Chemistry of aminoacylation and peptide bond formation on the 3′terminus of tRNA

ARTICLE *in* JOURNAL OF BIOSCIENCES · NOVEMBER 2006

Impact Factor: 2.06 · DOI: 10.1007/BF02705188 · Source: PubMed

1 AUTHOR:

Mathias Sprinzl
University of Bayreuth
274 PUBLICATIONS **9,826** CITATIONS

Chemistry of aminoacylation and peptide bond formation on the 3′terminus of tRNA

MATHIAS SPRINZL
Laboratorium für Biochemie, Universität Bayreuth, D 95440 Bayreuth, Germany
(Fax, 49-921-552432; Email, Mathias.Sprinzl@uni-bayreuth.de)

1. Introduction

There are 64 possible triplet codons which are translated into a polypeptide composed of defined sequence of twenty amino acids linked via peptide bonds. The polymerization of amino acids to form a polypeptide takes place in a sequential manner, defined by the sequence of triplet codons on the messenger RNA (mRNA). In order to react with an amino group of the incoming amino acid, the carboxyl group of the C-terminal amino acid of the growing polypeptide has to be activated by a sufficiently reactive leaving group. The role of the translational adaptor, which provides the hydroxyl group to activate the amino acid as an aminoacyl ester and at the same time recognizes the position of the mRNA that gives the signal for the incorporation of particular amino acid is fulfilled by the transfer RNA (tRNA). Each of about 40 different tRNAs present in the cell cytoplasm has two specific functions: (i) It recognizes with its anticodon triplet, a particular three-letter code of the mRNA. The translating machine is the ribosome, which together with the auxiliary translation factors takes care of the precise interaction of codon and anticodon. This complicated process is adjusted to physiological needs of the cell and proceeds with optimal fidelity. (ii) It accepts the incoming amino acid on its chemical "business site", which is always the 3′-terminal adenosine and carries it to the A-site of peptidyl transferase, a ribosomal centre where the new peptide bond is formed. The aminoacylation of the 3′-terminal adenosine occurs in the cytoplasm and requires the energy provided by ATP. The ribosome catalyses the peptide transfer from the donor peptidyl-tRNA, to the acceptor aminoacyl-tRNA.

This article reviews the history of the discovery of the chemical steps involved in biosynthesis of a peptide bond, tRNA aminoacylation catalyzed by the aminoacyl-tRNA synthetase and peptide bond formation catalyzed by the ribosome.

2. Aminoacylation of tRNA

Crick's adaptor hypothesis (Crick 1958) and the pioneering experimental work in Paul Zamecnik's laboratory at the Massachusetts General Hospital in Boston on *in vitro* protein synthesis are important milestones in our way to understand the role of tRNA in protein biosynthesis. The crucial discovery of Zamecnik and his colleagues was the notion that a radioactive amino acid, when incubated with the "pH 5 enzyme fraction", ATP and a ribonucleoprotein fraction, gets covalently attached to a small, "soluble RNA" (figure 1) (Hoagland *et al* 1957). The soluble RNA was later, in accordance with adaptor hypothesis, renamed to transfer RNA (tRNA). The "pH 5 enzyme" (E in figure 1) is the cell extract fraction that contains the aminoacyl-tRNA synthetases. Later, H Zachau and G Acs, working in Lipmann′s laboratory at the Rockefeller Institute in New York, conclusively demonstrated that the attachment of the ^{14}C-leucine to tRNA is achieved via an ester bond to the ribose residue of the 3′-terminal adenosine (Zachau *et al* 1958) . However, this adenosine has two vicinal hydroxyls available for esterification. Due to rapid transacylation the aminoacylation on either one of these hydroxyl groups results in a mixture of the 2′-, and 3′- isomers (figure 2). Somewhat higher stability of 3′-aminoacyl-adenosine and 3′-aminoacyl-tRNA over the corresponding 2′-isomers were later demonstrated by nuclear magnetic resonance spectroscopy (Sonnenbichler *et al* 1963; Taiji *et al* 1983).

3. The site of enzymatic aminoacylation of tRNA

The site of enzymatic aminoacylation of tRNA with respect to the 2′- or 3′- hydroxyl group of the 3′-terminal adenosine remained a puzzle for more then 10 years (Zamecnik1962). The thermodynamically higher stability of the 3′aminoacyl-, over the 2′-aminoacyl isomer was used as an argument for the 3′OH being the aminoacylation site. The structure and the function of antibiotic puromycin, being a minimal 3′-amido analogue of aminoacyl-tRNA also suggested that the 3′-isomer of aminoacyl-tRNA is an acceptor substrate during peptide bond formation in the ribosomal peptidyl transferase reaction (Maden 2003). A slightly higher reactivity of the

$$ATP+[^{14}C]Leucine+E \rightleftharpoons E(AMP\sim[^{14}C]Leucine+PP)$$

$$E(AMP\sim[^{14}C]Leucine)+RNA \rightleftharpoons RNA\sim[^{14}C]Leucine+E+(AMP)$$

Figure 1. P Zamecnik and coworkers at Massachusets General Hospital, Boston, demonstrated the enzymatic attachment of radioactive amino acid to small, soluble RNA.

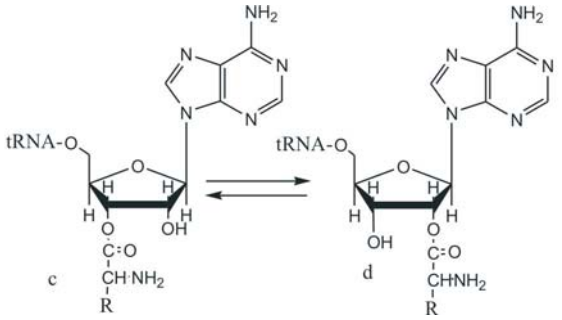
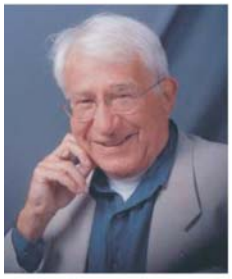


Figure 2. F Lipmann (left) and H Zachau (right), together with G Acs identified a mixture of the 2′-, 3′-isomers of aminoacyl-adenosine formed by enzymatic aminoacylation of tRNA.



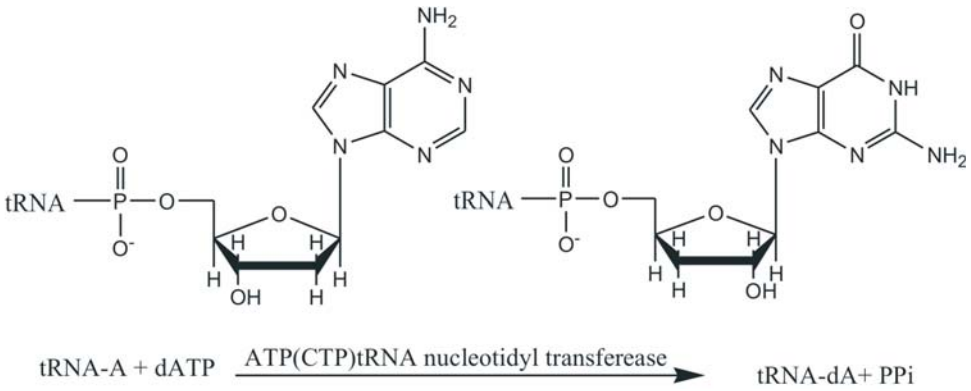


Figure 3. tRNAs with 2′- or 3′- deoxyadenosines at the 3′-end were enzymatically synthesized in F Cramer's laboratory at Max-Planck-Institute in Göttingen.

2′-OH led to an opposite suggestion that the aminoacylation by aminoacyl-tRNA synthetases may utilize this 2′-OH as an acceptor for tRNA aminoacylation (Zamecnik 1962). This problem was finally resolved when nonisomerisable aminoacyl-tRNAs became available. tRNAs with 3′-terminal 2′-deoxy- or 3′-deoxyadenosine were prepared by incorporation of corresponding nucleotides into the 3′-end by ATP(CTP)tRNA nucleotidyl transferase, the CCA adding enzyme (figure 3). Preparation of 'deoxy' tRNAs was first demonstrated in the laboratory of F Cramer in Göttingen (Sprinzl 1973). Enzymatic aminoacylation of $tRNA^{Phe}$-3′dA from yeast by yeast Phe-tRNA synthetase seemed to confirm the former hypothesis of Zamecnik that the more reactive 2′-OH group is the site of aminoacylation site of tRNA (Sprinzl and Cramer 1973). Moreover, the results of this work showed that the participation of the vicinal 3′-hydroxyl is not required for the phenylalanine transfer from phenylalanyl-AMP to $tRNA^{Phe}$. However, it soon became clear that the site of tRNA aminoacylation is not uniform for all aminoacyl-tRNA synthetases e.g. the seryl-tRNA synthetase aminoacylates the tRNA-2′dA (Cramer *et al* 1975). Later, systematic studies on enzymatic aminoacylation of 'deoxy' tRNAs by aminoacyl-tRNA synthetases of different specificity, mostly performed in the research groups of F Cramer (Sprinzl and Cramer 1975) and S Hecht (Chinault *et al* 1977) resulted in following

generalizations (table 1):

- One group of aminoacyl-tRNA synthetases (ARS) utilized only the tRNA-2′dA as substrates and could not aminoacylate the tRNA-3′dA. These ARS were classified as 3′-specific. The other group of ARS aminoacylated only the tRNA-3′dA and not the tRNA-2′dA. These tRNAs were classified as 2′-specific. Few ARS aminoacylated both 'deoxy' tRNA analogues.
- The distribution of 2′- and 3′-specificities of aminoacyl-tRNA synthetases, as determined by aminoacylation of 'deoxy' tRNAs, is retained among prokaryotes and eukaryotes.
- The 2′-, 3′-aminoacylation specificity is not determined by the structure of tRNA but is a feature of ARS, defined by the structure and mechanisms of these enzymes.

A slightly different approach to analyse the mechanistic differences of ARS in respect to the site of aminoacylation was applied by Fraser and Rich (1975). These investigators used a pair of tRNAs modified by 2′-amino-2′-deoxyadenosine and 3′-amino-3′-deoxyadenosine, respectively, on their 3′-ends (figure 4). The enzymatic aminoacylation provided almost identical results as obtained by the 'deoxy' tRNA pairs. However, one has to keep in mind that the use of 'deoxy' and 'amino' analogues of aminoacyl-tRNA to determine the site of aminoacylation may be misleading and can provide results that differ from the situation when native tRNAs are aminoacylated. Careful kinetic studies of the enzymatic reactions using these substrate analogues has to be performed in order to avoid ambiguities reported in the original data summarized in table 1.

The existence of two different classes of ARS discovered by aminoacylataion of 'deoxy' and 'amino' tRNAs was later convincingly confirmed by amino acid sequence comparison and X-ray structure analysis of several ARS (Eriani *et al* 1990). It became an accepted fact and a textbook knowledge, which shed light on the mechanism of aminoacylation and evolution of aminoacyl-tRNA synthetases (Ribas de and Schimmel 2001).

Table 1. Isomers of aminoacyl-tRNA formed by enzymatic aminoacylation of 'deoxy' tRNAs derived from *E. coli*, yeast and calf liver bulk tRNA.

Aminoacyl-tRNA	Attachement of amino acid			Group
	E. coli	Yeast	Calf	
Alanine	3′	3′	3′	II
Arginine	2′	2′	2′	I
Asparagine	2′	2′,3′	2′,3′	II
Aspartic acid	3′	2′,3′	3′	II
Cysteine	2′,3′	2′,3′	3′	I
Glutamine	2′	3′		I
Glutamic acid	2′			I
Glycine	3′	3′	3′	II
Histidine	3′	3	3′	II
Isoleucine	2′	2′	2′	I
Leucine	2′	2′	2′	I
Lysine	3′	3′	3′	II
Methionine	2′	2′		I
Phenylalanine	2′	2′	2′	II
Proline	3′	3′		II
Serine	3′	3′	3′	II
Threonine	3′	3′	3′	II
Tryptophane	2′	2′, 3′	3′	I
Tyrosine	2′, 3′	2′, 3′	2′, 3′	I
Valine	2′	2′	2′	I

The data are compiled from different publications (Sprinzl and Cramer 1975; Chinault *et al* 1977). Final group classification was done using the data of aminoacylation and X-ray structure analysis (Eriani *et al* 1990).

4. Function of the free vicinal hydroxyl group on the 3′-end of tRNA during aminoacylation

In the aminoacyl-tRNAs, one OH-group of the 3′- terminal adenosine is engaged in the ester bond and the other remains free (figure 2). This raises the question about the possible function of this free vicinal hydroxyl in the mechanism of aminoacylation and peptide bond formation. The kinetics of enzymatic aminoacylation of tRNA is not dramatically altered when the nonaccepting OH group is missing (Sprinzl and Cramer 1973). However, in some cases the absence of this free vicinal hydroxyl can cause a tRNA misaminoacylation (von der Haar and Cramer 1975).

The concept of kinetic proofreading (Hopfield 1974; Ninio 1977) initiated a search for experimental evidence to prove the existence of this suggestive mechanism for the maintenance of translational fidelity. A Fersht (Fersht and Kaethner 1976) demonstrated by fast kinetic experiments that the amount of erroneous aminoacylation by ARS may be transiently higher than that measured by chemical analysis of the final reaction products and explained this observation by a "kinetic proofreading mechanism". If an enzyme is designed in such a way that it is able to check the chemical structure of the product twice, in two thermodynamically almost irreversible reactions, then the overall fidelity will be the multiple of the fidelities of the two. Today, we know that some ARS are indeed equipped with two separately working active sites, one for aminoacyl transfer from aminoacyl-adenosine to tRNA and the other for hydrolytic correction, when a wrong amino acid was incorporated in the first step (Nureki *et al* 1998).

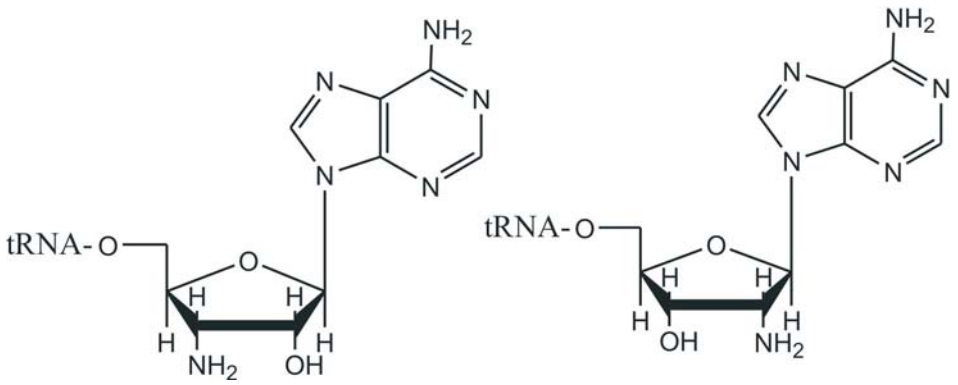


Figure 4. Structure of 'amino' tRNAs used to test the position of aminoacylation of tRNA.

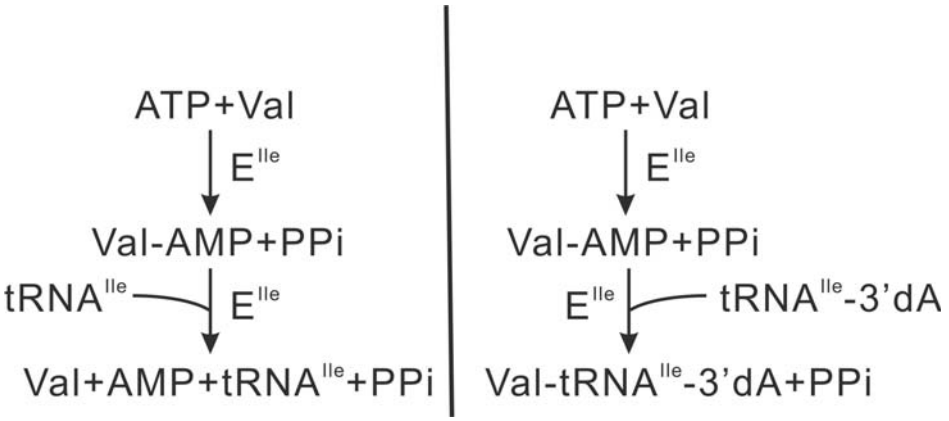


Figure 5. Valine is activated by Ile-tRNA synthetase but it is not transferred to $tRNA^{Ile}$. In the absence of 3′-OH this erroneous aminoacylation occurs in high yield (van der Haar and Cramer 1975).

Cramer's group investigated the role of the vicinal hydroxyls on the 3′-end of aminoacyl-tRNA on the efficiency of proofreading. Although, due to lack of a unifying common mechanism, it is difficult to provide a general scheme for a substrate assisted proofreading, in some cases, as in the case of isoleucine-tRNA synthetase-catalyzed misacylation of $tRNA^{Ile}$ by valine, the absence of the non accepting 3′-OH group completely knocked out the proofreading activity of this ARS, leading to efficient formation of misaminoacylated Val-$tRNA^{Ile}$ (figure 5). Thus, in this case the nonaccepting 3′-hydroxyl group on the terminal adenosine of tRNA is a substrate-provided catalyst that controls the proofreading and fidelity of aminoacylation (von der and Cramer 1975).

5. Recognition of 2′(3′)-aminoacyl-tRNA isomers by translation factors and ribosomal sites

Substrate specificities of the ribosomal tRNA binding sites were tested by aminoacyl-tRNA analogues as puromycin,

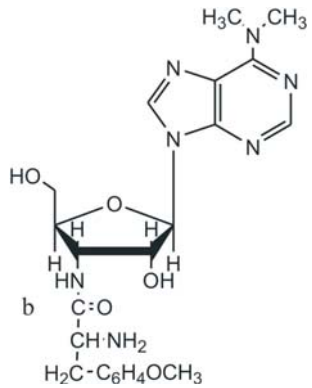


Figure 6. Structure of puromycin a nonisomerisable analogue of aminoacyl-tRNA.

nonisomerisable aminoacyl-oligonucleotides and enzymatically aminoacylated nonisomerisable aminoacyl-tRNA analogues. Very important for these studies were the assays developed by Monro and coworkers who introduced the

"fragment reaction" that allowed to test many synthetic aminoacyl-oligonucleotides and puromycin analogues for their activities in the ribosomal peptidyl transferase reaction. Results of these fundamental investigations, in which the concepts of ribosomal sites and the definition of the properties of peptidyl- and aminoacyl-tRNA located in the peptidyl-trasferase were established, were recently reviewed by Maden (2003). Important contributions using this approach for investigation of the structural requirements for substrates in the ribosomal peptidyl transferase were made by Chládek and coworkers and reviewed (Chládek and Sprinzl 1985). Later, as the non isomerisable aminoacyl-tRNAs became available, this work was extended by A Rich, S Hecht, M Sprinzl and their colleagues. The results are summarized in several articles and reviews by these authors (Fraser and Rich 1973; Sprinzl and Cramer 1973; Hecht 1977; Chládek and Sprinzl 1985).

6. Interaction of the positional isomers of aminoacyl-tRNA with EF-Tu and ribosomal sites

It was demonstrated by several independent investigations that both 2′- and 3′-aminoacyl isomers of 'deoxy' RNAs can bind EF-Tu·GTP, although, with about 50-fold lower efficiency as compared to the native 2′(3′)-aminoacyl-tRNA (Sprinzl *et al* 1977; Ringer and Chládek 1975; Alford *et al* 1979). The preference for the 3′-isomer of aminoacyl-tRNAs was later conclusively demonstrated by NMR experiments published by S Yokoyama and coworkers (Taiji *et al* 1985) and finally by X-ray structure analysis of aminoacyl-tRNA EF-Tu complexes (Nissen *et al* 1995). Thus, the EF-Tu·GTP is an isomerase that utilizes a mixture of the 2′(3′)-aminoacyl tRNA isomers as a substrate and stabilises the uniform 3′-complex. The ribosomal sites are not selective with respect to the positional isomers (Table 2). Especially, at high magnesium ion concentration they can accommodate both the isomers of aminoacyl-tRNAs, regardless of the presence or absence of the vicinal hydroxyl on the terminal ribose or the mode of attachment of the amino acid via ester or amide bond. The enzymatic, EF-Tu-dependent binding, of the aminoacyl-tRNA to the programmed ribosomes is also possible, although it is less efficient when one of the OH groups is missing. After peptide transfer uncharged tRNA is left in the P-site and before the next round of elongation starts it has to be translocated to the exit site (E-site). This translocation can not take place with tRNA-2′dA (Lill *et al* 1988). On the other hand the absence of the 2′-OH group does not hinder the translocation of the peptidyl-tRNA from the A- to the P-site (Weinger *et al* 2004).

7. Acceptor and donor activities and the mechanism of peptidyl transfer

First attempts to solve the question whether the 2′- or 3′-isomer of aminoacyl-tRNA is the substrate for peptidyl transferase were made by applying the fragment reaction with chemically synthesized nonisomerisable aminoacyl- and peptidyl-ribooligonucleotide fragments. The laboratory of S Chládek, first at the Academy of Sciences in Prague, and later at the Michigan Cancer Foundation in Detroit was leading this research (Chládek and Sprinzl 1985). As the nonisomerisable aminoacyl- and peptidyl-tRNA became available (figure 7) the positional isomers of whole aminoacyl-tRNA could be tested *in vitro* by using polyA or polyU as messengers. These assays derived from the classical

Table 2. Substrate properties of the native and nonisomerisable 'amino' and 'deoxy' tRNAs in different steps of elongation cycle.

	acyl-tRNA-A	Nonisomerisable acyl-		
		tRNA-2′dA	tRNA-3′dA	
Reaction	2′,3′mixture	3′-isomer	2′-isomer	Reference
A-site binding	Yes	Yes	Yes	Chládek and Sprinzl 1985; Hecht 1977
P-site binding	Yes	Yes	Yes	Chládek and Sprinzl 1985; Hecht 1977
Binding to EF-Tu•GTP	Yes	Yes	Yes	Sprinzl *et al* 1977; Ringer and Chladek 1975; Alford *et al* 1979
Acceptor activity	Yes	Yes	No	Chládek and Sprinzl 1985; Hecht 1977
Donor activity	Yes	No	No	Taiji *et al* 1985; Lill *et al* 1988; Weinger *et al* 2004
A-->P translocation	Yes	Yes	ND	Taiji *et al* 1985
P-->E translocation	Yes	No	ND	Alford *et al* 1979
Polypeptide synthesis	Yes	No	No	Chládek and Sprinzl 1985; Hecht 1977

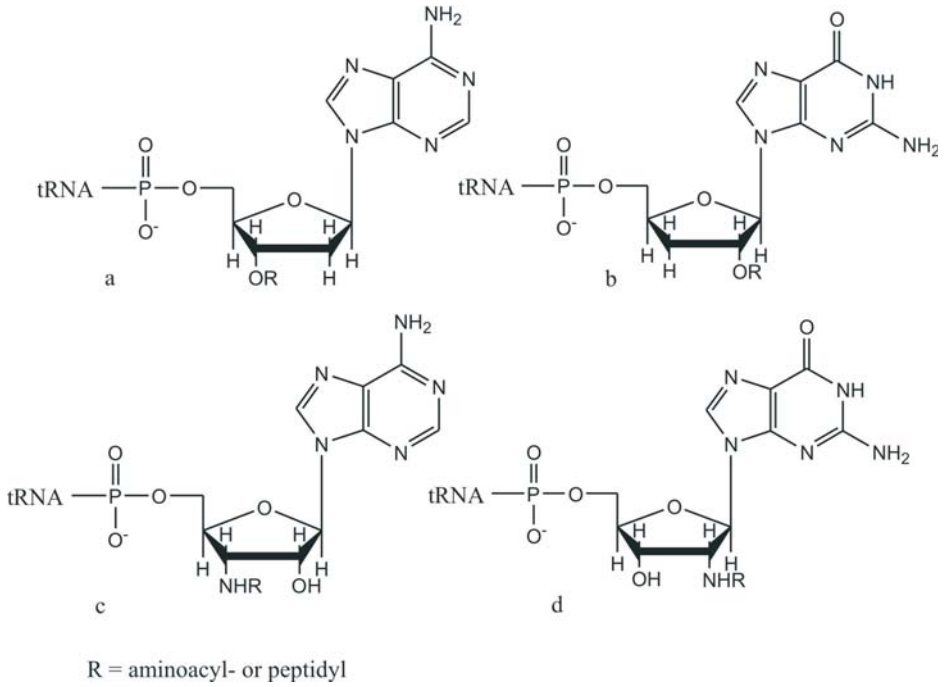


Figure 7. Structure of nonisomerisable aminoacyl- or peptidyl-tRNAs used to test the substrate specificity in the single steps of protein elongation cycle for positional isomers.

Matthaei-Nierenberg assay (Nirenberg and Matthaei 1961) did not allow to measure the kinetics of peptide bond formation with site-specific binding of particular isomers in the peptidyl transferase. Particularly, the donor activity of the peptidyl-tRNA-2′dA was not clear until very recently (Quiggle *et al* 1981; Hecht *et al* 1974; Wagner *et al* 1982). Finally, Strobel and coworkers (Weinger *et al* 2004) using short synthetic mRNA and kinetically controlled binding of tRNA substrates to the particular ribosomal sites convincingly confirmed the earlier observations (Quiggle *et al* 1981; Hecht *et al* 1974) that the 2′OH group of the peptidyl-tRNA is essential for peptide transfer. The data on 2′-, 3′-specificity of the peptidyl transferase in table 2 can be summarized as follows:

- Both isomers (2′ and 3′) can bind to the A- and P-sites.
- 2′-OH group is not essential for binding of tRNA to the ribosomal A- and P-sites.
- The acceptor (aminoacyl-tRNA) and the donor (peptidyl-tRNA) are active only when the acyl residue is on the 3′-position.
- Absence of the 2′-OH from the aminoacyl-tRNA (in the A-site) does not hinder the transfer of the peptidyl moiety from the P-site tRNA to the A-site tRNA.
- The 2′-OH group of the peptidyl-tRNA is essential for the transfer of peptide from the P-site tRNA to the A-site tRNA.
- Translocation of the peptidyl-tRNA from the A-site to the P-site does not require the 2′OH.
- Ribose with an intact *cis*-diol is required for translocation of tRNA from the P-site to the E-site.

8. 2′-OH group of peptidyl-tRNA is involved in substrate assisted catalysis of peptide bond formation in the peptidyl transferase

Chládek and coworkers who used the Monro′s fragment reaction to test the mechanism of peptidyl transferase suggested the possibility that the 2′-OH group of peptidyl-tRNA located in the P-site actively participates in the peptide transfer in a manner which we would name today as "substrate assisted catalysis" (Quiggle *et al* 2005). Strobel and coworkers proposed later a similar mechanism (Weinger *et al* 2004). Petkov and his colleagues (Changalor *et al* 2005) who studied the mechanism of hydrolysis of aminoacyl adenosines in details, made very important observations that reinforce the "substrate assisted catalysis" mechanism of the peptidyl transfer involving participation of the 2′-OH group of peptidyl-tRNA (figure 8). In this model, the 2′-OH serves as a proton relay, accepting a proton from an incoming α-amino group and passing it to the 3′ oxygen of the peptidyl-tRNA. Petrov suggests a transition state arranged in a six-membered ring stabilized by ribosomal RNA in the peptidyl transferase. If this mechanism is correct, then the

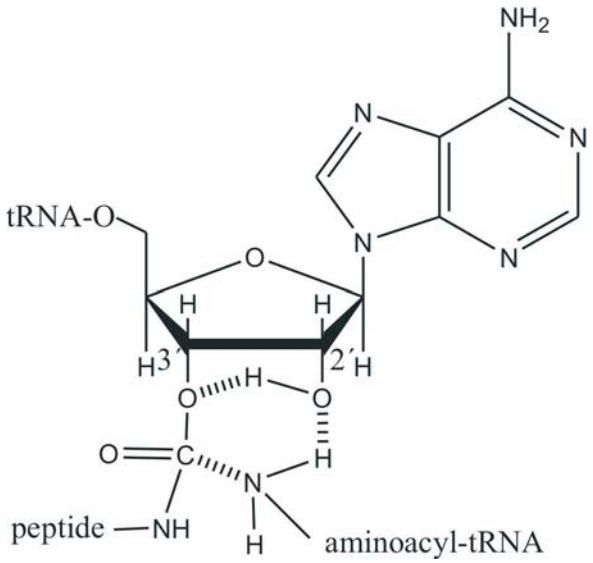


Figure 8. Transition state during the peptide bond formation in the peptidyl transferase. The 2′-OH group of the peptidyl-tRNA participates on proton transfer

conformation of the terminal ribose on both the aminoacyl- and the peptidyl-tRNA can efficiently control the peptidyl transferase reaction. There are several indications and some structural evidence reported in the literature that such kind of allosteric control indeed takes place (Schmeing *et al* 2005; Gnirke *et al* 1989) and the conformation of ribose provides the switch to trigger the substrate assisted catalysis by the 2′-OH of the peptidyl-tRNA (Schlosser *et al* 2001).

References

Alford B L, Pezzuto J M, Tan K H and Hecht S M 1979 Both positional isomers of aminoacyl-tRNA's are bound by elongation factor Tu; *J. Biol. Chem*. **254** 6894–6903

Changalov M M, Ivanova G D, Rangelov M A, Acharya P, Acharya S, Minakawa N, Foldesi A, Stoineva I B, Yomtova V M, Roussev C D, Matsuda A, Chattopadhyaya J and Petkov D D 2005 2′/3′-O-peptidyl adenosine as a general base catalyst of its own external peptidyl transfer: implications for the ribosome catalytic mechanism; *Chembiochem* **6** 992–996

Chinault A C, Tan K H, Hassur S M and Hecht S M 1977 Initial position of aminoacylation of individual *Escherichia coli*, yeast, and calf liver transfer RNAs; *Biochemistry* **16** 766–776

Chládek S and Sprinzl M 1985 The 3′-end of tRNA and its role in protein biosynthesis; *Angew. Chem. Int. Ed.* **24** 371–391

Cramer F, Faulhammer H, von der H, Sprinzl M and Sternbach H 1975 Aminoacyl-tRNA synthetases from baker's yeast: reacting site of enzymatic aminoacylation is not uniform for all tRNAs; *FEBS Lett.* **56** 212–214

Crick F H 1958 On protein synthesis; *Symp. Soc. Exp. Biol.* **12** 138–163

Eriani G, Delarue M, Poch O, Gangloff J and Moras D 1990 Partition of tRNA synthetases into two classes based on mutually exclusive sets of sequence motifs; *Nature (London)* **347** 203–206

Fersht A R and Kaethner M M 1976 Enzyme hyperspecificity. Rejection of threonine by the valyl-tRNA synthetase by misacylation and hydrolytic editing; *Biochemistry* **15** 3342–3346

Fraser T H and Rich A 1973 Synthesis and aminoacylation of 3′-amino-3′-deoxy transfer RNA and its activity in ribosomal protein synthesis; *Proc. Natl. Acad. Sci. USA* **70** 2671–2675

Fraser T H and Rich A 1975 Amino acids are not all initially attached to the same position on transfer RNA molecules; *Proc. Natl. Acad. Sci. USA* **72** 3044–3048

Gnirke A, Geigenmuller U, Rheinberger H J and Nierhaus L H 1989 The allosteric three-site model for the ribosomal elongation cycle. Analysis with a heteropolymeric mRNA; *J. Biol. Chem.* **264** 7291–7301

Hecht S M 1977 Utilization of isomeric aminoacyl transfer ribonucleic acids in peptide bond formation; *Account. Chem. Res.* **10** 239–245

Hecht S M, Kozarich J W and Schmidt F J 1974 Isomeric phenylalanyl-tRNAs. Position of the aminoacyl moiety during protein biosynthesis; *Proc. Natl. Acad. Sci. USA* **71** 4317–4321

Hoagland M B, Zamecnik P C and Stephenson M L 1957 Intermediate reactions in protein biosynthesis; *Biochim. Biophys. Acta* **24** 215–216

Hopfield J J 1974 Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity; *Proc. Natl. Acad. Sci. USA* **71** 4135–4139

Lill R, Lepier A, Schwagele F, Sprinzl M, Vogt H and Wintermeyer W 1988 Specific recognition of the 3′-terminal adenosine of tRNAPhe in the exit site of *Escherichia coli* ribosomes; *J. Mol. Biol.* **203** 699–705

Maden B E 2003 Historical review: Peptidyl transfer, the Monro era; *Trends Biochem. Sci.* **28** 619–624

Ninio J 1977 Are further kinetic amplification schemes possible?; *Biochimie* **59** 759–760

Nirenberg M W and Matthaei J H 1961 The dependence of cell-free protein synthesis in *E. coli* upon naturally occurring or synthetic polyribonucleotides; *Proc. Natl. Acad. Sci. USA* **47** 1588–1602

Nissen P, Kjeldgaard M, Thirup S, Polekhina G, Reshetnikova L, Clark B F and Nyborg J 1995 Crystal structure of the ternary complex of Phe-tRNAPhe, EF-Tu, and a GTP analog [see comments]; *Science* **270** 1464–1472

Nureki O, Vassylyev D G, Tateno M, Shimada A, Nakama T, Fukai S, Konno M, Hendrickson T L, Schimmel P and Yokoyama S 1998 Enzyme structure with two catlytic sites for double-sieve selection of substrate; *Science* **280** 578–582

Quiggle K, Kumar G, Ott T W, Ryu E K and Chladek S 1981 Donor site of ribosomal peptidyltransferase: investigation of substrate specificity using 2′(3′)-O-(N-acylaminoacyl) dinucleoside phosphates as models of the 3′ terminus of N-acylaminoacyl transfer ribonucleic acid; *Biochemistry* **20** 3480–3485

Ribas de P L and Schimmel P 2001 Two classes of tRNA synthetases suggested by sterically compatible dockings on tRNA acceptor stem; *Cell* **104** 191–193

Ringer D and Chladek S 1975 Interaction of elongation factor Tu with 2′(3′)-O-aminoacyloligonucleotides derived from the 3′

terminus of aminoacyl-tRNA; *Proc. Natl. Acad. Sci. USA* **72** 2950–2954

Schlosser A, Nawrot B, Grillenbeck N and Sprinzl M 2001 Fluorescence-monitored conformational change on the 3′-end of tRNA upon aminoacylation; *J. Biomol. Struct. Dyn.* **19** 285–291

Schmeing T M, Huang K S, Strobel S A and Steitz T A 2005 An induced-fit mechanism to promote peptide bond formation and exclude hydrolysis of peptidyl-tRNA; *Nature* **438** 520–524

Sonnenbichler J, Feldmann H, and Zachau H G 1963 Identifizierung der Aminoacyl-s-RNA als 3′-Ester des terminalen Adenosins; *Hoppe-Seyler's Z. Physiol. Chem.* **334** 283–286

Sprinzl M and Cramer F 1973 Accepting site for aminoacylation of tRNAphe from yeast; *Nat. New Biol* **245** 3–5

Sprinzl M and Cramer F 1975 Site of aminoacylation of tRNAs from *Escherichia coli* with respect to the 2′- or 3′-hydroxyl group of the terminal adenosine; *Proc. Natl. Acad. Sci. USA* **72** 3049–3053

Sprinzl M, Kucharzewski M, Hobbs J B and Cramer F 1977 Specificity of elongation factor Tu from *Escherichia coli* with respect to attachment to the amino acid to the 2′ or 3′-hydroxyl group of the terminal adenosine of tRNA; *Eur. J. Biochem.* **78** 55–61

Sprinzl M, Scheit K H, Sternbach H, von der H and Cramer F 1973 In vitro in corporation of 2′-deoxyadenosine and 3′-deoxyadenosine into yeast tRNA Phe using t RNA nucleotidyl transferase, and properties of tRNA Phe -C-C-2′dA and tRNA Phe -C-C-3′dA; *Biochem. Biophys. Res. Commun.* **51** 881–887

Taiji M, Yokoyama S and Miyazawa T 1983 Transacylation rates of (aminoacyl) adenosine moiety at the 3′-terminus of aminoacyl transfer ribonucleic acid; *Biochemistry* **22** 3220–3225

Taiji M, Yokoyama S and Miyazawa T 1985 Aminoacyl-tRNA exclusively in the 3′-isomeric form is bound to polypeptide chain elongation factor Tu; *J. Biochem. (Tokyo)* **98** 1447–1453

von der Haar F and Cramer F 1975 Isoleucyl-tRNA synthetase from baker's yeast: the 3′-hydroxyl group of the 3′-terminal ribose is essential for preventing misacylation of tRNAIle-C-C-A with misactivated valine; *FEBS Lett.* **56** 215–217

Wagner T, Cramer F and Sprinzl M 1982 Activity of the 2′ and 3′ isomers of aminoacyl transfer ribonucleic acid in the in vitro peptide elongation on *Escherichia coli* ribosomes; *Biochemistry* **21** 1521–1529

Weinger J S, Parnell K M, Dorner S, Green R and Strobel S A 2004 Substrate-assisted catalysis of peptide bond formation by the ribosome; *Nat. Struct. Mol. Biol.* **11** 1101–1106

Zachau H G, Acs G, and Lipmann F 1958 Isolation of adenosine amino acid esters from a ribonuclease digest of soluble, liver ribonucleic acid; *Proc. Natl. Acad. Sci. USA* **44** 885–889

Zamecnik P C 1962 Unsettled questions in the field of protein synthesis; *Biochem. J.* **85** 257–264

*e*Publication: 4 October 2006