

Where to begin? The mechanism of translation initiation codon selection in eukaryotes

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Selecting the codon at which to begin translation is a complicated event in an already complicated process. Many protein initiation factors (eIFs) have been implicated in start site selection, but the mechanistic details of their activities have remained obscure until recently. Biochemical and genetic studies of eIFs 1, 1A, 2 and 5 have suggested that the 43S pre-initiation complex exists in two conformations and that the changing interactions of the factors within the 43S pre-initiation complex in response to encountering an AUG codon regulates these conformations and, ultimately, the selection of the start codon.

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Introduction

The past 40 years of studies of translation initiation in eukaryotes have been synthesized into a model of the steps involved in the formation of an 80S ribosomal initiation complex (Figure 1). First, the eIF2•GTP•methionyl initiator tRNA (Met-tRNA_i) ternary complex (TC) is formed (see Box 1 for a list of eIFs involved in start codon recognition). In a process facilitated by eIFs 1, 1A and 3, the TC binds to the small (40S) ribosomal subunit, forming the 43S complex. The eIF4F complex binds to the 7-methylguanosine cap structure on the 5' end of the mRNA and the factor's RNA helicase activity is thought to facilitate loading of the ribosomal complex onto the message. This process is assisted by eIF3 and the poly(A) binding protein (PAB). The 43S complex presumably scans the mRNA, 5' to 3', in search of the AUG start codon in an ATP-dependent process. Usually the 5'-most AUG is selected as the start codon. In mammals, the start codon must also be in a good sequence context, called a 'Kozak' sequence, in order to be selected efficiently; the most important bases in this consensus

sequence are a purine at position –3 (relative to A in the AUG) and the G at position +4. AUG selection results in irreversible GTP hydrolysis by eIF2 in a reaction promoted by eIF5, a GTPase activating protein (GAP). This is the first irreversible step in the pathway and is thought to commit the complex to beginning translation at the selected codon (as no discard pathway for complexes assembled on the wrong codon has yet been demonstrated). eIF2•GDP is then thought to dissociate, leaving the Met-tRNA_i in the peptidyl (P) site of the 40S ribosomal subunit, base paired with the AUG start codon. In the final step, which may occur in concert with eIF2•GDP dissociation, the 60S ribosomal subunit is joined to the 40S subunit containing the Met-tRNA_i and mRNA in a process promoted by eIF5B, yielding the final 80S initiation complex awaiting the delivery of an aminoacyl-tRNA to the acceptor (A) site to begin elongation.

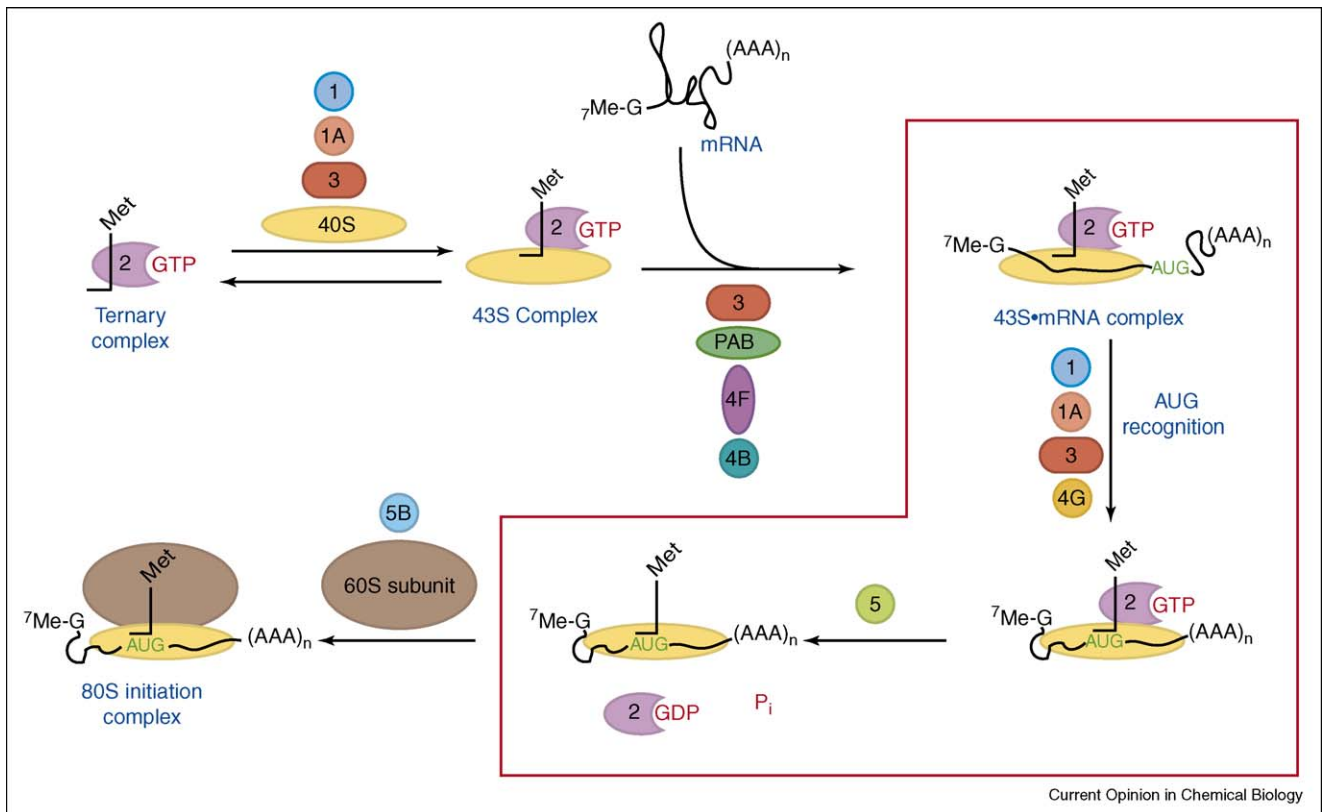
The current state of our knowledge of these events has been presented in many reviews encompassing the entirety of eukaryotic translation initiation, from ternary complex formation to subunit joining and beyond [1–5]. For this reason, the following review takes a more detailed look at a single, critical part of the pathway, selection of the start codon, and concentrates on the recent advances made in elucidating the molecular mechanics that make it possible.

Where to begin?

While all the steps leading to the formation of a translation-competent 80S initiation complex are important, perhaps the most critical of these is the correct selection of the codon at which to begin translation. Incorrect start site selection would at best result in an N-terminally truncated or extended protein, if translation is initiated in-frame, but more frequently would result in the synthesis of a completely miscoded polypeptide. Clearly, mistranslation of the genome to the proteome would be detrimental to the cell, and the cell has invested significant resources to make sure the correct start codon is selected. This is indicated by the fact that roughly half of the initiation factors in yeast have been implicated in start site selection (eIFs 1, 1A, 2, 3, 4G and 5) [6–12,13*, 14,15,16*,17**,18*,19**].

Although many of the protein factors involved in start site selection have been identified, it is not yet clear how the factors, and their interactions with the rest of the initiation complex, promote the identification of and the response to the start codon. Although the picture is far from

Figure 1



Overview of translation initiation in eukaryotes. The current model of translation initiation is depicted and is meant only to represent the core steps in the process. Ternary complex binds to the 40S subunit forming the 43S complex, which scans the mRNA, which scans the mRNA to locate the AUG codon. AUG selection results in irreversible GTP hydrolysis and presumably dissociation of eIF2. The resulting complex can then undergo eIF5B-promoted subunit joining, forming the 80S initiation complex. The boxed section represents the focus of this review. The stages at which various factors are thought to act, not when they are thought to bind, are indicated next to the arrows.

complete, important details of this complex process have recently been revealed.

eIF1 takes center stage

It is thought that all of the machinery required for the irreversible hydrolysis of GTP in response to start site identification is present in the scanning pre-initiation complex [17^{••},20,21]. Yet, complete GTP hydrolysis is

repressed until the start site is found, indicating a change must occur in the 43S•mRNA complex that permits irreversible hydrolysis upon start codon recognition. Several lines of evidence implicate eIF1 as central to this putative change in the 43S•mRNA complex *in vivo*. First, overexpression of eIF1 has been shown to suppress the start codon selection defect phenotype caused by a mutation in eIF5 that allows initiation at UUG codons. Second,

Box 1 List of eukaryotic initiation factors (eIFs) involved in start codon recognition

eIFs	Function(s)	Interactions (direct or indirect)
eIF1	Facilitates ternary complex (TC) binding. Dissociates from 43S•mRNA complex after AUG selection. Regulates the release of P _i from eIF2 after eIF5-promoted GTP hydrolysis.	40S ribosomal subunit, eIF1A, eIF2, eIF3, eIF5, eIF4G
eIF1A	Facilitates TC binding. Interaction with eIF5 is important in AUG selection.	40S ribosomal subunit, eIF1, eIF2, eIF5, eIF5B
eIF2	GTP-bound form binds initiator tRNA to form TC. Delivers tRNA to the 40S ribosomal subunit. Irreversibly hydrolyzes GTP in response to AUG selection.	40S ribosomal subunit, eIF1, eIF1A, eIF3, eIF5B
eIF3	Multi-subunit complex that interacts with numerous eIFs. Facilitates mRNA loading onto 43S complex.	40S ribosomal subunit, eIF1, eIF1A, eIF2, eIF4B, eIF4G, eIF5
eIF4G	Central protein in the 7-methyl-G cap binding complex (eIF4F).	eIF1, eIF3, eIF4A, eIF4E, eIF5, PAB
eIF5	GTPase activating protein for eIF2. Interaction with eIF1A is important in AUG selection.	eIF1, eIF1A, eIF2, eIF3, eIF4G

an eIF1 mutant that increases initiation at UUG codons has been shown to have reduced binding to the 43S pre-initiation complex *in vivo*, suggesting that its interaction with the complex is important for maintaining start site selection fidelity [7,8,17**].

In the mammalian system, eIF1 has been shown to promote rejection of mismatches between the mRNA codon and the anticodon of the Met-tRNA_i, and also to enhance discrimination between AUG codons in 'good' or 'bad' Kozak sequence contexts [22]. Pestova and colleagues proposed that the 43S complex exists in two conformations, a scanning-incompetent 'closed' form and a scanning-competent 'open' one. eIF1 may antagonize formation of the closed complex, setting the energetic bar higher for conversion from open to closed states such that only cognate AUG codons will induce a closed complex. It is unlikely that eIF1 senses the presence of the correct codon by directly monitoring the base pairing between the AUG in the mRNA and the anticodon of the Met-tRNA_i, because hydroxyl radical cleavage experiments suggest that while eIF1 is located near the P-site, it is not in a position to directly interact with the base pairs formed [23].

eIF2 α (one of the three subunits of eIF2) may also be involved in the 'open/closed' conformations of the 43S complex. In mammals, eIF2 α has been shown to interact with the -3 purine in the Kozak sequence suggesting it is responsible for recognizing the identity of this nucleotide. The interaction between eIF2 α and the -3 purine in a 'good' Kozak sequence is proposed to stabilize the closed conformation of the 43S complex during start codon recognition [24**].

A conformational change and movement of eIF1 upon AUG recognition

Maag *et al.* recently provided further evidence for a two state, 'open/closed' model for the conformations of the 43S initiation complex. By monitoring the stability of eIF1A in various initiation complexes using fluorescence spectroscopy, this study indicated an energetic interaction between eIF1A and eIF5 in the 43S•mRNA pre-initiation complex and implicated this interaction in maintaining the fidelity of start codon recognition [18*]. The interaction between the factors is strengthened upon AUG codon recognition, resulting in a significant shift in equilibrium between two states of the complex. A similar shift occurs at UUG when eIF1A and eIF5 have mutations that increase UUG recognition *in vivo*. These two states may be the 'open' and 'closed' complexes proposed earlier [22] and suggest a model for how this interaction acts in scanning and start codon selection. The 43S pre-initiation complex searches the 5' UTR of an mRNA and exchanges between an open, scanning-competent conformation and a closed, scanning-incompetent conformation. The scanning process is facilitated by eIF1A, either by stabilizing the open conformation or

by destabilizing the closed conformation. This effect is mediated by the unstructured C-terminal domain (CTD) of eIF1A, the removal of which increases UUG selection *in vivo* [19**]. Following the identification of an AUG codon, a direct or indirect interaction forms between eIF1A and eIF5 that results in movement of the eIF1A CTD and an increase in stability of the closed conformation relative to the open conformation. This change may stall the initiation complex at the AUG codon long enough for downstream events, such as irreversible GTP hydrolysis, to occur and may also prevent the complex from moving on after they have occurred.

Evidence that the 43S complex undergoes an AUG codon-dependent conformational change has also come from fluorescence resonance energy transfer (FRET) studies using fluorescently labeled eIFs 1 and 1A in a yeast-based system [25,26**]. In response to recognition of the start codon, the 43S•mRNA complex undergoes a rapid conformational change. Although the nature of the conformational change is unknown, it increases the distance between the C-termini of eIF1 and eIF1A and results in a reduction of the affinity of the 43S•mRNA complex for eIF1. This change in affinity leads to dissociation of eIF1 from the 43S•mRNA complex, most likely onto its binding site on eIF3 [15,27,28*].

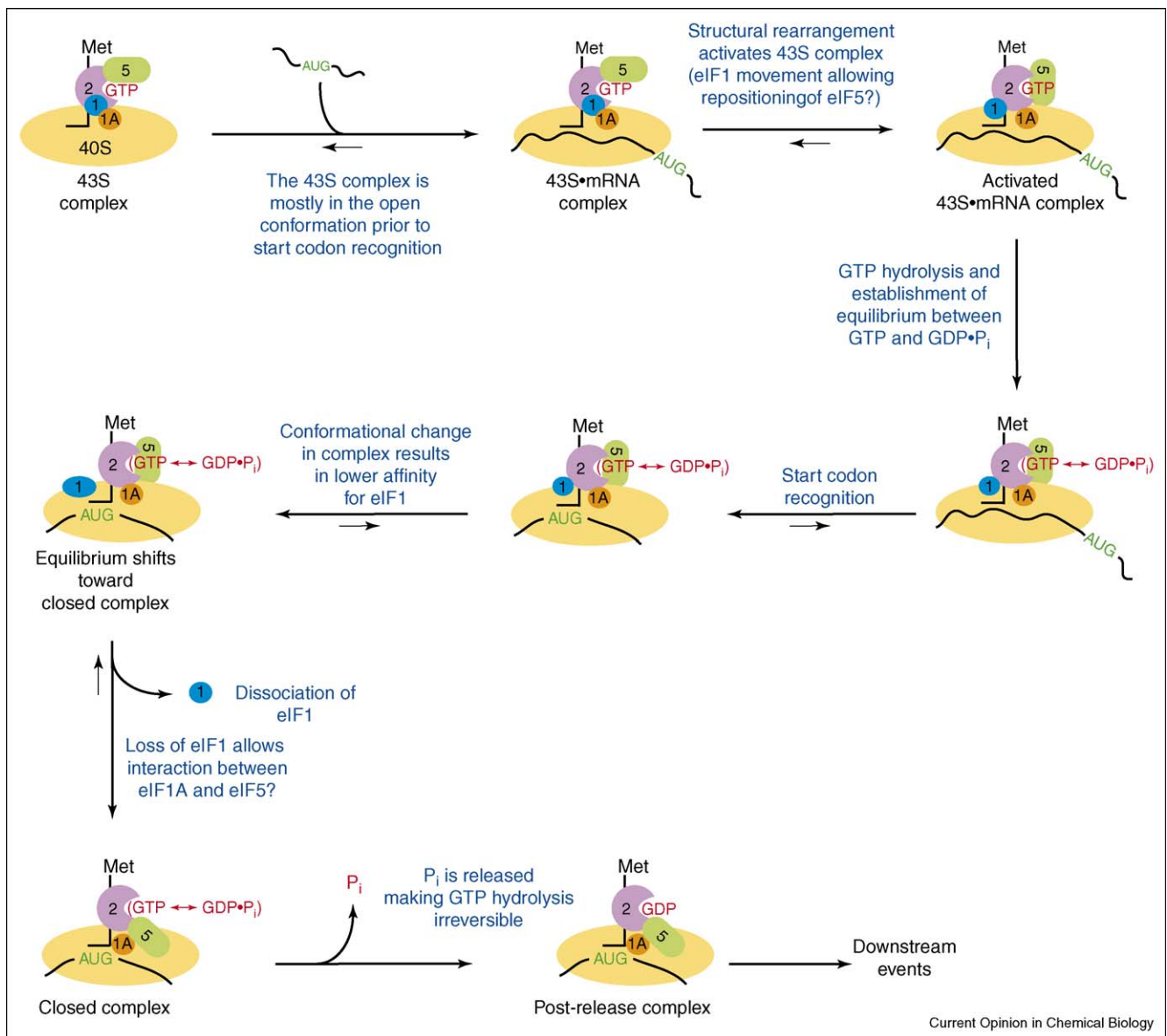
The importance of inorganic phosphate

The dissociation of eIF1 from the 43S•mRNA complex after AUG recognition has important implications for start site selection, as eIF1 has been suggested to act as a negative regulator of eIF5-promoted GTP hydrolysis by eIF2 [17**,26**,28*]. A recent kinetic dissection of eIF5-promoted GTP hydrolysis by eIF2 has shown that the AUG codon-dependence of GTP hydrolysis is small, only 2–4-fold [29**], consistent with previous results [28*,30]. The data also suggested that an equilibrium between GTP and GDP•P_i is established on eIF2 in the 43S complex in the absence of an AUG codon. The small dependence of hydrolysis on a start codon is unlikely to be sufficient to account for the fidelity of AUG selection *in vivo* (≥ 100 -fold) [31,32]. However, the release of inorganic phosphate (P_i) from the 43S•mRNA complex after GTP hydrolysis is highly dependent (170-fold) on the presence of an AUG codon [29**]. This large AUG dependence is significant because the release of P_i makes GTP hydrolysis irreversible. It was also shown that AUG codon-dependent release of eIF1 [26**] does not regulate the actual chemical step of GTP hydrolysis but instead regulates release of P_i (and therefore the reversibility of the hydrolysis reaction).

A new model

Taken together, the data suggest the following model for start codon selection in eukaryotes (Figure 2). Binding of an mRNA to the 43S complex accelerates a structural rearrangement that makes the complex fully competent

Figure 2



Model of steps leading to AUG recognition and irreversible GTP hydrolysis. mRNA binding (simplified in this model) accelerates a structural rearrangement in the 43S•mRNA complex that activates it for rapid eIF5-promoted GTP hydrolysis by eIF2. It has been proposed that the rearrangement is the movement of eIF1 from eIF2, allowing the catalytic domain of eIF5 to interact with eIF2. After initial GTP hydrolysis, but before AUG recognition, an equilibrium between GTP and GDP•P_i is established on eIF2. During the process of locating the AUG codon, eIF1 may destabilize the formation of the closed complex at non-cognate codons in the mRNA. Recognition of the start codon results in a rapid conformational change in the complex. This change alters the interactions of eIF1 with the complex, ultimately resulting in its dissociation. eIF1 then most likely moves fully onto eIF3 (not shown). Loss of eIF1 from the complex may allow the C-terminus of eIF1A to interact with eIF5, which may change eIF5's interaction with eIF2, possibly removing the 'P_i plug' and thereby allowing P_i to be released, and making codon selection irreversible.

to hydrolyze GTP [29^{••}]. One model proposes that this rearrangement is the movement of eIF1 away from eIF2 γ (the GTP-binding subunit of eIF2), which allows the N-terminal GAP domain of eIF5 to interact with eIF2 γ , resulting in GTP hydrolysis. This model is based on the NMR solution structure of the N-terminus of human eIF5, which shows a remarkable similarity to the overall

structure of eIF1 [33[•]]. The authors suggest that the similar folds may be used to interact with a common partner, in this case eIF2.

After the 43S complex is activated it scans the mRNA in search of an AUG codon. The presence of eIF5 results in reversible GTP hydrolysis occurring rapidly within the

complex, establishing an equilibrium between GTP and GDP•P_i [29^{••}]. eIF1 may keep the 43S complex in an 'open' or scanning-competent state at non-AUG codons [22], allowing the complex to continue along the mRNA rapidly, and also preventing P_i release from eIF2 before AUG recognition. When the AUG codon is found, base pairing occurs between the Met-tRNA_i and the mRNA, resulting in a fast conformational change in the complex that moves the C-termini of eIFs 1 and 1A away from each other. This change in the 43S complex reduces its affinity for eIF1, causing eIF1 to move onto eIF3 or dissociate [26^{••}]. This conformational change has been proposed to involve movement of eIF2β relative to eIF2αγ, affecting eIF1 stability in the complex [33[•]]. Because eIF1 and eIF1A interact on the 40S subunit [34], it is possible that release of eIF1 after start codon recognition alters the position or conformation of eIF1A. This would allow its C-terminus to interact with eIF5, increasing the stability of the 'closed' 43S complex, which prevents movement of the complex away from the AUG codon [18[•]]. Dissociation of eIF1 allows P_i to be released from eIF2, making GTP hydrolysis irreversible, which commits the 43S complex to initiation at the selected codon [29^{••}]. If eIF5's GAP activity requires physical contact with the GTP, it is possible that this contact could act as a barrier to P_i release. This scenario is appealing because mutations of a completely conserved arginine residue (R15) in eIF5 abolish GAP activity but do not affect the factor's binding to ternary complex [29^{••},35,36], suggesting R15 may be involved in transition state stabilization. Thus, eIF5 may have two roles in promoting GTP hydrolysis. First, eIF5 donates catalytic residues to the active site of eIF2, promoting the chemical hydrolysis step. Second, it may act as a 'plug', preventing premature P_i release. eIF5 may have GAP activity because premature GTP hydrolysis without the 'P_i plug' in place would be undesirable, even catastrophic, as it would allow initiation at non-AUG codons. It is possible that loss of its interaction with eIF1 [17^{••},21] or the gain of an interaction with eIF1A changes the position or conformation of eIF5, 'unplugging' eIF2 and allowing the P_i to be released.

Regulation of P_i release, as well as an internal equilibrium between GTP and GDP•P_i, has been observed in several other GAP-activated G proteins recently, including Rap, Ras and EF-G, a prokaryotic translation elongation factor [37[•],38^{••},39]. G proteins are known to undergo conformational changes when switching from the GTP-bound state to the GDP-bound state [40–45]. Because the difference between the conformations (and therefore activity) is induced by the loss of the gamma phosphate from GTP, rather than the actual hydrolysis step, there is a certain amount of structural logic for G proteins to control P_i release stringently.

Conclusions

Even with the recent advances in elucidating the molecular mechanics of start site selection, many questions

remain. What other interactions exist between the components of the pre-initiation complex and how do these interactions affect start site selection? How do previous steps, such as mRNA remodeling and scanning, alter AUG recognition? What are the natures of the different 43S complex conformations? And what within the complex moves during the conformational changes? Although the list of unanswered questions is long, continuing use of the powerful combination of biochemical, biophysical and genetic techniques should allow us to gain even more detailed insight into the molecular mechanism of start site selection. The field is poised and waiting for the structural revolution that energized the study of bacterial translation to begin for eukaryotic translation. Structural information about factor–factor, factor–ribosome, factor–mRNA and factor–tRNA interactions will be vital to completely understand how all of these components conspire to form the 80S initiation complex.

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