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REVIEW

Eukaryotic Type Translation Initiation Factor 2: Structure–Functional Aspects

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Abstract—Translation initiation factor 2 (IF2) is one of key components of the translation initiation system in living cells. In bacteria IF2 is a multidomain monomeric protein, while in eukaryotic and archaean cells e/aIF2 is heterotrimer ($\alpha\beta\gamma$). Data, including our own, on eukaryotic type translation initiation factor 2 (e/aIF2) structure and functioning are presented. There are also new data on initiation factors eIF5 and eIF2B that directly interact with eIF2 and control its participation in nucleotide exchange.

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In eukaryotes and archaeans, translation initiation factor 2 (IF2) is the heterotrimeric protein e/aIF2$\alpha\beta\gamma$ interacting with GTP and methionyl-$tRNA_i$ (Met-$tRNA_i$); it is functionally similar to bacterial IF2. In bacteria IF2 is a monomeric protein and has no homology with any of the e/aIF2 subunits. Sequence homologies between eukaryotic and archaean e/aIF2 subunits α, β, and γ reach 40-42, 27-30, and 50-57%, respectively. Subunits of archaean aIF2 are smaller than their eukaryotic homologs (Scheme).

Factor e/aIF2 plays the key role in initiation of protein biosynthesis. In the presence of GTP, e/aIF2 specifically recognizes initiator Met-tRNA and forms with it a stable triple complex Met-$tRNA_i$·e/aIF2·GTP. In this form e/aIF2 delivers Met-$tRNA_i$ into the P site of the small ribosomal subunit where exact recognition of initiating codon on mRNA takes place due to codon–anticodon interaction with subsequent hydrolysis of GTP. In complex with GDP, translation initiation factor 2 leaves the ribosome, then bound GDP is replaced by GTP and the factor is again able to form the stable complex with Met-$tRNA_i$. In eukaryotic cells eIF2 plays the role of total translation regulator at the stage of initiation. The regulatory function of eIF2 is mediated via phosphorylation–dephosphorylation of its subunit α. Most stresses cause almost immediate phosphorylation of eIF2 subunit α, which results in inhibition of intracellular translation initiation. In archaeans the role of aIF2 in translation regulation has not been identified, although the system of specific phosphorylation of this α subunit has been detected as well [1].

Although already in 1970s factor e/aIF2 had become the object of intense investigation, structural investigations of the factor began much later and significant progress in these studies was achieved only in the 2000s. First, structures of the separate factor subunits were determined [2-10], then structures of heterodimers [11, 12] and of an undersized heterotrimer [13]. However, the structure of the full-sized heterotrimer translation initiation factor 2 was determined for the first time in our laboratory in 2008 [14]. It should be noted that almost all structural investigations were done for the aIF2 factor from hyperthermophilic archaeans. Only the α subunit structure from both archaeans and eukaryotes was determined. The delay in X-ray investigations of eukaryotic eIF2 is due to technical difficulties in the isolation of this factor from eukaryotic cells, complications in eukaryotic gene cloning and generation of superproducers, as well as by problems connected with obtaining perfect crystals of eukaryotic proteins. Sufficiently high homology in sequences of archaean and eukaryotic e/aIF2 subunits enables the use of aIF2 crystallographic structures in its

Abbreviations: a.a., amino acid residue; CTD, *C*-terminal domain; ds, double-stranded; HCV, hepatitis C virus; IF2, translation initiation factor; MFC, multifactor complex; NTD, *N*-terminal domain; P_i, inorganic phosphate.

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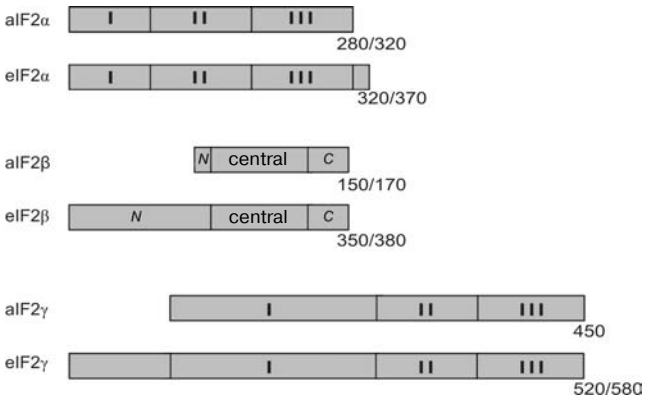

Primary structures of e/aIF2. *N* and *C* are *N*-terminal α-helix of aIF2β or *N*-terminal domain of eIF2β and *C*-terminal domains of e/aIF2β

different states for analysis of multiple biochemical data on eukaryotic eIF2 functioning, which significantly contributes to our understanding of protein synthesis initiation in archaeans and eukaryotes.

GENERAL ORGANIZATION OF THE HETEROTRIMERIC aIF2 MOLECULE

The aIF2 molecule resembles English letter L in its overall contour, the long side of which is formed by subunit α, the short by subunit β, and the angle by subunit γ [14] (Fig. 1). There is no contact between subunits α and β. The aIF2 subunit α consists of three domains. Domains I (OB-fold with the "Greek" key topology) and II (bundle of five α helices) are the compact "rigid" structure. This "rigid" block and domain III (αβ-fold), in turn, exhibit high mobility relative to each other. A long mobile loop, whose ends are flanked by 3_{10} helices, is located between the β3 and β4 strands of domain I. In the eukaryotic protein this loop is highly conservative and contains the invariant residue Ser51, the phosphorylation site for a number of serine protein kinases activated within cells in response to stress conditions [15-20]. The interaction of eIF2α with such protein kinases results in conformational alterations in the protein: the β1-β2 loop and *C*-terminus of the β3 strand of the α subunit are shifted by 1.6 and 1.8 Å, respectively [21]. In this case 3_{10} helices, closing the β3-β4 loop, are unfolded, and Ser51 undergoes phosphorylation. Phosphorylation of the eIF2 α subunit at this residue results in inhibition of protein biosynthesis. Unlike eukaryotes, in archaeans there are two invariant serine residues in positions 44 and 48 that are potentially capable of being phosphorylated. It was shown in *in vitro* experiments that Ser48 in aIF2 is phosphorylated by eukaryotic dsRNA-dependent PKR kinase activated upon infection by viruses as well as by the archaean homolog of this protein designated as Ph0512p [1]. Spatially, Ser48 occupies in aIF2α the place in which Ser51 is located in the eukaryotic subunit α. A similar translation regulation via α subunit phosphorylation-dephosphorylation might also exist in archaeans.

A distinctive feature of eukaryotic subunit α is the presence of mobile acidic *C*-terminal "tail" formed by a short α helix with a continuous disordered region of 30-40 a.a. (in the case of *Drosophila melanogaster* the length of subunit α reaches 60 a.a.). The functional role of this "addition" is not clear, but there are reports that the *C*-terminal "tail" of eIF2α contains a caspase cleavage site [22, 23].

The aIF2 subunit β can be also structurally divided to three parts. These are the disordered *N*-terminal part that acquires α helical conformation in complex with subunit γ [11, 13, 14] and two structurally independent domains: central (antiparallel β sheet has three α helices located on one side) and *C*-terminal (antiparallel β sheet). The *N*-terminal α helix is connected to the central domain by a flexible crosspiece, while the central and *C*-terminal domains are, in turn, joined by the long α4 helix. The *C*-terminal or zinc-binding domain contains the highly conservative and functionally important motif $CX_2CX_{17/19}CX_2C$, mutations in which result in pronounced defects in the function of eIF2 [24-26]. A zinc atom involved in maintenance of the *C*-terminal domain spatial structure is coordinately associated with two pairs of these cysteine residues [2, 4]. The archaean subunit β is almost half in size and homologous to the *C*-terminal part of eukaryotic subunit β. The *N*-terminal domain of subunit β, characteristic of eukaryotes, is variable in length and amino acid composition and contains three polylysine blocks of 6-8 lysine residues, each located at a

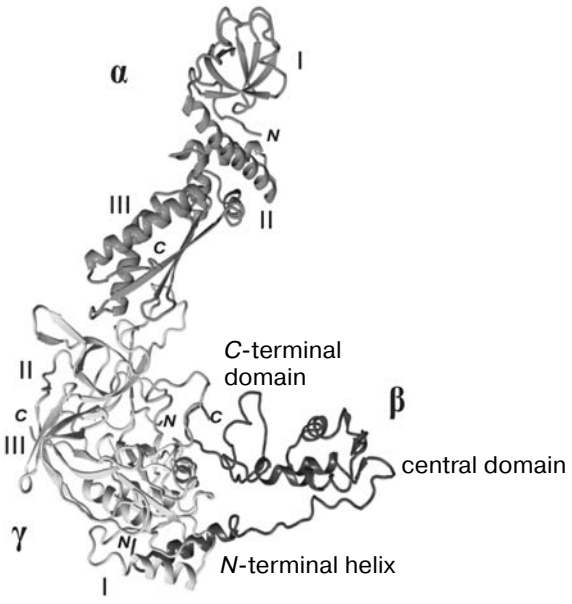

Fig. 1. Spatial structure of heterotrimeric aIF2 of *Sulfolobus solfataricus.*

significant distance from each other. It has been shown that these polylysine repeats are necessary for the interaction of eIF2 with mRNA [27], eIF2B [28], and eIF5 [29].

The e/aIF2γ central subunit is the ribosome-dependent GTPase and structurally belongs to the family of EF1A elongation factors (previous name is EF-Tu) and e/aEF1A that catalyze codon-dependent aminoacyl-tRNA binding to the ribosome A site. Subunit γ is a three-domain protein in which domains II and III (antiparallel β sheets) form a united structural block connected to the G domain (β sheet surrounded by α helices) by a flexible crosspiece. The distinctive feature of the G domain of e/aIF2 subunit γ is the presence of vitally important zinc-binding module, the "zinc ribbon" (β2-β3 loop) forming the projection between domains I and II. It was shown that the absence of zinc does not alter the "zinc ribbon" structure [13]. In eukaryotes this zinc-binding module is somewhat longer than in archaeans, and four cysteine residues are not strictly conservative. It should be noted that at the *N*-terminus in yeast eIF2γ there is a variable "tail" of from 12 (*S. pombe*) to 89 a.a. (*Saccharomyces cerevisiae*), deletion of which does not influence protein activity, whereas point mutations in this region result in decrease in cell growth [30].

In the G domain of e/aIF2γ there are functionally important loop switchers – switch1 and switch2 – and the nucleotide-binding "pocket" formed by conservative motifs DAPG, NKIE, SALH, and GHVDHGKT characteristic of GTP-binding proteins. It is known that upon transition from the GTP- to the GDP-bound state there is a significant shift of domains II and III relative to domain G in EF1A structure and simultaneous coordinated conformational changes in the loop switchers switch1 and switch2. In this case switch sites are converted from "ON" conformation into "OFF" conformation, while the "body" of the protein EF1A proper is transformed from active "closed" into inactive "open" conformation. Unlike EF1A structure, no significant changes were found in tertiary structure of the aIF2 subunit γ upon replacement of GTP by GDP. In all known structures of subunit γ [6, 8, 9, 11-14] domains G, II, and III are spatially drawn together and correspond to the "closed" GTP-bound active form of EF1A. However, the situation with conformational transitions of the switch sites of subunit γ is not so unequivocal. Thus, subunit γ without nucleotide and in complex with GDP or GTP [8, 9, 11], as well as within GDP-bound βγ dimer [11] and $\alpha_{\text{domain III}}\beta_{\alpha 1\text{-helix}}\gamma$-heterotrimer [13] contains switch sites in "OFF" position or close to this state. However, switch2 of subunit γ within the αγ dimer in complex with GTP [12] and within shortened heterotrimer $\alpha_{\text{domain III}}\beta\gamma$ complexed with GDP-P_i [13] acquires "ON" conformation, whereas switch1 in $\alpha_{\text{domain III}}\beta\gamma$·GDP-$P_i$ acquires a unique conformation that does not correspond to either "ON" and "OFF" state. In other structures [8, 11-13] atom coordinates for most amino acid residues of the switch1 loop of subunit γ are not detected in general due to high mobility of the polypeptide chain in this region.

It should be noted that different authors try to catch the correlation between conformational alterations in switch sites of subunit γ and the nature of the nucleotide bound to it, as well as with its interactions with subunits α and β within the heterotrimer. According to some authors, protein–protein interactions within heterotrimer help subunit γ achieve and hold the necessary conformations of switch sites at different stages of the nucleotide replacement cycle. To understand to what extent conformational alterations in the subunit γ switch1 and switch2 regions correlate with nucleotide binding to subunits α and β, our laboratory in cooperation with the group of structural investigations of ribosomal proteins of the Institute of Protein Research of the Russian Academy of Sciences compared conformations of these regions in all presently known structures of subunit γ [14]. It appeared that conformations of switch sites of subunit γ in all presently known structures are evenly distributed between two extreme states – "ON" and "OFF" – of the EF1A switch sites. Moreover, conformations of subunit γ switch sites in four aIF2 molecules [14] localized in an asymmetrical part of the crystal unit cell, differ only insignificantly from each other. Thus, our data indicate that flexible loops in switch sites of subunit γ randomly change their conformations independently of the bound nucleotide nature and/or interaction with subunits α and β.

Intersubunit interactions in aIF2 are mainly due to hydrogen bonding. Contact regions are formed in pairs between aIF2 subunits: between loops β6-β6, β7-β8 of subunit α domain III and β7-β8, β13-β6 of subunit γ domain II, respectively. The amphiphilic *N*-terminal α helix of subunit β forms a vast hydrophobic interface with the surface of the G domain of subunit γ formed by the "back" side of nucleotide-binding "pocket". There is also a second site for subunit β binding to subunit γ, but it differs in spatial structures of αβγ heterotrimer and βγ of heterodimer. In two presently known spatial structures of heterotrimeric aIF2, the *C*-terminal domain of subunit β forms an intersubunit interface with the G domain of aIF2γ [13, 14]. However, in the structure of aIF2 βγ dimer from the archaean *Pyrococcus furiosus*, the γ subunit interacts with central domain of subunit β rather than with the *C*-terminal domain [11]. The authors try to explain the resulting discrepancy as a possible alteration of subunit β orientation due to the transition aIF2·GDP to aIF2·GTP, i.e. subunit β interacts with subunit γ either by the central or by the *C*-terminal domain.

Comparison in our work [14] of all known structures of aIF2 and its subunits revealed high conformational mobility in subunits α and β. It appeared that in all structures domain III of subunit α and the *N*-terminal α helix of subunit β retain their positions relative to the γ subunit. In essence, the eukaryotic type IF2 can be considered as

a molecule consisting of a conformationally stable central part and two mobile "wings". The central part includes subunit γ, domain III of subunit α, and the *N*-terminal α helix of subunit β, while the "wings" include domains I and II of subunit α, on one side, and the central and *C*-terminal domains of subunit β on the other side. Evidently, such high intramolecular mobility of IF2 is necessary for its interactions with various ligands during its functioning.

INTERACTION OF e/aIF2 WITH INITIATOR Met-tRNA AND SMALL RIBOSOMAL SUBPARTICLE

The structure of triple complex Met-$tRNA_i$·e/aIF2·GTP has not yet been defined. Now it is only possible to imagine schematically the map of interactions between its components based on genetic and biochemical data. A necessary condition for initiator tRNA binding to e/aIF2 is the presence of GTP [31-35]. Unlike EF1A, whose affinity in the GDP-bound form to aminoacyl-tRNA is 10,000 times lower than in the GTP-bound form [36], the dissociation constants of Met-$tRNA_i$ with e/aIF2·GTP and e/aIF2·GDP differ by only one or two orders of magnitude [12, 37]. It was shown by Kapp and Lorsch [37] that eIF2·GDP interacts with Met-$tRNA_i$ with the same affinity as with deacetylated initiator tRNA. In this case eIF2·GTP interacts with deacetylated initiator tRNA with the same affinity as with eIF2·GDP, but the affinity of eIF2 to GTP increases in the presence of Met-$tRNA_i$ by one order of magnitude, whereas in the presence of deacetylated initiator tRNA remains practically unchanged: K_d eIF2·GTP = $1.7 \cdot 10^{-6}$ M; K_d [eIF2·Met-$tRNA_i$]·GTP = $2 \cdot 10^{-7}$ M; K_d [eIF2· $tRNA_i$]·GTP = $3.8 \cdot 10^{-6}$ M. This points to coupling of Met-$tRNA_i$ and GTP binding to eIF2. No coupled GDP and Met-$tRNA_i$ interaction with eIF2 is observed. It follows from the aggregate of these data that the $tRNA_i$ residue of initiator Met-tRNA remains bound without alterations with both the GTP-bound and GDP-bound eIF2 forms, whereas a methionine residue interacts only with the GTP-bound eIF2 form. It is assumed that in the case of GTP hydrolysis the affinity of eIF2 to the methionine residue disappears due to conformational alterations in the methionine-binding "pocket" of the factor.

A distinctive feature of initiator tRNA is the presence of the A1-U72 pair in the acceptor "stem". It was shown that initiator tRNA synthesized from corresponding cDNA within plasmid with the A1-U72 replaced by G1-C72 does not restore growth of yeast cells in which genes of all initiator tRNA were destroyed in advance [38]. It appeared that such mutation decreases the affinity of Met-$tRNA_i$ to e/aIF2 [37, 39, 40]. Evidently, strong G1-C72 pairing prevents initiator Met-$tRNA_i$ binding to e/aIF2, whereas the archaean factor aIF2 exhibits practically equal affinity both to its own Met-$tRNA_i$ and the bacterial initiator Met-$tRNA_f$ in which the 5′-terminal cytosine is not paired with the opposite adenine of the 3′-terminal site [12, 40]. The replacement of A1-U72 by A1-C72 and G1-U72 in yeast Met-$tRNA_i$ decreased its affinity to eIF2 factor by 10- and 14-fold, respectively [37]. The authors of that work believe that such mutations result in deviations in the *CCA* terminus direction relative to the acceptor "stem" due to alteration in geometry of nucleotide pairs in positions 1 and 72 in Met-$tRNA_i$. In other words, they believe that the A1-U72 pair in Met-$tRNA_i$ assigns a certain orientation of a methionine residue accepted at the *CCA* terminus, which is necessary for its optimal interaction with the methionine-binding "pocket" on eIF2. Thus, this indicates that a methionine residue together with the A1-U72 pair are the main elements of Met-$tRNA_i$ recognition by eukaryotic translation initiation factor 2.

The important role of the methionine residue in Met-$tRNA_i$ recognition by eIF2 factor was demonstrated in experiments with initiator tRNA acylated with a foreign amino acid [41, 42]. Thus, Ile-$tRNA_i$ [42] and Gln-$tRNA_i$ [41] did not initiate protein synthesis. However, Val-$tRNA_i$ was able to function in translation initiation, though it was four times less efficient compared to Met-$tRNA_i$ [41]. It was shown in experiments with archaean factor aIF2 that joining methionine to $tRNA^{Val}$ increased its affinity to aIF2·GTP by two orders of magnitude compared to Val-$tRNA^{Val}$ or Val-$tRNA_i$. The dissociation constant of the aIF2·GTP complex with elongater Met-$tRNA^{Met}$ only four times exceeded that with Met-$tRNA_i$ [40].

Based on the structure with Phe-$tRNA^{Phe}$·EF-Tu·GTP [43], on the e/aIF2 subunit γ the hypothetical methionine-binding "pocket" formed by amino acid residues of domain II chains β7, β8, and β14 was identified. Amino acid substitutions in the corresponding region (Gly235Asp (β8) in aIF2γ of *P. abyssi* [40] and Gly397Ala (β14) in eIF2γ of *S. cerevisiae* [8]) resulted in the loss of affinity to Met-$tRNA_i$ by translation initiation factor 2. Moreover, mutations Gly397Ile (β14) and Arg319Asp (β7) in eIF2γ of *S. cerevisiae* were lethal for cells [8]. Evidently, the replacement of Gly397 by the larger in size Ala or Ile results in steric conflict between these amino acid residues and ribose of A76 in $tRNA_i$. Site-directed mutagenesis also revealed the involvement of switch1 of the e/aIF2γ G domain in Met-$tRNA_i$ binding. For example, mutations Asn135Lys [26] and Asn135Asp [44] in eIF2γ of *S. cerevisiae* weakened the interaction of eIF2 with Met-$tRNA_i$ by 2.4- and 4-fold, respectively. Mutation Tyr142His resulted in almost complete loss of the ability of eIF2 factor to bind Met-$tRNA_i$ [45]. It should be noted that Tyr142 in eIF2γ corresponds to His67 in EF-Tu in *Thermus aquaticus*, which is involved in stacking interaction with an amino acid residue in Phe-$tRNA^{Phe}$. Upon replacement of the whole

sequence $_{39}$EELKR$_{43}$ of the switch1 site in aIF2γ of *S. solfataricus* by a cluster of five alanine residues, there was also complete loss of IF2 activity in the interaction with initiator Met-tRNA [11].

The use of cross-linking technique also showed that subunits β and γ are also involved in the initiator binding of tRNA to the eukaryotic factor eIF2 [46-48]. However, contradictory data concerning the involvement of this subunit in Met-tRNA$_i$ binding were obtained in experiments with preparations of eIF2 deficient in subunit β. According to data of works [15, 49, 50], the absence of the β subunit from eIF2 preparations had no effect on binding to GTP, GDP, Met-tRNA$_i$, and 40S ribosomal subparticle. However, the inverse situation was demonstrated [51, 52]: eIF2 preparations depleted in subunit β became incapable of forming the triple complex Met-tRNA$_i$·eIF2·GTP and complex with 40S subparticle, but in this case the interaction with the nucleotide remained at the previous level. The absence of subunit α from eukaryotic factor eIF2 decreases its constant of association with Met-tRNA$_i$ by only five-fold as compared with complete eIF2 [53]. However, in archaeans subunit α plays a role that is far from the last in formation of the triple complex Met-tRNA$_i$·aIF2·GTP. It was shown that the isolated γ subunit of aIF2 in complex with GTP interacts with Met-tRNA$_i$ and protects it against spontaneous deaminoacylation much more weakly than within the complete factor or αγ dimer [12, 40, 54]. Subunit α, namely its third domain, cardinally increases the affinity of aIF2γ to Met-tRNA$_i$, while subunit β does not influence this process [40]. Thus, this suggests that in eukaryotes and archaeans the firm binding of Met-tRNA$_i$ to e/aIF2 is provided in different ways. However, in both cases the γ subunit is responsible for recognition of the methionine residue by Met-tRNA$_i$.

There are now two hypothetical models of aIF2αγ interaction with initiator Met-tRNA that differently demonstrate the mechanism of participation of the α subunit in stabilization of Met-tRNA$_i$ binding. In essence, the first model repeats the known structure of the Phe-tRNA^{Phe}·EF-Tu·GDPNP complex where only subunit γ has contact with tRNA (Fig. 2a) [12]. The authors of this model supposed that subunit α indirectly influences the process of Met-RNA$_i$ binding to the aIF2 γ subunit, in some way helping the GTP-bound γ subunit to acquire in switch1 and switch2 regions conformations in which the γ subunit becomes able to form a stable complex with Met-RNA$_i$. In 2007 in our laboratory, in cooperation with the group of ribosomal protein structural investigations of the Institute of Protein Research, the structure of the *S. solfataricus* aIF2γ co-crystallized with GDPNP/GDP mixture was determined, where the pyrophosphate molecule was revealed in the groove, sterically suitable for the tRNA acceptor "stem". Analysis of this structure suggested an alternative model of the interaction of aIF2αγ with initiator Met-RNA [6]. The acceptor tRNA "stem" orientation in this model (Fig. 2b) is perpendicular to that in the above-described model (Fig. 2a). In such position of the acceptor "stem", tRNA forms a large zone of contact with the α subunit domain III, which explains stabilization of the triple complex in αγ heterodimer of aIF2. Moreover, this model demonstrates how specific recognition of initiator tRNA can occur: the unique protrusion at the initiator tRNA surface formed by two protruding nucleotides is sterically suitable for a site with concave surface on domain III of the α subunit.

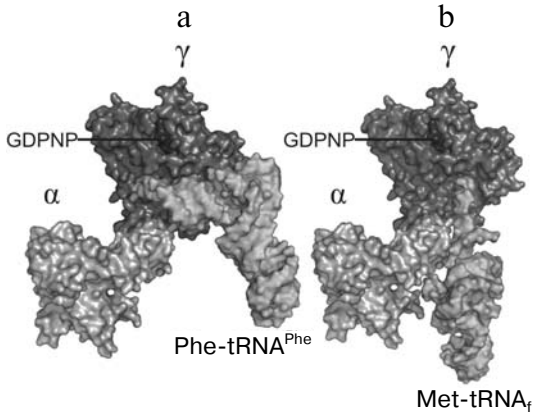

Fig. 2. Hypothetical models of tRNA·aIF2αγ·GTP complexes. a) Model mimicking the tRNA·EF-Tu·GTP triple complex [11]. b) Model designed by O. S. Nikonov on the basis of his own structural data [6].

The site of the triple complex Met-tRNA$_i$·eIF2·GTP binding on the small ribosomal subparticle was located using antibodies and covalent cross-linking technique. The Met-tRNA$_i$·eIF2·GTP cross links with 40S subparticle point to the closeness to the triple complex of such proteins as S3, S3a, S6, S13/16, S15, and S15a [48, 55]. Antibodies to proteins S3a, S6, S13/16, S19, and S24 inhibited the Met-tRNA$_i$·eIF2·GTP binding, while antibodies to S2, S3, and S17 had no effect on this function of 40S subparticles [56]. Comparison of these data with those on localization of ribosomal proteins S3a, S6, S13/16, S19, and S24 on the ribosome obtained by immune electron microscopy [57-59] suggests that eIF2 binds to the 40S subparticle between its "head" and the "platform".

INVOLVEMENT OF FACTORS eIF1, eIF1A, eIF2B, eIF3, AND eIF5 IN eIF2 ACTIVITY

In 2000 it was shown in Laboratory of Hinnebusch that triple complex Met-tRNA$_i$·eIF2·GTP together with factors eIF1, eIF3, and eIF5 form a stable multifactor complex (MFC) existing in the cell outside the ribosome [60]. It is seen in Fig. 3 that the platform for MFC assembly is the huge multisubunit factor eIF3 where subsequent

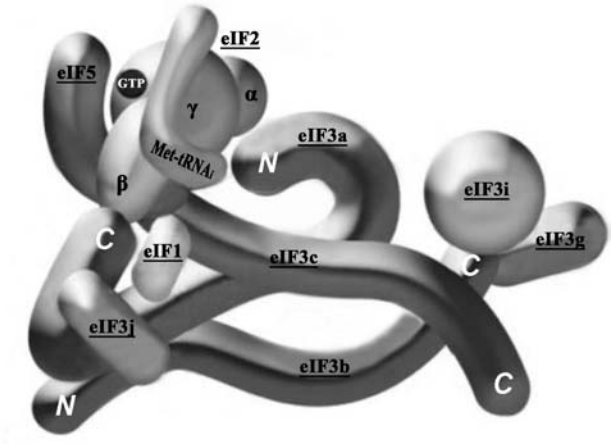

Fig. 3. Three-dimensional model of multifactor complex based on experimental data on binding of isolated subunits of translation initiation factors. Letters *N* and *C* correspond to the *N*- and *C*-termini of eIF3 subunits, respectively. The figure is from [62] with slight modifications.

protein–protein interactions take place: the *N*-terminal domain of the eIF2 subunit β directly interacts with the *C*-terminal domain of eIF3a and through eIF5CTD with the *N*-terminal domain of eIF3c; eIF1 binds factors eIF3cNTD, eIF3aCTD, eIF5CTD, and eIF2βNTD. Factor eIF1A is a labile component of the multifactor complex and can interact with factors eIF2, eIF3, and eIF5 due to the unstructured *N*-terminal "tail" [61]. It has been shown that the integrity of the multifactor complex is necessary for normal translation [60, 62]. It becomes evident that the MFC is a unique element that is extremely advantageous from the point of view of kinetics, energetics, reliability, and regulation of translation initiation. There are several examples of coordinated activity of MFC factors.

It is known that reduction in the active eIF2 pool in yeast cells upon amino acid deficiency causes translation of mRNA of transcription factor GCN4 [16]. It was shown that superproduction of the eIF3c *N*-terminal domain results in activation of GCN4 synthesis, while an increase in the content of full-sized eIF3c subunit in such cells inhibits translation of GCN4 mRNA [62]. It appeared that eIF3 promotes the binding of the triple complex Met-tRNA$_i$·eIF2·GTP to the 40S ribosomal subparticle. In the case of superproduction of the eIF3c *N*-terminal domain, the bulk of factor eIF2 passed into inactive non-full-sized complex (Met-tRNA$_i$·eIF2·GTP)·eIF5·eIF3cNTD·eIF1. As a result, the intracellular content of eIF2 decreased, and exactly this stimulated GCN4 translation.

Another example of coordinated functioning of MCF factors is recognition of the initiating AUG codon. Although initiator tRNA is responsible for the initiating codon recognition, genetic studies have shown that protein factors influence the accuracy and efficiency of this process. Thus, mutations in the yeast initiating ATG triplet of *his4* gene encoding an enzyme necessary for histidine biosynthesis provides for cellular His$^-$ phenotype. Such mutants survive only in the presence of histidine in the medium. Genetic manipulations made it possible to obtain revertants of these mutants able to grow in the absence of the corresponding metabolite [24-26, 63-65]. It appeared that compensation of mutations inactivating the *his4* gene is caused by suppressor mutations in the *sui* genes (suppressors of initiator codon mutations). Point mutations in *sui* genes resulted in initiation of translation from the UUG codon (position +3) in the absence of AUG in HIS4 mRNA. The genes *sui1*, *sui2*, *sui3*, *sui4*, and *sui5* encode eIF1, eIF2α, eIF2β, eIF2γ, and eIF5, respectively. *In vitro* experiments have shown that suppression mutations in each eIF2 subunit caused different character defects in formation of Met-tRNA$_i$·eIF2·GTP triple complex. For example, Lys or Asp substitution in Asn135 of yeast eIF2γ weakened the interaction of eIF2 with Met-tRNA$_i$ [26, 63]. Mutations in the zinc-binding domain of eIF2 subunit β, such as Ser264Tyr or Lys254Pro, resulted in eIF5-independent GTPase activity of eIF2 and subsequent premature dissociation of Met-tRNA$_i$ from eIF2 [24, 25]. The double mutation Tyr131Ala/Ser132Ala obtained by site-directed mutagenesis in the central part of the amino acid sequence of yeast eIF2 subunit β weakened the interaction between subunits β and γ and also resulted in HIS4 mRNA translation initiation from the UUG codon in the absence of AUG [66]. The Glu31Arg mutation in the *N*-terminal domain of eIF5 resulted in doubling of the activity of the mutant protein in stimulation of GTP hydrolysis compared to the wild-type protein, and it enabled initiation of translation from UUG codon in the absence of AUG in HIS4 mRNA [26].

Relatively recent kinetic studies have shown that eIF5-induced GTP hydrolysis in the presence of 40S subparticle is the two-step reaction, while inorganic phosphate release into solution is the "control passage point" for correct codon-anticodon pairing between AUG codon and initiator Met-tRNA rather than GTP hydrolysis proper [67]. The first stage of GTP hydrolysis occurs after MFC joins the 40S ribosomal subparticle. This reaction is rapid (rate constant is 11 sec^{-1}) with subsequent establishment of equilibrium. Thus, at this stage reversible GTP hydrolysis occurs. Factor eIF2 remains bound to inorganic phosphate and does not release it into solution until the codon–anticodon pairing between AUG codon and initiator Met-tRNA occurs. The second stage reaction of inorganic phosphate release or irreversible GTP hydrolysis is much slower (rate constant is 0.64 sec^{-1}) with equilibrium displacement towards formation of eIF2·GDP. The transition of the GTP hydrolysis reaction from the first to the second stage is regulated by scanning factor eIF1, working in conjunction with eIF1A, and in this case the action of these factors in the pair is mutually enhanced. Factor eIF1 stimulates moving

of the scanning 48S complex along mRNA whenever the P site of the ribosome is not occupied by the AUG codon [68]. In such cases the eIF1 factor adjacent to the P site blocks inorganic phosphate release from complex eIF2·GDP-P_i [67, 69]. The eIF1 factor stops acting as a "gate keeper" when the P site is occupied by an initiating codon. After correct codon–anticodon pairing, the 48S complex undergoes conformational alterations [69, 70]. As a result, eIF1 leaves its binding site, and inorganic phosphate is released into solution [67, 69, 71]. It was shown by FRET analysis (fluorescence resonance energy transfer) that the archaean factor aIF1 can inhibit binding of mRNA containing noncanonical initiating codon AUU to the 30S·aIF1A·Met-$tRNA_i$·aIF2·GTP complex [72]. Evidently, in archaeans factor aIF1 also controls recognition of the initiating codon AUG.

It is still not finally clear how factor eIF5 works; it activates the GTPase center of eIF2, or the GTPase center is formed in cooperation with eIF2, eIF5, and the small ribosomal subparticle. Thus, mutations in zinc-binding domain of eIF2 subunit β induced the GTPase activity of eIF2 in the absence of eIF5 and the 40S ribosomal subparticle [24, 25]. However, one cannot exclude the possibility that the GTPase center of eIF2 is not fully completed and some components of the small ribosomal subparticle and eIF5 are involved in its completion. Based on homology of the eIF5 *N*-terminal domain and zinc-binding domain of e/aIF2 subunit β, Thompson et al. proposed in 2000 a model that describes the mechanism of eIF5-dependent GTP hydrolysis [73]. According to this model, the zinc-binding domain of eIF2β prevents premature GTP hydrolysis. After the interaction of Met-$tRNA_i$·eIF2·GTP with eIF5, the latter displaces subunit β from factor eIF2 and thus induces GTP hydrolysis.

The mechanism of functioning of the five-subunit factor eIF2Bαβγδε also remains unclear. Two possible ways of eIF2B-dependent eIF2·GDP conversion to eIF2·GTP are described in the literature. According to the so-called triple complex mechanism, regeneration of eIF2·GTP should pass through the stage of provisional eIF2B·[eIF2·GDP]·GTP complex formation [74]. There are data that point to GTP-binding ability of eIF2B [74-76]. However, no motifs characteristic of GTP-binding proteins have been found in eIF2B subunits. A different mechanism, proposed by analogy with the EF-Ts (factor of nucleotide exchange in EF-Tu) mechanism, is based on kinetic data on nucleotide exchange in eIF2 [77]. A "ping-pong" type mechanism, called also the mechanism of enzyme replacement, implies that complex eIF2B·eIF2 is an intermediate that dissociates upon binding to GTP or GDP. However, the existence of stable eIF2B·(Met-$tRNA_i$·eIF2·GTP) complex upon centrifugation in the sucrose concentration gradient makes this mechanism doubtful [78].

It has been shown that GDP:GTP exchange is catalyzed by eIF2B subunit ε, namely by its *C*-terminal domain whose activity is stimulated by eIF2B subunit γ [79, 80]. The *C*-terminal domain of eIF2Bε is homologous to the *C*-terminal domain of eIF5 and also interacts with polylysine blocks of the *N*-terminal domain of eIF2 subunit β [28]. There are experimental data showing that factors eIF5 and eIF2B compete with each other for a binding site on eIF2 [81]. It was assumed for a long time that factors eIF2B and eIF5 regulate activity of eIF2γ domain G via the eIF2 subunit β. Recently, Alone and Dever [44] demonstrated a direct interaction of the G domain of eIF2 subunit γ with the catalytic domain of eIF2Bε and *N*-terminal domain of eIF5. However, the affinity of eIF2B and eIF5 to subunit γ was significantly lower than that to eIF2 subunit β. Evidently, eIF2 subunit β simply stimulates binding of eIF2B and eIF5 factors to the eIF2 subunit γ that creates conditions for their direct functioning.

To date no homologs of eIF5 and catalytic subcomplex eIF2Bγε have been found in archaeans. Comparative analysis of genomes of different archaeans has shown that a number of organisms of this kingdom also have no homologs of regulatory subcomplex eIF2Bαβδ [82]. Besides, the *N*-terminal domain with bound eIF5 and eIF2Bε is absent from archaean aIF2 subunit β. Therefore, the question concerning the mechanism of GTP hydrolysis and aIF2·GDP exchange for aIF2·GTP in archaeans is quite natural. It was shown [54] that archaean factor aIF2 binds GDP and GTP with equal affinity, and rates of the labeled GDP exchange for "cold" GTP or GDP are comparable. The authors supposed that in the archaean system the conversion of aIF2·GDP to aIF2·GTP is, spontaneous, i.e. without involvement of any additional protein factor. However, according to the same authors, no spontaneous ribosome-dependent GTP hydrolysis was observed in the Met-$tRNA_i$·aIF2·GTP complex, which points to possible involvement of an additional factor in this process.

PARTICIPATION OF eIF2 IN TRANSLATION REGULATION

The cell adaptation to sharp change in conditions (stress situations or launching certain programs requiring major rearrangement of cell physiology) includes immediate cessation of protein biosynthesis. In this case the cell retains expression of some templates against the background of stopped translation of the rest of the mRNA. The universal method of translation inhibition is phosphorylation of eIF2. Thus, upon amino acid starvation [16, 19], endoplasmic stress (large amount of incorrectly folded proteins, decrease in calcium concentration in endoplasmic reticulum) [18, 20], and in heme deficiency and viral infection [17, 83] kinases GCN2, PERK, HCI, and PKR are activated, respectively. All these protein kinases phosphorylate eIF2 subunit α at Ser51. It

should be noted that in the norm there is always a certain intracellular level of protein kinase opposed by eIF2 dephosphorylation by appropriate phosphatases [84, 85]. Therefore, there is equilibrium between eIF2α phosphorylation and dephosphorylation. Under stress conditions, the amount of protein kinase sharply increases and a shift in equilibrium to phosphorylated eIF2 occurs. Preparations of the original and phosphorylated eIF2 were equally active in triple complex Met-$tRNA_i$·eIF2·GTP formation, in the binding of this triple complex to 40S subparticle, and in final formation of initiator 80S complex [86, 87]. It appeared that phosphorylation of eIF2 subunit α sharply increases the affinity of this protein to eIF2B, namely to the regulatory subcomplex αβδ, which results in formation of a tight complex eIF2·eIF2B [77, 88-90]. Intracellular eIF2 concentration significantly exceeds that of eIF2B. This means that the binding of eIF2B by phosphorylated eIF2 results in eIF2B deficiency, which, in turn, decreases the rate of conversion of eIF2·GDP to eIF2·GTP and inhibits intracellular translation initiation.

Interesting data were obtained in May, 2010 by a group from Manchester University [91]. It appeared that in addition to the main GTP-hydrolyzing function, factor eIF5 is able to inhibit GDP dissociation from the eIF2·GDP complex. It was demonstrated using mutagenesis that the two activities of eIF5 are independent. It has shown that for the GDP-dissociation function, factor eIF5 needs the *C*-terminal domain with 88 a.a. "linker" region adjacent to the *N*-terminus. After W391F substitution in the "linker" region of yeast eIF5, such cells did not survive in the presence of inhibitor (3-aminotriazol (3AT)), the product of the *his3* gene involved in histidine biosynthesis. It is known that reduction in the eIF2 pool activity in yeast cells upon amino acid starvation stimulates translation of transcription factor GCN4 mRNA. Non-viability of cells containing mutant protein eIF5W391F shows that they are not able to induce GCN4 translation under the stress conditions. However, eIF5W391F superproduction restored growth of such cells and increased the amount of eIF2·GDP–eIF5 complex to the level in wild-type cells. Thus, the British researchers concluded that the GDP-dissociation activity of eIF5 is necessary for normal cell response to stress. Evidently, the eIF2·GDP–eIF5 complex is another critical component in the regulatory system of translation initiation involving phosphorylation of eIF2 subunit α.

However, under conditions of decreased total protein synthesis, some viral mRNA, such as those of hepatitis C virus (HCV), are efficiently translated [92]. In 2008 it was found in Shatsky's laboratory (Belozersky Institute of Physicochemical Biology, Lomonosov Moscow State University) that translation of HCV mRNA proceeds in two ways: eIF2-dependent and eIF2-independent [93]. Evidently, the latter way is used when the concentration of active eIF2 is sharply decreased due to its subunit α phosphorylation by PKR kinase. It appeared that factors eIF3, eIF5B, initiator Met-tRNA, and GTP are enough for this alternative pathway of HCV mRNA translation initiation. It is supposed that in this case eIF5B serves both as initiator of Met-tRNA placement in the ribosomal P site and the binding of ribosomal subparticles.

Below there are examples of the involvement of eIF2α phosphorylation in launching certain programs. Relatively recent genetic investigations on mice have shown that eIF2α phosphorylation is a critical step in memory formation [94]. It appeared that eIF2α phosphorylation by the GCN2 kinase homolog in mammals results in stimulation of synthesis of transcription factor 4 (ATF4) that is a repressor of CREB protein (cAMP-response element binding protein). CREB protein, in turn, regulates transcription of genes of many neuropeptides and proteins involved in neuron development and functioning: c-Fos (protooncogene), neurotrophin (also known as BDNF, brain derived neurotrophic factor), and tyrosine hydroxylase (catalyzes synthesis of dopamine precursor). In addition, it was reported that the low molecular weight inhibitor of eIF2α phosphatases Sa1003 causes memory blocking [95].

Another example is apoptosis, a genetically "programmed" protective mechanism aimed at launching self-destruction of pathologically altered mutant cells for preservation the integrity of the genome of the organism. Multiple different protein kinases and translation initiation factors are involved in the mechanism of apoptosis-dependent translation inhibition. In particular, it was shown that apoptosis inducers TNF (tumor necrosis factor) and TRAIL (apoptosis-inducing THF-like ligand) stimulate eIF2α phosphorylation resulting in inhibition of translation [96]. However, a usual stress-dependent mechanism of eIF2 inactivation might be involved in this case because TNF and TRAIL activate PKR kinase [97]. It is interesting that at later stages of the cell self-destruction program, the translation apparatus is partially reactivated [98, 99]. In this case translation blocking caused by eIF2α phosphorylation is eliminated by proteolysis of eIF2. The eIF2 subunit α is cleaved by caspases, which results in release of eIF2B from the eIF2·eIF2B complex.

It should be noted in conclusion that eIF2 subunit β is also a substrate for a number of serine protein kinases CK2, PKC, and PKA [100, 101]. However, phosphorylation of subunit β involves different serine residues, It was found that an increase in the 2,3-diphosphoglycerate (a CK-2 inhibitor) content in reticulocytes results in inhibition of eIF2β phosphorylation, which correlated with lowered synthesis of hemoglobin [102]. In another example sharp increase in the translation initiation rate and eIF2β phosphorylation were observed in rat liver during neonatal development (the first hour of life) [103]. Interesting subcellular distribution of eIF2 and CK-2 was observed during postnatal development of rat brain. The eIF2 and CK-2 activities were higher in the ribosome-

associated protein fraction [104]. The increase in eIF2 and CK-2 activities in post-microsomal supernatant, accompanied by parallel decrease in these activities in the ribosome-associated fraction, was observed during further development of animals from 5 to 60 days old. In this case total activity did not change during development of the organism. So, the phosphorylation of eIF2β might be an additional mechanism of translation regulation during cell differentiation.

mRNA-BINDING PROPERTIES OF e/aIF2

It was reported in a number of works that factor eIF2 is able to bind mRNA [105-112], and in this case the substrates were mRNA of cell and viral origin both single-stranded and double-stranded mRNA, capped mRNA, and mRNA devoid of cap structures. It was shown that the eIF2 subunit β, namely its polylysine repeats and the $CX_2CX_{17/19}CX_2C$ motif, are responsible for mRNA binding [27, 113]. Simultaneous mRNA and initiator tRNA binding by the factor are mutually exclusive events [114], although different eIF2 epitopes are involved in these two processes [115]. It is assumed that ATP, due to its interaction with subunit β, accounts for the mRNA-binding activity of eIF2 factor [75] because ATP binding inhibits formation of the Met-tRNA$_i$·eIF2·GTP triple complex [113, 116, 117].

However, it is still not clear whether there is any specificity in the interaction of eIF2 with mRNA. On one side, there are data showing that some viral and cellular mRNA having cap structures contain a site for eIF2 binding that is overlapped with the internal ribosome entrance site (IRES) [106, 110-112]. On the other side, there are reports concerning the involvement of eIF2 in cap structure recognition [109, 118, 119]. It was shown for *Penicillium chrysogenum* mRNA that up to 15 molecules of eIF2 protein join one molecule of double-helical template, mainly in the 5′-terminal region [107]. Most likely, in this case factor eIF2 recognizes simply A form of the helix.

It should be noted that mRNA differently bind eIF2, and template affinities to eIF2 correlate with their ability to compete with each other in translation [104, 120-122]. It was shown in Shatsky's laboratory that slight variations in secondary structure of 5′ untranslated region (5′-UTR) of β globin mRNA change the requirements for the factor eIF2 concentration [123]. Thus, increase in the number of paired bases without evolving of stable secondary structures in 5′-UTR of β globin mRNA sharply decreased the efficiency of this mRNA translation in rabbit reticulocyte lysate at high concentration of this mRNA. Addition of purified eIF2 to the lysate resulted in elimination of distinctions in translation of templates with different secondary structure in 5′-UTR of β-globin mRNA. It is known that mRNA with unstructured leader form a rather tight complex with ribosomal subparticle, while any secondary structures in 5′-UTR much weaken the interaction of the 40S subparticle with mRNA [124]. Dmitriev et al. [123] supposed that eIF2 stabilizes the interaction of 40S ribosomal subparticle with mRNA due to contacts with both components, and they proposed a model explaining their data. In the case of translation initiation on templates with unstructured leader, the eIF2 factor fulfils only a single function, Met-tRNA$_i$ delivery to the 40S subparticle stably associated with mRNA. Evidently, the lifetime of such complex is long enough to provide for binding triple complex at low eIF2 concentrations in the system. In the case of templates with structured leaders, eIF2 is used both for Met-tRNA$_i$ delivery and as factor stabilizing the interaction of the 40S ribosomal subparticle with mRNA. In this case the probability that AUG codon will appear in the necessary place on 40S subparticle directly depends on eIF2 concentration, and at its low values, like in the case of eIF2 phosphorylation, the rate of translation initiation on such templates is decreased (cited by [123]).

In 2008, it was found in laboratory of Blasi [125] that factor aIF2 also interacts with mRNA. However, in this case aIF2 subunit γ is responsible for binding, and the substrate is any mRNA with triphosphate at the 5′ end (mRNA mono- and dephosphorylated at the 5′ end do not bind aIF2γ). In this case aIF2 subunit γ interacts with the 5′ end of such mRNA and protects the latter against 5′→3′-directed degradation. It was also shown in this work [125] that mRNA and Met-tRNA are able to compete with each other for binding to aIF2. In this case the affinity of aIF2 to mRNA ($K_d = 10^{-8}$ M) exceeds that of aIF2·GTP to Met-tRNA$_i$ ($K_d = 15 \cdot 10^{-8}$ M), while aIF2·GTP in the presence of aIF1 and ribosomes interacts with Met-tRNA$_i$ with the same affinity as in the interaction of aIF2 with mRNA. The authors supposed that in the norm aIF2 preferably interacts with initiator tRNA and plays the role of the Met-tRNA$_i$ supplier to the ribosome. However, under stress conditions when synthesis of ribosomes is noticeably decreased, the presence of free aIF2 in the cells results this factor fulfilling its non-canonical function, protection of mRNA against degradation.

In conclusion, we would like to mention the work of a Japanese group published in April 2010 [126]. They managed to crystallize and determine structure of triple complex aIF2α (domains I, II)·aDim2p·16S rRNA (a fragment of 11 bases in length). In eukaryotes the Dim2p protein is the processing factor involved in maturation of rRNA of small ribosomal subparticle. They showed in this work that, on one side, the archaean homolog of Dim2p forms a firm complex with the 3′-terminal region of 16S rRNA in the region of the anti-Shine–Dalgarno sequence, and on the other side it interacts with the aIF2 subunit α. In this case the binding of aDim2p to aIF2α is independent of the presence of rRNA in the complex.

Additional binding experiments have shown that neither β nor γ subunits of aIF2 interact with aDim2p. The biological significance of the existence of the aIF2·aDim2p·16S rRNA complex is still not understood, but the authors believe that e/aDim2p is a multifunctional protein that while carrying out its functions evidently becomes involved in multiple interactions in eukaryotic and archaean cells.

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