Published in final edited form as:

Curr Opin Struct Biol. 2012 February ; 22(1): 119–126. doi:10.1016/j.sbi.2011.11.008.

Structural analyses clarify the complex control of mistranslation by tRNA synthetases

Min Guo¹ and Paul Schimmel²

Paul Schimmel: schimmel@scripps.edu

¹Department of Cancer Biology, The Scripps Research Institute, Scripps Florida, 130 Scripps Way, Jupiter, FL 33458

²The Skaggs Institute for Chemical Biology and the Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

Abstract

Proteins are precisely assembled with amino acids by matching the anticodons of charged transfer RNAs to nucleotide triplets in mRNA sequences. Accurate translation depends on the specific coupling of cognate amino acids and tRNAs – a step carried out by aminoacyl-tRNA synthetases (aaRSs) and that generates the genetic code. Due to their intrinsic similarity, aaRSs developed highly differentiated structures to discriminate between amino acids at the active site for aminoacylation. Because this discrimination is not sufficient to prevent toxic mistranslation, aaRSs developed separate structures to further refine recognition by proofreading. From comprehensive structural studies on aaRSs, many of the molecular details have been elucidated for the recognition of cognate amino acids and for the misactivation and editing of noncognate amino acids. Here we review recent advances in the structural description of the binding, activation and editing of amino acids, which collectively reveal many aspects of the fine-tuned systems that resulted in a robust and universal genetic code.

Introduction

Aminoacyl-tRNA synthetases (aaRSs) are the first resource for the production of proteins. The common function of aaRSs is to decipher the genetic code by accurate ligation of amino acids to their cognate tRNAs [1–3]. This reaction proceeds by the synthesis of an aminoacyl-adenylate, as an activated intermediate, from the amino acid and ATP (reaction 1); and the transfer of the aminoacyl moiety to the 3'-terminal adenosine (designated as A76) of the cognate tRNA to yield the aminoacyl-tRNA (reaction 2). aaRSs are divided into two classes of about 10 members each, defined by the distinct architectures of the aminoacylation domains [5]. It is thought that the genetic code initially had an ambiguous format, in which codons specified groups of similar amino acids [6,7]. As a consequence, the earliest proteins were not distinct chemical entities but were statistical in nature, because the earliest genes gave rise to families of closely related but diverse sequences. Strong selective pressure in favor of those species with the best activities generated pure chemical entities and forced the code into the precise form that it has today [8].

© 2011 Elsevier Ltd. All rights reserved.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The correct recognition of substrates by modern aaRSs is essential for the accuracy of translation. However, the affinity differences are not large enough for the enzymes to discriminate rigorously between similar amino acids. To achieve higher discrimination, amino acid recognition is split into two-steps, which include a first and inaccurate synthetic step followed by a second step that eliminates misactivated noncognate amino acids. This second step is called proofreading, or editing. For example, reaction 1 is not sufficiently accurate for isoleucyl-tRNA synthetase (IleRS) to discriminate between isoleucine vs. valine. In these instances, the misactivated amino acid (*aa*-AMP, pre-transfer, reaction 3), or the mischarged tRNA (*aa*-tRNA, post-transfer, reaction 4), is cleared by hydrolytic editing [9–11], at a second, discrete active site within an editing domain [12]. This domain has been identified for the class I IleRS, ValRS, and LeuRS and for the class II AlaRS, ThrRS, PheRS and ProRS [13]. Early structures of ValRS/IleRS/LeuRS re-capitulated the double-sieve (two active sites) model proposed by Fersht for the three close-related class I aaRSs [14].

This review focuses on the most recent structural advances that elucidate mechanisms in other proofreading aaRSs. When proofreading (editing) is impaired, mistranslation occurs because of the wrong amino acid being inserted at a specific codon. The consequences can result in toxicity, cell death, or a defined pathology (as seen in the mouse) [15–18]. As explained below, the challenge of recognizing cognate vs noncognate amino acids in the aminoacylation and post-transfer editing steps appears to involve more than just the stereochemistry of the relevant amino acids.

Thr/Ser recognition in editing domain of ThrRS

Due to the shared -OH group on the side chain of Ser and Thr, ThrRS recognizes them similarly and mis-activates Ser to Thr by a ratio of 1/1,000, which consequently requires an editing mechanism [19]. Two different editing domains were found at the N-terminus of ThrRS, one in bacteria and eukaryotes, which is homologous to the editing domain in AlaRS [19], and the other in archaeal ThrRS, which is homologous to D-amino acid deacylases [20].

A series of high-resolution structures of ThrRS showed how specific editing of noncognate amino acids occurs across the three kingdoms. In the *E. coli* ThrRS editing domain, the seryl moiety of a bound Ser-adenosine analog (designated as Ser-A76 (position 76 being the amino acid attachment site in the tRNA) that mimics the 3'-end of the mischarged Ser-tRNA^{Thr}) is positioned into a hydrophilic cavity by anchoring its α -amino group to an Asp¹⁸⁰ side chain, a His⁷⁷ imidazole ring and a nearby main chain carbonyl group (Fig. 1a) [21]. Three hydrogen bonds from His⁷³, Gly⁹⁶ and a water molecule coordinate the γ -OH group of the seryl moiety. With these constraints, the extra methyl group of threonine will clash with the narrow hydrophilic pocket formed by the side chains of His⁷⁷, Tyr¹⁰⁴ and Asp¹⁸⁰. Although this repulsion of Thr might not be as stringent as occurs in the active site of SerRS, the specificity is enough for removing the low ratio of Ser-tRNA^{Thr} [19].

In contrast to the deep amino acid binding pocket in the bacterial/eukaryotic ThrRS editing domain, the amino acid binding site in archaeal ThrRS editing domains is a rather shallow groove. Archaeal *Pyrococcus abyssi* ThrRS's editing domain provides the only system with structures of complexes with both cognate and the mischarged noncognate amino acid-A76 analogs [22]. A water molecule is involved in the recognition of the amino acid and in catalysis. This water molecule (W1) forms H-bonds to the conserved Lys side chain and to the ester bond between the seryl moiety and A76 (Fig. 1b). W1 was previously proposed to execute nucleophilic attack on the carbonyl carbon of the labile ester linkage between A76 and the amino acid (substituted by a stable amide linkage in the Ser-A76 analog) to trigger the hydrolytic reaction [23]. Interesting, when Thr-A76 was cocrystallized with this editing domain, W1 disappeared [22]. Structural analysis showed that the extra methyl group of the threonyl side chain pushed up the overlying Tyr¹²⁰ and moved the adjacent Lys¹²¹ side chain to bring it forward to occupy the position of W1. This loss of W1 may be the cause of chemical inactivation of hydrolysis of the cognate Thr-A76. This *k_{cat}* differentiation is interesting, especially when considering the only 10-fold difference of binding affinity of Ser-A76 and Thr-A76 to the editing domain. This result indicates that, in addition to the binding affinity, a chemical step may play an important role in the differentiation of amino acids for editing. The authors suggested a similar mechanism might also exist for other aaRSs' that edit mischarged amino acids [22].

Phe/Tyr recognition: amino acid variant recognitions of PheRS

Aminoacylation site of PheRS has broad specificity

PheRS is unique among aaRSs for its highly diverged structures. Structural and phylogenetic analysis revealed three different forms of PheRSs: a bacterial heterotetramer ($\alpha\beta$)₂; an archaeal/eukaryotic cytosolic heterotetramer ($\alpha\beta$)₂; and a mitochondrial monomer α [24–27]. While the ($\alpha\beta$)₂ prokaryote and eukaryote-cytosolic PheRSs have a similar structural organization, they share considerable sequence divergence even at their active sites. Mitochondrial PheRSs originate from a bacterial ancestor but evolved as a fusion of the catalytic α subunit and the B8-like domain from the β -subunit of bacterial PheRS [26,28]. PheRSs of all forms misactivate the noncognate amino acid tyrosine, while only the heterotetrameric PheRS contains the editing domain that further corrects this error at the post-transfer stage [25,29,30].

Extensive studies on the structures have been done on PheRSs from all three kingdoms [27,31,32]. Specific recognition of Phe at the aminoacylation site is achieved by two neighboring Phe residues (or one Tyr and one Phe in eukaryote cytosolic PheRSs) through a network of edge-to-face interactions [27]. With only one extra OH group, tyrosine makes similar interactions within this pocket. A conserved Ala residue (α Ala³¹⁴ in *T. thermophilus* PheRS) near the bottom of the pocket creates a limited steric hindrance for the *para*-hydroxyl of tyrosine (Fig. 2a). An α A294G mutation showed a 100-fold loss in specificity for Phe over Tyr in *E. coli* PheRS [34]. Certain cytosolic PheRSs (such as in yeast) have a Gly at this site. Correspondingly, yeast cytosolic PheRS activates tyrosine with more than a 10-fold higher efficiency than *E. coli* PheRS [34]. In addition, tyrosine is stabilized by interactions of its *p*-OH group with the main chain carbonyl oxygen of the same Ala (α Ala³¹⁴ in *T. thermophilus* PheRS), and a water molecule that is located within an extra space at the periphery of the Phe-pocket (Fig. 2a).

The extra space for phenylalanine causes an even broader specificity of PheRS. Although the indole ring of Trp does not fit the Phe pocket and cannot be activated by ATP, a series of unnatural amino acids with *para*-substitutions (*p*-fluoro, -chloro, -bromo, -amino, -ethynyl, and -azido-Phe) could be attached to tRNA^{Phe} by PheRS or its A294G mutant [35–37]. Structural analysis showed that the Cl atom of *p*-chloro-Phe binds to the Phe-pocket by

occupying the extra water space in *T. thermophilus* PheRS [38]. PheRS can also mis-activate a natural Phe-derivative, *meta*-tyrosine, which is generated from phenylalanine during oxidative stress and has the OH group in the *meta* position (*m*-Tyr) (Fig. 2a). Both *T. thermophilus* and human mitochondrial PheRSs bind *m*-Tyr with almost the same affinity as that of phenylalanine, and human cytoplasmic PheRS binds *m*-Tyr with only an ~ 10-fold lower affinity [39]. The conserved edge-to-face interactions and the promiscuous pocket shape place tremendous pressure on PheRS to differentiate Phe and the noncognate Tyr.

Editing: specific for Tyr

The editing site for tyrosine is located at the interface of the B3/B4 domain of the β subunit in PheRS [30], and ~ 35 Å away from the aminoacylation site [38]. While many of the *p*-substituted Phe analogs are misactivated by PheRS, only Tyr is a substrate for editing [40] [39]. The superior specificity of Tyr recognition is conferred by a conserved Glu residue in the editing pocket (β Glu³³⁴ in *T. thermophilus* PheRS, β Glu²⁵⁴ in human cytosolic PheRS) [27]. The OH group of Tyr is anchored by H-bonds with the carboxyl group of β Glu³³⁴ and another less conserved residue at the edge of the pocket (Fig. 2b). Mutation of the corresponding residue D243A in yeast cytoplasmic PheRS led to a loss in Tyr-tRNA^{Phe} deacylation activity [30,40]. (Similarly, TyrRS specifically binds the *p*-OH group of Tyr and is about 10⁵ more specific for Tyr than for Phe [41].) Editing domains in PheRSs also show kingdom-specific constitutions. In bacterial PheRS, a cluster of residues surround the conserved Glu to form a hydrophobic pocket for recognizing tyrosine. This cluster is replaced by hydrophilic residues in eukaryotic cytosolic PheRSs [27,33].

A recent study showed that the difference in PheRS specificity might be connected to the divergent requirement for quality control in different cells or organelles [34]. The monomeric mitochondrial PheRSs do not have the B3/B4 domain and therefore have no post-transfer editing activity [40]. However, its aminoacylation site has about 10-fold higher selectivity for Phe vs Tyr than that of the eukaryote cytosolic PheRS [40]. Mutation of the previously mentioned Ala (α Ala³¹⁴ in *T. thermophilus* PheRS) to Gly in the Phe-pocket decreases the selectivity (by ~ 10-fold) of yeast mitochondrial PheRS to the level of the cytosolic form. When introduced into cells, the specificity-reduced mitochondrial PheRS caused destabilization and subsequent loss of the mitochondrial genome in yeast. In contrast, the specificity-reduced mitochondrial PheRS, and even an editing-deficient yeast cytosolic PheRS, can (under the tested conditions) support protein synthesis in the cytoplasm and cell growth. Thus, by this criterion, yeast seemed more sensitive to translational fidelity in mitochondria than in the cytoplasm [34]. This result is distinct from, but consistent with, the cell environment-specific effects seen with a mildly editing-deficient AlaRS in mice, which is the most deleterious in neural cells compared with its lesser effects in other cells and tissues [18]. Another work on ProRS suggested that a missing editing domain in higher eukaryotes may be related to higher levels of proline relative to alanine [42–44]. Thus, the threshold for translational fidelity may be cell-, tissue-, development- and even environment-dependent.

Gly/Ala/Ser Recognition

Aminoacylation: the unconventional problem

Gly/Ala/Ser are the three smallest amino acids and have a single methylene or hydroxyl group difference between each other. Although neither GlyRS nor SerRS misactivates alanine, AlaRS can misactivate glycine and serine at a substantial level. Thus, AlaRS presents a unique paradox, where both a smaller and a larger amino acid are activated by one aaRS. Moreover, the larger serine is also chemically distinct from alanine. Even a mild

defect in the editing site of AlaRS leads to mistranslation of serine at alanine codons, resulting in severe neurodegeneration in the mouse [18].

Recent work systematically investigated this paradox with a series of crystal structures of AlaRS [45–47]. The results showed that the binding pocket for alanine is flexible and is shaped by the joint binding of the amino acid and ATP. Due to this flexible feature, AlaRS accommodates all three amino acids (glycine, alanine and serine) at almost the identical position. A universally conserved acidic residue–Asp²³⁵–uses its sidechain carboxyl to anchor the α -amino group of all three amino acids with a strong hydrogen bond spanning a distance of 2.6–2.7 Å. Surprisingly, this carboxyl also forms an extra hydrogen bond with the γ -OH side chain of serine (Fig. 3). This extra hydrogen bond turns out to be robust in retaining serine in AlaRS. A conserved residue in motif 2, Gly²³⁷, contacts the β -methyl group of alanine and defines the pocket size. (A conserved Ser in GlyRS prevents the extra methyl group side chain of alanine from binding to GlyRS.) A bulkier G237A mutation decreases the binding of Ala by 1500-fold, but only decreases the binding of Ser by ~ 2-fold. The structure of AlaRS-G237A with a substrate analog showed that, although the G237A mutation pushes up the bound Ser, the hydrogen bond between Asp²³⁵ and the γ -OH of the seryl moiety remains. Thus, the extra hydrogen bond plays a major role in causing the misrecognition of Ser in the binding pocket for Ala. However, Asp²³⁵ is critical both for binding and for activating Ala to form Ala-AMP. A D235A substitution decreased the *k*_{cat} for activation of Ala by 17-fold, increased the *K*_m for Ala by 10,000-fold, and was lethal when harbored by AlaRS in *E. coli*. Mutations of Asp²³⁵ to other residues (Asn, Gln, Glu) also caused a decrease in the rate of activation of Ala and dropped the differentiation ratio of Ser/Ala or of Gly/Ala to less than 1:19 (from the WT ratio of 1:300) [45]. Therefore, the universally conserved Asp that pins down the α -amino group of the bound Ala creates a serendipitous binding interaction with the serine γ -OH that is difficult to avoid.

AlaRS Editing and AlaXp's: a necessary redundancy

A unique feature of the alanine system is the widely distributed (all three kingdoms of life) freestanding AlaXp's [48,49]. These proteins encode an editing core homologous to that within AlaRS and proofread misaminoacylated tRNA^{Ala} by specifically hydrolyzing the ester linkage of Ser- and/or Gly-tRNA^{Ala}. Three types of AlaXp's are found: AlaXp-Ia (AlaXp-S) is the smallest, and contains the minimal domain for hydrolysis [50,51]. AlaXp-Ib (AlaXp-M) contains an additional motif at the N-terminus of the editing core [52,53]. Finally, AlaXp-II (AlaXp-L) most closely resembles the complete editing module of AlaRS, by fusing an extra C-terminal domain (C-Ala) to the editing core by a coiled-coil linker (Fig. 4a) [46,47,49].

Because the paradox-creating design of the aminoacylation site is present in every species, the proofreading activity for removing serine from tRNA^{Ala} must have developed early. Phylogenetic analysis of AlaXp's and of the editing domain of AlaRSs suggested that the transacting free-standing AlaXp's were evolved during primitive life and were later fused to the aminoacylation domain of AlaRS to create a *cis*-acting editing domain [58]. This fusion was accompanied by the co-appearance of a unique C-Ala domain in AlaXp-II and AlaRS (Fig. 4b). Binding to the elbow region of tRNA^{Ala}, this C-Ala domain helps to bring together aminoacylation and editing domains on one tRNA to facilitate their collaboration. The benefits of collaboration may be the impetus for the fusion process [58]. Recent crystal structures of archaeal AlaRSs showed that the editing domain is linked to the aminoacylation domain on the side opposite from that seen in ThrRS [46,47]. This unique mode for communicating aminoacylation and editing may be required for the two domains to separately recognize the critical G3:U70 base pair that is needed for specific aminoacylation and editing of tRNA^{Ala} [49,54–57].

Although the editing domain within AlaRS is robust for hydrolyzing both Ser- and Gly-tRNA^{Ala}, a combination of several studies revealed that AlaXp's are mainly specific for Ser-tRNA^{Ala}. Both AlaXp-I (Ia, Ib in *Pyrococcus horikoshii*) and -II (in yeast and mice) showed little or no activity for Gly-tRNA^{Ala} [45,50–52]. AlaXp's also share homology with a portion of the editing domain in ThrRS that hydrolyzes Ser-tRNA^{Thr} [52]. This substrate specificity suggests the primary role of AlaXp is to proofread mis-activated serine. A conserved His-His-Cys motif binds to Zn²⁺ in all solved structures of AlaXps and is found in the editing domains of AlaRSs (but is not found in ThrRSs). The structure of *P. horikoshii* AlaXp-Ia with free serine showed the zinc atom binds directly to the amino acid [51]. A Cys to Ala mutation in the zinc-binding motif of *A. fulgidus* and *E. coli* AlaRS and of mouse AlaXp-II abolished the editing activity [49,52,59,60], suggesting that the bound Zn²⁺ is involved in the recognition of serine and/or the catalysis of the hydrolysis reaction. A defined editing mechanism for AlaXp/AlaRS would require the structure of a complex of a Ser-tRNA^{Ala} analog, such as Ser-A76 or Gly-A76.

The unusual misrecognition of serine at the alanine binding pocket of AlaRS imposes enormous pressure on the co-evolution of AlaXp and AlaRS. Either a mild mutation in the AlaRS editing domain, or RNAi-directed suppression of AlaXp, led to serine-dependent accumulation of protein misfolding in murine cells [18,60]. Recent work also showed that AlaXp undergoes further evolution in mammals (Fig. 4b). Mammalian AlaXp genes are present as a multi-exon fusion that, by alternative splicing, can produce AlaXp, p23^H, and a fused p23^HAlaXp. Whether in *cis* or in *trans*, p23^H down-regulated the editing activity of AlaXp [60]. Interestingly, p23^H is a homologue of the co-chaperone p23 that forms a complex with the Hsp90 chaperone to suppress denatured protein aggregation. Interestingly, a lack of editing activity can be advantageous in certain circumstances (such as oxidative stress, pathogenesis) [61–65].

Epilogue

Selection operates to retain the modern genetic code for discrimination between cognate and noncognate amino acids and for preventing mistranslation. Extensive structural and functional studies showed that amino acid stereochemistry is not the sole determinant of a need for proofreading. This complex system evolved by taking account of the inherent competition between natural amino acids and their analogs and, at the same time, the historical and basic design of aaRSs, and the different requirements of cellular metabolic and signaling pathways. During this evolutionary process, many compromises were made, such as the 'serine paradox' of AlaRS, to sustain the growth of the entire Tree of Life with the same set of amino acids and aaRSs. Thus, recognition and proofreading of amino acids by aaRSs are unique mechanisms for preventing mistranslation and were essential for Nature to develop a universal genetic code.

Acknowledgments

This work is supported by grants GM 15539 and 23562 from the National Institutes of Health, a fellowship from the National Foundation for Cancer Research, a scholarship from Sidney Kimmel Foundation for Cancer Research and a fund of the state of Florida to Scripps Florida.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest

•• of outstanding interest

1. Francklyn C, Perona JJ, Puetz J, Hou YM. Aminoacyl-tRNA synthetases: versatile players in the changing theater of translation. *RNA*. 2002; 8:1363–1372. [PubMed: 12458790]
2. Carter CW Jr. Cognition, mechanism, and evolutionary relationships in aminoacyl-tRNA synthetases. *Annu Rev Biochem*. 1993; 62:715–748. [PubMed: 8352600]
3. Giegé R, Sissler M, Florentz C. Universal rules and idiosyncratic features in tRNA identity. *Nucleic Acids Res*. 1998; 26:5017–5035. [PubMed: 9801296]
4. Ibba M, Söll D. Aminoacyl-tRNA synthesis. *Annu Rev Biochem*. 2000; 69:617–650. [PubMed: 10966471]
5. Eriani G, Delarue M, Poch O, Gangloff J, Moras D. Partition of tRNA synthetases into two classes based on mutually exclusive sets of sequence motifs. *Nature*. 1990; 347:203–206. [PubMed: 2203971]
6. Jimenez-Sanchez A. On the origin and evolution of the genetic code. *J Mol Evol*. 1995; 41 :712–716. [PubMed: 8587115]
7. Schimmel P, Ribas de Pouplana L. Formation of two classes of tRNA synthetases in relation to editing functions and genetic code. *Cold Spring Harb Symp Quant Biol*. 2001; 66:161–166. [PubMed: 12762018]
8. Schimmel P. Development of tRNA synthetases and connection to genetic code and disease. *Protein Sci*. 2008; 17:1643–1652. [PubMed: 18765819]
9. Baldwin AN, Berg P. Transfer ribonucleic acid-induced hydrolysis of valyladenylate bound to isoleucyl ribonucleic acid synthetase. *J Biol Chem*. 1966; 241:839–845. [PubMed: 5324173]
10. Schreier AA, Schimmel PR. Transfer ribonucleic acid synthetase catalyzed deacylation of aminoacyl transfer ribonucleic acid in the absence of adenosine monophosphate and pyrophosphate. *Biochemistry*. 1972; 11:1582–1589. [PubMed: 4337554]
11. Eldred EW, Schimmel PR. Rapid deacylation by isoleucyl transfer ribonucleic acid synthetase of isoleucine-specific transfer ribonucleic acid aminoacylated with valine. *J Biol Chem*. 1972; 247:2961–2964. [PubMed: 4554364]
12. Ibba M, Söll D. Quality control mechanisms during translation. *Science*. 1999; 286:1893–1897. [PubMed: 10583945]
13. Jakubowski, H. Accuracy of aminoacyl-tRNA synthetases: Proofreading of Amino Acids. In: Ibba, M.; Francklyn, C.; Cusack, S., editors. *The Aminoacyl-tRNA Synthetases*. Eureka; 2005. p. 384–396.
14. Fersht AR. Editing mechanisms in protein synthesis. Rejection of valine by the isoleucyl-tRNA synthetase. *Biochemistry*. 1977; 16:1025–1030. [PubMed: 321008]
15. Nangle LA, Motta CM, Schimmel P. Global effects of mistranslation from an editing defect in mammalian cells. *Chem Biol*. 2006; 13:1091–1100. [PubMed: 17052613]
16. Karkhanis VA, Mascarenhas AP, Martinis SA. Amino acid toxicities of *escherichia coli* that are prevented by leucyl-tRNA synthetase amino acid editing. *J Bacteriol*. 2007; 189:8765–8768. [PubMed: 17890314]
17. Korencic D, Ahel I, Schelert J, Sacher M, Ruan B, Stathopoulos C, Blum P, Ibba M, Söll D. A freestanding proofreading domain is required for protein synthesis quality control in Archaea. *Proc Natl Acad Sci U S A*. 2004; 101:10260–10265. [PubMed: 15240874]
18. Lee JW, Beebe K, Nangle LA, Jang J, Longo-Guess CM, Cook SA, Davisson MT, Sundberg JP, Schimmel P, Ackerman SL. Editing-defective tRNA synthetase causes protein misfolding and neurodegeneration. *Nature*. 2006; 443:50–55. [PubMed: 16906134]
19. Dock-Bregeon A-C, Sankaranarayanan R, Romby P, Caillet J, Springer M, Rees B, Francklyn CS, Ehresmann C, Moras D. Transfer RNA-mediated editing in threonyl-tRNA Synthetase: The class II solution to the double discrimination problem. *Cell*. 2000; 103:877–884. [PubMed: 11136973]
20. Dwivedi S, Kruparani SP, Sankaranarayanan R. A D-amino acid editing module coupled to the translational apparatus in archaea. *Nat Struct Mol Biol*. 2005; 12:556–557. [PubMed: 15908961]
21. Dock-Bregeon AC, Rees B, Torres-Larios A, Bey G, Caillet J, Moras D. Achieving error-free translation; the mechanism of proofreading of threonyl-tRNA synthetase at atomic resolution. *Mol Cell*. 2004; 16:375–386. [PubMed: 15525511]

- 22••. Hussain T, Kamarthapu V, Kruparani SP, Deshmukh MV, Sankaranarayanan R. Mechanistic insights into cognate substrate discrimination during proofreading in translation. *Proc Natl Acad Sci U S A*. 2010; 107:22117–22121. The most complete structural studies of the archaeal ThrRS editing site with a series of cognate and noncognate post-transfer substrate analogs that reveal a chemistry-based discrimination mechanism. [PubMed: 21098258]
23. Minajigi A, Francklyn CS. RNA-assisted catalysis in a protein enzyme: The 2'-hydroxyl of tRNA^{Thr} A76 promotes aminoacylation by threonyl-tRNA synthetase. *Proceedings of the National Academy of Sciences*. 2008; 105:17748–17753.
24. Mosyak L, Safo M. Phenylalanyl-tRNA synthetase from *Thermus thermophilus* has four antiparallel folds of which only two are catalytically functional. *Biochimie*. 1993; 75:1091–1098. [PubMed: 8199244]
25. Sasaki HM, Sekine S, Sengoku T, Fukunaga R, Hattori M, Utsunomiya Y, Kuroishi C, Kuramitsu S, Shirouzu M, Yokoyama S. Structural and mutational studies of the amino acid-editing domain from archaeal/eukaryal phenylalanyl-tRNA synthetase. *Proc Natl Acad Sci U S A*. 2006; 103:14744–14749. [PubMed: 17003130]
26. Klipcan L, Levin I, Kessler N, Moor N, Finarov I, Safo M. The tRNA-induced conformational activation of human mitochondrial phenylalanyl-tRNA synthetase. *Structure*. 2008; 16:1095–1104. [PubMed: 18611382]
- 27•. Finarov I, Moor N, Kessler N, Klipcan L, Safo MG. Structure of human cytosolic phenylalanyl-tRNA synthetase: evidence for kingdom-specific design of the active sites and tRNA binding patterns. *Structure*. 2010; 18:343–353. The latest in a series of sophisticated structural studies that define the exceptional viability of architectural design of PheRS and its substrate recognition. [PubMed: 20223217]
28. Woese CR, Olsen GJ, Ibba M, Söll D. Aminoacyl-tRNA synthetases, the genetic code, and the evolutionary process. *Microbiol Mol Biol Rev*. 2000; 64:202–236. [PubMed: 10704480]
29. Lin SX, Baltzinger M, Remy P. Fast kinetic study of yeast phenylalanyl-tRNA synthetase: role of tRNA^{Phe} in the discrimination between tyrosine and phenylalanine. *Biochemistry*. 1984; 23:4109–4116. [PubMed: 6386044]
30. Roy H, Ling J, Irnov M, Ibba M. Post-transfer editing in vitro and in vivo by the beta subunit of phenylalanyl-tRNA synthetase. *EMBO J*. 2004; 23:4639–4648. [PubMed: 15526031]
31. Goldgur Y, Mosyak L, Reshetnikova L, Aniklova V, Lavrik O, Khodyreva S, Safo M. The crystal structure of phenylalanyl-tRNA synthetase from *Thermus thermophilus* complexed with cognate tRNA^{Phe}. *Structure*. 1997; 5:59–68. [PubMed: 9016717]
32. Reshetnikova L, Moor N, Lavrik O, Vassilyev DG. Crystal structures of phenylalanyl-tRNA synthetase complexed with phenylalanine and a phenylalanyl-adenylate analogue. *J Mol Biol*. 1999; 287:555–568. [PubMed: 10092459]
33. Mermershtain I, Finarov I, Klipcan L, Kessler N, Rozenberg H, Safo MG. Idiosyncrasy and identity in the prokaryotic Phe-system: crystal structure of *E. coli* phenylalanyl-tRNA synthetase complexed with phenylalanine and AMP. *Protein Sci*. 2011; 20:160–167. [PubMed: 21082706]
- 34••. Reynolds NM, Ling J, Roy H, Banerjee R, Repasky SE, Hamel P, Ibba M. Cell-specific differences in the requirements for translation quality control. *Proc Natl Acad Sci U S A*. 2010; 107:4063–4068. A particularly perceptive analysis of cell organelles-dependent sensitivity for error-prone PheRS mutants that provides new insight into the dynamics of translational quality control. [PubMed: 20160120]
35. Ibba M, Kast P, Hennecke H. Substrate specificity is determined by amino acid binding pocket size in *Escherichia coli* phenylalanyl-tRNA synthetase. *Biochemistry*. 1994; 33:7107–7112. [PubMed: 8003476]
36. Kirshenbaum K, Carrico IS, Tirrell DA. Biosynthesis of proteins incorporating a versatile set of phenylalanine analogues. *Chembiochem*. 2002; 3:235–237. [PubMed: 11921403]
37. Bentin T, Hamzavi R, Salomonsson J, Roy H, Ibba M, Nielsen PE. Photoreactive bicyclic amino acids as substrates for mutant *Escherichia coli* phenylalanyl-tRNA synthetases. *J Biol Chem*. 2004; 279:19839–19845. [PubMed: 15004015]

38. Kotik-Kogan O, Moor N, Tworowski D, Safo M. Structural basis for discrimination of L-phenylalanine from L-tyrosine by phenylalanyl-tRNA synthetase. *Structure*. 2005; 13 :1799–1807. [PubMed: 16338408]
39. Klipcan L, Moor N, Kessler N, Safo MG. Eukaryotic cytosolic and mitochondrial phenylalanyl-tRNA synthetases catalyze the charging of tRNA with the meta-tyrosine. *Proc Natl Acad Sci U S A*. 2009; 106:11045–11048. Combined enzymatic and structural characterization of PheRS for its ability to implant tRNA^{Phe} with meta-tyrosine – a derivative of Phe under oxidative stress. [PubMed: 19549855]
40. Roy H, Ling J, Alfonzo J, Ibba M. Loss of editing activity during the evolution of mitochondrial phenylalanyl-tRNA synthetase. *J Biol Chem*. 2005; 280:38186–38192. [PubMed: 16162501]
41. Fersht AR, Shindler JS, Tsui WC. Probing the limits of protein-amino acid side chain recognition with the aminoacyl-tRNA synthetases. Discrimination against phenylalanine by tyrosyl-tRNA synthetases. *Biochemistry*. 1980; 19:5520–5524. [PubMed: 7006687]
42. Beuning PJ, Musier-Forsyth K. Species-specific differences in amino acid editing by class II prolyl-tRNA synthetase. *J Biol Chem*. 2001; 276:30779–30785. [PubMed: 11408489]
43. SternJohn J, Hati S, Siliciano PG, Musier-Forsyth K. Restoring species-specific posttransfer editing activity to a synthetase with a defunct editing domain. *Proc Natl Acad Sci U S A*. 2007; 104:2127–2132. [PubMed: 17283340]
44. Splan KE, Ignatov ME, Musier-Forsyth K. Transfer RNA modulates the editing mechanism used by class II prolyl-tRNA synthetase. *J Biol Chem*. 2008; 283:7128–7134. [PubMed: 18180290]
45. Guo M, Chong YE, Shapiro R, Beebe K, Yang XL, Schimmel P. Paradox of mistranslation of serine for alanine caused by AlaRS recognition dilemma. *Nature*. 2009; 462:808–812. Systematic study of AlaRS misactivation of Ser and Gly with atomic level crystal structures, mutagenesis design and kinetic analyses, which clearly defines an unconventional enzyme-substrate interaction that is responsible for the paradox of misrecognition in AlaRS. [PubMed: 20010690]
46. Naganuma M, Sekine S, Fukunaga R, Yokoyama S. Unique protein architecture of alanyl-tRNA synthetase for aminoacylation, editing, and dimerization. *Proc Natl Acad Sci U S A*. 2009; 106:8489–8494. [PubMed: 19423669]
47. Sokabe M, Ose T, Nakamura A, Tokunaga K, Nureki O, Yao M, Tanaka I. The structure of alanyl-tRNA synthetase with editing domain. *Proc Natl Acad Sci U S A*. 2009; 106:11028–11033. [PubMed: 19549823]
48. Ahel I, Korencic D, Ibba M, Söll D. Trans-editing of mischarged tRNAs. *Proc Natl Acad Sci U S A*. 2003; 100:15422–15427. [PubMed: 14663147]
49. Beebe K, Mock M, Merriman E, Schimmel P. Distinct domains of tRNA synthetase recognize the same base pair. *Nature*. 2008; 451:90–93. [PubMed: 18172502]
50. Ishijima J, Uchida Y, Kuroishi C, Tuzuki C, Takahashi N, Okazaki N, Yutani K, Miyano M. Crystal structure of alanyl-tRNA synthetase editing-domain homolog (PH0574) from a hyperthermophile, *Pyrococcus horikoshii* OT3 at 1.45 Å resolution. *Proteins*. 2006; 62:1133–1137. [PubMed: 16374837]
51. Sokabe M, Okada A, Yao M, Nakashima T, Tanaka I. Molecular basis of alanine discrimination in editing site. *Proc Natl Acad Sci U S A*. 2005; 102:11669–11674. [PubMed: 16087889]
52. Fukunaga R, Yokoyama S. Structure of the AlaX-M trans-editing enzyme from *Pyrococcus horikoshii*. *Acta Crystallogr D Biol Crystallogr*. 2007; 63:390–400. [PubMed: 17327676]
53. Chong YE, Yang XL, Schimmel P. Natural homolog of tRNA synthetase editing domain rescues conditional lethality caused by mistranslation. *J Biol Chem*. 2008; 283:30073–30078. [PubMed: 18723508]
54. Hou YM, Schimmel P. A simple structural feature is a major determinant of the identity of a transfer RNA. *Nature*. 1988; 333:140–145. [PubMed: 3285220]
55. Musier-Forsyth K, Schimmel P. Functional contacts of a transfer RNA synthetase with 2'-hydroxyl groups in the RNA minor groove. *Nature*. 1992; 357:513–515. [PubMed: 1608452]
56. Kresge N, Simoni RD, Hill RL. Editing mischarged amino acids: the work of Paul R. Schimmel *J Biol Chem*. 2010; 285:e4–6.
57. McClain WH, Foss K. Changing the acceptor identity of a transfer RNA by altering nucleotides in a “variable pocket”. *Science*. 1988; 241:1804–1807. [PubMed: 2459773]

58. Guo M, Chong YE, Beebe K, Shapiro R, Yang XL, Schimmel P. The C-Ala domain brings together editing and aminoacylation functions on one tRNA. *Science*. 2009; 325:744–747. [PubMed: 19661429]
59. Beebe K, Ribas De Pouplana L, Schimmel P. Elucidation of tRNA-dependent editing by a class II tRNA synthetase and significance for cell viability. *EMBO Journal*. 2003; 22 :668–675. [PubMed: 12554667]
60. Nawaz MH, Merriman E, Yang XL, Schimmel P. p23H implicated as cis/trans regulator of AlaXp-directed editing for mammalian cell homeostasis. *Proc Natl Acad Sci U S A*. 2011; 108:2723–2728. The most recent discovery of a regulatory element in trans-editing AlaXp, which forecast a potential mechanism to directly coordinates translational fidelity with other cellular responses. [PubMed: 21285375]
61. Ling J, Söll D. Severe oxidative stress induces protein mistranslation through impairment of an aminoacyl-tRNA synthetase editing site. *Proc Natl Acad Sci U S A*. 2010; 107:4028–4033. [PubMed: 20160114]
62. Netzer N, Goodenbour JM, David A, Dittmar KA, Jones RB, Schneider JR, Boone D, Eves EM, Rosner MR, Gibbs JS, et al. Innate immune and chemically triggered oxidative stress modifies translational fidelity. *Nature*. 2009; 462:522–526. A revolutionary discovery of naturally present mischarged aminoacyl-tRNAs and their dynamic response to environmental stimuli, which sets a new base for active involvement of tRNA synthetases in broader cellular events. [PubMed: 19940929]
63. Jones TE, Alexander RW, Pan T. Misacylation of specific nonmethionyl tRNAs by a bacterial methionyl-tRNA synthetase. *Proc Natl Acad Sci U S A*. 2011; 108:6933–6938. [PubMed: 21482813]
64. Li L, Boniecki MT, Jaffe JD, Imai BS, Yau PM, Luthey-Schulten ZA, Martinis SA. Naturally occurring aminoacyl-tRNA synthetases editing-domain mutations that cause mistranslation in *Mycoplasma* parasites. *Proc Natl Acad Sci U S A*. 2011; 108:9378–9383. [PubMed: 21606343]
65. Rocha R, Pereira PJ, Santos MA, Macedo-Ribeiro S. Unveiling the structural basis for translational ambiguity tolerance in a human fungal pathogen. *Proc Natl Acad Sci U S A*. 2011; 108:14091–14096. [PubMed: 21825144]

Highlights

Accurate translation depends on the specific coupling of cognate amino acids and tRNAs – a step carried out by aminoacyl-tRNA synthetases (aaRSs) and that generates the genetic code. How selection operates to retain the modern genetic code is well illustrated by the evolved mechanisms for specific discrimination between cognate and noncognate amino acids. The extensive structural studies and functional analyses have shown that amino acid stereochemistry is not the sole determinant of a need for proofreading. This complex system evolved by taking account of the inherent competition between natural amino acids and their analogs and, at the same time, the historical and basic design of aaRSs, and the different requirements of the cellular metabolic and signaling pathways.

Figure 1. Thr/Ser recognition by ThrRS

(a) Structure of *E. coli* ThrRS (in complex with the Ser-A76 analog, *pdb1TKY*) represents the editing site for bacterial and eukaryotic ThrRS to hydrolyze mischarged Ser-tRNA^{Thr}. A hydrophobic pocket that recognizes the -OH group of Ser also excludes Thr [21]. (b) Editing domain of *P. abyssi* ThrRS shows that the editing site of archaeal ThrRS is homologous to D-amino acid deacylases. A chemical differentiation mechanism that only hydrolyzes Ser-tRNA^{Thr} is evidenced by its structures in complex with Ser-A76 and Thr-A76 (*pdb3PD2*, *3PD3*) [22].

Figure 2. Phe/Tyr recognition by PheRS

(a) Aminoacylation site of *T. thermophilus* PheRS in complex with Phe, *p*-Cl-phe, *m*-Tyr and Tyr (*pdb1B70*, *2AKW*, *3HFZ* and *2AMC*). The pocket envelope and the protein residue conformations are plotted from the structure of the complex with Phe. Two water molecules are located in the spacious pocket for amino acid activation in the α subunit of PheRS. One of the two Phe residues (F258) that forms the edge-to-face interactions with the benzene ring of the substrate is shown here. The other (F260) is on top of the binding pocket. (b) Editing site of *T. thermophilus* PheRS in complex with Tyr (*pdb2AMC*).

Figure 3. Gly/Ala/Ser recognition by AlaRS

Interactions with alanine and serine as shown in their ligand-bound structures (Ala-AMS, alanyl-adenylate sulfamoyl analog versus native Ala-AMP, and Ser-AMS, *pdb3HXU*, *3HXW*) [45]. The aromatic ring of Trp¹⁷⁰ stacks with the backbone of amino-acid substrates. Repulsion for Ser comes from a close contact between Gly237–Ser(OH). Asp²³⁵ forms a common hydrogen bond with the α -amino group of bound amino acids. One extra H-bond between Asp²³⁵ and the γ -OH of Ser retains Ser in the pocket for Ala.

Figure 4. AlaRS editing domain and AlaXps redundancy

(a) Structural model of AlaXp-II combined from the solved AlaXp-I and AlaRS structures [46,52]. C-Ala domain is linked to the editing active site by a long coiled-coil linker. The characteristic G3:U70 signature of tRNA^{Ala} (yellow) is recognized by the editing domain while the C-Ala domain anchors the elbow region of the L-shaped tRNA. (b) Continuous evolution of the editing activity for Ser-tRNA^{Ala}. Early addition of a Gly-rich motif to the editing core of AlaXp in primordial life is followed by the acquirement of the C-Ala domain and the subsequent fusion with the aminoacylation domain to form AlaRS. A regulated AlaXp editing activity is achieved with the addition of the p23^H domain in mammals. The p23^H polypeptide (gray-blue) is added as a covalent fusion or can bind in *trans* [58,60].