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Review

Cap-dependent, scanning-free translation initiation mechanisms

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ABSTRACT

Eukaryotic translation initiation is an intricate and multi-step process that includes 43S Pre-Initiation Complex (PIC) assembly, attachment of the PIC to the mRNA, scanning, start codon selection and 60S subunit joining. Translation initiation of most mRNAs involves recognition of a 5′end m7G cap and ribosomal scanning in which the 5′ UTR is checked for complementarity with the AUG. There is however an increasing number of mRNAs directing translation initiation that deviate from the predominant mechanism. In this review we summarize the canonical translation initiation process and describe non-canonical mechanisms that are cap-dependent but operate without scanning. In particular we focus on several examples of translation initiation driven either by mRNAs with extremely short 5′ leaders or by highly complex 5′ UTRs that promote ribosome shunting.

1. Introduction

Translation of mRNA to protein in eukaryotes requires a complex apparatus consisting of mRNA, ribosomes, tRNAs and protein factors. mRNA translation is a cyclic process which can be divided into initiation, elongation, termination and recycling. Within this framework the initiation stage is considered a major regulatory target. This stage involves different initiation factors (eIFs) and different mRNA features, all of which play important roles in translational control. The predominant form of eukaryotic translation initiation depends on the m7G cap structure, present at the 5′end of the mRNA, and on ribosomal scanning. In addition the translation process involves 43S Pre-Initiation Complex (PIC) assembly, attachment of the PIC to the mRNA, start codon selection and subunit joining, all of which are common to the various forms of translation. In recent years the number of eukaryotic mRNAs that utilize alternative mechanisms is growing. The distinction between canonical and non-canonical translation is primarily dictated by sequence elements and structural features present in the mRNA. This review focuses on several non-canonical translation initiation mechanisms described in recent years that are cap-dependent but operate without scanning.

2. Overview of cap-dependent translation initiation mechanism

Eukaryotic mRNAs are characterized by m7G cap structure at their 5′ end and a poly(A) tail at their 3′ end, both being added during transcription and playing essential roles in mRNA translation and stability. Most of these mRNAs are translated via the canonical cap-dependent scanning mechanism [1–4]. Translation initiation begins with formation of the 43S preinitiation complex (PIC) that is assembled from the ternary complex (eIF2–GTP–Met–tRNAi), several initiation factors (eIFs) that include eIF1, 1A, 3 and 5 and the 40S small ribosomal subunit (Fig. 1). This 43S PIC, through eIF3 and eIF1, then attaches the cap-proximal region of activated mRNAs. mRNA activation is driven by eIF4F which is composed of eIF4E — the cap-binding protein, eIF4GI — a scaffold protein, and eIF4A — a DEAD-box ATPase and RNA dependent helicase. The first step of activation occurs when eIF4E binds the m7G cap structure. Subsequently eIF4A unwinds the cap-proximal secondary structures (Fig. 1). The role of eIF4GI is to form a “closed-loop” mRNP complex consisting of eIF4F, mRNA and PABP [poly(A) binding proteins] since it interacts with the mRNA body directly and with the m7G cap and 3′-poly(A) tail indirectly through interactions with eIF4E and PABPs [5–8]. eIF4GI also interacts with eIF4A and strongly enhances its helicase activity [9–15]. In addition eIF4GI serves as a bridge between the cap-complex and the PIC through direct interaction with eIF3 and eIF1 [16–19].

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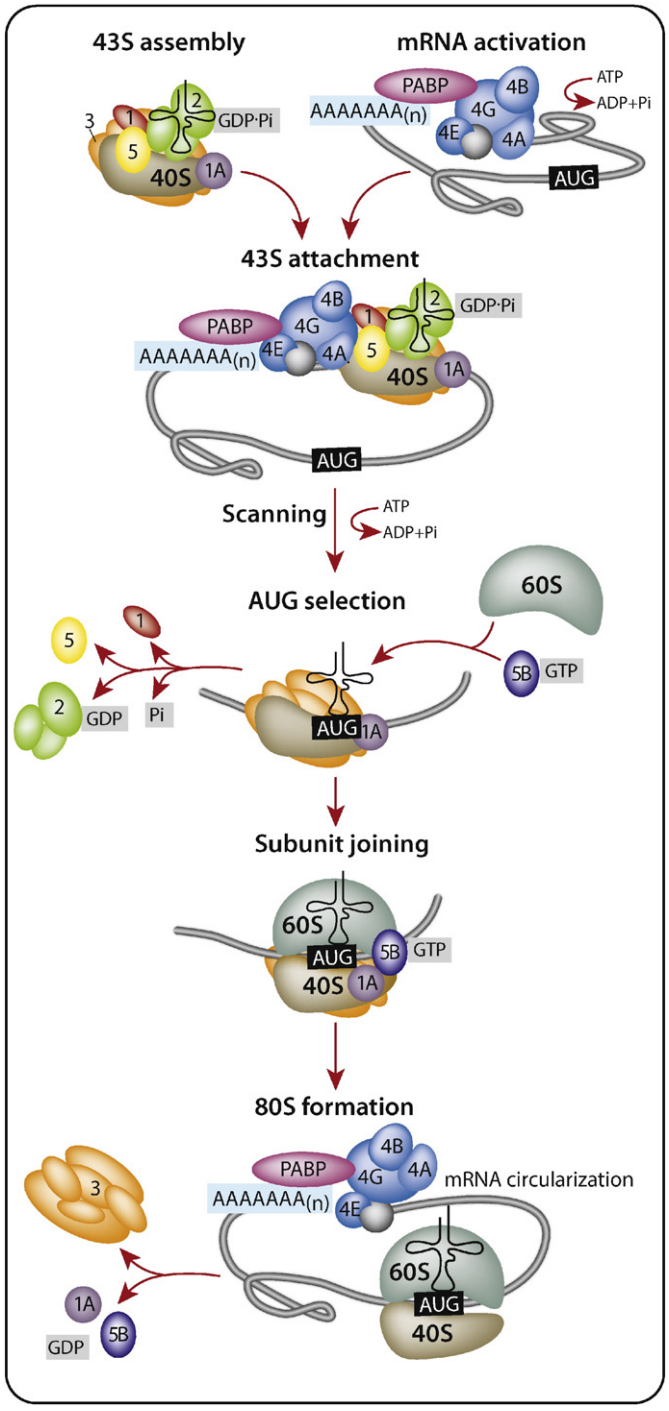

Fig. 1. Schematic representation of the eukaryotic cap-dependent translation initiation mechanism. The translation initiation is divided into several stages as indicated. The 43S PIC is assembled from the 40S subunit, a ternary complex consisting of eIF2–GTP–Met-tRNAi, eIF1, eIF1A, eIF3 and eIF5. The mRNA activation stage involves cap-binding and unwinding of cap-proximal region (an ATP-dependent process) by eIF4F subunits. RNA circularization, mostly occurring in polysomes, is mediated by PABP–eIF4GI interaction. Attachment of the PIC to the mRNA is mediated by the cap complex and is followed by an ATP-dependent scanning of the 5′ UTR in a 5′ to 3′ direction until an AUG is selected through codon-anti-codon base pairing with the Met-tRNAi. AUG recognition switches the scanning complex to a "closed" conformation and is accompanied by eIF5-assisted hydrolysis of eIF2-bound GTP, P_i release and eIF1 displacement. The 60S subunit joining to the 48S complex is associated with release of eIF2–GDP, eIF3, eIF4F and eIF5 and is mediated by eIF5B and eIF1A. GTP hydrolysis by eIF5B triggers its own and eIF1A release rendering the 80S ribosome ready to elongate.

Attachment of the ribosome to the mRNA is tightly linked to the cell physiology and is a major target for regulation. A central regulatory mechanism of this stage involves 4E-Binding Proteins (4E-BPs) which bind eIF4E with high affinity and interfere with its binding to eIF4GI, thereby inhibiting cap-dependent translation (for review see [4]). 4E-BP is controlled by the mammalian target of rapamycin (mTOR), a protein kinase that phosphorylates it and diminishes its ability to bind eIF4E. mTOR is active under conditions that permit cell growth. When mTOR activity is inhibited, as occurs under conditions of limited nutrients or certain stress conditions, 4E-BP activity is enhanced and cap-dependent translation is suppressed [4].

After mRNA binding, the 43S PIC travels along the mRNA 5′ UTR in a 5′ to 3′ direction, examining the RNA sequence for an AUG start codon through base pairing with the anticodon in the Met-tRNAi (Fig. 1). The 43S movement along the 5′ UTR is an ATP dependent process known as scanning. Secondary structures present in the mRNA 5′ UTR are unwound by eIF4A and other helicases such as yeast Ded1 and mammalian DExH-box helicase DHX29 [20–23]. The scanning compatible state of the 43S is an "open" conformation in which the Met-tRNAi does not fully occupy the P-site (P_{out} state). During scanning, eIF2 partially hydrolyzes its bound GTP with the assistance of eIF5 (GTPase activating protein), resulting in a stable eIF2 GDP Pi state. Complementation with the anticodon of the Met-tRNAi results in scanning arrest and commitment to the start codon [24]. Usually, translation initiates at the first 5′-proximal AUG codon. However, many mammalian mRNAs do not follow this "first-AUG" rule. In those mRNAs, translation initiates at a downstream AUG, a phenomenon known as leaky scanning. The extent of leaky scanning depends on the nucleotide context surrounding the AUG and on the 5′ UTR length [25–27]. For many years the optimal nucleotide context for translation initiation in mammals was the Kozak context **R**CCAUG**G**, in which the most significant nucleotides are the purine R in position −3 and G in position +4 relative to the A of the AUG codon that is designated as +1. These two positions distinguish between a "strong" and a "weak" translation initiation site. A weak AUG context allows a more leaky scanning, whereas a strong one prevents it [25,28]. The role of the purine in position −3 and the G in position +4 is probably to stabilize the 48S following recognition of the start codon [29].

It has been demonstrated that a 5′ UTR length of at least 20 nucleotides is needed for efficient recognition of an AUG with a favorable context [30]. The preferences for optimal AUG context and a minimal 5′ UTR length are conserved features of the translation apparatus [25]. By leaky translation the cell can produce two or more completely different proteins or protein variants from one single mRNA. The presence of an AUG upstream to the main ORF AUG in most cases reduces its translation [26,31,32], a finding which strongly supports the scanning mechanism. About half of all human and rodent mRNAs have at least one uAUG in their 5′ UTR [31,33,34].

Following scanning arrest eIF1, eIF2 GDP, its bound Pi and eIF5 are displaced from the PIC (Fig. 1). These rearrangements switch the "open" conformation to a "closed" committed 48S initiation complex in which the small ribosomal subunit is locked onto the mRNA and the Met-tRNAi is fully accommodated in the P-site (P_{in} state). eIF2B is a guanosine nucleotide exchange factor, which recycles eIF2-GDP to eIF2-GTP, in order to initiate another cycle of translation. The major regulators of the 48S conformational changes involved in scanning and start codon selection are eIF1 and eIF1A. The eIF5B GTPase and eIF1A promote joining of the 60S large subunit to create the 80S elongation competent ribosome complex. GTP hydrolysis by eIF5B stimulates its own dissociation and that of eIF1A. The 80S can now accept an appropriate aminoacyl-tRNA into the A site and begin the elongation phase.

Canonical and non-canonical forms of translation initiation share four major steps: 43S Pre-Initiation Complex (PIC) assembly, attachment of the PIC to the mRNA, start codon selection and subunit joining. A major difference between the various forms of cap-dependent translation initiation is the requirement of ribosomal scanning.