintaining the structure of a functionally active ribosome.

Besides the group of universal ribosomal proteins, there are also 68 other families of ribosomal proteins (Fig. 1). Among them proteins present only in the pairs bacteria and eucaryotes or bacteria and archaea were not found. Thirty-three ribosomal proteins are common for archaea and eucaryotes but are not found in bacteria [15]. One protein is specific for archaea and 11 proteins are specific for eucaryotes (Fig. 1). Thus, except for one protein, the ribosome of archaea from the viewpoint of protein composition can be considered as a reduced copy of the eukaryotic ribosome. The 33 proteins specific for archaea and eucaryotes are supposed to be important for stabilization of additional structural elements of rRNA that are absent in RNA of bacteria [14]. This hypothesis was confirmed by data of structural studies on ribosomes from yeast and the archaean *H. marismortui* [23, 24, 32]; these ribosomal proteins mainly contact with additional elements of ribosomal RNA.

As shown in Fig. 1, bacteria in addition to the group of universal proteins contain 23 proteins that are their specific feature. These proteins include eight proteins of the small (S1, S6, S16, S18, S20-S22, and S31e) and 14(15) proteins of the large (L9, (L16), L17, L19-L21, L25, L27, L28, L31-L36) ribosomal subparticles. The situation with the L16 protein is described above. The proteins S1, S21, S22, S31e, and L25 are found not in all known bacteria; therefore, they can be considered as not obligatory for functioning of the bacterial translation apparatus. Genes of the other 17 evolutionarily non-conservative ribosomal proteins are found in genomes of all known bacteria, but by now the appearance of these proteins in these organisms has not been definitely explained. There have been attempts to answer this question based on location of these proteins within the ribosome [15] or of their necessity for survival of the bacterial cell [33-35], but up to now there is no definite answer. Interesting results were recently obtained by analyzing structures of archaeal and bacterial 50S subparticles. Proteins were found without homology in both the primary and spatial structures but located identically within the ribosome [22, 26]. These were proteins L17/L31e, L19/L24e, L27/L21e, L31/L15e, L33/L44e, and L34/L37e. Now it is necessary to somewhat correct the statement concerning one of these proteins, L31. Recently, under a high resolution in the bacterial ribosome structure, not the L31 protein was detected but L28 in the same location [29]. The majority of the above-mentioned pairs is similar in size and interact with conservative elements of 23S rRNA [22]. Therefore, it was suggested that in ribosomes of different domains of life ribosomal proteins responsible for similar functions (e.g. stabilizing similar segments of ribosomal RNA) could appear independently.

Thus, comparative studies on the primary structures of ribosomal proteins significantly contributed to comprehension of the structural basis of their diversity but failed to answer an important question about the role of these proteins in formation and functioning of the ribosome. About 40 years ago, when studies on individual ribosomal proteins were started, the question of their spatial structure was very urgent. But because of difficulties in preparing crystals of isolated ribosomal proteins, this problem could not be solved by X-ray crystallography approaches for nearly 20 years. Studies on the spatial structure of individual ribosomal proteins and then of a whole ribosome were decisively advanced by the choice of extremophile procaryotes as an object for studies. In about 10 years, the spatial structures of ~20 individual ribosomal proteins were determined [36]. These data were a great help in determining the structure of the ribosome itself and of its subparticles from all domains of life [23-29, 37]. Thus, at present there is sufficient information for attempts to reveal structural characteristics of ribosomal proteins that determine their properties.

Analysis of spatial structures of ribosomes has shown that ribosomal proteins usually contain one or several globular domains with structures similar to the structure of these domains in the isolated protein [22, 38]. The structural topology of ribosomal proteins is rather diverse and similar to the structural packing of other known proteins, which seems to suggest their common origin. Because of shortage of space, we shall be limited here only to the general classification (α-proteins, β-barrel-containing proteins, α/β-proteins, and $\alpha+\beta$-proteins) of

the structural packing of ribosomal proteins (Fig. 3a). Thus, α-proteins (S13, S15, S18, S20, L20, L29, and L35) occur comparatively seldom among proteins of the bacterial ribosome. The majority of ribosomal proteins contain α-helices, β-strands that are packed into β-barrels (S12, S17, L2, L3, L14, L24, L25, and L27), α/β-sandwiches (S3, S5, S6, S9, S10, S11, L5, L6, L9, L12, L15, L16, L18, L22, L23, and L30), and other types of structural packing of proteins [22, 38]. In addition to the compact domain, nearly half of ribosomal proteins have elongated loops or N- and C-terminal "tails" (e.g. S5, S7, S9-S14, S17, L2-L5, L15, L22, and L24), which have a considerable intramolecular mobility (Fig. 3). Thus, about 50% of amino acid residues in protein L5 are located inside 10 loops and N- and C-terminal tails [39]. Within the ribosome, the majority of these segments are stabilized by contacts with RNA and with other ribosomal proteins.

Analysis of ribosomal protein structures has revealed that in the loops and tails the contents of positively charged lysines and arginines is two to three times higher than in the globular part of the protein [22, 38]. Moreover, loops, which comprise only 18% of the structure of all proteins, are found to occupy 44% of the total surface of RNA involved in the interaction with proteins. Comparatively to other RNA-binding proteins, ribosomal proteins mainly interact with the sugar-phosphate backbone of RNA through positively charged residues of the protein chain [22, 38, 40]. The interaction with RNA occurs with involvement of from 8% (S2) to 48% (S16) or 45-50% (L2-L4) of the total surface of different ribosomal proteins [22, 38]. The specificity of interaction of the majority of ribosomal proteins with RNA seems to be mainly determined by complementarity of the charge and shape of the surfaces and not by definite amino acid residues or their combination. It was first shown for the complex L11–rRNA [41]. Thus, the diversity of the surface relief of ribosomal proteins, as well as the presence of movable and positively charged structural elements, makes a great contribution to the specific interaction of ribosomal RNAs and proteins.

The comparative analysis of spatial structures of ribosomal proteins and their intermolecular contacts within the ribosome resulted in interesting findings. Some of these findings allow us to better understand the problem of diversity of ribosomal proteins and uniqueness of their structures. Thus, the globular parts of spatial structures of proteins from different ribosomal subunits L18 and S11 (Fig. 3a) were shown to be virtually identical [38, 42], and the structure of the β-barrel of ribosomal protein L3 to be very like the structure of domain II in the elongation factors EF-Tu or EF-G [43]. Comparison of spatial structures of a 5S rRNA-binding domain of proteins L25 from *E. coli* and TL5 from *Thermus thermophilus* showed that, despite a very low homology of their primary structures (18% identical

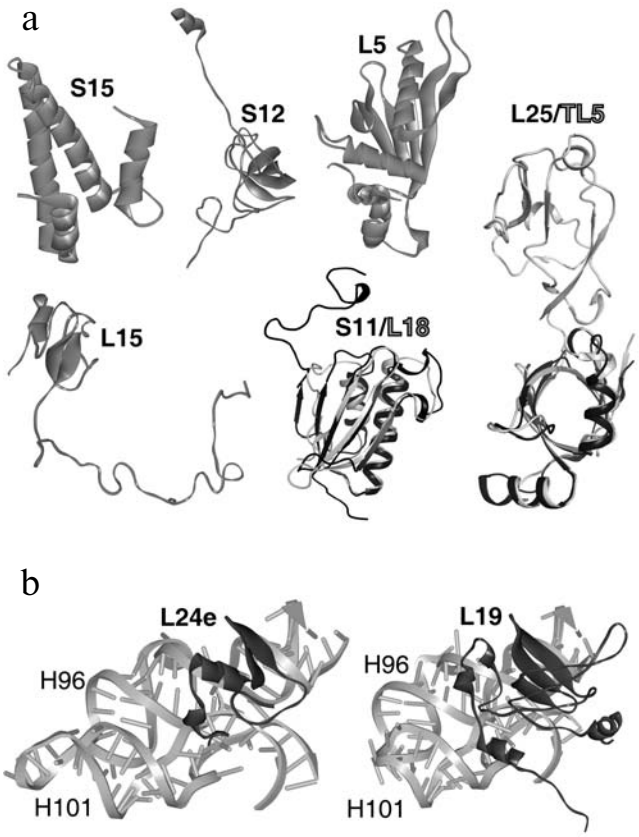

Fig. 3. Models of spatial structures of ribosomal proteins. a) Typical members of the main structural groups: S12 (β-barrels), S15 (α-proteins), L5 and L15 (α/β-sandwiches) of *Thermus thermophilus*. Comparison of structures of proteins S11/L18 and L25/TL5. b) Positions within the ribosome of functional analogs, protein L19 from *T. thermophilus* and protein L24e from *H. marismortui*. The 23S rRNA helices contacting the proteins are indicated. The models were constructed using structures of the 50S ribosomal subparticles from *T. thermophilus* (PDB code: 2J01) and from *H. marismortui* (PDB code: 1JJ2).

residues), they are spatially arranged similarly [44] (Fig. 3a). There were already mentioned six proteins of the bacterial ribosome (L17, L19, L27, L31(L28), L33, and L34) whose places in the ribosome of archaea and eucaryotes are occupied by proteins without structural homology with them. Figure 3b shows an example. One can see that proteins that are quite different in structure occupy virtually the same positions relative to structural elements of RNA.

In another recent work, interesting data were obtained about non-conservative proteins of the small ribosomal subparticle [45]. Amino acid residues of bacterial proteins S16, S18, and S20, which form contacts with RNA within the ribosome, were shown to be conservative in the archaeal and eucaryotic proteins S27e, S26e, and S25e, respectively. Therefore, it was supposed that these pairs of proteins could perform similar functions in ribosomes of different domains of life. Thus, now it can be

concluded that despite significant evolutionary changes in the protein composition of the ribosome, many principles of its structural organization retain their conservativeness.

ROLE OF PROTEINS IN FORMATION OF RIBOSOMAL SUBPARTICLES

The question about the role of individual ribosomal proteins in ribosome formation arose virtually immediately after the discovery of their diversity. Two main lines of the studies were followed: protein locating within the ribosome or on the ribosomal RNA [46-49]; elucidating the role of particular proteins in the formation of functionally active ribosomal subparticles [50-53]. In this section of the review, only the role of bacterial proteins in the assembly of ribosomal subparticles is discussed because the main results have been obtained just for them. A principal possibility of *in vitro* assembly of functionally active bacterial ribosomal subparticles was demonstrated already 40 years ago [54, 55] and opened new prospects for experimental studies on this process.

High molecular weight ribosomal RNA is capable of self-organized packing into the correct structure that determines the shape and some main morphological features of ribosomal subparticles. Thus, isolated 16S rRNA and 23S rRNA under conditions of compactization were shown to be similar in size and shape, respectively, with 30S and 50S subparticles, but in the absence of proteins this rRNA is significantly less compact than as a component of the ribosomal subparticle [56]. Thus, compactization of the ribosomal RNA structure was concluded to be a function of ribosomal proteins.

The proposed schemes of *in vitro* assembly of bacterial ribosomal subparticles [57-59] result in two main principles of this process: the proteins are incorporated step-by-step; the formation of ribosomal subparticles is accompanied by conformational changes in the structure of RNA. In the first step, a group of proteins is joined to RNA (15 proteins for the small subparticle or 22 proteins for the large one), and the majority of these proteins are universal ribosomal proteins (Fig. 4a), i.e. proteins that have been found within the ribosomes from members of all domains of life [15]. Within the bacterial ribosome structure [26-29] these universal proteins form direct contacts with rRNA (Fig. 4b), and some (S4, S7, S8, S15, S17 and L1, L2, L3, L4, L23, L24) are primary binding proteins [57-59]. In the *in vitro* reconstruction of both ribosomal subparticles (Fig. 4a), the joining of the first group of proteins is terminated by the conformational transition of ribonucleoprotein particles (reconstitution intermediate, RI) into the more compact state [57, 58].

These results were later confirmed by other researchers [56, 60-62]. Moreover, in bacterial cells precursors of both ribosomal subparticles were found, which were similar in compactness and protein composition to the *in vitro* assembled particles [63, 64]. The *in vitro* reconstruction of ribosomal subparticles clearly does not fully reproduce the conditions of their assembly within the cell. Biogenesis of bacterial ribosome occurs with involvement of dozens of special protein factors [65-67]. Nevertheless, the data mentioned above indicate that the *in vitro* assembly of ribosomal subparticles can be considered as a reproduction of this process in the bacterial cell. It seems that ribosomal components themselves contain the information that is necessary and sufficient for the correct assembly of the ribosome. Thus, in the first step of formation of bacterial ribosomal subparticles, the majority of universal proteins are joined, which leads to RNA compactization.

The recent analysis of structures of ribosomal RNAs from different organisms and organelles produced some interesting data on the possible evolution of these biological molecules [68-72]. Based on comparison of conservativeness of individual structural elements of high molecular weight RNAs and 5S rRNA, domain V of the high molecular weight RNA from the large ribosomal subparticle was concluded to be the most ancient. It seems that the precursor (domain III and a part of domain II) of contemporary RNA of the small ribosomal subparticle appeared nearly simultaneously with this RNA. A little later, the high molecular weight RNA of the large subparticle was completed by domain IV and a part of domain II. And nearly concurrently, 5S rRNA could appear in the large ribosomal subparticle [71]. The authors believe that the other domains of high molecular weight rRNAs appeared in the ribosome later. It follows from the structural data that all the universal proteins form immediate contacts with RNA in the ribosome (Fig. 4b), and this is in agreement with the earlier information concerning the binding sites of these proteins on RNA [46-49]. The majority of these intermolecular contacts occur with the evolutionarily conservative domains of RNA (Fig. 4b). These RNA–protein contacts are likely to be the most ancient in the ribosome, including the 5S rRNA contact with domain V of 23S(26S) rRNA, which is mediated by the universal proteins L5 and L18.

Note also that nearly half of the universal proteins (S2, S3, S5, S7, S11, S12, S17, L2-L4, L14-L16, L22, and L23) can interact with several domains of rRNAs (Fig. 4b), acting as interdomain clips. As noted above, evolutionarily conservative proteins S5, S7, S11, S12, S17, L2-L4, L15, and L22 have elongated loops or N- and C-terminal tails that significantly increase the area of their contact with RNA. Figure 5 exemplifies such interactions in the bacterial ribosome. It is obvious that the globular part of proteins S12, L2, and L15 interacts with one of the domains of ribosomal RNA, whereas the loops and tails of these proteins reach structural elements of the neighbor domains of RNA. Another situation is in the case of S17 (Fig. 5). This protein has a unique structure of

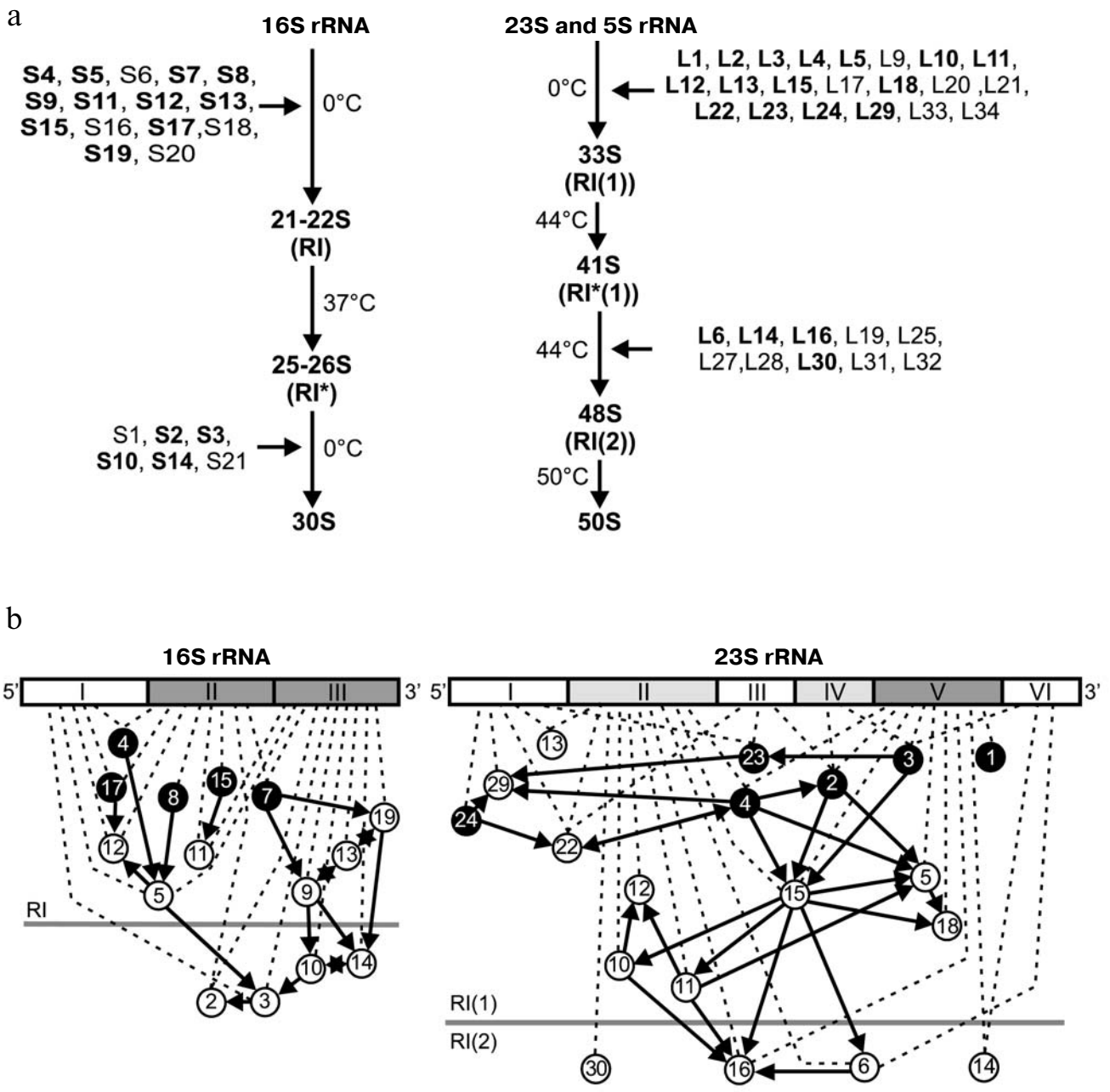

Fig. 4. Mutual influence of ribosomal proteins on their *in vitro* incorporation into the small and large ribosomal subparticles of *E. coli*. a) General schemes of the assembly of ribosomal subparticles. In the scheme there are the following indications: proteins that are joined to ribosomal RNA in different stages of the assembly (universal proteins are printed in bold); conditions of the assembly and sedimentation coefficient of the resulting ribonucleoprotein subparticles. The schemes are designed based on data from works [51, 57, 58]. b) Maps presenting interactions of universal proteins of the small and large subparticles with RNA. The mutual influence of proteins during *in vitro* assembly of the ribosomal subparticle are indicated by arrows in accordance with data of works [50, 51, 57, 59]. Main contacts of proteins with RNA within the ribosome are shown by interrupted lines, in accordance with data from works [22, 28, 29, 38]. RNAs are schematically divided onto domains colored according to their relative age: the darker are the more ancient (for details see text).

β-barrel with elongated loops and seems to be sprawled in the joint of three helices of 16S rRNA. Thus, the experimental data now available indicate that the function of at least some universal proteins, which appeared still in the proto-ribosome, is to stabilize interdomain contacts of RNA of both subparticles.

Another function of ribosomal proteins during the assembly of ribosomal subparticles is more local. The influence of some ribosomal proteins on the incorporation of other proteins into the ribosome was first shown on the *in vitro* assembly of bacterial ribosomal subparticles [57-59]. The universal proteins S5, S7-S10, S15, S17,

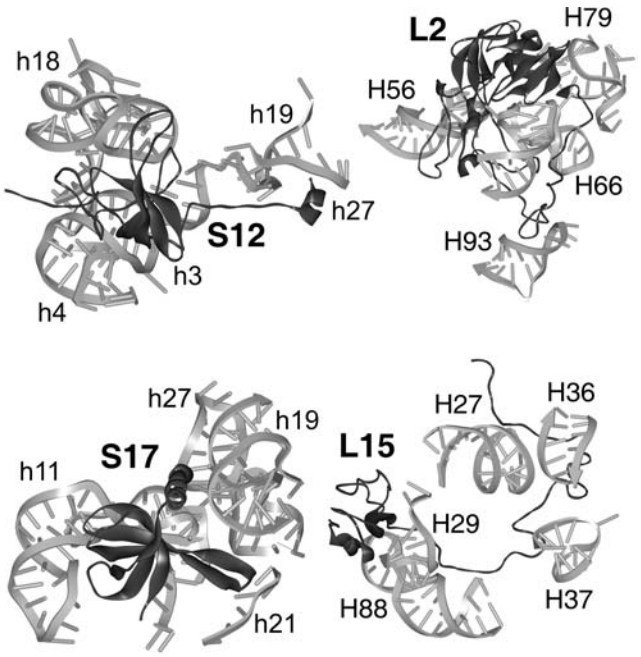

Fig. 5. Interactions of some universal ribosomal proteins from *T. thermophilus* (S12, S17, L2, and L15) with RNA in the ribosome. Helices of ribosomal RNAs (h and H for 16S and 23S, respectively) interacting with the proteins are indicated. The models are constructed using the structure of the ribosome from *T. thermophilus* (PDB codes: 2J00, 2J01).

L2-L4, L15, L23, and L24 were shown to influence the incorporation of other conservative (Fig. 4b) and also non-conservative proteins into the ribosomal subparticle. However, the mechanism of this influence is still unclear. Analysis of crystalline structures of ribosomal subparticles has somewhat elucidated this question [22, 38]. Extensive contacts found between some proteins within the ribosome (S3–S10–S14, S5–S8, L13–L20, and L23–L29) suggest that in these cases the effects detected during the assembly (Fig. 4b) are probably determined by direct contacts between the proteins. The smaller contacts in the ribosome between proteins S12–S17 and L17–L22 [22, 29, 38] also seem to confirm their mutual influence during assembly of the ribosomal subparticle. Most of these protein–protein contacts occur between universal proteins. Other cases of the influence of one protein on the binding of another are probably due to a local change in the RNA conformation during the binding of the first protein. Considering the structural conservativeness of the universal proteins, the main principles of ribosome assembly are supposed to be similar in all domains of life.

At present, general concepts are formulated concerning the role of individual groups of a protein in the ribosome formation and of fundamental mechanisms and structural bases of its assembly. However, to comprehend in detail such a complicated cooperative process as ribosome assembly, it is necessary to eliminate contradictions between the *in vitro* and *in vivo* experiments. Some conclusions based on the *in vitro* reconstruction of ribosomal subparticles are not confirmed by experiments performed on cells. Thus, the loss of one of ribosomal proteins (S9, S15, S17, L15, or L24), which play a key role in the *in vitro* assembly of ribosomal subparticles [57-59], was not lethal for the bacterial cell [33, 35, 73, 74]. Analysis of ribosomes from cells of these mutant strains revealed some defects in the ribosomal subparticles [73-76] but did not answer the question how the cells could at least partially compensate these deficiencies. Therefore, it was suggested that the ribosomal subparticle under *in vivo* conditions could be assembled even in the absence of one of the primary binding proteins. It was supposed that the bacterial ribosomal subparticle could be produced *in vivo* through alternative pathways compared to its map of *in vitro* assembly. Modern technical and methodical approaches can detect alternative pathways in the *in vitro* assembly of the 30S ribosomal subparticle [52, 53, 77]. Moreover, modern approaches of molecular genetics and gene engineering, which provide very accurate manipulations with the chromosomal genes, are now giving important new data for ribosome-associated problems [35, 67, 74, 78, 79]. Thus, we can hope that questions about specific roles of each ribosomal component in its formation will be solved in the nearest future.

INVOLVEMENT OF PROTEINS IN RIBOSOME FUNCTIONING

Studies on the role of individual proteins were started already 40 years ago, when it was shown that the elimination of protein L7/L12 from the ribosome inhibited the factor-dependent reactions of the elongation cycle [80]. The detection of diversity of ribosomal proteins stimulated long-term searches for proteins potentially responsible for specific functions of the ribosome. At present, the information accumulated about the ribosome structure and functions allows us to state that any functional center of the ribosome is not a privilege of RNA or a protein. The ability of the ribosome to synthesize polypeptides is determined by its three main functions: it can bind and move translation ligands and catalyze the generation of peptide bonds. Long-term studies revealed the ribosome regions (functional sites) responsible for these functions. Usually the following functional sites of the ribosome are discriminated: the site responsible for mRNA binding and the decoding center (the small subparticle); the site responsible for binding protein factors of elongation (the GTPase-associated center); and the peptidyl transferase center (PTC) (the large subparticle); A-, P-, and E-tRNA-binding sites (both subparticles). Another feature of ribosomal subparticles should also be noted, which is directly related with translation – they are capable of associating into a functioning ribosome.

Ribosomal subparticles are kept together mainly due to intersubunit bridges [27-29, 81]. In the structure of the bacterial ribosome there are 12 such bridges, and within the eucaryotic ribosome the majority of the bridges are also conservative [24, 32, 81]. Both RNA and proteins are involved in producing these contacts. Ribosomal proteins are involved in formation of more than half of the known intersubunit bridges: B1a (S13), B1b (S13 and L5), B4 (S15), B5 (L14), B6 (L19), B7b (L2), and B8 (L14) (Figs. 6a and 6b). Based on intermolecular crosslinks, all these proteins except for protein L14 were earlier believed to be involved in association of ribosomal subparticles [82]. Note that all the above-mentioned proteins, except L19, are universal proteins. As to protein L19, it was already mentioned (Fig. 3b) that its functional analog L24e [22] was found in ribosomes of archaea and eucaryotes, and this protein is also involved in formation of an intersubunit bridge in the eukaryotic ribosome [24, 32].

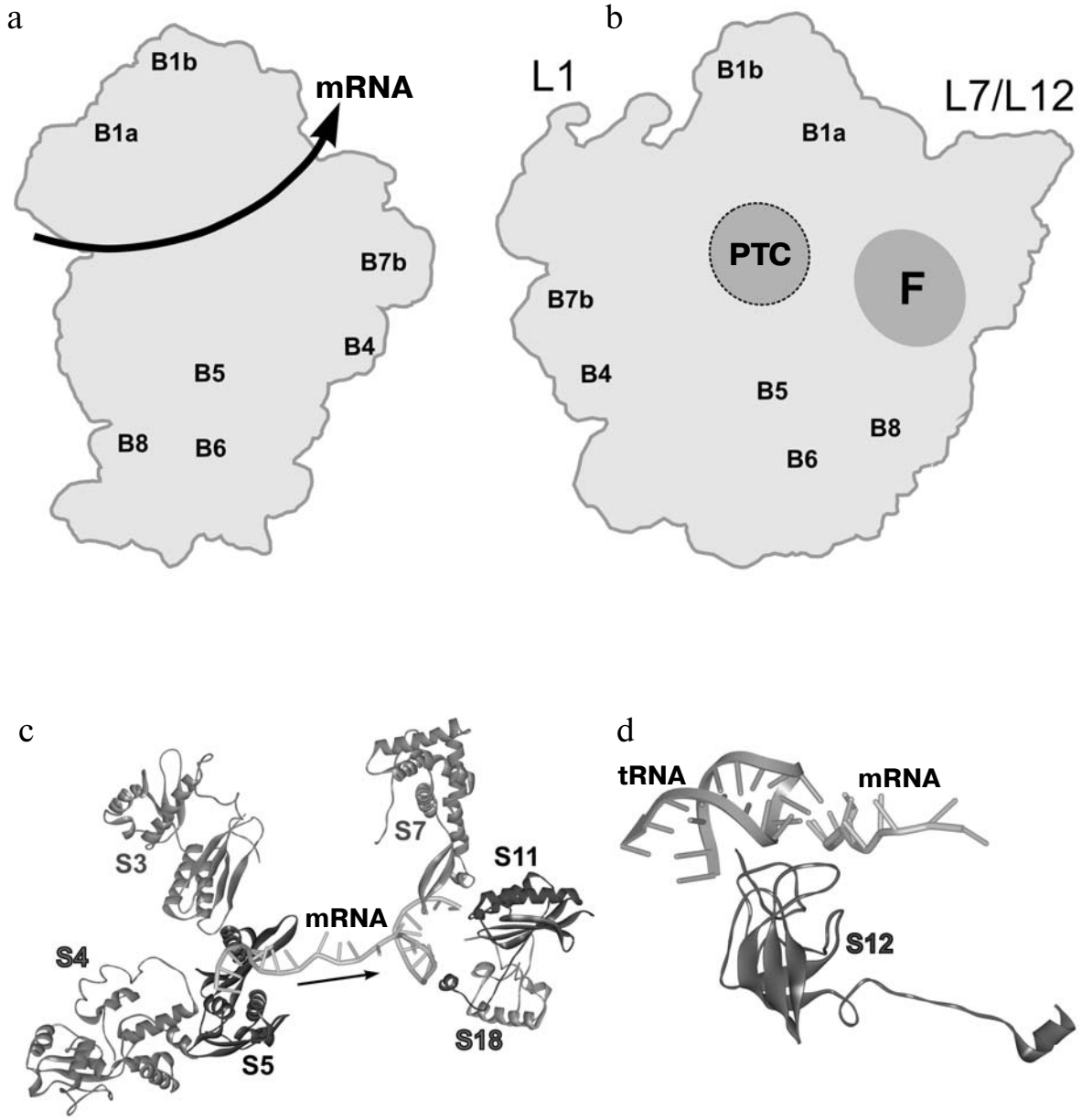

Fig. 6. a, b) Schemes of contacting surfaces of the bacterial small and large ribosomal subparticles. Positions of intersubunit bridges formed with involvement of proteins, the messenger RNA, and functional centers (F is the binding site of protein elongation factors, PTC is the peptidyl transferase center) are indicated on the surface of the subparticles. c) Mutual locations of mRNA and proteins of the small ribosomal subparticle interacting with it. The arrow indicates the mRNA direction from the 3′- to the 5′-terminus. d) Mutual locations of mRNA, tRNA (the anticodon stem), and protein S12 in the decoding center of the ribosome. The models were constructed using structures of the 30S ribosomal subparticle from *T. thermophilus* (PDB code: 2J00).

Two proteins, L5 and S13, which form an intersubunit bridge, must be specially noted. First, bridge B1b is the only protein–protein bridge within the bacterial ribosome. It is produced with involvement of the N-terminal tail of S13 protein and two long loops of protein L5 [27, 81]. Second, protein S13 forms two intersubunit bridges: one with protein L5 and the other (B1a) with helix 38 of 23S rRNA (the A-site finger). Third, both proteins produce contacts with tRNA located in the P-site of the ribosome [27, 29]. Based on structural studies of functional complexes of ribosomes [27-29, 81, 83, 84], it was supposed that intersubunit bridges produced by proteins and located on the ribosome periphery should play an important role in large-scale conformational rearrangements, e.g. in translocation of tRNA.

It was shown earlier that mRNA could bind with the 30S subparticle (Fig. 6a) in the cavity between the "head" and "body" [85]. Using the approach of intermolecular crosslinking, proteins S1, S3, S4, S5, S7, S9, S11, S13, S18, and S21 were shown to be located closely around mRNA [86-89]. Recent structural studies essentially confirmed these data [29, 90, 91]. Side chains of positively charged amino acid residues of proteins S3, S4, and S5 (Fig. 6c) were shown to be involved in formation of the so-called "entrance" for mRNA into the 30S subparticle. After mRNA enters into the small ribosomal subparticle, it is translocated on this cavity and at the exit produces a contact with three ribosomal proteins, S7, S11, and S18 (Fig. 6c) [90, 91]. Thus, mRNA is moved within the ribosome under the control of at least six proteins of the small ribosomal subunit, and the majority of these proteins are universal.

The selection of specific aminoacyl-tRNAs on their binding in the A-region according to the mRNA codon located within it is one of the main functions of the 30S subparticle (the decoding function). Nucleotides A1492, A1493, and G530 of 16S rRNA control the base-pairing in the first and second positions of the codon [92, 93], and amino acid residues of ribosomal protein S12 additionally control the base-pairing in the second and third positions (Fig. 6d). X-Ray crystallography data show that local changes in the decoding center on binding the specific aminoacyl-tRNA cause large-scale changes in the structure of the 30S subparticle, resulting in its transition from the "open" conformation into the "closed" one [93]. This conformational transition leads to changes in the interaction between proteins S4 and S5 and also protein S12 and 16S rRNA. This model of the participation of ribosomal proteins S4, S5, and S12 in functioning of the decoding center of the ribosome is in agreement with results of other studies [94-96]. The accuracy of translation was altered in cells of strains containing mutant forms of the above-mentioned proteins. Thus, on taking into account all these data, both immediate and RNA-mediated participation of proteins S4, S5, and S12 in providing translation accuracy can be considered as proved.

Now it is believed that during translation three tRNAs can be present in the ribosome. Based on intermolecular crosslinks, it was earlier shown that tRNA in the ribosome could have as neighbors nearly half of the proteins of the small ribosomal subparticle (S5, S7, S9, S10, S11, S12, S13, S19, and S21) and of the large one (L1, L2, L5, L11, L13, L14, L16, L27, and L33) [97-100]. Contacts of some of these proteins with tRNA were confirmed by crystallographic studies [27, 29, 101, 102]. Thus, in the A-site of the ribosome, tRNA has contacts with proteins S12, S13, L16, and L27 (Figs. 6d and 7). In the P-site of the ribosome, tRNA has contacts with ribosomal proteins S9, S13, L5, and L27 (Fig. 7). Long loops and N- or C-terminal tails of the proteins most often interact with tRNA. And on interacting with tRNA, the structure of these elements of the ribosomal proteins is stabilized. A possible involvement of protein L16 in tRNA binding with the A-site was reported earlier [100, 103]. Within the 70S ribosome structure, amino acid residues Arg51 and Arg56 of protein L16 are involved in contact with U54, G53, and G56 (the "elbow" region) of tRNA [102]. Conservative amino acid residues of protein L5 loop β2-β3 are involved in interaction with the "elbow" region of tRNA in the P-site [29]. Long C-terminal tails of ribosomal proteins S9 and S13 (Fig. 7) are located at the distance of contact with the anticodon stem of tRNA [27, 29, 102]. And protein S13 tail is located at nearly the same distance from tRNA in the A- and P-site. The deletion of some C-terminal residues in proteins S9 or S13

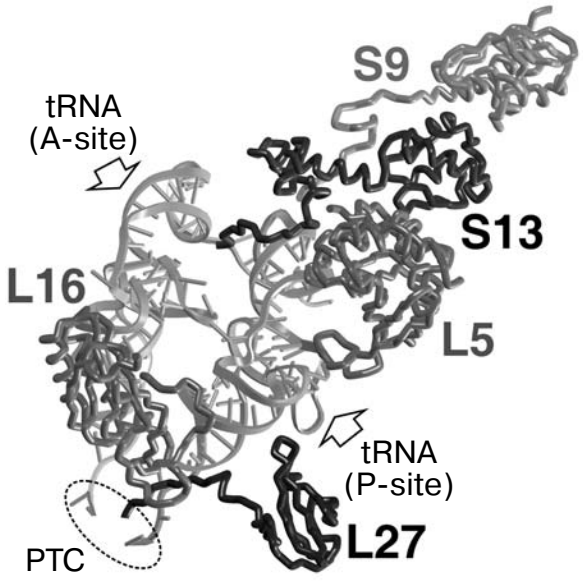

Fig. 7. Model of a structural fragment of the bacterial ribosome illustrating possible contacts of tRNA in the A- and P-sites with proteins of the small and large ribosomal subunits. Positions of the proteins, tRNA, and the region of the peptidyl transferase center (PTC) are indicated. The model was constructed using the structure of the ribosome from *T. thermophilus* (PDB codes: 2J00, 2J01).

influences insignificantly the growth of *E. coli* cells but decreases the affinity of tRNA for the 30S subunit and increases frameshift probability [104]. The distance between the L27 protein N-terminal tail and the 3′-ends of tRNA in the A- and P-sites (Fig. 7) is sufficient for producing the intermolecular contact [29, 101]. The possibility of such a contact is confirmed by inability of ribosomes containing protein L27 with deletions of three N-terminal residues to bind tRNA in the A-site [105].

According to the structural data, ribosomal proteins S7, S11, L1, L28, and L33 can contact with tRNA in the E-site of the bacterial ribosome [27, 29, 101]. Protein S7 has a contact with tRNA and/or mRNA in the E-site of the ribosome through a long β-hairpin. The shortening of this structural element in protein S7 or mutations destroying the contact of S7 and S11 proteins decrease translation accuracy [106, 107]. These data confirm the hypothesis that the E-site components can contribute to maintaining the reading frame [108]. Protein L1 forms a contact with the "elbow" region of tRNA in the E-site and promotes the elimination of deacylated tRNA from the ribosome. The absence of this protein in ribosomes of *E. coli* results in a decrease in their protein-synthesizing activity [109].

The peptidyl transferase center (PTC) is fully located on the large ribosomal subparticle (Fig. 6b) [110]. The ribosomal subparticle components related with the PTC were searched for by different approaches: intermolecular crosslinking, partial disassembly, or assembly of the ribosomal subparticle. More than ten proteins of the ribosomal subparticle were considered as candidates [103, 111, 112], but later the PTC was shown to be formed by 23S rRNA nucleotides [113, 114]. Only ribosomal protein L27 located rather far from the PTC can reach it through its N-terminal tail [26] (Fig. 7). Ribosomes lacking protein L27 [115] or containing a mutant protein [105] display markedly reduced peptidyl transferase activity, and the authors explain this to be due to the worsened ability of the mutant ribosomes to provide for factor-dependent binding of aminoacyl-tRNA in the ribosome A-site. It has been said above that the N-terminal tail of protein L27 can form a contact also with the 3′-end of tRNA in the A-site of the ribosome; therefore, this contact is likely to be favorable for the more accurate positioning of the 3′-ends of tRNA in the PTC and thus to promote the peptide transfer. However, it has been already said that protein L27 is specific for the bacterial ribosome, whereas its functional analog, protein L21e, occupying a similar place in the ribosomes of archaea and eucaryotes, does not have a tail capable of reaching the PTC. It seems that this situation is characteristic only for bacteria.

Elongation factors EF-Tu and EF-G interact with the 50S subparticle at the base of the L7/L12-protuberance (Fig. 6b), mainly with domains II (the GTPase-associated center) and VI (the sarcin-ricin loop) of 23S rRNA [116-118]. The protuberance L7/L12 is the only morphological element of the ribosome consisting of proteins (two dimers of protein L7/L12 and protein L10). A selective removal from the ribosome of tetramer L7/L12 inhibited factor-dependent reactions of the elongation cycle [80, 119]. And protein L11 binds with 23S rRNA at the base of the L7/L12 protuberance in the region of the GTPase-associated center [120]. The absence in the ribosome of protein L11 essentially inhibited the EF-G-dependent hydrolysis of GTP on the ribosome [121]. Based on intermolecular crosslinks, possible neighbors were determined for factors EF-Tu and EF-G, which included proteins L1, L3, L6, L7/L12, L11, L14, S3, S4, S12, and S19 [122, 123]. According to data of X-crystallography, only proteins L6, L7/L12, L11, and S12 can form contacts within the ribosome with the above-mentioned elongation factors [101, 124]. Taking into account locations of the protein factors on the ribosome and also the protein S12 contact with the anticodon stem of tRNA in the A-site (Fig. 6), just these ribosomal proteins are concluded to be involved in formation of the factor-binding center.

Thus, all the presented data indicate that the majority of universal proteins can play a key role not only in ribosome formation but also in its functioning.

To end the review, we would like to formulate the main conclusions and to say something about prospects in further studies on ribosomal proteins. Based on the available experimental data, we conclude that contemporary ribosomes of three domains of life contain a group of universal proteins that succeeded in retention of their structure and functions during evolution. The conservativeness of the majority of proteins of this group manifests itself on all levels, from the primary structures and to their functioning within the ribosome. It is reasonably to suppose that the universal ribosomal proteins appeared in the ribosomes already before the divergence of the main branches of life. It seems that the main principles of the structural organization of these proteins and their functions were determined already in the proto-ribosome. Moreover, comparative analysis of structures of ribosomes from different domains of life allows us to detect, at least, another group of ribosomal proteins that are functional analogs and the existence of which also indicates high evolutionary conservativeness of the ribosome structure as it is. Thus, it seems that the evolutionary conservativeness of the ribosome can be significantly higher than it was expected. Despite the half-century of studies, this unique molecular "machine" seems to retain many secrets for researchers. Thus, further complex comparative studies on universal proteins and their functions may elucidate problems of ribosome origin and evolution. At the same time, studies on proteins that have been acquired by archaea, bacteria, and eucaryotes during evolution have not only fundamental but also applied importance, because just the ribosome is a target for such agents as antibiotics and toxins.

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