

# **eIF-Three to Tango: Emerging Functions of Translation Initiation Factor eIF3 in Protein Synthesis and Disease**

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## Abstract

Studies over the past three years have substantially expanded the involvements of eIF3 in mRNA translation. It now appears that this multi-subunit complex is involved in every possible form of mRNA translation, controlling every step of protein synthesis from initiation to elongation, termination and quality control in positive as well as negative fashion. Through the study of eIF3, we are beginning to appreciate protein synthesis as a highly integrated process coordinating protein production with protein folding, subcellular targeting, and degradation. At the same time, eIF3 subunits appear to have specific functions that probably vary between different tissues and individual cells. Considering the broad functions of eIF3 in protein homeostasis, it comes as little surprise that eIF3 is increasingly implicated in major human diseases and first attempts at therapeutically targeting eIF3 have been undertaken. Much remains to be learned, however, about subunit- and tissue-specific functions of eIF3 in protein synthesis and disease and their regulation by environmental conditions and posttranslational modifications.

Keywords: eIF3, mRNA translation, ribosome, methyladenosine

## Introduction

Protein synthesis is a complex process accomplished by the ribosome and its associated factors that encompasses several highly coordinated steps, including the initiation of mRNA translation, translation elongation and termination as well as folding, targeting, and quality control of the nascent polypeptide. The canonical reactions of mRNA m7G cap-dependent translation have been studied for decades and have been fully reconstituted from purified components in vitro (see (Sonenberg and Hinnebusch, 2009) for review). In canonical initiation, mRNA is recruited to the ribosome through eIF4E-mediated cap binding. eIF4E interacts with eIF4G and eIF3 to attract the 43S pre-initiation complex consisting of the 40S ribosome loaded with methionyl-tRNA and additional eIFs. The 40S ribosome then scans the mRNA from its 5'-end to identify the start codon. Upon start codon recognition, the 60S ribosomal subunit joins to produce an actively translating 80S ribosome, whereas all eIFs are thought to be ejected.

Although all eukaryotic mRNAs possess a m7G cap, disabling the cap binding protein eIF4E through various strategies has surprisingly little impact on mRNA translation, affecting as few as ~200 mRNAs (Hsieh et al., 2012; Thoreen et al., 2012; Yanagiya et al., 2012; Morita et al., 2013; Truitt et al., 2015). This suggests the existence of additional cap binding proteins as well as cap-independent means of initiating translation. These alternative pathways might recruit ribosomes to mRNA that would then scan from the 5'-end for a start codon essentially in the same way as established for canonical eIF4E-dependent initiation (Shatsky et al., 2018). Recent evidence has implicated the multi-subunit eIF3 complex in several non-canonical modes of initiation that are discussed in this review.

eIF3 function is particularly well studied in the budding yeast, *S. cerevisiae*. Whereas the core functions of eIF3 in scanning and AUG recognition are well conserved between budding yeast and higher eukaryotes, *S. cerevisiae* eIF3 with six subunits (eIF3a, b, c, g, i, j) is considerably smaller than the 13-subunit mammalian complex, and thus misses several

components, including eIF3d, eIF3l and eIF3h, which have recently been attributed crucial functions.

Current reviews have provided comprehensive summaries of the roles of eIF3 in canonical translation initiation, including scanning, start codon recognition, and termination uniquely informed by structural insights obtained by X-ray crystallography and cryo-electron microscopy (Cate, 2017; Hinnebusch, 2017; Valášek et al., 2017). In addition, the sometimes conflicting involvements of eIF3 subunits in human cancer have been reviewed recently (e.g. (Hershey, 2015; Sesen et al., 2017; Gomes-Duarte et al., 2018)). In the present article, we are focusing on recent progress regarding canonical and non-canonical roles of eIF3 in translation initiation, elongation, and repression, and describe novel findings implicating eIF3 in diseases other than cancer.

## **New non-canonical mechanisms of translation initiation involving eIF3**

### *Methyladenosine modification as an eIF3 recruiting mRNA modification*

While all mRNAs carry a m7G cap, several thousand mRNAs are also modified by N6 methylation of adenosines (m6A). Dynamic m6A modification is enriched at the stop codon and the 3'-UTRs but also occurs in other regions of the mRNA, including the 5'-UTRs (Tong et al., 2018). m6A is known to broadly impact on mRNA metabolism, including splicing, nuclear export, translation, and mRNA decay. With respect to translation, it was shown that m6A can recruit eIF3 to induce 48S initiation complex formation independent of eIF4E cap binding (Meyer et al., 2015). Direct binding of eIF3 to m6A appears to occur within 5'-UTRs, especially under heat shock conditions when cap-dependent initiation is blocked and m6A relocates from 3'- to 5'-UTRs. Importantly, while cap-independent, the eIF3-m6A mediated initiation mechanism does

not operate as a cellular counterpart to viral internal ribosome entry sites (IRES), because, unlike viral IRESs, it requires an accessible mRNA 5'-end.

The m6A modification is added to mRNA by a family of methyltransferases (e.g. METTL3 “writers”), interpreted by a family of m6A binding YTH domain proteins (e.g. YTHDF1, 2, 3, “readers”), and removed by several demethylases (e.g. FTO; “erasers”). Readers of the YTHDF group have been implicated in m6A-dependent translation initiation. YTHDF1 was found to bind ~3,600 m6A-containing mRNAs and increase their ribosome occupancy (Wang et al., 2015b). In addition, artificial, m6A-independent, tethering of YTHDF1 to 3'-UTRs stimulated translation of a luciferase encoding reporter mRNA. Mechanistically, YTHDF1 was reported to bind to eIF3 in an RNA-independent and possibly direct manner, although it remains formally unproven that YTHDF1-mediated translation depends on interaction with eIF3. Unlike with direct eIF3-m6A binding, eIF3 recruitment via YTHDF1 is thought to occur through the m6A modification within the 3'-UTR. In turn, interaction of eIF3 with the 40S ribosome scanning along the 5'-UTR would lead to formation of the closed mRNA loop known to promote translation (Tarun et al., 1997).

Interestingly, while sharing ~50% of mRNA targets, YTHDF1 and its related YTHDF2 impart differential control. Whereas initial YTHDF1 binding promotes mRNA translation, subsequent YTHDF2 binding triggers mRNA decay (Wang et al., 2015b), thus suggesting a temporally ordered scheme of control of gene expression by m6A reader proteins. Under heat stress conditions, YTHDF2 was shown to play a different role, promoting the translation of HSP70 and possibly other heat-induced transcripts depending on m6A modification localized to the 5'-UTR, although this function was not linked to eIF3 (Zhou et al., 2015). Rather, YTHDF2, in this context, is thought to protect m6A modified mRNA from demethylation thus promoting cap-independent translation, possibly through direct recruitment of eIF3 to m6A as described above (Meyer et al., 2015).

Another m6A reader, YTHDF3, promotes translation apparently by serving as a specificity determinant of YTHDF1 interacting with m6A in the 3'-UTR (Shi et al., 2017). Whereas YTHDF3 does not interact with eIF3 directly, it appears to join the YTHDF1-eIF3 complex through binding to YTHDF1. On the other hand, YTHDF3 binding to m6A, apparently in a YTHDF1-independent fashion, was shown to mediate the translation of circular mRNAs (Yang et al., 2017). In this context, YTHDF3 functions in association with eIF4G2 and eIF3. Another factor that has recently been implicated in initiation mediated by m6A via the 5'-UTR is ABCF1, although its possible relationship to YTHDF readers and eIF3 remains unknown (Coots et al., 2017).

In addition to m6A readers, the m6A writer METTL3 has been implicated in translation initiation through eIF3. METTL3 binding to m6A modified 3'-UTRs reinforces the closed loop structure mediated by eIF4G-PABP interactions. This occurs through direct binding of METTL3 to eIF3h. Notably, this is independent of the catalytic methyltransferase activity of METTL3 and does not require YTH domain readers (Lin et al., 2016). The METTL3-eIF3 mechanism augments the translation of thousands of mRNAs, a subset of which is involved in tumor progression and apoptosis (Choe et al., 2018). Consequently, knockdown of METTL3 inhibits cell migration and tumor growth in mice, whereas overexpression of METTL3 can transform mouse fibroblasts. The newly discovered role of METTL3 in boosting translation through eIF3h potentially complicates the conclusions drawn from previous studies using METTL3 knockdown to experimentally manipulate m6A levels without awareness that METTL3 has an additional function (Meyer et al., 2015; Wang et al., 2015b; Coots et al., 2017).

#### *Direct cap binding of eIF3 subunits*

Using PAR-CLIP, eIF3 was shown to bind to the 5'-UTRs of hundreds of mRNAs with many of the binding sites overlapping with m6A modified sites (Lee et al., 2015; Meyer et al., 2015). Although only two of these binding sites, located in the c-JUN and BTG1 transcripts, have been

studied in detail, they can either activate or suppress translation (Lee et al., 2015). The stimulatory motif in the c-JUN 5'-UTR functions independent of the eIF4F but nevertheless requires the m7G cap. These disparate observations were reconciled when it was found that eIF3 subunit d has a cryptic cap binding activity that becomes activated upon binding to the structured motif within the c-JUN 5'-UTR (Lee et al., 2016). It thus appears that dual binding of eIF3 to the 5'-cap and the structured motif recruits the 40S ribosome to activate translation. However, whether eIF3 controls the synthesis of endogenous c-JUN protein through this mechanism remains to be demonstrated.

Another subunit of eIF3, eIF3l, was also shown to have cap binding activity (Kumar et al., 2016). Since eIF3l and eIF3k are not required for 48S complex formation in vitro, the function of eIF3l's cap binding activity is currently unknown.

#### *eIF3-PABP interaction in IRES-mediated initiation*

eIF3 was also implicated in translation initiation at the internal ribosome entry site (IRES) of the XIAP mRNA (Thakor et al., 2017). eIF3 interacts directly with the XIAP IRES in a fashion that is facilitated by poly-A binding protein (PABP) and results in 40S recruitment. The eIF3-PABP interaction is thought to promote closed loop formation. Since eIF4A and eIF2 $\alpha$  are not required, XIAP IRES initiation is proposed to be driven by direct eIF3-mediated positioning of the ribosome in the vicinity of the start codon, similar to what is observed for several viral IRESs.

### **Common themes emerging**

The above studies have substantially diversified the function of eIF3 in translation. It now appears that eIF3 is a central mediator of translation under both normal and stress conditions. Several common themes have emerged from these studies:

#### *m6A exerts distinct location-dependent modes of regulation*

m6A modification located in the 5'-UTR is stress-inducible and mediates cap-independent translation under stress conditions through direct recruitment of eIF3 to a limited set of stress response mRNAs (Meyer et al., 2015; Coots et al., 2017). In contrast, m6A modification located in the 3'-UTR appears to be constitutive and to provide a general boost to cap-dependent translation by promoting closed loop formation via interaction of eIF3 with m6A reader and writer proteins (Wang et al., 2015b; Lin et al., 2016; Shi et al., 2017; Choe et al., 2018).

#### *eIF3 is involved in IRES and non-IRES mediated initiation*

Cap-independent initiation through binding of eIF3 to m6A located in the 5'-UTR was shown to require 5'-end dependent scanning and was thus classified as non-IRES-mediated (Meyer et al., 2015; Coots et al., 2017). In contrast, m6A-YTHDF3-eIF3 mediated translation of circular RNA is by definition 5'-end independent and thus IRES-like (Yang et al., 2017). Likewise, initiation mediated by eIF3 cap binding or eIF3-PABP interactions proceeds independently of eIF4A and is thus scanning-independent (Lee et al., 2016; Thakor et al., 2017).

#### *eIF3 mediates translation under stress conditions*

Cap-independent initiation mediated by eIF3 may be particularly useful in stress conditions when canonical cap-dependent initiation is blocked. For example, amino acid starvation inactivates eIF4E. More generally, however, stress conditions such as heat shock and endoplasmic reticulum (ER) stress lead to inactivation of eIF2. During chronic ER stress with low eIF2 activity, translation initiation switches to an eIF3-dependent but eIF2-independent mechanism (Guan et al., 2017). However, translation of heat shock protein 70 (*HSP70*, *HSPA1A*) mRNA mediated by direct binding of eIF3 to m6A in the 5' UTR requires eIF2 (Meyer et al., 2015). In addition, binding of eIF2 to *HSP70* (*HSPA1A*) mRNA is increased by heat stress (Coots et al., 2017). Thus, despite reduced availability of eIF2 for ternary complex formation under heat stress, residual eIF2 appears to be preferentially reserved for delivering methionyl-



tRNA for m6A-mediated translation, at least in the case of *HSPA1A* mRNA. Whether other cap-independent mRNAs also use eIF2 during stress conditions or whether other factors deliver methionyl-tRNA – for example eIF5B (Guan et al., 2017) - remains to be established.

### **eIF3 and translational repression**

Whereas the eIF3 holo-complex typically activates mRNA translation in a reconstituted in vitro system, it was shown to act as a repressor in some instances. eIF3 PAR-CLIP studies identified a structured motif in the mRNA encoding BTG1 as a negative regulatory element (Lee et al., 2015). While eIF3 does not appear to bind this element directly, removal of the motif strongly de-represses the translation of luciferase reporters in vitro and in vivo. However, it remains unclear whether disabling eIF3 function will de-repress the synthesis of BTG1 protein in vivo and whether this is mediated through the structured eIF3 binding motif.

eIF3 was also described as a repressor of *nanos1* mRNA in developing *Xenopus* embryos (Aguero et al., 2017). In fertilized embryos where Nanos1 protein is synthesized, the repression is relieved by accumulation of the Dnd1 helicase and its binding to eIF3f. Dnd1 appears to interact with eIF3f to neutralize the repressive effect of holo-eIF3. Consistent with this idea and previous reports (Shi et al., 2006), ectopic eIF3f inhibits *nanos1* translation in vitro and in vivo. However, these dominant effects contrast with clear inhibition of protein synthesis observed upon knockdown of eIF3f in human HeLa cells (Wagner et al., 2016) and the dramatic effects of eIF3f deletion on protein synthesis and viability in the fission yeast *S. pombe* (Zhou et al., 2005).

Despite these uncertainties, many global datasets have documented positive as well as negative effects of individual eIF3 subunits on mRNA translation (Kim et al., 2007; Grzmil et al., 2010; Shah et al., 2016). It is thus likely that intricate subunit-selective mechanisms regulate eIF3 function in stimulation as well as repression of mRNA translation.

### **eIF3 in translation elongation and quality control**

Based on in vitro studies, it has long been believed that binding of eIF3 to the 40S inhibits 60S subunit joining and that eIF3 is therefore ejected from the 40S prior to subunit joining (Peterson et al., 1979; Trachsel and Staehelin, 1979). However, proteomic evidence has accumulated supporting the idea that eIF3 can form stable interactions with 80S ribosomes and translation elongation factors in various organisms (Guerrero et al., 2008; Sha et al., 2009; Reading et al., 2013; Wang et al., 2015a). eIF3-80S complexes also contain additional factors involved in protein quality control, including chaperones and the 26S proteasome to form a particle dubbed the “translasome” (Sha et al., 2009).

Recent cryo-EM structures of the 48S complex are consistent with the possibility that eIF3 remains bound to the ribosome after subunit joining (Simonetti et al., 2016; Eliseev et al., 2018). Whereas eIF3 is bound to the solvent exposed side of the 40S ribosome within the 43S pre-initiation complex, the eIF3b-eIF3g-eIF3i module appears to transiently move to the inter-subunit side during scanning only to move back to the solvent exposed side upon AUG recognition. Thus, there is no obvious steric hindrance that would prevent eIF3 to remain attached to the 80S ribosome.

Clear evidence that eIF3 remains associated with the 80S ribosome at least during translation of the first 5 – 10 codons has been provided recently. This was demonstrated for translation of upstream open reading frames (uORFs) of the budding yeast *GCN4* mRNA (Mohammad et al., 2017). The data showed that eIF3 remains associated with 80S ribosomes during translation of uORFs and that this facilitates reinitiation at downstream uORFs. Whether eIF3 remains associated with 80S ribosomes independently of uORF translation remains to be explored. The possibility was invoked that eIF3-80S ribosome interactions may mediate early translational slowdown thus giving rise to the “5'-ramp” of ribosome density widely observed within the first ~100 codons of *Saccharomyces cerevisiae* mRNAs (Weinberg et al., 2016). However, the 5'-ramp is not typically observed in studies of human cells (Ingolia et al., 2011;

Shalgi et al., 2013), and the wider applicability of this proposed function of eIF3-80S interactions to eukaryotic gene expression remains questionable.

In the context of the translasome, eIF3-80S ribosome interactions may have to be considered more broadly with respect to the fate of the nascent chains emerging from translating ribosomes. Genetic loss-of-function screening has firmly implicated eIF3 in protein quality control (Pegoraro et al., 2012). Given the important role of early elongation speed in determining the fate of nascent membrane proteins (Kramer et al., 2009; Acosta-Sampson et al., 2017), it is not inconceivable that eIF3's potential role in controlling elongation is linked to its role in protein quality control. These connections invoke a scenario in which eIF3 and its associated proteins cooperate in an integrated fashion to orchestrate the folding and targeting of nascent chains to appropriate locales. If proper folding/targeting fails, eIF3's association with the proteasome and ubiquitylating enzymes may trigger ubiquitin-mediated co-translational degradation (Turner, 2000; Medicherla and Goldberg, 2008; Duttler et al., 2013; Wang et al., 2013).

### **eIF3 in diseases: cancer and beyond**

With respect to the anabolic nature of cancer cell metabolism, mechanisms controlling protein synthesis have been at the center of attention for many years. The cancer promoting activity of eIF4E which acts downstream of mTORC1 signaling has been well documented (Siddiqui and Sonenberg, 2015). Likewise, eIF3 has been placed at the cross-roads of mTOR and S6K signaling (Holz et al., 2005). Since then, many subunits of eIF3 have been implicated in cancer in various positive as well as negative ways as discussed in detail by Hershey (Hershey, 2015). In addition, eIF3 was shown to control the translation of proliferation-related mRNAs (Lee et al., 2015). Nevertheless, no unified mechanism has arisen as to the oncogenic or tumor suppressive roles of eIF3. These roles are likely multifaceted with different subunits playing distinct roles in different tissues. Although a preponderance of evidence suggests a positive role

of eIF3 in cancer, rigorous genetic evidence for tissue-specific oncogenic or tumor suppressive functions of individual eIF3 subunits remains outstanding. Conditional eIF3 mutant mice are beginning to be established that will enable such testing (Zeng et al., 2013; Sadato et al., 2018).

A recent study has suggested a possible involvement of eIF3 in cystic fibrosis. Integration of genetic and proteomic screening data implicated eIF3a as well as some other eIF3 subunits in the synthesis and folding of the cystic fibrosis transmembrane conductance regulator (CFTR) (Hutt et al., 2018). Counterintuitively, knockdown of eIF3a which probably caused partial impairment of the eIF3 holo-complex, led to increased production of functional CFTR containing disease causing mutations. The study proposed that the functional rescue of mutant CFTR by eIF3 attenuation is due to decreased translation initiation thus reducing the load of nascent peptides. This in turn would free chaperone capacity for the folding of mutant CFTR. However, the study does not provide data sufficient to rule out alternative scenarios consistent with previous protein interaction studies according to which knockdown of eIF3 function would slow down translation elongation and prevent the recruitment of the proteasome to the ribosome (see above paragraphs). Both events may promote the expression of mutant CFTR: Reduced translation elongation rate may provide additional time for folding during synthesis, whereas compromised proteasome recruitment may decelerate co-translational degradation of slowly folding mutant protein. Like the proteasome inhibitor MG132, knockdown of eIF3a strongly inhibits the proteasomal degradation of mutant CFTR (Hutt et al., 2018). In fact, eIF3a knockdown is considerably more efficient than MG132, consistent with the above notion that impairment of eIF3 may promote CFTR expression in multiple proteasome-dependent and independent ways. Regardless, these recent findings nominated eIF3 as a potential drug target in cystic fibrosis.

## Exploring eIF3 as a therapeutic target

Targeting of eIF3 with small molecules and other approaches is in its infancy. Both in cancer and cystic fibrosis, inhibition of eIF3 function would appear desirable. However, eIF3 does not have any intrinsic enzymatic activities amenable to inhibitor development. A screen for chemicals able to disrupt the interaction of the recombinant eIF3 octameric core complex with the hepatitis C virus (HCV) IRES identified two crude extracts isolated from marine actinobacteria (Zhu et al., 2017). The fractions also modestly inhibited cap-dependent and IRES-mediated translation in vitro as assessed by reporter assay. Assuming a molecular mass of 400 for a single active ingredient present in the the crude fractions, inhibition of the eIF3-HCV IRES interaction in an electrophoretic mobility shift assay requires ~100 uM compound. The potency of these reagents is thus limited, in addition to their molecular structure remaining unknown. Nevertheless, these studies provide initial proof-of-concept for small molecule targeting of eIF3. Interestingly, the same study also identified compound HP-3 as a promoter of the eIF3-HCV IRES interaction and a stimulator of IRES-selective translation in vitro and in vivo. Mechanistically, HP-3 was shown to bind to the HCV IRES and alter its structure, apparently in ways that facilitate 40S recruitment. Nevertheless, the utility of a compound stimulating HCV IRES activity remains presently unclear.

Based on the findings that eIF3f inhibits protein synthesis and is downregulated in multiple cancers, including pancreatic adenocarcinoma, melanoma, and breast cancer among others, cell penetrating eIF3f protein was studied for potential anti-cancer activity. Nanomolar doses of either full-length eIF3f or a truncation mutant encompassing the MPN domain were effectively internalized by cells and induced apoptosis, apparently in a cancer cell selective fashion (Marchione et al., 2015). Whereas the mechanism of induction of apoptosis by eIF3f remains uncharacterized and the protein delivery approach meets with several practical challenges, an eIF3f-based protein drug may potentially be developed in the future. Once again,

these studies provide early proof-of-concept for potentially targeting eIF3 function through biologics.

### **Concluding remarks**

Whereas the findings summarized above have implicated eIF3 in all known steps of protein synthesis, targeting and quality control (Figure 1), much remains to be learned. For one, we still know very little about the biochemistry of the eIF3-m6A interaction. Which subunit(s) of eIF3 bind m6A and YTH domain reader proteins? Likewise, there is very little mechanistic understanding of how eIF3 inhibits the translation of certain mRNAs. In addition, it is unknown whether eIF3 has a role in translation elongation beyond uORF translation in select yeast mRNAs. If eIF3 has a more general role in translation elongation, is this role confined to certain regions of the mRNA (such as the 5' ramp, for example) or does it extend along the entire mRNA length? Furthermore, what is the structure of the apparent eIF3-80S complex? Many questions also remain regarding the potential oncogenic or tumor suppressive functions of eIF3 and its individual subunits. Are these linked to mRNA-selective translation or to unexplored moonlighting functions of eIF3 subunits outside of holo-eIF3? Likewise, several eIF3 subunits were shown to localize to the nucleus, but their potential nuclear functions remain entirely elusive. Considering these important unresolved questions, eIF3 promises to remain an exciting multifunctional protein complex with many more unexpected roles in cellular physiology and pathophysiology ready to be unearthed in years to come.

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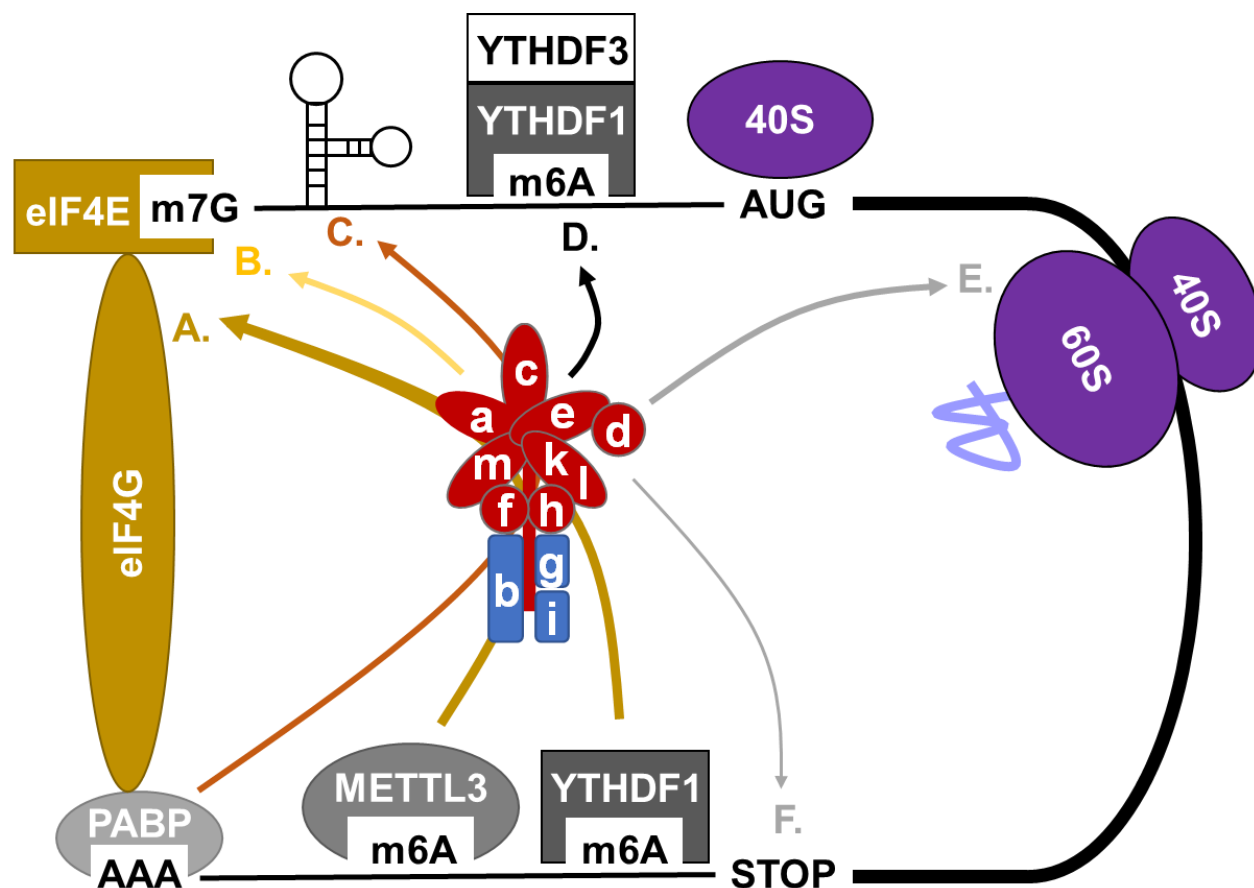
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**Figure 1 Multiple roles of eIF3 in canonical and non-canonical translation**

- A. Promotion of closed loop formation through 3'-UTR m6A binding of writer and reader proteins and eIF3 recruitment.
- B. Direct cap and stem loop binding of eIF3 via eIF3d.
- C. Promotion of closed loop formation through PABP facilitated recruitment of eIF3 to cellular IRES elements
- D. Direct or YTH reader-mediated recruitment of eIF3 to 5'-UTR m6A sites to promote cap-independent but scanning-dependent initiation.
- E. Potential role of eIF3 in translation elongation through direct interaction with translating 80S ribosomes.
- F. Role of eIF3 in translation termination/ribosome recycling (not discussed here).