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The Many Roles of the Eukaryotic Elongation Factor 1 Complex

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Abstract

The vast majority of proteins are believed to have one specific function. Throughout the course of evolution, however, some proteins have acquired additional functions to meet the demands of a complex cellular milieu. In some cases changes in RNA or protein processing allow the cell to make the most of what is already encoded in the genome to produce slightly different forms. The eukaryotic Elongation Factor 1 (eEF1) complex subunits, however, have acquired such moonlighting functions without alternative forms. Here, we discuss the canonical functions of the components of the eEF1 complex in translation elongation as well as the secondary interactions they have with other cellular factors outside of the translational apparatus. The eEF1 complex itself changes in composition as the complexity of eukaryotic organisms increases. Members of the complex are also subject to phosphorylation, a potential modulator of both canonical and non-canonical functions. Although alternative functions of the eEF1A subunit have been widely reported, recent studies are shedding light on additional functions of the eEF1B subunits. A thorough understanding of these alternate functions of eEF1 is essential for appreciating their biological relevance.

Keywords

translation; elongation; actin; virus; phosphorylation

Introduction

The process of eukaryotic protein synthesis is traditionally defined as occurring in three phases: initiation, elongation and termination. Each phase requires the action of not only the ribosome, mRNA and aminoacylated-tRNAs (aa-tRNA) but also a series of soluble protein factors that facilitate each step. Once translation is initiated at the first codon by the assembled 80S ribosome, it continues onto the elongation phase wherein the peptide chain increases its length cyclically one amino acid at a time. The aa-tRNA delivery step is

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catalyzed by the eukaryotic Elongation Factor 1 (eEF1) complex. In bacteria two factors, EF-Tu and EF-Ts, are involved in making the appropriate aa-tRNA available to the elongating ribosome. The eukaryotic Elongation Factor 2 (eEF2, the homolog of bacterial EF-G) acts as the translocase, allowing the ribosome to move down the mRNA one codon at a time. Peptide elongation continues until the ribosome reaches the stop codon at which point termination is triggered by the release factors. Aside from their function in translation elongation, components of the eEF1 complex have been implicated in a wide variety of cellular and viral processes. In this review, we present data from a number of laboratories in support of the secondary functions of the eEF1 subunits and consider their biological significance.

THE eEF1 COMPLEX

The search for eukaryotic counterparts involved in the first step of translation elongation has led to the isolation of various multi-protein macromolecular complexes in different species. For the sake of clarity and functional consistency across different kingdoms, we are adopting the modified nomenclature as proposed by Le Sourd et al (Table 1) to describe the various players involved in the first step of translational elongation.¹

Canonical Function of eEF1 in Translation Elongation—eEF1A in its GTP-bound form binds and delivers aa-tRNAs to the A site of the ribosome.² Upon formation of a correct codon-anticodon pair, a conformational change in the ribosome leads to GTP hydrolysis and release of eEF1A•GDP.³ Since the spontaneous rate of GDP dissociation from eEF1A is very slow with an equilibrium dissociation constant of 10×10^{-7} M, GDP must be actively exchanged for GTP by the guanine nucleotide exchange factor (GEF) eEF1B in order for eEF1A to participate in another round of elongation.⁴ In the yeast *Saccharomyces cerevisiae*, one of the simplest eukaryotes, the eEF1B complex is made up of two subunits: eEF1B α and eEF1B γ . Early work by Moller and colleagues indicated that the C-terminal domain of eEF1B α was involved in eEF1A binding and the nucleotide exchange activity.⁵ This observation was later confirmed by the co-crystal structure of the yeast complex.⁶ The N-terminal domain of eEF1B α forms a strong binding interface with the eukaryote specific structural protein eEF1B γ .⁷ Unlike eEF1A and eEF1B α , eEF1B γ is not essential for viability in yeast.⁷ It has been shown to bind to the endoplasmic membrane and is thought to have an anchoring role, localizing the eEF1B complex to the endoplasmic reticulum (ER), a major site of protein synthesis.⁸

In higher eukaryotes, the eEF1B complex takes on a third factor that has its own GEF activity, called eEF1B β in plants and eEF1B δ in metazoans. The C-terminal domain of eEF1B β and eEF1B δ is highly similar to the corresponding region of eEF1B α and accounts for its nucleotide exchange activity.¹ Metazoan eEF1B has often been isolated as a complex bound to valyl tRNA synthetase (ValRS) via the metazoan-specific 200 amino acid N-terminal extension on ValRS.⁹ As tRNA aminoacylation precedes tRNA binding to eEF1A, the presence of ValRS in complex with eEF1B supports the channeling hypothesis according to which translation in eukaryotes is streamlined by coupling the charging of tRNA to its delivery to the ribosome.¹⁰ eEF1B δ seems to be present primarily as two isoforms, eEF1B δ 1 and eEF1B δ 2, both of which can bind to eEF1B α • γ complex.

Phosphorylation of the eEF1 Complex—Studies on the regulation of translation by phosphorylation have traditionally focused on the initiation step. There is, however, growing evidence that the elongation step is also regulated to achieve a more robust control of the cellular translational machinery in response to environmental cues. Seminal work, particularly by the Traugh laboratory, demonstrated that the subunits of the eEF1 complex are phosphorylated by a number of serine/threonine protein kinases (Table 2). Protein kinase

C (PKC), a key stimulator of cell proliferation and differentiation, has been shown to upregulate both transcription and translation rates via its kinase activity. PKC phosphorylates eEF1A, eEF1B α , eEF1B δ as well as ValRS causing a two fold increase in the GDP/GTP exchange activity.¹¹ This correlates well with the reported two- to three- fold stimulation of elongation activity in response to PKC activation.¹² Insulin also causes a rapid upregulation of protein synthesis.¹³ Studies using serum deprived 3T3-L1 cells showed that insulin stimulates phosphorylation of all the components of the eEF1 complex, possibly through S6 kinase. Thus there is evidence from multiple systems implicating phosphorylation of the eEF1 complex in increased translation rates.

While most of the studies on eEF1 suggest a stimulatory role for phosphorylation, there are reports that phosphorylation by certain kinases may result in translational repression. eEF1A was identified in a screen as a phosphorylation substrate for type I transforming growth factor β receptor (T β R-I).¹⁴ T β R-I was shown to phosphorylate eEF1A1 at Ser300 *in vitro* and *in vivo*. Mutation of this residue significantly decreased the amount of phosphorylated eEF1A1 *in vitro*, while a phosphomimetic substitution resulted in inhibition of cell proliferation. As Ser300 is located near the region of eEF1A proposed to interact with the 3'-end of the aa-tRNA, its phosphorylation could disrupt the interacting surface for the aa-tRNA eventually leading to a reduction in translation and proliferation.¹⁴ Although, phosphopeptide mapping of eEF1A has revealed phosphorylation on at least five different sites,¹³ the exact residues that enhance eEF1A activity when phosphorylated have not been determined. Future experiments should address this issue so that a direct comparison of the similarities or differences among the phosphorylation sites that affect eEF1A function can be made. It has also been reported that casein kinase II phosphorylates both eEF1B α and eEF1B δ without an associated effect on eEF1A activity as measured *in vitro*.¹⁵ This phosphorylation event might be required to mediate unknown protein-protein interactions or changes in the distribution of eEF1 complex within the cell. It has also been reported that the C-terminus of eEF1B γ contains a conserved LAMMER kinase phosphorylation site that is important for its normal function.¹⁶

Kinases also play an important role in the tight regulation of cell cycle progression. The plant specific eEF1B β has a conserved cell cycle dependent kinase (CDK) phosphorylation site.¹⁷ A mutant form of plant eEF1B β lacking the CDK phosphorylation site, but not the wild type, was able to complement the loss of eEF1B α in yeast implying that phosphorylation is most likely used to reduce the GEF activity of eEF1B β .¹⁷ In metazoan oocytes, eEF1B γ and eEF1B δ have been identified as a target for the CDK1.^{18, 19} Additionally, CDK1 activation during M-phase has been correlated with a decrease in the translation elongation rate.^{20, 21} eEF1B δ has been shown to undergo mitosis dependent spatial redistribution which could potentially be driven by CDK1 dependent phosphorylation. A fraction of the eEF1B δ pool was shown to form a ring around the nucleus and following the breakdown of the nuclear membrane, accumulated around the mitotic spindle pole.²⁰ It is tempting to speculate that this cell cycle specific localization pattern might be the result of isoform specific mobilization and degradation of eEF1B δ transcripts.²² Changes in the subcellular localization of the translation factors caused by CDK1 phosphorylation may lead to localized decreases in protein synthesis. Wild type eEF1B δ has been shown to pull down a significantly lower amount of eEF1A when compared to its non-phosphorylatable mutant form, eEF1B δ -Ser133Ala.²¹ Such a reduced interaction may result in reduced GEF activity which in turn can decrease eEF1A activity leading to downregulation of translation. Interestingly the same site was shown to be phosphorylated in HSV infected cells by a viral kinase.²³ A global reduction of elongation might be advantageous for the translation of viral mRNA in infected cells.

As shown herein, a number of different metabolic signals converge at the level of translational elongation validating it as a target of regulation. Whether the changes in translation observed in response to extracellular signals reflects changes specific to a subset of mRNAs or against the total transcriptome of the cell remains unknown. The use of these modifications to release eEF1 complex members from the translational apparatus to perform other functions is another intriguing possibility.

Architecture of the eEF1 complex—Several models have been proposed to explain the structural organization of the eEF1 complex (Figure 1). Initial attempts to purify eEF1A from calf brain produced a novel form 5 to 20 times heavier than free eEF1A. This complex aggregate of eEF1A contains cholesterol and phospholipids, and was proposed to have a storage or regulatory role.^{24, 25} Our understanding of the organization of eEF1 complex has changed over the years with the discovery of the multiple subunits of the eEF1B complex. The complete eEF1 complex structure was initially proposed in *Artemia salina* to consist of the four subunits eEF1A, eEF1B α , eEF1B γ and eEF1B δ in a molar ratio of 2:1:1:1. eEF1B δ was shown to bind to the N terminal domain of eEF1B γ which in turn is bound to eEF1B α . The presence of two molecules of eEF1A in the complex is not surprising since there are the two GEFs in the complex.²⁶ Since the sequences of the eEF1 subunits are largely conserved across various species including humans, the interaction pattern uncovered in *A. salina* may hold true for mammalian systems as well. In the same study, a second distinct eEF1 complex lacking the eEF1B δ subunit was also isolated, containing eEF1A, eEF1B α and eEF1B γ in a 1:1:1 molar ratio. In *S. cerevisiae*, an organism lacking eEF1B δ , the presence of such a 1:1:1 complex was supported by crystallization and gel filtration studies of the N terminal half of eEF1B γ .⁶ In addition, eEF1B γ was determined to facilitate the dimerization of this complex. Such dimerization by eEF1B γ was also suggested in a rabbit eEF1 complex model proposed by Chang and Traugh.¹³

Early characterization of the eEF1 complex in mammalian cells involved *in vitro* reconstitution experiments with purified rabbit proteins. Waller and colleagues analyzed different combinations of eEF1B $\alpha\gamma$, eEF1B δ and native or truncated ValRS with each other by chromatography and demonstrated that the N-terminal extension of ValRS was required for binding to eEF1B δ . eEF1B $\alpha\gamma$ was proposed to prevent eEF1B δ from forming high molecular weight aggregates, thereby giving the complex a defined quaternary structure. The entire complex was also proposed to dimerize via a leucine heptad repeat within the N-terminal region eEF1B δ .⁹ The ability of eEF1B δ to dimerize along with its distinct binding interface with eEF1B γ indicates that eEF1B α and eEF1B δ might have different regulatory roles in metazoans. This model is also supported by the interactions uncovered using the yeast two-hybrid system, which demonstrated eEF1B γ can interact with eEF1B δ .²⁷ Finally, 3D reconstruction studies using electron microscopic images of the complex shows the presence of two symmetrical domains allowing two eEF1B δ subunits to be placed near the center of the complex.²⁸

Work from Yu and colleagues suggest that there is a unique organization of eEF1B complex in human fetal brain cells.^{29, 30} According to this model, kinectin anchors the eEF1B complex to the ER by binding to eEF1B δ . Kinectin is an integral membrane protein whose primary role is to anchor the microtubule associated cargo protein kinesin on intracellular organelle membranes. However, the brain specific isoform of kinectin lacks the kinesin binding domain pointing to its possible role in a non-canonical process.²⁹ A conserved 60 amino acid fragment of kinectin was shown to co-immunoprecipitate eEF1B δ from cell lysates. Moreover, both kinectin and eEF1B subunits were found to co-localize in the ER as would be expected of a spatially organized translation system. It is of note that overexpression of the eEF1B δ binding domain of kinectin caused displacement of the eEF1B subunits from the ER which argues against eEF1B γ being the primary membrane

binding component of the eEF1B complex. In the absence of eEF1B δ -kinectin interaction, cytoplasmic protein synthesis was favored of that of membrane proteins.³⁰ The distribution of the elongation factors might, therefore, potentially be used to regulate synthesis of different types of proteins.

Interactions with the Cytoskeleton—Work from a number of laboratories has firmly established a link between cytoskeletal organization and translation. Most of the work bridging these processes has placed actin as the major cytoskeletal component involved. Actin plays a significant role in regulating the efficiency of translation, based on the observation that slight perturbations of the actin cytoskeleton in permeabilized cells produces a profound negative effect on global translation even though the levels of key translation components remained similar to those of intact cells.³¹

Since the discovery of eEF1A as an actin binding protein,³² an extensive number of *in vitro* studies have characterized this interaction. Competitive binding experiments with eEF1A, F-actin and aa-tRNA showed that as pH increases, the affinity of eEF1A for F-actin decreased while that for aa-tRNA increased.³³ eEF1A has two pH-sensitive actin binding domains that could be important for modulating the cellular response to external stimuli by allowing the reorganization of the actin cytoskeleton and the associated translational machinery.³³ pH also influences the ability of eEF1A to inhibit actin polymerization *in vitro*, which may be important in regulating processes like the transport, localization and translation of mRNAs.³⁴ Such is the case in sea urchins, where an increase in intracellular pH acts as a signal in activating protein synthesis at fertilization.³⁵

Genetic analysis in *S. cerevisiae* revealed that overexpression of eEF1A disrupts the organization of the actin cytoskeleton without causing appreciable effects on protein synthesis.³⁶ In a genetic screen designed to separate the actin binding/bundling and translation elongation functions of eEF1A, two classes of mutants were identified (Figure 2). The first class of eEF1A mutants exhibited defects in actin cytoskeleton organization due to reduced actin bundling activity while maintaining normal translation activity.³⁷ The second class of mutants displayed severe defects in actin organization and surprisingly translation initiation.³⁸ Several actin associated protein mutants also exhibit reduced initiation providing further evidence that the cytoskeleton and the translational machinery are intimately linked. As eEF1A interacts with Bni1p/She5p, a downstream target of Rho1p that can regulate actin reorganization,³⁹ it is possible that eEF1A acts as bridge between the cytoskeleton and actin modulators. The findings also suggest that regulation of the actin cytoskeleton by eEF1A is crucial for efficient protein synthesis.

A number of reports have shown that eEF1A can also interact with microtubules. eEF1A was identified as a component of the mitotic apparatus,⁴⁰ and was shown to bind, bundle and stabilize microtubules *in vitro* in a Ca²⁺/calmodulin dependent manner.^{41, 42} In contrast, a different study found that eEF1A acts to sever microtubules, as microinjection of eEF1A into cultured fibroblasts led to a rapid decline in tubulin polymers.⁴³ The specific role eEF1A plays in the regulation of microtubule organization remains unclear. The same domains of eEF1A that interact with actin (domains I and III) also interact with microtubules,⁴⁴ raising the question of how the interactions between eEF1A, actin, microtubules and other eEF1A binding partners are regulated or distributed in the cell.

Genetic and biochemical data suggest that eEF1B α modulates the equilibrium of eEF1A between translation and actin organization.^{4, 45} In addition, eEF1B α itself has been implicated in direct binding to actin. A 17 kDa fragment of eEF1B α was isolated as a protein that bound tightly with actin on gel filtration and ion-exchange chromatography columns. However, the effect on eEF1B α on actin filaments is unclear. While intact

eEF1B α γ complex isolated was shown to stimulate initial rate of actin assembly *in vitro*, indicating a role in the nucleation phase of the reaction, recombinant *D. discoideum* eEF1B α exhibited a concentration dependent negative effect on actin assembly.⁴⁶ This later finding is more consistent with the demonstration that eEF1A bundling of actin bundling is mutually exclusive to eEF1B α binding.⁴⁷ It remains to be seen whether eEF1B γ may alter the confirmation of eEF1B α and affect actin assembly.

On the basis of a yeast two-hybrid screen, eEF1B γ has been proposed to bind to keratin, an intermediate filament of the cytoskeleton.⁴⁸ It has since then been shown to bind and bundle keratin filaments in human epithelial cells, the disruption of which resulted in decreased translation.⁴⁹ The presence of large amounts of eEF1B γ in keratin bundle rich hair fibers supports its biological role in the intermediate filament organization. Keratin filaments not only provide mechanical strength to cells, but also regulate cell signaling, apoptosis and the response to stress. The integration of translational factors with the keratin cytoskeleton therefore opens up the potential for pathways that centrally regulate cellular processes by modulating the translational apparatus or *vice versa*.⁵⁰

The observation that translation factors associate with the cytoskeleton has led to the hypothesis that protein synthesis and cytoskeletal organization undergo reciprocal regulation. The actin cytoskeleton in particular is proposed to serve as a scaffold for components of the translation machinery, since a number of initiation factors as well as a large proportion of translating ribosomes have been found in association with the actin cytoskeleton.^{51, 52} However, it remains unclear how changes in actin organization would lead to changes in the rate of translation. One possibility may be that the cytoskeleton provides a spatial ordering of the components such that it allows coordinated interactions between the mRNA, ribosome, and the various translation factors to take place and thus give rise to efficient protein synthesis. The role microtubules would play in such a model remains to be determined. Thus, future experiments designed to separate the effects of the eEF1 complex on the different components of the cytoskeleton are needed.

MOONLIGHTING FUNCTIONS OF eEF1A

Nuclear Export—The role of eEF1A in the nuclear export of tRNAs and the shuttling of eEF1A between the nucleus and cytoplasm have been debated for some time. The initial work on the function of eEF1A in nuclear export of tRNAs was based on a multi-copy suppressor screen of a synthetic lethal *los1 arc1* mutant in *S. cerevisiae*.⁵³ Overexpression of eEF1A was able to restore the growth of this mutant in the tRNA export pathway. Likewise, a reduction in the levels of eEF1A led to a decrease in nuclear export of several aa-tRNAs.⁵³ Mutations in the aa-tRNA binding domain of eEF1A (Glu286Lys or Glu291Lys) show defects in nuclear export of aa-tRNA, leading to an accumulation of tRNAs in the nucleus.⁵⁴ The abundance of eEF1A in the cell would suggest that all aa-tRNA could be bound by eEF1A as a ternary complex with GTP, thus not only protecting the labile aa-tRNA linkage but also effectively eliminating the possibility of aa-tRNA diffusion into the nucleus. Previous work by the Hopper lab has demonstrated that retrograde import of tRNAs into the nuclei of yeast cells occurs when they are deprived of nutrients.⁵⁵ However, it remains unclear whether eEF1A is involved in the initial export of tRNAs from the nucleus or their re-export during recovery from suboptimal growth conditions.

Most of the studies examining the subcellular localization of eEF1A have found the protein to be predominantly localized in the cytoplasm.^{53, 56, 57} Exportin-5, a member of the β -importin family of nuclear transport receptors, has been implicated in keeping eEF1A outside of the nucleus in a manner dependent on the presence of aa-tRNAs.^{56, 57} However, a recent study demonstrated that eEF1A could be found in the nucleus of the *msn5 Δ* nuclear export mutant.⁵⁸ Although eEF1A appeared to localize around the nuclear membrane of

wild-type cells upon nutrient deprivation, a greater proportion of eEF1A was found to localize within the nucleus of *msn5Δ* cells under the same conditions. Since eEF1A is found in high levels in cell, localization studies can be challenging. Therefore, more studies using advanced imaging techniques will help address the question of nuclear roles of eEF1A.

Proteolysis—Although there are examples of different isoforms of the same protein performing opposing functions, not many examples are known of a single protein having opposing functions. For eEF1A, this dichotomy exists for the proposed functions in both protein synthesis and protein degradation. Because of its central role in translation elongation, eEF1A has been proposed to be a good candidate for recognizing damaged proteins and shuttling them to the proteasome for degradation. eEF1A was initially identified as a factor required for the degradation of N- α -acetylated proteins.⁵⁹ Later experiments showed that eEF1A could directly interact with nascent polypeptides while they are being synthesized.⁶⁰ eEF1A was shown to not only interact with an unfolded protein after its release from the ribosome, but also to help mediate refolding. The idea that eEF1A could possess a chaperone-like activity is not too surprising as the bacterial homolog EF-Tu has been shown to have chaperone properties.⁶¹ *In vivo* evidence supporting a role for eEF1A in protein degradation came from its identification as a high copy suppressor of the slow growth phenotype caused by deletion of the *RAD23* and *RPN10* genes involved in the proteolytic pathway.⁶² eEF1A was subsequently shown to interact directly with Rpt1p, a subunit of the 19S regulatory particle. Deletion of *RPT1* significantly reduced the interaction between eEF1A and the proteasome. An association between eEF1A and ubiquitinated proteins following ATP depletion was also demonstrated. Interestingly, resistance to the arginine analog canavanine, which leads to misfolding of proteins and subsequent ubiquitin-dependent degradation, was conferred by a single amino acid mutation (Asp156Asn) in the GTP-binding domain of eEF1A.⁶² Taken together, these data support a model in which eEF1A acts by directing damaged or misfolded proteins from the ribosome to the proteasome.

Apoptosis—The first report of the involvement of eEF1A in apoptosis showed a direct correlation between the levels of eEF1A and the rate of apoptosis.⁶³ In another study, eEF1A was isolated in a cDNA screen to identify factors that give resistance to apoptosis following growth factor withdrawal.⁶⁴ How is it possible for eEF1A to have both pro- and anti-apoptotic effects? The answer may lie in the fact that higher eukaryotes have two different isoforms of eEF1A. Although roughly 93% identical at the protein level, these two eEF1A isoforms vary not only in their expression pattern but also in their involvement in apoptosis. eEF1A1 is ubiquitously expressed throughout all tissues, whereas eEF1A2 seems to only be expressed in brain, heart and muscle.^{65, 66} Analysis of the expression levels of the eEF1A isoforms during muscle fiber differentiation revealed that eEF1A1 acts as a pro-apoptotic factor whereas eEF1A2 is anti-apoptotic.⁶⁷ Interestingly, when the levels of eEF1A1 declined, eEF1A2 levels increased during the differentiation process providing further support for the opposing roles of the two isoforms. This has led to speculation that the switch between eEF1A1 and eEF1A2 in brain, heart and muscle is a critical component of the developmental cycle of these tissues.^{66, 67} Other studies have shown that increased eEF1A2 expression or decreased eEF1A1 expression results in resistance to apoptosis induced by ER stress.^{25, 64} Findings from a more recent study suggest that interferon α (IFN α) may induce an anti-apoptotic response by modulating the expression of eEF1A through the serine/threonine kinase C-Raf, pointing to a link between the phosphorylation status of eEF1A and apoptosis.⁶⁸ Although the details of how phosphorylation affects the role of eEF1A in apoptosis are yet to be determined, the finding further supports the idea that non-canonical functions of eEF1A could be affected by post-translational modifications.

Viral Propagation—Viruses count not only on proteins encoded within their genome but also on host proteins in order to replicate. Given that eEF1A is one of the most abundant cellular proteins, it makes sense that viruses would hijack eEF1A for their own benefit. To date, eEF1A has been shown to affect positive-strand RNA viruses such as Dengue virus,⁶⁹ turnip yellow mosaic virus (TYMV),⁷⁰ tobacco mosaic virus (TMV),⁷¹ turnip crinkle virus (TCV)⁷² and West Nile Virus (WNV).⁷³ A common theme among these viruses is that eEF1A can interact with the 3' C-terminal end of the viral RNA. This result most likely stems from the fact that the proposed secondary structure of the 3'-ends of the viral RNA resembles that of tRNA, a canonical eEF1A binding partner. eEF1A can also interact with their respective viral encoded RNA-dependent RNA polymerase (RdRp). eEF1A was able to stimulate the RdRp activity, or minus strand synthesis, of TCV *in vitro*.⁷² Interestingly, the efficiency of synthesis appears to increase when the RdRp and eEF1A were combined prior to the addition of the viral RNA suggesting the possibility of a sequential order in the interactions. In the case of TYMV, eEF1A appears to antagonize minus strand synthesis presumably by preventing the binding of RdRp to the viral RNA dependent on the latter's aminoacylation status⁷⁰, the tRNA-like structure at the 3'-end of the TYMV RNA resembles that of tRNA^{Val} and has been shown to be valylated *in vitro*.⁷⁴ Although eEF1A associates primarily with positive-strand viruses, it is worth noting that it has also been found to interact with the RdRp of vesicular stomatitis virus (VSV), a negative single strand RNA virus.⁷⁵

Though the role of eEF1A in translation of the viral RNA is straightforward to understand, what could be the significance of the role it plays in the replication of these RNA viruses? Given that the viral replication cycle is composed of a sequential series of events, some have speculated that eEF1A could be involved in helping maintain that order. In the case of TYMV, eEF1A could prevent the viral RNA from being copied by allowing time for the viral proteins like the RdRp to be synthesized first. Once sufficient viral encoded proteins are produced, RdRp competes eEF1A off the 3'-end of the viral RNA positive strand to allow for minus strand synthesis⁷⁰ (Figure 3). For viruses where eEF1A interacts with both the viral RNA and RdRp, eEF1A may help sequester these components of the replicase complex into vesicles.⁷⁶ eEF1A could help in anchoring to the ER membrane via a phospholipid modification at Asp306 and possibly mediate vesicle formation.⁷³ Findings from a more recent study suggest that See1p, a methyltransferase involved in vesicular transport, methylates eEF1A providing further support to this idea.⁷⁵

THE eEF1B COMPLEX OUTSIDE THE TRANSLATION SYSTEM

Although about 10 fold less abundant than eEF1A, the eEF1B complex is an abundant protein by cellular standards. Though fewer non-canonical functions have been ascribed to the eEF1B complex proteins, more examples are recently emerging. These alternate functions are most apparent for the eEF1B γ and eEF1B δ subunits, perhaps separating the highly conserved catalytic GEF function of eEF1B α from these additional roles.

The primary role attributed to eEF1B γ in translation elongation is as a structural scaffold for eEF1B α . Given that the deletion of eEF1B γ in *S. cerevisiae* has no significant effect either on total protein synthesis or translational fidelity *in vivo*,⁷⁷ it is possible that eukaryotic evolution has selected eEF1B γ as a part of the translation complex for its additional functions. Expression patterns during embryogenesis also suggest that it might have a role in the formation of anterior/posterior axis.¹⁶ The N terminal domain of eEF1B γ is structurally similar to the theta class of Glutathione S-transferases (GST).⁷⁸ GSTs catalyze the conjugation of glutathione to electrophilic substrates such as peroxides and hence play an important role in cellular detoxification of reactive oxygen species. While the structural details of the GST catalytic sites seem to be conserved in eEF1B γ , attempts to detect its

GST activity have been largely unsuccessful.⁶ However rice recombinant eEF1B γ , as well as the entire eEF1B complex, was shown to possess a low GST activity.⁷⁹ On a similar note the eEF1B complex isolated from *Leishmania major* was shown to conjugate a variety of electrophilic substrates to trypanothione, a substrate similar to glutathione. The Trypanothione-S-Transferase pathway is used by *L. major* in its defense against chemical and oxidative stress.⁸⁰ An alternative possibility is that eEF1B γ , with its lower transferase activity, could be involved in coupling the redox balance of the cell to the translation complex.⁷⁹

eEF1B γ plays a significant role in the oxidative stress response pathway. Deletion of the two genes encoding eEF1B γ in *S. cerevisiae* gave an additive resistance to oxidative stress.⁷⁷ Total protein analysis by 2D gel electrophoresis indicated a constitutively active stress response pathway in the absence of eEF1B γ .⁸¹ It was also shown to exhibit defective vacuolar morphology resulting from defective ER-Golgi transport and consequentially had a defective protein turnover mechanism.⁸¹ Mislocalization of eEF1B α γ has previously been shown to decrease the synthesis of membrane proteins.³⁰ It is therefore interesting to speculate that in the absence of eEF1B γ , synthesis of certain membrane proteins essential for vesicle formation is severely affected, which in turn, can lead to defective vacuole and ER-Golgi transport.

eEF1B γ has also been shown to have a potential role in the transcription, localization and translation of vimentin mRNA.⁸² Passananti and colleagues were able to show by co-immunoprecipitation (co-IP) and chromatin immunoprecipitation (ChIP) experiments that eEF1B γ physically interacts with Rpb3p, a subunit of the RNA polymerase II, as well as the promoter region of the vimentin gene. Knockdown of eEF1B γ , however, resulted in mitochondrial fragmentation and a concomitant increase in cellular superoxide levels as would be expected with its previously established role in cellular stress response. However eEF1B γ knockdown by siRNA did not seem to have a major effect on vimentin mRNA levels leaving unanswered the role it plays at the vimentin promoter. Although there are reports suggesting that eEF1B γ binds to the 3' UTR of vimentin mRNA, studies have indicated that the binding is non-specific.⁸³ Hence, further evidence is required to prove that eEF1B γ can affect the localization of specific mRNAs in the cell.

In a recent study, eEF1B δ was revealed to undergo alternate splicing to form a novel heat-shock response (HSR) transcription factor.⁸⁴ While the common isoforms of eEF1B δ are around 30-40 kDa a larger splicing variant termed eEF1B δ L was detected in mouse brain and testis. Unlike the short isoform, the long isoform includes a putative nuclear localization signal and localization studies indicated that eEF1B δ L was found both in the nucleus and the cytoplasm. eEF1B δ L expression induced expression from a heat shock element (HSE) containing reporter. The association of eEF1B δ L with the HSE was confirmed by ChIP analysis and was shown to regulate HSE responsive gene induction in response to heat shock, thereby implicating eEF1B δ L as a HSR transcription factor. In response to heat shock, there was a simultaneous up-regulation of eEF1B δ L levels and a downregulation of eEF1B δ isoform levels. This points to a novel two-pronged effect on cellular metabolism in response to heat shock, in the form of increased molecular chaperone levels and decreased total protein synthesis.

Yeast two hybrid screen and co-immunoprecipitation analysis recently demonstrated that eEF1B δ also interacts with SIAH-1, an E3 ubiquitin ligase.⁸⁵ Overexpression of eEF1B δ caused an inhibition of the E3 ligase activity as evidenced by the decrease in ubiquitination of its substrate HPH2. While it is not immediately obvious the physiological requirement for such an interaction in the cell, it is probable that the endogenous interaction between the two proteins is regulated by specific cellular cues.

Conclusion

Translation elongation factors are among the most abundant proteins in the cell and play an essential role in assuring the proper decoding of mRNA to produce cellular proteins. A common theme emerging from the proposed models of the eEF1 complex is that this system has acquired compositional and organizational complexity with evolution from bacteria to yeast to metazoans. The extensive interactions between the elongation factors and the cytoskeleton also point to the existence of a higher order structural organization of the protein synthesis machinery. This supramolecular organization not only leads to a highly efficient translational system, but also opens up multiple avenues for regulatory control. Although the eEF1 complex can be phosphorylated at multiple sites, many aspects of the *in vivo* consequences of this modification remain largely unknown. The central role played by translation in producing the appropriate cellular response to external stimuli may have driven the elongation factors into acquiring multiple functions. For eEF1A, sustained work has demonstrated the importance of these alternate functions in viral systems and the actin cytoskeleton. However, far less is known about the non-canonical functions of the eEF1B complex. It will be interesting to see if more roles for these proteins emerge in the future in the context of the various post translational modifications in higher eukaryotes. One of the major challenges that remains is to determine the mechanism underlying these secondary functions. Gathering mechanistic data on the non-canonical functions of the eEF1 subunits, particularly eEF1A, is challenging as they are some of the most abundant proteins in the cell. This makes the post-translational modifications of the eEF1 complex an attractive venue to explore how canonical and non-canonical functions converge to regulate the cellular processes. Future work will provide a better picture of the role of the eEF1 complex within the global cellular context.

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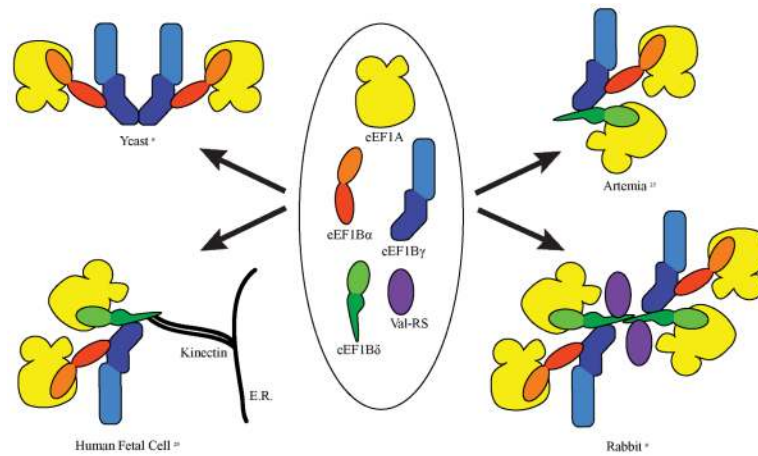


Figure 1. Model of eEF1 complex

The variation in the organization of the eEF1 complex in different organisms illustrates the differences in the composition as well as the interactions of the various subunits. Domain I, the largest domain of eEF1A (gold) is the GTP binding domain. For the eEF1B subunits α (red) γ (blue) δ (green), the N and C terminal domains are indicated in darker and lighter shades respectively. Val RS (purple) interacts with the eEF1 complex in metazoans.

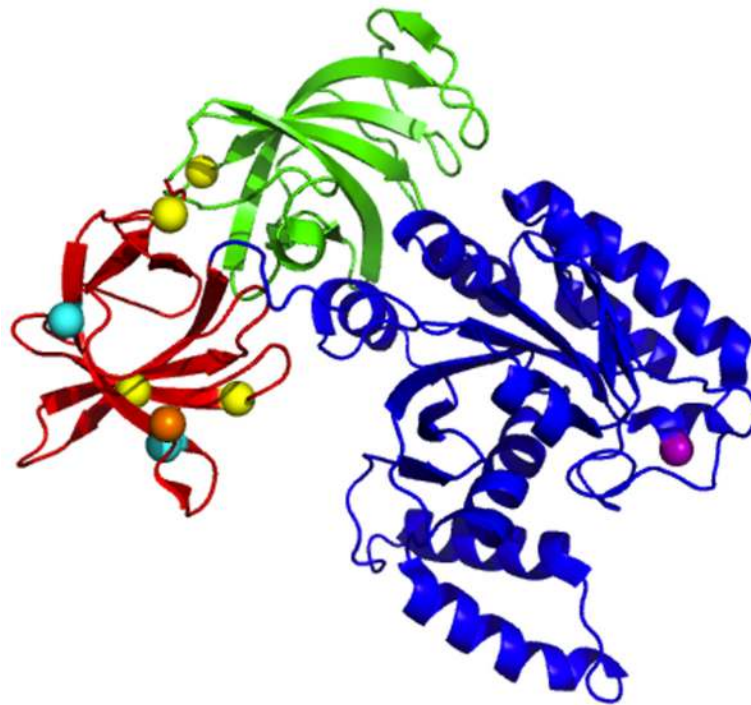


Figure 2. Location of mutations that affect non-canonical functions and known phosphorylation sites on eEF1A

eEF1A from *S. cerevisiae* is composed of three well-defined domains as determined from the co-crystal structure with the C terminus of eEF1B α . Domain I (blue) binds GTP, domain II (red) is proposed to interact with the aminoacyl end of the aa-tRNA, and domains II and III (green) are linked to actin binding and bundling. eEF1B α binds eEF1A in domains I and II. Colored spheres indicate mutations that affect the non-canonical functions of eEF1A. In domain I, Asp156Asn (purple) affects protein turnover. In domain II, Glu286Lys and Glu291Lys (cyan) affect nuclear transport. Mutations that affect actin organization, Asn305Ser, Asn329Ser, Phe308Lys and Ser405Pro, (yellow), are shown in domains II and III. Phosphorylation of Glu298 (the yeast equivalent of human Ser300) (orange) may lead to downregulation of translation. The figure was prepared with PyMol using Protein Data Bank (PDB) 1F60.⁸⁶

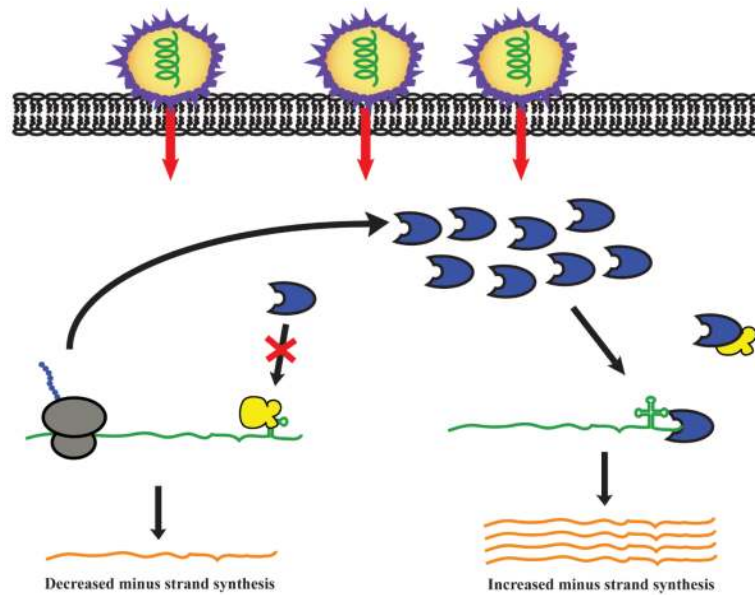


Figure 3. The role of eEF1A in minus strand synthesis of positive strand viral RNAs

Upon release of the viral RNA (green) into the cytoplasm, eEF1A (yellow) binds at the tRNA-like structure at the 3'-end of the RNA preventing the RNA-dependent RNA polymerase (RdRp, blue) from initiating minus strand synthesis. By inhibiting minus strand synthesis, eEF1A allows viral proteins to be synthesized first. Once sufficient RdRp is made, RdRp competes eEF1A off of the viral end allowing for minus strand synthesis to occur.

Table 1

Components of the eEF1 complex:

| Current Nomenclature | | Prior Nomenclature | Canonical Function |
|----------------------|---------------------------|--|----------------------------------|
| eEF1A | | eEF1 α | aa-tRNA delivery to the ribosome |
| eEF1B | eEF1B α | eEF1 β (yeast and metazoan) eEF1 β' (plant) | GEF for eEF1A |
| | eEF1B β (plant) | eEF1 β (plant) | GEF for eEF1A |
| | eEF1B γ | eEF1 γ | Structural component |
| | eEF1B δ (metazoan) | eEF1 δ (metazoan) | GEF for eEF1A |
| Val-RS (metazoan) | | Val-RS | Valyl tRNA synthetase |

The eukaryotic elongation factor 1 (eEF1) complex consists of eEF1A (EF-Tu in bacteria), the Gprotein involved in delivering aa-tRNA to the elongating ribosome and eEF1B (EF-Ts in bacteria), a multiprotein complex that acts as the guanine nucleotide exchange factor (GEF). In animals, Val-RS is found bound to the eEF1 complex and this complex is often called the eEF1H complex. In mammals, multiple aminoacyl-tRNA synthetases have been suggested to interact with eEF1 complex.⁸⁷

Table 2

Kinases, their targets in the eEF1 complex and effects of protein synthesis

| Kinases | Effect on Translation | Subunits Phosphorylated | References |
|---------------------------|-------------------------|---|--|
| Protein Kinase C | Upregulation | eEF1A, eEF1B α , eEF1B δ | Peters et al. ¹¹ ; Venema et al. ¹² |
| Multipotential S6 Kinase | Upregulation | eEF1A, eEF1B α , eEF1B δ | Chang and Traugh ¹³ |
| Cyclin Dependent Kinase 1 | Possible Downregulation | eEF1B β , eEF1B γ , eEF1B δ | Janssen et al. ¹⁸ ; Mulner-Lorillon et al. ¹⁹ ; Sivan et al. ²¹ |
| Casein Kinase II | No Effect | eEF1B α , eEF1B δ | Sheu and Traugh ¹⁵ |
| TGF- β Receptor I | Downregulation | eEF1A | Lin et al. ¹⁴ |
| C-Raf | Unknown | eEF1A | Lamberti et al. ⁶⁸ |
| PAS Kinase | Upregulation | eEF1A | Eckhardt et al. ⁸⁸ |
| DOA Kinase | Unknown | eEF1B γ | Fan et al. ¹⁶ |