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Review

A structural view on the mechanism of the ribosome-catalyzed peptide bond formation

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ABSTRACT

The ribosome is a large ribonucleoprotein particle that translates genetic information encoded in mRNA into specific proteins. Its highly conserved active site, the peptidyl-transferase center (PTC), is located on the large (50S) ribosomal subunit and is comprised solely of rRNA, which makes the ribosome the only natural ribozyme with polymerase activity. The last decade witnessed a rapid accumulation of atomic-resolution structural data on both ribosomal subunits as well as on the entire ribosome. This has allowed studies on the mechanism of peptide bond formation at a level of detail that surpasses that for the classical protein enzymes. A current understanding of the mechanism of the ribosome-catalyzed peptide bond formation is the focus of this review. Implications on the mechanism of peptide release are discussed as well.

The ribosome is a large RNA–protein machine that catalyzes protein synthesis in all living organisms. It is composed of two subunits in all kingdoms of life. In eubacteria and archaea the large subunit comprises ~3000 nucleotides of ribosomal RNA (rRNA), >30 proteins and sediments at 50S, whereas the ~1500 nucleotides and >20 proteins of the smaller subunit sediment at 30S. These conveniently different sedimentation coefficients are used as descriptors of the large (50S) and the small (30S) ribosomal subunits, while the entire ribosomal particle is referred to as the 70S ribosome (in eukaryotes these are 60S, 40S and 80S, respectively). Not only do the two subunits differ in their size and morphology, as evidenced by the early micrographs of the entire *E. coli* 70S particle [1], but they also perform distinct roles during protein synthesis [2]. The 50S subunit contains the peptidyl-transferase center (PTC) that catalyzes the synthesis of peptide bond, whereas the 30S subunit contains the decoding center that ensures that the tRNA with the correct anticodon is bound to the ribosome and paired with the mRNA codon.

The early studies of the ribosome and the mechanism it utilizes to make proteins were confined within the realm of basic biochemistry and the low-resolution electron microscopy. However, by the early 1990s both ribosomal subunits and the entire 70S ribosome from several organisms were crystallized [3–5]. The major challenge to determining their structures, however, was to correctly solve a heavy atom derivative. This was finally accomplished in 1998 using heavy atom cluster compounds at very low resolution (15 Å), which allowed Steitz and colleagues to phase the crystals of the 50S ribosomal subunit from the archaeon *Haloarcula marismortui* (Hma50) and produce the first map, at 9 Å resolution, showing continuous RNA helices [6]. Ramakrishnan and colleagues rapidly took this approach and obtained an electron density map of the 30S ribosomal subunit from *T. thermophilus* at 5.5 Å [7], while that of the Hma 50S subunit was extended to 5.0 Å resolution [8]. In only another year the resolution of the large ribosomal subunit map was extended to 2.4 Å [9] and that of the small subunit to 3.0 Å resolution [10]. These important successes were followed by a crystal structure of the 50S subunit from *Deinococcus radiodurans* at 3.3 Å resolution in 2001 [11], and a 5.5 Å electron density map obtained for the 70S ribosome from *T. thermophilus* (Tth70) complexed with three tRNA molecules and mRNA [12] that allowed fitting of the atomic structures of the 30S and 50S subunits to make a 70S ribosome model. Then, the 3.5 Å resolution apo-structure of the *E. coli* 70S ribosome was determined [13]. Finally, Ramakrishnan and coworkers were able to determine the crystal structure of the entire *T. thermophilus* 70S ribosome complexed with mRNA, partially ordered A-site tRNA, the P-site tRNA and the E-site tRNA at 2.8 Å resolution [14], while Noller and colleagues determined the crystal structure of a similar complex at 3.7 Å resolution [15].

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A rapid accumulation of structural data was paralleled by a series of elegant mutational, kinetic and computational experiments that had a common goal of deciphering the mechanism of the ribosome-catalyzed peptide bond formation. Upon binding of suitable substrates, the 70S ribosome catalyzes the peptide bond formation rapidly with the rate of $>300\ s^{-1}$ [16]. The 50S subunit alone is also able of catalyzing the peptidyl transfer reaction at a similar rate when fragments of the tRNA substrates are used [17]. This is the essence of the fragment assay that was used in the biochemical studies of the PTC for decades. The advantage of the fragment assay is that it is not dependent on the complete tRNA substrates and translation factors, which are otherwise necessary if the 70S ribosome is used. An approach analogous to the classical fragment assay was employed in the detailed structural studies of the PTC in which the 50S subunit of *H. marismortui* was used as a model system. A series of small mimics of the tRNA substrates were soaked into the Hma50 crystals with the goal to capture the heart of the ribosome in distinct stages of the catalytic cycle. Indeed, the approach produced a series of high-resolution snapshots of the PTC in various stages of the peptidyl transfer reaction. The current detail of structural data acquired for this large RNA–protein machine outshines that of the many classical protein enzyme systems. However, one should be aware that this method has its natural limitations. For instance, all the processes where the communication between the tRNA molecule, the translational factor(s) and the two subunits is essential for ribosomal function, such as accommodation or translocation, could not be addressed using this approach.

After a brief description of the elongation phase of the ribosome-catalyzed translation, the detailed description of the structure of the ribosomal PTC in complex with substrates, products and intermediates along the reaction pathway of peptide elongation is presented. Also, the current consensus on the mechanism of the ribosome-catalyzed peptide bond formation is summarized.

1. Translation

The ribosome "reads" mRNA in a 5′–3′ direction and synthesizes the corresponding protein from its N-terminus. Both ribosomal subunits contain three binding sites for tRNA molecules that are in distinct functional states: (*i*) the A site binds the aminoacyl-tRNA, which brings a new amino acid that is to be incorporated into the growing polypeptide, (*ii*) the P site binds the peptidyl-tRNA (as well as the initiator fMet-tRNA^{fmet}), and (*iii*) the E site binds the deacylated tRNA that is to be soon dissociated from the ribosome (Fig. 1). The 30S subunit binds mRNA and it ensures the fidelity of translation through close monitoring of the anticodon–codon interactions [18]. The 50S subunit, on the other hand, binds the acceptor ends of substrate tRNAs and it catalyzes the peptide bond formation in which an α-amino group of the aminoacyl-tRNA attacks the carbonyl carbon of the peptidyl-tRNA. From a chemical standpoint the reaction is an aminolysis of an acyl-ester link formed between the carbonyl carbon of the peptidyl moiety and the O3′ atom of the P-site A76. Upon peptide bond synthesis, the lengthened peptidyl-tRNA is bound to the A site, whereas the deacylated tRNA is in the P site. Peptide elongation is further promoted by the GTP-dependent protein elongation factors EF-G and EF-Tu. EF-G promotes translocation of the A-site bound peptidyl-tRNA into the P-site and of the P-site bound deacylated tRNA into the E site. Consequently, the ribosome moves down the mRNA filament with an active site that is ready for a new reaction cycle. Then, the elongation factor EF-Tu delivers the aminoacyl-tRNA to the A-site. The proper codon–anticodon interactions stimulate the GTP-ase activity of EF-Tu, leading to its dissociation from the complex. The acceptor end of the A-site aminoacyl-tRNA reorients and positions an incoming amino acid for the reaction with the peptidyl moiety attached to the P-site peptidyl-tRNA in a process known as accommodation. The rate of accommodation is significantly slower than the rate of peptide bond formation and is measured to be

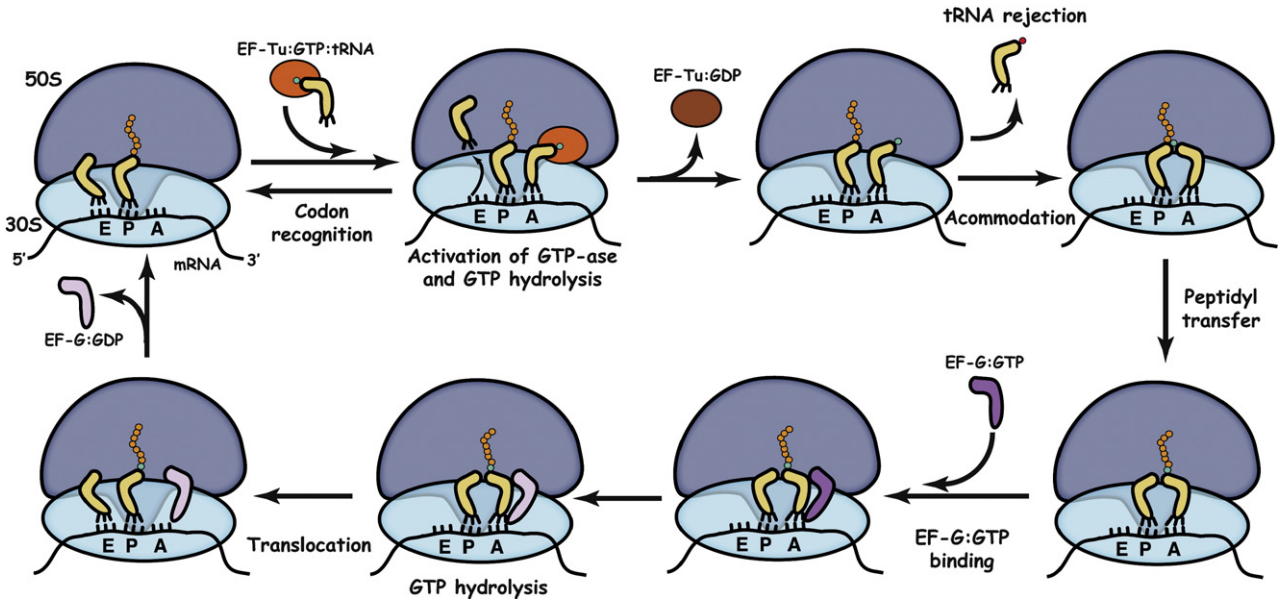

Fig. 1. A schematic diagram of the elongation phase of the ribosome-catalyzed translation. A peptidyl-tRNA is bound to the P site and the deacylated tRNA is in the ribosomal E site. The elongation factor EF-Tu complexed with GTP (orange) delivers an aminoacyl-tRNA to the A site. The deacylated tRNA dissociates from the E site on binding of the aminoacyl-tRNA to the A site. Upon codon recognition the GTP-ase activity of EF-Tu is stimulated and this causes a conformational change in EF-Tu upon which the factor dissociates from the ribosome. If the appropriate codon–anticodon interaction is established the CCA-end of the A-site aminoacyl-tRNA undergoes conformational change in a process known as accommodation, whereas the non-cognate tRNA is rejected at this point. After accommodation a free α-amino group of the aminoacyl-tRNA is oriented properly for the nucleophilic attack onto the acyl-ester link of the peptidyl-tRNA in the P site. The peptidyl transfer reaction occurs rapidly yielding a lengthened peptidyl-tRNA bound to the A site and the deacylated tRNA in the P site. The translocation of the reaction products and mRNA is promoted by the elongation factor EF-G in a GTP-dependent manner. The peptidyl-tRNA moves from the A site into the P site, whereas the deacylated tRNA moves from the P site into the E site. Also, the ribosome has now shifted in the 3′ direction of the mRNA and a new codon occupies the A site on the 30S subunit. After dissociation of the EF-G:GDP complex from the ribosome a new round of peptide synthesis ensues. Once the ribosome encounters the translational stop codon the termination phase of protein synthesis is initiated (not shown).