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REVIEW

Translation Factor eIF5A, Modification with Hypusine and Role in Regulation of Gene Expression. eIF5A as a Target for Pharmacological Interventions

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Abstract—Translation factor eIF5A participates in protein synthesis at the stage of polypeptide chain elongation. Two eIF5A isoforms are known that are encoded by related genes whose expression varies significantly in different tissues. The eIF5A1 isoform is a constitutively and ubiquitously expressed gene, while the eIF5A2 isoform is expressed in few normal tissues and is an oncogene by a number of parameters. Unique feature of eIF5A isoforms is that they are the only two proteins that contain amino acid hypusine. Modification with hypusine is critical requirement for eIF5A activity. Another distinctive feature of eIF5A is its involvement in the translation of only a subset of the total population of cell mRNAs. The genes for which mRNAs translation requires eIF5A are the members of certain functional groups and are involved in cell proliferation, apoptosis, inflammatory processes, and regulation of transcription and RNA metabolism. The involvement of eIF5A is necessary for the translation of proteins containing oligoproline fragments and some other structures. Modification of eIF5A by hypusine is implemented by two highly specialized enzymes, deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH), which are not involved in other biochemical reactions. Intracellular activity of these enzymes is closely associated with systems of protein acetylation, polyamine metabolism and other biochemical processes. Inhibition of DHS and DOHH activity provides the possibility of pharmacological control of eIF5A activity and expression of eIF5A-dependent genes.

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Eukaryotic translation initiation factor 5A (eIF5A, synonyms: IF-M2Bα and eIF-4D) was discovered in the mid-70s as a protein that stimulates the synthesis of polypeptide chain on a polyuridine RNA template [1]. Mammalian eIF5A is a small (16.7 kDa) acidic protein with a high level of expression in cells of different types [2]. Two isoforms of this factor are known, eIF5A1 and eIF5A2, which are encoded by different genes. Amino acid sequences of eIF5A1 and eIF5A2 are 84% identical and coincide by 94% considering amino acids of the same type. Unlike the ubiquitous eIF5A1 isoform, eIF5A2 is expressed in a limited number of tissues, for example, some parts of the brain and some tumor cells [3-6]. eIF5A1 and eIF5A2 isoforms differ greatly in the importance for cell viability. Inactivation of the *eIF5A1* gene is lethal at the early stages of embryogenesis, while animals with *eIF5A2* gene knockout remain viable [7].

Contrary to the conventional name, the main function of eIF5A is the elongation of polypeptide chains. The assumption about the involvement of eIF5A in the initiation of translation was based on the stimulating effect of purified eIF5A on the synthesis of methionyl puromycin [8]. This reaction reproduces the formation of the first peptide bond but does not fully correspond to real intracellular processes. According to the later studies, eIF5A does not affect the assembly of ribosomal subunits and binds to the already formed 80S complex (K_d = 9 nM) [9]. eIF5A binds to the ribosome in the region between the E and P sites and promotes peptidyl transferase reaction, presumably, in cooperation with the elongation factor

Abbreviations: DFMO, difluoromethylornithine; DHS, deoxyhypusine synthase; DOHH, deoxyhypusine hydroxylase; eIF5A, eukaryotic translation initiation factor 5A; eIF5A(Dhp), deoxyhypusine-modified eIF5A; eIF5A(Hyp), hypusine-modified eIF5A; eIF5A(Lys), unmodified eIF5A; GC7, N1-guanyl-1,7-diaminoheptane; iNOS, inducible nitric oxide synthase; shRNA, small hairpin RNA.

eEF2 [9-11]. Participation of eIF5A in the process of elongation was confirmed in numerous experiments, for instance, in the studies of the polysomal profiles of yeast cells with temperature-sensitive mutations of *eIF5A* gene. According to recent observations, eIF5A is involved in the termination of protein synthesis in yeast cells, which is manifested in stimulation of hydrolysis of peptidyl-tRNA catalyzed by the termination factors eRF1 and eRF3 [12, 13]. The role of eIF5A in the translation termination in higher eukaryotes has not been studied. Moreover, a number of studies indicate that eIF5A is involved in processes that are not directly related to protein synthesis, such as the transport of a newly generated mRNAs from the nucleus to the cytoplasm and proteins across the membranes of endoplasmic reticulum [14]. eIF5A participation in nuclear-cytoplasmic transport was shown for *CD83* and *iNOS* mRNAs [15, 16]. In general, the transport function of eIF5A remains poorly understood.

THE SELECTIVITY OF eIF5A FOR TRANSLATION OF DIFFERENT mRNA POPULATIONS

A characteristic feature of eIF5A is that it is required for translation of a limited part of the mRNA. In yeast cells and in higher eukaryotes the majority of mRNAs are translated without the participation of eIF5A. In HeLa cells, a decrease in the level of eIF5A to less than 10% of the initial level, achieved by adenoviral transfection of small hairpin RNA (shRNA), leads to suppression of the total protein synthesis to less than 30% of the initial level. Along with this, there is a detectable decrease in translation of 104 of the 972 studied proteins [17]. It remains unclear, which structural characteristics of proteins and coding mRNAs determine the necessity of eIF5A participation in their translation. The most common view is that eIF5A-dependent proteins contain amino acid triplets formed by either three proline residues or two consecutive prolines and glycine (Pro-Pro-Pro or Pro-Pro-Gly) [18]. This empiric rule is consistent with the fact that at the stage of elongation the formation of peptide bond between certain amino acids occurs with relatively low efficiency. Glycine and proline are ineffective as acceptors of the growing polypeptide chain, and the efficiency of proline as a donor is also low [19, 20]. According to recent findings [12, 13], the presence of oligoproline triplets cannot be considered as absolute, necessary and sufficient condition for eIF5A participation in the synthesis of a corresponding protein. In yeasts (*Saccharomyces cerevisiae*), the composition of eIF5A-dependent triplets in addition to proline and glycine may also include basic (arginine, lysine) and acidic (aspartate, glutamate) amino acids, and the consensus of most frequent amino acids has the following structure: (Pro/Asp/Gly)-(Pro/Asp/Gly)-(Pro/Gly/Lys). In a whole, yeast genome has 29 amino acid triplets which determine the dependence of translation from eIF5A; of them 11 contain at least two proline residues [12]. Apparently, yeast eIF5A is less selective regarding the composition of amino acid triplets of a synthetized protein than eIF5A of higher eukaryotes. Accordingly, in yeasts the total protein synthesis depends on eIF5A to a greater extent than in higher eukaryotes. Genetic inactivation of eIF5A in yeast cells results in 75% decrease in total protein synthesis [21].

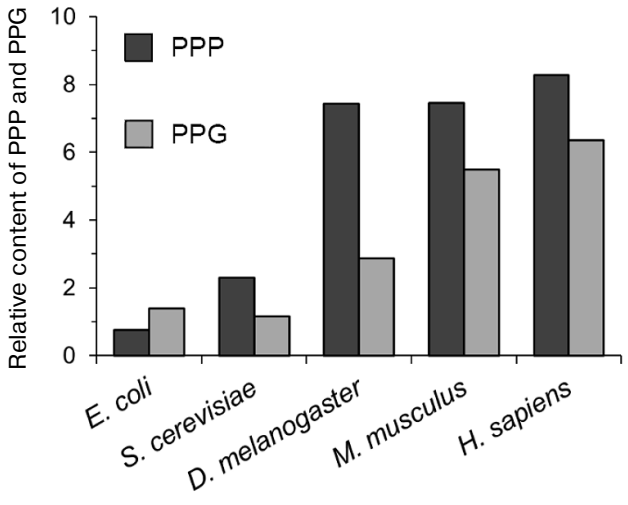

Fig. 1. The content of oligoproline fragments in species representing different stages of evolution. The frequency of Pro-Pro-Pro (PPP) and Pro-Pro-Gly (PPG) amino acid triplets in five proteomes. The histogram shows the number of PPP and PPG fragments per 10^4 amino acid residues [17].

Comparative bioinformatic analysis reveals that the portion of proteins containing the Pro-Pro-Pro or Pro-Pro-Gly triplets increases considerably during the evolution (Fig. 1). It should be noted that the level of proline-rich conservative structures as PPII and functional domains as SH3, WW, and EVH1 in proteomes increases significantly as organisms become more complex [22]. In bacteria (*E. coli*), amino acid triplets Pro-Pro-Pro or Pro-Pro-Gly are present in approximately 5% proteins, whereas in *S. cerevisiae* the content of such proteins is ~12%. In humans, already ~24% proteins contain at least one Pro-Pro-Pro site and ~20% of proteins contain one or more Pro-Pro-Gly sites. For comparison, in course of evolution the frequency of homogenous triplets formed by amino acids other than proline either does not change or slightly increases (Glu_3 and Leu_3). Bioinformatic analysis of gene networks shows that genes of oligoproline proteins are assembled in functional clusters. During the evolution, the total number of such groups has increased, and their organization has become more complex. For example, mouse (*Mus musculus*) genome contains 28 clusters of proteins with triplets Pro-Pro-Pro and 21 clusters of proteins with triplets Pro-Pro-Gly, whereas the

genome of *S. cerevisiae* contains only four clusters of Pro-Pro-Pro proteins and one cluster of Pro-Pro-Gly proteins [13, 18, 23].

The occurrence of Pro-Pro-Pro/Gly triplets is especially high among signaling proteins and their receptors, factors involved in cytoskeleton organization and regulation, RNA transcription and metabolism, chromatin modification and DNA replication [4, 24, 25]. Proteins containing the Pro-Pro-Pro/Gly sequences are characterized by multiple functional interactions and participate in various regulatory systems. In species at different stages of evolution the number of functional interactions between such proteins increases significantly as biological objects become more complex. The largest number of functional interactions is characteristic of oligoproline-containing proteins Abl1, Abl2, CREBP, Notch1, JunD, and MAPK7/ERK5 that play key roles in different regulatory systems. It should be noted that eIF5A inactivation (for instance, in HeLa cells transfected with adenoviral Ad-eIF5A^{shRNA} construction) leads not only to inhibition but also to stimulation of expression of a large group of proteins. The amount of such proteins is comparable with the number of proteins whose expression is reduced after eIF5A inactivation. This activation appears to be a secondary effect of eIF5A inactivation, due to the fact that suppression of the translation of eIF5A-dependent regulatory proteins can cause an induction of another group of genes. Remarkably that only part of proteins whose expression is stimulated by eIF5A inactivation contains oligoproline sequences. Therefore, the requirement in eIF5A for translation of proteins with oligoproline sequences is not absolute [13, 17, 26, 27].

HYPUSINATION AND OTHER COVALENT MODIFICATIONS OF eIF5A

A unique feature of eIF5A it that its activity is crucially dependent on hypusination, the post-translational transformation of specific lysine residue to hypusine (hypusine is an abbreviation of: **hy**droxy**pu**trescine + ly**sine**) [28]. Initially the amino acid hypusine was revealed in cells in a free state before eIF5A has been discovered [29]. Reaction of hypusination involves a transfer of the aminobutyl residue, generated after the cleavage of spermine, on the specific lysine residue of eIF5A (Lys50 in mice and humans, Lys51 in yeasts) with the formation of deoxyhypusine residue, which after oxidation is transformed to hypusine (Fig. 2). eIF5A1 and eIF5A2 are the only two proteins containing hypusine residues, and their modification is a unique example of covalent attachment of the polyamine fragment to proteins [4, 24, 25, 30].

The modification of eIF5A by hypusine is catalyzed by two enzymes: deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH) [28, 31]. Participation in transformation of eIF5A(Lys) to eIF5A(Hyp) is the only function of these enzymes. DHS is a tetrameric protein consisting of four 41-kDa subunits. DOHH is a monooxygenase containing nonheme iron (two Fe^{II} atoms) in a complex with His and Glu residues of the polypeptide chain. A specific feature of DOHH that distinguishes it from other monooxygenases is the high stability of the peroxo-$(Fe^{III})_2$ intermediate generated in the reaction of reduced (Fe^{II}) DOHH with O_2 [32]. In the formation of the DOHH holoenzyme, the inclusion of iron ions in the apoenzyme occurs with the participation of PCBP1 and PCBP2 chaperones, which are also involved in the incorporation of iron in prolyl hydroxylases and ferritin [33].

The first stage of hypusine synthesis is covalent binding of diaminobutane produced by spermidine cleavage to the ε-amino group of Lys329 residue of DHS with the formation of imine intermediate. Diaminobutane is then transferred from DHS to eIF5A. At the final stage of the hypusine synthesis DOHH in the presence of O_2 and NADH hydroxylates deoxyhypusine with the formation of eIF5A(Hyp) [34] (Fig. 2). Remarkably that unmodified eIF5A(Lys) is inactive as a translation elongation factor, although, according to some data, it accumulates in the cell nucleus and can act as an RNA-binding protein [35, 36]. Besides, studies with yeast cells showed that unmodified eIF5A retains its ability to participate in termination of protein synthesis and may stimulate the hydrolysis of peptidyl-tRNA, although less efficiently than eIF5A(Hyp) [12]. Intermediate eIF5A(Dhp) is active as a translation factor. Worth to note that eIF5A(Dhp) can return to the eIF5A(Lys) state. The reverse reaction is catalyzed by DHS in the presence of NADH and either diaminobutane (putrescine) or diaminopropane with the formation of spermidine or homospermidine, respectively [37]. Inactivation of the *DOHH* gene in mice (but not in yeast cells) leads to the complete loss not only of eIF5A(Hyp) but also eIF5A(Dhp) and is lethal for all higher eukaryotes [38]. According to all available data, Dhp50 transformation into Hyp50 is completely irreversible, and the transformation of modified eIF5A(Hyp) to the original eIF5A(Lys) is impossible. Therefore, after the hydroxylation of the deoxyhypusine residue the activation of eIF5A becomes irreversible. Taking into account the high stability of eIF5A ($\tau_{1/2}$ ~ 24 h), its activation should be long-lasting, and the activity of eIF5A can be regulated only at the level of synthesis and modification. Apparently, under normal conditions, hypusination of eIF5A occurs shortly after translation of EIF5A, which is present in cells mainly in active hypusine-modified state [27, 36, 39]. Nevertheless, intracellular content of eIF5A1(Hyp) can be increased by several factors, such as proinflammatory cytokines and inducers of endoplasmic reticulum stress, or, on the contrary, reduced by hypoxia [4, 15]. It was shown that in peritoneal macrophages, the ligands of TLR2 (receptor of bacterial lipoproteins and glycolipids)

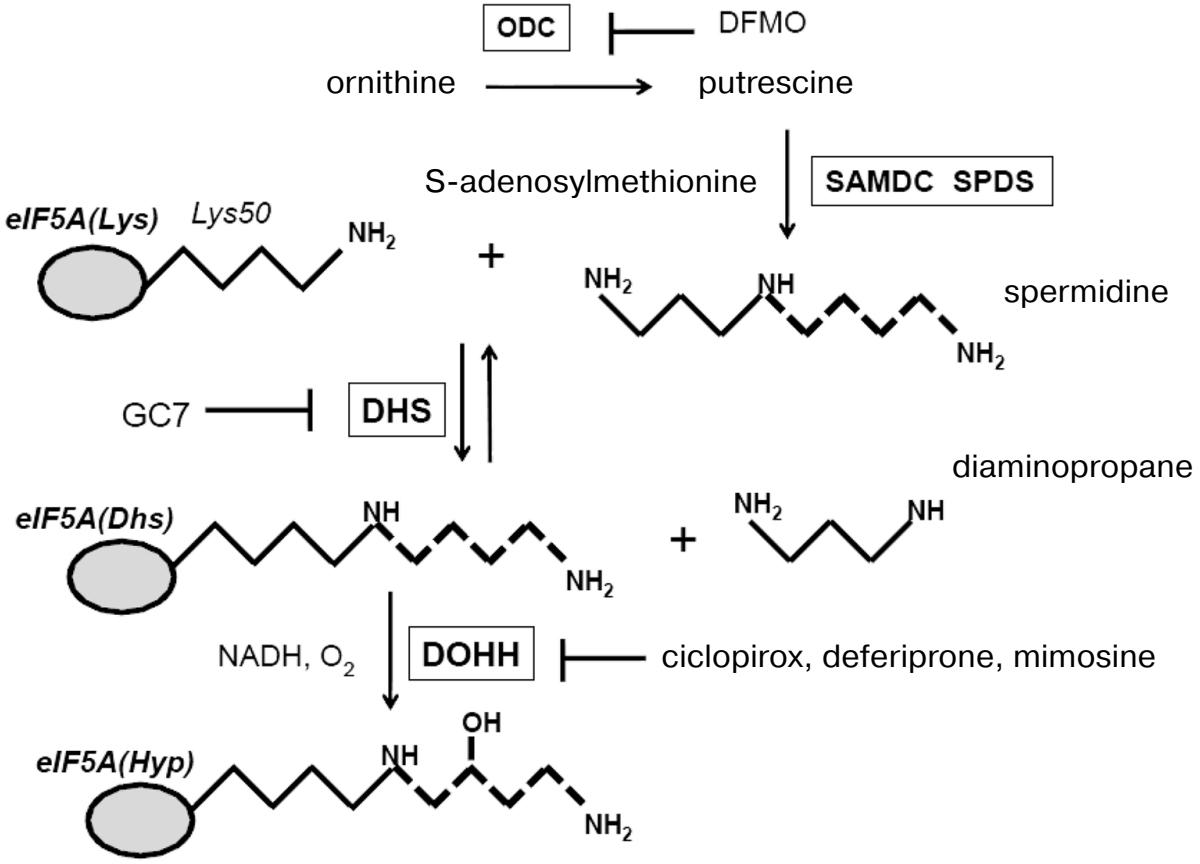

Fig. 2. The scheme of hypusine synthesis and modification of translation factor eIF5A. In both eIF5A isoforms the transformation of Lys50 residue to hypusine (Hyp50) occurs via two consecutive enzymatic reactions. First, deoxyhypusine synthase (DHS) transfers the aminobutyl fragment of spermidine on the ε-amino group of lysine residue with the formation of deoxyhypusine intermediate. After the final stage deoxyhypusine hydroxylase (DOHH) in the reaction with O_2 and NADH oxidizes deoxyhypusine with formation of hypusine residue. After that, eIF5A becomes an active translation factor. eIF5A modification can be suppressed by inhibitors of DHS and DOHH as well as by inhibition of ornithine decarboxylase (ODC), which is responsible for the synthesis of spermidine. DFMO, difluoromethylornithine (an inhibitor of ODC); SAMDC, S-adenosylmethionine decarboxylase; SPDS, spermidine synthase.

and Mincle (lectin receptor) cause an increase in the level of eIF5A(Hyp). Stimulation of eIF5A hypusination is mediated by MAP kinase p38 and is critically required for translation of the *iNOS* mRNA [16, 40].

Along with hypusination, there are some other covalent modifications of eIF5A. For example, phosphorylation of the Ser2 residue and transglutamination [41, 42]. The effect of these modifications on the eIF5A activity remains unclear. The acetylation of Lys47 residue of eIF5A by acetyl transferase PCAF was studied more carefully. Acetylation and hypusination of eIF5A are mutually exclusive processes. Acetylation is only possible for unmodified eIF5A(Lys) but not for eIF5A1(Hyp), and, on the contrary, there is no hypusination of eIF5A(Ac). Unlike hypusination, acetylation is a reversible modification. eIF5A(Ac) deacetylation is catalyzed by Zn-dependent lysine deacetylase HDAC6 and NAD-dependent lysine deacetylase SIRT2 [35, 43]. The action of SIRT2 inhibitors, for example, nicotinamide, increases the intracellular content of eIF5A(Ac) [44]. Acetylation affects eIF5A intracellular distribution: eIF5A(Ac) is localized in cell nucleus, eIF5A(Lys) is present in entire cell volume, whereas eIF5A(Hyp) is present only in the cytoplasm (Fig. 3) [4]. The eIF5A(Lys)/eIF5A(Hyp)/eIF5A(Ac) ratio depends on the cell type. The intracellular levels of unmodified and modified eIF5A can be measured by isoelectric focusing and two-dimensional electrophoresis, since eIF5A1(Hyp), eIF5A1(Lys), and eIF5A1(Ac) have different isoelectric points (p*I* 5.2, 5.1, and 5.0, respectively). In cells of many types, the dominating form is eIF5A1(Hyp) with a lower level of eIF5A1(Lys), whereas eIF5A1(Ac) is the least abundant form of eIF5A1. In cardiac muscle the two-dimensional electrophoresis reveals only eIF5A1(Hyp) [45]. However, in HeLa and A549 cell lines under hypoxic conditions (1% O_2), the level of eIF5A1(Ac) is significantly increased, whereas the level of eIF5A1(Hyp) is decreased. Apparently, this effect of hypoxia is universal for all types of cells and is mediated by a decrease in DOHH activity utilizing oxygen as a substrate in the reaction of

eIF5A1(Dhu) hydroxylation [46]. In cells of different types, unmodified eIF5A(Lys) is implicated in regulation of apoptosis. In epithelial cell lines HeLa and HT-29, apoptosis is similarly induced by exogenously overexpressed wild-type eIF5A1(Lys50) and mutant factor eIF5A(Lys50Arg) that is resistant to hypusine modification [47]. For some unknown reason, cellular DHS and DOHH are unable to recognize exogenous eIF5A(Lys), which remains unmodified and acts as a pro-apoptotic stimulus [48].

In bacterial cells, the modification of factor EF-P (a prokaryotic analog of eIF5A) occurs in a process similar to eIF5A hypusination. This modification is implemented by three enzymes – YjeK, YjeA, and YfcM. The first step is isomerization of α-lysine to β-lysine. The next step is the attaching of β-lysine to Lys34 residue of the EF-P polypeptide chain with the formation of β-lysyl-lysine. The final stage is hydroxylation of Lys34 (Fig. 4) [49, 50]. Worth noting that besides eIF5A several other elongation factors undergo unique covalent modifications. For instance, eEF1A is modified by ethanolamine phosphoglycerol attachment to the Glu residue, and eEF2 is modified by diphthamide attachment to the His residue [51].

REGULATION OF ACTIVITY AND PHARMACOLOGICAL MODULATION OF eIF5A

eIF5A is one of the most highly expressed genes. In HeLa cells, the content of eIF5A corresponds to about three copies per ribosome [52]. According to mass-spectral analysis of *S. cerevisiae* proteome, eIF5A is among ~100 most abundant proteins, and the level of eIF5A in yeast cells is ~9-15 μM [9, 12, 53]. Such a high content of eIF5A in cells is probably required for maintaining the optimal intensity of protein synthesis. Expectably that even a partial decrease in the level of eIF5A(Hyp) caused by inhibitors of hypusination will considerably reduce the rate of translation of mRNAs of oligoproline-containing proteins. Elevated expression of eIF5A1 is common to some tumors, such as glioblastoma and adenocarcinomas

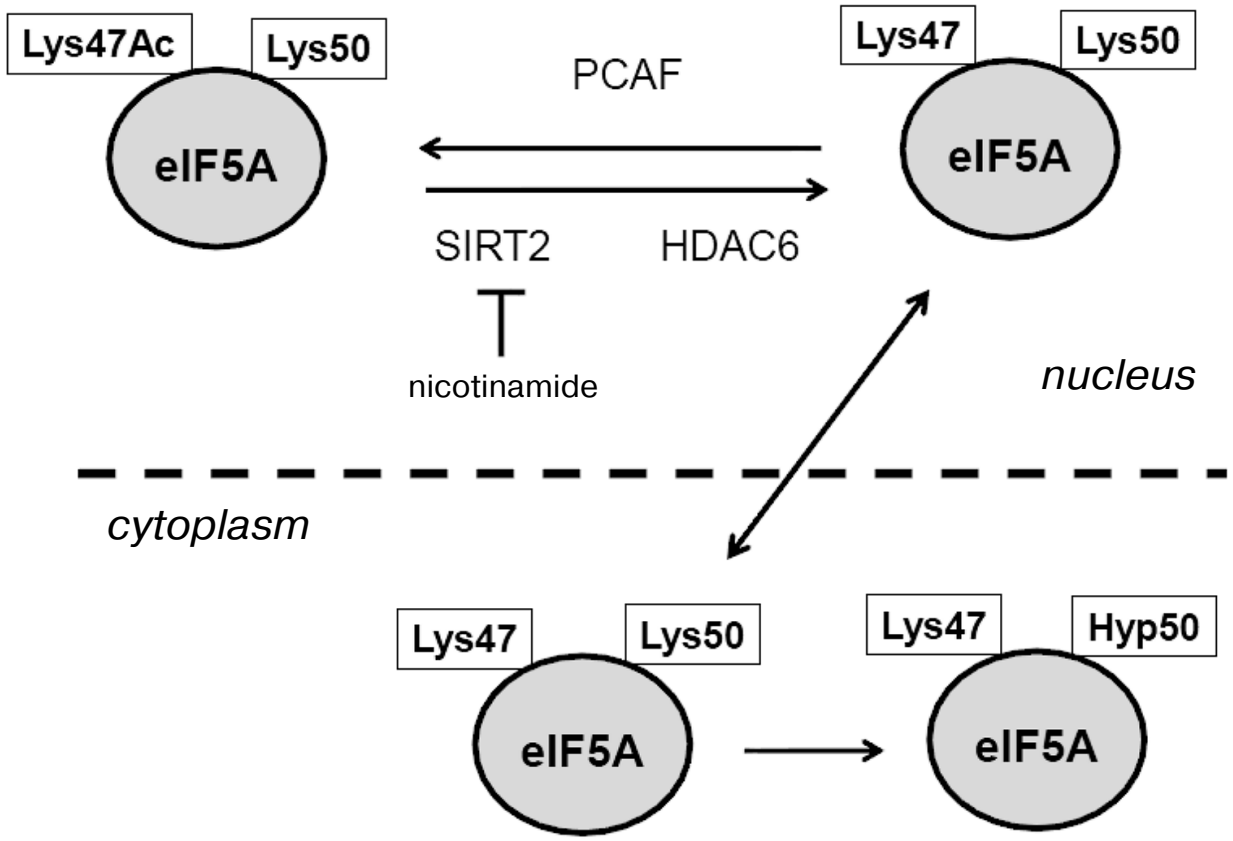

Fig. 3. Modification and intracellular localization of eIF5A. Acetylation and hypusination determine the intracellular localization of eIF5A. The unmodified eIF5A is distributed throughout the entire cell volume, eIF5A(Lys47Ac) is localized in the cell nucleus, eIF5A(Hyp50) is present only in the cytoplasm. Acetylation and hypusination are alternative processes. eIF5A acetylated at Lys47 cannot be hypusinated at Lys50 and, on the contrary, eIF5A hypusinated at Lys50 cannot be acetylated at Lys47. eIF5A acetylation is catalyzed by acetyltransferase PCAF. eIF5A(Ac) is deacetylated by the Zn-dependent lysine deacetylase HDAC6 and NAD-dependent lysine deacetylase SIRT2. A large number of inhibitors of these enzymes are known (the figure reveals only nicotinamide, inhibitor of SIRT2).

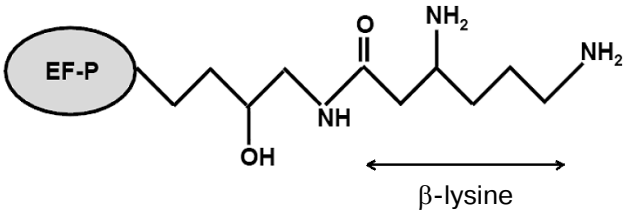

Fig. 4. The structure of the bacterial translation factor EF-P, a prokaryotic analog of eIF5A. EF-P modification (Lys34 residue of the polypeptide chain of *E. coli* EF-P) consists in formation of β-lysyl-lysine and the subsequent hydroxylation of Lys34.

of different localization, and may be explained, probably, by participation of oncoproteins c-Myc, K-Ras, and Bcr-Abl in the regulation of the *eIF5A1* gene expression [25, 54].

Pharmacological inhibition of eIF5A1 is a possible approach for suppression of the growth of malignant tumors, prevention of diabetes progression and inflammatory processes, and treatment of retroviral and protozoan infections [4, 24, 25, 30, 54-57]. First of all, eIF5A activity can be suppressed by lowering the level of eIF5A(Hyp) by means of DHS inhibitors, such as GC7, CNI-1493 (semapimod), deoxyspergualin (gusperimus), and recently developed DHSI-15, along with inhibitors of ornithine decarboxylase (e.g., difluoromethylornithine, DFMO), which suppress the synthesis of the spermine serving as substrate for DHS. The inhibitors of DOHH (ciclopirox, deferiprone, and mimosine) can also be used (Fig. 5). Of these compounds, ciclopirox is the most efficient inhibitor of eIF5A(Hyp) production (IC_{50} is ~5 μM in the epithelial cells HUVEC) [58]. Under experimental conditions, eIF5A activity can be suppressed through inactivation of the *eIF5A*, *DHS*, and *DOHH* genes by knockout of these genes or by transfection of short interfering RNAs or small nuclear RNA miR-434-3p, which is a negative regulator of *eIF5A1* mRNA expression. Moreover, the level of eIF5A(Hyp) can be reduced by stimulating the eIF5A(Ac) accumulation via using lysine deacetylases inhibitors. All these approaches lead to suppression of cell growth and inflammatory processes in experiments on cell lines and animals [4, 7, 24, 39, 45, 46, 59].

Further study of already known eIF5A inhibitors such as DHS and DOHH along with search for new compounds is a task with a perspective of clinical application.

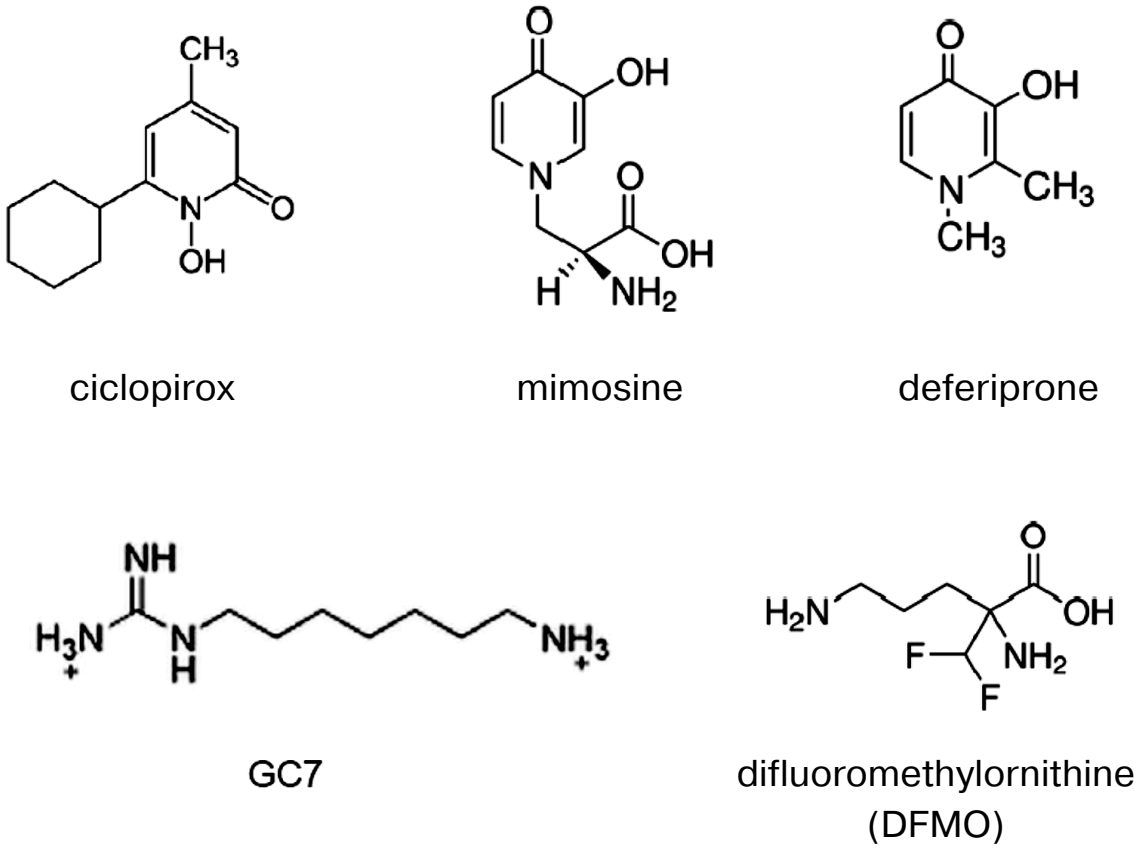

Fig. 5. The structure of compounds that suppress eIF5A hypusination. The inhibitors of key metabolic pathways involved in eIF5A modification by hypusine are shown. GC7 is an inhibitor of DHS; ciclopirox, mimosine, and deferiprone are inhibitors of DOHH; DFMO is an inhibitor of ornithine decarboxylase. Also, the cellular level of eIF5A hypusination is affected by inhibitors of lysine deacetylases that prevent conversion of the inactive eIF5A(Ac) to eIF5A(Lys) and then to eIF5A(Hyp) (not shown).

One can expect that transformed cells, which are characterized by elevated activity of eIF5A, DHS, and DOHH, are more sensitive to such inhibitors than normal cells. Since the 1990s, a significant number of spermidine analogues have been synthesized and analyzed for their ability to suppress DHS activity and eIF5A hypusination. Compound GC7 (N1-guanyl-1,7-diaminoheptane) was found to be the most potent inhibitor of DHS, with K_i = 10 nM, which is 450 times lower than K_m for spermidine [60]. GC7 inhibits hypusination of both eIF5A isoforms. However, all currently known DHS inhibitors are not sufficiently selective and have side effects or limited bioavailability. Under physiological conditions the effectiveness of GC7 is restricted by activity of polyamine oxidases present in blood. Another known inhibitor, CNI-1493, suppresses the transmembrane transfer of arginine and, as a consequence, metabolic processes involving this amino acid, including iNOS activity [61]. Other DHS inhibitors are also known to have adverse side effects. For example, deoxyspergualin inhibits protein kinase Akt [62]. The effect of all known inhibitors of iron-containing hydroxylase DOHH (ciclopirox, deferoxamine, deferiprone, mimosine) is based on the chelation of Fe^{2+} ions, and the use of such compounds induces multiple side effects. For example, ciclopirox-mediated inhibition of ribonucleotide reductase and prolyl hydroxylase leads to the suppression of DNA replication and activation of the transcription factor HIF1α, respectively [63]. One can expect that more selective inhibitors of DHS and DOHH can be developed in the future.

THE CELL RESPONSE TO THE SUPPRESSION OF eIF5A

Partial genetic inactivation of eIF5A1 isoform increases the resistance of islet β-cells to the damaging effects of proinflammatory cytokines, suppresses the activation of the iNOS expression and improves insulin secretion in streptozotocin-induced type 1 diabetes. In INS-1 β-cell line the inhibition of the DHS activity by GC7 prevents eIF5A(Hyp) accumulation, the activation of the proapoptotic factor CHOP and subsequent apoptosis caused by thapsigargin, an inducer of endoplasmic reticulum stress [36, 64]. Apparently, insulin-producing islet β-cells are highly sensitive to factors affecting eIF5A hypusination, which may be associated with a short lifespan of eIF5A in these cells in comparison with other types of cells ($\tau_{1/2}$ ~6 and ⩾24 h, respectively) [36, 65]. Protection of β-cells from death by means of eIF5A down-regulation may be mediated by a decrease in the level of tyrosine protein kinase Abl [66]. At the same time, it was shown that Abl inhibitors (e.g., imatinib) increase the resistance of islet β-cells to cytotoxic stimuli [67].

As noted above, many genes whose translation depends on eIF5A(Hyp) are involved in functional clusters identified by bioinformatics analysis suggesting a coordinated participation of these genes in metabolic and signaling processes. By all appearances, even a relatively small but synchronous change in the expression of these interdependent proteins will lead to a significant change in the cell status. There are several examples how changes in the intracellular level of eIF5A affect the expression of a number of key regulatory factors. Overexpression of eIF5A or DHS causes an increase in intracellular levels of the G-protein RhoA and protein kinases ROCK2 and PEAK1 resulting in an increase in cell mobility and invasiveness and subsequent intensification of angiogenesis and metastasis [26, 68]. On the contrary, suppression of eIF5A achieved in various experimental conditions, such as RNA interference or treatment with GC7, prevents activation of the hypoxia-sensitive transcriptional factor HIF-1α and induction of dependent genes (*VEGFA*, *BNIP3*, etc.) [46]. In cultured primary lymphocytes of NOD mice prone to the development of autoimmune diabetes, GC7 suppresses proliferation and differentiation of Th1 lymphocytes, as evidenced by the reduction of the expression of the CD25 antigen (cytokine IL-2 receptor) [69]. In RAW 264.7 monocytes GC7 inhibits TNF secretion stimulated with lipopolysaccharides [70] and shows strong cytotoxic effect on the human neuroblastoma cell line MYCN2. The latter effect may be due to the changes in the state of important cell growth regulators: a decrease in the total content and phosphorylation level of the retinoblastoma factor Rb, induction of the inhibitor of cyclin-dependent protein kinases p21 and decrease in the level of protein kinase Cdk4 [71]. The reduced DHS expression in heterozygous $Dhs^{+/-}$ mice (homozygous $Dhs^{-/-}$ mice are not viable) and the resulting decrease in the level of eIF5A(Hyp) lead to the suppression of iNOS translation without affecting induction of the *iNOS* mRNA by proinflammatory cytokines (IL-1β, IFNγ, TNF) in pancreatic β-cells [56]. Moreover, animals heterozygous by the *Dhs* gene are more resistant to development of hyperglycemia and induction of diabetes by streptozotocin. Ornithine decarboxylase inhibitor DFMO provides a similar protective effect mediated by decrease in the level of eIF5A hypusination [30]. It is noteworthy that eIF5A-dependent proteins RhoA and iNOS do not contain canonical oligoproline triplets. It is still unclear why eIF5A inhibition suppresses translation of mRNAs of these genes.

eIF5A inhibitors in combination with a number of known chemotherapy agents produce a cumulative cytotoxic effect on transformed cells. For example, GC7 in combination with cetuximab (an inhibitor of EGFR, receptor of epidermal growth factor) has a cumulative cytotoxic action on carcinoma cells [72]. In the epidermal KB cells, GC7 enhances IFNα-mediated cell growth inhibition and apoptosis [73]. It was also shown that GC7 enhances cytotoxic effects of cisplatin on eIF5A2-expressing carcinoma cells OSCC [74]. GC7 in combina-

tion with DFMO has a pronounced cumulative cytotoxic effect on the neuroblastoma cells. This effect may be mediated by the key role of ornithine decarboxylase in the synthesis of spermidine, which is critically required for hypusination and activation of eIF5A. It should be noted that neuroblastoma is characterized by increased expression of eIF5A and ornithine decarboxylase [75].

Targeted inhibition of eIF5A2 may be a new approach for selective suppression of tumor cell growth and induction of apoptosis. The *eIF5A2* gene is localized in the region 3q26.2 of chromosome 3, in which mutations cause malignant transformation of cells. Based on a number of parameters, *eIF5A2* can be considered as an oncogene. The mRNA of *eIF5A2* gene is represented by a large number of isoforms [48]. eIF5A2 expression is critically required for the proliferation of some lines of transformed cells, in particular, carcinoma cells [76]. eIF5A2 is a diagnostic marker of the presence of transformed cells for different types of tumors as carcinomas of different localization and melanoma [77]. It can be assumed that eIF5A1 is involved in processes occurring in normal cells, whereas eIF5A2 is involved in processes typical for embryonic and transformed cells, which may be due to the possible functional differences between eIF5A1 and eIF5A2 [6]. Moreover, the isoforms may differ in selectivity to oligoproline and other amino acid triplets and, consequently, mediate the translation of different mRNA populations.

In conclusion, eIF5A has a number of unique characteristics, of which the most remarkable is hypusine modification that is inherent only in the two eIF5A isoforms among endless variety of proteins of all biological objects. Another distinctive feature of eIF5A is involvement in translation of only part of the overall cellular mRNA population. According to bioinformatic analysis, the genes requiring eIF5A for their mRNA translation are not scattered randomly in the gene network but included in certain functional groups. Proteins whose synthesis depends on eIF5A are involved in cell proliferation and response to proinflammatory cytokines. eIF5A modification with hypusine is catalyzed by two highly specialized enzymes, DHS and DOHH, which are not involved in other biological reactions. Indirectly, the activity of these enzymes is associated with other metabolic processes. DHS uses spermidine as a substrate, and, therefore, the reaction of hypusination is involved in the network of polyamine metabolism. The monooxygenase DOHH contains non-heme iron, and its activity depends on the cell saturation with oxygen and intracellular iron level. As far as it is known, under physiological conditions the activity of eIF5A is maintained at the maximal level. eIF5A activity may be reduced by relatively few endogenous factors, such as IFNα and hypoxia. However, there are many opportunities for pharmacological regulation of eIF5A, the enzymes responsible for eIF5A hypusination being suitable targets for this. The activity of eIF5A can be suppressed by compounds that inhibit DHS and DOHH, used separately or in combination with inhibitors of other signaling systems, leading to synergistic effect. Remarkably that according to a number of features, the *eIF5A2* isoform is an oncogene. eIF5A2 expression is a characteristic of transformed cells, and genetic inactivation of eIF5A2 slows down proliferation and causes death of such cells. At present, no inhibitors acting directly on eIF5A and, moreover, selectively suppressing the eIF5A2 isoform are known. The development of such inhibitors can serve as an aim for future research.

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