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tRNA synthetase: tRNA Aminoacylation and beyond

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Abstract

The aminoacyl-tRNA synthetases are prominently known for their classic function in the first step of protein synthesis, where they bear the responsibility of setting the genetic code. Each enzyme is exquisitely adapted to covalently link a single standard amino acid to its cognate set of tRNA isoacceptors. These ancient enzymes have evolved idiosyncratically to host alternate activities that go far beyond their aminoacylation role and impact a wide range of other metabolic pathways and cell signaling processes. The family of aminoacyl-tRNA synthetases have also been suggested as a remarkable scaffold to incorporate new domains that would drive evolution and the emergence of new organisms with more complex function. Because they are essential, the tRNA synthetases have served as pharmaceutical targets for drug and antibiotic development. The recent unfolding of novel important functions for this family of proteins offers new and promising pathways for therapeutic development to treat diverse human diseases.

The aminoacyl-tRNA synthetases (aaRSs) comprise an ancient family of enzymes that are responsible for the first step of protein synthesis. This diverse set of proteins is united by a common aminoacylation reaction, which attaches an amino acid to its cognate tRNA. Each aaRS is adapted to activate a single amino acid (aa) via an adenylate intermediate (aa-AMP) and then covalently link it to one of the ribose hydroxyls at the 3' end of a set of tRNA isoacceptors.

Identity elements within each tRNA serve as molecular determinants that confer specificity of the aaRS for tRNA [1]. Accurate amino acid selection is challenging for about half of the aaRSs and is compensated by a hydrolytic editing domain to clear mistakes [2]. Thus, each aaRS has been finely tuned through a lengthy evolutionary period to ensure accurate protein synthesis.

The aaRSs have also been recruited for a myriad of other idiosyncratic activities that are unrelated to their primary role in protein synthesis [3, 4]. These include supporting RNA splicing, cell signaling, as well as transcriptional and translational regulation. Paralogs of

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aaRSs also exist, which have lost their aminoacylation function [5]. However, in many cases, the aaRS maintains dual roles in protein synthesis and its alternate activities. Some of these activities are unleashed by variations in splicing events, proteolytic cleavage, or post-translational modifications [6]. Remarkably, the incorporation of novel domains into members of the aaRS family has been evolutionarily linked with increased complexity that yields new organisms.

aaRS Classifications and Distinctions

The family of aaRSs has been divided into two classes based on their chemical properties, architecture of their catalytic domains and consensus sequences [7-9] (Table 1). Each class can be further sub-divided into subclasses based on their unique mechanistic properties, anticodon-binding domain characteristics, as well as the organization of conserved structural motifs (Table 1) [2, 9-13]. With only one exception, lysyl-tRNA synthetase (LysRS) [14], the division of the class 1 and 2 aaRSs has been strictly conserved.

A Rossmann ATP binding fold defines the canonical core of class I aaRSs, which carries out the aminoacylation reaction [15]. The active site is marked by two consensus sequences: “KMSKS” (Lys-Met-Ser-Lys-Ser) [16-18] and “HIGH” (His-Ile-Gly-His) [19] (Figure 1). The KMSKS sequence functions to stabilize ATP during amino acid activation [20], in addition to stabilizing the 3' end of tRNA for amino acid transfer [20-23]. This short KMSKS peptide is found in loops that bridge the β -strand S6 and the α -helix H11 [16]. During amino acid activation, the KMSKS sequence moves approximately 8 Å from an “opened” to a “closed” conformation in order to bring the KMSKS polypeptide in close proximity to stabilize the pyrophosphate moiety of ATP [24]. The N-terminal HIGH sequence also plays a role in amino acid activation [19], whereby the histidines bind to and stabilize the phosphate backbone of ATP [25]. The HIGH sequence is found in loops between the β -strand S2 and the α -helix H3 [16]. Its movement is synchronous with the “opened” and “closed” conformations of the KMSKS sequence [24].

In contrast to the class I enzymes, which tend to be monomeric, class II aaRSs are almost exclusively oligomeric and are usually in dimeric or tetrameric form. The topology of the class II aaRS catalytic core is only found in a few non-aaRS proteins and is comprised of seven antiparallel β -strands flanked by α -helices [7, 8, 26]. Three conserved sequence motifs define the class II aaRSs (Figure 1). Motif 1 [g ϕ xx ϕ xxp ϕ ϕ where ϕ represents a hydrophobic amino acid] is located at the interface of the dimer. It has been hypothesized to provide a communication mechanism between the active sites of the two subunits [27]. Motif 2 [fRx ϕ -h/rxxxFxxx(d/e)] and motif 3 [g ϕ g ϕ g ϕ (d/e)R ϕ ϕ ϕ ϕ ϕ] comprise part of the aminoacylation active site [7, 8, 28]. In the past decade, two new aaRSs that activate O-phosphoserine (SepRS) and pyrrolysine (PylRS) have been added to the set of class II aaRSs [29, 30] (Table 1).

Historically, LysRS had been associated with the class II aaRSs, but a few surprising cases have been found in archaea that represent the class I enzymes [14]. For example, there is not a class II LysRS in *Methanococcus maripaludis* LysRS [14]. Rather, a 62 kDa class I protein has been identified as a LysRS. In just one organism (*Methanosarcineae*) identified so far,

both the class I and II LysRSs co-exist within a single organism. In this case, it is hypothesized that the two LysRSs collaborate to promote the aminoacylation of the rare amino acid pyrrolysine [31].

The two distinct cores of the aaRS classes confer different mechanisms to carry out the two-step aminoacylation reaction. Class I aaRSs bind ATP in an extended conformation [16, 17, 32], while class II aaRSs bind ATP in a bent conformation [21, 33] (Figure 1) due to a difference in active site structure of the aaRS. In addition, the class I aaRSs bind the tRNA stem via the minor groove side [17], thus orienting the 2'-hydroxyl group of the A76 ribose for attachment of amino acid [34]. In contrast, class II aaRSs bind the tRNA stem via the major groove side [28], resulting in accessibility of the 3'-hydroxyl group for charging [34].

Some aaRSs have acquired insertions or appendages to their catalytic canonical cores. In many cases, these inserted domains enhance enzyme specificity and fidelity for the aminoacylation reaction [5]. About half of the aaRSs rely on an extra domain with a hydrolytic active site to edit mistakes that were made in the aminoacylation active site of the canonical core domain [2]. The connective polypeptide 1 (CP1) [35] hydrolytic editing domain ensures amino acid fidelity of the class I leucyl- (LeuRS), isoleucyl- (IleRS), and valyl- (ValRS) tRNA synthetases [36, 37]. The class II prolyl-tRNA synthetase (ProRS) possesses a hydrolytic editing domain that can either be inserted or appended to the main body of the enzyme depending on the origins of the ProRS [38]. In bacterial ProRS, the editing domain (INS) is inserted between motifs 2 and 3 and enables ProRS to hydrolyze Ala-tRNA^{Pro} [39, 40]. In contrast, ProRS from lower eukaryotes possess an appended editing domain that has moderate homology to the INS [41].

An anticodon binding domain typically provides added specificity for tRNA substrate interactions with the aaRS. With the exception of LeuRS, seryl-tRNA synthetase (SerRS), and alanyl-tRNA synthetase (AlaRS) [1, 42-46], the aaRS interacts with the anticodon loop of tRNA to identify its cognate isoacceptors. Interestingly, co-crystal structures of LeuRS with tRNA indicate that a C-terminal domain extension of LeuRS interacts with the tRNA^{Leu} elbow [47, 48]. The anticodon-binding domains are varied in their structure and sequence. For example, those from glutamyl-tRNA synthetase (GluRS) [49] and glutaminyl-tRNA synthetase (GlnRS) [17] are comprised primarily of α -helices and β -strands, respectively. Likewise, sequence alignments of five anticodon binding domains of IleRS ranging from *Escherichia coli* (*E. coli*) to humans reveal that they are very divergent depending on their origin [50]. The anticodon binding domains only share a 7% identity residue score that is calculated by dividing the number of identical residues by the total length of the shortest sequence used in the comparison analysis [50].

A small distinctive N-terminal domain in *Neurospora crassa* (*N. crassa*) mitochondrial tyrosyl-tRNA synthetase (TyrRS) adds a second essential function to this aaRS that aids in group I intron splicing [51, 52]. The N-terminal region [52, 53] and two short inserts [54] of TyrRS are believed to promote splicing by binding to the group I intron with a newly evolved surface for group I intron binding that is different from where it binds tRNA^{Tyr} during aminoacylation [55]. In addition, the human TyrRS can also cleave itself to form two distinct cytokines, which are only active after the splitting of TyrRS occurs [56, 57]. The C-

terminal domain fragment is conserved with the proinflammatory cytokine known as endothelial-monocyte-activating polypeptide II (EMAP II) [58] and carries out similar immunological activities [57, 59, 60].

An emerging frontier of aaRS research has identified unique non-aminoacylation related domains in eight aaRSs from higher organisms [3]. Some aaRSs idiosyncratically also incorporate known domains including GST [61-67], WHEP [68-75], EMAP II [57, 76-79] and leucine zipper [80-83] domains. Remarkably, the addition of these domains in the tree of life has been correlated with increasing the complexity of organisms and driving the evolution of new more sophisticated species [6].

Amino Acid Fidelity

Accurate discrimination of amino acids by the aaRSs in the first step of protein synthesis is critical to the cell. Charging the wrong amino acid to tRNA would result in statistical mutations in the proteome [84, 85]. Failure to clear these mistakes can result in cell death for microbes and neurodegenerative diseases in mammals [86-89]. Thus, some aaRSs have evolved amino acid editing mechanisms to ensure the highest level of fidelity during protein synthesis [2, 90, 91].

In 1958, Pauling first predicted an error rate of 1 in 20 during protein synthesis when he projected that enzymes would not distinguish fully between structurally similar amino acids that differed by only a single methyl group [92] (Figure 2). Subsequently, Loftfield measured the misincorporation of valine for isoleucine in chicken ovalbumin to be much lower with an error rate of less than 1 in 3000 [93]. It was later found that IleRS could hydrolyze a valyl-adenylate intermediate with the help of tRNA in order to maintain fidelity [94].

Fersht proposed that some aaRSs possess a secondary editing site where error correction promotes higher specificity [95]. In collaboration then, these two separate active sites would form a “double sieve” for amino acid selection to maintain fidelity (Figure 3). The aminoacylation active site would serve as a coarse sieve for amino acid activation in the canonical core, for example, to exclude bulky amino acids, but allow smaller isosteric amino acids to pass through. The second hydrolytic active site operates as a fine sieve to bind to mischarged amino acid for hydrolysis, while blocking correctly charged tRNAs for release to elongation factors. The second sieve was first identified by mutational and deletion analysis of the class I enzyme's editing domain [96-98]. X-ray crystal structure for the class I IleRS [99] and class II threonyl-tRNA synthetase (ThrRS) [100, 101] showed how the double sieve was separated into two clear protein domains.

Most of the editing aaRSs correct amino acid errors in *cis* via a hydrolytic domain that is inserted into the catalytic core [2]. However, some archaea as well as bacterial aaRSs lack an editing domain that ensures fidelity during protein synthesis. In order to compensate for this, independent paralogs and homologues of editing domains that have hydrolytic activity for amino acid correction have been found to occur naturally. An example is the ProRS editing domain homologue YbaK that edits mischarged Ala-tRNA^{Pro} and Cys-tRNA^{Pro} in *Haemophilus influenzae* [40, 102]. Additionally, ProX, a paralog of the ProRS editing

domain from *Clostridium sticklandii* hydrolyzes Ala-tRNA^{Pro} [41], while AlaX, an AlaRS homolog from *Methanosarcina barkeri* and *Sulfolobus solfataricus*, cleaves mischarged Ser-tRNA^{Ala} and Gly-tRNA^{Ala} [41, 103].

In either the case of *cis* or *trans* editing, the mischarged 3' end of the tRNA must be transferred from the aminoacylation to the editing active site [104]. Comparison of the crystal structures of LeuRS in various states of binding to tRNA, including an aminoacylation and editing complex of *E. coli* LeuRS [105] demonstrate that the CP1 domain rotates through the different stages of aminoacylation and its movement is dependent on the flexibility of the β -strands that tether the editing CP1 domain to the main body of the enzyme [2, 48, 106]. Substitution of a specific glycine within the β -strands disrupted tRNA translocation [107]. A “translocation peptide” within the CP1 domain of *E. coli* LeuRS also facilitates transfer of tRNA from the canonical core to the CP1 domain [104]. Additionally, mutation of a specific residue in IleRS that binds the amino group of mischarged tRNA to the enzyme for editing, prevents translocation of charged tRNA from the aminoacylation active site of the enzyme to its editing pocket [108].

The aaRSs edit by different pathways: pre-transfer and post-transfer of charging to the tRNA. Post-transfer editing hydrolyzes mischarged tRNA to yield free tRNA and non-cognate amino acid (Figure 4) [109]. As discussed above, cleavage of the amino acid from tRNA occurs in the separate hydrolytic editing domain [37, 96] or in some cases via an independent hydrolytic protein [40, 102]. By comparison, pre-transfer editing targets the aminoacyl-adenylate for hydrolysis to release the free amino acid and AMP [94]. Pre-transfer editing can also be dependent on a substrate cyclization reaction such as homocysteine's conversion to form thiolactone by methionyl-tRNA synthetase (MetRS) [110]. The site where pre-transfer editing occurs is poorly defined and has been hypothesized to be associated with the canonical core [111-114] or the hydrolytic editing domain [25, 108, 115]. A natural LeuRS from *Mycoplasma mobile* that is completely missing its CP1 domain was shown to have a weak pre-transfer editing activity [116, 117], which was enhanced by a fusion mutant that incorporated a CP1 domain from *E. coli*. Ironically, CP1 domains originating from ValRS and IleRS, as well as LeuRS enhanced pre-transfer editing activity in *M. mobile* LeuRS and suggested that this editing domain profoundly influences a pre-transfer editing mechanism that occurs in the LeuRS canonical core [117].

Different aaRSs have been suggested to have either pre- or post-transfer editing dominate as the primary fidelity mechanism [95, 118-121]. However, in a backdrop of post-transfer editing, it has been difficult to assess the contribution of pre-transfer editing to achieve amino acid fidelity [120, 122]. In some cases, the aaRS will activate a second editing pathway in the failure of the dominant editing pathway. For example, when the post-transfer editing active site of *E. coli* LeuRS is removed, the enzyme maintained overall fidelity by activation of a pre-transfer editing pathway [111]. Activation of separate pathways can also be dependent on the amino acid that is misactivated [123, 124]. Specific residues have also been hypothesized to enhance pre-transfer editing [121]. Likewise, when post-transfer editing in the human cytoplasmic LeuRS (hscLeuRS) is mutationally abolished, the enzyme

fails to mischarge tRNA, indicating the activation of a second editing pathway to maintain overall accuracy [125].

Cellular mechanisms to ensure amino acid fidelity have been duplicated and even triplicated [2, 120] demonstrating its importance to the cell. Indeed, disruptions to fidelity that lead to even low levels of statistical mutations in the proteome are consequential to the health and viability of cells in both microbial and mammalian systems [84-89]. Yet paradoxically, new examples are emerging, where organisms have naturally evolved to facilitate statistical proteomes. These include *Mycoplasma* pathogens in which LeuRS, PheRS, and ThrRS contain mutationally disrupted editing domains. *M. mobile* LeuRS is completely missing its CP1 editing domain [116]. In *Candida* pathogens, Ser-Leu codon ambiguity persists via tRNA mischarging and also results in statistical proteomes [126, 127]. These examples, suggest that statistical proteomes provide an evolutionary benefit to cells in certain physiological environments.

Aminoacyl-tRNA Synthetase Recognition of tRNA

In general, tRNAs have similar secondary and tertiary structures (Figure 5) [128]. Thus, the aaRSs depend on embedded identity elements within the tRNA substrate to differentiate their cognate substrates for aminoacylation [129-131]. These tRNA identity elements tend to cluster in the anticodon and acceptor stem regions of the tRNA, where there is typically direct interaction with the aaRS [1]. Crystal structures have also identified distal regions, such as the L-shaped tRNA elbow as important recognition sites too [47, 48, 132].

Identity elements include single nucleotide variations, such as the discriminator base at position 73 that is located at the end of the tRNA acceptor stem [1, 133]. Based on co-crystal structure information, the tRNA discriminator base is in close proximity to the aaRS active site, providing an opportunity for specific protein-RNA interactions. In addition, unique base pairs such as G3:U70 in the acceptor stem of tRNA^{Ala} [129, 134] confer specificity for AlaRS. In other cases, for example tRNA^{Ser}, the enzyme relies on recognition elements in its D-stem and extra long variable loop [135]. For most aaRSs, distal interactions with the tRNA anticodon nucleotides are required for recognition. The only exceptions are tRNA^{Leu}, tRNA^{Ser}, and tRNA^{Ala} [1, 42, 43] as these tRNAs have multiple isoacceptors with too many different anticodon nucleotide combinations to provide a unique anticodon-aaRS match.

Antideterminants prevent productive interactions between the aaRS and noncognate tRNAs. The first antideterminant was identified at position 34 of *E. coli* tRNA^{Ile}, which contains lysidine, a modified cytosine, that blocks misaminoacylation by MetRS [136]. Other examples of antideterminants include modified nucleotide m¹G37 in yeast tRNA^{Asp} that hinders aminoacylation by arginyl-tRNA synthetase (ArgRS) [137, 138]. In addition, the G37 nucleotide on tRNA^{Ser} prevents charging by yeast LeuRS [139]. In contrast, the A73 nucleotide on tRNA^{Leu} inhibits aminoacylation by SerRS [140].

A growing base of crystal structures for the family of aaRSs has provided atomic details of the interface between tRNA and the aaRS that facilitate recognition and specificity [1]. However, interpretation using these static structures are limited to specific points of contacts between the protein:RNA pair and fail to provide insight into long-distance effects of distal

regions of the tRNA that influence the aaRS active site. A computational approach based on community network analysis has identified communities that are linked by correlated motions and define amino acid and nucleotide nodes that although distal, appear to be critical to tRNA binding and aminoacylation [141].

Macromolecular Complexes of aaRSs Harbor Non-Aminoacylation Activities

In higher eukaryotes, nine aaRSs and three non-synthetase proteins are assembled into a multi-aaRS complex. It was originally hypothesized that assembly of the aaRSs would localize these protein synthesis enzymes for more efficient translation Fox[142, 143]. Recently however, it has been shown that this multi-aaRS complex operates as a “functional depot” for alternate activities of its members [144]. Many of these proteins trigger specific cell signaling activities when freed from the complex.

The macromolecular aaRS complex is comprised of ArgRS, GlnRS, IleRS, LeuRS, LysRS, MetRS, aspartyl- (AspRS), and glutamyl-prolyl-tRNA synthetase (Glu-ProRS) (Figure 7). The Glu-ProRS is an unusual fused aaRS that is linked by three tandem repeating peptides [145, 146]. Three auxiliary non-synthetase proteins called AIMP1, AIMP2, and AIMP3 (aminoacyl-tRNA synthetase interacting multifunctional proteins) formally known as p43, p38, and p18 respectively, are at the core of the multi-aaRS complex [142, 147, 148] (Figure 7).

A 30 Å cryo-electron microscopy (EM) structure of the multi-aaRS complex is shaped as an asymmetric triangle with dimensions of approximately $19 \times 16 \times 10$ nm. A central cleft that is 4 nm deep extends about two-thirds of the length of the complex [149, 150]. Biochemical investigations indicate that the large complex can be partitioned into three sub-complexes. Based on tandem affinity purification from human cells, AIMP2 interacts with AspRS and LysRS and provides a foundation for complex assembly [66, 80] by connecting the other two complexes [147, 151]. The AIMP1 protein interacts with GlnRS and the N-terminus of ArgRS to hold them together with the rest of the complex [147, 152, 153]. The AIMP3 anchors MetRS and also interacts with the other multi-aaRS components such as Glu-ProRS, IleRS, and LeuRS [147].

Certain events prompt the release of individual aaRSs or one of the AIMP proteins to elicit a specific activity or cell signaling cascade [154] (Table 2). In at least one case, phosphorylation was critical to the release of AIMP2 from the multi-synthetase complex in response to DNA damage [155]. Phosphorylation of AIMP1 is also important to its regulation of an immune reaction [156, 157].

In human cells, LysRS generates diadenosine tetraphosphate (Ap_4A), a critical signalling molecule, in order to activate mast cells. The Ap_4A binds to the tumor suppressor gene Hint to decrease interactions between Hint and the transcription factor MITF [158]. This causes MITF to dissociate from LysRS as well as Hint, thus releasing it to transactivate its responsive genes [158]. The LysRS can also act as a pro-inflammatory cytokine [159] if its release is induced by tumor necrosis factor α (TNF- α). This secreted LysRS can bind

macrophages in order to enhance TNF- α production and migration in a positive feedback loop [159, 160].

Glu-ProRS can be switched from its classical role in translation by phosphorylation of one of the linking WHEP domains to become a gene-specific modulator of translation [161]. It forms a complex called GAIT (gamma-interferon-activated inhibitor of translation) with ribosomal subunit L13a and glyceraldehyde-3-phosphate (GAPDH) in response to interferon- γ to trigger translational silencing in ceruloplasmin. The Glu-ProRS complex binds to the 3'-untranslated region of ceruloplasmin to block mRNA translation [162]. Specific translational silencing of ceruloplasmin has been hypothesized to serve as a feedback mechanism to prevent over-accumulation of the protein, which might result in oxidative damage [162].

Lower eukaryotes such as *S. cerevisiae* also contain multi-synthetase complexes although comprising fewer components than those found in mammals [163]. In the case of *S. cerevisiae*, Arc1p binds to tRNA and associates with MetRS as well as GluRS in order to function as a co-factor for these two aaRSs [163, 164]. Similarly, the archaea *Methanothermobacter thermautotrophicus* harbors a small multi-synthetase complex comprising LeuRS, LysRS and ProRS that enhances tRNA^{Pro} aminoacylation [165, 166]. In addition, kinetic experiments indicate that the catalytic turnovers of LysRS and ProRS increased when in complex as compared to the free enzyme [167].

The three auxiliary proteins, AIMP1, AIMP2, and AIMP3 associated with the macromolecular aaRS complex in the cell possess signaling activities when freed from the complex. For example, AIMP1 is secreted as a cytokine and stimulates fibroblast proliferation [168]. It also has pro-apoptotic function [169], and is involved in angiogenesis [170]. AIMP2 displays pro-apoptotic function [171] and is the target of parkin for ubiquitination, leading to neurodegeneration [172]. Finally, AIMP3 acts as a tumor suppressor by activating ataxia-telangiectasia, mutated/ATM and Rad3-related (ATM/ATR) which is an upstream kinase of p53 [173]. This is essential for subsequent p53 induction and DNA repair [64, 173].

Secondary and Alternate Functions of aaRSs

The aaRSs are considered to be one of the most ancient families of proteins. Throughout their long evolutionary history, these enzymes have adapted to carry out dual roles in the cell that include non-protein synthesis activities (Figure 6) [174]. In some cases, aaRS-like proteins or paralogs of aaRSs that originate from complete or partial gene duplications of the aaRS gene have also adapted for diverse cellular functions [5] (Figure 6). A number of these alternate functions have been implicated in disease states [175]. They have also been proposed to drive organism evolution by conferring increased complexity [6].

The secondary activities of aaRSs capitalize on their existing binding sites for RNA, amino acid and ATP [3]. They also take advantage of inserted domains or peptides to adapt for alternate activities [3]. As Figure 6 shows, these alternate roles are broad in scope and include mitochondrial RNA splicing [51, 176-179], immunology [56, 57, 154], translational [180], and transcriptional regulation [181, 182]. In many cases, the alternate function is

masked and the aaRS requires modification and/or proteolysis to unleash the unconventional activity [144]. Some disease states including neurodegenerative and autoimmune disorders have implicated or identified an aaRS alternate functions when it has been compromised or failed [183].

The glycyl-tRNA synthetase (GlyRS) [184] as well as TyrRS [185] have been associated with Charcot-Marie-Tooth (CMT) disease which is a heritable disorder of the peripheral nervous system [186]. Interestingly, CMT-causing mutations lie at the dimer interface of GlyRS, and presumably affect interactions between the two subunits [187]. In addition, mutations of a highly conserved glycine (G41R) involved in tyrosyl-adenylate formation as well as the Glu196 residue in human TyrRS that make contact with tRNA^{Tyr} result in this disease phenotypes too [185]. While the mechanism of GlyRS and TyrRS involvement remains unclear, investigations with differentiating motor neurons indicate that wild type TyrRS localizes to the axonal termini. In contrast, in the case of neurons expressing mutant TyrRS proteins, their normal function and axonal distribution is disrupted leading to axonal loss, degeneration and peripheral neuropathy.

Some auto-immune disorders that are associated with aaRSs include chronic myopathies and interstitial lung diseases. For example, auto-antibodies against HisRS are associated with chronic muscle inflammatory disease or polymyositis a form of idiopathic inflammatory myopathy [188, 189]. Antibodies to AsnRS are linked to auto-immune interstitial lung disease [190, 191]. IleRS and GlyRS were also shown to be antigenic in screens for auto-antibodies generated in myositis patients [192-194].

Many of the aaRSs or a fragment of the aaRS are involved in cell signaling activities. This includes an immunology activity during apoptosis for eukaryotic TyrRS [56, 57], when it is cleaved into its N- and C- terminal halves to yield two distinct cytokines. The TyrRS C-terminal fragment up-regulates TNF- α , while its N-terminal fragment mimics the functions of interleukin-8 (IL-8) that is released by macrophages in an antigen response [56, 57]. In the case of parasite protozoan *Entamoeba histolytica*, LysRS is up-regulated by TNF- α and then is cleaved by proteases to release a cytokine-like domain that functions in chemotaxis [195]. A TrpRS signal transducer activity mediates interactions between DNA-dependent protein kinases and poly ADP ribose polymerase leading to downstream signaling events for p53 activation [196]. GlyRS can be secreted from macrophages and exhibits cytokine-like properties that down-regulate ERK MAP kinases to inhibit the Ras activated tumor cells. In particular, GlyRS binding to CDH6 (cadherin) releases suppressed phosphatase 2A (PP2A) that dephosphorylates activated ERK [197]. As another example, the HisRS amino terminal domain functions as a chemokine through CCR5 receptor-mediated interactions [198].

As introduced above, the eukaryotic multi-synthetase complex has been termed a depot that sequesters signaling functions for these housekeeping proteins [144]. For example, when LysRS, is released from the complex upon MAPK-dependent phosphorylation, it enters the nucleus to activate transcription of immune response genes [154]. Then, in response to immunogenic cell death inducers, LysRS translocates to the cell surface along with calrectulin that acts as an engulfment signal for antigen-presenting cells [199]. In a second example, Glu-ProRS is phosphorylated by Cdk5, which is responsible for translational

control of macrophage inflammatory gene expression [200]. Additionally Glu-ProRS also is involved in the translational silencing of mRNAs encoding vascular endothelial growth factor A (VEGF-A). Under INF- γ stimulus, the phosphorylated Glu-ProRS is recruited to the GAIT complex for the translational silencing event. However, VEGF-A is required for vessel maintenance and in order to maintain its basal expression, a C-terminally truncated form of Glu-ProRS protects the mRNA from the GAIT complex assembly [201].

AsnRS was found to be one of the important components of survival signaling cascade induced by fibroblast growth factor 2(FGF2) in osteoblasts [202]. In addition, filarial parasite *Brugia malayi* AsnRS interacts with and activates human IL-8 cytokine receptors CXCR1 and CXCR2. It also stimulates MAP kinases like ERK1 and ERK2 in cell migration and signal transduction [203].

In lower eukaryotes, two different mitochondrial aaRSs adapted to support RNA splicing, but via distinct mechanisms. The *N. crassa* TyrRS has acquired a small idiosyncratic N-terminal α -helical domain [52] as well as two short inserts [54] for splicing activity. Its X-ray co-crystal structure shows that TyrRS binds to the group I intron with a newly evolved surface for group I intron binding that is different from where it binds tRNA^{Tyr} during aminoacylation [55]. In contrast, *Saccharomyces cerevisiae* (*S. cerevisiae*) mitochondrial LeuRS appears to require no special adaptations to confer RNA splicing of group I introns [204]. The CP1 editing domain is important to its activity and can function in splicing independent of the full-length enzyme [205]. In both examples of aaRS-dependent group I intron splicing however, it is proposed that they bind introns and promote folding of their cognate introns into an active conformation [205-207].

In *E. coli*, regulation of gene expression at transcription and translational levels is aided in part by AlaRS and ThrRS. Biochemical studies showed that AlaRS binds to a palindromic sequence that flanks its promoter site to repress transcription of its own gene [182]. Also, ThrRS regulates its production at the translational level by preventing ribosome binding for translation [180]. The enzyme binds to two stem-loop structures upstream of the ribosome binding site, which mimic the anticodon arm of tRNA^{Thr} [180].

Human LysRS is packaged into human immunodeficiency virus type 1 (HIV-1) together with its cognate tRNA^{Lys} [208, 209]. Packaging depends on a specific interaction of LysRS with the viral Gag protein [210, 211] and Gag-Pol has also been implicated in LysRS recruitment and packaging [212-215]. Interestingly, the binding of tRNA^{Lys3} to LysRS rather than aminoacylation of tRNA^{Lys3} is critical for tRNA^{Lys3} packaging into HIV [208, 212]. Once tRNA^{Lys3} is inside the virion, it anneals to the genomic RNA of the virus and primes reverse transcription [216].

Several aaRSs charge non-canonical tRNA substrates that have unusual functions. For example, *E. coli* AlaRS aminoacylates transfer-messenger RNA (tmRNA). The charged tmRNA binds to stalled ribosomes and provides its own template to translate a specific C-terminal fusion peptide that targets the truncated peptide for degradation [217]. Additionally, aminoacylated tRNAs can be used for peptide cell wall biosynthesis. For example, a species

of tRNA^{Gly} [218] is involved in peptidoglycan synthesis, while Lys-tRNA^{Lys} can be used to remodel lipids [219].

Some plant RNA viral genomes possess tRNA like structures (TLS) [220] at their 3' ends that can be specifically aminoacylated by valine [221, 222], histidine [223-225] or tyrosine [226, 227]. The TLSs lack the modified bases, conserved residues, D loop and T loop sequences characteristic of tRNAs [228]. However, the TLSs contain a pseudo-knotted aminoacyl acceptor stem which places the 5' end of the TLS away from the acceptor end [229, 230]. Interaction of the TLS with the tRNA synthetase predominantly occurs via the anticodon and the acceptor arms despite the presence of the pseudoknot [227, 231].

Paralogs of aaRSs or aaRS-like proteins have been identified that take part in diverse cellular activities (Figure 6) [5]. For instance, *E. coli* YadB protein resembles GluRS and attaches glutamate to a queuosine base (position 34) at the first anticodon position of tRNA^{Asp} [232]. This modification expands and alters tRNA decoding of codons [232, 233]. HisZ, a paralog of HisRS, is one component of the enzyme catalyzing the first step of histidine biosynthesis [234]. As indicated above, aaRSs that lack an editing domain such as ProRS [40] and AlaRS [103], have paralogs and homologs of editing domains in order to maintain overall fidelity [41].

aaRSs as Drug Targets

As essential enzymes, the aaRSs are ideal targets for antibiotics, with the caveat that these drugs must be species-specific to avoid inhibiting the human counterpart. The most successful example is mupirocin (pseudomonic acid A), a topical antibiotic for *Staphylococcus aureus* [235] that inhibits IleRS [236, 237]. It functions by interfering with isoleucine as well as ATP binding to IleRS [238, 239]. Mutational analysis has identified two residues in the IleRS active site that play a significant role in conferring mupirocin resistance [25].

More recently, a small boron-containing molecule called AN2690 (5-fluoro-1,3-dihydroxy-2,1-benzoxaborole) was discovered that targets the editing active site of LeuRS [240] (Figure 8). The mechanism of action for AN2690 relies on the boron to trap tRNA^{Leu} within the editing active site of LeuRS. AN2690 binds specifically to the editing site and the boron attacks the ribose *cis* diols at the 3' end of the tRNA to form an oxaborole adduct. This potent therapeutic kills a range of organisms including yeasts, molds and dermatophytes [241] and has just emerged from clinical trials for commercialization to treat onychomycosis.

Other potential therapeutics involving aaRSs include a natural fragment of the TyrRS enzyme (mini TyrRS) that contains the aminoacylation catalytic domain. It is involved in angiogenesis by functioning as a proangiogenic cytokine [242]. Mini-TyrRS binds to endothelial cells and activates an angiogenic signaling pathway that can potentially be harnessed to combat cardiovascular disease [242]. In addition, an alternative splice fragment of TrpRS [243] as well as a natural proteolytic fragment (T2-TrpRS) [244] have strong anti-angiogenic activity and function by inhibiting vascular endothelial growth factor-induced angiogenesis [244]. The aminoacylation active site of T2-TrpRS interacts with two

tryptophan residues of VE-cadherin which is a main constituent of adherens (protein complexes at cellular junctions) junctions of endothelial cells [245]. During new blood vessel formation, T2-TrpRS exhibits anti-angiogenic activity by binding to the exposed Trp residues on VE-cadherin unlike in the case of developed blood vessels where the Trp2 and Trp4 of VE-cadherins are interlocked, buried and unavailable for T2-TrpRS binding. Anti-angiogenic therapy studies suggest that TrpRS is a promising natural therapeutic to prevent blindness caused by abnormal angiogenesis in the eye [246].

Summary and Perspective

The aaRSs have long been known for their prominent role in protein synthesis and have served as an extraordinary model to investigate the rules that govern RNA-protein interactions, enzyme catalysis, protein structure-function relationships, and the principles of evolution. Recent advances suggest that they will also offer profound insight into the functional expansion of the proteome and systems biology as the depths of the aaRSs' alternate activities are further unraveled and characterized. It is clear that nature has repeatedly called upon the aaRSs throughout the evolution of the cell to adapt as the physiology and metabolic pathways of its environment became more sophisticated. Many of the resultant novel complexities and idiosyncracies that are associated with each of the members of the aaRS family have potential for development into therapeutics to combat human diseases.

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Figure 1.

Conserved structure motifs and ATP binding in class I and II aaRSs. A. Class I LeuRS enzyme [*Thermus thermophilus* (*T. thermophilus*)] binds to an extended conformation of a sulphonamoyl analogue of leucyl-adenylate (black) within the synthetic active site. The conserved signature sequences "HIGH" (HMGH) and "KMSKS" (MSKSK) are highlighted in blue and green respectively (PDB: 2V0C) [240]. While these signature sequences can vary as shown in this example, they are readily recognizable in a sequence alignment of all class I aaRSs. B. Class II glycyl-tRNA synthetase (GlyRS) binds ATP (black) in a bent conformation. The three conserved sequences, motifs 1, 2, and 3 of class II aaRSs are highlighted orange, purple, and blue respectively (PDB: 1B76) [247].

Figure 2.
Structurally similar amino acids. A. IleRS discriminates isoleucine from valine. B. AlaRS discriminates alanine from glycine. The colored balls represent as follows: red, oxygen; blue, nitrogen; and gray, carbon. (Hydrogens are not shown.)

Figure 3.

The double sieve of LeuRS. A. The double sieve for LeuRS contains a coarse sieve (red) for aminoacylation that excludes larger amino acids and a “fine sieve” (blue) that blocks cognate amino acid, but allows non-cognate amino acids to be hydrolyzed. B. The crystal structure of *T. thermophilus* LeuRS has an ancient canonical aminoacylation core (red) and CP1 hydrolytic editing domain (blue) that are linked by β -strands (green). Residues that impact editing within the hydrolytic active site are in orange. Cartoon on the left is adapted from [248] and structure on right (PDB: 2BTE) is adapted from [48].

Fig 4.

Pathways for aaRS editing. Post-transfer editing occurs when the incorrectly charged tRNA is hydrolyzed, while pre-transfer editing cleaves activated aminoacyl-adenylate.

Figure 5. Secondary and tertiary structure of tRNA. The secondary cloverleaf structure (left) and tertiary structure of tRNA (right) show how the dihydrouridine (D; red) and TΨC (green) loops interact for folding. The colors represented on the tRNA are: orange, 3' acceptor stem; purple, acceptor stem; green, TΨC stem-loop; red, D stem-loop; light green, variable loop; blue, anticodon stem-loop; and black, anticodon trinucleotide.

Figure 6.

Non-canonical activities and paralogs of aaRSs. A. The aaRSs are adapted for dual roles that coexist with their aminoacylation activity. B. Paralogs of aaRSs and their domains can provide important non-aminoacylation functions within the cell [183].

Figure 7.

Organization of the multi-aaRS complex. The aaRS complex consists of nine aaRSs (gray) and three auxiliary proteins (green). The varied sizes of the proteins are schematically indicated by different sized balls. Dashed lines indicate sub-complexes. Solid lines represent protein-protein interactions with the exception of Glu-ProRS, which is covalently fused by a repeating peptide motif.

Figure 8.
Structure of boron-containing AN2690

Table 1

Classification of aaRSs based on structural and chemical properties [7, 8].

Class I	Class II
Ia	IIa
LeuRS [*]	SerRS
IleRS [*]	ThrRS [*]
ValRS [*]	ProRS [*]
MetRS [*]	HisRS
CysRS	GlyRS (Dimer)
ArgRS	
	IIb
Ib	AspRS
GlnRS	AsnRS
GluRS	LysRS-II [*]
LysRS-I	
	IIc
Ic	PheRS [*]
TyrRS	AlaRS [*]
TrpRS	GlyRS (Tetramer)
	PylRS
	SepRS

* These aaRSs have a hydrolytic editing pathway to clear misactivated and mischarged amino acids [2].

Table 2

Representative activities associated with aaRSs and AIMP3 when free of the human macromolecular complex

Proteins	Activities
LysRS-II	Inflammatory cytokine (Extracellular) Viral assembly (Plasma membrane) Transcriptional control (Nuclear)
Glu-ProRS	Translational silencing
GlnRS	Anti-apoptosis
MetRS	rRNA transcription
IleRS	Autoimmune response
AIMP1	Cytokine activation of macrophages
AIMP2	Degradation of FBP, a transcriptional activator of <i>c-myc</i>
AIMP3	p53 induction and DNA repair by activating ATM/ATR in the nucleus