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Review

The mechanics of ribosomal translocation

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ABSTRACT

The ribosome translates the sequence of codons of an mRNA into the corresponding sequence of amino acids as it moves along the mRNA with a codon-step width of about 10 Å. The movement of the million-dalton complex ribosome is triggered by the universal elongation factor G (EF2 in archaea and eukaryotes) and is termed translocation. Unraveling the molecular details of translocation is one of the most challenging tasks of current ribosome research. In the last two years, enormous progress has been obtained by highly-resolved X-ray and cryo-electron microscopic structures as well as by sophisticated biochemical approaches concerning the trigger and control of the movement of the $tRNA_2 \cdot mRNA$ complex inside the ribosome during translocation. This review inspects and surveys these achievements.

1. Introduction

Ribosomal protein-synthesis is divided in three functional phases: initiation, the elongation cycle and termination. Each phase is governed by specialized factors. Accordingly we distinguish factors for initiation, elongation and termination. The elongation cycle is controlled by two universal factors, EF-Tu and EF-G (EF1 and EF2 in eukaryotes), present in all three domains and therefore developed before domain separation around 3 billion years ago. The other two phases, *viz*. initiation and termination, contain domain-specific factors and thus have been tuned after domain separation. It follows that the elongation phase developed first, and that in early stages of life on this planet a ribosome just bound to the 5'-end of an mRNA and started translation, which all kinds of ribosomes can still do today, *e.g.* translating the artificial mRNA poly(U) during poly(Phe) synthesis (for review see Ref. [1]).

A ribosome separates into two subunits, the large subunit (50S in bacteria, 60S in eukaryotes) and the small subunit (30S/40S, respectively). The large 50S subunit contains two ribosomal RNAs (rRNAs) and 33 L-proteins (L-for **l**arge), the 30S subunit one rRNA and 21 S-proteins (S-for **s**mall). Fig. 1A shows both subunits from the interface with some structural landmarks.

A ribosome decodes an mRNA with the help of adapters, the transfer RNAs (tRNAs). Up to 20 different aminoacyl-tRNA synthetases (aaRS) specifically recognize their cognate tRNA and ligate the corresponding proteinogenic amino acid to the 3′-end of the tRNA, forming aminoacyl-tRNA. The anticodon of the tRNA is located at the other distal end of the L-shaped tRNA and can form three consecutive base pairs with a complementary codon of the mRNA within the ribosome, thereby decoding the genetic code.

The elongation factor EF-Tu·GTP binds to aminoacyl-tRNA in a ternary complex (aa-tRNA·EF-Tu·GTP), and carries the aminoacyl-tRNA to the first ribosomal tRNA binding site, the A site (A for aminoacyl-tRNA) (Fig. 1B). The ribosome harbors two further tRNA binding sites, the P-site (P for peptidyl-tRNA) and the E site (E for exit), through which each tRNA will be stepped in this order before it is released from the E site.

At the beginning of an elongation cycle, the P site is occupied by a peptidyl-tRNA and the E site by a deacylated tRNA. The ternary complex aa-tRNA·EF-Tu·GTP interacts with the decoding center on the small subunit, which is part of the A site, and triggers the decoding process. If successful, the GTPase center of EF-Tu is activated. Following GTP cleavage, EF-Tu changes it conformation and leaves the ribosome as EF-Tu·GDP, whereas the aa-tRNA accommodates into the A site (Fig. 1B). Next, the peptidyl residue is transferred from the peptidyl-tRNA at the P site to the aminoacyl-tRNA at the A site *via* formation of a peptide bond. This reaction is catalyzed by the peptidyltransferase center located on the large ribosomal subunit. As a result, the nascent peptide is elongated by

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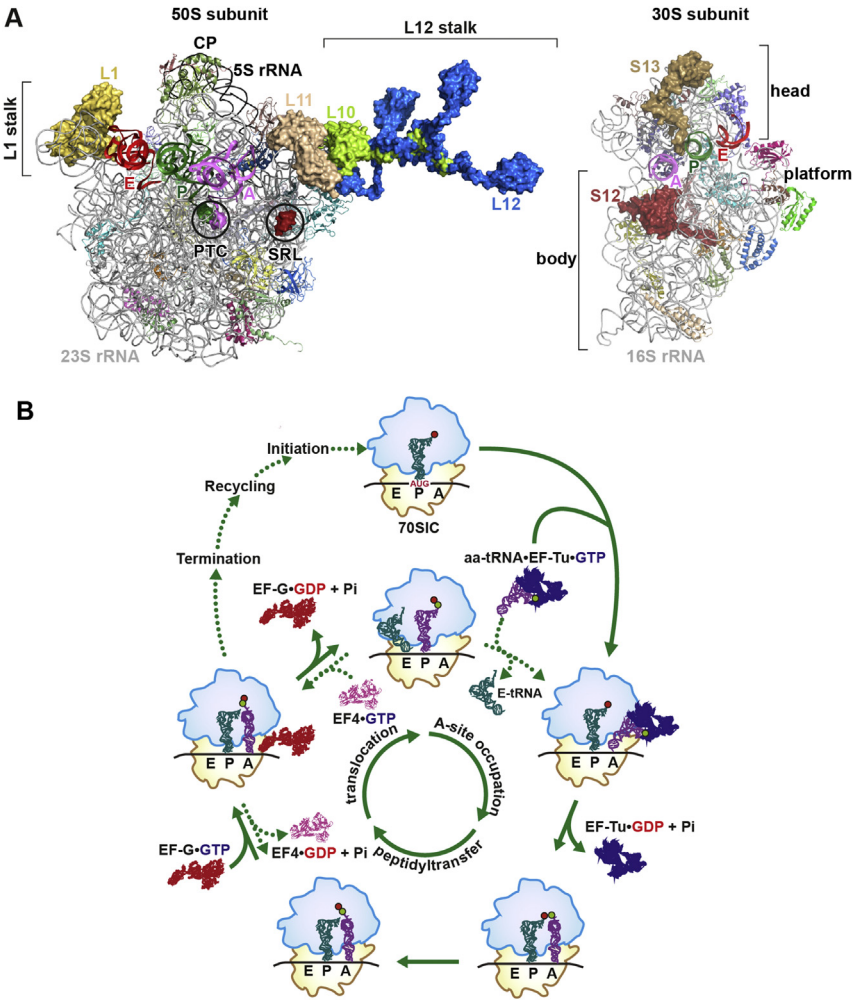

Fig. 1. Ribosomal landmarks and the elongation cycle. **A**, both subunits are shown from the interface. The large 50S subunit contains the 23S ribosomal RNA (rRNA) and 5S rRNA (light gray and dark gray, respectively), and the small 30S subunit the 16S rRNA (light gray). Ribosomal proteins are represented as colored ribbons, and those that have specific roles in translocation, as well as the sarcin–ricin loop (SRL) of the 23S rRNA and the acceptor ends of A- and P-site tRNAs within the peptidyl-transferase center (PTC), are highlighted by surface representation. The A-site, P-site and E-site tRNAs are also shown. For clarity, only the anticodon stem-loops of the tRNAs are shown on the 30S subunit. CP, central protuberance of the 50S subunit; PTC, peptidyltransferase center; SRL, α-sarcin-ricin loop. Taken from Ref. [29], modified. **B**, elongation cycle. At top, an aminoacyl-tRNA (aa-tRNA) is delivered to the ribosomal A site by the elongation factor EF-Tu–GTP. Next, the nascent peptide chain is transferred from the peptidyl-tRNA to the A-tRNA *via* a peptide bond, leaving a deacylated tRNA at the P site. EF-G then catalyzes the translocation of peptidyl-tRNA from the A site and deacylated tRNA from the P site to P and E sites, respectively. 50S subunits in blue, 30S in yellow. Taken from [28].

one aminoacyl-residue and now attached to the A-site tRNA, whereas the P-site tRNA is deacylated. The next step is the EF-G·GTP catalyzed translocation, during which the $tRNA_2$·mRNA complex is moved by one codon length inside the ribosome, shifting the tRNAs from A and P to P and E sites, respectively. Translocation finishes an elongation cycle, through which a ribosome runs for each amino-acid incorporation into the nascent peptide chain. During an elongation cycle a ribosome oscillates between two main states, *viz*. the pre-translocational (PRE) and the post-translocational (POST) state, which are separated by high activation-energy barriers of about 80–100 kJ/mol [2]. The PRE state is characterized by tRNAs at the A and P sites and the POST state by tRNAs at the P and E sites.

Several lines of evidence indicate that the tRNA translocated to the E site is released during the PRE-state of the next elongation cycle. Accordant with biochemical and genetic data [3,4], cryo-electron microscopic (cryo-EM) structures of bacterial and mammalian POST-state ribosomes [5,6] and an X-ray crystal structure of bacterial POST-state ribosomes [7] showed that the ribosome maintains the E site tRNA after completion of an elongation cycle. In bacteria, tRNA is released from the E site during an early contact of the ternary complex aa-tRNA·EF-Tu·GTP with the ribosomal A site after decoding and before aa-tRNA accommodation [8] (for review, see Ref. [9]), whereas in higher eukaryotes the release happens probably after the accommodation step [6].

2. An important ribosomal gross movement before translocation: tRNAs in hybrid sites

In the PRE state ribosomal subunits can rotate against each other by 7° in the interface plane [10–13]. If we fix the large subunit and look onto the solvent side of the small one, the small subunit rotates counterclockwise (Fig. 2A). This rotation – sometimes called ratcheting – is accompanied by a movement of the tRNAs only on the large subunit from A and P sites into P and E sites, respectively. On the small subunit, the tRNAs stay at A and P sites, respectively. The consequence is that the tRNAs are now in hybrid sites annotated A/P and P/E hybrid sites, where the first letter indicates the location on the small subunit and the second that on the large

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