Aminoacyl-tRNA Synthesis in Methanogenic Archaea

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Summary

Aminoacyl-tRNA synthetases (AARSs) are essential for faithful translation of the genetic code and have long been studied intensively. Major discoveries explained basic principles of how amino acids are paired to their cognate tRNAs to ensure high fidelity of translation. However, advances in genomics instigated identification of novel enzymes and pathways to aminoacyl-tRNA synthesis. In that respect methanogenic Archaea are particularly prominent, most of which possess non-canonical routes to synthesis of Asn-tRNA, Cys-tRNA, Gln-tRNA and Lys-tRNA. Additionally, some methanogenic seryl-tRNA synthetases are only marginally related to their homologues outside the archaeal kingdom, while other AARSs exhibit multiplicity of their genes (LysRS, SerRS, PheRS). Therefore, methanogens represent an exciting group of organisms regarding aminoacyl-tRNA synthesis, attesting to high degree of evolutionary diversity.

Key words: aminoacyl-tRNA, methanogenic Archaea, evolution, tRNA, translation

Recent studies arising from the analysis of completed genomes have shown a significant degree of evolutionary diversity in aminoacyl-tRNA synthesis. This was first indicated by the analysis of the genome sequence of *Methanocaldococcus jannaschii* (*1*), and further supported by the advances in the number of sequenced genomes of methanogenic Archaea. Accordingly, methanogens have been shown to be a particularly interesting group of organisms with respect to aminoacyl-tRNA synthesis. Here we summarize our present knowledge of this subject.

Aminoacyl-tRNA Synthetases

Successful translation of genetic information contained within mRNA sequence relies on pairing of codons in mRNA with tRNA anticodons. In order to perform this action, a ribosome requires a full complement of tRNAs, correctly charged with appropriate amino acids. The formation of the ester bond between an amino acid and a 3' end of tRNA is catalyzed by a family of enzymes, collectively known as the aminoacyl-tRNA synthetases (AARSs).

Despite their conserved mechanism of catalysis, AARSs are divided into two unrelated classes (I and II), each of which consists of 10 enzymes. This partition is generally conserved throughout the living kingdom (Class rule; *2*). Structural studies have shown that the active sites of class I enzymes share sequence motifs HIGH and KMSKS, and have topology based on a parallel β-sheet nucleotide-binding fold (Rossmann fold). In contrast, class II enzymes contain characteristic signa-

ture motifs (motifs 1,2 and 3), with their active sites built on an antiparallel β-sheet surrounded by α-helices (*3,4*). Such distinction in the active site architecture is reflected as a difference in the mode of binding of substrates: ATP is bound in either extended (class I), or bent (class II) conformation. Furthermore, two classes of enzymes approach the acceptor stem of tRNA from different sides.

Since the accuracy of protein synthesis is essentially dependent on the process of aminoacyl-tRNA synthesis, AARSs are highly specific with respect to both tRNA and amino acid. A logical assumption, proposed by Crick in 1958 (*5*), that every organism should contain 20 AARSs, was based on the firm belief that each amino acid is charged to its cognate tRNA by the specific AARS. This notion appeared to be confirmed by identification of 20 AARSs in *Escherichia coli* (*6*), only to be confounded by completion of genomic sequences of *Methanocaldococcus jannaschii* (*1*) and *Methanothermobacter thermautotrophicus* (*7*), which lacked open reading frames (ORFs) for particular AARSs. This observation immediately raised questions as to how these organisms synthesize a full complement of aminoacyl-tRNAs; consequently, novel enzymes and pathways have been discovered and analyzed, the result of which is our finer understanding of diversity of aminoacyl-tRNA synthesis.

Methanogenic Archaea

Methanogenic Archaea are strictly anaerobic organisms responsible for the final step of anaerobic degradation of organic matter. They derive their metabolic energy from the conversion of the restricted number of substrates, such as $H_2 + CO_2$, formate, acetate, methanol, and methylamines to methane (*8*). These strict anaerobes are present in most anaerobic environments on earth, including deep-sea hydrothermal vents, rice paddies, lake or marine sediments, marshes and the gastrointestinal tracts of animals including humans (*9–11*), and can be found at temperatures ranging from 20 to 98 °C (*12,13*).

Methanogens likely represent a monophyletic group (*14*) that belongs to the archaeal domain of Euryarchaeota (*15*). Phylogenetically they can be divided into five orders: *Methanobacteriales, Methanococcales, Methanosarcinales, Methanopyrales* and *Methanomicrobiales* (*13*). In some aspects of their molecular biology, such as their transcription apparatus, methanogens are closer to Eukarya than to Bacteria, whereas other features of methanogens (and of Archaea in general) are more similar to Bacteria. Due to their methanogenic metabolism, they possess enzymatic pathways, as well as coenzymes and cofactors not found in other organisms (*16*). Moreover, the unusual genomics of their AARSs greatly challenged Crick's Adaptor hypothesis (*5*), and still receives a great amount of attention, as research on methanogens rapidly progresses.

Aminoacyl-tRNA Synthetases in Methanogenic Archaea

With completion of the genomic sequence of *M. jannaschii* (*1*), four of the AARS homologues were identi-

fied as 'missing': asparaginyl-tRNA synthetase (AsnRS), cysteinyl-tRNA synthetase (CysRS), glutaminyl-tRNA synthetase (GlnRS) and lysyl-tRNA synthetase (LysRS). Since 20 canonical amino acids are all utilized in archaeal protein synthesis, it is a sensible presumption that pathways for the formation of corresponding aminoacyl-tRNAs undeniably exist. Ultimately, through studies which helped unravel the mystery of 'missing' synthetases, it became evident that routes of aminoacyl-tRNA synthesis are more versatile than it was once thought.

tRNA-dependent transamidation

Already in the late sixties Wilcox and Nirenberg postulated the existence of indirect way of aminoacyl-tRNA synthesis. They showed that Gln-tRNA^{Gln} formation in some species of *Bacilli* occurs in a two-step process: first, mischarged Glu-tRNA^{Gln} is formed, which is subsequently amidated into correct aminoacyl-tRNA, Gln-tRNA^{Gln} (*17,18*). Other reports have demonstrated the indirect pathway to be a standard route to Gln-tRNA^{Gln} formation in many organisms and organelles (*19*). Furthermore, some microorganisms have been shown to employ similar strategies for Asn-tRNA^{Asn} synthesis (*20*). Finally, identification of enzyme constituents involved in indirect aminoacylation pathways clarified why the formation of Gln-tRNA^{Gln} and Asn-tRNA^{Asn} in many organisms readily occurs in the absence of their respective AARSs (GlnRS and AsnRS; *21,22*).

Since indirect routes to both Gln-tRNA^{Gln} and Asn-tRNA^{Asn} syntheses rely on the presence of mischarged intermediates (Fig. 1), the existence of AARSs of relaxed specificity (non-discriminating GluRS and AspRS) is a prerequisite to their formation. A non-discriminating GluRS catalyzes the synthesis of both Glu-tRNA^{Glu} and Glu-tRNA^{Gln}; likewise, a non-discriminating AspRS is responsible for both Asp-tRNA^{Asp} and Asp-tRNA^{Asn} formation. Exactly how these mischarged species of tRNA evade the danger of being incorporated into the growing polypeptide chain during translation process is still
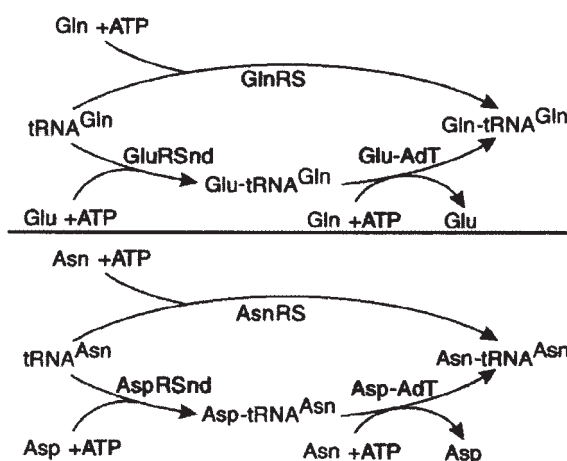

Fig. 1. Pathways for the synthesis of Asn-tRNA^{Asn} and Gln-tRNA^{Gln}. Direct (top of each diagram) and indirect (transamidation) pathways (bottom of each diagram) are shown. The suffix nd indicates non-discriminating aminoacyl-tRNA synthetases.

obscure, but indications exist that they are rejected by translation elongation factors (*23*).

Conversion of Glu-tRNA^{Gln} and Asp-tRNA^{Asn} into correctly aminoacylated tRNAs is catalyzed by a tRNA--dependent amidotransferase (AdT) in an ATP-dependent reaction, in the presence of the amide donor (tRNA-dependent transamidation, *21*). Two distinct AdT enzymes have been identified in Archaea: a heterotrimeric GatCAB amidotransferase, capable of generating both Gln-tRNA^{Gln} and Asn-tRNA^{Asn}, and a heterodimeric GatDE, exclusive to archaeal domain, with only Glu-tRNA^{Gln} amidotransferase activity. The distribution of AdTs in Archaea suggests differences in their function: quite possibly, the role of GatCAB is Asn-tRNA^{Asn} formation, as its presence has been detected only in Archaea lacking AsnRS. Similarly, ubiquitous distribution of GatDE in archaeal domain is in accordance with the absence of GlnRS enzyme. As regards methanogens, they are deficient in recognizable ORFs for GlnRS and AsnRS; consequently, they all possess GatCAB and GatDE homologues.

Synthesis of cysteinyl-tRNA

Among all the absences of recognizable AARS orthologs from the genomes, the most difficult to explain was the lack of genes encoding CysRS (*cysS* genes). Analysis of the completed genomes from three methanogenic Archaea, *M. jannaschii* (*1*), *M. thermoautotrophicus* (*7*), and most recently, *Methanopyrus kandleri* (*14*), showed that there was no recognizable gene for CysRS in these organisms. In contrast, the sequenced genomes of more than 90 other organisms from all three kingdoms possess easily recognizable CysRS. Furthermore, CysRS seems to be one of the most conserved AARSs, with the singular and distinct domain architecture (*24*).

There is a notable correlation between the absence of CysRS and the presence of an unusual, highly diverged SerRS in methanogenic Archaea (see below), which led to the hypothesis that this enzyme could be involved in tRNA-dependent thiolation of Ser-tRNA^{Cys}, resembling the synthesis of selenocysteinyl-tRNA^{Sec} (*25*). However, attempts to serylate tRNA^{Cys} using purified *M. thermoautotrophicus* SerRS were unsuccessful (*26*). Instead, direct enzymatic charging of cysteine onto tRNA was detected in aminoacylation assays in *M. jannaschii* S100 extracts (*27*). Standard biochemical purification of this cysteinylation activity led to identification of ProRS, capable of charging cysteine onto tRNA, in addition to its cognate activity (*28,29*). Although the structural basis for binding of tRNA^{Cys} is still unclear, mutagenesis of the active site residues suggests that the binding sites for cysteine and proline greatly overlap (*30*).

More recently, an unconventional synthetase (ORF MJ1477) has also been proposed to form Cys-tRNA in *M. jannaschii* (*31*). Homologues of this protein were found only in a limited number of organisms (*Thermotoga maritima* and *Deinococcus radiodurans*) and thus cannot be responsible for Cys-tRNA formation in other methanogens lacking *cysS*, such as *M. thermoautotrophicus*, as well as *Methanococcus maripaludis*, which was shown to be viable after disruption of its canonical *cysS* gene (*32*).

Class I lysyl-tRNA synthetase

Initial analyses of the first completed archaeal genome sequences failed to identify ORFs encoding LysRS; however, a direct charging of lysine onto tRNA was observed in cell-free extracts, which allowed for purification of a single protein from *M. maripaludis* (*33*). Following N-terminal sequencing, the gene encoding *M. maripaludis* LysRS was cloned and demonstrated to possess canonical LysRS activity. The predicted amino acid sequence of this LysRS was unrelated to canonical LysRS proteins (class II), but showed high similarity to open reading frames of unassigned function in both *M. thermautotrophicus* and *M. jannaschii*. Moreover, the presence of amino acid motifs characteristic of the Rossmann dinucleotide binding domain identified *M. maripaludis* LysRS as a class I aminoacyl-tRNA synthetase, in contrast to the known class II examples of this enzyme. Despite their lack of sequence similarity, both class I and class II LysRSs are able to recognize the same amino acid and highly similar tRNA substrates (*34*).

Although class I LysRS was first identified in Archaea, it is not confined to this domain and is present in some bacteria (*Streptomyces coelicolor*, Spirochetes and some α-proteobacteria; *35,36*). It has been identified in all of the currently available genome sequences of methanogenic Archaea, among which three (*Methanosarcina mazei*, *Methanosarcina acetivorans* and *Methanosarcina barkeri*) contain both class I and class II homologues (see below).

SerRS in methanogenic Archaea

With the exception of *M. mazei* and *M. acetivorans*, all of the presently sequenced genomes of methanogenic Archaea exclusively possess an ORF for a highly diverged SerRS. As a class II enzyme, it is characterized by the presence of three sequence motifs (*26*); however, sequence alignments of such methanogenic SerRSs with SerRSs present in Bacteria, Eukarya and even other Archaea show conspicuously low similarity. Most notably, a gap existing in the motif II of these unusual SerRSs is absent from SerRS proteins of other organisms (Fig. 2). Furthermore, the alignment reveals insertions present elsewhere in the sequence of methanogenic SerRSs (not shown). Despite initial doubts regarding SerRS identity of these proteins, they have been demonstrated to possess canonical SerRS activity (*26*).

Multiplicity of AARSs in Methanosarcina sp.

In addition to absences of specific AARSs and examples of their atypical architecture, some methanogens display peculiarities through multiplicity of different AARS genes that they possess. *M. barkeri* is remarkable in that respect, as it contains two genes encoding two SerRSs: one is of a standard bacterial type, while the other highly resembles other methanogenic SerRSs. Both enzymes were shown to have the ability to efficiently serylate *M. barkeri* tRNA (unpublished results).

Interestingly, recent reports show coexistence of three LysRSs in *M. acetivorans*, *M. barkeri* and *M. mazei*. Apart from class I and class II enzymes, both of which have been cloned, purified and shown to possess canonical LysRS activity (unpublished data), additional LysRS

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Dme    GFKLISVPDI    YRAETSGL-QEEKGIYRVHQFNKVE    GTATAIPRLLIAL ⎤
Sce    GYIPLQAPVM    FRREAGSHGKDAWGVFRVHAFEKIE    STLAATQRALCCI ⎥ Eukarya
Hsa    GYIPIYTPFF    FRQEVGSHGRDTRGIFRVHQFEKIE    ATMCATTRTICAI ⎦


Eco    GYSENYVPYL    FRSEAGSYGRDTRGLIRMHQFDKVE    GSGLAVGRTLVAV ⎤
Bsu    NYTEVIPPYM    FRSEAGSAGRDTRGLIRQHQFNKVE    GSGLAVGRTVAAI ⎥ Bacteria
Taq    GFLPMTPLSY    FRSEAGSFGKDVRGLMRVHQFHKVE    NTALATPRILAML ⎦


Pho    GFTPVIPPYM    FRKEAGTAGKDTKGIFRVHQFHKVE    STAIATSRAIVAI ⎤
Mb2    GYLEVFPPVL    FRREAGKHGQDTRGIIRQHQFNKVE    GSGLAVGRTVVAI ⎥ Archaea
Afu    DFTIVSPPYM    FRKEAGAHGKDTKGIFRVHQFNKVE    STAIATTRAITAI ⎦


Mja    GFQECLFPKL    YRWEGGGA----RGLDRVNEFLRVE    CTGYGITRWVVGY ⎤
Mma    GFDECLFPKL    YRWEGGGA----KGLDRVNEFLRGE    CTGYGLSRWLIGF ⎥
Mth    GFVECLFPKL    YRWEAGGS----KGLDRVHEFQRVE    CTGIGLSRWIYGF ⎥ Methanogenic
Mka    GFEPALFPKL    YRWEGGAA----KGLERVNEFQRIE    CAGLGVTRWVVGL ⎥ Archaea
Mb1    GYREMIFPKL    HRYESGGI----HGIERVDEFHRIE    CSGVGLERWAAVF ⎦
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Fig. 2. Alignment of motifs 1, 2 and 3 from a number of representative SerRSs. The sequences (accession numbers) are from *Saccharomyces cerevisiae* (X04884) (Sce), *Drosophila melanogaster* (Y14823) (Dme), *Homo sapiens* (X91257) (Hsa), *Escherichia coli* (X04017) (Eco), *Bacillus subtilis* (D26185) (Bsu), *Thermus aquaticus* (sp:P34945) (Taq), *Archaeoglobus fulgidus* (AE000962) (Afu), *Pyrococcus horikoshii* (AB009490) (Pho), *Methanocaldococcus jannaschii* (U67550) (Mja), *Methanothermobacter thermautotrophicus* (AF009823) (Mth), *Methanococcus maripaludis* (AF009822) (Mma), *Methanopyrus kandleri* (AE010438) (Mka), *Methanosarcina barkeri* – methanogenic SerRS (Mb1) and *Methanosarcina barkeri* – bacterial-type SerRS (from http://www.jgi.doe.gov/JGI_microbial/html/methanosarcina/methano_homepage.html) (Mb2). They were aligned with the Clustal program (*55*), and the motif regions are presented.

gene (*pylS*) is present in these organisms (*37*). Its sequence shows some degree of similarity to specific class II AARSs (PheRS and SerRS), but does not group with sequences of known classII LysRSs. The enzyme encoded by *pylS* is proposed to be responsible for the translational regulation of the genes involved in methanogenesis (*37*). In particular, it was reported to charge the specific amber supressor tRNA with lysine, thus possibly enabling translation of in-frame stop codons of certain genes (*38,39*) as either lysine or its modified derivative pyrrolysine (*40*). While detailed analysis is not yet available, indications exist that pyrrolysine represents the 22nd genetically encoded amino acid to be identified in nature.

Lastly, all methanogenic genomes sequenced as yet indicate the presence of two genes encoding α-subunits of phenylalanyl-tRNA synthetase (PheRS). PheRS is a class II synthetase with heterotetrameric $\alpha_2\beta_2$ structure in most organisms, although monomeric α-forms are also found (*41*). It was demonstrated in *M. thermautotrophicus* that only one of the α-subunits constitutes $\alpha_2\beta_2$ tetramer (*42*), but the function of the additional gene encoding α-subunit homologue remains unknown.

Evolutionary Aspects of Aminoacyl-tRNA Synthetases

The central role of AARSs in translation suggests an early origin of the process of aminoacyl-tRNA synthesis during the evolution of contemporary gene expression. Consequently, the evolution of AARSs has been largely speculated and while definite answers remain elusive, some theories seem to find their support, as will be discussed.

The existence of the two classes of AARSs was widely assumed to suggest that the tRNA-charging function evolved at least twice (*43*), as two classes of the enzymes are quite unrelated. Furthermore, the same

amino acid specificity could have evolved in any of the two backgrounds of the AARS classes, support of which can be found in the existence of class I and class II LysRS. Despite their lack of sequence similarity, both classes of LysRS are able to recognize the same amino acid and a highly similar tRNA substrate, hence providing an example of functional convergence by different enzymes (*33*) and supporting the hypothesis that tRNA^Lys itself predates at least one of the two extant forms of LysRS (*34,44*).

Alternatively, two classes of the primordial AARSs could have coexisted in a putative RNA world (*45–47*), where aminoacylation reaction was catalyzed by ribozymes of a limited amino acid specificity. The pairs of primordial AARSs (class I and class II) could have acted as molecular chaperones to cover acceptor stem of tRNA and preserve aminoacylation in an environment (such as high temperature) where the structure of tRNA was especially vulnerable (*48*). At that point, emerging class I and class II synthetases might have been under strong selective pressure to develop amino acid or ATP affinities to increase the efficiency of the aminoacylation reaction. Rationale for this hypothesis comes from the docking experiments, where LysRS class I and LysRS class II could simultaneously be docked onto the tRNA^Lys molecule without any steric clashes (*49*). In this context, several other combinations of class I and II AARSs could also be docked to a single tRNA molecule, possibly relating to the origin of tRNA synthetases (*48,50*).

Phylogenetic and structural considerations suggest that GlnRS and AsnRS were the last AARSs to emerge, resulting from duplication and diversification of ancestral GluRS and AspRS, respectively (*51,52*). Whether the comparatively recent emergence of GlnRS and AsnRS also reflects a late recruitment of glutamine and asparagine to coded protein synthesis remains an open question, as it seems likely that Gln-tRNA^Gln and Asn-tRNA^Asn were originally synthesized by tRNA-dependent transamidation. Nevertheless, unlike many other Archaea that

acquired gene for AsnRS, methanogenic Archaea use exclusively tRNA-dependent transamidation to produce both Gln-tRNA^{Gln} and Asn-tRNA^{Asn}, and quite possibly these organisms never had genes for GlnRS and AsnRS.

Evolutionary scenarios for CysRS and SerRS remain uncertain. Regardless of the exact model of the relationships between Archaea, Bacteria and Eukaryota, phylogenetic analysis provides evidence that evolution of AARSs involved a variety of horizontal gene transfers. These events were relatively limited between Archaea, especially methanogens, and Bacteria. The most straightforward explanation, which allows direct experimental verification, is that archaeal AARSs are generally poorly compatible with bacterial tRNAs (24). Horizontal gene transfer from Bacteria to methanogens seems to be a possibility only for CysRS, SerRS and LysRS II. It is plausible that *M. maripludis* and *Methanosarcina* species independently acquired the bacterial *cysS* gene, unlike their hyperthermophilic relatives, which lack CysRS (51). In the case of SerRS, highly diverged methanogenic SerRS is replaced by bacterial-type SerRS in *M. mazei* and *M. acetivorans*, possibly aquired from Gram-positive bacteria. In *M. barkeri*, which might be an intermediate case, both types of SerRS coexist. Class II LysRS, present in addition to class I enzyme in all three sequenced *Methanosarcina* species, might have come from the same Gram-positive bacterial group as SerRS. In general, species from genus *Methanosarcina* are the most metabolically diverse methanogens, with a number of duplications of genes (not only encoding AARSs) and methabolic pathways (53,54). This diversity is reflected in the size of the genomes of *Methanosarcinae*, which are notably bigger than the genomes of other methanogens.

Horizontal gene transfer seems to have been the major force in the evolution of AARSs (24). The fact that methanogenic Archaea were less exposed to it is probably reflected in their ancient-looking genomics of AARSs. Still, having a full set of 20 modern AARSs seems to be advantageous for the organisms in the world that we know today, and methanogens might have been just slower on this path than the other groups. It seems that especially hyperthermophilic, non-symbiotic Archaea, which are in general more isolated in their environmental niches, use a particularly slow pace. Further genome sequencing of nonthermophilic and particularly symbiotic Archaea should be revealing in this terms.

References

1. C. J. Bult, O. White, G. J. Olsen, L. Zhou, R. D. Fleischmann, G. G. Sutton, J. A. Blake, L. M. FitzGerald, R. A. Clayton, J. D. Gocayne, A. R. Kerlavage, B. A. Dougherty, J. F. Tomb, M. D. Adams, C. I. Reich, R. Overbeek, E. F. Kirkness, K. G. Weinstock, J. M. Merrick, A. Glodek, J. L. Scott, N. S. M. Geoghagen, J. C. Venter, *Science, 273* (1996) 1058–1073.

2. G. Eriani, M. Delarue, O. Poch, J. Gangloff, D. Moras, *Nature, 347* (1990) 203–206.

3. J. G. Arnez, D. Moras, *Trends Biochem. Sci. 22* (1997) 211–216.

4. S. Cusack, *Curr. Opin. Struct. Biol. 7* (1997) 881–889.

5. F. H. C. Crick, *Symp. Soc. Exp. Biol. 12* (1958) 138–163.

6. G. Eriani, G. Dirheimer, J. Gangloff, *Nucleic Acids Res. 19* (1991) 265–269.

7. D. R. Smith, L. A. Doucette-Stamm, C. Deloughery, H. Lee, J. Dubois, T. Aldredge, R. Bashirzadeh, D. Blakely, R. Cook, K. Gilbert, D. Harrison, L. Hoang, P. Keagle, W. Lumm, B. Pothier, D. Qiu, R. Spadafora, R. Vicaire, Y. Wang, J. Wierzbowski, R. Gibson, N. Jiwani, A. Caruso, D. Bush, J. N. Reeve, *J. Bacteriol. 179* (1997) 7135–7155.

8. J. G. Ferry, *Science, 278* (1997) 1413–1414

9. W. J. Jones, D. P. Nagle Jr., W. B. Whitman, *Microbiol. Rev. 51* (1987) 135–177.

10. W. E. Balch, G. E. Fox, L. J. Magrum, C. R. Woese, R. S. Wolfe, *Microbiol. Rev. 43* (1979) 260–296.

11. S. Zinder: Physiological ecology of methanogens. In: *Methanogenesis*, J. G. Ferry (Ed.), Chapman and Hall, New York (1993) pp. 128–206.

12. K. R. Sowers: Methanogenic Archaea: An overview. In: *Archaea: A Laboratory Manual*, F. T. Robb, A. R. Place, K. R. Sowers, H. J. Schreier, S. DasSarma, E. M. Fleischmann (Eds.), Cold Spring Harbor Laboratory Press, Plainview (1995) pp. 3–13.

13. D. Boone, W. Whitman, P. Rouviere: Diversity and taxonomy of methanogens. In: *Methanogenesis*, J. G. Ferry (Ed.), Chapman and Hall, New York (1993) pp. 35–80.

14. A. I. Slesarev, K. V. Mezhevaya, K. S. Makarova, N. N. Polushin, O. V. Shcherbinina, V. V. Shakhova, G. I. Belova, L. Aravind, D. A. Natale, I. B. Rogozin, R. L. Tatusov, Y. I. Wolf, K. O. Stetter, A. G. Malykh, E. V. Koonin, S. A. Kozyavkin, *Proc. Natl. Acad. Sci. USA, 99* (2002) 4644–4649.

15. C. R. Woese, O. Kandler, M. L. Wheelis, *Proc. Natl. Acad. Sci. USA, 87* (1990) 4576–4579.

16. L. Daniels: Biochemistry of methanogens. In: *The Biochemistry of Archaea (Archaebacteria)*, M. Kates, D. J. Kushner, A. T. Matheson (Eds.), Elsevier, Amsterdam (1993) pp. 41–112.

17. M. Wilcox, M. Nirenberg, *Proc. Natl. Acad. Sci. USA, 61* (1968) 229–236.

18. M. Wilcox, *Cold Spring Harbor Symp. Quant. Biol. 34* (1969) 521–528.

19. A. Schön, C. G. Kannagara, S. Gough, D. Söll *Nature, 331* (1988) 187–190.

20. A. W. Curnow, M. Ibba, D. Söll, *Nature, 382* (1996) 589–590.

21. A. W. Curnow, K. W. Hong, R. Yuan, S. I. Kim, O. Martins, W. Winkler, T. M. Henkin, D. Söll, *Proc. Natl. Acad. Sci. USA, 94* (1997) 11819–11826.

22. D. L. Tumbula, H. D. Becker, W. Z. Chang, D. Söll, *Nature, 407* (2000) 106–110.

23. F. J. LaRiviere, A. D. Wolfson, O. C. Uhlenbeck, *Science, 94* (2001) 165–168.

24. Y. I. Wolf, L. Aravind, N. V. Grishin, E. V. Koonin, *Genome Res. 9* (1999) 689–710.

25. S. Commans, A. Böck, *FEMS Microbiol. Rev. 23* (1999) 335–351.

26. H. S. Kim, U. C. Vothknecht, R. Hedderich, I. Celic, D. Söll, *J. Bacteriol. 180* (1998) 6446–6449.

27. C. S. Hamann, K. R. Sowers, R. S. Lipmann, Y. M. Hou, *J. Bacteriol. 181* (1999) 5880–5884.

28. C. Stathopoulos, T. Li, R. Longman, U. C. Vothknecht, H. D. Becker, M. Ibba, D. Söll, *Science, 287* (2000) 479–482.

29. R. S. Lipman, K. R. Sowers, Y. M. Hou, *Biochemistry, 39* (2000) 7792–7798.

30. C. Stathopoulos, C. Jacquin-Becker, H. D. Becker, T. Li, A. Ambrogelly, R. Longman, D. Söll, *Biochemistry, 40* (2001) 46–52.

31. C. Fabrega, M. A. Farrow, B. Mukhopadhyay, V. de Crecy-Lagard, A. R. Ortiz, P. Schimmel, *Nature, 411* (2001) 110–114.

32. C. Stathopoulos, W. Kim, T. Li, I. Anderson, B. Deutsch, S. Palioura, W. Whitman, D. Söll, *Proc. Natl. Acad. Sci. USA, 98* (2001) 14292–14297.

33. M. Ibba, S. Morgan, A. W. Curnow, D. R. Pridmore, U. C. Votknecht, W. Gardner, W. Lin, C. R. Woese, D. Söll, *Science, 278* (1997) 1119–1122.

34. M. Ibba, H. C. Losey, Y. Kawarabayasi, H. Kikuchi, S. Bunjun, D. Söll, *Proc. Natl. Acad. Sci. USA, 96* (1999) 418–423.

35. M. Ibba, J. L. Bono, P. A. Rosa, D. Söll, *Proc. Natl. Acad. Sci. USA, 94* (1997) 14383–14388.

36. D. Söll, H. D. Becker, P. Plateau, S. Blanquet, M. Ibba, *Proc. Natl. Acad. Sci. USA, 97* (2000) 14224–14228.

37. G. Srinivasan, C. M. James, J. A. Krzycki, *Science, 296* (2002) 1459–1462.

38. L. Paul, D. J. Ferguson, J. A. Krzycki, *J. Bacteriol. 182* (2000) 2520–2529.

39. C. M. James, T. K. Ferguson, J. F. Leykam, J. A. Krzycki, *J. Biol. Chem. 276* (2001) 34252–34258.

40. B. Hao, W. Gong, T. K. Ferguson, C. M. James, J. A. Krzycki, M. K. Chan, *Science, 296* (2002) 1462–1466.

41. A. Sanni, C. Hountondji, S. Blanquet, J. P. Ebel, Y. Boulanger, F. Fasiolo, *Biochemistry, 30* (1991) 2448–2453.

42. R. Das, U. C. Vothknecht, *Biochimie, 81* (1999) 1037–1039.

43. G. M. Nagel, R. F. Doolittle, *J. Mol. Evol. 40* (1995) 487–498.

44. L. Ribas de Pouplana, R. J. Turner, B. A. Steer, P. Schimmel, *Proc. Natl. Acad. Sci. USA, 95* (1998) 11295–11300.

45. G. F. Joyce, L. E. Orgel: Prospects for understanding the origin of the RNA world. In: *The RNA World*, R. F. Gesteland, J. F. Atkins (Eds.), Cold Spring Harbor Laboratory Press, Plainview (1993) pp. 1–26.

46. M. Illangasekare, G. Sanchez, T. Nickles, M. Yarus, *Science, 267* (1995) 643–647.

47. N. Maizels, A. M. Weiner, *Proc. Natl. Acad. Sci. USA, 91* (1994) 6729–6734.

48. L. Ribas de Pouplana, P. Schimmel, *Cell, 104* (2001) 191–193.

49. T. Terada, O. Nureki, R. Ishitani, A. Ambrogelly, M. Ibba, D. Söll, S. Yokoyama, *Nat. Struct. Biol. 9* (2002) 257–262.

50. L. Ribas de Pouplana, P. Schimmel, *Trends Biochem. Sci. 26* (2001) 591–596.

51. C. R. Woese, G. J. Olsen, M. Ibba, D. Söll, *Microbiol. Mol. Biol. Rev. 64* (2000) 202–236.

52. V. Lamour, S. Quevillon, S. Diriong, V. C. N'Guyen, M. Lipinski, M. Mirande, *Proc. Natl. Acad. Sci. USA, 91* (1994) 8670–8674.

53. J. E. Galagan, C. Nusbaum, A. Roy, M. G. Endrizzi, P. Macdonald, W. FitzHugh, S. Calvo, R. Engels, S. Smirnov, D. Atnoor, A. Brown, N. Allen, J. Naylor, N. Stange-Thomann, K. DeArellano, R. Johnson, L. Linton, P. McEwan, K. McKernan, J. Talamas, A. Tirrell, W. Ye, A. Zimmer, R. D. Barber, I. Cann, D. E. Graham, D. A. Grahame, A. M. Guss, R. Hedderich, C. Ingram-Smith, H. C. Kuettner, J. A. Krzycki, J. A. Leigh, W. Li, J. Liu, B. Mukhopadhyay, J. N. Reeve, K. Smith, T. A. Springer, L. A. Umayam, O. White, R. H. White, E. Conway de Macario, J. G. Ferry, K. F. Jarrell, H. Jing, A. J. Macario, I. Paulsen, M. Pritchett, K. R. Sowers, R. V. Swanson, S. H. Zinder, E. Lander, W. W. Metcalf, B. Birren, *Genome Res. 12* (2002) 532–542.

54. M. Graupner, R. H. White, *J. Bacteriol. 183* (2001) 5203–5205.

55. J. D. Thompson, T. J. Gibson, F. Plewniak, F. Jeanmougin, D. G. Higgins, *Nucleic Acids Res. 25* (1997) 4876–4882.

Sinteza aminoacil-tRNA u metanogenih arhebakterija

Sažetak

Aminoacil-tRNA-sintetaze su enzimi prijeko potrebni za vjernu translaciju genetskoga koda. Već su godinama cilj intenzivnih znanstvenih istraživanja, zahvaljujući kojima su razjašnjena osnovna načela sparivanja aminokiseline i pripadne molekule tRNA. Međutim, napredak u sekvenciranju novih genoma potaknuo je identifikaciju dotad nepoznatih enzima i puteva sinteze aminoacil-tRNA. Po tome su metanogene arhebakterije skupina osobitih svojstava jer nerijetko sadržavaju neuobičajene puteve sinteze asparaginil-tRNA, cisteinil-tRNA, glutaminil-tRNA te lizil-tRNA. Nadalje, pojedine metanogene seril-tRNA-sintetaze pokazuju vrlo malu sličnost s vlastitim homolozima izvan arhejskog carstva, dok druge metanogene aminoacil-tRNA-sintetaze (lizil-tRNA-sintetaza, seril-tRNA-sintetaza i fenilalanil-tRNA-sintetaza) pokazuju višestrukost gena kojima su kodirane. Metanogene arhebakterije su zbog toga izrazito zanimljiva skupina organizama što se tiče sinteze aminoacil-tRNA te svjedoče o značajnoj evolucijskoj raznolikosti spomenutih biosintetskih puteva.