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Functional Interactions by Transfer RNAs in the Ribosome

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Abstract

Recent X-ray crystal structures of the ribosome have revolutionized the field by providing a much-needed structural framework to understand ribosome function. Indeed, the crystal structures rationalize much of the genetic and biochemical data that have been meticulously gathered over 50 years. Here, we focus on the interactions between tRNAs and the ribosome and describe some of the insights that the structures provide about the mechanism of translation. Both high-resolution structures and functional studies are essential for fully appreciating the complex process of protein synthesis.

Keywords

translation; decoding; translocation; termination; ribosome

Introduction

Ribosomes are the cellular complexes responsible for protein synthesis in all organisms. The 30S subunits of bacterial ribosomes consist of 16S rRNA and about 20 proteins and their 50S large subunits consist of 23S rRNA, 5S rRNA and about 30 proteins. During protein synthesis, ribosomes bind aminoacylated tRNAs, in a codon-dependent manner, to synthesize proteins encoded by the mRNAs. The anticodon arm of the tRNA interacts with the 30S ribosomal subunit, while the acceptor arm interacts with the 50S ribosomal subunit. There are three tRNA binding sites on the ribosome: aminoacyl (A), peptidyl (P), and exit (E) site. Recent X-ray crystal structures of the ribosome complexes have revealed in exquisite detail the interactions of tRNAs with the A, P and E sites [1-3]. These structures have revolutionized the field and a deeper understanding of the mechanism of translation is beginning to emerge by combining structural data with results obtained by traditional genetic and biochemical experiments. This review focuses on the interactions between the tRNAs and the ribosome that have provided insights into the mechanism of protein synthesis.

We first give a brief overview of the steps in protein synthesis before discussing the functional significance of the ribosome structure. Protein synthesis consists of four major phases: initiation, elongation, termination, and ribosome recycling (Figure 1). During initiation, the 30S subunit binds an mRNA and the initiator tRNA with the help of initiation factors 1, 2, and

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3 (IF1, IF2, and IF3). The position of the mRNA on the 30S subunit depends on base pairing interactions between the Shine-Dalgarno (SD) sequence in the mRNA and a complementary sequence present in the 3'-end of 16S rRNA known as the anti-Shine-Dalgarno (ASD) sequence. The initiator tRNA binds directly to the 30S subunit P site and the anticodon of the initiator tRNA forms base pairs with the start codon in the mRNA. Proper interactions between the initiator tRNA and the start codon signals the rapid association of the 50S subunit to form the 70S ribosome. The association of the 50S subunit with the 30S subunit triggers GTP hydrolysis by IF2 and the release of initiation factors from the ribosome.

The ribosome, with an initiator-tRNA in the P site, is now ready for the elongation phase of protein synthesis. Aminoacyl-tRNAs bind to the ribosome as a ternary complex with elongation factor Tu and GTP (EF-Tu•GTP•aa-tRNA). The anticodon arm of the tRNA, in the ternary complex, interacts with the 30S subunit A site, while the acceptor arm remains bound to EF-Tu•GTP and is not available for interactions with the 50S subunit A site [4]. This intermediate of the decoding process is called the A/T state and has been visualized by cryogenic electron microscopy and three-dimensional image reconstructions (cryo-EM) [5]. Importantly, non-cognate and near-cognate ternary complexes do not form stable A/T states on the ribosome and are rejected in a process called "initial selection" [6]. When cognate codon-anticodon pairing does occur, GTP is hydrolyzed by EF-Tu and the aminoacyl-tRNA is rapidly accommodated into the 50S subunit A site. During the tRNA accommodation step, near-cognate tRNAs are generally rejected by the ribosome in a process called "proofreading" [6]. Accommodation of the aminoacyl-tRNA into the 50S subunit A site leads to peptide bond formation by the peptidyl transferase center (PTC) that is largely composed of conserved bases from the 23S rRNA [7-9]. Peptidyl transferase reaction results in a deacylated-tRNA in the P site and a dipeptidyl-tRNA in the A site. Interestingly, the acceptor arm of deacylated-tRNA fluctuates between the 50S subunit P and E sites, while the anticodon arm remains firmly bound to the 30S subunit P site resulting in the P/E hybrid state [4,10]. Similarly, the acceptor arm of dipeptidyl-tRNA can move from the 50S subunit A site to the P site, while retaining interactions with the 30S subunit A site, resulting in the A/P hybrid state.

The final step in the elongation cycle is the translocation of the deacylated- and dipeptidyl-tRNAs into the E and P sites of the 30S subunit, respectively. This reaction is catalyzed by elongation factor G (EF-G), which hydrolyzes GTP to accelerate the movement of the tRNAs [11]. Binding of EF-G•GTP stabilizes the tRNAs in the P/E and A/P hybrid states [12]. Formation of the hybrid states is loosely coupled with the 30S subunit undergoing a ratchet-like rotation with respect to the 50S subunit [13]. Movement of the tRNAs into the hybrid states is thought to lower the activation energy barrier for translocation [14]. EF-G then completes the process by catalyzing movement of P/E and A/P tRNAs with respect to the 30S subunit to form the E/E and P/P states. Translocation of the mRNA-tRNA complex by EF-G places the next mRNA codon in the A site, which allows a new round of the elongation cycle to proceed. This process continues until a stop codon (UAA, UAG or UGA) enters the A site.

Entry of the stop codon into the A site triggers the termination phase of protein synthesis. Stop codons are recognized by release factors 1 and 2 (RF1 and RF2). RF1 recognizes UAA and UAG, while RF2 recognizes UAA and UGA. RF1 or RF2 binds to a site that overlaps with the ribosomal A site and catalyzes the release of the newly completed polypeptide attached to the tRNA in the P site. Peptide hydrolysis by RF1 or RF2 occurs in the PTC and conserved bases in 23S rRNA are required for promoting the hydrolysis reaction.

The next steps are devoted to removing mRNA, tRNA and RF1 (or RF2) from the ribosome and splitting the ribosome into the 30S and 50S subunits. This final phase of protein synthesis is known as recycling. RF1 or RF2 are removed from the ribosome by the activity of release factor 3 (RF3), which exchanges GDP for GTP on the ribosome [15]. Binding of GTP to RF3

triggers the dissociation of RF1 or RF2 from the ribosome [15]. RF3 then hydrolyzes GTP and dissociates from the ribosome. Next, ribosome recycling factor (RRF) and EF-G•GTP bind and split the ribosome into 30S and 50S subunits. Re-association is prevented by IF3 binding to the 30S subunit. The mRNA and the deacylated-tRNA are also removed from the 30S subunit. The recycled 30S subunit is now free to initiate translation on a new mRNA.

Functional Interactions in the P site

As described above, during translation initiation the initiator tRNA^{fMet} binds directly to the 30S subunit P site. An important question is how are elongator tRNAs prevented from binding to the P site during initiation, especially elongator tRNA^{Met}, which can form base pairs with the start codon in the mRNA? Initiator tRNA^{fMet} has unique structural features such as the C-A mismatch at position 1-72 in the acceptor stem that is important for formylation. IF2 specifically recognizes the formyl group of initiator tRNA^{fMet} and stabilizes it on the 30S subunit. Another feature of initiator tRNA^{fMet} and some elongator tRNAs is the three adjacent G-C base pairs in the anticodon arm. Structural data and biochemical studies recently indicated that interactions between the anticodon arm of initiator tRNA^{fMet} and the 30S subunit P site are important for IF3-dependent discrimination of initiator tRNA^{fMet} from elongator tRNAs [1-3,16-19]. The interaction of bases G1338 and A1339 of 16S rRNA with the conserved G-C base pairs at positions 29-41 and 30-40, respectively in the anticodon arm of the initiator tRNA^{fMet} stabilizes the tRNA in the 30S subunit P site (Figure 2). Any mutation at position A1339 resulted in an 18-fold decrease in translation of a reporter protein consistent with a Type I minor interaction with the P-tRNA [18]. By contrast, mutation of G1338 to A showed no effect on translational activity, and instead seemed to stabilize P-tRNA [18,19]. This is consistent with a Type II minor interaction between G1338 and the G-C base pair at position 29-41 in the tRNA. In a Type II minor interaction a G can substitute for an A [20]. However, mutation of G1338 to C or U decreased translational activity by 11-fold and 4.5-fold, respectively [18,19].

In addition to G1338 and A1339, the anticodon stem-loop (ASL) of P site tRNA interacts extensively with several 16S rRNA bases and the small subunit proteins S9 and S13 [1-3]. Base A790 contacts the backbone of nucleotides 37-38 in P-tRNA. Mutation of A790 to the other three bases only modestly decreased translation (5-fold maximum decrease), indicating that interaction of A790 with P site tRNA is not critical for protein synthesis [18]. A790G decreases the affinity of IF3 for the 30S subunit [19]. Base G926 interacts with the phosphate group at the first position of the P site codon. Mutation of G926 results in an 8-fold decrease in translation [18]. Base m²G966 interacts with the backbone of P-tRNA at position 34. Mutations at m²G966 decreased translation by 3- to 10-fold [18]. The backbone of nucleotides 1229-1230 of 16S rRNA packs against the backbone of nucleotides 29-30 of P-tRNA (Figure 2B). Base C1400 of 16S rRNA stacks with base 34 of P-tRNA. Mutation of C1400 to U had very little effect on translation; however, mutation to A or G reduced translation by 12-fold and 20-fold, respectively [18]. The larger purine substitutions at position 1400 may cause steric hindrance with the codon-anticodon helix in the P site. Bases 1401-1403 of 16S rRNA are located close to the P site codon and 1401-1402 coordinates a metal ion that interacts with the codon. Finally, the phosphate at position 1498 of 16S rRNA interacts with the first position of the P site codon. The C-terminus of ribosomal proteins S9 and S13 interact with the anticodon arm of P-tRNA. Deletion of the C-terminal tails of S9 and S13 only modestly affected the growth rate indicating that they are not essential for translation [21]. These results show that most base specific contacts in the 30S subunit P site are redundant and not absolutely essential for translation.

Ribosomal protein L5 is close to position C56 in the elbow region of P site tRNA [1-3]. On the opposite side, helix 69 of 23S rRNA contacts positions 12-13 in the D-stem of P-tRNA. In

addition, base A1913 in helix 69 interacts with positions 37-38 of A-tRNA. Helix 69 also interacts with helix 44 of 16S rRNA to form an intersubunit bridge (bridge B2a). Ribosomes with helix 69 deleted, surprisingly, can accurately synthesize a full-length protein [22]. Nevertheless, helix 69 deleted ribosomes are defective in peptide release by RF1, and can recycle in the absence of RRF possibly due to weakened subunit association [22]. A more recent study showed that mutations at A1912 and A1919 in helix 69 strongly inhibited subunit association, initiation and ribosomal processivity implicating helix 69 in the initiation and elongation phases of protein synthesis [23].

Functional Interactions in the A site

The anticodon arm of A site tRNA interacts with the decoding center in the 30S subunit (Figure 3). A X-ray crystal structure of the 30S subunit with a cognate ASL in the A site showed that universally conserved 16S rRNA bases G530, A1492 and A1493 interact with the minor groove of the codon-anticodon helix [24]. Base G530 undergoes rotation from a *syn*- to an *anti*-conformation, while bases A1492 and A1493 flip out of helix 44 to contact the codon-anticodon helix (Figure 3B). A1493 forms a Type I minor interaction with the codon-anticodon base pair at the first position of the A site codon. Both G530 and A1492 interact with the second position of the A site codon. A1492 also interacts with G530, C518, and with Ser50 of ribosomal protein S12. Finally, the third position of the A site codon is contacted by G530. In addition, the 2'-hydroxyl group of the codon forms a metal ion-mediated interaction with C518 and Pro48 of S12.

Bases G530, A1492, and A1493 were proposed to discriminate between cognate and non-cognate tRNAs by monitoring the shape of the cognate codon-anticodon helix [24]. Mutations at G530, A1492, and A1493 show a dominant lethal phenotype [25]. These mutations in the decoding center decreased the rate of GTP hydrolysis by EF-Tu and the rate of peptide bond formation by 20- to 40-fold [25]. In addition, mutations at G530, A1492, and A1493 reduced the rate of translocation by ≈ 20 -fold [26]. Furthermore, 2'-deoxy or 2'-fluoro substitutions at position 35 or 36 in the anticodon of an A site ASL inhibited translocation due to disruption of the interactions with G530 and A1493 [27]. These results show that interactions in the decoding center are not only important for tRNA selection, but also for rapid translocation.

Base C1054 of 16S rRNA stacks on the ribose at position 34 in the anticodon arm of A site tRNA [1,3]. Mutation of C1054 to U had very little effect on translation indicating that this base is not essential [18]. Ribosomal protein S12 interacts with the backbone of the A site codon, as described above (Figure 3B). Historically, several mutations in S12 have been isolated that affect tRNA selection by the ribosome [28]. Most mutations in S12 increase the fidelity of translation and this is proposed to be due to the inhibition of conformational changes in the 30S subunit that follows tRNA binding to the A site [29]. S13 contacts the backbone of A-tRNA at position 41. Interestingly, the lack of S12 or S13 in reconstituted ribosomes promotes spontaneous, EF-G-independent translocation [30]. S13 forms two intersubunit bridges (1a and 1b) with the 50S subunit that are broken by the ratchet-like rotation during translocation [31]. The loss of S13 may, therefore, favor formation of the ratcheted state and promote spontaneous translocation. Furthermore, a S13 deletion mutant showed defects in subunit association and translation initiation possibly due to weakened subunit association [32].

Ribosomal protein L16 interacts with the elbow region of A site tRNA [1]. Arg51 and Arg56 of L16 interact with the backbone of tRNA at positions 53-54. Previous biochemical studies have shown that L16 is important for the binding of tRNA to the A site and for peptidyl transferase activity [33,34]. L16 may stabilize the interaction of tRNA with the A site. In addition, helix 38 of 23S rRNA lies between the elbows of A and P site tRNAs and is known

as the A site finger. Bases G882 and G883 in helix 38 interact with position 19 in the D-loops of both A and P site tRNAs. Shortening helix 38 enhances +1 frameshifting and EF-G-dependent translocation suggesting that the role of helix 38 is to maintain the pre-translocation state of the ribosome [35].

Interaction of A and P site tRNAs with the Peptidyl Transferase Center

The universally conserved 3'-CCA ends of A and P site tRNAs interact with the peptidyl transferase center of the 50S subunit (Figures 2C and 3C). Conserved bases C2063, G2251, G2252, G2255, A2450, A2451, U2506, U2585, and A2602 in 23S rRNA form the active site [1-3, 7-9]. Surprisingly, mutations at A2451, U2506, U2585, and A2602 in the PTC do not inhibit peptide bond formation [36-38]. These bases, however, are important for peptide release by RF1 [36, 38]. Bases C74 and C75 of P-tRNA form base pairs with 23S rRNA bases G2252 and G2251, respectively. Disruption of the C74-G2252 base pair increased the rate of translocation by 2- to 4-fold, which is consistent with the idea that formation of the P/E hybrid state facilitates translocation [39, 40]. Furthermore, disruption of the C74-G2252 base pair did not inhibit peptidyl transfer but reduced the rate of peptide hydrolysis by RF1 by ≈ 100 -fold indicating that this base pair is important for peptide release [39]. The terminal base A76 of P-tRNA stacks with bases C74 and C75 and forms an A-minor interaction with the A2450-C2063 base pair in 23S rRNA. The 2'-hydroxyl group of A76 is within hydrogen bonding distance of the 2'-hydroxyl and N3 of A2451. Recent data show that the 2'-hydroxyl group of A2451 is important for peptidyl transfer possibly by stabilizing the ribose C2'-endo conformation of A76 [41]. Interestingly, substitution of the 2'-hydroxyl group at A76 of P site tRNA with 2'-deoxy or 2'-fluoro groups inhibits peptidyl transferase reaction by as much as 10^6 -fold demonstrating that it plays a critical role in the reaction [42]. The related reaction of peptide release by RF1 is also inhibited by 10^3 -fold with 2'-deoxy and 2'-fluoro substitutions suggesting that the ribosome uses a similar strategy to catalyze both reactions [43].

The α -amino group of the aminoacylated-tRNA in the A site contacts the N3 and 2'-hydroxyl of A2451 in 23S rRNA and the 2'-hydroxyl of A76 of P-tRNA [1]. Base A76 of A-tRNA forms a Type I minor interaction with G2583 and the ribose of A76 contacts U2585. Base C75 of A-tRNA forms a base pair with G2553 of 23S rRNA. Interestingly, formation of the C75-G2553 base pair by A site tRNA induces conformational changes in the peptidyl transferase center that are necessary for properly orienting the ester link of P site tRNA for peptidyl transfer [44]. A recent X-ray crystal structure of the 70S ribosome revealed that the N-terminal tail of ribosomal protein L27 is close to the CCA end of A and P site tRNAs [1]. Consistent with the structural data, previous biochemical studies have shown that deletion of the first three amino acids from the N-terminus of L27 inhibits peptidyl transfer [45]. Thus, L27 by stabilizing the 3'-ends of the P and A site tRNAs in the peptidyl transferase center may promote peptide bond formation by the ribosome.

Functional Interactions in the E site

The anticodon of E site tRNA is loosely monitored by the ribosome (Figure 4). Bases G693 and A695 of 16S rRNA interact with the E-tRNA [1-3]. G693 contacts position A35 of E-tRNA and also packs against the ribose at the third position of the E site codon. The ribose and phosphate of A695 interact with the phosphate at position 39 of E-tRNA. Proteins S7 and S11 interact with the anticodon arm of E site tRNA (Figure 4B). Arg76 and Arg146 in the C-terminal tail of S7 contact positions 33 and 41 of E-tRNA, respectively. Truncation of S7 by deleting Arg77 to Tyr 84 increased both -1 and +1 frameshifting [46]. The rate of spontaneous reverse translocation was increased; however, the rate of EF-G-dependent translocation was not affected by truncation of S7 [46]. It thus appears that the interactions in the 30S subunit E site do not contribute significantly to the process of EF-G-dependent translocation. It is unclear

whether the anticodon of E site tRNA forms Watson-Crick base pairs with the mRNA codon. Most of the high-resolution crystal structures of the ribosome have a non-cognate tRNA in the E site, which clearly cannot form Watson-Crick base pairs with the E site codon. However, the crystal structure of a ribosomal complex representing the post-initiation state indicates that the E site codon can relax into a conformation that is favorable for forming base pairs with the tRNA [47]. This question will be settled definitely when structures of the ribosome with a cognate tRNA in the E site are solved in the future.

In contrast to the 30S subunit, the acceptor arm of E site tRNA forms extensive interactions with the 50S subunit [1-3]. Base A76 of E site tRNA intercalates between 23S rRNA nucleotides G2421 and A2422 and forms hydrogen bonds with the universally conserved base C2394 (Figure 4C). Surprisingly, mutations at C2394 decreased the growth rate only moderately [48,49]. Ribosomes with mutations at C2394 did not inhibit GTP-hydrolysis by EF-G but reduced the rate of translocation (2- to 8-fold inhibition). In addition, the apparent affinity of EF-G for the pre-translocation complex was much reduced for the mutant ribosomes (4-fold to 23-fold decrease in affinity) [48]. These results suggest that rapid and reversible GTP hydrolysis precedes movement of tRNA into the hybrid P/E state [48].

The ribose of bases 70 and 71 in E site tRNA interact with the backbone of bases C1893 and C1892, respectively [1-3]. Substitution of the 2'-hydroxyl group at positions 66, 70, 71, or 76 with a 2'-O methyl group in the acceptor arm of P-tRNA inhibited translocation [50]. Single 2'-deoxy substitutions at 71 and 76 also inhibited translocation, while no inhibition was observed with single 2'-deoxy substitutions at 66 and 70. Inhibition of translocation with the bulkier 2'-O methyl group at positions 66 and 70 may be caused by steric hindrance within the ribosome. The backbone of base 71 does not interact with the P site; however it interacts with C1892 in the E site as described above. These results suggest that substitutions in the P site tRNA that block interactions with the 50S subunit E site inhibit translocation. Indeed, earlier studies showed that deletion or modification of A76 reduces E site affinity and inhibits translocation of P-tRNA into the E site [51]. Interaction between the 2'-hydroxyl group at position 71 in the P tRNA and C1892 in the 50S subunit E site may stabilize the P site tRNA in the P/E state. This will lower the activation energy barrier for translocation.

The backbone of bases 3 and 5 in E site tRNA interacts with the backbone of bases 1850-1853 in 23S rRNA. Surprisingly, these interactions do not appear to be important for translocation because single 2'-deoxynucleotide substitutions in the 5'-acceptor arm of P site tRNA (positions 1-17) does not inhibit translocation [52]. The E site tRNA makes many other interactions with the 50S subunit as revealed by the recent crystal structures. These include interactions of the backbone of bases 73 and 74 of E site tRNA with Lys38 and Arg39 of ribosomal protein L28. Also, position 74 of E site tRNA interacts with Arg28 of ribosomal protein L33. Bases G19 and C56 in the elbow region of E site tRNA stack with bases G2112 and A2169 of 23S rRNA. Furthermore, the elbow region of E site tRNA at positions 55, 62, and 63 interacts with Lys167, Arg52, and Arg53, respectively of ribosomal protein L1. Thus, the elbow region of E site tRNA interacts intimately with the L1 stalk of the ribosome (L1 and helices 76, 77, and 78 in 23S rRNA). Cryo-EM data [31], X-ray crystallography [1-3] and recent single molecule fluorescence resonance transfer experiments [53-55] showed that the conformation of the L1 stalk is very dynamic and is influenced by the absence or presence of a tRNA in the E site. In the pre-translocation ribosome, with the tRNA in the P site fluctuating between P/P and P/E states, the conformation of L1 switches between a fully open and a fully closed state. In the post-translocation ribosome, with a tRNA in the E/E state, L1 switches to a half-closed conformation. In ribosomes with a vacant E site, L1 switches between the fully open and fully closed states. Interestingly, the binding of EF-G•GDPNP to the ribosome favors the fully closed conformation of L1 possibly by stabilizing the P site tRNA in the P/E state [55]. These findings have led to the proposal that during translocation, the L1 stalk guides the

movement of the deacylated-tRNA from the P site to the E site and subsequently ejects the tRNA from the E site by switching to the fully open state.

As summarized in this review, the X-ray crystal structures of the ribosome provide a firm foundation to evaluate the vast amount of genetic and biochemical data that have been acquired over the years. The structures have provided valuable insights into the mechanism of decoding, peptidyl transferase reaction, translocation, and translation termination. However, there are instances when it is difficult to rationalize the biochemical data with the structural information. For example, the universally conserved base A2451 of 23S rRNA is located in the heart of the peptidyl transferase center and is within hydrogen bonding distance of the P and A site substrates [1,7,8]. Mutations at A2451 are lethal to *E. coli*; yet, biochemical experiments showed that peptide bond formation is inhibited by less than 2-fold [36,37]. Although peptide release by RF1 is inhibited by as much as 6-fold with the A2451G and the A2451C mutations, A2451U mutation showed no defects in peptide release [36]. So why is A2451 universally conserved? Similarly, there are other elements of the ribosome that appear to be important from a structural perspective but show only nominal effects in *in vitro* assays. Perhaps, this implies that sensitive biochemical techniques need to be developed to more rigorously interrogate each and every step of protein synthesis.

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Abbreviations

ASL	anticodon stem-loop analog of tRNA
IF	initiation factor
EF-Tu	elongation factor Tu
EF-G	elongation factor G
RF	release factor
RRF	ribosome recycling factor

References

1. Voorhees RM, Weixlbaumer A, Loakes D, Kelley AC, Ramakrishnan V. Insights into substrate stabilization from snapshots of the peptidyl transferase center of the intact 70S ribosome. *Nat Struct Mol Biol* 2009;16(5):528–33. [PubMed: 19363482]
2. Korostelev A, Trakhanov S, Laurberg M, Noller HF. Crystal structure of a 70S ribosome-tRNA complex reveals functional interactions and rearrangements. *Cell* 2006;126(6):1065–77. [PubMed: 16962654]
3. Selmer M, Dunham CM, Murphy F.V.t. Weixlbaumer A, Petry S, Kelley AC, Weir JR, Ramakrishnan V. Structure of the 70S ribosome complexed with mRNA and tRNA. *Science* 2006;313(5795):1935–42. [PubMed: 16959973]
4. Moazed D, Noller HF. Intermediate states in the movement of transfer RNA in the ribosome. *Nature* 1989;342(6246):142–148. [PubMed: 2682263]
5. Valle M, Zavialov A, Li W, Stagg SM, Sengupta J, Nielsen RC, Nissen P, Harvey SC, Ehrenberg M, Frank J. Incorporation of aminoacyl-tRNA into the ribosome as seen by cryo-electron microscopy. *Nat Struct Biol* 2003;10(11):899–906. [PubMed: 14566331]
6. Pape T, Wintermeyer W, Rodnina M. Induced fit in initial selection and proofreading of aminoacyl-tRNA on the ribosome. *Embo J* 1999;18(13):3800–7. [PubMed: 10393195]

7. Ban N, Nissen P, Hansen J, Moore PB, Steitz TA. The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* 2000;289(5481):905–920. [PubMed: 10937989]
8. Nissen P, Hansen J, Ban N, Moore PB, Steitz TA. The structural basis of ribosome activity in peptide bond synthesis. *Science* 2000;289(5481):920–30. [PubMed: 10937990]
9. Harms J, Schlutzen F, Zarivach R, Bashan A, Gat S, Agmon I, Bartels H, Franceschi F, Yonath A. High resolution structure of the large ribosomal subunit from a mesophilic eubacterium. *Cell* 2001;107(5):679–88. [PubMed: 11733066]
10. Munro JB, Altman RB, O'Connor N, Blanchard SC. Identification of two distinct hybrid state intermediates on the ribosome. *Mol Cell* 2007;25(4):505–17. [PubMed: 17317624]
11. Rodnina MV, Savelsbergh A, Katunin VI, Wintermeyer W. Hydrolysis of GTP by elongation factor G drives tRNA movement on the ribosome. *Nature* 1997;385(6611):37–41. [PubMed: 8985244]
12. Spiegel PC, Ermolenko DN, Noller HF. Elongation factor G stabilizes the hybrid-state conformation of the 70S ribosome. *Rna* 2007;13(9):1473–82. [PubMed: 17630323]
13. Frank J, Agrawal RK. A ratchet-like inter-subunit reorganization of the ribosome during translocation. *Nature* 2000;406(6793):318–22. [PubMed: 10917535]
14. Semenov YP, Rodnina MV, Wintermeyer W. Energetic contribution of tRNA hybrid state formation to translocation catalysis on the ribosome. *Nat Struct Biol* 2000;7(11):1027–31. [PubMed: 11062557]
15. Zavialov AV, Buckingham RH, Ehrenberg M. A posttermination ribosomal complex is the guanine nucleotide exchange factor for peptide release factor RF3. *Cell* 2001;107(1):115–24. [PubMed: 11595190]
16. Berk V, Zhang W, Pai RD, Cate JH. Structural basis for mRNA and tRNA positioning on the ribosome. *Proc Natl Acad Sci U S A* 2006;103(43):15830–4. [PubMed: 17038497]
17. Lancaster L, Noller HF. Involvement of 16S rRNA nucleotides G1338 and A1339 in discrimination of initiator tRNA. *Mol Cell* 2005;20(4):623–32. [PubMed: 16307925]
18. Abdi NM, Fredrick K. Contribution of 16S rRNA nucleotides forming the 30S subunit A and P sites to translation in *Escherichia coli*. *RNA* 2005;11(11):1624–32. [PubMed: 16177132]
19. Qin D, Abdi NM, Fredrick K. Characterization of 16S rRNA mutations that decrease the fidelity of translation initiation. *Rna* 2007;13(12):2348–55. [PubMed: 17942743]
20. Doherty EA, Batey RT, Masquida B, Doudna JA. A universal mode of helix packing in RNA. *Nat Struct Biol* 2001;8(4):339–43. [PubMed: 11276255]
21. Hoang L, Fredrick K, Noller HF. Creating ribosomes with an all-RNA 30S subunit P site. *Proc Natl Acad Sci U S A* 2004;101(34):12439–43. [PubMed: 15308780]
22. Ali IK, Lancaster L, Feinberg J, Joseph S, Noller HF. Deletion of a conserved, central ribosomal intersubunit RNA bridge. *Mol Cell* 2006;23(6):865–74. [PubMed: 16973438]
23. Kipper K, Hetenyi C, Sild S, Remme J, Liiv A. Ribosomal intersubunit bridge B2a is involved in factor-dependent translation initiation and translational processivity. *J Mol Biol* 2009;385(2):405–22. [PubMed: 19007789]
24. Ogle JM, Brodersen DE, Clemons WM Jr, Tarry MJ, Carter AP, Ramakrishnan V. Recognition of cognate transfer RNA by the 30S ribosomal subunit. *Science* 2001;292(5518):897–902. [PubMed: 11340196]
25. Cochella L, Brunelle JL, Green R. Mutational analysis reveals two independent molecular requirements during transfer RNA selection on the ribosome. *Nat Struct Mol Biol* 2007;14(1):30–6. [PubMed: 17159993]
26. Garcia-Ortega L, Stephen J, Joseph S. Precise alignment of peptidyl tRNA by the decoding center is essential for EF-G-dependent translocation. *Mol Cell* 2008;32(2):292–9. [PubMed: 18951096]
27. Phelps SS, Jerinic O, Joseph S. Universally conserved interactions between the ribosome and the anticodon stem-loop of A site tRNA important for translocation. *Mol. Cell* 2002;10:799–807. [PubMed: 12419224]
28. Ogle JM, Ramakrishnan V. Structural insights into translational fidelity. *Annu Rev Biochem* 2005;74:129–77. [PubMed: 15952884]
29. Ogle JM, Murphy FV, Tarry MJ, Ramakrishnan V. Selection of tRNA by the ribosome requires a transition from an open to a closed form. *Cell* 2002;111(5):721–32. [PubMed: 12464183]

30. Cukras AR, Southworth DR, Brunelle JL, Culver GM, Green R. Ribosomal proteins S12 and S13 function as control elements for translocation of the mRNA:tRNA complex. *Mol Cell* 2003;12(2):321–8. [PubMed: 14536072]
31. Gao H, Sengupta J, Valle M, Korostelev A, Eswar N, Stagg SM, Van Roey P, Agrawal RK, Harvey SC, Sali A, Chapman MS, Frank J. Study of the structural dynamics of the E coli 70S ribosome using real-space refinement. *Cell* 2003;113(6):789–801. [PubMed: 12809609]
32. Cukras AR, Green R. Multiple effects of S13 in modulating the strength of intersubunit interactions in the ribosome during translation. *J Mol Biol* 2005;349(1):47–59. [PubMed: 15876367]
33. Moore VG, Atchison RE, Thomas G, Moran M, Noller HF. Identification of a ribosomal protein essential for peptidyl transferase activity. *Proc Natl Acad Sci U S A* 1975;72(3):844–8. [PubMed: 1055382]
34. Hampl H, Schulze H, Nierhaus KH. Ribosomal components from *Escherichia coli* 50 S subunits involved in the reconstitution of peptidyltransferase activity. *J Biol Chem* 1981;256(5):2284–8. [PubMed: 7007380]
35. Komoda T, Sato NS, Phelps SS, Namba N, Joseph S, Suzuki T. The A-site finger in 23 S rRNA acts as a functional attenuator for translocation. *J Biol Chem* 2006;281(43):32303–9. [PubMed: 16950778]
36. Youngman EM, Brunelle JL, Kochaniak AB, Green R. The active site of the ribosome is composed of two layers of conserved nucleotides with distinct roles in peptide bond formation and peptide release. *Cell* 2004;117(5):589–99. [PubMed: 15163407]
37. Polacek N, Gaynor M, Yassin A, Mankin AS. Ribosomal peptidyl transferase can withstand mutations at the putative catalytic nucleotide. *Nature* 2001;411(6836):498–501. [PubMed: 11373685]
38. Polacek N, Gomez MJ, Ito K, Xiong L, Nakamura Y, Mankin A. The critical role of the universally conserved A2602 of 23S ribosomal RNA in the release of the nascent peptide during translation termination. *Mol Cell* 2003;11(1):103–12. [PubMed: 12535525]
39. Feinberg JS, Joseph S. A conserved base-pair between tRNA and 23 S rRNA in the peptidyl transferase center is important for peptide release. *J Mol Biol* 2006;364(5):1010–20. [PubMed: 17045291]
40. Dorner S, Brunelle JL, Sharma D, Green R. The hybrid state of tRNA binding is an authentic translation elongation intermediate. *Nat Struct Mol Biol* 2006;13(3):234–41. [PubMed: 16501572]
41. Lang K, Erlacher M, Wilson DN, Micura R, Polacek N. The role of 23S ribosomal RNA residue A2451 in peptide bond synthesis revealed by atomic mutagenesis. *Chem Biol* 2008;15(5):485–92. [PubMed: 18439847]
42. Weinger JS, Parnell KM, Dorner S, Green R, Strobel SA. Substrate-assisted catalysis of peptide bond formation by the ribosome. *Nat Struct Mol Biol* 2004;11(11):1101–6. [PubMed: 15475967]
43. Brunelle JL, Shaw JJ, Youngman EM, Green R. Peptide release on the ribosome depends critically on the 2' OH of the peptidyl-tRNA substrate. *Rna* 2008;14(8):1526–31. [PubMed: 18567817]
44. Schmeing TM, Huang KS, Strobel SA, Steitz TA. An induced-fit mechanism to promote peptide bond formation and exclude hydrolysis of peptidyl-tRNA. *Nature* 2005;438(7067):520–4. [PubMed: 16306996]
45. Maguire BA, Benaminov AD, Ramu H, Mankin AS, Zimmermann RA. A protein component at the heart of an RNA machine: the importance of protein l27 for the function of the bacterial ribosome. *Mol Cell* 2005;20(3):427–35. [PubMed: 16285924]
46. Devaraj A, Shoji S, Holbrook ED, Fredrick K. A role for the 30S subunit E site in maintenance of the translational reading frame. *Rna* 2009;15(2):255–65. [PubMed: 19095617]
47. Jenner L, Rees B, Yusupov M, Yusupova G. Messenger RNA conformations in the ribosomal E site revealed by X-ray crystallography. *EMBO Rep* 2007;8(9):846–50. [PubMed: 17721443]
48. Walker SE, Shoji S, Pan D, Cooperman BS, Fredrick K. Role of hybrid tRNA-binding states in ribosomal translocation. *Proc Natl Acad Sci U S A* 2008;105(27):9192–7. [PubMed: 18591673]
49. Sergiev PV, Lesnyak DV, Kiparisov SV, Burakovsky DE, Leonov AA, Bogdanov AA, Brimacombe R, Dontsova OA. Function of the ribosomal E-site: a mutagenesis study. *Nucleic Acids Res* 2005;33(18):6048–56. [PubMed: 16243787]
50. Feinberg JS, Joseph S. Identification of molecular interactions between P site tRNA and the ribosome essential for translocation. *Proc Natl Acad Sci U S A* 2001;98(20):11120–11125. [PubMed: 11562497]

51. Lill R, Robertson JM, Wintermeyer W. Binding of the 3' terminus of tRNA to 23S rRNA in the ribosomal exit site actively promotes translocation. *EMBO J* 1989;8(12):3933–3938. [PubMed: 2583120]
52. Feinberg JS, Joseph S. Ribose 2'-hydroxyl groups in the 5' strand of the acceptor arm of P-site tRNA are not essential for EF-G catalyzed translocation. *RNA* 2006;12(4):580–8. [PubMed: 16489185]
53. Cornish PV, Ermolenko DN, Staple DW, Hoang L, Hickerson RP, Noller HF, Ha T. Following movement of the L1 stalk between three functional states in single ribosomes. *Proc Natl Acad Sci U S A* 2009;106(8):2571–6. [PubMed: 19190181]
54. Fei J, Kosuri P, MacDougall DD, Gonzalez RL Jr. Coupling of ribosomal L1 stalk and tRNA dynamics during translation elongation. *Mol Cell* 2008;30(3):348–59. [PubMed: 18471980]
55. Fei J, Bronson JE, Hofman JM, Srinivas RL, Wiggins CH, Gonzalez RL Jr. Allosteric collaboration between elongation factor G and the ribosomal L1 stalk directs tRNA movements during translation. *Proc Natl Acad Sci U S A*. 2009

Figure 1. Schematic illustration of the major steps in protein synthesis

In the initiation step of protein synthesis the 30S subunit (yellow) binds mRNA, initiator tRNA (purple stem-loop), IF1 (magenta), IF2 (cyan) and IF3 (dark green) before binding the 50S subunit (salmon). During the elongation cycle the 70S ribosome binds EF-Tu•GTP-tRNA ternary complex (pink and green stem-loop) and catalyze peptide bond formation. The mRNA-tRNAs are translocated by EF-G (blue). In the termination step, RF1 (light green) or RF2 recognize the stop codons and catalyze the release of the newly completed polypeptide (colored circles). RF1 and RF2 are removed from the ribosome by RF3 (purple). In the recycling step, the ribosome is dissociated into 30S and 50S subunits by the action of RRF (orange), EF-G and IF3 (see text for more details).

Figure 2. Interactions of P site tRNA with the ribosome

(A) Overview of the P site tRNA (magenta) interacting with bases in 16S rRNA (yellow), 23S rRNA (salmon) and proteins S9, S13, L5, L16, and L27 (orange tubes). Specific bases in the rRNAs are shown in cyan color and the mRNA is colored black. (B) Interactions between the anticodon arm of P site tRNA and the 30S subunit. (C) Interactions between the 3' CCA end of P site tRNA and the 50S subunit.

Figure 3. Interactions of A site tRNA with the ribosome

(A) Overview of the A site tRNA (green) interacting with bases in 16S rRNA (yellow), 23S rRNA (salmon) and proteins S12, S13, L16, and L27 (orange tubes). Specific bases in the rRNAs are shown in red color and the mRNA is colored black. (B) Interactions between the anticodon arm of A site tRNA and the 30S subunit. (C) Interactions between the 3' CCA end of A site tRNA and the 50S subunit.

Figure 4. Interactions of E site tRNA with the ribosome

(A) Overview of the E site tRNA (blue) interacting with bases in 16S rRNA (yellow), 23S rRNA (salmon) and proteins S7, S11, L1, L28, and L33 (orange tubes). Specific bases in the rRNAs are shown in violet color and the mRNA is colored black. (B) Interactions between the anticodon arm of E site tRNA and the 30S subunit. (C) Interactions between the 3' CCA end of E site tRNA and the 50S subunit.