Quality Control During Aminoacyl-tRNA Synthesis

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The fidelity of translation is determined at two major points: the accuracy of aminoacyl-tRNA selection by the ribosomes and synthesis of cognate amino acid/tRNA pairs by aminoacyl-tRNA synthetases (aaRSs) in the course of the aminoacylation reaction. The most important point in aminoacylation is the accurate recognition of cognate substrates coupled with discrimination of non-cognates. While this is generally accomplished by a single enzyme, we have recently found that discrimination against lysine analogues requires the existence of two unrelated lysyl-tRNA synthetases. For other amino acids, initial recognition is not sufficiently accurate with errors being corrected by an intrinsic editing activity. Recent studies indicate how editing prevents the misinterpretation of phenylalanine as tyrosine in the genetic code and have shown the importance of this process *in vivo*. More recent studies indicate that while these editing reactions are critical in the cytoplasm, some are absent from mitochondria suggesting that the overall fidelity of protein synthesis might be reduced in this compartment.

Keywords: *Aminoacyl-tRNA, editing, genetic code, transfer RNA, translation*

Introduction

The correct functioning of living systems requires maintaining a certain level of fidelity in all processes dealing with the transfer of information. Translation is the process by which genetic information is transferred from a nucleic acid sequence into the amino acid sequence of a protein. The fidelity of translation is determined at two major points: the accuracy of aminoacyl-tRNA (aa-tRNA) selection by the ribosomes and synthesis of cognate amino acid/tRNA pairs in the course of the aminoacylation reaction. Aminoacyl--tRNAs are made by the aminoacyl-tRNA synthetases (aaRSs), a family of twenty proteins each of which pairs a particular amino acid with the correct tRNA, thereby defining the genetic code.[1,2] After aa-tRNAs are synthesized they are screened by elongation factor Tu[3] (EF-Tu) which also delivers them to the ribosome where further checking may also occur[4] (Fig. 1). Quality control steps after aa-tRNA synthesis are not sufficient on their own to completely prevent aminoacylation errors from being propagated during translation. This focus of quality control on aa-tRNA synthesis has allowed the design of *in vitro* systems for the site-specific co-translational insertion of synthetic amino acids in response to in-frame stop codons.[5-8] These systems all rely on aaRSs with modified substrate specificities[9] and no other modifications to the cellular protein synthesis machinery are necessary for their function. The ability to manipulate the genetic code in such a straightforward way illustrates the pivotal contribution of quality control to translational fidelity during aa-tRNA synthesis.

Quality control and aminoacyl-tRNA

The synthesis of non-cognate aa-tRNAs is potentially catastrophic for cellular viability. A variety of quality control strategies are employed by the cell to ensure that typically only about one in every 10^4 codons is mistranslated, even though much higher rates can be tolerated at some codons.[10-12] In addition to codon-anticodon pairing,[13] the mechanisms of translational quality control are broadly of three types; specificity of substrate selection by aa-tRNA synthetases, proofreading, and exclusion from the ribosome. Exhaustive studies over the last four decades have described in intricate detail the molecular recognition strategies, that allow synthetases to select particular canonical amino acids and tRNAs to generate correctly matched aa-tRNAs (reviewed in Ibba and Söll, 2000). These include a variety of different editing mechanisms designed to ensure that misactivated amino acids or mischarged aa-tRNA will be hydrolyzed.[14-17] It has recently become clear that amino acid specificity is also enhanced by the existence of a natural reservoir of diverse synthetase alleles. This pool of synthetases displays differences in specificity towards molecules outside the canonical set of amino acids and can exclude amino acid analogues from translation[18] and provide resistance against inhibitory amino acid mimics.[19] Representative examples of several of the strategies for quality control during aa-tRNA synthesis are discussed in more detail below.

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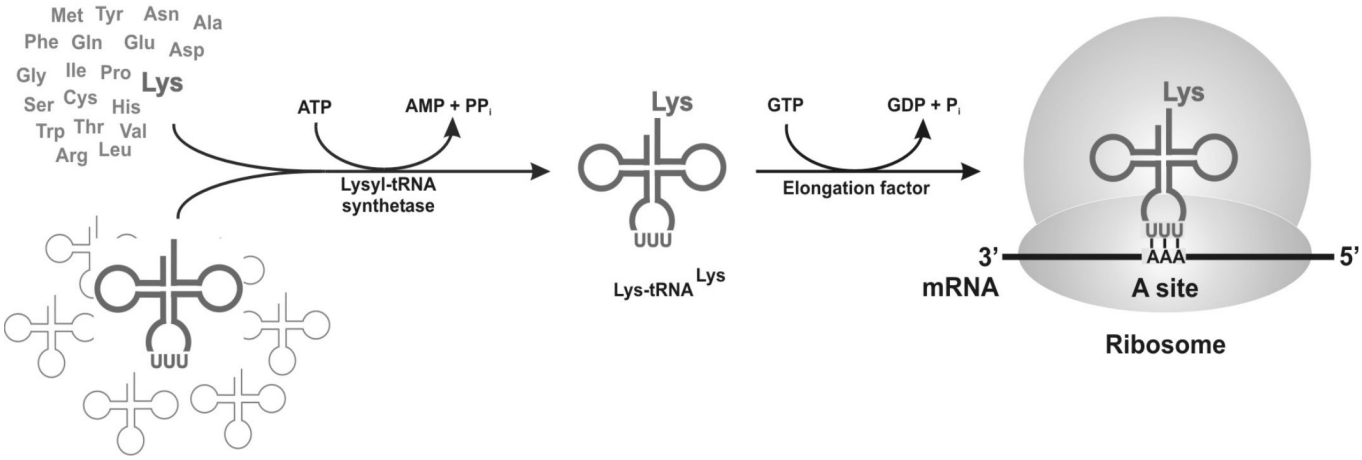
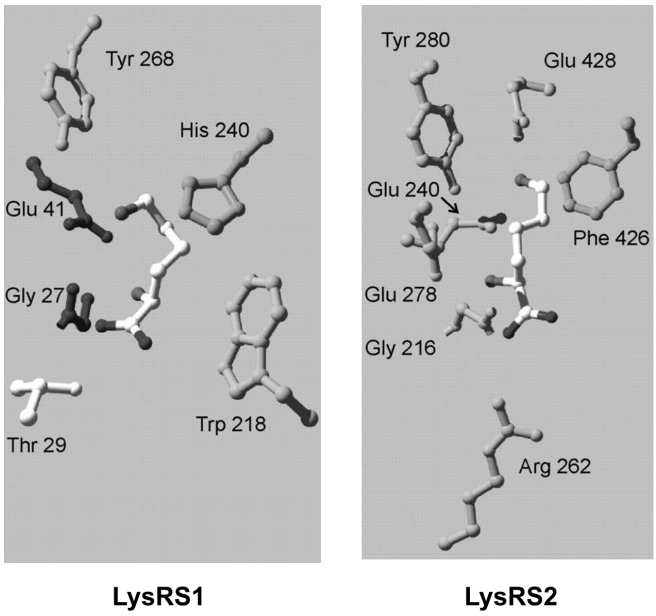


F i g. 1 – *Translation of the codon AAA as lysine during the elongation phase of protein synthesis. Uncharged tRNA^{Lys} and lysine are first selected from the cellular pools of similar molecules by lysyl-tRNA synthetase (LysRS). After synthesis and release from LysRS, lysyl-tRNA^{Lys} is delivered to the ribosome, where its anticodon can then interact with the corresponding codon in mRNA.*

S l i k a 1 – *Translacija kodona AAA kao lizina za vrijeme elongacijske faze u sintezi proteina. Nenabijena tRNA^{Lys} i lizin najprije su selektirani iz celularne skupine sličnih molekula pomoću lizil-tRNA sintetaze (LyRS). Nakon sinteze i oslobađanja iz LysRS, lizil-tRNA^{Lys} dolazi do ribosoma, gdje njegov anti-kodon tada može reagirati s odgovarajućim kodonom u mRNA.*



F i g. 2 – *L-lysine recognition by LysRS1 and LysRS2. L-lysine in the active site of* Pyrococcus horikoshii *LysRS1[31] (left panel) and* Escherichia coli *LysRS2[71] (right panel).*

S l i k a 2 – *LysRS1 i LysRS2 prepoznaju u aktivnom mjestu* Pyrococcus horikoshii *LysRS1[31] (lijevo) i* Escherichia coli *LysRS2[71] (desno).*

Substrate discrimination by lysyl-tRNA synthetase

AaRSs can be divided into two structurally unrelated classes, I and II, with 10 canonical members in each.[20] The divergent structures lead to functional differences with respect to ATP and tRNA binding in each class.[21] An aaRS from each class is designated to each amino acid with only one exception known to date, lysyl-tRNA synthetase[22] (LysRS), for which examples from, both, class I (LysRS1) and class II (LysRS2) are known. Analysis of the distribution of LysRS has so far shown that LysRS2 is found in all eukaryotes, most bacteria and some archaea, and LysRS1 is present in some bacteria and most archaea.[23,24] LysRS1 and LysRS2 are not generally found together, their co-existence being restricted to a few organisms. Although, structurally different, LysRS1 and LysRS2 are able to recognize lysine and tRNA^{Lys} *in vivo* and *in vitro* in much the same way. For example, the elements recognized by, both, LysRS1 and LysRS2 in tRNA^{Lys} are the same, namely the anticodon, acceptor stem, and discriminator bases.[25-27] In contrast, the activation mechanism for lysine is significantly different between each LysRS. LysRS2 forms lysyl-adenylate after binding only lysine and ATP, while LysRS1 additionally requires the prior binding of tRNA^{Lys}. tRNA binding prior to amino acid activation is a feature shared by only a small sub-group of class I aaRS.[28-30] The crystal structures of LysRS1 and LysRS2 complexed with lysine reveal, that while the mechanisms of recognition of the R-group of L-lysine rely on similar arrangements of amino acids in each binding pocket, the active sites are different[31] (Fig. 2). These structural differences lead to divergent patterns in non-cognate substrate discrimination between LysRS1 and LysRS2.[18,32] Based on the structure of L-lysine complexed with *E. coli* LysRS2 (*lysS*), residues implicated in amino acid recognition and discrimination were systematically replaced. Steady-state kinetic parameters for these variants showed reductions in the catalytic efficiency (k_{cat} / K_M) of 1 to 3 orders of magnitude, allowing the assignment of specific roles for key residues in the active site of LysRS2. To further investigate the role of each residue in discrimination against non-cognate amino acids, steady-state kinetic parameters were determined for the non-protein amino acid *S*-(2-aminoethyl)-L-cysteine, a potent inhibitor of LysRS2. While a number of variants showed reductions of several hundred fold in efficiency of *S*-(2-aminoethyl)-L-cysteine utilization, this was uniformly accompanied by similar reductions in the efficiency of lysine utilization. Thus, manipulation of the amino acid binding site only allowed up to a four fold improvement in *S*-(2-aminoethyl)-L-cysteine discrimination.[33]

This is in contrast to the highly effective discrimination against *S*-(2-aminoethyl)-L-cysteine by class I LysRS, and correlates with the fundamentally different roles of conserved aromatic residues in the two LysRS active sites. These data indicate that the lysine-binding site is more open in LysRS2 than in LysRS1, in agreement with previous structural studies. The physiological significance of divergent amino acid recognition was reflected by the *in vivo* resistance to growth inhibition imparted by LysRS1 against *S*-(2-aminoethyl)-L-cysteine and LysRS2 against -amino butyric acid. These differences in resistance to naturally occurring non-cognate amino acids illustrate how the distribution of LysRS1 and LysRS2 contributes to quality control during protein synthesis by excluding lysine analogues.

Editing of misactivated tyrosine by phenylalanyl-tRNA synthetase

The accuracy of aa-tRNA synthesis is generally assured by the existence of aaRSs specific for each particular amino acid:tRNA pair, as for example in the case of LysRS described above. Cognate tRNA recognition, and discrimination of non-cognate RNAs, is achieved by sequence-specific direct and indirect readout of the numerous combinations of bases present in tRNAs.[34-37] The relative structural simplicity of the amino acid substrates makes their accurate recognition and discrimination more challenging. While some amino acids, such as cysteine and tyrosine (Tyr), are distinct enough to allow their specific recognition by a particular aaRS,[38,39] others, such as valine and isoleucine, are less easily distinguished. For example the class I aaRS isoleucyl-tRNA synthetase (IleRS) is only able to poorly discriminate against valine, which has a misactivation rate of about 1:200 compared to the cognate substrate isoleucine. Despite this significant rate of misactivation and misaminoacylation, the accuracy of translation is not compromised due to the existence of an intrinsic proofreading and editing mechanism in IleRS, that specifically hydrolyzes both misactivated Val-AMP and misaminoacylated Val-tRNA^{Ile}.[14,40] In addition to IleRS, it has been found that many other class I and class II aaRSs also employ editing to prevent release of non-cognate aa-tRNA and subsequent loss of translational accuracy (reviewed in[41,42]). With a few notable exceptions,[43] editing generally occurs in specialized domains distal from the active site such as the class I specific CP1 region of IleRS, leucyl- (LeuRS) and valyl-tRNA synthetases (ValRS). The editing domains of class II aaRSs are more diverse than their class I counterparts and include the "HxxxH" domain found in, both, alanyl-[44] and threonyl-tRNA synthetase (ThrRS),[15] an unrelated domain in archaeal ThrRS,[45,46] and a Ybak-like domain in prolyl-tRNA synthetase (ProRS).[47-49]

The editing domains of aaRSs are normally found in the same polypeptide as the active site, the only exceptions being *trans*-editing enzymes that are believed to compensate for the lack of editing in some archaeal ThrRSs and bacterial ProRSs and PheRS. PheRS is usually an $(\alpha\beta)_2$ heterotetramer, with the active site located in the α-subunit and tRNA binding sites in both subunits. Phenylalanyl-tRNA synthetase mis-activates tyrosine and subsequently corrects such errors through hydrolysis of tyrosyl-adenylate and Tyr-tRNA^{Phe}. Structural modeling combined with an *in vivo* genetic screen identified the editing site in the B3/B4 domain of the β-subunit, 40 Å from the active site in the α-subunit.[50] Replacements of residues within the editing site had no effect on Phe-tRNA^{Phe} synthesis but abolished hydrolysis of Tyr-tRNA^{Phe} *in vitro*. Expression of the corresponding mutants in *Escherichia coli* significantly slowed growth, and changed the activity of a recoded β-galactosidase variant by misincorporating tyrosine in place of phenylalanine. This loss in aromatic amino acid discrimination *in vivo* revealed that editing by phenylalanyl-tRNA synthetase is essential for faithful translation of the genetic code.

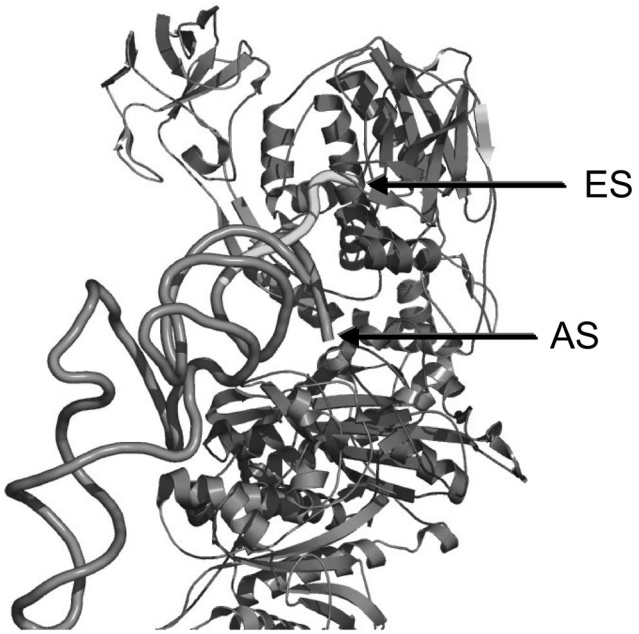


Fig. 3 – *Model for RNA-dependent editing by phenylalanyl-tRNA synthetase. The 3′-end of tRNA^{Phe} is shown in the active site (AS) as depicted in the crystal structure,[72] and modeled into the editing site (ES).*

Slika 3 – *Model za uređivanje ovisno o RNA pomoću fenilalanil-tRNA-sintetaze. Pokazan je 3′-završetak tRNA^{Phe} u aktivnom mjestu (AS) kao što je opisan u kristalnoj strukturi[72], i modeliran na uređivačko mjesto (ES).*

It is less clear whether mitochondrial PheRSs also have the potential to edit misacylated tRNAs, as they are monomers and thus lack the conventional $(\alpha\beta)_2$ oligomeric form.[51,52] Mitochondrial PheRS sequences are most closely related to the bacterial-type, and are chimeras of the α-subunit with an inserted domain between motifs 2 and 3, and the C-terminal tRNA anticodon binding domain (B8) of the β-subunit.[51] Despite their similarity to bacterial PheRSs, the mitochondrial versions do not contain regions analogous to the known editing domain.[50] While it was originally suggested that mitochondrial PheRSs were active in editing,[53] later studies questioned these findings.[51] Yeast cytoplasmic PheRS contains an editing site functionally analogous to that of *Escherichia coli*, and disruption of the site abolishes editing of Tyr-tRNA^{Phe}, both, in *cis* and *trans* but does not diminish Phe-tRNA^{Phe} synthesis. Wild-type mitochondrial PheRS lacks both *cis* and *trans* editing and can synthesize Tyr-tRNA^{Phe}, an activity enhanced in active site variants with improved tyrosine recognition. These findings indicate that the mitochondrial protein synthesis machinery lacks the ty-

rosine proofreading activity characteristic of cytoplasmic translation. Functional analyses and sequence-based predictions suggest that other non-cognate aa-tRNA editing pathways, such as that for Ile-tRNA^{Leu}, [54] may also be absent from mitochondria. These differences between mitochondrial and cytoplasmic aaRS functions suggest that, either organelle protein synthesis quality control is focused on another step, or that translation in this compartment is inherently less accurate.

Higher order complexes in aminoacyl-tRNA synthesis

In bacteria aaRSs typically perform their role as individual enzymes, found either as monomers, homo-dimers, or homo- or hetero-tetramers. However, in eukaryotes several aa-tRNA synthetases exist in multi-enzyme complexes[55-57] and two different types have so far been found in mammalian cells. One is composed of only one aaRS, ValRS, and EF-1H, the heavy form of translation elongation factor 1.[58] The second complex is considerably larger and includes nine aaRSs, IleRS, leucyl-(LeuRS), prolyl-(ProRS), methionyl-(MetRS), glutaminyl-, glutamyl-(GluRS), LysRS, arginyl-, and aspartyl-(AspRS) tRNA synthetases. In addition, the polypeptide carrying the ProRS activity is multi-functional in that the protein also comprises the catalytic domain and activity of GluRS.[59] Three auxiliary proteins, p18, p38, and p43 are also part of the multi-synthetase complex. Although, the structural and functional significance of the complex still remains to be elucidated, it is known that N- and C-terminal extensions of the mammalian synthetases mediate association of the components. The accessory components p18, p38 and p43 assist complex formation and stability, and promote tRNA binding by the complex.[60-62]

The only other multi-aaRS complex so far identified in eukaryotes was discovered in the yeast *Saccharomyces cerevisiae*. The complex consists of MetRS, GluRS and the non-synthetase protein Arc1p, which has homology to the mammalian protein p43.[63,64] The association with Arc1p was shown to increase the catalytic efficiency of the two synthetases and enhance nuclear export of tRNA. The bacterial homologue of Arc1p, trbp111, was first found in the extreme thermophile *Aquifex aeolicus* and was shown to promote tRNA binding by aaRSs.[65,66] Factors unrelated to the translation machinery have also been found to associate with aaRSs. In one case a two-hybrid screen revealed interaction between yeast seryl-tRNA synthetase and Pex21p, a protein involved in peroxisome biogenesis.[67] In a similar screen yeast tyrosyl-tRNA synthetase was isolated as a protein associating with Knr4p, a protein involved in regulation of cell wall assembly.[68]

In archaea much less is known about aaRS complexes, and to date only two studies have reported their possible existence. *Methanocaldococcus jannaschii* ProRS was co-purified with the H_2-forming N^5-N^{10}-methylene tetrahydromethanopterin dehydrogenase (HMD), a component of the methanogenesis pathway.[69] A yeast two-hybrid screen for interactions between *Methanothermobacter thermautotrophicus* proteins, using ProRS as the bait, identified components of methanogenesis, protein-modifying factors, and LeuRS.[70] The association of ProRS with LeuRS was confirmed *in vitro* by native gel electrophoresis and size exclusion chromatography. Determination of the steady-state kinetics of tRNA^{Pro} charging showed that the catalytic efficiency (k_{cat}/K_M) of ProRS increased 5-fold in the complex with LeuRS compared to the free enzyme, while the K_M for proline was unchanged. No significant changes in the steady-state kinetics of LeuRS aminoacylation were observed on addition of ProRS. These findings indicate that ProRS and LeuRS associate in *M. thermautotrophicus*, and suggest that this interaction contributes to translational fidelity by enhancing tRNA aminoacylation by ProRS. Further studies using other components of archaeal protein synthesis suggest that the interaction between LeuRS and ProRS is in fact part of a larger complex containing, both, aaRSs and other factors.

Conclusions

Advances over the last few years have provided new insights into the mechanisms that determine quality control and fidelity during aa-tRNA synthesis. These studies have primarily focused on the processes by which single proteins accurately recognize cognate substrates, discriminate against non-cognate substrates, and correct errors when they arise. More recently it has started to become clear that protein:protein interactions also contribute to fidelity, both, by enhancing cognate substrate selection and expanding substrate specificity. Investigating the mechanisms of quality control within these larger complexes, and within the cell itself, now offers the opportunity to understand more clearly how the cell maintains fidelity during protein synthesis.

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References
Literatura

1. *C. R. Woese, G. J. Olsen, M. Ibba, D. Söll*, Microbiol. Mol. Biol. Rev. **64** (2000) 202.
2. *P. O'Donoghue, Z. Luthey-Schulten*, Microbiol. Mol. Biol. Rev. **67** (2003) 550.
3. *F. J. LaRiviere, A. D. Wolfson, O. C. Uhlenbeck*, Science **294** (2001) 165.
4. *R. P. Fahlman, T. Dale, O. C. Uhlenbeck*, Mol. Cell **16** (2004) 799.
5. *L. Wang, A. Brock, B. Herberich, P. G. Schultz*, Science **292** (2001) 498.
6. *R. A. Mehl, J. C. Anderson, S. W. Santoro, L. Wang, A. B. Martin, D. S. King, D. M. Horn, P. G. Schultz*, J. Am. Chem. Soc. **125** (2003) 935.
7. *J. W. Chin, T. A. Cropp, J. C. Anderson, M. Mukherji, Z. Zhang, P. G. Schultz*, Science **301** (2003) 964.
8. *C. Kohrer, J. H. Yoo, M. Bennett, J. Schaack, U. L. RajBhandary*, Chem. Biol. **10** (2003) 1095.
9. *T. Kobayashi, O. Nureki, R. Ishitani, A. Yaremchuk, M. Tukalo, S. Cusack, K. Sakamoto, S. Yokoyama*, Nat. Struct. Biol. **10** (2003) 425.

10. *T. Ruusala, M. Ehrenberg, C. G. Kurland,* EMBO J. **1** (1982) 741.
11. *C. G. Kurland*, Annu. Rev. Genet. **26** (1992) 29.
12. *B. Min, M. Kitabatake, C. Polycarpo, J. Pelaschier, G. Raczniak, B. Ruan, H. Kobayashi, S. Namgoong, D. Söll*, J. Bacteriol. **185** (2003) 3524.
13. *J. M. Ogle, A. P. Carter, V. Ramakrishnan*, Trends Biochem. Sci. **28** (2003) 259.
14. *O. Nureki, D. G. Vassylyev, M. Tateno, A. Shimada, T. Nakama, S. Fukai, M. Konno, T. L. Hendrickson, P. Schimmel, S. Yokoyama*, Science **280** (1998) 578.
15. *A. Dock-Bregeon, R. Sankaranarayanan, P. Romby, J. Caillet, M. Springer, B. Rees, C. S. Francklyn, C. Ehresmann, D. Moras*, Cell **103** (2000) 877.
16. *T. L. Lincecum, M. Tukalo, A. Yaremchuk, R. S. Mursinna, A. M. Williams, B. S. Sproat, W. Van Den Eynde, A. Link, S. Van Calenbergh, M. Grøtli, S. A. Martinis, S. Cusack*, Mol. Cell **11** (2003) 951.
17. *M. L. Ferri-Fioni, E. Schmitt, J. Soutourina, P. Plateau, Y. Mechulam, S. Blanquet*, J. Biol. Chem. **276** (2001) 47285.
18. *B. Jester, J. Levengood, H. Roy, M. Ibba, K. Devine*, Proc. Natl. Acad. Sci. U. S. A **100** (2003) 14351.
19. *J. R. Brown, D. Gentry, J. A. Becker, K. Ingraham, D. J. Holmes, M. J. Stanhope*, EMBO Rep. **4** (2003) 1.
20. *L. Ribas De Pouplana, P. Schimmel*, Cell **104** (2001) 191.
21. *M. Ibba, D. Söll*, Annu. Rev. Biochem. **69** (2000) 617.
22. *M. Ibba, S. Morgan, A. W. Curnow, D. R. Pridmore, U. C. Vothknecht, W. Gardner, W. Lin, C. R. Woese, D. Söll*, Science **278** (1997) 1119.
23. *M. Ibba, J. L. Bono, P. A. Rosa, D. Söll*, Proc. Natl. Acad. Sci. U.S.A **94** (1997) 14383.
24. *A. Ambrogelly, D. Korencic, M. Ibba*, J. Bacteriol. **184** (2002) 4594.
25. *M. Ibba, H. C. Losey, Y. Kawarabayasi, H. Kikuchi, S. Bunjun, D. Söll*, Proc. Natl. Acad. Sci. U.S.A **96** (1999) 418.
26. *D. Söll, H. D. Becker, P. Plateau, S. Blanquet, M. Ibba*, Proc. Natl. Acad. Sci. U.S.A **97** (2000) 14224.
27. *A. Ambrogelly, M. Frugier, M. Ibba, D. Söll, R. Giege*, FEBS Lett. **579** (2005) 2629.
28. *B. Delagoutte, D. Moras, J. Cavarelli*, EMBO J. **19** (2000) 5599.
29. *L. D. Sherlin, J. J. Perona*, Structure.(Camb.) **11** (2003) 591.
30. *S. Sekine, O. Nureki, D. Y. Dubois, S. Bernier, R. Chenevert, J. Lapointe, D. G. Vassylyev, S. Yokoyama*, EMBO J. **22** (2003) 676.
31. *T. Terada, O. Nureki, R. Ishitani, A. Ambrogelly, M. Ibba, D. Söll, S. Yokoyama*, Nat. Struct. Biol. **9** (2002) 257.
32. *J. Levengood, S. F. Ataide, H. Roy, M. Ibba*, J. Biol. Chem. **279** (2004) 17707.
33. *S. F. Ataide, M. Ibba*, Biochemistry **43** (2004) 11836.
34. *W. H. McClain, J. Schneider, S. Bhattacharya, K. Gabriel*, Proc. Natl. Acad. Sci. U.S.A **95** (1998) 460.
35. *R. Giege, M. Sissler, C. Florentz*, Nucleic Acids Res. **26** (1998) 5017.
36. *M. E. Saks, J. R. Sampson, J. Abelson*, Science **279** (1998) 1665.
37. *S. Hauenstein, C. M. Zhang, Y. M. Hou, J. J. Perona*, Nat. Struct. Mol. Biol. **11** (2004) 1134.
38. *A. R. Fersht, J. S. Shindler, W. C. Tsui*, Biochemistry **19** (1980) 5520.
39. *K. J. Newberry, Y. M. Hou, J. J. Perona*, EMBO J. **21** (2002) 2778.
40. *A. N. Baldwin, P. Berg*, J. Biol. Chem. **241** (1966) 839.
41. *T. L. Hendrickson, P. Schimmel*, Transfer RNA-dependent Amino Acid Discrimination by Aminoacyl-tRNA Synthetases, in J. Lapointe, L. Brakier-Gingras (Ed.), Translation Mechanisms, Landes Bioscience, 2003, pp 34.
42. *H. Jakubowski*, Accuracy of Aminoacyl-tRNA Synthetases: Proofreading of Amino Acids, in M. Ibba, C. S. Francklyn, S. Cusack (Ed.), The Aminoacyl-tRNA Synthetases, Landes Bioscience, Georgetown, 2005, pp 384.
43. *H. Jakubowski*, EMBO J. **10** (1991) 593.
44. *K. Beebe, L. Ribas De Pouplana, P. Schimmel*, EMBO J. **22** (2003) 668.
45. *K. Beebe, E. Merriman, L. Ribas De Pouplana, P. Schimmel*, Proc. Natl. Acad. Sci. U.S.A **101** (2004) 5958.
46. *D. Korencic, I. Ahel, J. Schelert, M. Sacher, B. Ruan, C. Stathopoulos, P. Blum, M. Ibba, D. Söll*, Proc. Natl. Acad. Sci. U.S.A **101** (2004) 10260.
47. *P. J. Beuning, K. Musier-Forsyth*, Proc. Natl. Acad. Sci. U.S.A **97** (2000) 8916.
48. *F. C. Wong, P. J. Beuning, C. Silvers, K. Musier-Forsyth*, J. Biol. Chem. **278** (2003) 52857.
49. *I. Ahel, D. Korencic, M. Ibba, D. Söll*, Proc. Natl. Acad. Sci. U.S.A **100** (2003) 15422.
50. *H. Roy, J. Ling, M. Irnov, M. Ibba*, EMBO J. **23** (2004) 4639.
51. *A. Sanni, P. Walter, Y. Boulanger, J. P. Ebel, F. Fasiolo*, Proc. Natl. Acad. Sci. U.S.A **88** (1991) 8387.
52. *J. M. Bullard, Y. C. Cai, B. Demeler, L. L. Spremulli*, J. Mol. Biol. **288** (1999) 567.
53. *H. J. Gabius, R. Engelhardt, F. R. Schroder, F. Cramer*, Biochemistry **22** (1983) 5306.
54. *S. W. Lue, S. O. Kelley*, Biochemistry **44** (2005) 3010.
55. *C. Behrens, J. N. Nielsen, X. J. Fan, X. Doisy, K. H. Kim, M. Prætorius-Ibba, P. E. Nielsen, M. Ibba,* Tetrahedron **56** (2000) 9443.
56. *M. Mirande*, Multi-aminoacyl-tRNA Synthetase Complexes, in *M. Ibba, C. S. Francklyn, S. Cusack* (Ed.), The Aminoacyl-tRNA Synthetases, Landes Bioscience, Georgetown, 2005, pp 298.
57. *C. L. Wolfe, J. A. Warrington, S. Davis, S. Green, M. T. Norcum*, Protein Sci. **12** (2003) 2282.
58. *G. Bec, P. Kerjan, J. P. Waller*, J. Biol. Chem. **269** (1994) 2086.
59. *C. Cerini, P. Kerjan, M. Astier, D. Gratecos, M. Mirande, M. Semeriva*, EMBO J. **10** (1991) 4267.
60. *S. Quevillon, J. C. Robinson, E. Berthonneau, M. Siatecka, M. Mirande*, J. Mol. Biol. **285** (1999) 183.
61. *S. Quevillon, M. Mirande*, FEBS Lett. **395** (1996) 63.
62. *J. Y. Kim, Y. S. Kang, J. W. Lee, H. J. Kim, Y. H. Ahn, H. Park, Y. G. Ko, S. Kim*, Proc. Natl. Acad. Sci. U. S. A **99** (2002) 7912.
63. *G. Simos, A. Segref, F. Fasiolo, K. Hellmuth, A. Shevchenko, M. Mann, E. C. Hurt*, EMBO J. **15** (1996) 5437.
64. *K. Deinert, F. Fasiolo, E. C. Hurt, G. Simos*, J. Biol. Chem. **276** (2001) 6000.
65. *A. J. Morales, M. A. Swairjo, P. Schimmel*, EMBO J. **18** (1999) 3475.
66. *T. Nomanbhoy, A. J. Morales, A. T. Abraham, C. S. Vortler, R. Giege, P. Schimmel*, Nat. Struct. Biol. **8** (2001) 344.
67. *S. Ročak, I. Landeka, I. Weygand-Đurašević*, FEMS Microbiol. Lett. **214** (2002) 101.
68. *A. Dagkessamanskaia, H. Martin-Yken, F. Basmaji, P. Briza, J. Francois*, FEMS Microbiol. Lett. **200** (2001) 53.
69. *R. S. Lipman, J. Chen, C. Evilia, O. Vitseva, Y. M. Hou*, Biochemistry **42** (2003) 7487.
70. *M. Prætorius-Ibba, T. E. Rogers, R. Samson, Z. Kelman, M. Ibba*, J. Biol. Chem. **280** (2005) 26099.
71. *S. Onesti, G. Desogus, A. Brevet, J. Chen, P. Plateau, S. Blanquet, P. Brick,* Biochemistry **39** (2000) 12853.
72. *Y. Goldgur, L. Mosyak, L. Reshetnikova, V. Ankilova, O. Lavrik, S. Khodyreva, M. Safro,* Structure **5** (1997) 59.

SAŽETAK

Kontrola kvalitete pri biosintezi aminoacil-tRNA

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Vjernost translacije bitno ovisi o točnosti dvaju koraka: odabiru aminoacil-tRNA na ribosomu i sintezi ispravnih aminoacil-tRNA pomoću odgovarajućih aminoacil-tRNA-sintetaza u reakciji aminoaciliranja. Najvažniji događaj u aminoaciliranju precizno je prepoznavanje pripadnih supstrata (tRNA i aminokiseline) i diskriminacija nepripadnih. Iako taj posao uglavnom obavlja po jedan enzim za svaki par tRNA : aminokiselina, nedavno smo ustanovili da su za diskriminaciju analoga lizina potrebne dvije različite lizil-tRNA-sintetaze. U nekim drugim slučajevima otkriveno je da su pogreške u odabiru tRNA i njihovih pripadnih aminokiseline i suviše velike, pa je nužan naknadni popravak pogrešnih produkata u reakciji aminoaciliranja, koji također mogu katalizirati neke aminoacil-tRNA-sintetaze. Na primjeru krivog odabira tirozina umjesto fenilalanina, te naknadnog popravka, pokazano je kako je mogućnost korekcije važna u sprečavanju pogrešne translacije genetičkog koda *in vivo*. Najnovija istraživanja pokazala su da su mehanizmi popravka od ključne važnosti u citoplazmi, no neki se ne zbivaju u mitohondriju, ukazujući na smanjenu ukupnu točnost biosinteze proteina u ovom staničnom odjeljku.

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