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Major structural rearrangements of the canonical eukaryotic translation initiation complex

Ewelina Guca and Yaser Hashem

Translation initiation in eukaryotes is a complex multistep process that requires the interplay of over a dozen protein factors together with the small ribosomal subunit (SSU) and the mRNA. During all these steps, the SSU serves as a platform for attachment, displacement and release of different molecules. In recent years, the great number of high-resolution X-ray and cryo-EM structures provided unprecedented insights into the molecular mechanism of this important process in eukaryotes. More specifically, cryo-EM became a leading technique in uncovering the structural details of this process due to exceptional advances in resolution and in image processing. Here, we briefly review cap-dependent eukaryotic translation initiation with an emphasis on its major conformational changes at several key steps during the process, unraveled thanks to the recent advances in the structural biology field.

Address
INSERM U1212, Institut Européen de Chimie et Biologie, University of Bordeaux, Pessac 33607, France

Corresponding author: Hashem, Yaser (yaser.hashem@u-bordeaux.fr)

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Introduction

Protein synthesis is an essential process required for growth and development in all living organisms. It relies on the translation of the messenger RNA (mRNA) into amino acids that fold and become functional proteins. Translation can be divided into four stages: initiation, elongation, termination and recycling. Each stage is composed of multiple steps that require extensive rearrangements of subunits of its key players around the ribosome, a hybrid RNA–protein complex with a size ranging from ~ 2.5 MDa (in bacteria) to ~ 4 MDa (in mammals) [1].

The ribosome is composed of two subunits: small (SSU or 40S) and large (LSU or 60S). The space between the subunits comprises three binding sites called: A(aminoacyl), P (peptide) and E (exit) sites, where different translation factors and the transfer RNAs (tRNAs) carrying the amino acids interact during the different stages of translation. The rate-limiting and principally regulated step of the translation process is the initiation step, where the mRNA is loaded into the SSU, leading eventually through a complex sequence of interactions to the accommodation of the mRNA start-codon at the ribosomal P-site and the joining of both subunits. The SSU is responsible for RNA-decoding and mRNA binding [2,3], whereas LSU possesses mainly the amino acid polymerization function, as it contains the peptidyl transferase center [4]. Therefore, SSU is a central actor during the translation initiation (TI) process.

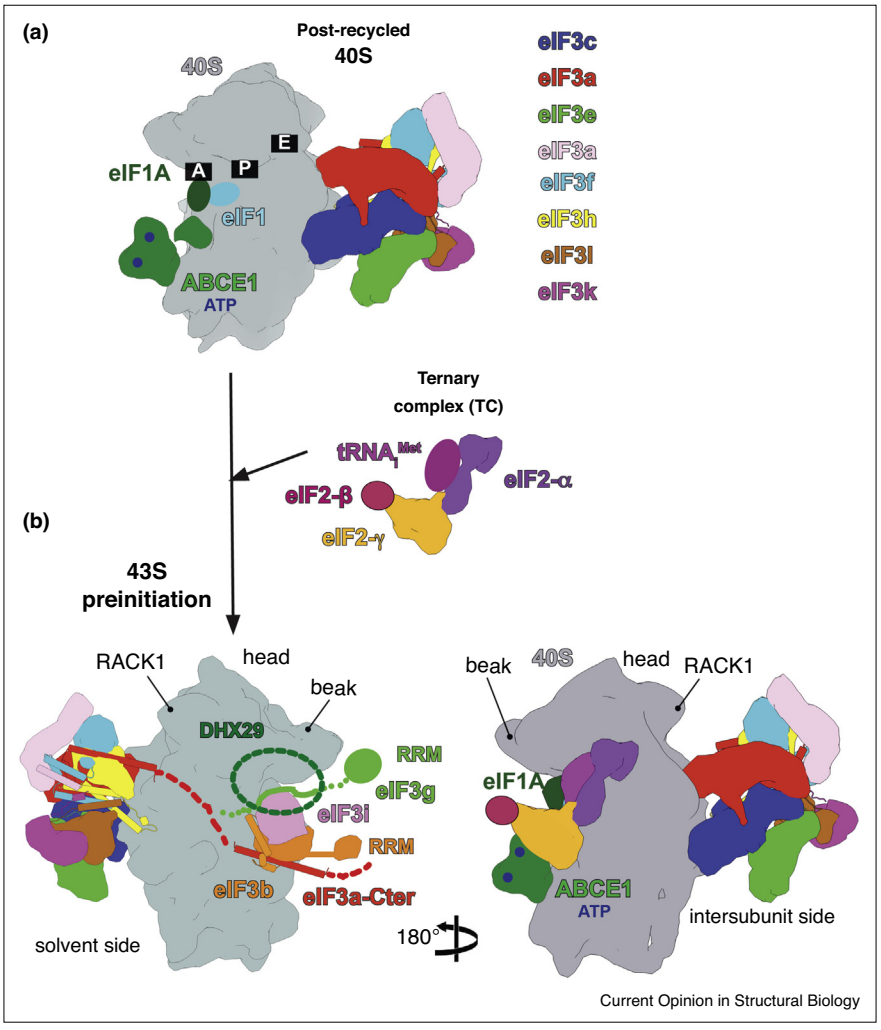
TI in eukaryotes requires the interaction of a dozen protein eukaryotic initiation factors (eIFs) at different stages of the process. The canonical TI mechanism is based on the recruitment of a 5′ 7-methyl guanosine (m^7G) cap leading the coding mRNA to the ribosome that is the most common process through which mRNA is translated in eukaryotes. The cap is linked to the first 5′ nucleotide of the mRNA, and in higher eukaryotes two riboses of the following nucleotides are 2′-O methylated. This canonical pathway is composed of four main steps: firstly preinitiation, secondly mRNA recruitment, thirdly scanning and start-codon recognition and fourthly ribosomal subunit joining.

Structural biology provided a crucial contribution to the understanding of translation mechanism in bacteria and eukaryotes at a molecular level. Up-to-date, over 2400 structures of ribosomal translation-related complexes in the Protein Data Bank (PDB) are available, representing different stages of the translation process. Among them, over 800 structures come from eukaryota and around 25% are solved by the cryo-electron microscopy (cryo-EM). In recent years, cryo-EM became a very powerful and versatile technique that enabled several groups around the world to study the TI mechanism due in part to major advances in resolution [5••], but also in image processing thus making possible to obtain a detailed structural image of multiple stages of the process [6]. Recent exhaustive reviews highlighting different aspects of the translation initiation process already exist [7•,8,9••,10–12], therefore in this review we will focus on other aspects of the process and attempt to briefly summarize the recent achievements by structural biology in characterizing the major structural rearrangements between different transient complexes in cap-dependent initiation, mainly during the late-steps of the process in eukaryotes.

Pre-initiation

The earliest step of translation relies on the formation of a 43S pre-initiation complex (43S PIC). Firstly, a ternary complex (TC) is formed by the binding of the heterotrimeric eukaryotic initiation factor 2 (eIF2) to a molecule of guanosine triphosphate (GTP) and the initiatior methionylated Met-tRNA ($tRNA_i^{Met}$). Once formed, TC recruitment to the 40S is in part mediated by initiation factors that increase its affinity to the 40S and to mRNA in the later step (eIF1, eIF1A, eIF3 and eIF5 (Figure 1)), thus forming the 43S PIC [13–18]. Recent studies demonstrated the role of ATP binding cassette E1 (ABCE1) in the 43S PIC assembly by acting as an anti-ribosomal-subunit association factor [19•,20]. In addition, another factor specific to translation initiation in higher eukaryotes such as mammals, DHX29 [14] a DExH-box helicase that in cooperation with eIF3 is required for translation of mRNAs possessing highly structured 5′UTRs. The exact molecular mechanism underlying its role in unwinding mRNA remains an open question [21,22].

Figure 1



The pre-initiation step. Schematic/cartoon representation of the pre-initiation complexes. **(a)** Post-recycled 40S subunit (in grey) with A (aminoacyl), P (peptide) and E (exit) sites (black boxed). eIF1A (a ~17 kDa globular protein with unstructured N-terminal and C-terminal tails, shown in dark green) and eIF1 (a ~13 kDa protein with similar topology, shown in skyblue) are bound close to the A and P -sites. ABCE1 (an ABC-type NTPAse, in green) comes in this specific context as a posttermination factor bound to the 40S, after is has served as a key ribosomal recycling factor in the context of the 80S after the elongation termination step. eIF3 (an ~800 kDa complex, in mammals composed of 13 subunits, the subunits with known structures are shown in different colors) is present all along the translation initiation process. **(b)** Formation of 43S PIC upon binding of the TC (ternary complex composed of ~130 kDa eIF2 protein complex and $tRNA_i^{Met}$). The location of DXH29 helicase (155 kDa NTP-depended RNA helicase) is shown at the solvent side (dark green dashed line).
Abbreviations: eIFeukaryotic initiation factor; RRMRNA recognition motif
Adapted from Ref. [8].