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Review

The new aspects of aminoacyl-tRNA synthetases✪

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Aminoacyl-tRNA synthetases (AARS) are essential proteins found in all living organisms. They form a diverse group of enzymes that ensure the fidelity of transfer of genetic information from the DNA into the protein. AARS catalyse the attachment of amino acids to transfer RNAs and thereby establish the rules of the genetic code by virtue of matching the nucleotide triplet of the anticodon with its cognate amino acid. Here we summarise the effects of recent studies on this interesting family of multifunctional enzymes.

The universal genetic code is established in a single aminoacylation reaction of transfer ribonucleic acids (tRNAs). The reaction is catalysed by the family of aminoacyl-tRNA synthetases (AARS) each of which activates an amino acid by binding to ATP and transfers it to the 3′ end of the cognate tRNA. The conservation of the genetic code suggests that AARS evolved early and were probably among the first protein enzymes to emerge from the RNA world [1, 2]. Interestingly, AARS are the only components of the gene expression apparatus that function at the interface between nucleic acids and proteins. This leads to three interesting aspects of studies on aminoacyl-tRNA synthetases: (i) the mechanism of amino acid recognition and chemical activation, (ii) the specificity of tRNA recognition, and (iii) the origin and evolution of AARS [3].

AARS constitute a family of 20 cellular enzymes that are responsible for specific esterification of tRNAs with their cognate

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Abbreviations: AARS, aminoacyl-tRNA synthetases (e.g. GlnRS, glutaminyl-tRNA synthetase); aa-tRNA, aminoacyl-transfer ribonucleic acid; Ap_nA, diadenosine oligophosphates; EDD, embryo-defective development; EMAP II, endothelial monocyte-activating polypeptide II; NLS, nuclear localization signal.

amino acids, and thus are essential in maintaining the fidelity of the protein biosynthesis process. In Prokaryota their number varies due to doubling of some aminoacyl-tRNA synthetase genes, post-aminoacylation recharging of aa-tRNA as an alternative decoding mechanism and, finally, presence of bifunctional AARS. In contrast, in all eukaryotes a full set of AARS genes has been found [4–11]. The aminoacyl-tRNA synthetases database Y2K is available at http://biobases.ibch.poznan.pl. Recent investigations have shown that AARS are capable of a broad repertoire of functions that not only affect protein biosynthesis, but also extend to a number of other cellular activities (Fig. 1).

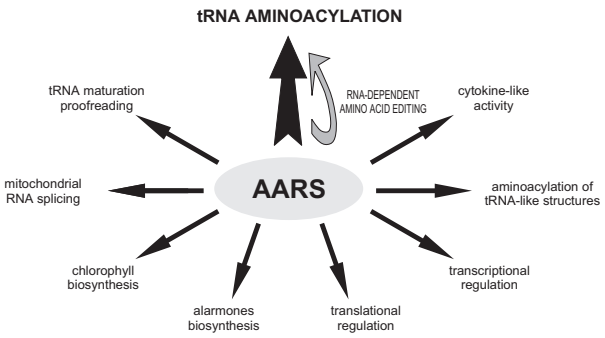

Figure 1. The cellular activities of aminoacyl-tRNA synthetases.

The functions of AARSs are described and discussed in the text, except for chlorophyll biosynthesis [6], binding to DNA [76] and aminoacylation of tRNA-like structures [77–79].

Specific AARS are involved in translational fidelity, tRNA processing, RNA splicing, RNA trafficking, apoptosis and transcriptional and translational regulation [12–14]. The aminoacylation reaction catalysed by AARS is achieved in two steps. First, the amino acid is activated by attacking a molecule of ATP at the α-phosphate, giving rise to a mixed anhydride intermediate, aminoacyl-adenylate, and inorganic pyrophosphate. In the second step, the activated amino acid moiety is transferred to the 3′-terminal ribose of the cognate tRNA, yielding the specific aminoacyl-tRNA and AMP. The first step takes place in the absence of tRNA, however, in some cases (GlnRS, GluRS and ArgRS) amino acid activation is tRNA dependent [6, 7]. tRNA binding to AARS is thought to proceed through an initial broad specificity interaction that is followed by more precise recognition that involves conformational changes of both AARS and cognate tRNA. This allows entry into the translation state of catalysis and provides a major contribution to tRNA specificity, as bound noncognate tRNAs normally are not charged [15, 16].

The fidelity of aminoacylation is controlled by positive (identity) and negative regulatory elements in tRNAs and AARS which permit both recognition and productive binding of the cognate pairs as well as discrimination against a non-productive binding of non-cognate pairs. The accuracy of this process is at least 10000:1, and constitutes one of the fundamental phenomena in protein–nucleic acid molecular recognition [5–9, 12–16]. Identity residues, properly located in several regions of the tRNAs, trigger specific recognition and charging by the cognate AARS [6, 7].

AARS — DIVERSITY OF STRUCTURES AND FAMILY CLASSIFICATION

Although aminoacyl-tRNA synthetases catalyse the same basic reaction and share the common substrate (ATP) and cofactor (magnesium), they have long been known to differ in their size, amino-acid sequences and subunit structure. The quaternary structures of AARS include monomers (α), homodimers and tetramers (α_2, α_4) and heterotetramers ($\alpha_2\beta_2$). The peptide size of the subunits in *Escherichia coli* varies from 344 aa for TrpRS to 951 aa for ValRS [6–9]. AlaRS is an α_4 tetramer while both PheRS and GlyRS are $\alpha_2\beta_2$ tetramers. PheRS adopts the $\alpha_2\beta_2$ tetrameric structure in all prokaryotic and eukaryotic cytoplasmic sources known. A

crystal structure of PheRS from *Thermus thermophilus* indicates that this tetrameric structure actually behaves as an $(\alpha\beta)_2$ structure, that is a dimer built from two heterodimers [17]. A different situation is observed for yeast and human mitochondrial PheRS, which are active in a monomeric form [18]. They constitute a class of PheRS distinct from the enzymes found in prokaryotes and the eukaryotic cytoplasm [18]. The eukaryotic enzymes are usually larger than their prokaryotic counterparts. This is due to the presence of the carboxy- and amino-terminal extensions that are dispensable for aminoacylation, but their function is still unclear [19–21]. However, it has been shown that the N-terminal appended domain of yeast GlnRS increases the stability as well as concentration of productive complexes between *E. coli* GlnRS and yeast $tRNA^{Gln}$. The fused domains do not distort or reorient the complexes away from a productive mode but increase the "on" rate and decrease the "off" rate for association of tRNA with the AARS [22, 23]. Since amino acids share a common core structure and tRNAs show the same basic fold, aminoacyl-tRNAs can be specifically recognised by other components of the protein biosynthesis pathway, e.g. elongation factors.

The AARS can be divided into two classes (I and II) of 10 members each, based on the presence of mutually exclusive amino-acid sequence motifs [20, 21, 24]. This division reflects structurally distinct topologies within the active site and two different structural frameworks evolved independently to catalyse the aminoacylation reaction. The catalytic domain of class I enzymes is formed by a five-handed parallel β-sheet (Rossman fold), which was already known as a nucleotide binding element. This group of related enzymes was originally assigned by identification of two signature sequences: HIGH and KMSKS. These sequence motifs are critical elements in the structure of the active site for aminoacyl-adenylate synthesis. The class I AARS approach the end of tRNA acceptor helix from the minor groove side and catalyse attachment of the amino acid to the 2′-OH at the end of tRNA chain. On the other hand, class II enzymes have a seven-stranded β-structure with three α-helices, that was identified for the first time in the structure of SerRS, and three sequence motifs, numbered 1, 2 and 3 [20, 21, 24]. Class II AARS (with one exception), approach the end of tRNA from the major groove, attaching the amino acid to 3′-OH. The crystallographic studies on aminoacyl-tRNA synthetases in free and complexed forms allow getting insight into the specificity of substrate recognition and the catalysis itself [24]. Many idiosyncratic domains are attached to and/or inserted in the class defining catalytic core and are responsible for binding and recognition of cognate RNAs.

A broader evolutionary interest in the AARSs stems from their biological function being one of the core requirements for progression from the RNA world to the universal common ancestor in numerous schemes for the origin of life [5, 25, 26]. In *Methanococcus jannaschii* genome 16 aminoacyl-tRNA synthetase gene homologues have been identified. The genes of GlnRS, AsnRS, LysRS and CysRS were missing. In addition, the assignment of an open reading frame encoding SerRS was based on rather low homology, while there appeared to be three reasonable candidates to encode the two subunits of PheRS. Components of the selenocysteinyl-tRNA synthesis pathway could not be identified [27]. Recently it was shown that cysteinylation in certain *Archaea* is performed by synthetase of unique structure — bifunctional ProCysRS [10, 11].

It is generally assumed that an AARS of a given specificity will always belong to the same class regardless of its biological origin, reflecting the ancient evolution of this enzyme family. The only known exceptions to this rule are LysRSs, which are composed of two unrelated families, class I enzymes in certain *Archaea* and bacteria and class II enzymes in all other organisms. Although the class I and

class II LysRS recognise the same sites in their tRNA substrates, they differ in their exact mechanism of lysine activation [28, 29]. The class I LysRS binds to the minor groove of the helix, whereas the class II enzyme approaches from the major groove side. The class II LysRS of *E. coli* accommodates major groove determinants encoded by G–C, but is blocked by the presence of a G–U pair. In contrast, the class I LysRS of *Borrelia burgdorferi* accepts the minor groove determinants of G–C or G–U. In this organism the presence of blocking determinants for a class II LysRS could explain its displacement by its class I counterpart [28, 29].

AMINOACYLATION PROOFREADING

The accurate flow of genetic information during DNA replication and protein biosynthesis cannot be explained in terms of conventional specificity-related mechanisms. Therefore additional proofreading mechanisms operating at several levels are needed. Such mechanisms control the fidelity of the DNA and RNA synthesis and of aminoacylation as well. A requirement for proofreading is evident in the case of AARS that have isosteric or smaller competing substrates. One of the strongest factors determining the specificity of aminoacylation is steric repulsion. A smaller substrate can always rattle around in a larger cavity. On the other hand, it is energetically unfavourable to cram a larger substrate into the active site built for a smaller one. The active site of aminoacyl-tRNA synthetase acts as a coarse sieve, allowing activation at a substantial rate of only those amino acids that are of the same size as, or smaller than the desired one. The active site responsible for hydrolysis of the aminoacyl-adenylate is a fine sieve that destroys the products of activation of amino acids smaller that the correct ones [15]. The "double sieve" mechanism for proofreading (editing) has been confirmed by solving the crystal structure of isoleucyl-tRNA synthetase from *Th. thermophilus* [30]. In this mechanism the selectivity is achieved by sterical excluding at the synthetic active site of amino acids larger than the correct one, and only smaller amino acids are sterically allowed to bind at a hydrophobic editing active site. The two active sites of IleRS form separated domains and therefore they have to be brought closer together by rotation of the editing domain upon addition of tRNA [30]. Analysis of the crystal structure of *Staphylococcus aureus* IleRS suggests the existence of an alternative mechanism involving a tRNA-dependent shuttling of the incorrect product between the two active sites [31].

High selectivity of AARS is a prerequisite for their pivotal role in the implementation and translation of the genetic code. When differences in binding energies of amino acids to an AARS are inadequate, editing is used as a major mechanism of the enzyme selectivity. It is known that AARS belong to the most accurate group of enzymes. Although numerous non-cognate amino acids are accepted as substrates by many AARS in the first step of aminoacylation, in most cases they are not transferred to tRNA, e.g. homocysteine is mischarged by IleRS, LeuRS and MetRS at frequencies exceeding the frequency of translation errors *in vivo* but is never transferred to tRNA. In a few cases where a non-cognate amino acid is transferred to tRNA, the misacylation is 10^3–10^6 fold less efficient than the correct charging, e.g. IleRS promotes one reaction of $tRNA^{Ile}$ with Val per 350 000 correct acylations with Ile; ValRS promotes one aminoacylation of one molecule of $tRNA^{Val}$ with Ile and Thr per 5 000 and 350 000 cognate chargings with Val, respectively. LysRS catalyses one mis-aminoacylation of $tRNA^{Lys}$ with Arg, Thr, Met, Leu, Ala, Cys, and Ser per 1 600, 16 000, 32 000, 132 000, 265 000, 560 000 and 750 000 correct acylations with Lys, respectively [32]. Purified Arg-$tRNA^{Lys}$, Thr-$tRNA^{Lys}$ and Met-$tRNA^{Lys}$ were essentially not deacetyla-

ted by LysRS. On the other hand, LysRS possesses an efficient editing mechanism which prevents misacylation with ornithine. In this latter reaction ornithine lactam is formed [33].

SOME ADDITIONAL FUNCTIONS AND UNEXPECTED FEATURES OF AARS

In addition to aminoacylation some AARS have been also found to be involved in other cellular processes. LysRS, SerRS and PheRS were shown to be responsible for the synthesis and turnover of diadenosine tetraphosphate (Ap_4A) that plays an important role in the response of bacterial and eukaryotic cells to a variety of stress conditions [34–36]. The diadenosine oligophosphates (Ap_nA) including Ap_4A were recently shown to function within the cell as a new class of signalling molecules, called alarmones [37, 38]. Mitochondrial TyrRS from *Neurospora crassa* was shown to be a key component in splicing of group I intron of pre-rRNA, by substitution of the missing RNA domain of this otherwise self-splicing intron [39]. In some instances, the aminoacyl-tRNA synthetases are involved in autoregulation of their expression at the level of translation, by binding the tRNA-like structures within the mRNA [16].

In *E. coli,* the biosynthesis of ThrRS (encoded by *thrS* gene) is autoregulated by a feedback mechanism at the translational level [40]. The enzyme binds to the leader of its own mRNA close to the translation initiation site, thereby inhibiting ribosome attachment and, in consequence, translation. The translational operator of the *thrS* gene contains two stem-loop structures that both mimic the anticodon arm of *E. coli* $tRNA^{Thr}$ and interact with the monodimeric ThrRS. As for $tRNA^{Thr}$, the two last bases of the anticodon-like triplets are essential determinants for ThrRS recognition. The fact that $tRNA^{Thr}$ and the mRNA operator share common specificity determinants argues in favour of structural analogies between the two recognition modes. The macromolecular mimicry model predicts that the two anticodon-like domains of the *thrS* operator recognise the anticodon-binding domain of each monomer of the ThrRS. This accounts for a competition between the two RNAs for ThrRS binding and therefore links aminoacylation and regulation in a coherent manner [40]. An other example of mimicry can be identified between *E. coli* ribosomal protein L25 and GlnRS [41]. Tertiary structure comparisons indicate that the six-stranded β-barrels of L25 and the tRNA anticodon-binding domain of glutamyl-tRNA synthetase are similar. A recent model for the evolution of GlnRS family suggests that a subgroup of the eubacterial kingdom acquired GlnRS *via* horizontal gene transfer from an eukaryotic source [3, 42, 43]. Therefore it is possible that the six-stranded β-barrel, which constitutes an RNA-binding fold for L25 and the anticodon-domain of GlnRS, represent a case of convergent evolution [41].

AARS COMPLEXES AND HOMOLOGUES OF THEIR COMPONENTS

AARS do not require additional protein factors for activity, however, in higher eukaryotes, nine AARS are associated together forming a supramolecular multienzyme complex [44]. The function of the whole complex, which contains in addition to the AARS three auxiliary peptides: p43, p38 and p18, remains unknown. The exact structure and composition of these complexes is still controversial. Recently it has been shown that p43 may be a precursor of a tumour-specific cytokine, endothelial monocyte-activating polypeptide II (EMAP II), a conclusion based on over 80% sequence identity between the two proteins [45]. EMAP II was identified in the culture medium of murine fibrosarcoma cells treated by methylchilanthrene A [46]. It triggers an acute inflammatory response and is involved

in development-related apoptosis. The precursor of EMAP II is associated with the N-terminal extension of human ArgRS facilitating aminoacylation. It is processed with releasing C-terminal cytokine domain of 23 kDa, which shares homology with the C-terminal parts of MetRS of prokaryotes, archaea, nematode and plants [47], and also a yeast Arc1p/G4p1, which interacts with MetRS and GluRS [48–51] (Fig. 2).

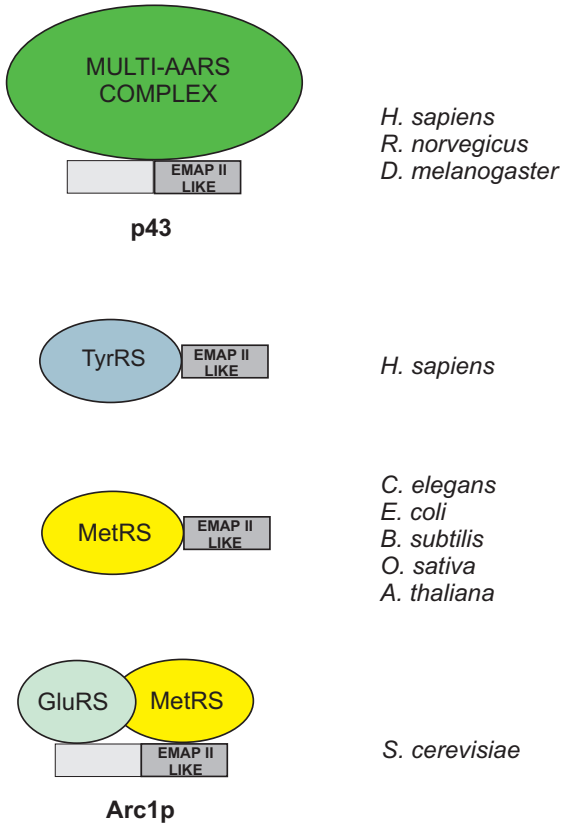

Figure 2. Different organizations of endothelial monocyte-activating polypeptide II(EMAP II)-like domains.

A sequence with strikingly high identity to EMAP II has been found in C-terminal part of p43 protein (a precursor of EMAP II [45]), which is present in multisynthetase complexes isolated from some animals and humans. Sequences with high homology to EMAP II were also found in the C-terminal domains of: human TyrRS; this domain shows a cytokine activity after being released from enzyme by specific proteolysis [52, 53]; MetRSs from some bacteria (e.g. *E. coli* and *Bacillus subtilis*), nematode *Caenorhabditis elegans* and plants (*Oryza sativa* – rice and *Arabidopsis thaliana*) [45, 47], Arc1p protein, which forms a complex with yeast MetRS and GluRS; it delivers tRNAs to those enzymes, increasing the efficiency of aminoacylation [49, 50].

Human TyrRS can be split into two fragments with distinct cytokine activities [52]. The EMAP II-like carboxy-terminal domain has potent leukocyte and monocyte chemotaxis activity and stimulates production of myeloperoxidase, tumour necrosis factor-α and MP tissue factor (tissue factor produced by mononuclear phagocytes – MPs). Its catalytic amino-terminal domain binds to the interleukin-8 type A receptor and functions as an interleukin-8-like cytokine [52]. Under apoptotic conditions, specific proteolysis of TyrRS leads to two cytokines [52, 53]. p38 is a non-synthetase component of the multienzyme complex and encodes a 320 amino-acid protein which has no homologue in yeast, bacteria or archaea. It is a moderately hydrophobic protein, displays a putative leucine-zipper motif and is able to associate with p43 and various AARS [54].

p18 is likely to be involved in association of the multisynthetase complex with other components of the protein biosynthesis pathway. Overall mass of a multiprotein particle is approximately 1.1–1.2 MDa. Different forms have been isolated, depending on the purification method. The results of application of computational microscopy for studying multisynthetase complexes support a model of a cup or elongated "U". The understanding of the structure of these multienzymatic complexes is important for finding of the functional link between the aminoacylation and other cellular processes [55].

AARS ARE PRESENT IN THE NUCLEUS

In 1998 evidence was provided for aminoacylation of tRNA in the nucleus of *Xenopus laevis*, which implies that AARS exist in nucleus [56, 57]. Since these enzymes are too large to be imported by passive diffusion, they are thought to use sequence signatures to enter the nucleus specifically. AARS probably bind through a nuclear localisation signal

(NLS) to the importins and translocate to nucleus by an energy dependent step [58]. Analysis of the databases identified NLS-like motifs in 15 cytoplasmic aminoacyl-tRNA synthetases from yeast *Saccharomyces cerevisiae* [58]. Comparison of those data with AARS sequences from *Arabidopsis thaliana* and *Caenorhabditis elegans* shows that potential NLS sequences and their localisation in protein have not been conservative through evolution (Table 1). Although uncharged tRNA is also exported, it seems that nuclear acylation makes the export more efficient [56]. Intranuclear aminoacylation of tRNA is therefore suggested to be a proof-reading mechanism ensuring that only correctly mature and aminoacylated tRNA will be exported [59].

AARS ROLE IN EMBRYO DEVELOPMENT

Recent investigations have shown that functions played by aminoacyl-tRNA synthetases are important for plant and animal embryogenesis. Embryo formation is the first

Table 1. Nuclear localization signals found in some eukaryotic aminoacyl-tRNA synthetases

AARS	Species	GenBank AccNo.	Potential NLS
AlaRS	*S. cerevisiae*	P40825	^{780}PI**KEKK**L
	A. thaliana	CAA80381	—
	C. elegans	CAA91396	—
ArgRS	*S. cerevisiae*	Q05506	—
	A. thaliana	CAB38959	—
	C. elegans	Q19825	—
AsnRS	*S. cerevisiae*	P38707	^{75}**KKK**AV**K**Q**K**EQEL**KK**Q**QK**
	A. thaliana	CAA10906	^{101}**RKRKK**
			^{569}**RKRRR**
			^{659}P**KE**K**R**
			^{738}PKPG**KKRK**
	C. elegans	Q19722	^{60}**KK**AV**KK**YEAEV**KK**LE**K**
AspRS	*S. cerevisiae*	P04802	^{31}**KK**AL**KK**LQ**K**EQE**K**Q**RKK**
	C. elegans	Q03577	^{10}P**K**LS**KK**ELN**K**LA**RK**A**KK**
CysRS	*S. cerevisiae*	P53852	—
	A. thaliana	AAD20662	^{236}**RKK**H
	C. elegans	AAC64633	^{19}**KKK**P
			^{303}PD**KKK**
			^{828}**KKRK**
GlnRS	*S. cerevisiae*	P13188	^{677}**K**P**KK**P**K**
	C. elegans	CAB08998	^{184}P**KKKEKK**
GluRS	*S. cerevisiae*	P46655	^{554}P**K**H**KK**
	A. thaliana	AAC36469	^{549}P**K**H**KK**
	C. elegans	CAB00060	—
GlyRS	*S. cerevisiae*	P38088	^{145}**KKKRKKK**
			^{436}P**K**F**RK**DA
	A. thaliana	O23627	^{545}**KK**SVNI**KK**NMVSIS**KEKKK**
	C. elegans	Q10039	—
HisRS	*S. cerevisiae*	SYBYHC	^{26}P**KK**G**K**LQ
	C. elegans	P34183	—
IleRS	*S. cerevisiae*	P09436	^{267}**KK**P**K**
	C. elegans	Q21926	—

LeuRS	*S. cerevisiae*	P26637	^{931}**KKKK** ^{968}**RK**LFSEQTLDDN**KK**V**R**E
	A. thaliana	AAD36946	—
	C. elegans	Q09996	^{408}**KKKK**P ^{937}P**KKK**
LysRS	*S. cerevisiae*	P15180	^{33}**KKR**I**K**Q**R**QVEA**KK**AA**KK** ^{56}PAS**KKK**T
	A. thaliana	AAD17333	^{43}**KK**EQ**K**M**K**Q**K**EEE**KRRK**
	C. elegans	Q22099	—
MetRS	*S. cerevisiae*	P00958	—
	A. thaliana	CAA74281	^{591}**KK**P**K**
	C. elegans	Q20970	^{733}P**KK**A**K**EQ**KK** ^{871}P**KKK**
PheRS	*S. cerevisiae* (β chain)	P15625	^{163}**KKRK**
	A. thaliana (β chain)	CAB43643	^{163}PE**RKK**
	C. elegans	CAA90360	—
ProRS	*S. cerevisiae*	P38708	^{375}PVV**K**G**RK** ^{492}**RK**HIHETA**R**SVES**R**L**KK**
	C. elegans	AAA50660	^{63}**K**P**KK**
SerRS	*S. cerevisiae*	P07284	^{54}**KK**FN**K**LQ**K**DIGL**K**F**K**N**K** ^{87}**KK**ELTE**K**EQQED**K**DL**KK** ^{458}**KKKK**
	A. thaliana	Q39230	—
	C. elegans	Q18678	—
ThrRS	*S. cerevisiae*	P04801	—
	A. thaliana	O04630	^{50}P**KR**I**K**
	C. elegans	P52709	—
TrpRS	*S. cerevisiae*	Q12109	—
TyrRS	*S. cerevisiae*	P36421	^{243}P**K**QV**KKK** ^{360}PQ**K**S**KK**A ^{367}**KK**P**K**
	A. thaliana	AAC69137	^{377}PQE**KKK**P
	C. elegans	AAB09162	—
ValRS	*S. cerevisiae*	P07806	^{65}**KK**P**KKKK** ^{38}**KKK**AE**K**LL**K**FAA**K**QA**KK** ^{53}**KK**NAAATTGASQ**KK**P**KK**
	A. thaliana	P93736	^{60}**RKKKK**EE**K**A**K** ^{436}**KR**H**K**

From the eukaryotic AARSs sequences present in computational data bases we have chosen those of *Arabidopsis thaliana* and *Caenorhabditis elegans* to compare them with NLS-like motifs of *Saccharomyces cerevisiae* (53).

— potential NLS was not found.

patterning process during vegetative plant growth. To identify "embryonic" genes a normal embryo development of *A. thaliana* has been disrupted by mutation [60]. A transposon element used as insertional mutagen enabled identification of the mutant *edd1* that leads to embryo-defective development (EDD). The insertion mutation was lethal, arresting embryo growth between the globular and heart stages. Sequences flanking the transposed element have been isolated and used to clone a full-length cDNA representing the wild-type EDD1 gene. Based on homology to *E. coli*, the EDD1 gene has been predicted to encode a novel glycyl-tRNA synthetase (GlyRS), not identified previously in higher plants. Its N-terminal portion was able to direct a marker protein into the pea chloroplast.

Thus, the EDD1 gene encodes a GlyRS homologue, probably a plastidic one [60].

In *Drosophila melanogaster* expression of the *Sex-lethal (Sxl)* gene dictates the choice between male and female development [61]. Early in embryogenesis the *Sex-lethal* gene expression is regulated at the level of transcription and then, later in embryogenesis, *Sxl* regulation switches to an autoregulatory RNA splicing. It has been shown that successful activation of *Sxl* requires both maternally and zigotically provided gene products, many of which are essential for viability and have other, non-sex specific functions. One of a novel maternally expressed genes *l(2)49Db* encodes aspartyl-tRNA synthetase [62]. Furthermore, mutations in tryptophanyl-tRNA synthetase are also gene dosage sensitive maternal modifiers of *Sxl*. Those data suggest that stable activation of *Sxl* in the embryo may be particularly sensitive to change in the translation machinery [62]. It has also been found that TrpRS is expressed to high levels in the developing *Drosophila* salivary gland [63]. Its expression is dependent on the homeotic gene Sex combs reduced (Scr) and is required for embryonic survival.

AARS IN MEDICINE

Several aminoacyl-tRNA synthetases have been found to act as autoantigens in a subgroup of patients with the idiopathic inflammatory myopathies, polymyositis and dermatomyositis [64]. Autoantibodies against synthetases are found almost exclusively in these cases, with a patient having usually antibodies against only one synthetase. Most commonly, the antibodies are directed against HisRS, labelled "anti-Jo-1" autoantibodies, but the antibodies to threonyl-, asparaginyl-, alanyl- or glycyl-tRNA synthetases or the multienzymatic complex have also been found [64, 65].

Recently, a subset of familial and sporadic amyotrophic lateral sclerosis cases have been found to be associated with the mutations in the gene encoding Cu, Zn superoxide dismutase (SOD1), that binds to lysyl-tRNA synthetase [66].

AARS have been used as a model for screening of synthetase-directed drugs [66]; their advantages are solubility, relative stability, possibility of expression from recombinant genes and of purification, easiness of assay by several methods as well as a large body of data on their X-ray structures [67–71] and chemistry of aminoacylation. However, design of a drug is limited by its potential toxic effects which could appear due to insufficient specificity of a drug for the pathogen as opposed to its human host. The present efforts are directed at development of new antibiotics, which would inhibit the activity of one or more AARS from such pathogens as *Enterococcus faecalis*, *S. aureus*, *Streptococcus pneumoniae*, *Helicobacter pylori*, *Mycobacterium tuberculosis* or *Candida albicans*. Vancomycine is now the drug of choice in treatment of potentially lethal infections caused by *S. aureus* [72], but resistance to this antibiotic has already started to emerge [73]. As an alternative for vancomycine could serve pseudomonic acid (mupirocine), synthesized by *Pseudomonas fluorescens* [74], which is an inhibitor of isoleucyl-tRNA synthetases from Gram positive infectious pathogens, including *S. aureus*, *Staphylococcus epidermalis*, *Staphylococcus saprophyticus,* and from Gram-negative organisms such as *Haemophilus influenzae*, *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Pseudomonic acid has an approximately 8000-fold higher selectivity for pathogen IleRS *vs.* mammalian one [75]. However its use is limited by the lack of systemic bioavailability, so that the drug can be ordered only for external applications (in skin infections). An other promising future drug is a synthetic adenylate-like inhibitor of pathogenic IleRSs, called CB432 [67]. It shows a 60–1100-fold higher discrimination of the pathogen than the human enzyme. It is able to arrest cell growth of *S. aureus*, *Streptococcus*

pyogenes and *E. coli* (permeability mutant) in culture at minimum inhibitory concentrations (MIC) of 10, 0.5, and 10 μg/ml, respectively (cited by [67]). On the other hand, high dosage of CB432 is correlated with its low bioavailability, due at least in part to binding to serum albumin. However, these data provide a starting point for further efforts leading to design of a fully efficient and available drug.

CONCLUSIONS

Aminoacyl-tRNA synthetases are a fascinating family of enzymes, playing an essential role in genetic code deciphering during protein biosynthesis. Moreover, they act as an important factor in many other cellular processes, which lead e.g. to stress response, apoptosis and embryo development. However, some problems related to AARS structure and functions are still unsolved. This concerns such questions as the mechanism of synthetases transport into the nucleus, function(s) played by multisynthetase complexes in higher eukaryotes, AARS organisation or selenocysteinylation pathway. The design of drugs targeted precisely against pathogenic synthetases provides an important challenge and raises great hope for efficient therapy of bacterial infections.

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