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REVIEW

5S rRNA and Ribosome

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Abstract—5S rRNA is an integral component of the ribosome of all living organisms. It is known that the ribosome without 5S rRNA is functionally inactive. However, the question about the specific role of this RNA in functioning of the translation apparatus is still open. This review presents a brief history of the discovery of 5S rRNA and studies of its origin and localization in the ribosome. The previously expressed hypotheses about the role of this RNA in the functioning of the ribosome are discussed considering the unique location of 5S rRNA in the ribosome and its intermolecular contacts. Based on analysis of the current data on ribosome structure and its functional complexes, the role of 5S rRNA as an intermediary between ribosome functional domains is discussed.

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Key words: 5S rRNA, ribosomal proteins, intermolecular interactions, ribosome, translation

The discovery of ribosomal 5S RNA (5S rRNA) is directly associated with studies on another low molecular weight cellular RNA, transfer RNA (tRNA). By the beginning of the 1960s there was already rather abundant information about features of tRNA (see [1] for a review). In particular, just tRNA was known to deliver into the ribosome amino acids required for synthesis of a polypeptide. Three 3′-terminal nucleotides of tRNA and a special enzymatic fraction "pH 5 enzyme" (tRNA synthase) were required for binding the amino acid residue to this RNA. During studies on the tRNA and ribosome interaction a low molecular weight RNA fraction was detected that was very tightly kept by the large ribosomal subparticle [2-4]. The nucleotide composition and size of molecules of this RNA fraction (about 100 nucleotides) and of tRNA (70-80 nucleotides) proved to be similar [2, 5, 6]. The sedimentation coefficients of these molecules also were close: 5S and 4S, respectively. Based on these characteristics, the newly detected RNA was called "transfer-like RNA" [2, 6], and it was even supposed to be a precursor of tRNA [6, 7]. However, not all authors adopted this explanation of the findings. Thus, R. Rosset and R. Monier found that the difference between these RNAs was more pronounced than the resemblance [8]. In fact, as opposed to tRNA, 5S RNA (or the so-called "transfer-like RNA") did not contain modified bases, had no 3′-terminal CCA triplet, and was unable to form aminoacyl-RNA, i.e. to bind an amino acid residue [6, 8]. As opposed to tRNA, 5S rRNA was very tightly kept by the large ribosomal subparticle and could be detached from it only under severe conditions (in the presence of SDS or at high concentrations of salts) [3, 4, 9, 10]. In *Escherichia coli* cells 5S RNA was found in the ribosomes but not in the cytoplasm [5, 8, 11]. Later, 5S RNA was also found in the ribosomes of other organisms [12-14]. The uniqueness of the 5S RNA molecule was also confirmed in experiments with DNA–RNA hybridization [15] and mapping of its oligonucleotides [16]. The last doubts on the 5S RNA being an independent type of cellular molecules disappeared after its primary structures was determined [17, 18]. Primary structures of 5S RNA from *E. coli* and from eukaryotic cells displayed a pronounced homology [19]. But no sufficiently extended homology between nucleotide sequences of 5S RNA and tRNA was found. Thus, by the end of the 1960s the first question was answered. The low molecular weight RNA found in the ribosomes was shown to be a new type of ribosomal RNA, 5S rRNA.

However, this answer, or more exactly, just the existence of 5S rRNA, raised a number of other quite reasonable questions. But any question concerning ribosomal 5S RNA during the last half-century was always associated with the apparently most important problem: what is this RNA required for in the ribosome? The three following sections of this review consider the triunity of the problem of this small molecule. For a more complete picture

of the problem and approaches to its solution, the literature data are presented chronologically. The future seems to be promising for young enthusiasts: despite studies for a half of century, the question what 5S rRNA is required for the ribosome is still open.

LOCATION OF 5S rRNA IN THE RIBOSOME

As mentioned above, the fist step in locating 5S rRNA in the cell was simultaneous with its discovery. 5S RNA was found only in the large ribosomal subparticle [3, 4, 9, 10]. Concurrently two fundamental discoveries were made that became a starting point for studies on the role of 5S rRNA in the formation of the ribosome. First, the possibility of step-by-step *in vitro* disassembly of ribosomal subparticles was demonstrated [20-23], as well as the assembly of functionally active ribosomal subparticles from separate components of *E. coli* and *Bacillus stearothermophilus* [24-27]. Second, the bacterial cell was shown to contain precursors of ribosomal subparticles [28-31] possessing features and components that corresponded to those of particles prepared in the *in vitro* reconstruction experiments [32]. Experimental approaches were developed for elucidation of the role of 5S rRNA in the disassembly and assembly of the ribosome. It was already known that 5S rRNA was released from the large ribosomal subparticle at the initial stages of unfolding or disassembly of the latter [9, 10, 33-35]. Analysis of natural precursors of the large ribosomal subunit found in *E. coli* cells revealed that 5S rRNA was present not only in the late precursor (the 43S or $p_2$50S particles) of the 50S ribosomal subunit [36] but even in its early precursor (32S or $p_1$50S) [30, 37, 38]. This suggested that 5S rRNA could be incorporated into the ribosomal subunit at the early stage of its assembly in the cell. These data were mainly confirmed by experiments on the *in vitro* reconstruction of the 50S ribosomal subparticle [32, 39]. The ability of the 5S rRNA to incorporate into the ribosomal subparticle at any stage of the reconstruction of the functionally active subparticle was shown. However, the early precursor of the ribosomal subparticle ($p_1$50S) already containing 5S rRNA [30, 37] possessed only half of the ribosomal proteins [38]. Among proteins of these particles the proteins L5 and L18 [40] or even three proteins were capable of binding 5S rRNA [38]. Similar results were obtained on the *in vitro* reconstruction of the 50S ribosomal subparticle and its intermediates [32, 39, 41]. Moreover, the large ribosomal subparticle of *E. coli* reconstructed *in vitro* in the absence of 5S rRNA did not have the three 5S rRNA-binding proteins (L5, L18, and L25) and the protein L16 [32, 42]. Based on these findings, it was suggested that the incorporation of protein L16 into the ribosomal particle should depend on the presence of the 5S rRNA–protein complex. Other researchers also reported that at least the proteins L5 and L18 were sufficient and necessary for the interaction of 5S rRNA with 23S rRNA [43, 44]. At the same time, Rohl and Nierhaus observed that on the *in vitro* assembly of the 50S subparticle other proteins, in addition to proteins L5 and L18, were required for the interaction of 5S rRNA with 23S rRNA [42]. For the *in vitro* incorporation of 5S rRNA into ribonucleoprotein particles the protein L15 (Fig. 1) was also needed, whereas protein L2 and possibly L3 and L4 had an influence on this process [42]. However, in the precursor ($p_1$50S) of the *E. coli* ribosomal subparticle possessing the 5S rRNA–protein complex these proteins were not found earlier [38]. Moreover, a viable mutant of *E. coli* was found that did not have the ribosomal protein L15 [45, 46]. Studies on the ribosomes of this mutant strain [47-49] somewhat corrected the scheme of the assembly of the 50S ribosomal subparticle. Because there were no data on the direct interaction of the above-mentioned proteins (L15, L2, L3, and L4) with the 5S rRNA–protein complex, it was concluded that these proteins could only accelerate the necessary conformational changes in 23S rRNA [41, 49] that could also occur in their absence. These findings suggested that 5S rRNA, or more likely the 5S rRNA–protein complex, should be rather an independent structural element (domain) of a large ribosomal subunit, and only the proteins L5 and L18 were required for its interaction with 23S rRNA.

For the next step, namely, for locating 5S rRNA in the ribosomal subparticle, it was necessary to associate it with the ribosome topography. In the mid-1970s several research groups nearly concurrently published works describing in detail the morphology of the *E. coli* 50S ribosomal subparticle [50-52]. A typical projection of the 50S ribosomal subparticle, a crown-like projection, had three characteristic protuberances. Ribosomal subparticles of both prokaryotes and eukaryotes appeared to have a similar morphology [51, 53, 54]. Advances in analytical

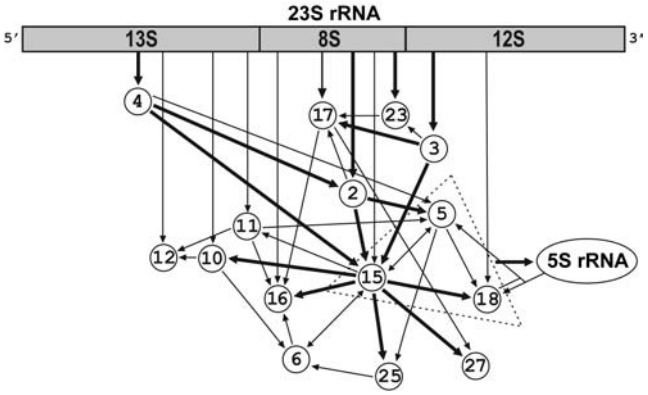

Fig. 1. Reciprocal influences of some ribosomal proteins on their *in vitro* incorporation into the 50S ribosomal subparticle of *E. coli*. The arrows indicate influences of some proteins on incorporation of the others (thick and thin lines show, respectively, strong and weak effects). The scheme is compiled using data from work [42].

approaches of molecular biology during that period allowed researchers to identify all of the individual proteins of the bacterial ribosome [55]. All these achievements promoted the next step in the study of ribosome structure, the mapping of the ribosomal components. Immunological approaches (using specific antibodies to individual ribosomal components) combined with electron microscopy resulted in a new approach – immunoelectron microscopy. This approach provided a burst in the study on morphology and structure of such a huge macromolecule as a ribosome. Two lateral protuberances were called L1 protuberance and L7/L12 stalk, according to the ribosomal proteins located inside them [52, 56-59]. The 5S rRNA was detected in the third, central protuberance of the 50S ribosomal subparticle. The 3′-5′-end of the 5S rRNA molecule was located nearly simultaneously by the groups of A. Bogdanov and V. Vasiliev [60] and of G. Stoffler [61]. For this locating different haptens covalently linked to ribose of the 3′-terminal nucleotide of 5S rRNA were used. In the two works the 3′-terminal nucleotide was located virtually on the apex of the central protuberance of the ribosomal subparticle (Fig. 2a). The Russian authors continued their study for locating 5S rRNA in the ribosome [62] and located the nucleotides A_{39} and U_{40} of the 5S rRNA loop C (Fig. 2a). The distance determined between these two regions of 5S RNA (less than 50 Å) was in good agreement with the Y-like model of this molecule proposed earlier by P. Osterberg [63]. And V. Vasiliev and O. Zalite found that morphological features of the 50S subparticle (Fig. 2b) including its protuberances were determined by the shape of 23S rRNA [64]. Under conditions of compactization, the isolated 23S rRNA had shape and size comparable to those of the ribosomal subparticle. The central protuberance of these particles had similar size. Consequently, the central protuberance of the 50S subparticles is produced with involvement not only of 5S rRNA but also 23S rRNA. This conclusion was confirmed by results of studies on protein-deficient 50S ribosomal particles of *E. coli* [65]. Ribonucleoprotein particles consisting only of 23S rRNA and nine ribosomal proteins were shown to be very like the 50S ribosomal subparticle in the size and shape. Thus, already by the mid-1980s two conclusions could be made: (i) 5S rRNA was located in the central protuberance of the large ribosomal subparticle; (ii) 5S rRNA was only one of the structural elements of the central protuberance of the ribosomal subparticle.

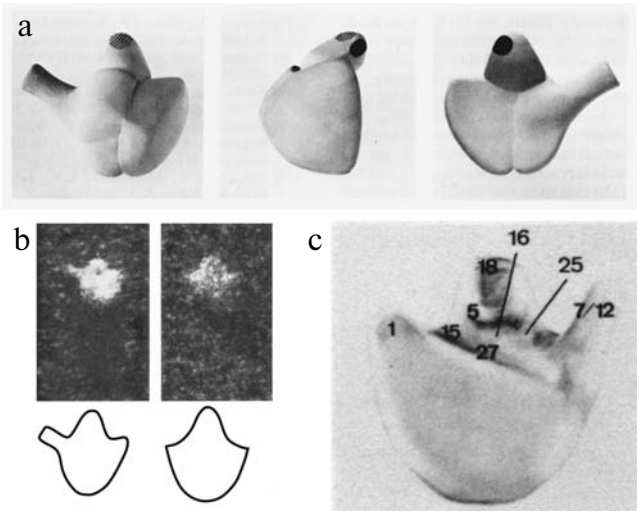

Fig. 2. Location of 5S rRNA–protein complex in 50S ribosomal subparticle. a) Positions of 5S rRNA regions in the central protuberance (positions of A_{39} and U_{40} of the C loop are indicated by darkening; the 3′-terminal nucleotide position is shown by hatching). b) Contribution of 23S rRNA to formation of the central protuberance. The right panel presents an isolated 23S rRNA under conditions of compactization, the left panel presents the 50S ribosomal particle. Figures 2a and 2b are taken from works [62, 64] under the kind permission of V. D. Vasiliev. c) Location of proteins in the central protuberance of the 50S ribosomal subparticle of *E. coli* (positions of proteins of lateral protuberances are given for orientation). The figure based of work [68] is modified according to the problem under consideration.

After the 5S rRNA was located in the ribosome, it was important to determine its intermolecular contacts. This could be performed using direct or indirect approaches. Certainly, the most direct approach would be X-ray crystallography, but crystallographic data on the ribosome structure appeared much later. Other more or less direct approaches for determination of intermolecular contacts of 5S rRNA in the ribosome were very difficult because of the multicomponent composition of this ribonucleoprotein consisting of several RNAs and dozens of proteins. Therefore, during this period researchers often had to use indirect approaches. These indirect approaches, which were also used for detecting other ribosomal components interacting with 5S rRNA, include immunoelectron microscopy and intermolecular chemical cross-linking. As mentioned above, from immunoelectron microscopy the central protuberance of the 50S subparticle was shown to contain not only 5S rRNA but also 23S rRNA. Protein L18 was one of the first proteins found in the protuberance of the large ribosomal subparticle [50]. However, the data of this work concerning the subparticle protuberances were insufficiently clear (the preliminary model of the 50S subparticle did not yet have a pronounced asymmetry of protuberances). Therefore, the authors suggested that protein L18 should be located in a lateral protuberance. Further studies, including those of the same authors, corrected the location of protein L18 in the ribosome. In 1983 the group of G. Stoffler found that protein L18 located in the central protuberance of the 50S subparticle [66] (Fig. 2c). A little later two other 5S rRNA-binding proteins, L5 and L25, were also located in the central protuberance [67, 68]. These data were a good confirmation of earlier findings that ribosomal proteins L5, L18, and L25 could specifically bind with the isolated 5S rRNA [69]. In Fig. 2c the

location of proteins of the central protuberance of the 50S ribosomal subparticle is indicated in accordance with the data of the above-mentioned works. Protein L25 is located at the base of the central protuberance of the 50S ribosomal subparticle at the side of the L7/L12 prominence. Protein L18 is located nearly at the apex of the central protuberance from the side of the L1 prominence. Protein L5 is placed near protein L18 but on the protuberance surface contacting the 30S ribosomal subparticle. As shown in Fig. 2c, some ribosomal proteins (L15, L16, and L27) are located at the base of the 50S subparticle central protuberance. At the same time, using limited enzymatic hydrolysis of rRNA within the ribosome, P. Brimacombe's group found a region of 23S rRNA (the region 450-1000 nucleotides from the 3′-end of the molecule) interacting with the 5S rRNA–protein complex [70]. Similar results were also obtained by the group of P. Zimmermann [71]. Later, the 50S ribosomal subparticle of *E. coli* was topographically mapped in detail using bifunctional reagents, which permitted RNA cross-linking with protein [72]. In particular, both 5S rRNA-binding proteins, L5 and L18, were shown to have a similar cross-linking region on the 23S rRNA molecule (helix H84, U_{2305}–C_{2310}, and G_{2307}–U_{2320}, respectively). In the same region (helix H81-H87) of domain V of 23S rRNA protein L27, which was another component of the central protuberance of the large ribosomal particle, was also cross-linked. The development of photo-activated bifunctional reagents made it possible to visualize special intermolecular RNA–RNA cross-linking within the ribosome. Thus, the cross-linking of 5S rRNA with domains II and V of 23S rRNA in the *E. coli* ribosome was found in the collaboration of Russian and American researchers (the groups of A. Bogdanov and O. Dontsova and of P. Brimacombe, respectively) [73-76]. Figure 3 schematically presents the results of all these investigations. Data on intermolecular cross-linking were obtained using different photo-activated uridine derivatives randomly incorporated into the 5S rRNA molecule. The most active in the 5S rRNA molecule was U_{89} located in the apical loop of the second domain (helices IV and V, loops E and D). This nucleotide was cross-linked with 23S rRNA nucleotides located at a significant distance in the nucleotide sequence (domain II – U_{958}, A_{960}, G_{1022}, G_{1138} and domain V – C_{2475}(U_{2477})). Moreover, the first domain (helices I, II, and III, the loops B and C) of the 5S rRNA containing photo-activated U_{40}, U_{48}, U_{55}, and U_{65} was cross-linked with two regions of 23S rRNA (helices H81-H85 (U_{2272}–G_{2345}) in domain V and the distal part H38 (C_{865}–U_{929}) of domain II) (Fig. 3). Thus,

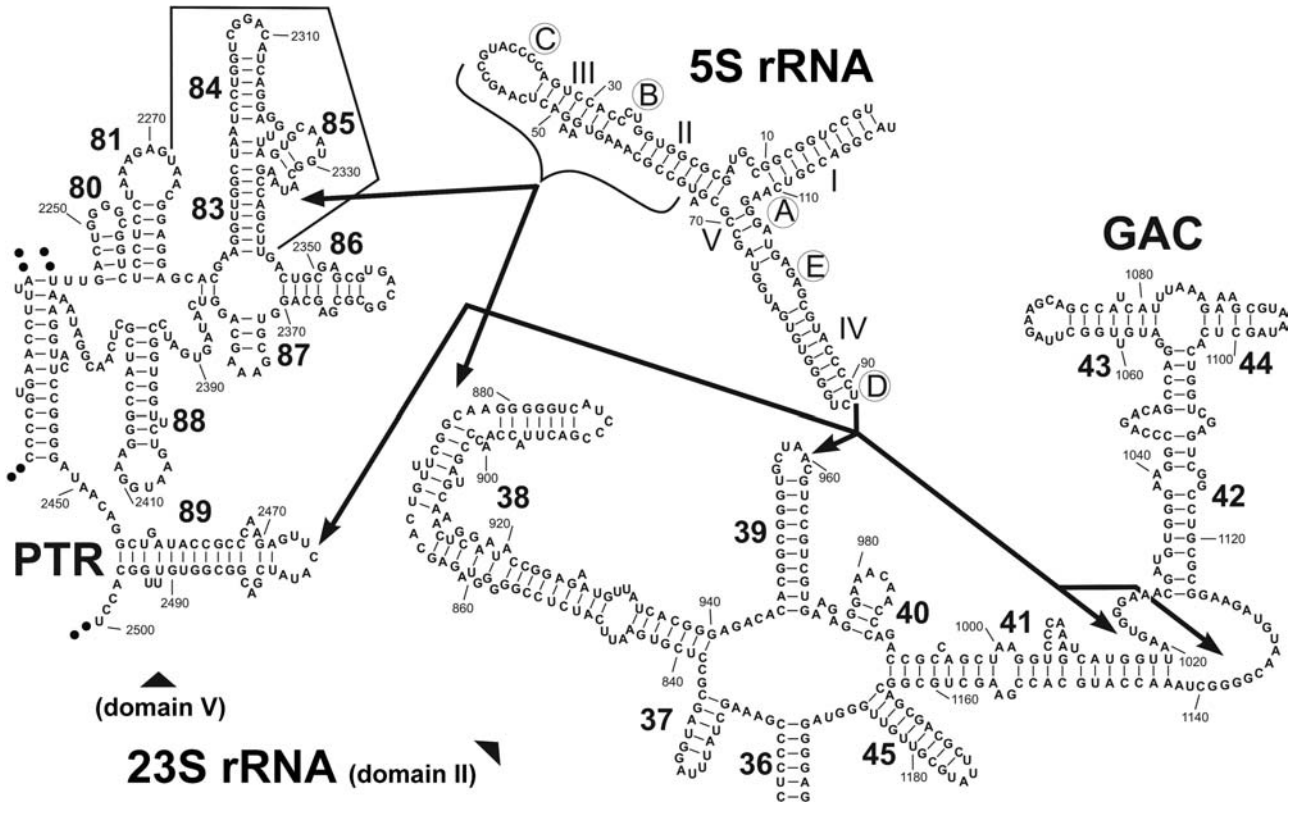

Fig. 3. Scheme of secondary structure of ribosomal RNAs with indicated regions of cross-links between 5S rRNA and 23S rRNA in the *E. coli* ribosome. Large Arabian ciphers indicate the numbers of helices in 23S rRNA, Roman numerals show their numbers in 5S rRNA. For the scheme data of works [73-76] are used.

some regions of domains II (helices H38, H39, and loop H41-H42) and V (helices H81-H85 and H89) of 23S rRNA were shown to be the closest neighbors of 5S rRNA (Fig. 3). These findings led to two conclusions: (i) the above-mentioned structural elements of the 23S rRNA together with 5S rRNA seemed to form the central protuberance of the 50S ribosomal subparticle; (ii) the 5S rRNA could serve a link between two domains of 23S rRNA.

Thus, by the second half of the 1990s components of the central protuberance of the bacterial ribosome large subparticle had been identified completely. The central protuberance of the large ribosomal subparticle was considered to be a 5S rRNA–protein complex with attached structural elements of domain II (helices H38, H39, and loop H41-H42) and of domain V (helices H81-H85 and H89) of 23S rRNA, with proteins L15, L16, and L27 in the bottom of the protuberance.

Contemporary crystallographic data on the structure of ribosomes from different domains of life obtained during the last decade [77-82] allowed researchers to dot many i's, in particular, in locating 5S rRNA in the ribosome. First, the majority of the data obtained earlier by different methods [60-76] was supported by data of crystallography. Figure 4 shows complete coincidence of the 5S rRNA location in the central protuberance of the large ribosomal subparticle with its location determined earlier. The conclusions that the central protuberance of the bacterial ribosome should include proteins L16 and L27, as well as structural elements of domains II and V of 23S rRNA were confirmed (Fig. 4a). However, the locating in the central protuberance of some small ribosomal proteins (L30, L33, and L35) must be considered to be an achievement of the crystallographic studies.

Second, analyses of all structures of ribosomes and ribosomal subparticles that are now known indicate that the location of 5S rRNA is conservative in ribosomes of all three domains of life (Fig. 4). Such location of 5S rRNA was mainly determined by two groups of conservative contacts in the ribosomes of various organisms. The conservativeness of these intermolecular contacts was earlier revealed on analyzing structures of the ribosomes of bacteria and archaea [83-85]. At present, the same can be shown for representatives of all domains of life (Fig. 4, b-d). One of these contacts is located in the first domain of 5S rRNA and the region of helices H83-H85 of 23S rRNA and is mediated through proteins L5 and L18. The contact of these 5S rRNA-binding proteins with the indicated region of 23S rRNA was predicted earlier based on results of intermolecular cross-linking [72]. The modern crystallographic data show that this intermolecular interaction is essentially retained in ribosomes of bacteria, eukaryotes, and archaea (Fig. 4, b-d). Another conservative contact is realized directly between helix H38 of 23S rRNA and the inner loop E of 5S rRNA, thus fixing the location of the second domain of the small ribosomal RNA. This RNA–RNA contact, detected for the first time in the structure of 50S ribosomal subparticle of *Haloarcula marismortui* [77, 86], seems to be typical for ribosomes of the other domains of life (Fig. 4, b-d). Thus, it seems that these conservative contacts not only determine the unique location of 5S rRNA in the ribosome but are also responsible for functioning of this small RNA as a mediator between domains II and V of the high molecular weight RNA of the large ribosomal subparticle.

Third, it is now possible to compare the contribution of proteins to determination of the unique and conservative location of 5S rRNA in the central protuberance of the large ribosomal subparticle from evolutionally distant organisms. The involvement of ribosomal proteins of the L5 and L18 families in the interaction of two rRNAs has been mentioned above. Although the immediate contact of helix H38 and 5S rRNA is rather extensive, it seems to need an additional stabilization in the functioning ribosome. In the ribosomes of *Saccharomyces cerevisiae* at least three proteins (L10e, L21e, and L7e-L30p) interact simultaneously with 5S rRNA and helix H38 of 25S rRNA [82]. The bacterial protein L16 (136 residues in *E. coli*), which is a homolog of the archaeal protein (163 residues in *H. marismortui*) and of the eukaryotic protein L10e (221 residues in *S. cerevisiae*) [87, 88], is significantly smaller than the latter two proteins [89] and does not interact with 5S rRNA (Fig. 4b), but it contacts helix H38 of 23S rRNA [80, 81]. Protein L21e is absent in the bacterial ribosome [89], but it is present in the ribosome of archaea, where it also forms contacts with ribosomal RNAs [77, 88]. Moreover, the eukaryotic ribosome also contains a large protein of the L7e-L30p family (244 residues in *S. cerevisiae*), which similarly to its homolog from archaea (154 residues in *H. marismortui*) can form contacts with 5S rRNA and helix H38 of a high molecular weight RNA of the large ribosomal subparticle [77, 82, 88]. In the bacterial ribosome the small protein L30 (58 residues in *E. coli*) interacts with helix H38 of 23S rRNA [80, 81] and seems to form the single contact with 5S rRNA (Fig. 4b), which is unlikely to be stabilizing.

Thus, in the ribosome from archaea and eukaryotes there are three proteins that stabilize a direct contact between helix H38 of 23S (26S) rRNA and 5S rRNA. In the bacterial ribosome the situation is different: these proteins are either absent or are insufficiently large to realize this function [85]. Therefore, an alternative scenario of stabilization of the direct interaction of two rRNAs can occur in the bacterial ribosome. In bacteria a new 5S rRNA-binding protein appears (protein L25 in *E. coli* from the CTC family [85]) which was absent in archaea and eukaryotes [85, 89]. In bacterial ribosomes the C-terminal region of protein L16 interacts with a protein of the CTC family (Fig. 4b). This interaction with the multidomain protein of the CTC family (e.g. ribosomal protein TL5 from *Thermus thermophilus* or CTC from *Deinococcus radiodurans*) involves two domains that sig-

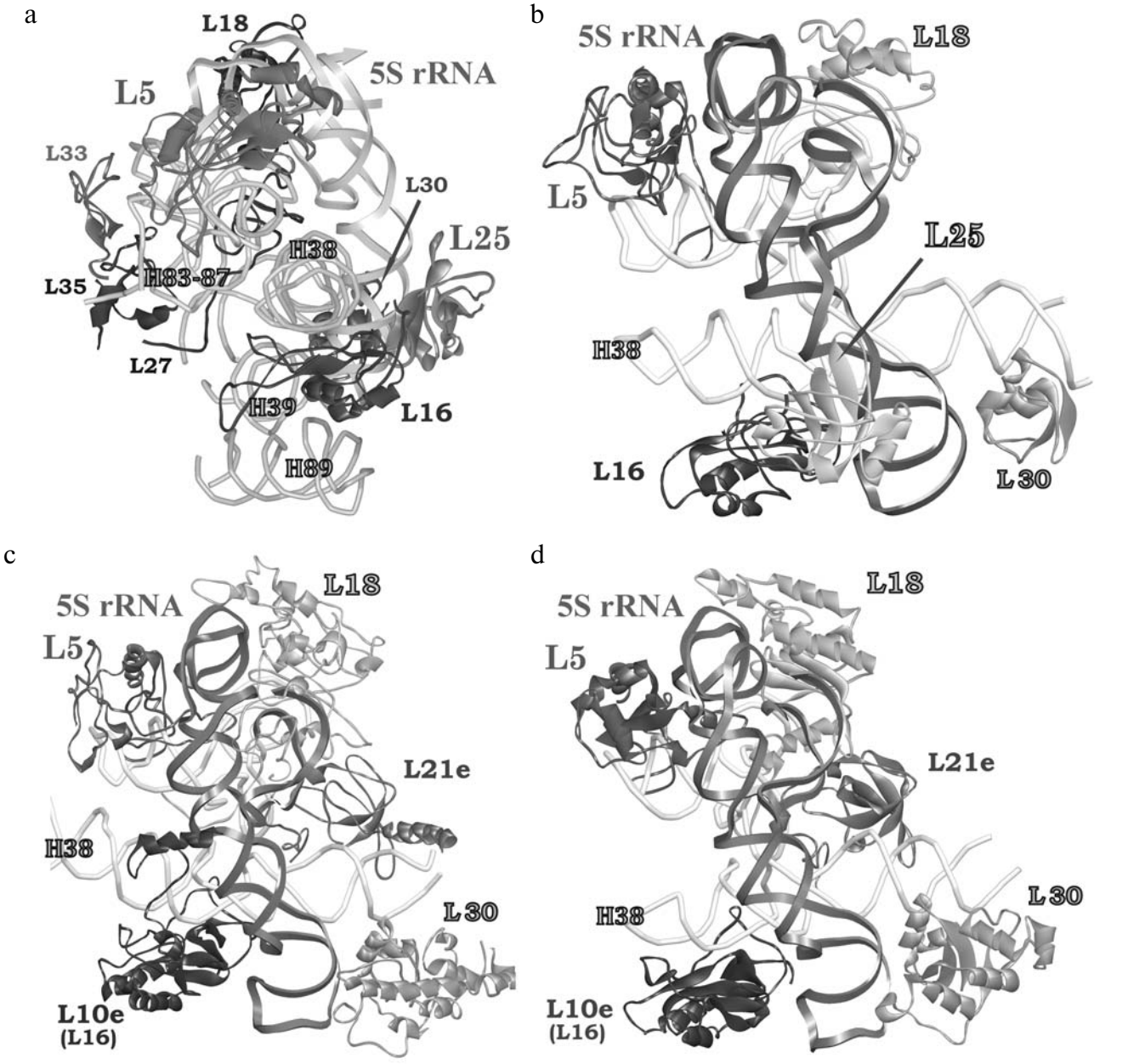

Fig. 4. Model of structural organization of the central protuberance of the large ribosomal subparticle according to crystallographic data [77, 80, 82]. a) The central protuberance of the 50S ribosomal subparticle of *E. coli*. b-d) Fragments of the central protuberance of the large ribosomal subparticle of bacteria, eukaryotes, and archaea, respectively. These models show the main intermolecular contacts of 5S rRNA in the ribosome. The models were designed using the ribosomal structures (PDB codes: 2AW4, 1JJ2, and 3O58).

nificantly increase the region of the contact as compared to the single-domain protein L25 from *E. coli* (Fig. 4b) [78-81, 85]. In addition the second domain of protein TL5 forms hydrogen bonds with helix H38. Thus, in the bacterial ribosome a protein of the CTC family, in particular a multidomain protein, functions as several proteins from archaea and eukaryotes in stabilizing the conservative contact between 5S rRNA and helix H38 of 23S rRNA. Moreover, it is now clear that proteins of the central protuberance play an important role in creating and supporting the unique location and possibly also the functioning of 5S rRNA in the ribosome.

5S rRNA–PROTEIN COMPLEX AND RIBOSOME FUNCTIONING

The question of 5S rRNA functioning arose significantly earlier than details of its structural organization and the determination of the above-described intermole-

cular interactions were ascertained. Searches for biological functions of this molecule started even before it was discovered. As noted in the beginning of this review, 5S rRNA was initially termed "transfer-like RNA" [2, 6] and was even supposed to be a precursor of tRNA [6, 7]. Later, 5S RNA was proved to be an independent ribosomal RNA inherent in ribosomes of all known organisms, and in all features it was quite different from tRNA. However, for some years some enthusiasts still tried to find a resemblance between these RNAs in structure, origin, and functions. Thus, assuming that 5S rRNA could have a structure similar to that of tRNA, I. Raacke supposed that the 3′-terminal nucleotide of the 5S rRNA should act as a mediator in the peptidyl transferase reaction of the ribosome [90, 91]. Certainly, this idea was rather attractive, although slightly adventurous because it had no experimental base. It was not surprising that a simple experiment with inactivation of the 3′-terminal nucleotide of 5S rRNA in the ribosome revealed the failure of this idea [92, 93]. The ribosome containing 5S rRNA with oxidized 3′-terminal nucleotide fully retained its activity during polypeptide synthesis and, respectively, during the peptidyl transferase reaction. Moreover, only based on a slight homology between the primary structures of 5S rRNA and tRNA, some authors proposed a hypothesis about the common origin of these molecules [94, 95], but this hypothesis was rather emotionally rejected by other authors [96]. Quite naturally, the majority of other hypotheses about the functions of 5S rRNA were associated with the known functions of the large ribosomal subparticle. The involvement of 5S rRNA was speculated in virtually all functions of the 50S ribosomal subparticle. There was a period of attempts to assign individual functions of the ribosome to some specific ribosomal components (a protein or RNA). And 5S rRNA did not escape such a fate. In addition to the idea that 5S rRNA should be a mediator in the peptidyl transferase reaction [90, 91], this ribosomal RNA was also supposed to have the GTPase and ATPase activities, to be necessary for association of ribosomal particles, and also to be involved in the binding of tRNA with the 50S subparticle (see [97] for a review).

It seems that the idea about the GTPase activity of 5S rRNA was first expressed by V. Erdmann with colleagues based on their findings [98]. The 50S ribosomal subparticle of *Bacillus stearothermophilus* reconstructed in the absence of 5S rRNA was found to be unable to bind the EF-G·GTP complex. This naturally resulted in the idea that 5S rRNA should be responsible for this function. In subsequent works these authors demonstrated that isolated bacterial and eukaryotic 5S rRNA–protein complexes possessed GTPase and even the ATPase activity [99-103]. A similar phenomenon was also described by other authors [104]. It was also shown that in the ribosome·EF-G·GTP complex the photo-activated analog of GTP was cross-linked with a number of proteins (L5, L11, L18, and L30) including 5S rRNA-binding proteins [105]. It was also shown that in the ribosome·EF-2·GMPPCP complex the eukaryotic elongation factor EF-2 (an analog of EF-G) was linked with 5S rRNA [106]. At first glance, these findings could appear quite definitive and very important if they were not contradicting all other experimental data [107, 108] about the GTPase activity of the translation apparatus that were already available. First, the specific GTPase activity of the 5S rRNA–protein complex was, at least, hundreds of times lower than the EF-G (EF-Tu)-dependent activity of ribosomes described in some works [109-113]. Such a low enzymatic activity could be associated not with the 5S rRNA–protein complex itself but with contaminations of the sample. Second, by that time it was already known that just the translation protein factor (EF-G or EF-Tu) but not the ribosome (not ribosomal components) could bind [114-117] and cleave GTP [118, 119]. The GTPase or ATPase activities of the 5S rRNA–protein complex manifested themselves in the absence of EF-G [99] and were not stimulated on its addition. Third, ribosomal 50S subparticles or their derivatives lacking the 5S rRNA–protein complex retained the EF-G-dependent GTPase activity [39, 120]. Such ambiguous data resulted in a very short life of the hypothesis about the GTPase or ATPase activity of the 5S rRNA–protein complex. It should also be added that further studies completely excluded any contribution of the 5S rRNA–protein complex to the cleavage of the GTP molecule by the translation apparatus during protein biosynthesis.

The idea of a possible involvement of 5S rRNA in association of ribosomal subparticles was first expressed by R. Rosset et al. in 1964 [5]. This involvement could be direct and indirect. A highly effective *in vitro* hybridization between eukaryotic 5S rRNA and 18S rRNA was detected by a group of researchers [121-123]. Based on these findings and on detecting complementary regions in many prokaryotic and eukaryotic ribosomal RNAs (Fig. 5), A. Azad supposed that 5S rRNA could be directly involved in association of ribosomal subparticles [124]. However, further studies did not confirm the existence of such RNA–RNA interactions in the ribosome. In experiments with different approaches (formation of complexes from isolated components, chemical probing of rRNA, and intermolecular RNA–RNA cross-linking in the ribosome) no results were obtained in favor of existence of

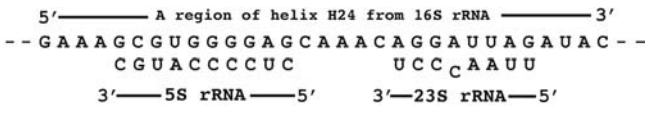

Fig. 5. Supposed regions of interaction of three ribosomal RNAs. The figure taken from work [124] is modified.

contacts between 5S rRNA and 16S rRNA [125-130]. Moreover, results of the most recent crystallographic studies on ribosomes [77-82] finally rejected a possibility of such an RNA–RNA interactions in the ribosome. The denoted regions of ribosomal RNAs, helix IV in 5S rRNA and helix H24 in the central domain of 16S rRNA, are located in the ribosome at a distance exceeding 100 Å and in no case could produce a supposed double helix. Thus, the presence of complementary regions in 5S rRNA and 16S rRNA seems to be accidental and having no biological reason. However, the question about a possibility of an indirect involvement of 5S rRNA in association of ribosomal subparticles mediated through other ribosomal components is still open. The matter is that as early as in the 1970s two fundamental results were obtained. First, the central protuberance of the 50S ribosomal subparticle containing 5S rRNA is obviously in contact with the 30S subparticle [51]. Second, the ability of large ribosomal subparticles arranged *in vitro* in the absence of 5S rRNA to associate with 30S subparticles was shown to be very strongly reduced [39]. Based on these data, it was concluded that 5S rRNA should play a very important if not a crucial role in association of ribosomal subparticles. However, results of further studies on the large ribosomal subparticle lacking the 5S rRNA–protein complex slightly corrected this conclusion [131]. It was shown that, although these ribosomal subparticles are associated with 30S subparticles less tightly than with intact 50S particles, they form a 70S ribosome with subparticles accurately oriented relatively to one another. Moreover, the absence of the 5S rRNA–protein complex in the ribosomal subparticle resulted in a strong deformation of the central protuberance but not in its disappearance. Therefore, it was concluded that the intersubunit contact formed by the central protuberance of the 50S subunit with the 30S subunit should be an important but not a decisive factor. As noted above, no direct interaction of 5S rRNA with 30S subparticle was detected. Therefore, considering the known intermolecular contacts of 5S rRNA inside the 50S ribosomal subunit (see the section "5S rRNA Location in the Ribosome" of this review) it was reasonable to suppose that the central protuberance could interact with the 30S subparticle via 5S rRNA-binding proteins and/or via 23S rRNA. Such a "mediated involvement of 5S rRNA in association of ribosomal subparticles" was confirmed by recent data of crystallographic studies [77-82]. It was found that two intersubunit bridges, B1a and B1b, were formed, respectively, by helix H38 of 23S rRNA interacting with 5S rRNA and by the 5S rRNA-binding protein L5. Thus, 5S rRNA was shown to be involved in association of ribosomal subparticles not as a direct participant but as a kind of mediator.

The idea of the relation of 5S rRNA to the interaction of tRNA with the large ribosomal subparticle seemed to be the most popular among all hypotheses. Possibly, just this was the reason for the "survival" of this idea up to now, although it has undergone significant changes. For the first time a possibility of the involvement of 5S rRNA in the binding of tRNA to the ribosome was supposed by the above-mentioned R. Rosset et al. [5], and in the work of B. Forget and S. Weissman this idea acquired a sufficiently concrete form [18]. The discovery in 5S rRNA of a region ($_{47}$CAAG$_{44}$) complementary to GTΨC (T-loop) of tRNA induced the idea that 5S rRNA could directly interact with tRNA in the ribosome. This idea of the direct interaction of two RNAs was adopted with enthusiasm. During the following years many works appeared concerning this problem (see for review [12, 108, 132, 133]). First, results of some studies on features of ribosomes lacking 5S rRNA [27, 34, 39, 98, 134] supported the idea that 5S rRNA was necessary for providing the interaction of tRNA with the ribosome in the P-site. Moreover, it was shown that just the nucleotides in the mentioned regions of 5S rRNA or tRNA in the ribosome–tRNA complex changed their own availability to various modifying agents [135-138]. Second, the existence of this contact was confirmed by an inhibitory effect of the oligonucleotide TΨCG$_p$ on the binding of tRNA with the ribosome [139-146]. Moreover, cross-linking of the modified tRNA was shown to occur in the ribosome mainly with 5S rRNA and not with 16S rRNA or with 23S rRNA [147]. The idea about the possibility of contact between 5S rRNA and tRNA in the ribosome (Fig. 6) was supported also by authors of other works [94, 148-150]. However, it should be noted that all these data only indirectly indicated to the possibility of 5S rRNA contact with tRNA in the ribosome. Only in work [151] was a direct binding of the tRNA fragment (TΨCG$_p$) with the isolated 5S rRNA reported. But the association constant of these molecules was so low (K_{app} $8 \cdot 10^3$ M^{-1}) that it was difficult to estimate the reality of existence of such a complex. Some results were also obtained that contradicted the hypothesis about the direct interaction of 5S rRNA and tRNA in the ribosome. Thus, deletions or mutations in the corresponding region (loop C) of 5S rRNA were shown to have no effect on the binding of tRNA with the ribosome [152, 153]. Tritium exchange revealed that the incorporation of tRNA in the ribosome did not cause changes in this region of 5S rRNA [154]. Moreover, the substitution of C by A in the T loop of tRNA was shown to have no influence on its biological activity [155]. These findings forced the authors to doubt the correctness of this hypothesis. Thus, all experimental data obtained during 15 years did not give an unambiguous answer to the question of the possibility of a direct interaction of 5S rRNA with tRNA in the ribosome. It would seem that the idea was untenable. However, this story received an unexpected although rather logical continuation. During the same period, i.e. the 1970-1980s, tRNA was shown to selectively interact with isolated ribosomal proteins. One of these works was the above-mentioned work by V. Erdmann et al. [151], which demonstrated this interac-

tion. They found that the complex of the tRNA fragment ($T\Psi CG_p$) with 5S rRNA became 10-fold more stable if the 5S-rRNA–protein complex was used. But then the influence of the protein on RNA association was not yet given its due attention. Later, use of immobilized tRNA and 5S rRNA resulted in interesting and important data on the selectivity of binding of ribosomal proteins with different RNAs. Thus, on a decrease in the magnesium ion concentration in the medium not only three already known ribosomal proteins L5, L18, and L25 could be detected in the complex with 5S rRNA, but also the proteins L2, L15, L16, L17, L22, L33, and L34 [156]. This set of proteins had a resemblance with the protein composition of the complex with tRNA [157-161]. Note that all above-mentioned complexes contained proteins L18 and L16 (sometimes the complex also included proteins L5 and L15). The resulting RNA–protein complexes had features similar to those of the ribosome (binding of tRNA or of ribosomal 30S subparticle) [156, 161, 162]. Using photo-activated derivatives of tRNA, puromycin, or other appropriate antibiotics, other researchers identified ribosomal proteins cross-linked with them in the ribosome. Among the proteins cross-linked with tRNA and with the listed antibiotics there were proteins of the central protuberance of the 50S subparticle, in particular, 5S rRNA-binding proteins. Thus, proteins L18, L27, L15, L16, and L25 cross-linked with the acceptor part of tRNA or with puromycin [163-167]. Proteins L5, L16, and L27 cross-linked with nucleotides of the T and D hairpins, i.e. in the region of the angle of the L-shaped tRNA molecule [168-171].

Thus, by the mid-1990s from the totality of experimental data it followed that 5S rRNA-binding proteins and other proteins of the central protuberance of the large ribosomal subparticle could be involved in the binding of tRNA with the ribosome. Some of these data were recently confirmed by crystallographic studies on ribosomes and their functional complexes [77-82].

To continue the theme about the possibility of the involvement of 5S rRNA in formation of the functional centers of the ribosome, consider one more relatively new hypothesis that was proposed by A. Bogdanov et al. 15 years ago [172, 173]. As already described in detail in the section "Location of 5S rRNA in the Ribosome", the environment of 5S rRNA in the ribosome was carefully studied by these researchers [73-75, 173]. Structural elements of 23S rRNA were found to be the closest neighbors of 5S rRNA (Fig. 7) and to form two functional centers of the ribosome, peptidyl transferase and GTPase-associating ones; therefore, it was supposed that 5S rRNA could be a mediator between these functional centers. The authors suggested that 5S rRNA can be responsible for coordination of operating of these functional centers of the ribosome. At present, based on the accumulated experimental data, this hypothesis about the functional role of 5S rRNA in the ribosome seems the most realistic. The modern data based on crystallographic studies on ribosomes and their functional complexes [77-82, 86-88] in general confirm the earlier findings concerning the topography and intermolecular contacts of the 5S rRNA–protein complex in the ribosome (see the section "Location of 5S rRNA in the Ribosome"). Moreover, it follows from the abundant literature data that the 5S rRNA–protein complex and other components of the central protuberance of the large ribosomal subparticle can be also involved in formation of intersubunit bridges and of tRNA-binding sites [78, 80-82, 156-159, 163-171]. Therefore, we think that the involvement of 5S rRNA (5S rRNA–protein complex) in synchronization of activities of the ribosome functional centers is a very interesting and promising idea.

THE PROBLEM OF THE ORIGIN OF 5S rRNA

The problem of the origin of 5S rRNA arose concurrently with its discovery (see beginning of the review) and

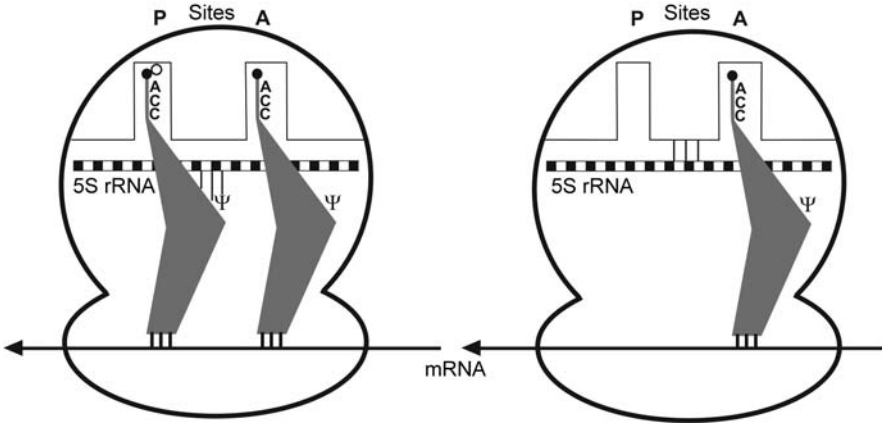

Fig. 6. Scheme illustrating possible contact of 5S rRNA with tRNA in the P-site of the ribosome. The scheme is modified from work [148].

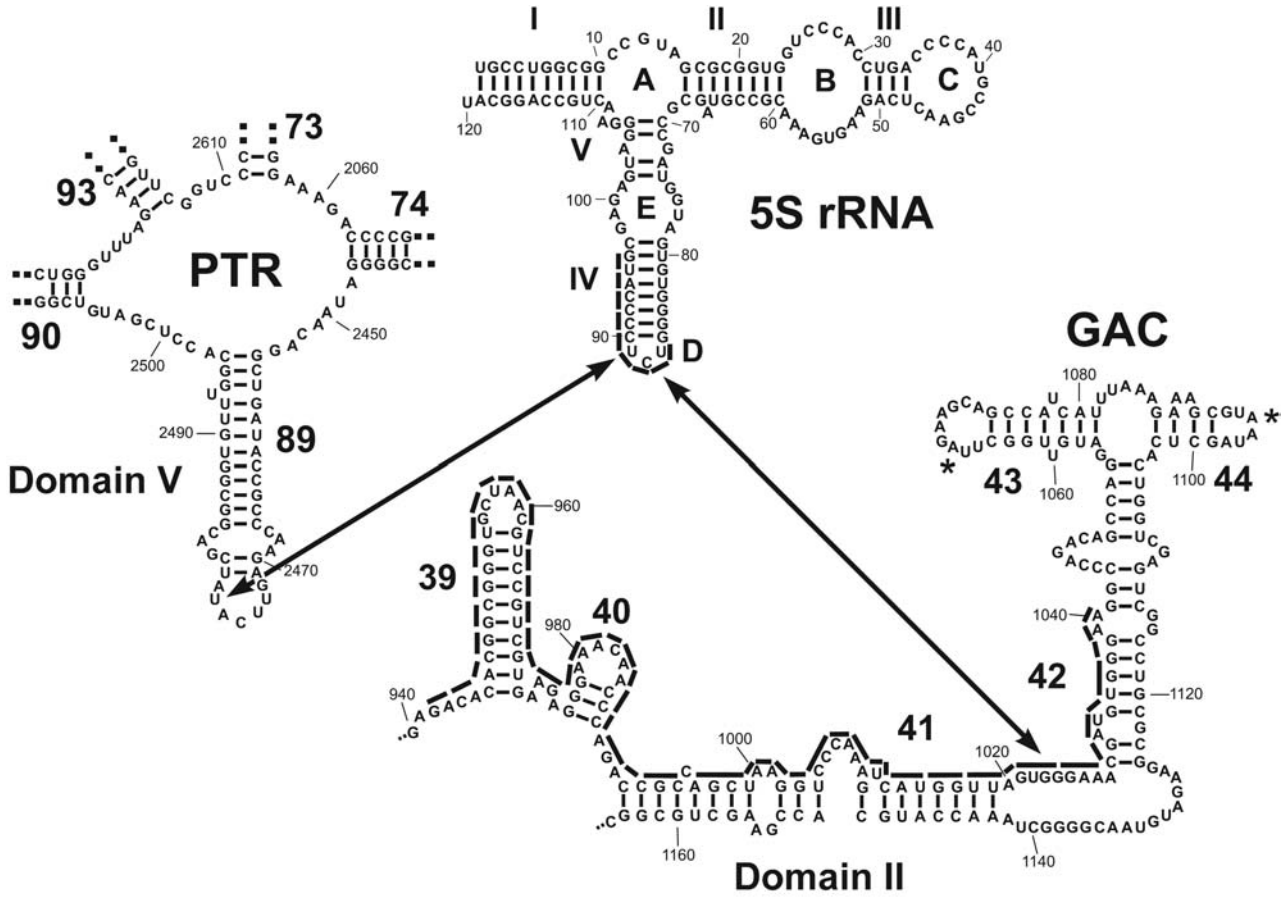

Fig. 7. Scheme illustrating possible contacts of 5S rRNA with the GTPase-associated and peptidyl transferase regions of 23S rRNA in the large subparticle of the *E. coli* ribosome based on intermolecular cross-linking. PTR, peptidyl transferase ring; GAC, GTPase-associated center. The figure taken from works [172, 173] is changed according to the problem under discussion.

includes at least two questions. First, do other molecules exist with known functions and possessing a resemblance (relation) with 5S rRNA? Second, at what step of the evolution of living organisms and their translation apparatus did 5S rRNA appear in the ribosome? An answer to each of these questions could give important information about the function of this ribosomal RNA. However, answering these questions is not simple. Thus, the question of relation between 5S rRNA and other RNAs that first time appeared even before the official discovery of 5S rRNA (see the beginning) was under consideration for more than ten years and failed to result in a definite conclusion. The idea of the common origin of 5S rRNA and tRNA was the most popular [2, 6, 7]. Resemblance was looked for either in their primary structures [94, 95] or in the possible spatial structure of the molecules [90, 174]. Also, other small RNAs such as 5.8S rRNA were not forgotten [103]. The most accepted conclusion for today is that ribosomal 5S RNA has no parental or related molecules in contemporary organisms. This confirms the idea about the uniqueness of this small ribosomal RNA and its function.

The other question was still more difficult. To get a clear answer to the question of when 5S rRNA appeared in the ribosome is difficult because of the billions of years of the evolution of living beings and the absence of any information concerning the events in the very beginning of this process. Nevertheless, there have been some attempts to determine the "age of 5S rRNA", such attempts continue at present, and they may be continued in the future. Molecular evolutionary biology is relatively young [175], and much time has been required for development of approaches for studies and for accumulation of the initial experimental findings and experience. Earlier, to answer the question about the origin of 5S rRNA its primary structures were compared [176-179], conservative structural elements in RNAs from different organisms were analyzed [177, 179], the organization of ribosomal RNA genes in the genomes were compared [180, 181], and regions homologous to 5S rRNA in high molecular weight rRNAs were searched for [182]. These studies have provided much information about 5S rRNA structure and its specific features, but there are very few data that would lead to the answer to the question about the origin of this

molecule. The only reliable knowledge is that 5S rRNA was already existent by the moment of divergence of the three branches of life (1.8-2.4 billion years ago) [176, 178]. At present, the most modern analytical approaches and the huge experimental material accumulated during decades are used in attempts to elucidate the problem of the origin of biological molecules. During recent years, this problem has been analyzed using a combined approach: comparative analysis of the primary and spatial structure of ribosomal RNAs and their structural elements and ribosomal proteins and the intermolecular contacts between them in the ribosome [183-191]. We believe that progress is already achieved in attempts to solve the question about the origin of 5S rRNA. Summarizing the results of these studies, some interesting conclusions can be made.

First, the evolutionary analysis of structural elements of 5S rRNA [189, 190] shows that the 3′-5′-terminal helix is the most ancient element, the first domain hairpin is slightly "younger" (helices II and III, loops B and C), and the second domain (helices IV and V, loops E and D) is "the youngest" element. The evaluated age of the 5S rRNA-binding proteins appears to correspond to the age of the RNA structural elements that they are bound with [189].

Second, the evolutionary analysis of structural elements of high molecular weight RNA of the large ribosomal subparticle has revealed that domain V is the most ancient, domains IV and II are somewhat younger, and domains I and III are the youngest [183, 187]. As follows from the structural analysis of present-day ribosomes (see section "Location of 5S rRNA in the Ribosome", Fig. 4), one of conservative contacts is between the first domain of 5S rRNA and domain V of 23S (26S) rRNA via proteins L5 and L18. Therefore, it can be supposed that a small RNA on its first entrance into the ribosome was only a hairpin (the first domain) of the contemporary 5S rRNA. The time of appearance of this small RNA in the "protoribosome" determined based on relative evaluations of the age of the participants [185-189, 191] could be approximately 2.5-3.0 billions of years ago. This was followed by finishing the building of the large ribosomal subparticle of the "protoribosome" by adding domain II of 23S rRNA and the second domain of 5S rRNA, which formed another conservative contact. Thus, it seems that the complication of the "protoribosome" structure and its function even then required a coordinator to provide for operating of its structural–functional domains. This was the reason for appearance of 5S rRNA in the ribosome.

In this review an attempt has been made to consider from different standpoints the same question: "What is 5S rRNA required for in the ribosome?" The data presented above allowed us to conclude that 5S rRNA appeared in the ribosome for connection, at least, of two functional domains of the ribosome, the peptidyl transferase and GTPase-associated ones. No other direct functional contacts of 5S rRNA have been detected in the ribosome up to now. Nevertheless, just 5S rRNA-binding proteins and structural elements of high molecular weight ribosomal RNA contacting with 5S rRNA are involved in association of ribosomal subparticles and in the interaction of tRNA with the ribosome. Thus, 5S rRNA is indirectly connected with all functional centers of the large ribosomal subparticle. Therefore, the idea expressed 15 years ago that 5S rRNA could coordinate the work of functional domains of the large ribosomal subparticle is by now to a great extent confirmed experimentally. Such a viewpoint on this problem allows us to explain a specific feature of 5S rRNA, namely, its independence and conservativeness of the structure in representatives of all domains of life. It seems that such a complicated function destined for 5S rRNA could not be solved by a simple elongation of a hairpin of high molecular weight RNA. Certainly, billions of years of evolution of living beings have significantly corrected the structure and functioning of their ribosomes. Thus, in bacteria, archaea, and eukaryotes their specific 5S rRNA-binding proteins appeared. However, 5S rRNA and some conservative and functionally important intermolecular contacts formed by it in the ribosome remained virtually unchanged. Many details of the functional activity of this small RNA in the ribosome are still unclear, but the main steps have already been made. It can be expected that the mechanism of functioning of 5S rRNA will soon be discovered, and considering its location in the ribosome, also of a significant part of the protein-synthesizing apparatus. Moreover, having in mind the importance of 5S rRNA for the functioning of the ribosome and specific features of the nearest environment of this small RNA in representatives of various domains of the life, we suppose that the central protuberance of the large ribosomal subparticle could be a very promising target for inhibitors of protein biosynthesis.

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