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REVIEW

Noncanonical Functions of Aminoacyl-tRNA Synthetases

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Abstract—Aminoacyl-tRNA synthetases, together with their main function of covalent binding of an amino acid to a corresponding tRNA, also perform many other functions. They take part in regulation of gene transcription, apoptosis, translation, and RNA splicing. Some of them function as cytokines or catalyze different reactions in living cells. Noncanonical functions can be mediated by additional domains of these proteins. On the other hand, some of the noncanonical functions are directly associated with the active center of the aminoacylation reaction. In this review we summarize recent data on the noncanonical functions of aminoacyl-tRNA synthetases and on the mechanisms of their action.

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Aminoacyl-tRNA synthetases (aaRS) are enzymes catalyzing the first key reaction of protein biosynthesis – the covalent binding of an amino acid to an appropriate tRNA molecule. The aminoacylation reaction is completed in two stages.

The first stage is activation of the amino acid molecule:

$$AA + ATP \leftrightarrow AA\text{-}AMP + PPi,$$

where AA is a free amino acid molecule, AA-AMP is aminoacyl adenylate (which stays associated with the aaRS), and PPi is pyrophosphate.

The second stage is the attachment of the amino acid molecule to the tRNA molecule:

$$AA\text{-}AMP + tRNA \leftrightarrow AA\text{-}tRNA + AMP,$$

where AA-tRNA is aminoacyl-tRNA. At this stage the amino acid binds to the 2′- or 3′-hydroxyl group of the ribose at 3′-end of the tRNA molecule [1].

In the course of evolution aaRS have acquired additional domains and insertions in addition to preexisting domains, which expanded the range of functions performed by these enzymes. Table lists the noncanonical functions that are discussed in this review.

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EXTRACELLULAR FUNCTIONS OF aaRS

aaRS as cytokines. Substances that enter the extracellular space during apoptosis may be harmful to surrounding cells. For this reason, monocytes and macrophages have to quickly remove dying cells. In this regard, when in apoptosis cells secrete factors that have the ability to induce chemotaxis of leukocytes and monocytes. One such factor is the proinflammatory cytokine EMAP II (endothelial monocyte-activating polypeptide II). Its precursor (pro-EMAP) is cut and secreted from apoptotic cells, which results in appearance of active EMAP II (22 kDa) in the intercellular space. The mature cytokine causes phagocyte and leukocyte migration and stimulates phagocytes to synthesize α-tumor necrosis factor. EMAP II also has antiangiogenic activity [2].

Tyrosyl-tRNA synthetase (TyrRS, Fig. 1) of insects and vertebrates has an extended C-terminus in comparison with TyrRS of prokaryotes and lower eukaryotes. The sequence of this additional domain is 49% identical to the sequence of mature human EMAP II protein [3].

Full-length TyrRS is secreted in culture under apoptotic conditions and is cleaved by extracellular proteases. As a result, two cytokines are generated: a C-terminal fragment which is an EMAP II-like protein with cytokine properties and an N-terminal fragment (mini-TyrRS) which harbors a catalytic aminoacylation domain and is capable of stimulating leukocyte migration [4]. The mini-

Noncanonical functions of aminoacyl-tRNA synthetases

aaRS	Performed function	Organism
AlaRS	transcription regulation	*E. coli*
	tRNA import into mitochondria	higher plants (tobacco)
GlyRS	transcription termination	*Saccharomyces cerevisiae*
GlnRS	apoptosis regulation	*Homo sapiens*
GluProRS	translation regulation	*H. sapiens*
IleRS	cleavage of glycosidic bond	different organisms
LeuRS	splicing	*S. cerevisiae*
LysRS	cytokine	*H. sapiens*
	assembly of retrovirus particles	mammals
	involvement in DNA replication	*E. coli*
	synthesis of diadenosine oligophosphates	different organisms
	intracellular transport	*H. sapiens*
	tRNA import into mitochondria	*S. cerevisiae*
MetRS	homocysteine thiolactone formation	*E. coli, S. cerevisiae*
	involvement in synthesis of rRNA	mammals
SerRS	angiogenesis regulation	*Danio rerio*
	translation regulation	mammals (*Murinae*)
TyrRS	cytokine	higher plants (tobacco)
	angiogenesis regulation	–"–
	tRNA export from nucleus	different organisms
	splicing	*Neurospora crassa*
ThrRS	translation regulation	*E. coli*
TrpRS	angiogenesis regulation	mammals
	signal transmission inside the cell	*Drosophila melanogaster*
	γ-phosphate cleavage	higher eukaryotes

TyrRS molecule contains a conserved Glu-Leu-Arg motif typical for interleukin-8 (IL-8), a representative of the leukocyte chemoattractant protein family. The mini-TyrRS molecule is also similar to IL-8 in tertiary structure [4].

After cleavage, the C-terminal domain of TyrRS attracts a large number of macrophages to the apoptosis site. The macrophages remove remnants of cells and release their elastase into the extracellular space. The elastase, in turn, cleaves TyrRS. This attracts a larger

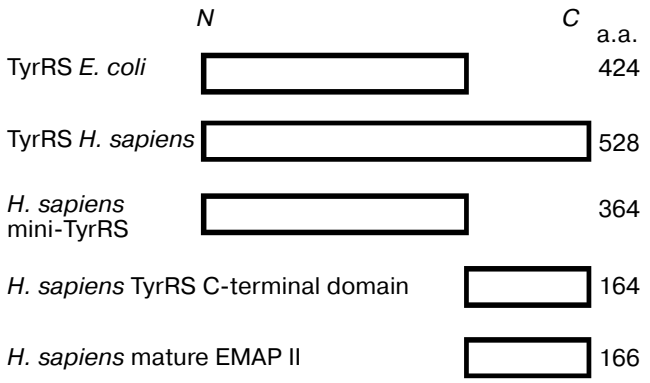

Fig. 1. Schematic comparison of the lengths and amino acid sequences of *E. coli* TyrRS, human TyrRS, human mini-TyrRS, C-terminal domain of human TyrRS, and human EMAP II.

number of macrophages to the site of apoptosis. Thus, the secretion of TyrRS provides timely liquidation of apoptosis products [3].

Another aaRS capable of recognition and elimination of dying cells is LysRS.

In the course of cell death, various cell death-associated molecules (CDAM) are exposed on the cell surface. The combination of exposed CDAM determines whether a body's immune response is specific or nonspecific [5].

In tumor cells in response to certain therapeutic agents (anthracyclines, ionizing radiation), calreticulin can be transferred from the lumen of the endoplasmic reticulum to the surface of the cell membrane. This results in the absorption of antigens by dendrite cells that present the antigens to the immune cells [6]. Treatment of tumor cells with the doxorubicin agent anthracycline leads to exposure not only of calreticulin, but also of LysRS, and their colocalization have been shown too. Decreased expression of LysRS results in decreased amount of exposed calreticulin [7]. The mechanism of LysRS secretion is still unknown since the protein lacks an export signal. It is thought that posttranscriptional modifications of LysRS play a role of the signal. Secreted LysRS functions as a cytokine and activates macrophages.

aaRS as regulators of angiogenesis. a) As described above, EMAP II (as well as the C-terminal domain of TyrRS) possesses an anti-angiogenesis activity. This protein was shown to inhibit angiogenesis stimulated by basic fibroblast growth factor. Significant antitumor activity of this protein was also demonstrated in the models of primary and metastatic tumors [8].

In contrast, mini-TyrRS possesses a pro-angiogenic activity, like α-chemokines that contain the Glu-Leu-Arg motif [2, 9]. The tertiary structure of TyrRS is organized in such a way that the EMAP II-like domain prevents the binding of the Glu-Leu-Arg motif to its receptors and blocks the pro-angiogenic activity of mini-TyrRS. After TyrRS is secreted and proteolytically cleaved in the extracellular space, the blocking effect is removed.

b) Tryptophanyl-tRNA synthetase (TrpRS) is a close homolog of TyrRS. Like mammalian TyrRS, mammalian TrpRS contain an additional domain in the N-terminus of the molecule (Fig. 2). Similar domains are found in mammalian His-, Gly-, and MetRS [10]. The domain has helix-turn-helix structure, which is known to take part in tRNA binding [11]. Perhaps this domain of aaRS facilitates the binding of tRNA. However, it is not necessary for aminoacylation, so its main function is unclear.

Alternative splicing of TrpRS mRNA leads to the formation of T1-TrpRS mRNA, which lacks the N-terminus of TrpRS.

Full-length TrpRS is inactive as an anti-angiogenesis factor, but T1-TrpRS is capable of blocking angiogenesis *in vivo*. In turn, T1-TrpRS can also be proteolytically cleaved, which results in formation of its shortened version, T2-TrpRS. Both T1- and T2-TrpRS bear antiangiogenic activity, but the activity of T2-TrpRS is higher. Specifically, this effect is manifested in retinal angiogenesis, the study of which showed that T2-TrpRS is associated with preexisting blood vessels in primary retinal layers and inhibits further angiogenesis [12].

Products of 15 of 27 genes with the expression profile similar to TrpRS are involved in signal transduction. Apparently, TrpRS is somehow involved in the transmission of signals from the environment into the cell. This was also evidenced by the increased expression of TrpRS during embryogenesis (for example, in the development of salivary glands in *Drosophila melanogaster*) [13].

Thus, the additional domains of TyrRS and TrpRS are similar in structure to many cytokines and growth factors. They are inactive in full-length aaRS, but under certain conditions the domains are split off from the aaRS molecules by proteases or synthesized separately from short mRNAs that were formed as result of alternative splicing. As a result, they get activated and function as regulators of angiogenesis: mini-TyrRS stimulates angiogenesis, and the C-terminal domains of TyrRS and TrpRS and T1-/T2-TrpRS, in contrast, inhibit this process.

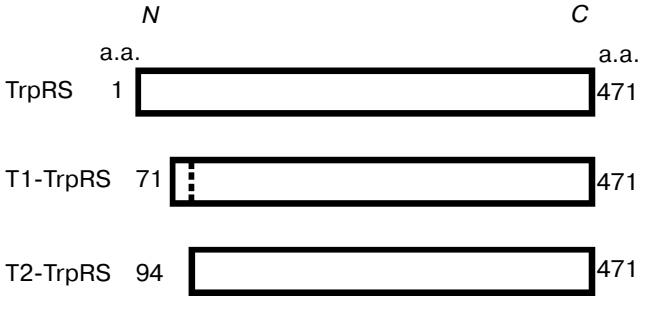

Fig. 2. Schematic comparison of lengths and amino acid sequences of human TrpRS, T1-TrpRS, and T2-TrpRS. The site of proteolytic cleavage is indicated by the dotted line.

These domains have the opposite effect on development of blood vessels. As a result, a coordinated mechanism of angiogenesis regulation is achieved [2].

c) Another aaRS that affects angiogenesis is seryl-tRNA synthetase (SerRS) [14]. Zebrafish SerRS mutants (the SerRS gene is present with the s277 allele) are characterized by the dilation of aortic arch vessels [15] and abnormal branching of established intersegmental and cranial vessels 60-h post-fertilization [15, 16]. The restoration of the normal phenotype is achieved not only by injection of wild-type SerRS mRNA, but also with the expression of mutant SerRS (with the T429A mutation) that is incapable of tRNA aminoacylation, which confirms that the involvement of SerRS in angiogenesis is not associated with the canonical aminoacylation function [16].

The vascular endothelial growth factor (VEGF) signaling pathway plays a central role in the development and function of blood vessels in vertebrates [17]. By activating tyrosine kinase receptors, VEGF stimulates the proliferation and migration of endothelial cells. Hyperfunctioning of this signaling pathway leads to a phenotype similar to the mutant SerRS. The inhibition of VEGF receptors in SerRS mutant embryos results in the suppression of the phenotype and the restoration of vascular development [15, 16]. Thus, hyper-functioning of the VEGF signaling pathway leads to the phenotype observed in SerRS mutants.

Based on these data we can conclude that SerRS controls vessel proliferation caused by the operation of the VEGF signaling pathway, but the mechanism of action of SerRS has not been studied yet.

Participation of aaRS in retroviral particle assembly. Retroviruses carry their genetic information in the form of single-stranded RNA. In the cytoplasm of the host cell, the process of viral RNA reverse transcription takes place, resulting in synthesis of double-stranded DNA (pro-viral DNA), which is then transported into the nucleus and integrated into the host cell genome. As a result, transcription of viral DNA produces either mRNA that can be spliced and translated to yield viral enzymes and structural proteins, or daughter genomic RNA. The tRNA are used to prime the process of reverse transcription, and different families of retroviruses use different tRNA: $tRNA^{Trp}$ is used by the group of avian sarcoma and avian leucosis viruses such as Rous sarcoma virus, $tRNA^{Lys}$ by HIV-1 virus, and $tRNA^{Pro}$ by Moloney murine leukemia virus. Primer tRNA are selectively packaged into retroviruses during their assembly, i.e. their relative concentration in the low molecular weight RNA population increases in moving from the cytoplasm to the virus. For example, the relative concentration of $tRNA^{Trp}$ has been reported to increase from 1.4 to 32%, while both $tRNA^{Lys}$ and $tRNA^{Pro}$ increase from 5-6% in the cytoplasm to 50-60 and 12-24% in virions, respectively [18].

The HIV-1 virion contains all three human isoacceptor lysine tRNA, but only one of them is used to prime the reverse transcription of viral RNA [18]. Packaging of lysine tRNA occurs independently from both the packaging of two genomic viral RNA and processing of Gag and GagPol precursor proteins. However, both of these proteins are required for packaging of tRNA. The tRNA is almost absent in the cell in free form, as it is normally transmitted from one component of the translational apparatus to another and is constantly in association with one of them, so the packaging of tRNA into viral particles requires the interaction of viral proteins with a translational apparatus protein – LysRS [19]. Both human LysRS (cytoplasmic and mitochondrial) are also packaged into the virions [20]. Gag interacts with LysRS, and GagPol is essential for the selective binding of complexes between tRNA and LysRS: in the absence of GagPol LysRS is only included into the virion [19, 21]. The aminoacylation state of tRNA is not important for its packaging [22].

Thus, LysRS is a factor responsible for the interaction of HIV-1 proteins with lysine tRNA and the selective packaging of tRNA into the virion.

CYTOPLASMIC FUNCTIONS OF aaRS

aaRS as regulators of apoptosis (GlnRS). Fas-proteins take part in transmembrane signaling. Upon binding the receptors, they trigger the apoptotic process, which takes place through the activation of apoptosis signal-regulating kinase 1 (ASK1) and c-Jun N-terminal kinase (JNK; also known as stress-activated protein kinase (SAPK)). Glutamine-tRNA synthetase (GlnRS) is associated with ASK1: complex formation involves the C-terminal catalytic domains of the proteins. GlnRS strongly represses the kinase activity of ASK1. The presence of glutamine further enhances the repression, contributing to the stabilization of GlnRS and ASK1 complex. In this system, glutamine can be considered as a signal molecule that blocks ASK1 activation by binding to GlnRS. Thus, GlnRS is a direct inhibitor of the apoptotic signaling pathway [23].

aaRS as regulators of translation. Control of gene expression can occur at different stages of this process. As for translation, the stage of initiation is the most frequently regulated. Regulatory molecules are usually proteins or RNA that bind the mRNA operator site and block the initiation or, alternatively, contribute to the release of the ribosome-binding site. A very common type of translation control is feedback control. With such type of control the final product of a gene (protein synthesized from mRNA, or even a compound synthesized by this protein) binds to mRNA and inhibits translation initiation (e.g. thiamine binds to several mRNA encoding enzymes involved in certain stages of its biosynthesis, and thus inhibits the translation initiation of these mRNA [24]).

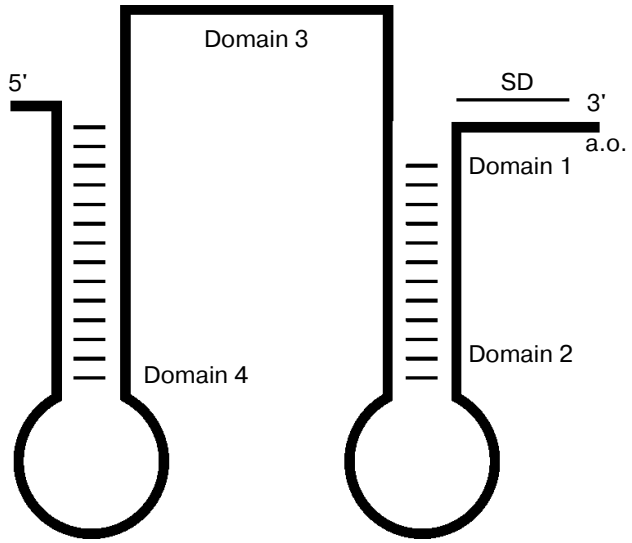

Fig. 3. Schematic representation of the operator site of *E. coli* ThrRS mRNA. SD, Shine–Dalgarno sequence.

a) *Feedback regulation of thrS (ThrRS gene) mRNA translation in E. coli*. ThrRS binds to the operator site located in the leader region of its own mRNA upstream from Shine–Dalgarno sequence. Thus, ThrRS binding prevents the binding of 30S-subunit of the ribosome to mRNA and inhibits translation initiation [25]. The operator site consists of four domains (Fig. 3). Domain 1 and the 3′-part of domain 3 together form a ribosome-binding site. Domains 2 and 4 are hairpins recognized by ThrRS exactly in the same way as ThrRS recognizes the anticodon loop of threonyl-tRNA [26].

Replacing the second (G) or the third (U) nucleotide in the anticodon of threonyl tRNA leads to defects of aminoacylation of this tRNA. The substitutions of corresponding nucleotides in the anticodon-like structures of domains 2 and 4 in ThrRS mRNA leader sequence lead to serious breaches of ThrRS synthesis control. After replacing the anticodon sequences in the hairpins with methionyl-tRNA anticodon, ThrRS synthesis will be controlled by methionyl-tRNA synthetase [27]. ThrRS binds two molecules of threonyl-tRNA, but only one molecule of ThrRS mRNA. Since ThrRS is active in a homodimeric form, it is might be assumed that each of the second and the fourth anticodon-like domains of mRNA leader sequence is associated with one subunit of the complex [28]. This assumption was confirmed by the comparison of crystal structures of complexes between ThrRS and threonyl-tRNA and between ThrRS and domain 2 of the ThrRS mRNA operator site [29, 30]. The same amino acid residues are involved in the bond formation with both the nucleotides of the tRNA anticodon loop and the nucleotides of domain 2 in the mRNA leader sequence.

Judging by the findings of recent studies, the binding of the ThrRS mRNA leader sequence leads to blocking the correct placement of mRNA in the "neck" of a small ribosomal subunit. The case of ThrRS mRNA translation regulation is interesting because it is based on similarity between the mechanisms of the basic and regulatory functions.

b) In mouse cells, SerRS is associated with mRNP (messenger ribonucleoprotein). It is assumed that SerRS is one of the proteins that bind to a cap structure and is involved in translation initiation. This hypothesis is also supported by the fact that the molecular weight of this aaRS corresponds to the weight of one of the cap-binding proteins, and that SerRS is accumulated in the 48S ribosomal pre-initiator complex in the presence of Edein (inhibitor of translation initiation). Nevertheless, it remains unclear if SerRS itself is capable of complex formation with mRNA or whether it occurs in an mRNP complex via protein–protein interactions [31].

c) In five aaRS of higher eukaryotes (TrpRS, HisRS, GlyRS, MetRS, and GluProRS) WHEP-domain is found, which consists of 50 amino acid residues forming a helix-turn-helix structure [32]. This structure (two closely packed antiparallel α-helices) is similar to the RNA-binding domains of the S15 ribosomal protein and the first nonstructural protein (NS1) of influenza A virus. Removal of the WHEP-domain only slightly reduces the aminoacylation activity of aaRS, and, apparently, it is responsible for the noncanonical functions of these enzymes [33, 34]. GluProRS contains three WHEP-domains. They are positioned in the linker part of the protein that connects the domains catalyzing glutamyl- and prolyl-tRNA synthetase activities (Fig. 4). The GluProRS linker region has the ability to bind the GAIT-element (IFN-γ-activated inhibitor of translation element) in the 3′-untranslated region (3′-UTR) of some mRNA (e.g. mRNA of the ceruloplasmin gene) [35]. Ceruloplasmin is a copper-containing plasmatic protein synthesized and secreted by hepatocytes and

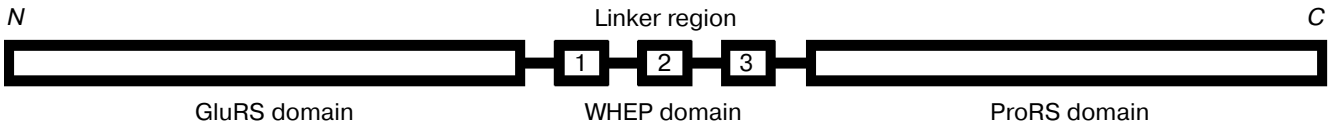

Fig. 4. Topological scheme of higher eukaryotes GluProRS.

activated macrophages. It is a multifunctional oxidase that plays an important role in the metabolism of iron and in the development of inflammation [36]. Ceruloplasmin synthesis is induced by interferon-γ (INF-γ) and is subsequently suppressed so as not to damage surrounding tissues with the large amount of oxidase in the extracellular space [37]. The GAIT protein complex binds the GAIT-element, and this binding stops translation [38].

In monocytes that are not stimulated by interferon-γ, GluProRS is present as part of a multi-synthetase complex [39]. In 2-4 h after stimulation with interferon, GluProRS is phosphorylated by a still unidentified kinase and released from the complex [40]. Phosphorylation seems to occur on serine residues in the linker section that connects the second and the third WHEP-domains [41]. The phosphorylated aaRS interacts with NSAP1 (NS1-associated protein-1) [35]. This complex is called the pre-GAIT-complex. It is unable to bind the GAIT-element and inhibit translation because the NSAP1 binding site in GluProRS overlaps with the GAIT-element binding site. In 10-12 h after pre-GAIT complex formation the ribosomal protein L13a becomes phosphorylated and is released from the ribosomal 60S subunit. Phosphorylated L13a and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) bind to the pre-GAIT-complex with the formation of the mature GAIT-complex, which binds to the GAIT-element in the 3′-UTR of mRNA and inhibits the translation of mRNA [35]. The binding of phosphorylated L13a and GAPDH leads to conformational changes of the pre-GAIT complex. As a result, NSAP1 releases the GAIT-element binding site in GluProRS [35].

Thus, under the influence of interferon-γ both the synthesis of ceruloplasmin and the activation of inhibitors of its translation take place. In this way the time of ceruloplasmin synthesis is limited, and thus the side effects of its long-term impact are avoided [40].

Unusual reactions catalyzed by aaRS. a) *Synthesis of diadenosine oligophosphates.* A long time ago it was observed that LysRS can catalyze the formation of Ap_4A from lysyl adenylate and ATP. However, the synthesis of diadenosine oligophosphates can also be catalyzed by other aaRS (e.g. PheRS). This reaction is greatly accelerated by the removal of pyrophosphate (in presence of pyrophosphatase). At least three substrates compete for the binding aminoacyl adenylate located in the complex with aaRS, namely tRNA (in this case, the reaction leads to the formation of aminoacyl-tRNA), pyrophosphate (reaction proceeds in the opposite direction with the formation of a free amino acid and ATP), and nucleoside di- or triphosphate (dinucleoside oligophosphate is formed). The balance between the reactions is regulated by the concentration of zinc ions: the higher the concentration, the more effective the reaction of diadenosine oligophosphate formation proceeds [42]. Recently a new mechanism of Ap_4A synthesis was discovered and described: GlyRS was shown to synthesize this compound directly from two molecules of ATP, without the presence of glycyl adenylate. Since, apparently, GlyRS is the only aaRS that is able to catalyze this reaction, it is suggested to influence the maintenance of a certain concentration of Ap_4A [43].

Dinucleoside oligophosphates perform a variety of functions: inhibition of potassium channels (human myocardium), activation of glycogen phosphorylase (rat hepatocytes), cell cycle control (*E. coli*), metabolic stress alarming (*E. coli*), and many others [44].

b) *Cleavage of the glycosidic bond* [45]. IleRS from *E. coli* can catalyze the cleavage of a glycosidic bond in 5-bromouridine. Analogs of this compound (5-fluoro-, 5-chloro-, and 5-iodouridine) can also be cleaved by the enzyme. The enzyme is also able to hydrolyze uridine to uracil and ribose, though the efficiency of this process is significantly lower. Other aaRS (AlaRS, ValRS, and TyrRS from *E. coli*; bovine pancreas TrpRS; rat liver LysRS [46]) can also catalyze the reaction of 5-bromouridine cleavage. At the same time, neither cytidine nor 6-azauridine can be split by these enzymes.

The efficiency of glycosidic bond cleavage by aaRS is rather low. It has not been studied yet whether this reaction is related to the process of tRNA aminoacylation.

c) *Cleavage of γ-phosphate from purine nucleosides* [47]. Mammalian TrpRS can cleave γ-phosphate from ATP and GTP with the formation of ADP and GDP, respectively. This reaction proceeds slowly and requires magnesium ions; the reaction is inhibited by zinc ions. The reaction is catalyzed by the N-terminus of the protein molecule, which is not involved in the catalysis of the aminoacylation reaction.

Bacterial and yeast TrpRS lack the domain corresponding to the N-terminal domain of mammalian TrpRS. Therefore, bacterial and yeast enzymes cannot cleave off γ-phosphate. Such a feature is inherent in TrpRS only in higher eukaryotes. The role of this process is still unknown.

d) *Homocysteine thiolactone formation* [48]. Homocysteine is an intermediate of methionine synthesis in prokaryotes and eukaryotes. Since methionine and homocysteine are similar in structure, homocysteine can also be aminoacylated by MetRS. However, an editing reaction takes place: after the first stage of the aminoacylation, homocysteine adenylate is cyclized to yield AMP and homocysteine thiolactone. Homocysteine thiolactone is accumulated in cells since homocysteine cannot be recovered from the compound (in contrast to other amino acids, whose adenylate can easily be split to AMP and a free amino acid). Such a mechanism of protection against incorrect aminoacylation and inclusion of homocysteine in the polypeptide chain is found in *E. coli*, *S. cerevisiae*, and in malignant cells of mammals, but it is not described for normal mammalian cells. The latter

probably use a different mechanism of protection against homocysteine aminoacylation.

aaRS are involved in DNA replication in bacteria. The initiation of replication of the bacterial plasmid ColE1 is a complicated and not fully understood process that involves many proteins and RNA molecules [49]. The synthesis of the primer (RNA II) for replication begins at the –555 position and extends to the *ori* site, where proper replication begins. Several single base-changes that alter the conformation of the primary transcript (primer for replication), RNA II, lead to a decrease in the efficiency of replication of ColE1 [50]. Replication of the plasmid carrying the *cer114* mutation can be restored by the *herC180* mutation. It was found that the *herC* gene in *E. coli* encodes lysyl-tRNA synthetase [51].

The *cer114* mutation, a single base pair substitution 95 bp upstream from the replication starting point, is found in the region of the non-transcribed strand of DNA that can be folded into a stem–loop structure mimicking that of a $tRNA^{Lys}$ molecule. The mutation (the point thymine to cytosine substitution) destroys a TTT triplet, which occupies the position of the anticodon in the tRNA-like structure, resulting in the TTC "anticodon" [52].

Other mutations that lead to the defects of ColE1 replication also affected the structure of this tRNA-like region (for example, they destroy the pairing of nucleotides in the "anticodon branch"). Suppressor mutations restored the structure of the "clover leaf". Perhaps the similarity of the site that is upstream to *ori* with the lysine tRNA is functionally necessary [52].

Based on indirect evidence, LysRS is involved in the replication of ColE1: the enzyme apparently binds to the tRNA-like structure, which contributes to the stabilizing of single-strand DNA.

aaRS as regulators of gene transcription (alanyl-tRNA synthetase (AlaRS) in *E. coli*) [53]. *Escherichia coli* AlaRS inhibits the transcription of its own *alaS* gene. Elevated concentrations of the cognate amino acid increase repression.

According to the results of DNA footprinting, AlaRS protects DNA nucleotides from nuclease action in the –69...–79 and –92...–102 regions. Between these regions the promoter and transcription starting point are found (the latter corresponds to position –79). Protected areas contain palindrome sequences whose centers are separated by 19 bp, which almost corresponds to two turns of DNA helix in B-form. *Escherichia coli* AlaRS, which has four subunits (molecular mass of each is 95,000 Da), is the largest prokaryotic aaRS. It is large enough to simultaneously interact with regulatory sites separated by two turns of DNA helix. One molecule of the enzyme interacts with both regulatory sites at the same time, which results in the repression of the *alaS* gene transcription.

NUCLEAR FUNCTIONS OF aaRS

aaRS are involved in the synthesis of rRNA. Human cytoplasmic methionyl-tRNA synthetase (MetRS) consists of about 900 amino acid residues [54]. This aaRS is one of the components of the multi-synthetase complex [55]. However, human cells also possess a population of free MetRS. The core domain of the synthetase is homologous to that in prokaryotes. In addition, human MetRS contains an N-terminal extension of 297 amino acid residues that is not required for catalytic activity but is involved in protein–protein interactions for the formation of the multi-synthetase complex [56]. The C-terminus of human MetRS is extended in comparison to prokaryotic MetRS by about 40 amino acid residues, which also provide for interaction with proteins and nucleic acids [32]. In addition, the C-terminus of human MetRS has a nuclear localization signal [57], which is unusual since aaRS are mainly localized in the cytoplasm.

In mammalian cells, MetRS is found both in the cytoplasm and the nucleolus [58]. Finding MetRS in the nucleolus depends on the presence of cell growth signals, but does not depend on the type of the signal and is induced by serum, epidermal growth factor (EGF), platelet derived growth factor (PDGF), and insulin [58].

Nucleolar localization of MetRS is directly related to the synthesis of rRNA. This is evidenced by the fact that in the absence of RNA (after treatment with RNase) MetRS also disappeared from the nucleolus. The same effect was observed in the inhibition of RNA-polymerase I, which is responsible for the synthesis of ribosomal RNAs. Synthesis of 18S and 28S rRNA is also directly correlated with the amount of MetRS in the nucleolus [58]. Therefore, MetRS is involved in protein synthesis both as an enzyme and as a positive regulator of ribosome biogenesis [59]. Despite the fact that the involvement of MetRS is necessary for rRNA synthesis, the mechanism and the reasons for its involvement in the synthesis are unknown.

Involvement of aaRS in 3′-end formation of mRNA molecules. The 3′-end formation of eukaryotic mRNA is a complicated process that involves factors recognizing and processing pre-mRNA as well as factors facilitating the dissociation of RNA-polymerase II from the DNA template. Many participants in this process are already known, but there is still no complete model of the process. The termination mechanism is supposed to be the recognition of the polyadenylation signal, which leads to the cut of the mRNA molecule at the appropriate site and to the consequent dissociation of the enzyme and, as a result, termination of transcription [60].

Transcription factors may be a part of the polyadenylation complex [61], suggesting the close association of transcription and mRNA processing *in vivo*. To coordinate the termination of transcription and transcript processing, additional factors that affect both processes are

essential. Changing the pattern of RNA polymerase modifications (e.g. reducing the degree of phosphorylation of the C-terminal domain) affects the fidelity of the enzyme [62]. Besides, there are additional transcription factors that can directly modify the transcription complex so that the dissociation of the complex from the matrix occurs, or alternative ways of transcription termination may be involved, such as chromatin remodeling [63].

During the study of the transcription termination mechanism additional interactions have been found between the components of the transcription complex and trans-acting factors. It turned out that one of the mutations (P552F) in the *S. cerevisiae* glycyl-tRNA synthetase (GlyRS) gene leads to RNA polymerase not recognizing the functional transcription termination signals [64]. This results in a pronounced temperature-sensitive phenotype, which is manifested in decrease of growth rate at elevated temperature (37°C).

The assumption that aaRS can interact with the 3′-end of mRNA was supported by data on the presence of tRNA-like structures at the 3′-ends of some yeast mRNA (*ADH2*, *CYC1*) [64]. It was confirmed that GlyRS binds tRNA-like structures, and this binding occurs by the same part of the protein as the binding of tRNA. However, mutant GlyRS was unable to bind tRNA-like structures. At the same time, mutant mRNA, whose transcription termination was disrupted, were not bound by wild-type GlyRS. Since the aminoacylation ability of mutant GlyRS was only slightly reduced in comparison with wild-type GlyRS, these are transcription termination disorders that cause a pronounced phenotype in yeast [65].

Involvement of aaRS in tRNA export to the cytosol. Eukaryotic pre-tRNAs are synthesized in the nucleus, and there are several stages of processing required for the formation of mature tRNA molecules from the precursor: the removal of introns and extra nucleotides at the 3′- and 5′-ends, nucleotide modification, the joining of the CCA sequence to the 3′-end [66, 67], and only then the export of tRNA to the cytoplasm takes place. Some tRNA that contain introns are exported to the cytoplasm, where the excision of introns occurs, and then they are imported back into the nucleus [68, 69]. After this the quality assurance step occurs and mature tRNA are exported to the cytoplasm. The aminoacylation of tRNA in the nucleus is one of the ways to test the maturity of tRNA. This stage occurs in the nucleolus [70]. However, the selection of tRNA can be accomplished in other ways, since aminoacylation is not absolutely necessary for the export of tRNA either in *X. laevis* or in *S. cerevisiae* [71, 72]. The export of tRNA from the nucleus to the cytoplasm occurs in two ways: in one case tRNA aminoacylation is essential, in the other it is not [70]. To date, some of the components required for the export are identified. Among them there is Ran GTPase and its regulators involved in the export from the nucleus and import into the nucleus of many proteins and RNA (including tRNA) [73, 74]. Other regulators include exportin-t in mammals and its yeast homolog Los1p. Both of them are able to directly bind tRNA [75, 76].

In the study of the efficiency of tRNA export to the cytoplasm in yeast strains mutant for methionyl-, isoleucyl-, or tyrosyl-tRNA synthetase, it was found that each mutation led to significant defects in export of relevant tRNA, which means that aminoacylation is important for the export [67]. Mutation of MetRS did not destroy the export of isoleucine and tyrosine tRNA. However, in cells of yeast strains carrying mutations in the IleRS or TyrRS gene, defects in the export of all three tested tRNA – isoleucine, tyrosine, and methionine – were observed. When replacing the wild-type TyrRS with mutant TyrRS whose catalytic activity was not broken, but which was unable to be imported into the nucleus, it turned out that not only the export of tyrosine tRNA is disrupted, which could be due to the absence of aminoacylation in the nucleus, but also the export of isoleucine and methionine tRNA, which indirectly confirms the involvement of aaRS in tRNA export from the nucleus to the cytosol [72]. The mechanism of export is still unknown.

MITOCHONDRIAL FUNCTIONS OF aaRS

Involvement of aaRS in splicing of group I introns of mitochondrial RNA. The first group of introns includes the introns of nuclear rRNA from *Tetrahymena* sp. and *Physarum polycephalum*, the majority of fungal mitochondrial RNA introns, some chloroplast introns, and introns of T-even phages [77]. All of these introns are characterized by the same mechanism of splicing, consisting of two consecutive *trans*-esterification reactions: the joining of guanosine to the 5′ end of the intron (accompanied by splitting in the 5′-site splice) and exon ligation (followed by splitting of 3′-splice site). Some of these introns are capable of self-splicing *in vitro*, but their splicing *in vivo* requires the participation of proteins that are probably necessary for the folding of RNA into a catalytically active conformation [78].

Baker's yeast mitochondrial LeuRS has been shown to participate in the splicing of group I introns of mitochondrial mRNA. LeuRS-mediated splicing involves also bI4 maturase (encoded by the fourth intron of the cytochrome *b* gene). The absence of bI4 maturase destroys splicing, but this defect can be suppressed by LeuRS mutations in the so-called "suppression sites" [79]. Other mutations affecting DNA regions next to the "suppression sites" have been shown to strongly reduce the efficiency of splicing mediated by LeuRS, but they do not affect the tRNA aminoacylation ability of the enzyme [80]. Splicing efficiency is measured by the ratio of mature mRNA to its precursor, determined by RT-PCR. All of the described mutations (both the absence of sup-

pressors of bI4 maturase and splicing destroyers) are localized at a site of LeuRS called CP1 (connective polypeptide 1), which forms a separate domain in the tertiary structure of LeuRS, ValRS, and IleRS [81, 82]. The CP1 domains of all of these aaRS have been shown to participate in aminoacylation editing [83], but only in the case of LeuRS the CP1 domain is necessary for mRNA splicing. However, the CP1 domain of IleRS does not complement mutations in the CP1 domain of LeuRS. Apparently, the CP1domain of LeuRS interacts with introns in the same manner as with leucine tRNA [84].

Neurospora crassa mitochondrial TyrRS was shown to take part in the splicing of the large mitochondrial rRNA intron by stabilizing the catalytically active structure of the RNA. TyrRS binds first with a single RNA domain (P4-P6), contributing to the formation of its stable helical structure, and then with the second domain (P3-P9 that consists of the P3, P7, P8, and P9 helices), stabilizing their relative position to form a catalytically active splice site [85].

The C-terminal domain of the synthetase interacts with the P4-P6 domain of the intron, which is similar in structure to the anticodon loop of tRNA, and the N-terminal domain binds to the secondary structure P5 and P9 elements that form a structure similar to the amino acceptor stem of tRNA. Thus, TyrRS interacts with the introns in the same way as with tRNA [86]. Indeed, the results of TyrRS point mutagenesis showed that the regions of the protein required for splicing are distributed over the amino acid sequence and overlap with regions of the protein responsible for binding of tRNA [87]. Studies on the structure of the group I introns showed a distinct similarity in structures of introns and tRNA [85].

In *N. crassa* and *P. anserina* TyrRS (TyrRS of the latter is also shown to participate in the splicing of mitochondrial RNA) domains were found that are absent in TyrRS not involved in splicing (*E. coli*, *S. cerevisiae*) [88]: additional α-helix in the N-terminus of the protein (denoted H0) and a small domain in the nucleotide binding site [89]. Moreover, in the C-terminal domain of *N. crassa* TyrRS a large number of insertions and extensions were found, which make it more than twice longer in comparison with the C-terminal domain of bacterial TyrRS.

Involvement of aaRS in tRNA import into mitochondria. In the yeast *S. cerevisiae* there are three iso-acceptor lysine tRNA, two of which are encoded by the nuclear genome, and one by the mitochondrial genome. One of the tRNA encoded by nuclear DNA ($tRNA_1^{Lys}$, with the CUU anticodon) is partially imported into mitochondria (95% of the molecules are found in the cytosol, 5% in the mitochondrial matrix) [90, 91]. The second lysine tRNA ($tRNA_2^{Lys}$, with the UUU anticodon) is found exclusively in the cytoplasm, and the third ($tRNA_3^{Lys}$, with the UUU anticodon) is encoded in the mitochondrial genome and functions in mitochondrial translation only.

To be imported, it is necessary for $tRNA_1^{Lys}$ to be aminoacylated (this apparently allows it to take a specific tRNA conformation required for its import) [92]. Then $tRNA_1^{Lys}$ binds to Eno2p (one of two isoforms of the enolase glycolytic enzyme) and delivered to the surface of mitochondria [93], where it binds to the precursor of mitochondrial lysyl-tRNA synthetase (pre-mitoLysRS) [94]. The presence of Eno2p significantly reduces the dissociation constant of the complex between $tRNA_1^{Lys}$ and pre-mitoLysRS (from 180 to 40 nM). It is assumed that $tRNA_1^{Lys}$ is transferred into the mitochondrial matrix in complex with pre-mitoLysRS. In the matrix the complex dissociates, the mitochondrial localization signal of pre-mitoLysRS is cut off, and the protein begins to function as a synthetase, namely to aminoacylate $tRNA_3^{Lys}$ [95]. The $tRNA_1^{Lys}$ is necessary for mitochondrial translation at elevated (non-permissive) temperatures, since in this case $tRNA_3^{Lys}$ is hypo-modified at the first position of the anticodon and is not able to recognize effectively the lysine codon that is not completely complementary (AAG) [96].

The involvement of aaRS in tRNA import into plant mitochondria is also expected. The number of imported tRNA greatly varies from species to species: for example, in *A. thaliana* mitochondria six tRNA are imported, in *T. aestivum* – four tRNA, and in *S. tuberosum* – more than a dozen different tRNA species. Import mechanisms also differ depending on the species, and there are no reliable models of the process so far, but we know that aaRS are essential components. An indirect proof of this lies in the fact that mutations in alanine tRNA preventing its binding to AlaRS also block the import of tRNA into the mitochondria [97]. A similar effect was observed in the case of valine tRNA [98]. Other mutations that do not affect the determinants of tRNA recognition by aaRS did not affect the import of tRNA into mitochondria.

As described above, aaRS have a great variety of functions that are not related to their canonical function of the synthesis of aminoacyl-tRNA. In the case of eukaryotes, it may be related to an evolutionary strategy to maximize the functional diversity of proteins without increasing the number of genes. The aaRS of all organisms can be considered as "hotspots" of the regulation system: aaRS as a class of proteins are functionally related to the processes of splicing, apoptosis, transcription, translation, and transport of proteins and tRNA. Due to this diversity of functions aaRS have a tremendous impact on the life of cells and contribute to the regulation and coordination of processes in the cell and the organism as a whole.

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