



The eEF1 family of mammalian translation elongation factors

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ABSTRACT

The eEF1 family of mammalian translation elongation factors is comprised of the two variants of eEF1A (eEF1A1 and eEF1A2), and the eEF1B complex. The latter consists of eEF1B α , eEF1B β , and eEF1B γ subunits. The two eEF1A variants have similar translation activity but may differ with respect to their secondary, “moonlighting” functions. This variability is underlined by the difference in the spatial organization of eEF1A1 and eEF1A2, and also possibly by the differences in their post-translational modifications. Here, we review the data on the spatial organization and post-translation modifications of eEF1A1 and eEF1A2, and provide examples of their involvement in various processes in addition to translation. We also describe the structural models of eEF1B subunits, their organization in the subcomplexes, and the trimeric model of the entire eEF1B complex. We discuss the functional consequences of such an assembly into a complex as well as the involvement of individual subunits in non-translational processes.

1. Introduction

The eEF1A1 and eEF1A2 variants of eEF1A, and the eEF1B α , eEF1B β and eEF1B γ subunits of the eEF1B complex comprise the eEF1 family of mammalian translation elongation factors. A key role of the eEF1 family in protein biosynthesis is the delivery of aminoacyl-tRNA to a corresponding codon of the mRNA-programmed ribosome. Though the basic principles of eukaryotic eEF1A function resemble those of prokaryotic EF1A (also known as EF-Tu), there are some important differences in their actions [1]. One reason for this may be the compartmentalization of the translation apparatus that serves as a structural basis for tRNA/aminoacyl-tRNA channeling during protein synthesis in mammalian cells. eEF1 is a central partner in this process, and transfers aminoacyl-tRNA from aminoacyl-tRNA synthetase to the ribosome, and then a deacylated tRNA back to aminoacyl-tRNA synthetase [2]. Also, the members of the eEF1 family were found to interact with several non-translational proteins, and are probably involved in carrying out other independent moonlighting functions in the cell. Both eEF1A1 and eEF1A2 have been implicated in the development of numerous human diseases through their enhancement of oncogenesis, the blockade of apoptosis, and increased viral pathogenesis. All these features have prompted an increasing interest in the eEF1 family. In this review, we

focus on the structure of the eEF1 family members as the basis for understanding their involvement in mammalian translation and other cellular functions.

2. eEF1A1 and eEF1A2

eEF1A1 was considered to be the only mammalian analogue of bacterial EF-Tu factor until the tissue-specific eEF1A2 variant in rat and human tissues was discovered by Eugenia Wang's [3] and Brian Clark's [4] labs, respectively. The variants were 97% homologous and 92% similar, suggesting their full functional resemblance. The expression of eEF1A1 and eEF1A2 was mutually exclusive. eEF1A2 was found in muscle and brain tissue, whereas eEF1A1 was expressed in all other tissue types. The *EEF1A1* and *EEF1A2* genes were assigned to different human chromosomes [5]. The developmental role of eEF1A1/eEF1A2 substitution in neuronal tissue and muscle was illuminated by the detection of a wasted mutation in mice, disabling the expression of the *EEF1A2* gene, which is normally observed in postnatal mouse development [6]. eEF1A2 was described as an oncogene in ovarian and lung cancer [7, 8] whereas it is absent in corresponding healthy tissue. This raised the question of the selective regulation of eEF1A1 and eEF1A2 expression, which may be disrupted by cancer. We concluded that the

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uncontrolled increase of the eEF1A2 expression in cancerous tissue may result from the loss of miRNA-mediated control. Indeed, we found that miR-663 and miR-744 negatively influenced the amount of eEF1A2 in breast cancer cells, and inhibited their proliferation. Apart from indicating the role of these microRNA as anticancer agents, this suggests that one of the possible mechanisms of tissue-specific eEF1A2 suppression could be related to miRNA [9]. This is especially important, considering the recently observed co-expression of eEF1A1 and eEF1A2 mRNAs in mature hippocampal neurons and the predominant expression of eEF1A1 mRNA in dendrites [10].

What is so specific about the eEF1A variants with 92% similar amino acids sequences, which makes it impossible to replace one with another? One way to answer this question is to physically examine differences between the eEF1A1 and eEF1A2 proteins. A comparison of the physical properties of the eEF1A1 and eEF1A2 proteins became possible after development of the procedures of their isolation in preparative quantities from mammalian tissues [11, 12], as the mammalian proteins expressed in *E. coli* were not functionally active. The differential scanning calorimetry values of transition temperature, half-width, and enthalpy change for the eEF1A1 and eEF1A2 proteins suggested that the latter had a more compact state. Subsequent CD measurements of eEF1A1 and eEF1A2 displayed the difference in their near-UV region spectra, indicating the difference in the tertiary-structure organization of the proteins [13]. A comparison of the surface hydrophobicity of the protein variants revealed that eEF1A1 displayed more hydrophobic properties than eEF1A2 [14]. In line with this, small-angle X-ray scattering, and analytical ultracentrifugation showed eEF1A1's increased ability to self-associate. Also, small-angle X-ray scattering measurements at various urea concentrations revealed a lower resistance of the A1 protein structure to denaturation by urea [14]. All these data permitted us to conclude that the eEF1A2 protein is more compact and stable than eEF1A1. Therefore, eEF1A2, rather than eEF1A1, was chosen for structural studies by X-ray crystallography. eEF1A2 crystals were successfully obtained [15], and the X-ray structure of the dimeric form of eEF1A2 was solved [16]. The first X-ray structure of mammalian eEF1A revealed major differences in the organization of its nucleotide-binding domain, when compared to bacterial analogue EF-Tu, namely, a lack of involvement of a Mg^{2+} ion in stabilizing GDP. The availability of the structure of yeast eEF1A in the complex with nucleotide exchange factor eEF1B α [17] permitted the modeling of structural rearrangements of eEF1A during the nucleotide exchange process. Understanding the intramolecular movements of eEF1A allowed us to suggest that the first step of eEF1A*GDP dissociation from the 80S ribosome is the rotation of its nucleotide-binding domain, induced by GTP hydrolysis [16]. The structure obtained proved to be useful for modeling eEF1A2*GDP*aminoacyl-tRNA/tRNA complexes that may contribute to an understanding the overall translation elongation control in eukaryotic cells [18] and mechanisms of the aminoacyl-tRNA/tRNA channeling process [19–21].

Along with substitutions of certain amino acid residues, the structures of eEF1A1 and eEF1A2 may differ in the number of post-translational modifications. Our earlier observation that more compact eEF1A2 may be less modified post-translationally than eEF1A1 [22] is consistent with the recent data. The PhosphositePlus database [23] mentions twenty seven phosphorylated, nineteen acetylated, and twenty three ubiquitinated amino acid residues in eEF1A1, and twelve phosphorylated, ten acetylated, and seven ubiquitinated residues in eEF1A2 (accessed July 26, 2022). Here, the sites with more than three independently confirmed modifications were considered. These data may provide independent evidence that the spatial structure of eEF1A1 is more “open,” as it is more accessible to the enzymes that catalyze various post-translational modifications.

Interestingly, the number and location of methylated amino acid residues in eEF1A1 and eEF1A2 were quite similar, which may be explained by the localization of methylated residues on the surfaces of eEF1A1 and eEF1A2 proteins. In 1989, William Merrick's lab identified

five methylated residues of rabbit eEF1A1 [24], but the mammalian eEF1A methylation studies practically came to a standstill for twenty five years, until corresponding methyltransferases were identified (for a review see [25, 26]). Consequently, it became clear that eEF1A is the only known target of these enzymes. Dimethylation of Lys55 was found to promote tumorigenesis by increasing translation efficiency [27], probably by stabilizing a certain conformation of the nucleotide binding site, caused by the strengthening of cation- π interaction with neighboring aromatic residues [28]. A possible, important role of other methylated lysine residues in both variants of eEF1A is not yet known. Recently we verified whether methylation may have an effect on the interaction of eEF1A2 with the subunits of nucleotide exchange complex eEF1B [29]. We produced a set of eEF1A2 mutants in which potentially methylated Lys-residues were replaced with Arg. BRET analysis and pull-down experiments have shown no difference in the interaction of eEF1B α , eEF1B β , and eEF1B γ subunits of eEF1B with eEF1A2 and its unmethylable mutants in HEK293 cells [29]. This suggests that the methylation of Lys35, Lys55, Lys79, Lys165, and Lys318 does not influence the interaction of eEF1A2 with the eEF1B complex.

We have demonstrated that in contrast to eEF1A1, eEF1A2 interacts with the SH2 domains of Grb2, RasGAP, Shc and the C-terminal part of Shp2, and with the SH3in domains of Crk, Fgr, Fyn, and phospholipase C- γ 1 [30]. Tyrosine phosphorylation normally promotes the interactions of signaling proteins via SH2 domains, while for SH3 domains, the post-translational modification prevents interaction. The specific phosphorylated tyrosine residues responsible for this interaction remain unidentified. A recent theoretical analysis led to the interesting conclusion that all Tyr-residues, reported to be phosphorylated in eEF1A2, cannot be phosphorylated in a crystal dimer structure, owing to chemical constraints [28]. The straightforward explanation for this apparent contradiction is the difference in the spatial structures of an individual monomer, and a monomer that is part of a dimer. Besides, it seems possible that the crystal and in-solution structures of the dimer differ. Also, we cannot exclude the existence of a set of various solution conformations of both monomeric and dimeric forms of eEF1A2. Finally, the 56th and 177th Tyr-residues found to be phosphorylated, according to the PhosphoSitePlus database, were not included in the analysis [28], for unknown reasons. A possible conformational variety of eEF1A variants should be particularly investigated in the future studies. If we consider Ser- and Thr, there were seventeen additional phosphorylated residues in eEF1A1, and three additional phosphorylated residues in eEF1A2, compared to the opposite isoform (no search limits in PhosphoSitePlus were imposed). It is worth mentioning an eEF1A1-specific phosphorylation pattern in domain 1, including S157, T158, S163, S175, and T176, which may be linked to the increased structural lability of eEF1A1. Prospective roles of eEF1A phosphorylation and other post-translational modifications of the protein were described by Mills and Gago [28].

Both eEF1A1 and eEF1A2 promoted actin bundling. The dimerization of eEF1A2 in the crystal permitted us to specify a requirement for a dimeric structure of eEF1A to interact with F-actin. Peculiarly, removing the N-terminal part of eEF1A completely eradicated eEF1A's actin-bundling property, despite this element not being necessary to the interaction of eEF1A with actin [31]. On the other hand, according to the crystal structure, the N-terminal part was important for the formation of eEF1A dimers [16], which is why it has been concluded that the dimers are responsible for actin bundling. The model of actin-bundle formation in the presence of eEF1A dimers was advanced, and it was suggested that domain 3 of each monomer in the eEF1A dimer interacts with different actin chains [31]. Subsequent modeling and molecular dynamic simulation studies showed that it is specifically the C-terminal 445 to 463 α -helical region of each eEF1A2 monomer that is most probably involved in actin bundling [32]. In contrast to eEF1A1, eEF1A2 induced formation of short and thick actin bundles [33], which was eventually explained by the different contacts of the actin-interacting C-terminal region, triggered by the presence of a penultimate glycine

residue in eEF1A2, rather than in eEF1A1 [32]. Interestingly, in mice, the overexpression of eEF1A2—but not eEF1A1—leads to cytoskeleton rearrangements that may contribute to corticospinal axon repair following injury [34].

There is an indication that eEF1A2 mutant with four eEF1A2-specific phosphomimetic substitutions in domain 3 (S342E, S358E, S393E, and S445E) loses both F-actin binding and bundling activities, and shows increased mobility in spines and dendrites [35]. Ser 445 is an element of the C-terminal region that was predicted to provide eEF1A2–actin interaction, which suggests that its phosphorylation may be most crucial for preventing the eEF1A2–actin interaction. On the other hand, Ser 358 is the only residue of the four mentioned, whose phosphorylation has been confirmed experimentally (PhosphositePlus, accessed July 26, 2022). Also, the exclusive phosphorylation of Ser 454 in eEF1A1 may have an effect on the eEF1A1-induced shape of actin bundles. However, eEF1A1 may also have a different mechanism for regulating its binding to actin. We have found that Ca^{2+} /calmodulin is capable of binding eEF1A1 [36], and interferes with its tRNA-binding and actin-bundling activities *in vitro*, whereas eEF1A2 showed no signs of interacting with calmodulin [33]. As Ca^{2+} /calmodulin dislocates tRNA from its complex with eEF1A1, and suppresses the actin-bundling activity of the latter, it is possible to hypothesize that the replacing eEF1A1 by eEF1A2 in muscle, myocardial, and neuronal tissues is necessary for protecting ribosomal polypeptide elongation from the effect of quick changes in $[\text{Ca}^{2+}]$ observed in these tissues [37,38].

Our further investigation of the possible functional effects of structural differences between eEF1A1 and eEF1A2 revealed that eEF1A1 rather than eEF1A2 interacted with the Sgt1 (suppressor of G2 allele of Skp1) protein. The interaction sites were identified, and we modeled a protein–protein complex that demonstrated both the shape and charge complementarities of the eEF1A1–Sgt1 interface, stabilized by a few salt bridges [39]. Sgt1 is involved in antiviral defense systems [40], whereas eEF1A interacts with viral RNA to enhance virus performance [41]. Therefore, we suggested that Sgt1 can compete with viral RNA, disrupting the eEF1A1–RNA complex, and provided *in vitro* evidence that such a mechanism may be functional [39].

Apart from eEF1A's involvement in viral propagation, including SARS-CoV-2 [42] and HIV1 [43], and cytoskeleton rearrangement, which may also be virus-related [44], it may be linked to a few other non-translational processes. The eEF1A variants participate in protein renaturation [45], contribute to spermatogenesis [46], are involved in cell-cycle progression [47], apoptosis [48], and autophagy [49], participate in the heat shock response [50] and protect against toxin-induced neuronal death [51]. They are associated with carcinogenesis [52], myocardial hypertrophy [53], Parkinson's disease [54], Alzheimer's disease [55], fatal familial insomnia [56], and neurodevelopmental disorders [57], may exacerbate insulin resistance [58], and act as an antigen for the autoantibodies production in type 1 diabetes [59]. We realize that a full understanding of the exact role of the eEF1A variants in non-translational cellular processes and diseases still has a way to go, but recent progress in structural and proteomics studies gives us hope that there will be rapid progress toward this goal.

3. eEF1B

Mammalian complex eEF1B comprises three subunits – eEF1B α (EEF1B2), eEF1B β (EEF1D), and eEF1B γ (EEF1G) – according to the established nomenclature [60]. In the UniProtKB database, eEF1B α is described as Elongation factor 1-beta (EF1B_human, accession number P24534), eEF1B β as Elongation factor 1-delta (EF1D_human, accession number P29692), and eEF1B γ as Elongation factor 1-gamma (EF1G_human, accession number P26641). eEF1B α and eEF1B γ have been well-preserved during evolution, whereas eEF1B β is a metazoan-specific protein [61]. The N-terminal domains of all eEF1B subunits are responsible for their involvement in the complex, whereas the C-terminal domains of eEF1B α and eEF1B β carry out nucleotide

exchange activity [62]. The complete spatial structures of the subunits are not known, however, there are some structural data on their domains.

The part of the human eEF1B α C-terminal domain was resolved by NMR [63]. The unpublished structure of the human eEF1B α N-terminal domain, crystallized in the complex with the eEF1B γ N-terminal domain, is present in PDB (5DQS). The structure of the human eEF1B β C-terminal fragment was determined by NMR [64], whereas the unpublished structure of the human eEF1B β N-terminal domain fragment complexed with the eEF1B γ N-terminal domain is available in PDB (5JPO). Also known are the NMR-derived structure of the 19 kDa C-terminal fragment of human eEF1B γ [65], the crystal structure of the individual N-terminal domain of yeast eEF1B γ [66], and the aforementioned structures of the human eEF1B γ N-terminal domain complexed with its eEF1B α or eEF1B β counterparts.

We have found that human eEF1B α possesses an elongated shape in solution [67]. The structures of the folded N- and C-terminal domains of eEF1B α are known, and represent rigidly structured elements of this protein. We used hydrogen–deuterium exchange coupled with mass spectrometry (HDX–MS) [68] to show that, in contrast, the linker and central acidic region are highly dynamic [69]. Hence, eEF1B α has a structure wherein two structured domains are connected by a long dynamic linker (Fig. 1A).

This may contribute to the general dynamic properties of eEF1B α . Interestingly, N-terminally truncated eEF1B α showed increased nucleotide exchange activity, which was unexpected, considering that catalytic activity of eEF1B α resides in its C-terminal part. A conventional role of the N-terminal domain of eEF1B α is to bind eEF1B γ . Addition of eEF1B γ has a similarly positive effect on the activity of full-sized eEF1B α . All these data led to the conclusion that the N-terminal domain negatively influences eEF1A binding to the C-terminal catalytic domain of the individual eEF1B α subunit, owing to the overall flexibility of the molecule, whereas the formation of a stable complex of the eEF1B γ and eEF1B α N-terminal domains establishes a way to overcome this inhibitory effect [67]. It may have some regulatory consequences in cancer cells, where eEF1B α is present in both unbound and bound to eEF1B statuses. Interestingly, the self-inhibitory action of a distant member of a protein was previously observed in a different member of eukaryotic translation machinery: methionyl-tRNA synthetase [70,71].

We have revealed that the N-terminal protein-binding domain of eEF1B β (1–77 residues) is a monomer in solution [72]. This eEF1B β fragment has been found to bind eEF1B γ in an equimolar ratio. The secondary structure of eEF1B β (1–77) is mostly α -helical, with a portion of disordered region. The flexibility of this entity was confirmed by rapid hydrogen/deuterium exchange for all eEF1B β (1–77) peptides measured with HDX–MS. Taking into account a computational modeling of eEF1B β (1–77), we suggested the existence of several conformation states of eEF1B β (1–77), each composed of three labile α -helices connected by flexible linkers.

Together, the data suggest that the protein-binding domain of eEF1B β shows a highly dynamic spatial organization, which may be needed for interaction with eEF1B γ or other protein partners [72]. Further research was directed at elucidating the exact oligomeric status of the eEF1B β molecule, as the purified protein was seen to form oligomers in solution [73]. The analytical ultracentrifugation in sedimentation velocity mode, and the sedimentation equilibrium data showed that recombinant eEF1B β self-associates in a stable trimer with a highly elongated shape [69]. In the subsequent experiments we unequivocally attributed a trimer-forming role to a heptad repeat that contains six leucine residues that occupy every seventh position, and create a hydrophobic path along the helix. The trimeric coiled-coil conformation of the leucine-zipper motif region was successfully modeled [69]. The internal dynamics of the eEF1B β trimer were estimated by HDX–MS. The leucine-zipper motif region and most of the guanine exchange (GEF) domain were shown to be rigidly structured. In contrast, the N-terminal domain, linker, and central acidic region were demonstrated to be

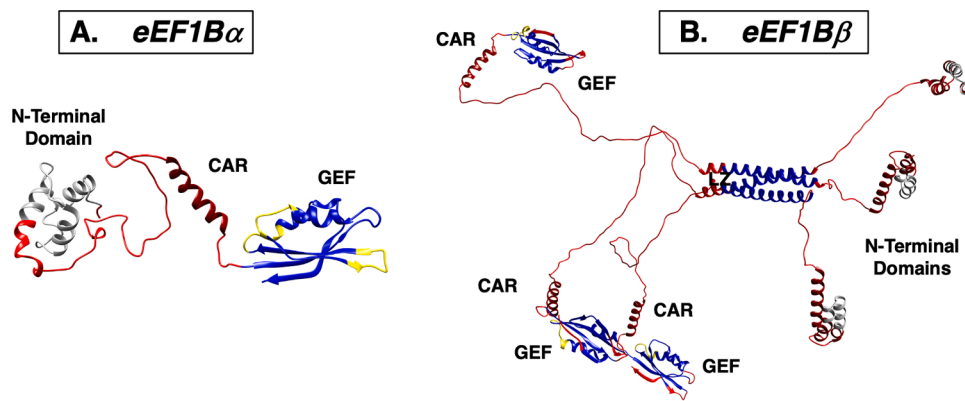


Fig. 1. Structural dynamic organization of (A) eEF1B α and (B) eEF1B β subunits of the eEF1B complex. The structural models are depicted using previously published PDB file [69] and are colored in accordance with the dynamic data from the H/D exchange experiments. Abbreviation of the structural domains: GEF – guanine nucleotide exchange factor, LZ – leucine zipper motif, CAR – central acidic region. Rigidly structured regions with high protection from H/D exchange are indicated in blue, dynamic regions with weak and no protection from H/D exchange are indicated in yellow and red, respectively. CAR region of both proteins and the N-terminal domain of eEF1B β that display no protection from H/D exchange but were modeled to have α -helical organization are shown in dark red. Gray color indicates the region of eEF1B α and eEF1B β

which undergo additional protection from H/D exchange upon interaction with eEF1B γ subunit. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

unprotected, indicating highly dynamic structures. An atomistic model of full-sized eEF1B β was developed by combining a homology modeling approach with HDX-MS and analytical ultracentrifugation data (Fig. 1B) [69].

As mentioned above, the crystal and NMR-derived structures of the individual eEF1B γ domains are known. We combined the homology-modeling approach and HDX analysis to develop an atomistic model that shows that eEF1B γ is a non-globular protein with a moderately elongated shape, which consists of two rigidly structured domains connected by a long, highly dynamic linker (Fig. 2) [69]. The eEF1B γ protein is known for its elevated self-aggregation capacity [74,75], so it is rather difficult to estimate its actual oligomeric state *in vitro* and *in vivo*. The sedimentation velocity experiments revealed that the minor and major eEF1B γ sedimenting species had masses close to the monomeric and dimeric forms of the factor, whereas analytical ultracentrifugation in the sedimentation equilibrium mode showed a mixture of monomeric and variously-sized oligomeric forms of eEF1B γ in solution [69].

Until now, there was no information about the structure of the whole eEF1B complex, except for low-resolution EM images of ValRS/EF-1 complexes published in 2005 [76]. At approximately that time, a few biochemical studies by various laboratories time proposed various structural models of the eEF1B complex. One of the first models was developed by the Wim Möller lab, and suggested that the N-terminal domains of eEF1B α and eEF1B β bind to the eEF1B γ subunit to form the eEF1B $\alpha\beta\gamma$ complex, whereas each of their C-terminal domains binds one molecule of eEF1A [77]. The structural role of the leucine-zipper motif

of eEF1B β in a possible dimerization of eEF1B $\alpha\beta\gamma$ was first proposed by the Jean-Pierre Waller lab [78], whereas Minella et al. hypothesized about the trimerization of an eEF1B $\alpha\beta\gamma$ monomer [79]. Charlotte Knudsen's lab demonstrated the independent binding of eEF1B α and eEF1B β to eEF1B γ , using a three-hybrid yeast system [80]. Jolinda Traugh suggested the dimerization of eEF1B γ , with eEF1B α and eEF1B β binding to various molecules of eEF1B γ in the eEF1B $\alpha\beta\gamma$ complexes, which, in turn, could be dimerized via the eEF1B α subunit [81].

We have determined the regions of the subunits involved in the interaction within the eEF1B complex by using HDX-MS. A whole N-terminal domain of eEF1B α (Fig. 1A) and the only 19-amino-acid-long region of eEF1B β (Fig. 1B) increased their protection against deuterium incorporation when interacting with eEF1B γ [69]. eEF1B γ is known to bind eEF1B α and eEF1B β concurrently [80], which makes it interesting to understand how eEF1B γ binds both proteins at the same time. As expected, according to HDX-MS, the N-terminal part of eEF1B γ participated in the interaction with both proteins. Amino acid residues 144–161 and 170–190 participated in the interaction with eEF1B α (Fig. 2A), whereas most of the remaining peptides of the N-terminal region were more strongly protected when they interacted with eEF1B β (Fig. 2B). The C-terminal domain and the linker region of eEF1B γ were not involved in this interaction. The same interaction patterns for the eEF1B α , eEF1B β , and eEF1B γ proteins were observed for the whole eEF1B complex [69].

The stoichiometry of the binary eEF1B $\alpha\gamma$ and eEF1B $\beta\gamma$ and ternary eEF1B $\alpha\beta\gamma$ complexes was studied by analytical ultracentrifugation in various modes. eEF1B $\alpha\gamma$ revealed the presence of eEF1B $\alpha\gamma$ and eEF1B

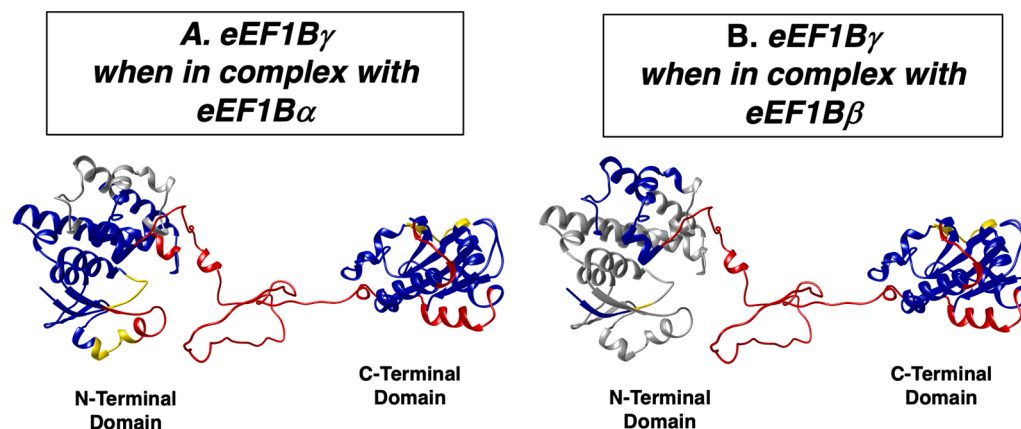


Fig. 2. Structural dynamic organization of eEF1B γ subunit. The structural model of each subunit is depicted using previously published PDB file [69] and are colored in accordance with the dynamic data from the H/D exchange experiments. Rigidly structured regions with high protection from H/D exchange are indicated in blue, dynamic regions with weak and no protection from H/D exchange are indicated in yellow and red, respectively. Gray color indicates the region of eEF1B γ that undergoes additional protection from H/D exchange upon interaction with eEF1B α (A) and eEF1B β (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

$(\alpha\gamma)_2$ forms. eEF1B $\beta\gamma$ displayed peaks that corresponded to the molecular masses of heterotrimeric eEF1B $(\beta\gamma)_3$ and heterohexamer eEF1B $(\beta\gamma)_6$. Sedimentation velocity analysis of the ternary eEF1B $\alpha\beta\gamma$ complex indicated the presence of the major species corresponding to the heterotrimeric eEF1B $(\alpha\beta\gamma)_3$, whereas sedimentation equilibrium analysis revealed the presence of the heterotrimer–heterohexamer mixture in solution. We concluded that the α , β , and γ subunits of eEF1B preferentially associate in a stable heterotrimeric complex with the possibility of further oligomerization *in vitro*, mediated by the eEF1B γ subunit [69]. The existence of a heterotrimeric eEF1B $(\alpha\beta\gamma)_3$ complex indicates the presence of six guanine nucleotide exchanging domains within one complex, which raises the question of whether they function concurrently. We found that eEF1B $(\alpha\beta\gamma)_3$ was saturated by eEF1A2 in a 1:6 molar ratio, demonstrating that one molecule of the eEF1B complex can successfully bind up to six molecules of eEF1A2 [69]. The supercomplex of eEF1B and eEF1A has historically been called the heavy complex, or eEF1H. The model of spatial organization of eEF1H is shown in Fig. 3.

An obvious role of the stable trimeric eEF1B complex may be to increase the local concentration of the GEF domains, which may be important for maintaining efficient GDP/GTP exchange in the eEF1A molecules in the vicinity of the translating ribosomes. The question whether eEF1B α and eEF1B β show identical functions *in vivo* remains unanswered. An exclusive role of eEF1B β may be to serve as a sole nucleotide exchange factor for eEF1A interacting with valyl-tRNA synthetase and forming valyl-tRNA–eEF1A–GTP complexes [82] which does not exclude eEF1B β interaction with eEF1A molecules that are not routed to valyl-tRNA synthetase. In turn, eEF1B α may be a preferential nucleotide exchange factor for eEF1A2 in neurons. Neuronal and muscle-tissue-specific eEF1A2 depends more significantly on nucleotide exchange factors than does eEF1A1 [67]. Local translation of specific mRNAs in different regions of neurons is well known, thus the presence of trimeric eEF1B in the neuronal translational compartments may be especially important to supporting efficient translation of specific mRNAs. There is also an indication of a possibility of localized translation in a sarcomere [83].

Recently-published translatomes of synapse/axon- and neuron-body-enriched sections of hippocampi [84] revealed that the distribution of newly synthesized eEF1A2 among these fractions was different than the distribution of eEF1B β and eEF1B γ . However, the distribution of eEF1B α still followed eEF1A2-like trends. If the glial and neuronal cells were compared, eEF1A1, eEF1B β , and eEF1B γ showed very similar glial

preference, whereas eEF1A2 demonstrated neuronal localization, and eEF1B α occupied an intermediate position. Additional observation that favored possible eEF1A2–eEF1B α coupling came from the study of eEF1B subunit expression in the brain and liver throughout late embryonic and postnatal mouse development [85]. The amount of eEF1B α in late embryonic tissue was very low. However, it increased, especially in brain tissue, and stabilized during postnatal development, to some extent resembling the changes in the expression profile of eEF1A2 in the mouse brain. In contrast, eEF1B β was abundantly expressed in late embryonic tissue, whereas the amount rapidly decreased and stabilized after 10 days of post-embryonic brain and liver development. Interestingly, the phosphomimetic eEF1A2 mutants of 342, 358, 393, and 445 serine residues showed a significant reduction in the interaction with eEF1B α in the spines of hippocampal neurons and in the soma of mouse neuroblasts Neuro-2A cell line, with no significant effects on translation [35]. The idea that eEF1B α may be a local nucleotide exchange factor of eEF1A2*GDP is not new (see [85] for discussion). A plausible explanation is that some portion of just-synthesized eEF1B α does not enter the eEF1B complex, and in some cases may serve as a local nucleotide exchange factor for eEF1A2*GDP. In any case, these findings raise a question concerning the extent of integrity of the whole eEF1B complex *in vivo*.

We addressed the existence of individual subunits of the eEF1B complex in the organism by studying their expression level in various human cancer tissues. The changes in the expression of various eEF1 subunits in human cancers, compared to those in the tumor-surrounding tissue, were found to be unbalanced. The independent overexpression of at least one eEF1 component was observed in 72% of 25 clinical samples of cardioesophageal cancer [86] and 52% of 25 samples of lung cancer [87]. Peculiarly, a concomitant cancer-related increase of both eEF1B β and eEF1B γ was found in four cases of cardioesophageal cancer and five cases of lung carcinomas whereas, no cases of a coordinated increase in eEF1B α and eEF1B γ were revealed. The coordinated up-regulation of eEF1B β and eEF1B γ was also found in oral squamous cell carcinoma [88]. An associated increase in eEF1B β and eEF1B γ expression was noticed in the prenatal (compared to postnatal) mouse brain and liver [85]. These data hint at the possibility of the separate functioning of pools of individual eEF1B α , and the eEF1B β –eEF1B γ subcomplex in brain tissue. However, if eEF1B γ was not present in the complex with eEF1B α , it would not overcome the self-inhibitory effect of the latter, described above. In this case, the guanine nucleotide exchange activity

Proposed Structural Organization of the eEF1H Complex

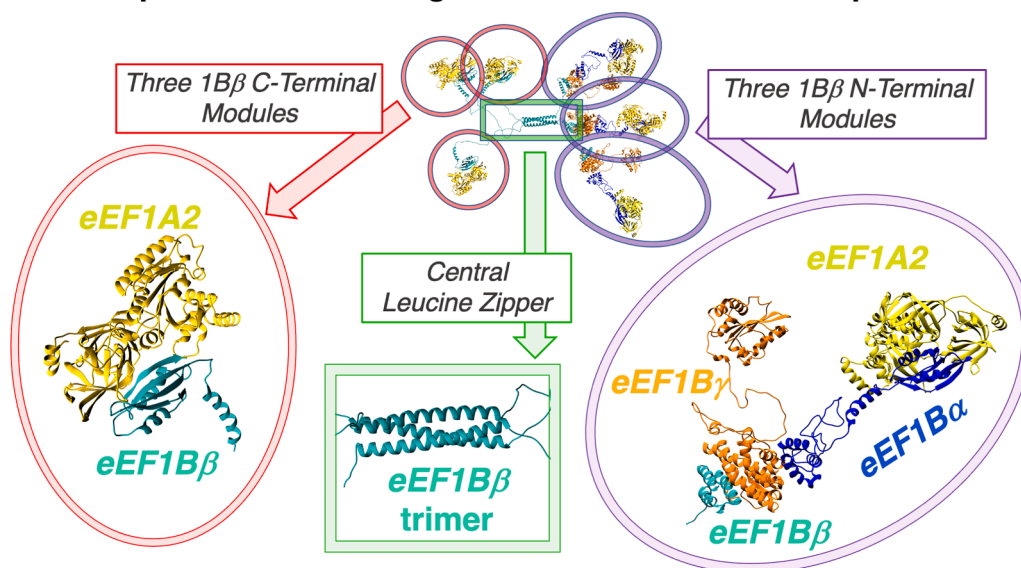


Fig. 3. Structural organization of the eEF1H complex. eEF1B α , eEF1B β and eEF1B γ subunits are painted in blue, green and orange, respectively. eEF1A2 is painted in yellow. The structural model of the eEF1B complex is depicted using previously published PDB file [69]. The structural model of eEF1A2 was developed using the PDB structures 4COS and 6RA9. The proposed structure is assembled upon a template of a trimer of eEF1B β subunits, held together by a Central Leucine Zipper region (green rectangle). The C-terminal domain of each eEF1B β subunit interacts with one eEF1A2 molecule (pink ovals). The N-terminal domain of each eEF1B β subunit interacts directly with one eEF1B γ subunit, with the latter engaging a complex of eEF1B α subunit and another eEF1A2 molecule (purple ovals). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

of eEF1B α decreased, and the GDP-bound form of the neuron-specific eEF1A2 variant may accumulate in the cell, perhaps fulfilling some non-translational role specific to eEF1A2*GDP.

Immunohistochemical analysis of eEF1B subunits in normal and cancerous tissue indicated the possibility of their non-overlapping localization. In normal cardioesophageal tissue, localization of eEF1B α , eEF1B β and eEF1B γ subunits was nuclear-cytoplasmic, whereas in the cancerous tissue the only eEF1B γ subunit was revealed in the nucleus [86]. eEF1B α was present in the nuclei of normal lung cells, eEF1B β was found in the nuclei of lung carcinoma cells, and eEF1B γ showed nuclear localization in both normal and carcinomatous tissues [87]. However, all three subunits of eEF1B were present in both the cytoplasm and the nucleus of lung carcinoma cell line A549, suggesting cell/tissue-specific effects [87].

Hence, a pool of individual subunits can exist separately from the eEF1B complex in human tissues. We believe that this is mainly the result of the impossibility of newly synthesized subunits entering the complex, which could be modulated by their post-translational modification. Given the known regions of interaction between the subunits [69], we have analyzed data on modifications of eEF1B subunits presented by the PhosphositePlus database (accessed 18 July 2022). Five phosphorylated residues were found in the N-terminal domain of eEF1B α , whereas in eEF1B γ the post-translational modifications were found only in the interaction region comprising residues 144–161. Of those, no modifications in eEF1B α , and the modifications of K147 and R149 in eEF1B γ are promising candidates, as they may affect eEF1B γ –eEF1B α interaction, according to the eEF1B structure [69]. Unexpectedly heavy phosphorylation of the linker region of eEF1B α was detected, where 8 residues were found to be phosphorylated in the short region comprising 79 to 95 residues. The phosphorylation of the linker may directly or indirectly influence the overall structural dynamics of eEF1B α , and affect the self-inhibitory interaction between N- and C-terminal parts of the protein in its non-complexed state [67].

In eEF1B β , a short region comprising 11 to 29 residues interacts with eEF1B γ [69]. Remarkably, it contains a few modified residues, including phosphorylated tyrosines, monomethylated arginine, acetylated or ubiquitylated lysines. From these, modifications of K15, R24, and Y26 may have an effect on eEF1B β binding to eEF1B γ . So far, in eEF1B γ , sixteen post-translationally modified residues were found in its extended region, interacting with eEF1B β , however the only K17 modification may influence the eEF1B γ –eEF1B β interaction, according to the eEF1B structure [69].

All the residues whose modification may influence the interaction of

eEF1B α and eEF1B β with eEF1B γ are depicted in Fig. 4. It should be noted that the PhosphositePlus database reports on post-translational modifications of the subunits most likely involved in the eEF1B complex rather than the free forms. One may suggest that more sites are open to modification in the individual subunits than in complexed ones. To address this question, one needs to conduct special studies of the eEF1B subunit modifications under conditions where a portion of individual subunits is increased, as in some human cancer tissues.

Individual eEF1B subunits or their subcomplexes can participate in translation, albeit with less efficiency in the case of individual eEF1B α . However, several processes that show little or no relation to translation are known to be linked to eEF1B subunits [62]. For instance, eEF1B α may be involved in repair of DNA double-strand breaks [89,90] and cytoskeleton rearrangements [91]. eEF1B β is related to adipose tissue inflammation and diabetes [92,93]. eEF1B γ negatively regulates the motor-mediated transport of membrane organelles along microtubules [94]. It is involved in the retrograde transport from Golgi apparatus to endoplasmic reticulum [95] and microRNAs transfer to extracellular fluid [96]. It is not clear yet whether eEF1B α , eEF1B β , and eEF1B γ fulfill these and other functions in an individual or complex state.

To understand a variety of non-translational processes coupled to eEF1B β and eEF1B γ subunits in human cells, we identified their possible protein partners by co-immunoprecipitation with subsequent mass spectrometric analysis. Several bioinformatic approaches were used to classify the biological functions of the partners [97–100]. Based on the similarity of the processes in which the subunits are involved, we could predict that the eEF1B β –eEF1B γ complex is involved in cell-cycle regulation, chromatin and nucleosome remodeling, mRNA splicing and turnover. Individual eEF1B β may be linked to DNA replication and repair, adipose tissue biology, epithelial-to-mesenchymal transition, microRNA turnover, protein chaperoning and degradation. Individual eEF1B γ may be coupled to viral RNA transcription, oxidative stress response, cytoskeleton–membrane linking, and cellular trafficking. We do not consider this list to be in any way complete, however, it gives some idea of what to expect from the further development of eEF1B studies.

4. Concluding remarks

In this short review, we present a summary of our knowledge of the eEF1 family of mammalian translation elongation factors, attained through years of studies by several generations of researchers. We analyzed the data on spatial organization and post-translation

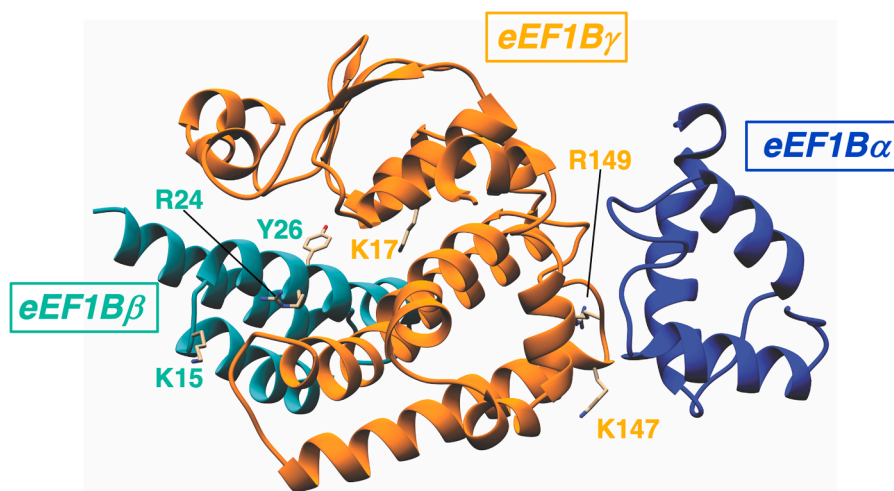


Fig. 4. Structural model of the interface of the N-terminal domains of eEF1B α , eEF1B β and eEF1B γ subunits in the eEF1B complex. The eEF1B α , eEF1B β and eEF1B γ domains are painted in blue, green and orange, respectively. The indicated residues can undergo posttranslational modifications, potentially influencing protein-protein interactions in the complex. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

modifications of eEF1A1 and eEF1A2, and provided examples of their various involvements in a variety of non-translational processes. The structural models of eEF1B subunits, their organization in the sub-complexes, and the trimeric structural model of the whole eEF1B complex are described. The possibility of the individual subunits' involvement in some non-translational processes is also discussed.

We believe that this description of the structure and possible functions of the members of the eEF1 family during and beyond translation will encourage further studies. These could include investigating the role of post-translational modifications of eEF1 proteins, deciphering the structural organization of eEF1A1 and eEF1A2 protein variants, research on non-translational functions of the members of the eEF1 group, and identification of auxiliary members of this group, together with further study of their possible synergistic actions in health and disease.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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