Starting the protein synthesis machine: eukaryotic translation initiation

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Summary

The final assembly of the protein synthesis machinery occurs during translation initiation. This delicate process involves both ends of eukaryotic messenger RNAs as well as multiple sequential protein–RNA and protein–protein interactions. As is expected from its critical position in the gene expression pathway between the transcriptome and the proteome, translation initiation is a selective and highly regulated process. This synopsis summarises the current status of the field and identifies intriguing open questions. ***BioEssays*** **25:1201–1211, 2003.**

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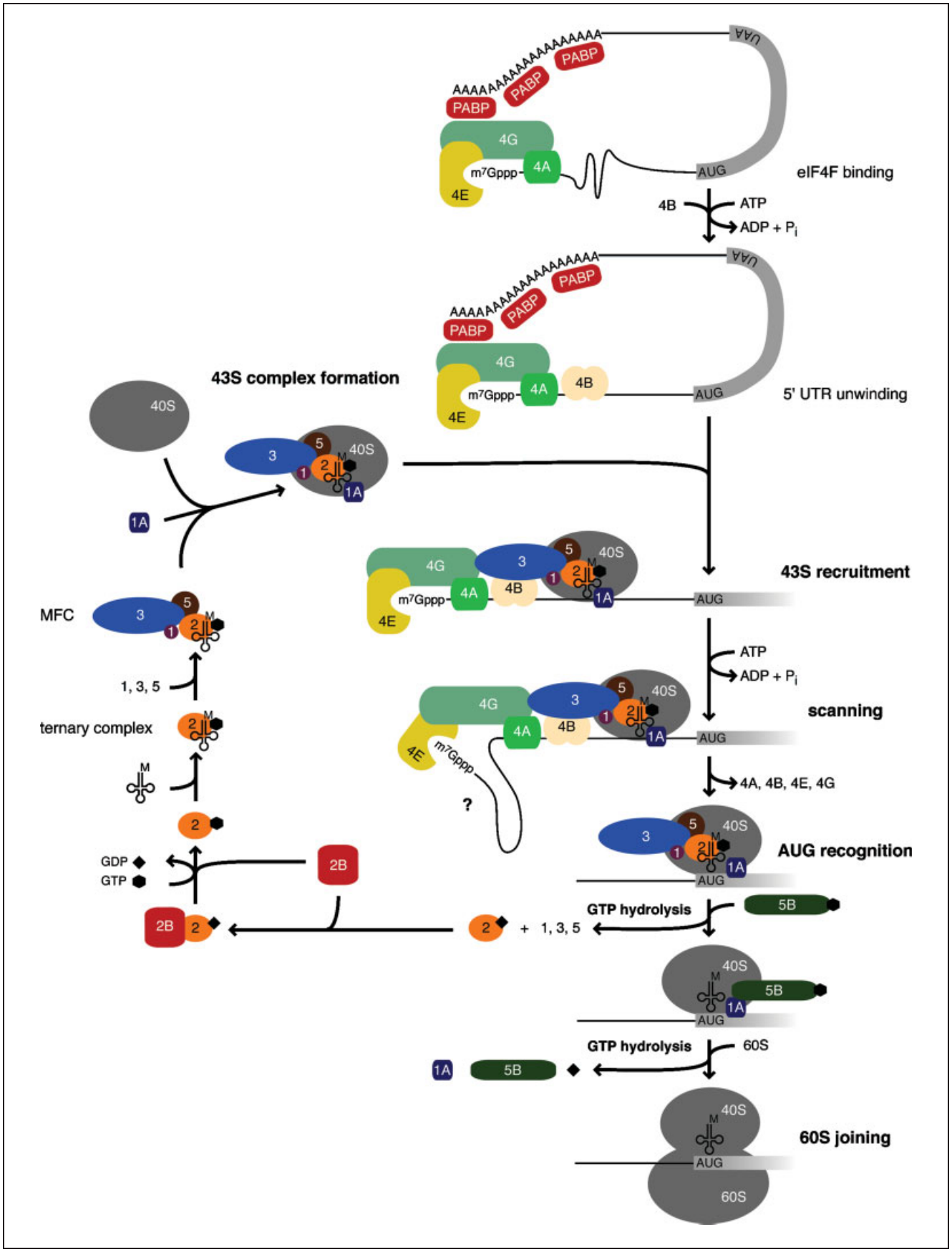
Abbreviations: 4E-BP, eIF4E-binding protein; ATP, adenosine triphosphate; ATPase, adenosine triphosphatase; AUG, translation start codon; CTD, carboxy-terminal domain; C-terminal/terminus, carboxy-terminal/terminus; Cys, cysteine; DEAD box, signature motif of a RNA helicase family; eIF, eukaryotic (translation) initiation factor; eRF, eukaryotic release factor; GAP, GTPase-activating protein; GDP/GTP, guanosine di/triphosphate; GEF, guanine nucleotide exchange factor; G-protein, guanyl-nucleotide-binding protein; GTPase, guanosine triphosphatase; HEAT-motif, huntingtin-, elongation factor 3-, a subunit of protein phosphatase 2A-, and target of rapamycin-motif; IF, (bacterial translation) initiation factor; IRES, internal ribosome entry site; $m^7G(5')ppp(5')N$, mRNA cap structure; MA3 domain, protein domain first identified in the mouse MA3 protein; MDa, mega-Dalton, unit of mass; Met, methionine; MFC, multifactor complex; MIF4G domain, Middle portion of eIF4G domain; Mnk-1, MAP kinase-interacting kinase-1; mRNA, messenger RNA; NMR, nuclear magnetic resonance; nt, nucleotide; NTD, N-terminal domain; N-terminal, amino-terminal; OB-domain, oligonucleotide-binding domain; oligo/poly(A), oligo/poly(adenylate); p97, alternative name- DAP5 (death-associated protein 5); PABC, PABP C-terminal domain; PABP, poly(A)-binding protein; Paip, PAPB-interacting protein; PAM, PABP-interacting motif; P-site, peptidyl-site (on the ribosome); RRM, RNA-recognition motif; rRNA, ribosomal RNA; S, Svedberg, unit of sedimentation constant; tRNA, transfer RNA; UTR, untranslated region; W2 domain, protein domain named after two conserved tryptophans (W).

Introduction

Protein synthesis is accomplished by ribosomes, large ribonucleoprotein assemblies of approximately 4 MDa acting in concert with a considerable number of accessory factors to 'translate' the genetic information contained in messenger RNA (mRNA) molecules. The dynamic process of mRNA translation is usually divided into three phases: initiation, elongation and termination.[1] The initiation phase represents all processes required for the assembly of a ribosome with a initiator-methionyl-transfer-RNA (Met-$tRNA_i^{Met}$) in its peptidyl (P-) site at the start codon of the mRNA. The actual polypeptide synthesis takes place during the elongation phase. Peptide bond formation occurs on catalytic centres that are fundamentally formed by the ribosomal RNA (rRNA) of the large subunit.[2,3] When the ribosome reaches the stop codon, this signals termination, comprising the release of the completed polypeptide and, presumably, the ribosome from the mRNA. Thus, the title of '*protein synthesis machine*' clearly goes to the ribosome itself, although it cannot perform its functions alone. Additional 'devices' are those translation factors that help it to engage the mRNA template, to select the activated building blocks for polypeptide synthesis, and to mediate termination. This is particularly true during the process of translation initiation on eukaryotic mRNAs in the cytoplasm. To accomplish this, cells use the 5′ $m^7G(5')ppp(5')N$ cap structure and the 3′ poly(A) tail of the mRNA and at least 12 eukaryotic initiation factors (eIFs). Translation initiation is accomplished in four subsequent steps: (i) formation of a 43 Svedberg (S) preinitiation complex from the small (40S) ribosomal subunit, initiation factors, and Met-$tRNA_i^{Met}$, (ii) recruitment of the 43S complex to the (capped) 5′ end of the mRNA, (iii) 'scanning' of the 5′ untranslated region (UTR) of the mRNA and start codon recognition, and (iv) joining of a large (60S) subunit to assemble a complete (80S) ribosome (Fig. 1).


AAAAAAAAAAAAAAAA
PABP
PABP
PABP
UAA
4G
4A
m7Gppp
4E
AUG
eIF4F binding
4B
ATP
ADP + Pi
5' UTR unwinding
43S complex formation
40S
3
5
2
M
1
1A
43S recruitment
MFC
1, 3, 5
ternary complex
scanning
4A, 4B, 4E, 4G
?
AUG recognition
GDP
GTP
2B
GTP hydrolysis
5B
+ 1, 3, 5
60S
60S joining

Ground work into translation initiation included the purification of the factors involved, a characterisation of their biochemical activities and the cloning of corresponding cDNAs. More recently, yeast genetics, interaction studies, structure determination and reconstitution experiments using purified and recombinant components have yielded insights into detailed functions. A central question has been to understand how multiple protein–protein and protein–RNA interactions are established between translation initiation factors, the mRNA and the ribosome, and how these interactions are remodeled during the stepwise process of translation initiation. Due to space limitations, this review focuses on current knowledge regarding the predominant cap-dependent 'scanning' mechanism of translation initiation (Fig. 1) and the contribution of the poly(A) tail to this process. The initiation phase of translation is also a major target for global and mRNA-specific regulation. We will refer in passing to major pathways of regulating initiation factor function; however, several recent review articles cover this topic more comprehensively.[4–8] An important alternative pathway for initiating translation, by internal ribosome entry, is also not covered here. Internal ribosome entry sites (IRES) were initially discovered in certain viral RNAs but this mode of initiation is also employed by some cellular mRNAs (see Refs. 9–11 for current reviews). Finally, other colleagues have recently reviewed the pathway of eukarotic initiation of translation. We refer the reader to these articles, for an alternate take on the topic and a more comprehensive citation of the primary research literature.[1,12–16]

Formation of the 43S preinitiation complex

Physiological conditions favour the association of 40S and 60S ribosomal subunits to form complete 80S ribosomes. Thus, the first requirement for initiation is to promote a dissociation of vacant ribosomes into their subunits. This is thought to be promoted by eIF3 and eIF1A (the homologue of bacterial initiation factor 1, IF1) by largely unknown means (see chapter by JWB Hershey and WC Merrick in Ref. 1). The dissociated 40S subunit then binds several initiation factors as well as Met-tRNA$_i^{Met}$. The latter is delivered to the P-site of the ribosome by the G-protein eIF2 (Fig. 1).

eIF2, Met-tRNA$_i^{Met}$ and GTP jointly bind the 40S subunit as a ternary complex (Fig. 1; see chapter by AG Hinnebusch in Ref. 1). Since the GDP-bound form of eIF2 generated by each initiation cycle cannot bind Met-tRNA$_i^{Met}$, it requires the action of the guanine nucleotide exchange factor (GEF) eIF2B (Fig. 1). eIF2 is a heterotrimer of α, β, and γ subunits. Biochemical and genetic analyses suggest that the eIF2 γ-subunit binds both GTP and Met-tRNA$_i^{Met}$. The β-subunit promotes GTPase activity and modulates initiator tRNA binding of eIF2γ. Furthermore, eIF2β possesses three polylysine stretches near the amino terminus and a putative Cys2-Cys2 zinc finger motif in the carboxy terminal domain (CTD). Both motifs have been implicated in mRNA binding and could aid in codon–anticodon interactions.[17] The lysine repeats furthermore mediate the mutually exclusive interaction of eIF5 and the ε-subunit of eIF2B with eIF2β.[18] The α-subunit of eIF2 functions predominantly as a regulator of translation initiation. It is required for interactions between eIF2 and eIF2B that promote nucleotide exchange. Phosphorylation of the α-subunit of eIF2 at the conserved residue Serine-51 is carried out by specific eIF2-kinases and constitutes a major means of regulating translation in response to different forms of cellular stress.[8] It converts GDP-bound eIF2 into a competitive inhibitor of its GEF, eIF2B. eIF2B is a heteropentamer of α, β, γ, δ, and ε subunits. It can be divided into a catalytic subcomplex consisting of the δ and ε subunits, responsible for recycling GDP-bound eIF2, and a regulatory subcomplex comprising the other three subunits, responsible for proper response to eIF2α phosphorylation.[19] eIF2Bδ and eIF2Bε exhibit sequence homology with each other throughout their length and the GEF activity resides in the C-terminal region of the ε subunit. The α, β and γ subunits of eIF2B are also homologous to each other and bind to eIF2α and discriminate between its phosphorylated and non-phosphorylated status.

Figure 1. The translation initiation pathway. The 40S ribosomal subunit is primed for initiating translation by binding of the ternary complex comprising eIF2, Met-tRNA$_i^{Met}$, and GTP (see left side of depicted scheme). In yeast, this is aided by the multifactor complex (MFC), an intermediate with roles in several steps of translation initiation. The resulting 43S preinitiation complex is recruited to the mRNA via interactions with the eIF4 factors bound at or near the cap structure of the mRNA (see top of schematic). The 43S complex then scans the 5′UTR to locate the initiator codon (centre-right of scheme). Following recognition of the AUG, involving base-pairing with the anticodon loop of Met-tRNA$_i^{Met}$, release of the bound factors accompanies two distinct GTP hydrolysis steps and joining of the 60S subunit to form an elongation competent 80S ribosome (see bottom of figure), poised to start the first peptide elongation cycle. GDP-bound eIF2 is recycled by eIF2B to allow further ternary complex formation (see bottom left of the diagram). For clarity, eukaryotic initiation factors are labelled with the unique portion of their respective names only, omitting the general 'eIF' prefix i.e., '4E' instead of 'eIF4E'). Every effort was made to design this schematic such that it accurately reflects the many interactions between factors. Due to geometric constraints, however, the figure fails to show the interaction between eIF1A, and eIF2 as well as eIF3. The '?' near the looped-out 5′ UTR in the centre of the scheme indicates the speculative nature of this scanning intermediate. To simplify the bottom part of the diagram, the distinct requirements of GTP-bound eIF5B for 60S subunit joining and GTP hydrolysis after 80S ribosome formation for eIF5B release are not shown. The recently identified factor eIF4H is also not represented in the scheme (see main text for more information).

Binding of the ternary complex to the 40S subunit is aided by eIF1, eIF1A, and the multisubunit factor eIF3 (Fig. 1), which consists of a core of five non-identical subunits (eIF3a, eIF3b, eIF3c, eIF3i and eIF3g in yeast)[20] and up to six additional subunits in mammals (see Ref. 21 for a current nomenclature of eIF3 subunits). In yeast, a multifactor complex (MFC) of eIF1, eIF2, eIF3, eIF5, and Met-tRNA$_i^{Met}$ assembles independently of the ribosome and may be an important functional unit during several stages of translation initiation[22] (Fig. 1). Interactions within the MFC have been extensively studied, thus allowing the construction of a provisional model of its architecture (Fig. 2; summarised in Ref. 23). These studies show that each of the three largest subunits of eIF3 (a, b, and c) has a binding site for the other two subunits. The extreme CTD of eIF3b additionally interacts with the eIF3i and eIF3g subunits. eIF3j (a substochiometric component in yeast) binds simultaneously to both the N-terminal domain (NTD) of eIF3b and the CTD of eIF3a. eIF1 is tethered to the MFC through interactions with the eIF3a-CTD and eIF3c-NTD. The eIF3c-NTD also binds the CTD of eIF5. The β-subunit of eIF2 makes two critical contacts with eIF3, a direct interaction with the extreme CTD of eIF3a and an indirect association with the eIF3c-NTD via the eIF5-CTD. In further work, it was established that eIF3a-NTD and eIF3c can interact with the ribosmal protein RPS0A located on the solvent side of the 40S subunit. Furthermore, the eIF3a-CTD can specifically bind to a short segment from domain I of 18S rRNA. Taken together, these findings led Valasek et al.[23] to propose a 'wrap-around' model for binding of the MFC to the 40S subunit. In this model, eIF3 binds to the solvent side but has access to the 60S-interface side of the 40S subunit, via the eIF3a-CTD-18S rRNA interaction. eIF2, eIF5 and eIF1 are placed at the interface side of the 40S subunit (Fig. 2).

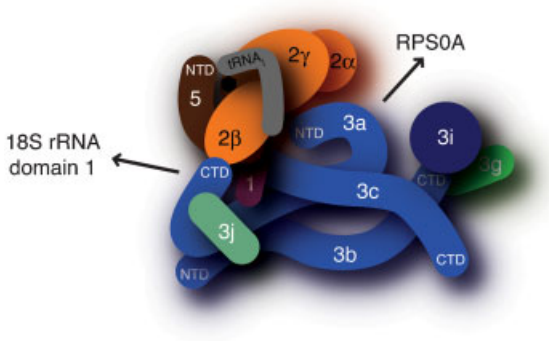


Figure 2. Summary of subunit interactions within the yeast multifactor complex (MFC). A combination of interaction data and molecular modelling studies suggests that eIF1, eIF2, and eIF5, the NTD of eIF3c, and the CTD of eIF3a (see Ref. 21 for a current nomenclature of eIF3 subunits) interact with the 60S interface side of the 40S ribosomal subunit, while the remainder of eIF3 wraps around the solvent side of the 40S subunit. The figure depicts a working model of the MFC architecture that may require revision once direct structural data is available. See text for further details. (Adapted from Ref. 23).

Recruitment of the 43S complex to the 5′ end of the mRNA

eIF3 is also required for binding of the 43S complex to the mRNA (Fig. 1). Mammalian eIF3 interacts with the central region of eIF4G[24] (Fig. 3).To date, it is not known which subunit of eIF3 mediates this interaction and binding of eIF3 to eIF4G has not been found in yeast. Other interactions may act as alternative or additional links between eIF3 and the mRNA: yeast eIF5 can bind to eIF4G[25] and eIF4B interacts with eIF3 subunits in mammals[26] and yeast.[27]

eIF4G is a subunit of the heterotrimeric eIF4F complex that binds to the cap structure at the 5′ end of the mRNA. Additional components are the cap-binding protein eIF4E and the ATP-dependent RNA helicase eIF4A. eIF4E resembles a cupped hand and its concave side provides a small hydrophobic slot for insertion of the cap structure and a contiguous region for mRNA binding. The opposite convex face of the protein is the contact region with eIF4G.[28–30] Its primary function in translation is to enhance the binding of the eIF4F complex to the 5′ end of the mRNA. eIF4A is a DEAD box helicase that can unwind RNA secondary structure in the cap-proximal region of the mRNA. It does so much more efficiently as part of the eIF4F complex and aided by eIF4B, although the two proteins probably do not interact directly. eIF4A has a dumbbell structure consisting of two domains connected by a flexible linker.[31] It probably undergoes a series of conformational changes as it binds its substrates, RNA and ATP, hydrolyses ATP, and releases products. An important feature of eIF4A's function is also an apparent need to exchange eIF4F-bound with free factor for efficient unwinding.[32,33] eIF4B is a homodimer that can bind RNA by virtue of an N-terminal RNA recognition motif (RRM) and a C-terminal arginine-rich motif.[12] Another recently identified factor, eIF4H, has homology to the RRM domain of eIF4B and has also been shown to stimulate ATPase and helicase activity of eIF4A.[34]

The centrepiece of eIF4F is the multivalent adapter molecule eIF4G.[35] The binding regions for several interaction partners of eIF4G have been mapped by deletion and mutation analysis (Fig. 3). The N-terminal third harbours binding sites for eIF4E and the poly(A)-binding protein PABP (see below) that serve to latch it onto both mRNA ends, implying the potential to pseudo-circularise the mRNA.[14] The eIF4E-binding region changes from an unstructured state to an α-helix with two turns when bound to the factor.[36,37] The phylogenetically conserved central third of eIF4G[38,39] interacts with eIF4A, RNA and eIF3 (at least for mammalian eIF4G) and appears to constitute the primary ribosome recruitment

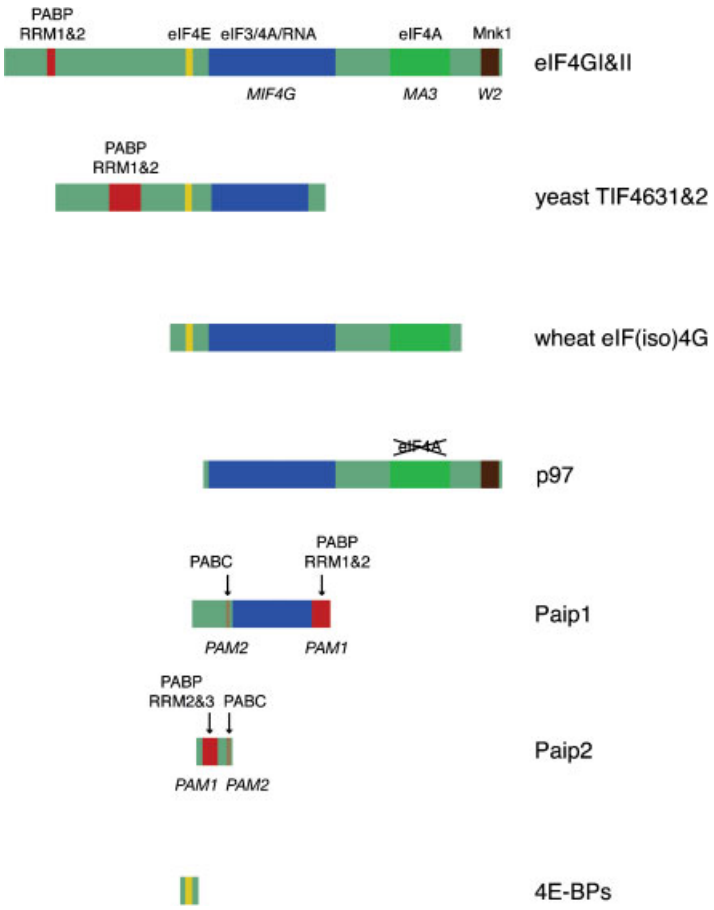


Figure 3. Domain structure of eIF4G and selected proteins with roles in translation initiation. Mammalian cells contain two isoforms of eIF4G (I&II), with analogous domain structure and modes of interaction with other proteins (indicated above the schematic protein sequence). Most of these interaction sites have conserved amino acid and/or structural signatures (named below the eIF4G schematic: MA3, first identified in the mouse MA3 protein; MIF4G, middle portion of eIF4G; W2, named after two conserved tryptophan residues) that are also found in other proteins involved in translation. The yeast and plant homologues of eIF4G lack some of the domains found in the mammalian factor, suggesting that eIF4G has evolved around a conserved core region. p97 is a mammalian protein with homology to the C-terminal two thirds of eIF4G. Apparently it binds eIF4A only through its MIF4G portion and it may function as a specialised initiation factor for IRES-containing mRNAs during cellular stress. Paip1 is a further MIF4G-domain protein with two PABP-interacting motifs (PAM1 and PAM2). These motifs are also found in Paip2 and both proteins bind to overlapping regions of PABP (see Fig. 4). The 4E-BPs are molecular mimics of the eIF4E-binding region of eIF4G and regulate translation.

module.[40,41] The structure of this segment of eIF4G was recently solved. A region of 259 amino acids folds into ten α-helices, which are arranged into five HEAT motifs.[42] The C-terminal third has an additional binding site for eIF4A.[43] There is no consensus as to whether eIF4G binds simultaneously to just one or two eIF4A molecules.[44,45] At the extreme C terminus of metazoan eIF4G is another conserved region termed W2 domain (Fig. 3), with homology to eIF5 and eIF2Bε.[18,38] In mammals, this region of eIF4G binds the eIF4E-kinase Mnk-1.[46,47] Phosphorylation of eIF4E at serine 209 by Mnk-1 generally correlates with increased translation rates[48] and may serve as a marker of eIF4F integrity. It has long been thought that phosphorylation increases the cap-binding affinity of eIF4E.[49] Recent studies have now challenged this view[50,51] (discussed in Ref. 48). Phosphorylation also regulates the activity of the 4E-binding proteins (4E-BPs), which act as a molecular mimic of the eIF4E-binding region of eIF4G and hence as competitive inhibitors of eIF4G binding to eIF4E. Growth stimulatory signals lead to increased 4E-BP phosphorylation and dissociation from eIF4E, allowing for increased eIF4F complex formation and enhanced translation.[5,12]

In summary, the assembly of the 43S preinitiation complex at the 5′ cap structure of the mRNA is principally directed by the cap-binding protein eIF4E and co-ordinated by eIF3 and eIF4G, which provide multiple contact points to other initiation factors, the small ribosomal subunit, and the mRNA.

Scanning of the 5′ untranslated region and AUG recognition

Once assembled near the 5′ end of the mRNA, the 43S complex has to locate the appropriate start codon on the mRNA. A linear movement along the mRNA 5′ UTR in search of an AUG triplet, termed 'scanning', was originally proposed by Kozak[16,52] (Fig. 1). It explains well the adherence of most known mRNAs to the 'first-AUG' rule, which predicts that initiation should occur on the start codon closest to the mRNA 5′ end. It is also consistent with a large body of genetic and biochemical data,[52] although it needs to be noted that 'scanning' has not been directly demonstrated to date. At least in

mammals, recognition of an AUG as a translation start codon also critically depends on its surrounding sequence. The consensus sequence GCC(**A/G**)CCAUG**G** (positions where changes have the most pronounced effect are marked in bold) provides an optimal context for initiation[16,52–54] (see chapter by JWB Hershey and WC Merrick in Ref. 1). Despite its plausibility and conceptual relevance, information about the molecular nature of the scanning process and what drives it has long been lacking (discussed by RJ Jackson in Ref. 1).

Experiments using highly pure or recombinant mammalian initiation factors and ribosomal subunits to reconstitute initiation intermediates have now shed more light on the elusive scanning process. Primer extension by reverse transcriptase is arrested at the leading edge of mRNA-associated ribosomal complexes ('toe-printing'), providing information about their presence and position on the mRNA. In this assay, addition of 40S subunits, ATP, eIF2, eIF3, eIF4A, eIF4B, eIF4F, to native, capped globin mRNA allowed the formation of a cap-proximal complex I (leading edge 21–24 nt from the 5′ end). Complex I remained in a cap-proximal position and could not be chased towards the initiator codon. Inclusion of the two small factors eIF1 and eIF1A, however, led to formation of an authentic 48S complex, centred over the AUG (complex II, leading edge 15–17 nt 3′ of the AUG). eIF1 and eIF1A act synergistically in this assay and, when added to complex I formed in their absence, require a cycle of dissociation–reassociation to assemble into complex II. Thus, although complex I is not a direct precursor of complex II, eIF1 and eIF1A are initiation factors intimately linked to the positioning of the small ribosomal subunit at the translation initiation codon[55] (Fig. 1). eIF1A has also been implicated in other aspects of translation initiation. It was shown to promote binding of the ternary complex to the 40S subunit,[56] it binds to eIF5B (the homologue of bacterial IF 2),[57] primarily through their CTDs, and the NTD of eIF1A binds to eIF2 and eIF3.[58] The NMR solution structure of eIF1A shows a modular organisation with an unstructured, basic NTD, a central five-stranded β-barrel oligonucleotide-binding (OB) domain, responsible for sequence-independent binding to single-stranded RNA, and an additional acidic CTD comprising two α-helices followed by an unstructured tail. Mutations along the RNA-binding surface interfere with preinitiation complex formation at the AUG codon in vitro.[59] eIF1 contains a five-stranded β-sheet packed against two α-helices.[60] Mutation of several conserved residues of eIF1 in yeast leads to a lower fidelity in initiation codon selection (summarised by TF Donahue in Ref. 1) and structural studies suggest that they form part of an interaction surface with other molecules.[60] Via a simultaneous binding to eIF3c, it is in close proximity to eIF5 and could affect its role as a GTPase-activating protein (GAP) for eIF2,[22] thus indicating a role for eIF1 in AUG selection.

Further analyses in the reconstitution system using a series of engineered mRNA templates provided more insight into the scanning process.[61] A minimal complex comprising a 40S subunit, eIF3, and the eIF2 ternary complex can bind to the 5′ end of an mRNA with a wholly single-stranded 5′ UTR, and in the presence of eIF1 reach and identify the initiation codon. Attachment of a 43S complex to a structured 5′ UTR, however, does require eIF4A, eIF4B, and eIF4F in addition. In the absence of eIF4A, eIF4B, eIF4F, and ATP, 43S complexes could not move through even weak internal secondary structures embedded within an otherwise unstructured 5′ UTR. Thus, eIF4F is normally required not only for ribosomal attachment to the mRNA but also appears to contribute to the scanning process. Although 43S complexes retained some capacity to scan along mRNA in the absence of eIF1, they could no longer discriminate between cognate and non-cognate initiation codons or sense their sequence context. Addition of eIF1 can even dissociate complexes preassembled at AUG codons in poor context in its absence, and stimulate positioning of preinitiation complexes at the appropriate start codon. This activity of eIF1 is abolished after 60S subunit joining, as completely assembled 80S complexes are resistant to eIF1 addition.[61]

Thus, these experiments provide biochemical evidence for a critical role of eIF1 in start codon selection. Pestova and Kolupaeva[61] suggest a model whereby 43S complexes exist in two conformations: a 'closed', scanning-incompetent form in the absence of eIF1 and an 'open', scanning-competent form in the presence of eIF1. In the closed configuration, the anticodon loop of initiator tRNA can establish (partial) base-pairing interactions even with sub-standard initiation regions. In the open form, it can form stable interactions only with cognate AUG triplets surrounded by a proper nucleotide context. The same study also shows that eIF1, eIF1A, eIF4A, eIF4B, and eIF4F all contribute to the processivity of scanning, although most likely they have complementary roles. eIF4A, eIF4B and eIF4F may serve to restructure the mRNA, while eIF1 and eIF1A may influence the structure of the mRNA-binding cleft of the 40S subunit and/or the position of initiator tRNA in these complexes. In summary, the biochemical reconstitution experiments have defined a minimal set of factors required to assemble a 43S complex at the initiation codon of a typical mRNA. The observation of a role for eIF4F during scanning through a structured 5′ UTR favours the view that it (or at least a part of it) continues to interact with the scanning complex as it departs from the 5′ end, perhaps up to the 60S subunit joining step (Fig. 1). The cycling of eIF4A through eIF4F could then potentially act as the ATP-dependent 'motor' of scanning, by providing a coupling between mRNA unwinding and the scanning movement.[61] Critical questions that remain to be addressed experimentally include the identification of 'en-route' scanning intermediates, and the definition of when and where the various interactions between the cap-structure, associated eIFs and 40S subunit are released during scanning.

Assembly of the 80S ribosome

Once the 40S subunit and associated factors have reached the initiator codon and base-pairing between the AUG and the anticodon loop of the initiator tRNA has occurred, a further series of events take place that result in the joining of the 60S subunit to form the active synthesis machine, the 80S ribosome. 60S subunit joining necessitates the release of initiation factors from the 40S subunit and requires GTP hydrolysis (Fig. 1).

The codon–anticodon interaction probably triggers a conformational change in 40S-subunit-bound eIF2, which leads to GTP hydrolysis, aided by the GAP protein eIF5 that is bound to the β-subunit of eIF2. This is followed by the release of eIF2 and possibly other initiation factors (see chapter by AG Hinnebusch in Ref. 1). GTP hydrolysis by eIF2 serves as a checkpoint for proper identification of the mRNA start codon. Until recently, hydrolysis of one molecule of GTP by eIF2 was thought to suffice for 60S joining. However, in vitro reconstitution experiments revealed that a second GTPase termed eIF5B is required for 80S ribosome assembly.[62,63] The GTPase activity of eIF5B is activated by 60S subunits and more strongly by the combination of 40S and 60S subunits. GTP-bound eIF5B stimulates 60S subunit joining but GTP hydrolysis only occurs after 80S formation and is required for the release of eIF5B.[62] Consistent with this interpretation, mutational studies with yeast eIF5B succeeded in uncoupling the translation and GTPase activities, suggesting that GTP hydrolysis by eIF5B serves as a final checkpoint for correct 80S assembly rather than having a mechanical role.[64] The structure of eIF5B resembles a chalice, with three N-terminal domains (I–III) forming the cup, connected by a long helix (the stem), to domain IV, which forms the base.[65] The GTP-binding motif resides in domain I and hydrolysis results in a modest structural change in domain I that is transduced through a lever-type mechanism into a more significant movement of domain IV. This switch-like conformational change regulates the ribosome affinity of eIF5B.[64] Domain IV is essential for eIF5B function and interacts with eIF1A, suggesting that the release of eIF1A and eIF5B from the ribosome could be coupled.[57] Thus, formation of an elongation-competent 80S ribosome requires two distinct GTP hydrolysis steps, which predominantly serve as checkpoints for proper AUG codon identification and 80S assembly.

The mechanistic role of the poly(A) tail during initiation of translation

So far, translation initiation has been discussed mostly in the context of the cap structure and the 5′ UTR. The 3′ poly(A) tail of mRNAs is, however, another critical determinant for translation initiation. Early experiments in mammalian cell extracts indicated that the addition of a poly(A) tail to a test mRNA yielded a modest stimulation of its translation resulting from an enhancement of 60S subunit joining (for a review of this early work see Ref. 66). Furthermore, a tight correlation between cytoplasmic polyadenylation of maternal mRNAs and their translational activation in vertebrate oocytes and developing embryos had been established (for a review of early work on maternal mRNA translation see Ref. 67). Later, a strong synergism between the cap structure and the poly(A) tail during translation initiation was discovered, first in electroporated cells[68] and subsequently in cell-free translation systems of different origins.[69–72]

In a yeast cell-free system, the poly(A) tail, like the cap structure, was able to support the recruitment of the 40S ribosomal subunit by itself to an uncapped mRNA.[73] This function of the poly(A) tail as well as the functional synergy with the cap structure requires the poly(A)-binding protein (PABP)[73] and its interaction with the N-terminal part of eIF4G[74,75] (Fig. 3). This interaction between the N terminus of eIF4G and PABP has been reported to induce an increase of the affinity of the cap-binding protein eIF4E for the cap structure[76] (see also below). In some in vitro systems, investigators have also seen stimulated translation of capped mRNAs when poly(A) was added in *trans*, raising the possibility that there may not be a mandatory requirement for the poly(A)-PABP-eIF4G-eIF4E-cap interaction to occur in *cis*,[66,77] although this phenomenon seems difficult to place into the physiological context of cellular mRNA translation. On balance, these observations suggest a model where the mRNA adopts a pseudocircular conformation during translation through simultaneous binding of eIF4E and PABP to eIF4G (Figure 1, for recent reviews see the chapter by AB Sachs in Ref. 1, or Ref. 78). This model could also explain earlier observations of circular polyribosomes[79] and has been further substantiated by functional data[80] and direct visualisation by atomic force microscopy. Adding eIF4E, eIF4G, PABP, and a capped and polyadenylated mRNA together in vitro resulted in the formation of pseudocircular complexes.[81]

PABP and eIF4G also interact in plant[82] and mammalian cells.[83,84] Interestingly, there is no apparent sequence homology between the PABP-binding regions of yeast and mammalian eIF4G, despite the evolutionary conservation of the eIF4G–PABP interaction. Mammalian cells have evolved additional PABP-interacting proteins, termed Paip1 and Paip2, with apparent roles in translation (Fig. 3). Paip1 exhibits homology to the central third of eIF4G.[85] It forms complexes of 1:1 stochiometry with PABP,[86] interacts with eIF4A, and was reported to co-activate cap-dependent translation—despite having no eIF4E binding motif.[85] By contrast, Paip2 is a small acidic protein that acts as a translational repressor, with a preferential effect on the translation of polyadenylated mRNAs.[87] Two molecules of Paip2 can simultaneously bind to PABP.[88] It reduces the affinity of PABP for oligo(A) and disrupts the periodicity with which multiple PABP molecules bind to poly(A). Furthermore, Paip1 and Paip2 compete with each other for binding to PABP (Fig. 4A).[87]

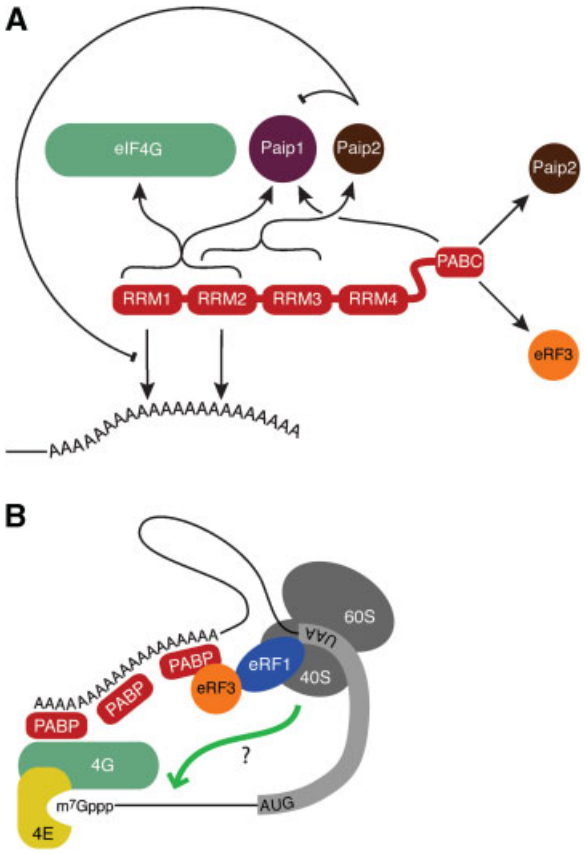


Figure 4. The role of PABP and the poly(A) tail of mRNAs during translation. **A:** PABP consists of four conserved RRMs, and a PABC domain at the C terminus. The arrows indicate interactions with various binding partners as discussed in the main text. eIF4G and poly(A) can bind simultaneously to RRM1 and RRM2, while the available evidence suggests that only one partner can bind to the PABC at any given time. Paip2 can interact with RRM2 and RRM3 of PABP as well as with the PABC domain and forms complexes of 2:1 stochiometry with PABP. Paip2 binding to RRM2 and RRM3 of PABP can disrupt the binding of PABP to poly(A) and inhibit translation. The inhibitory effects of Paip2 are indicated by lines with a short bar at the end. Paip1 interacts with RRM1 and RRM2 as well as with the PABC domain in complexes of 1:1 stochiometry. **B:** The ribosome-recycling concept. A possible function of mRNA circularization could involve facilitation of a direct recycling of ribosomes or ribosomal subunits, after termination at the stop codon, back to the 5′ region of the same mRNA (indicated by the green arrow). This speculative model is supported by the observation of circular polyribosomes, as well as interactions between PABP, initiation factors bound to the cap structure, and the translation termination factor eRF3.

All known PABP sequences reveal a conserved domain organisation.[89] The N-terminal part of PABP contains four highly conserved RRMs, joined together by conserved linker sequences. The proline-rich C terminus exhibits another conserved domain, termed PABC (i.e., PABP C-terminal domain), for additional protein–protein contacts (Fig. 4A). RRM1 and RRM2 of PABP together bind poly(A) and also interact with eIF4G.[14,89,90] X-ray crystallography of the PABP RRM1 and RRM2 fragment bound to poly(A) show that single-stranded poly(A) interacts with the RNA-binding motifs of both RRMs. The surface of the PABP-fragment facing away from the RNA forms a phylogenetically conserved region, which probably contacts eIF4G.[89] This hypothesis is further supported by targeted mutational studies of conserved amino acids in this region.[91] Structural data is also available for the PABC domain.[92,93] This conserved sequence of 74 amino acids in length consists of five α-helices arranged in the shape of an arrow and binds specifically through a hydrophobic region to an approximately 12 amino acid peptide motif present in a number of confirmed interaction partners. These include Paip1 and Paip2, and interestingly also the eukaryotic release factor (eRF) 3 (Fig. 4, see below).[94] In the case of Paip2, this PABC-interacting motif (or PAM2, for PABP-interacting motif 2), resides in the CTD. In addition to that, Paip2 exhibits a second PABP-binding site termed PAM1 in its central region, which interacts specifically with a region encompassing RRM2 and RRM3 of PABP (Figs 3, 4). The latter interaction displays the higher affinity and is sufficient to promote the characteristic disruption of the PABP-poly(A) complex, as well as repress translation in a poly(A)-responsive in vitro system.[87] Paip1 also has functional PAM1 (in the CTD) and PAM2 motifs (in the NTD). Paip1/PAM-1 interacts with the PABC domain and Paip1/PAM2 binds to RRM1 and RRM2 of PABP (Fig. 3).

Collectively, this leads to the following working model for the function of the PABP–poly(A) complex in translation initiation (Fig. 4): the two N-terminal RRMs of PABP are responsible for binding to poly(A) and for contacting the 5′ end of the mRNA through binding of eIF4G. This stimulates 43S complex recruitment to the mRNA and leaves the PABC region available to provide further protein–protein contacts, which may also serve translational purposes (i.e., eRF3, see below) or affect other aspects of mRNA metabolism. In mammalian cells, Paip1 and Paip2 will very likely compete with other interaction partners for binding to the PABC domain. With regard to interactions with the RRM region of PABP, we know that Paip2 antagonises poly(A) binding. It remains to be seen whether Paip1, eIF4G and poly(A) can simultaneously interact with the RRM1 and RRM2 region of PABP. Potential advantages of a pseudocircular structure of mRNAs are easily recognised (summarised by AB Sachs in Ref. 1). The error rate of translation could be reduced since only intact mRNAs act as efficient templates. A well-characterised mRNA decay pathway starts with a deadenylation step, followed by decapping and exonucleolytic degradation of the mRNA body (see chapter by DC Schwartz and R Parker in Ref. 1). The association of both mRNA ends with the translation machinery may therefore stabilize the mRNA. In addition to this protective

effect, the spatial proximity could also have a direct positive effect on translation. Ribosomes may not dissociate away from the mRNA after termination but instead initiate a new round of translation at the 5′ end of the same mRNA molecule. So far, there is no direct published evidence for this attractive concept of ribosome recycling. It is tantalising to speculate that the documented interaction between eRF3 and PABP serves to loop out the 3′UTR of mRNAs and bring terminating ribosomes into the vicinity of the cap structure (Fig. 4B).[94] In yeast, the synergistic stimulatory effect of the cap structure and the poly(A) tail originates at least in part from a competition for limiting components of the translation machinery.[80,95] Such competition effects could arise at the level of the first or the subsequent rounds of translation. Multiple aspects of co-operative binding involving the cap–eIF4E–eIF4G–PABP–poly(A) assembly[76,82,96–99] suggests that the observed synergistic effects occur at least in part on the level of initial recruitment of the mRNA.

Conclusions

Translation initiation is a dynamic, stepwise process. It is critical in determining qualitatively and quantitatively which proteins are made, when and where. While most components of the initiation machinery are now likely to be known, the biochemical details of their assembly are only incompletely understood. The 3′end of the mRNA (3′ UTR and poly(A) tail) plays an unexpectedly important role in this process. Important areas for future work include a better understanding of the 'scanning' process, of 'internal ribosome entry' and of 'recycling' of ribosomes for multiple rounds of translation on the same mRNA.

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