ACTIVE SITES IN *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

Ribosomes consist of two different subunits. In *E. coli*, the smaller 30 S subunit is composed of one 16 S RNA molecule and 21 proteins. The larger 50 S subunit consists of one 23 S RNA molecule, one 5 S RNA molecule and 34 proteins (for reviews see refs. [1–5]). Proteins are synthesized on the ribosome as a result of a large number of highly coordinated events (for reviews see refs. [6–13]). Initiation of protein synthesis commences with the proper binding of mRNA to a 30 S subunit. Subsequently, f-Met-$tRNA_F^{Met}$ in response to the initiation codon, GTP, and initiation factors are attached. This initiation complex then combines with a 50 S subunit. The f-Met-$tRNA_F^{Met}$ becomes positioned in a puromycin sensitive site which is identical or analogous to the ribosomal P-site.

Initiation of protein biosynthesis is followed by a many times repeated cycle: In response to the respective codon, the ternary complex EF–Tu~GTP~aminoacyl-tRNA is bound in the ribosomal A-site, which is not sensitive to puromycin. Peptide bond formation and peptidyl (f-Met) transfer then occur and are followed by translocation of the newly formed peptidyl-tRNA from the A-site into the P-site. Translocation requires elongation factor G and again GTP. This ribosomal cycle commences anew, with the binding of another EF–Tu~GTP~aminoacyl-tRNA complex to the next codon. It is repeated until the termination signal on the mRNA is reached. The binding of release factors in response to the termination signal leads to hydrolysis of peptidyl-tRNA and to release of the newly synthesized protein molecule from the ribosome. Protein synthesis on the ribosomes thus requires many ribosomal and supernatant components.

Several ribosomal models [14–20] describing sites and their functions in codon recognition, transpeptidation and translocation have been proposed. Major common features of these models are: (a) There are one or more decoding sites for tRNA on the 30 S subunit. (b) There are two (and possibly more) sites for tRNA binding on the 50 S subunit. The two sites on the 50 S subunit are functionally not identical. (c) Translocation involves movement of mRNA along the 30 S subunit, realignment of the 30 S decoding site with the 50 S A-site, and removal of deacylated tRNA.

At present, most experiments on protein synthesis can be interpreted by the classic two-site donor–acceptor model. The discussion in this paper is based on this model.

2. Initiation

2.1. *Binding of mRNA*

Initiation of protein synthesis begins with binding of mRNA to 30 S subunits. This step apparently depends upon the presence of protein S1, which does not occur on every 30 S subunit [21,22]. Addition of S1 to 30 S enhances mRNA binding [23]. Protein S1 alone is capable of binding poly(U). Both ribosomal and non-ribosomal mRNA binding capacities are inhibited by aurin-tricarboxylic acid [24]. The involvement of protein S1 in mRNA binding is also indicated by the observation that it is the only 30 S protein protected by poly(U) against trypsin digestion [25].

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In vitro S1 is readily exchangeable between 30 S and supernatant [23]. The fate of protein S1 during ribosomal protein synthesis remains unclear. It was reported that free 30 S subunits lose proteins S1 and S2 after formation of the 30 S initiation complex [26]. On the other hand, polysomes contain higher amounts of proteins S1 and S2 (S6, S21, L7 and L12 as well) than run-off ribosomes [27]. Furthermore, polysomes generated under the direction of poly(U) contain approximately one molecule of S1 per ribosome [28]; whereas, this is not the case with 30 S subunits. These data are not contradictory to a report that 30 S particles reconstituted with all proteins except protein S1 showed 100% activity with respect to poly(U) dependent ribosomal poly(Phe) synthesis [29]. The supernatant enzyme fraction used in the assay could have contained protein S1 which entered the ribosome and stimulated its activity [30].

The control of correct initiation of mRNA translation involves 30 S protein(s). Ribosomes of *E. coli* recognize all three initiator regions of bacteriophage R17 RNA, whereas ribosomes of *B. stearothermophilus,* almost exclusively, recognize only one region [31]. Heterologous reconstitution experiments showed that cistron specificity is a property of the 30 S subunit [32] and that protein S12 plays a critical role in correct initiation of protein synthesis [33]. Another example that messenger specificity resides in the 30 S subunit was reported for ribosomes of *Caulobacter crescentus* [34].

2.2. *Binding of f-Met-tRNA*$_F^{Met}$

The f-Met-tRNA$_F^{Met}$ binds, together with initiation factors (IF), to the 30 S mRNA complex. IF-dependent f-Met-tRNA$_F^{Met}$ binding is stimulated by the addition of the fractional proteins S2, S3, S14 [35]. This binding is strongly inhibited by specific antibody fragments (Fab) against the ribosomal proteins S3, S10, S14, S19 and S21 [36]. A less pronounced, but significant inhibition was obtained with Fab's against protein S2, S5, S6, S12 and S13. This second group inhibited f-Met-tRNA$_F^{Met}$ binding only, but had no effect on enzymatic Phe-tRNA^{Phe} binding (see section 3.2.). Reconstituted 30 S particles, deficient in either protein S12, S13, S20, S21 (and S6) have almost completely lost the capacity to form the IF-2 dependent A–U–G~f-Met-tRNA$_F^{Met}$~30 S complex [37]. However, it has proved difficult to assign a direct role to a particular protein by these single-component-omission experiments [4].

The presence or absence of protein S21 may control initiation and/or elongation of protein synthesis. f-Met-tRNA$_F^{Met}$ binding is inhibited by protein S21 in the presence of 50 S subunits [35]. Accordingly, protein S21 was not detected on native 30 S subunits in contrast to monosomes and polysomes [35,38]. It is present in higher amounts in polysomes than in run off ribosomes [27]. Ribosomes of cells grown in rich medium contain two to three times more S21 than those of cells grown in minimal medium [39].

2.3. *Binding of initiation factors*

Polypeptide chain initiation requires three complementary protein factors (IF-1, IF-2, IF-3) which promote the binding of f-Met-tRNA$_F^{Met}$ to ribosomes in the presence of mRNA and GTP (for a recent review see ref. [9]). IF-2 catalyzes the binding of f-Met-tRNA$_F^{Met}$ and the concomitant hydrolysis of GTP. Removal of proteins L7 and L12 results in great reduction of IF-2 dependent activities [40]. Addition of IF-1 stabilizes the IF-2 dependent initiation complex formation. IF-3 prevents association of 30 S and 50 S subunits and apparently functions in directing ribosomes to recognize initiation signals on mRNA. The binding site for IF-3 on the 30 S subunit is mainly provided by a segment of 16 S RNA. Ribosomal proteins only play an auxiliary role in this binding [41]. IF-3 binds stoichiometrically to 30 S, but not to 50 S or 70 S at selectively high Mg^{2+} concentrations (10^{-3} to 10^{-2} M) [42–44]. However, appreciable binding of IF-3 to free 50 S subunits was observed at low Mg^{2+} concentration (2×10^{-4} M) [45].

3. Elongation

3.1. *Binding of aminoacyl-tRNA*

In response to the respective codon on mRNA, the EF–Tu~GTP~aminoacyl-tRNA complex binds to the ribosome in the A-site. The fidelity of proper codon–anticodon interaction between mRNA and tRNA is governed by several ribosomal proteins and can be disturbed by the addition of streptomycin.

Reconstitution experiments showed that proteins S3 and S5 are part of one binding site of dihydrostreptomycin [46]. Furthermore, affinity labeling

experiments with a streptomycin analog indicated that protein S4 is near the site where the streptose moiety of this antibiotic is bound [47,48]. Protein S12 is the ribosomal component which confers resistance against or dependence on streptomycin [49,50]. Mutational alterations in protein S4 and S5 counteract that in S12 as shown by ram (= ribosomal ambiguity) mutants [51–54] and those which suppress streptomycin dependence [55–61].

Reconstituted 30 S particles deficient in protein S12 show a pronounced reduction in translational error frequency; whereas, 30 S particles deficient in protein S11 show an increase in translational error frequency [37].

In summary, the effect of streptomycin on misreading can be explained in the following way: Binding of streptomycin to proteins S3 and S5 blocks the translational fidelity exerted by proteins S3, S4, S5 and S11. Hence, the influence of protein S12 on translation becomes dominant, giving rise to a significant increase of improper codon–anticodon recognition. Since protein S12 is a major component of ribosomal control on cistron specificity and codon–anticodon interaction, one would expect that it is located where mRNA–tRNA interaction takes place.

Several experiments indicate that 30 S ribosomal proteins are also involved in EF–Tu dependent aminoacyl-tRNA binding to the A-site on 70 S ribosomes. Particles, deficient in protein S3, S10 or S14, lost their capacity to bind Phe-tRNA^{Phe} non-enzymically in response to poly(U) [37,62]. Poly(U) directed binding of Phe-tRNA^{Phe} protected proteins S3, S6, S14, S18, S19 and S21 against tryptic digestion [25]. Incubation of ribosomes with the fractional proteins S2, S3 and/or S14 stimulated the EF–Tu dependent Phe-tRNA^{Phe} binding [63]. Furthermore, Fab's against proteins S3, S9, S11, S18, S19 and S21 strongly inhibited EF-Tu dependent poly(U) directed Phe-tRNA^{Phe} binding [36]. Fab's against three of these proteins (S3, S19, S21) also inhibit binding of f-Met-tRNA$_F^{Met}$ (see section 3.2.). However, one should bear in mind that inhibition of enzymic Phe-tRNA^{Phe} binding is also affected indirectly by inhibition of mRNA or EF–Tu binding and of subunit association. None of the cited experiments prove in which of the various steps the proteins are involved.

The 3′-terminus of aminoacyl-tRNA is bound by 50 S ribosomal components in the peptidyltransferase center [64]. Chloramphenicol inhibits binding of respective aminoacyl-tRNA fragments, such as C–A–C–C–A–Leu or C–C–A [65–67]. Therefore, it has been concluded that chloramphenicol binds to the A-site of the peptidyltransferase center. Partial reconstitution experiments have shown that protein L16, a 23 S RNA binding protein [68], is involved in chloramphenicol binding [67]. Affinity-labeling studies with monoiodoamphenicol, a chloramphenicol analog, demonstrated that monoiodoamphenicol reacted exclusively with protein L16 [69]. From these data it can be concluded that protein L16 is located at the A-site of the peptidyltransferase center.

Another chloramphenicol analog, monobromamphenicol, when irreversibly reacted with *E. coli* ribosomes, partially inactivates peptidyltransferase activity [70]. However, under the reaction conditions employed, chloramphenicol failed to protect ribosomes against reaction with the synthetic analog. The label was predominantly associated with proteins L2 and L27, which have been recently identified as part of the ribosomal P-site [71,72].

Puromycin is another antibiotic which binds in the A-site of the peptidyltransferase center and competes with the 3′-terminus of aminoacyl-tRNA [64,66,73]. Affinity-labeling experiments with *N*-iodoacetylpuromycin which can be regarded as an analog to the product of the puromycin reaction, namely *N*-peptidylpuromycin, were carried out in order to identify ribosomal proteins close to the puromycin binding site [74,75]. In these reactions protein L6 was predominantly labeled and to a minor extent protein L2. Partial reconstitution experiments indicate that protein L6 is needed together with protein L16 in order to obtain competition between chloramphenicol and C–A–C–C–A–Leu binding [76]. These data show that proteins L6 and L2 are in the neighborhood of protein L16, i.e. a ribosomal A-site protein, and thus near, or at, the peptidyltransferase center of the ribosome. Furthermore, N-substituted phenylalanyl-tRNA's have been employed as affinity labels. Again, protein L16 could be identified as one of the labeled proteins [71,72]. The proximity of protein L2 to the A-site of the peptidyltransferase center is particularly interesting, since it influences binding of 5 S RNA–protein complexes to 23 S RNA [77]. Protein L16 binds towards the 5′-end of 23 S

RNA and protein L2 towards the 3′-end of 23 S RNA [78]. This would mean that both parts of 23 S RNA contribute to the structure of the peptidyltransferase center and possibly to its function as well.

Besides proteins, ribosomal RNA is involved in binding to tRNA. Modification of 6–7 guanine residues of 16 S RNA by treatment with kethoxal results in the loss of ribosomal capacity to bind tRNA. Messenger RNA binding is not impaired by this modification [79]. Furthermore, it has been shown that the tRNA-fragment TpΨpCpGp specifically interacts with 5 S RNA [80] and that the presence of 5 S RNA in the 50 S subunit is crucial for ribosomal activity [81]. It has also been found that TpΨpCpGp inhibits enzymic binding of aminoacyl-tRNA to ribosomes and magic spot formation [82]. The sequence TpΨpCpPu is common to all tRNA.s except eukaryotic initiator tRNA's [83–85] and tRNA's needed in cell wall synthesis [86,87]. This and other evidence [88] suggest that 5 S RNA interacts with TpΨpCpPu of aminoacyl-tRNA in the ribosomal A-site.

3.2. *Binding of peptidyl-tRNA*

After the translocation step in the elongation cycle, peptidyl-tRNA is located in the P-site of the ribosome. This site is similar to the f-Met-$tRNA_F^{Met}$ binding site in the 70 S initiation complex, since in both cases the substrate can react with puromycin. This finding might indicate that 30 S ribosomal proteins involved in f-Met-$tRNA_F^{Met}$ binding also participate in the binding of peptidyl-tRNA.

Inhibition experiments with specific Fab's showed that there is one class of Fab's which exclusively interferes with f-Met-$tRNA_F^{Met}$ binding (anti-S2, -S5, -S6, -S12 and -S13) and another class of Fab's (anti-S8, -S9, -S11 and -S18) which only interferes with EF–Tu dependent poly(U) directed Phe-tRNA binding [36]. These results indicate that on 30 S the f-Met-$tRNA_F^{Met}$ binding site (or P-site) is not identical to the EF–Tu dependent aminoacyl-tRNA binding site (A-site). On the other hand, there are indications that both 30 S tRNA binding sites overlap: a) Fab's against proteins S1, S3, S10, S14, S19, S20 and S21 interfere with f-Met-$tRNA_F^{Met}$ binding as well as with aminoacyl-tRNA binding [36]. b) Proteins S2, S3 and S14 stimulate both types of tRNA binding [35,63]. c) Binding of streptomycin to the ribosome apparently distorts the A- and P-site [89,90]. This suggests that proteins at the streptomycin binding site, such as protein S3 [46], are important for proper functioning of both the A- and P-site.

N-substituted phenylalanyl-$tRNA^{Phe}$ [71,72] and methionyl-$tRNA_F^{Met}$ analogs [91] have been constructed as very specific affinity-labels of the 50 S P-site in the peptidyltransferase center. These labels were predominantly associated with proteins L2 and/or L27. Protein L2 also reacted with *N*-iodoacetyl-puromycin [48]. Modification of proteins L2 and L27 by unspecific sulfhydryl reagents does not inactivate peptidyltransferase activity [70]; whereas, the affinity-labeling reactions do. All these data indicate that proteins L2 and L27 are located at, or near, the P-site of the peptidyltransferase center.

Erythromycin binds to the 50 S subunit in the vicinity of peptidyl-tRNA [92]. Ribosomal mutants which are resistant to this antibiotic are altered in protein L4 or L22 [93]. Peptidyltransferase activity is normal in L22 mutants but strongly reduced in mutants with altered L4. These data suggest that at least protein L4 is located at the P-site and is involved in binding of the growing peptide chain.

3.3. *Peptidyltransferase*

There still remains the question, which ribosomal protein actually forms the peptide bond. Partial reconstitution experiments indicate that this enzymatic activity is carried out by protein L11. 50 S derived particles deficient in protein L11 exhibited no peptidyltransferase activity and addition of L11 restored the enzymatic activity [94]. Recent affinity-labeling experiments support this observation. After binding the photo affinity-label [^{1}H] SNAP-tRNA to the P-site and [^{3}H] Phe-$tRNA^{Phe}$ to the A-site and subsequent irradiation with UV-light, protein L11 was found as one of the major products of the reaction [95]. These results indicate that protein L11 is near the 3′-terminus of peptidyl-tRNA and responsible for peptidyl-transfer.

Protein L11 is the peptidyltransferase and, therefore, should neighbor 50 S proteins in the A-site, such as L16 and L6. Reconstitution experiments indicated that these three proteins influence each other's functions [76]: a) Addition of protein L16 to 50 S derived particles which lack this protein but which contain L11 resulted in a pronounced increase

of peptidyltransferase activity. Vice versa, chloramphenicol binding to L16 containing particles devoid of protein L11, is stimulated by the addition of protein L11. These findings indicate that proteins L11 and L16 are neighbors. b) Addition of protein L6 to particles which contain the chloramphenicol binding protein L16 but no L6, stimulates chloramphenicol binding, which implies that proteins L6 and L16 are neighbors in the A-site. This conclusion is also supported by genetic data that protein L6 is altered in ribosomal mutants resistant to chloramphenicol and lincomycin [96]. c) Protein L6 stimulates peptidyltransferase activity of respective particles deficient in L6. However, this stimulation is not observed with 50 S particles missing L6 and L16. Thus, protein L6 stimulates peptidyltransferase activity only in the presence of protein L16.

3.4. *EF–Tu and EF–G*

In the elongation cycle, binding of aminoacyl-tRNA is mediated by the supernatant factor EF–Tu. Another supernatant factor, EF–G, is necessary for translocation. Both steps in protein synthesis require energy which is provided by GTP-hydrolysis. Ribosomal proteins involved in these energy requiring steps are different from those which are involved in the peptidyltransferase center, because antibiotics like thiostrepton or siomycin inhibit GTP-hydrolysis at a site which is distinct from this center [97,98]. After peptidyltransfer, EF–G mediates translocation of the newly formed peptidyl-tRNA from the A-site to the P-site and only then can EF–Tu dependent aminoacyl-tRNA binding take place anew. Since EF–G prevents binding of the ternary EF–Tu~GTP~aminoacyl-tRNA complex to the A-site, it is concluded that EF–G and EF–Tu bind to overlapping sites of the ribosome [99–104].

Proteins L7 and L12 are necessary for both, EF–G and EF–Tu dependent functions as shown by removal and readdition of L7 and L12 as well as by inhibition tests with antibodies against these proteins [40,105–111]. They are located at the EF–G binding site as shown by cross-linking experiments [112]. In the absence of proteins L7 and L12, EF–G and EF–Tu-dependent GTP hydrolysis can be restored by methanol [108]. Interestingly proteins L6 as well as L10 were shown to be important for EF–G-dependent ribosomal GTP hydrolysis [113,114].

It was observed that 5 S RNA is important for ribosomal functions which are linked to the A-site [80,82] and that according to reconstitution experiments, proteins L18 and L25 (and to lesser extent L5, L20 and L30) bind to 5 S RNA [115,116]. Furthermore, it was shown that such 5 S RNA–protein complexes could also be isolated as a native complex from *B. stearothermophilus* 50 S ribosomal components [116]. 23 S RNA firmly binds to the *E. coli* 5 S RNA–protein complex if proteins L2 and L6 are added [77]. Therefore, experiments were run to see if proteins associated with 5 S RNA already exhibit enzymic activities relevant to factor-mediated steps in protein synthesis. Indeed, hydrolysis of GTP as well as ATP was observed by these complexes [117]. These enzymic activities are inhibited by fusidic acid and thiostrepton but not by antibiotics which bind in the A-site or P-site of the ribosome [118].

The data strongly suggest that 5 S RNA binding proteins are part of the site in which EF–G and EF–Tu exert their function although the GTPase activity of the 5 S RNA–protein complex does not depend on the presence of EF–G. This conclusion is supported by affinity labeling experiments which involves a fusidic acid stabilized complex between ribosomes, EF–G and the photo-affinity label APh-GDP (1-(4-azidophenyl)-2-(5′-guanyl)pyrophosphate) [119]. The results of these experiments showed that proteins L5, L18, L30 and L11 are involved in GDP binding and they are in good agreement with the results cited above.

Since the 30 S subunit is intimately involved in mRNA- and tRNA-binding, it can be expected that 30 S ribosomal proteins are also important for EF–G and EF–Tu-dependent ribosomal activities. It was shown by partial reconstitution experiments that proteins S2, S9 (and to a lesser extent S5) are needed for EF–Tu-dependent GTP hydrolysis [120]. Correspondingly, proteins S5, S9 (and to a lesser extent S2) are necessary for EF–G-dependent GTPase hydrolysis [121]. The mode of action of these 30 S proteins seems to be rather indirect. They enable the formation of active 70 S ribosomes and this formation is fundamental for EF–G and EF–Tu-dependent activities [122].

30 S and 50 S proteins which are involved in similar functions should neighbor each other at the ribosomal interface. This concept is supported by evi-

dence obtained from binding Fab's to ribosomal proteins in the 30 S, 50 S or 70 S particle [123]. The following conclusions can be drawn from these results: The 30 S proteins S9 (GTPase activity), S11 and S12 (translational fidelity), S14 and S20 (tRNA binding) and the 50 S proteins L1, L6 (A-site), L14, L15, L19, L20 (binding to 5 S RNA), L23, L26, L27 (P-site) are at the ribosomal interface. As shown independently [111], at least six ribosomal proteins (S9, S11, L14, L19, L23, L27) are at the interface and promote subunit association necessary for GTPase activity.

4. Termination

The elongation cycle is terminated when the ribosome reaches a termination signal on the mRNA. In response to this termination signal, the ribosomal binding of special release factors leads to hydrolysis of peptidyl-tRNA and to subsequent release of the synthesized protein [10,13]. The release factors apparently bind at a site similar to, or near, the binding site of EF–G and EF–Tu because antibodies against L7 and L12 also inhibit release factor binding [124]. The proteins responsible for fixation of the 3′-end of aminoacyl-tRNA in the A-site and for peptidyl-transfer are apparently identical to those involved in peptidyl-tRNA hydrolysis [125], since antibodies against proteins L11 and L16 inhibit peptidyl-tRNA hydrolysis [124].

When bacteria under stringent control are starved of amino acids, premature termination of protein synthesis occurs and guanosine tetra- and pentaphosphates are accumulated [126]. These compounds (magic spots) are synthesized on the ribosomes as a product of an idling step in protein synthesis. It has been shown that ATP, GTP, stringent factor, mRNA and deacylated aminoacyl-tRNA in the A-site are needed for magic spot synthesis [126–129]. The stringent factor catalyzes pyrophosphate transfer from ATP to GDP [130]. So far the only ATPase activity associated with the ribosome has been that correlated with 5 S RNA–protein complexs [117]. Ribosomal requirements for magic spot synthesis are: Presence of 5 S RNA and correct positioning of intact uncharged tRNA in the ribosomal A-site [128,129, 131]. Proteins L7 and L12 do not seem to be required for the synthesis of magic spots [131,132].

5. Summary

In the figure, 30 S ribosomal proteins have been arranged according to their functional role: Protein S1 is required for mRNA binding. Proteins S3, S4,

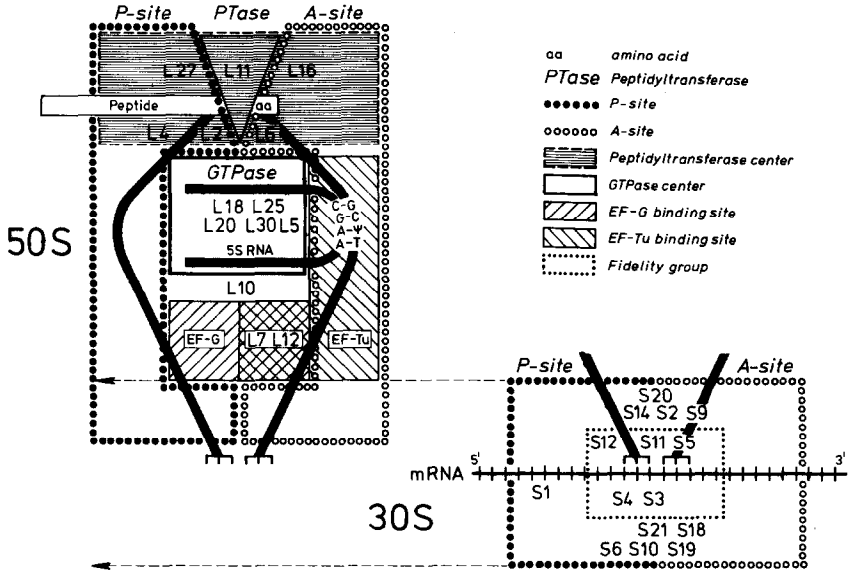

Fig. 1. Scheme of the functional arrangement of components in the active sites of *E. coli* ribosomal subunits.

S5, S11 and S12 are involved in cistron and/or codon–anticodon recognition. They must be close to the decoding sites on the 30 S subunit. Furthermore proteins S2, S3, S10, S14, S19 and S21 function in f-Met-tRNA binding. Proteins S1, S2, S3, S10, S14, S19, S20 and S21 are important for the function of both decoding sites, whereas proteins S9, S11 and S18 are only needed for EF–Tu-dependent aminoacyl-tRNA binding. Proteins S2, S5, S9 and S11 would be close to the GTPase center of the 50 S subunit, since they are important for this activity.

The present available data concerning the 50 S subunit allow the following picture to be drawn: Protein L16 is involved in binding the 3′-terminus of aminoacyl-tRNA in the A-site. Next to it in the A-site, there is protein L6. The P-site is located adjacent to the A-site of the peptidyltransferase center. Accordingly, protein L2 is near protein L6 and is located in the P-site as well as proteins L27 and L4. Protein L11, which is intimately involved in peptide bond formation, would have to border parts of both A- and P-sites. Proteins L6 and L2 stimulate binding of 5 S RNA–protein complexes to 23 S RNA. The 5 S RNA–protein complex has GTPase and ATPase activities. The proteins in this complex (L5, L18, L20, L25 and L30) seem to be located close to the A-site of the peptidyltransferase center. These proteins together with protein L11 are involved in GDP binding. Proteins L10 and L6 are implicated in reconstitution of protein L7 and L12 mediated EF–G-dependent ribosomal GTP hydrolysis. This observation is supported by the fact that the aminoacyl-tRNA binding site, e.g. proteins L16 and L6, is connected with EF–G and EF–Tu binding site, e.g. proteins L7 and L12, as well as the GTPase center. Furthermore, if one of the functional roles of 5 S RNA is to bind aminoacyl-tRNA via T–Ψ–C, then those ribosomal proteins which bind to 5 S RNA (or are close to it) would be located near or at the A-site.

The model of active sites in *E. coli* ribosome illustrated in the figure is based on the presently available experimental results. It is far from being complete and should not be overinterpreted as an accurate topographical model. More data on the functional role of ribosomal components and on the topography of the subunits can be expected in the near future and will add to the knowledge on the active sites in ribosomes.

Note added in proof

Recently published data provided further evidence that the factors for initiation, elongation and termination have overlapping binding sites on the ribosome: It was shown that L7 and L12 are part of the binding site for IF-2 [133]. These proteins are also required for peptide chain termination [134] Interaction of RF-1 and RF-2 with ribosomes is inhibited by binding of EF–G to ribosomes [135]. In addition to L7 and L12 another (not yet identified) protein is also required for the action of EF–G as well as EF–Tu. This protein probably functions by facilitating the binding of L7 and L12 to the ribosomes [136].

Affinity labeling studies have yielded further results: Proteins L24 and L33 were identified as part of the P-site by affinity labeling with peptidyl-tRNA analogs [137]. Puromycin was modified at the 5′-OH group such that it bound irreversibly to ribosomes, but could still undergo peptide bound formation. Preliminary results indicate that this affinity label reacted with ribosomal RNA instead of protein [138]. Proteins L27 and L15 were labeled with an f-Met-tRNA$_F^{Met}$ analog and were therefore concluded to belong to the binding site for the aminoacyl end of tRNA$_F^{Met}$ [139]. That protein L15 is possibly involved in the peptidyltransferase centre has also been concluded from chemical modification studies [140].

Based on oligonucleotide binding studies to 30 S ~ poly (U) ~ EF–Tu ~ GTP ~ Phe-tRNA^{Phe} complex it was concluded that codon–anticodon interaction induces a conformational change in the tertiary structure of aminoacyl-tRNA such that base pairing between the T–Ψ–C–G– sequence of tRNA^{Phe} and the C–G–A–A– sequence of 5 S RNA becomes facilitated [141].

In contrast to an earlier report [35], it was found that S21 does not inhibit AUG-directed f-Met-tRNA binding. S21 is required for full activity of ribosomes in initiation of polypeptide synthesis [142].

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