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Review

IRES-induced conformational changes in the ribosome and the mechanism of translation initiation by internal ribosomal entry

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ABSTRACT

Translation of the genomes of several positive-sense RNA viruses follows end-independent initiation on an internal ribosomal entry site (IRES) in the viral mRNA. There are four major IRES groups, and despite major differences in the mechanisms that they use, one unifying characteristic is that each mechanism involves essential non-canonical interactions of the IRES with components of the canonical translational apparatus. Thus the ~200nt.-long Type 4 IRESs (epitomized by Cricket paralysis virus) bind directly to the intersubunit space on the ribosomal 40S subunit, followed by joining to a 60S subunit to form active ribosomes by a factor-independent mechanism. The ~300nt.-long type 3 IRESs (epitomized by Hepatitis C virus) binds independently to eukaryotic initiation factor (eIF) 3, and to the solvent-accessible surface and E-site of the 40S subunit: addition of eIF2-GTP/initiator tRNA is sufficient to form a 48S complex that can join a 60S subunit in an eIF5/eIF5B-mediated reaction to form an active ribosome. Recent cryo-electron microscopy and biochemical analyses have revealed a second general characteristic of the mechanisms of initiation on Type 3 and Type 4 IRESs. Both classes of IRES induce similar conformational changes in the ribosome that influence entry, positioning and fixation of mRNA in the ribosomal decoding channel. HCV-like IRESs also stabilize binding of initiator tRNA in the peptidyl (P) site of the 40S subunit, whereas Type 4 IRESs induce changes in the ribosome that likely promote subsequent steps in the translation process, including subunit joining and elongation.

1. Introduction

Initiation of translation of a number of eukaryotic viral mRNAs occurs by non-canonical end-independent mechanisms that are mediated by an internal ribosomal entry site (IRES) in the mRNA [1–3]. IRESs are functionally defined RNA elements that are classified into four major structural groups, epitomized by poliovirus (PV; Type 1), encephalomyocarditis virus (EMCV; Type 2), hepatitis C virus (HCV; Type 3) and cricket paralysis virus (CrPV; Type 4). (This nomenclature extends the generally accepted designation for Type 1 and Type 2 IRESs [4]). Although evidence to date suggests that these groups of IRESs mediate initiation by distinct mechanisms, a characteristic of all of these mechanisms is that they involve direct and essential interactions with components of the translational apparatus (i.e. eukaryotic initiation factors (eIFs) and the ribosomal 40S subunit). Recent structural and biochemical studies on Type 3 and Type 4 IRESs have revealed a second important characteristic of IRES-mediated initiation, which is that these IRESs induce conformational changes in the ribosome that include influencing the entry, positioning and fixation of mRNA in the ribosomal decoding channel, stabilizing binding of initiator tRNA in the peptidyl (P) site of the 40S subunit, and priming the resulting initiation complex for subsequent steps in the translation process, including subunit joining and ribosomal translocation during the first elongation cycle.

2. The canonical mechanism of translation initiation in eukaryotes

Initiation of translation on the majority of mRNAs can be described by the scanning model, and occurs in two stages: 48S complex formation, followed by subunit joining to form active 80S ribosomes [5]. Initiation requires a free pool of ribosomal 40S and 60S subunits, which are generated by recycling of post-termination ribosomes by eIF3, eIF3j (a loosely associated subunit of eIF3), eIF1 and eIF1A [6]. The first step in initiation is assembly of an initiator tRNA (Met-tRNA$^{Met}_i$)/eIF2.GTP ternary complex that, with eIF3, eIF1, eIF1A and a 40S subunit forms a 43S preinitiation complex. Attachment of the 43S complex to the cap-proximal region of mRNA is promoted by eIF4F, a heterotrimer that comprises eIF4E (which binds the mRNA m^{7}GTP 'cap'), eIF4A (a DEAD-box RNA helicase) and eIF4G (which binds eIF4E, eIF4A, and eIF3). The mechanism of attachment involves coordination of unwinding of the cap-proximal region of mRNA by eIF4F, eIF4A and its cofactor eIF4B to prepare an attachment site, and establishment of interactions between eIF3, eIF4G, mRNA and the 40S subunit. 43S complexes need eIF1 and eIF1A for the subsequent

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scanning step [7]; scanning on all 5′ UTRs is enhanced by the DExH-box protein DHX29, and scanning on highly structured 5′ UTRs requires this factor [8]. The 43S complex stops when it recognizes the initiation codon (which is usually the first AUG triplet with a favorable nucleotide context) forming a 48S complex in which the initiation codon is base-paired with the anticodon of Met-tRNA$^{Met}_i$. Initiation codons that deviate from the optimum context **A**ccAUG**G**, particularly at the −3 and +4 positions (bold) may be bypassed by scanning ribosomal complexes, leading to initiation downstream [9].

Hydrolysis of eIF2-bound GTP/P_i release are key steps in commitment of a scanning ribosomal complex to arrest at an initiation codon, and eIF1 plays an important role in this process, by inhibiting premature hydrolysis of eIF2-bound GTP/P_i release during scanning [10]. Repression is relieved by displacement of eIF1 following establishment of codon–anticodon base-pairing, which is therefore linked by eIF1 to this commitment step [10–12]. eIF1 also contributes to the fidelity of initiation by dissociating 48S complexes assembled on non-AUG codons and AUG codons with poor context [7,14].

The transition from the scanning conformation of 43S complexes to the arrested conformation of 48S complexes is associated with conformational changes in the 40S subunit, with changes in the relative positions of Met-tRNA$^{Met}_i$ and mRNA (see below) and with changes in the interactions and relative positions of initiation factors. eIF1A occupies the ribosomal A-site, but its N- and C-terminal tails (NTT and CTT, respectively) extend into the P-site in 43S complexes [15], near to eIF1, which binds to the interface surface, close to Met-tRNA$^{Met}_i$ and the P-site [16]. The eIF1A-NTT is thought to promote the 'closed', arrested conformation of the 40S subunit, whereas the CTT promotes the 'open' scanning-competent conformation [15,17]. Start codon recognition leads to dissociation of eIF1 from the 40S subunit [10–12] and ejection of the eIF1A-CTT from the P-site [15,17], whereas the folded domain of eIF1A binds more tightly in the aminoacyl (A)-site [18]. These changing interactions have been hypothesized to change the position and interactions of eIF5, enabling it to promote hydrolysis of eIF2-bound GTP, and/or triggering release of P_i from the ribosome-associated eIF2/GDP/P_i complex [19,20]. Cross-linking studies have shown that in 48S complexes, nucleotides at the −3 and +4 positions establish specific interactions with eIF2α and $AA_{1818–1819}$ of 18S rRNA, respectively, and biochemical data indicate that the eIF2α/-3nt. interaction is an important determinant of initiation codon selection [21]. In addition to promoting scanning and influencing initiation codon selection, eIF1A also interacts with eIF5B, enhancing its activity in subunit joining [22,23]. eIF5B.GTP displaces the remaining eIFs from the 40S subunit and promotes joining of a 60S subunit to form an 80S ribosome [10,24]. The eIF5B C-terminal domain may replace eIF2 in interacting with the acceptor end of Met-tRNA$^{Met}_i$, leading to displacement of eIF2.GDP and likely reorienting Met-tRNA$^{Met}_i$ so that it can enter the P-site of the 60S subunit correctly and that subunit joining can proceed. Finally, hydrolysis of the bound GTP leads to release of eIF5B.GDP, yielding a ribosome that can enter the elongation phase of translation [24].

3. Comparisons between initiation of translation in eukaryotes and prokaryotes

Translation initiation mechanisms in eukaryotes and in prokaryotes are fundamentally similar: both begin with separated ribosomal subunits, involve binding of a unique initiator tRNA in the P-site of the small ribosomal subunit, and proceed via formation of an intermediate complex containing mRNA and this tRNA before the stage of subunit joining. The core of the ribosome is highly conserved, and major differences are restricted to rRNA expansion segments and additional proteins at peripheral locations [25]: accordingly, insights gained from studies of prokaryotic ribosomes can (cautiously) be extrapolated to eukaryotic translation. The small ribosomal subunit consists of the body, which contains major morphological features such as the shoulder and the platform, and is connected via the narrow neck to the head (Fig. 1). tRNA is held tightly in the P-site by multiple interactions with the prokaryotic small (30S) subunit so that its anticodon can base-pair accurately with each P-site codon and maintain the reading frame [26,27]. Prokaryotic mRNA binds in a channel that wraps around the neck of the 30S subunit, passing through two non-covalently closed tunnels, one as it enters between the head and shoulder and the other as it exits between the head and platform [26,28,29]. mRNA is anchored onto the platform by base-pairing between the mRNA Shine–Dalgarno (SD) sequence upstream of the initiation codon and the anti-SD sequence at the 3′-end of 16S ribosomal rRNA (rRNA). This sequence is absent from eukaryotic ribosomes, but the path of mRNA on the eukaryotic 40S subunit is in most respects analogous to that in prokaryotes [30].

However, the initiation process in prokaryotes is considerably simpler than in eukaryotes: mRNA and initiator tRNA can bind directly to the 30S subunit in the complete absence of the three single-subunit initiation factors IF1, IF2 and IF3. Their functions are to accelerate the rate and to control the fidelity of initiation. IF2, the bacterial homolog of eIF5B, accelerates binding of initiator tRNA to the 30S subunit, positions its acceptor end for insertion into the 50S subunit during subunit joining and accelerates joining [31,32]. IF1, which is homologous to the central domain of eIF1A, binds in the ribosomal A-site [33] whereas the C-terminal domain of IF3 (a functional analog of eIF1) binds to the interface surface of the platform near the P-site [34]. IF1 and IF3 promote the accuracy of initiation, accelerating dissociation of mispaired tRNA–mRNA complexes from the subunit, likely by a mechanism that includes factor-induced conformational changes in the 30S subunit [33,35]. eIF1 binds to a similar location as IF3 and also promotes the accuracy of initiation [14,16]. The observation that eIF1 and IF3 can play these roles in heterologous systems is indicative of a conserved mode of action that may involve similar induced conformational changes in the small ribosomal subunit [36].

4. Conformational changes in ribosomal complexes during the canonical initiation process

The small ribosomal subunit is a metastable structure that undergoes large and small-scale conformational changes in response to the binding or release of ligands during successive steps in the translation process. Conformational changes in bacterial 30S subunits induced by mRNA, tRNA and factors have been visualized by X-ray crystallography and cryo-electron microscopy (cryo-EM) [31,37]. The neck connecting the head to the body consists of a single helix (h) 28 of 16S rRNA, and it permits hinge-like and rotational movement of the head relative to the body: binding of mRNA and the anticodon stem-loop (ASL) of tRNA in the P-site lead to tilting of the head to close the P-site cleft, and to deeper binding of the ASL in the P-site [27], binding of IF1 induces tilting of the head towards the A-site and changes in the conformation of h44 [33], and binding of cognate tRNA in the A-site alters the conformation of the universally conserved rRNA bases A_{1492}/A_{1493} and G_{530} (which line the floor of the A-site) and induces closure of the small subunit around the A-site by movements that include rotation of the head and shoulder towards each other [38,39]. IF2 alters the conformation and orientation of initiator tRNA on the 30S subunit, anchoring the tRNA acceptor end until subunit joining occurs [31].

The head of the eukaryotic 40S subunit is similarly mobile [40,41]: several conformational changes can be hypothesized to occur in it during initiation, and experimental data to support them is accumulating. Thus to account for eIF1's dual roles in promoting scanning and in ensuring the fidelity of initiation codon selection, a model was suggested in which eIF1A and particularly eIF1 are required for preinitiation complexes to adopt an 'open' scanning-competent conformation that isomerizes to form a stable 'closed' complex following initiation codon recognition [14,42]. The entry channel of the mRNA-binding cleft is occluded in vacant 40S subunits [43], so