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Co-evolution of the Archaeal tRNA-dependent Amidotransferase GatCAB with tRNA^{Asn}

Suk Namgoong[†], **Kelly Sheppard**[†], **R. Lynn Sherrer**[†], and **Dieter Söll**[†,‡,*]
†*Departments of Molecular Biophysics and Biochemistry, New Haven, CT 06520 8114, USA*
‡*Department of Chemistry Yale University, New Haven, CT 06520 8114, USA*

Abstract

The important identity elements in tRNA^{Gln} and tRNA^{Asn} for bacterial GatCAB and in tRNA^{Gln} for archaeal GatDE are the D-loop and the first base pair of the acceptor stem. Here we show that *Methanothermobacter thermautotrophicus* GatCAB, the archaeal enzyme, is different as it discriminates Asp-tRNA^{Asp} and Asp-tRNA^{Asn} by use of U49, the D-loop and to a lesser extent the variable loop. Since archaea possess the tRNA^{Gln}-specific amidotransferase GatDE, the archaeal GatCAB enzyme evolved to recognize different elements in tRNA^{Asn} than those recognized by GatDE or by the bacterial GatCAB enzyme in their tRNA substrates.

Keywords

tRNA; transamidation; Asn-tRNA; Gln-tRNA; evolution

1. Introduction

Attaching the correct amino acid to its cognate tRNA species is an essential step for the fidelity of protein synthesis, catalyzed usually by the aminoacyl-tRNA synthetases (aaRS) [1]. However, many bacterial and archaeal genomes do not encode an asparaginyl-tRNA synthetase (AsnRS) and instead use an indirect pathway for Asn-tRNA synthesis. These organisms take advantage of a non-discriminating aspartyl-tRNA synthetase (ND-AspRS) to form Asp-tRNA^{Asn} and an Asp-tRNA^{Asn}-dependent amidotransferase (Asp-AdT) that converts the mischarged tRNA by amidation to Asn-tRNA^{Asn} [2-8]. Prokaryotes lacking a glutaminyl-tRNA synthetase use a similar two-step tRNA-dependent transamidation pathway to form Gln-tRNA with the aid of a ND-glutamyl-tRNA synthetase and a Glu-AdT [3,9,10].

Two AdTs exist in nature, the heterotrimeric GatCAB [10] and the heterodimeric GatDE [3]. The former is found in both archaea and bacteria [3]. *In vitro* GatCAB is capable of acting as both a Glu-AdT and an Asp-AdT [3,5,7]. GatDE occurs only in archaea and functions solely as a Glu-AdT [3]. Given the presence of GatDE in every archaeon the archaeal GatCAB enzyme may function *in vivo* as an Asp-AdT; this is a plausible but still unproven assumption. The GatC protein is small, about 100 aa long, and is likely a chaperone for GatA [10], helping it bind to GatB [11]. GatA turned out to be an amidase [12] whereas GatD was shown to be an asparaginase [3,13]. GatE and GatB belong to an isolated protein family and have been

*To whom correspondence should be addressed. Tel: +1 203 432 6200; Fax +1 203 432 6202; Email: soll@trna.chem.yale.edu

implicated as tRNA binding domains catalyzing the ATP-dependent activation of the mischarged tRNA and subsequent amidation into the correct aa-tRNA [11,13-15].

AdTs must recognize specific elements of a tRNA to discriminate the mischarged species (Asp-tRNA^{Asn} or Glu-tRNA^{Gln}) from the correctly charged ones (Asp-tRNA^{Asp} or Glu-tRNA^{Glu}). Studies with *Staphylococcus aureus* GatCAB and *Methanothermobacter thermautotrophicus* GatDE showed that the D-loop and the first base-pair of the acceptor stem are important recognition elements for tRNA^{Gln} identity [10,15]. Recent work revealed similar recognition elements in use by bacterial GatCAB to discriminate tRNA^{Asn} from tRNA^{Asp} [16]. The same work suggested the variable loop would be the major identity element recognized by the archaeal GatCAB to differentiate tRNA^{Asn} from tRNA^{Asp} [16]. In *M. thermautotrophicus*, the first base pair of the acceptor stem is invariant between tRNA^{Asp} and tRNA^{Asn}. Given that the AdT tRNA recognition work to date focused on the bacterial GatCAB enzyme and on the archaeal specific Glu-AdT, GatDE, we decided to study the *M. thermautotrophicus* GatCAB as an Asp-AdT. We demonstrate a new assay for measuring aa-tRNA recognition by AdTs and report on the identity elements used by GatCAB to discriminate tRNA^{Asn} from tRNA^{Asp}.

2. Materials and Methods

2.1 General

Oligonucleotide synthesis and DNA sequencing were performed by the Keck Foundation Biotechnology Resource Laboratory at Yale University. [^{14}C]Asp (213 mCi/mmol) and [^{3}H] Asp (26 Ci/mmol) were purchased from Amersham Biosciences (Piscataway, NJ) and [^{14}C] Asn (228.4 mCi/mmol) was purchased from Perkin-Elmer Life Sciences (Boston, NE).

2.2 Preparation of enzymes and tRNAs

The heterotrimeric *M. thermautotrophicus* GatCAB enzyme was overexpressed as a C-terminal His_6-tag fusion at the GatB subunit, purified by nickel affinity chromatography as previously described [17] föllowed by size-exclusion chromatography on a Superdex HR200 column (Amersham Biosciences) in buffer A (50 mM HEPES-KOH (pH 7.4), 10 mM $MgCl_2$, 1 mM DTT, 100 mM NaCl) with 10% glycerol, then dialyzed in buffer A containing 50 % glycerol and stored at −20 °C before use.

For preparation of *in vitro* tRNA transcripts, chemically synthesized oligonucleotides corresponding to wild-type and mutant tRNA^{Asn} and tRNA^{Asp} sequences were cloned into pUC19. The transcription templates were digested with *Bst*NI (tRNA^{Asn}) or *Nsi*I (tRNA^{Asp}) at 55 °C for 16 hr. *In vitro* T7 RNA polymerase run-off transcriptions were conducted as described [18] and the RNA purified using a Qiagen (Valencia, CA) RNA-DNA kit according to manufacturer's instructions.

2.3 tRNA aminoacylation

For amidotransferase assays, tRNA^{Asp} and tRNA^{Asn} generated by *in vitro* transcription was charged with [^{14}C]Asp (50 μM) or [^{3}H]Asp (50 μM) by 4 μg purified *M. thermautotrophicus* AspRS as described [17]. After aminoacylation, phenol:chloroform (1:1) extraction, and precipitation with two volumes of ethanol the Asp-tRNA preparations were stored at −20 °C.

2.4 Amidotransferase assay

GatCAB activity was measured as described earlier [5] with slight modifications. [^{14}C] or [^{3}H] labeled Asp-tRNAs were suspended in 100 μl amidation buffer (50 mM HEPES-KOH, pH 7.4, 25 mM KCl, 10 mM $MgCl_2$, 2 mM dithiothreitol) and 10 nM of *M. thermautotrophicus* GatCAB enzyme was added and incubated at 37 °C for 5 min. Aliquots

of 20 μl were removed every 60 seconds and quenched by the addition of 20 μl of 50 mM KOH. The tRNAs were deacylated by a 15 min incubation at 25 °C, dried in a Speedvac, and then resuspended in 3 μl of 50 nM of unlabeled Asp and Asn. An aliquot (1.5 μl) was spotted onto a cellulose TLC plate (Sigma-Aldrich, St. Louis, MO) and developed in ammonia: water: chloroform: methanol (2:1:6:6). The [^{14}C]-radioactive amino acids were detected in a Storm 860 Bioimager and analyzed with ImageQuaNT V4.0 program (Amersham Biosciences). When [^{3}H]-aminoacyl-tRNAs were used, the [^{3}H] amino acids were detected after spraying with 0.02 % ninhydrin and excising the portions of the TLC plate corresponding to the Asp and Asn spots, and finally liquid scintillation counting.

2.5 RNase Protection Assay

To investigate binding between Asp-tRNAs and GatCAB, an RNase protection assay [19] was used. 40 nM of [^{3}H] Asp-tRNA was preincubated for 30 min at 4 °C in the presence of 40-2000 nM GatCAB, 50 mM HEPES-KOH (pH 7.4), 10 mM $MgCl_2$, 25 mM KCl and 20 % glycerol to form a ternary complex of Asp-tRNA and GatCAB protein. RNase digestion was started by the addition of 1 μg pancreatic RNase mix (Roche Biochemical, Indianapolis, IN) and was stopped by withdrawing 20 μl and spotting onto a 3mm paper filter (Whatman, NJ) immersed with 10 % of trichloroacetic acid (TCA) solution. The paper filters were washed twice with 10 % and 5 % of TCA solution respectively and residual radioactivity was counted by liquid scintillation counting.

3. Results

3.1 Modified bases in tRNA^{Asn} and tRNA ^{Asp} do not play significant roles in recognition by GatCAB

Previously, it was shown that Asp-tRNA^{Asn} derived from *in vitro* transcription could be transamidated by recombinant *M. thermautotrophicus* GatCAB enzyme [3], suggesting that modified bases in tRNA^{Asn} transcripts do not play significant roles in the recognition of Asp-tRNA^{Asn} by GatCAB. In order to determine whether the same enzyme still discriminates against the unmodified tRNA^{Asp} transcript we employed two methods. The first, a standard *in vitro* transamidation assay [10] based on the separation of amino acids by TLC was carried out with *in vitro* transcribed Asp-tRNA^{Asn} and Asp-tRNA^{Asp} (Fig. 1A). The second technique was an RNase protection assay [18], which has been used for investigation of binding properties of aa-tRNAs with EF-Tu, adapted for the investigation of interactions between GatCAB and Asp-tRNA. EF-Tu protects bound aa-tRNAs from RNase digestion, and the amount of TCA-insoluble radioactivity correlates with the level of ternary complex between EF-Tu and radiolabed aa-tRNAs [18]. This was used to measure Asp-tRNA^{Asn}:GatCAB complex formation. As expected, Asp-tRNA^{Asn} (as transcript) was converted to Asn-tRNA^{Asn} by the addition of *M. thermautotrophicus* GatCAB [3], while the Asp-tRNA^{Asp} transcript was not transamidated (Fig. 1A). The amount of [^{3}H]Asp-tRNA^{Asn} protected in this assay increased with increasing GatCAB concentration (Fig. 1B). Using linear regression analysis, we estimated the K_D of *M. thermautotrophicus* GatCAB for Asp-tRNA^{Asn} to be approximately 0.6 μM. Asp-tRNA^{Asp} was not protected from RNase digestion by any amount of GatCAB tested. These results suggest that GatCAB specifically binds Asp-tRNA^{Asn}. Therefore, modified nucleotides do not play an essential role in GatCAB discrimination of Asp-tRNA^{Asn} from Asp-tRNA^{Asp}.

D-loop and T-arm are important for GatCAB recognition of Asp- tRNA^{Asn}—We constructed eight chimeric tRNA^{Asn} variants (tRNA^{Asx}) in which different structural portions of tRNA^{Asp} replaced the corresponding ones in tRNA^{Asn} (sequences in Fig. 2A). These tRNA^{Asx} variants (depicted schematically in Fig. 2B) were then aspartylated with *M. thermautotrophicus* AspRS. Seven out of eight of these tRNA^{Asx} mutants could be

aspartylated; exchange of the $tRNA^{Asn}$ D-arm for that of $tRNA^{Asp}$ gave rise to an unchargeable tRNA. The remaining Asp-$tRNA^{Asx}$ variants were tested for GatCAB-catalyzed amidation.

For five of the other Asp-$tRNA^{Asx}$ swap mutants, a 0-4 fold decrease of conversion efficiency was observed. The T-stem swap mutant retained the A49-U65 base pair found in $tRNA^{Asn}$ (see below). The largest effects were seen with the T-arm and D-loop Asp-$tRNA^{Asx}$ mutants; they supported the amidotransferase 10-fold less than did wild-type Asn-$tRNA^{Asn}$.

A similar effect was seen when the interaction of selected Asp-tRNAs was investigated with the RNase protection assay. While wild-type Asp-$tRNA^{Asn}$ and anticodon swapping mutants had nearly similar binding affinity to GatCAB, the protection level of the T stem-loop mutant was significantly reduced (Fig. 2C), indicating the $tRNA^{Asn}$ T-arm is critical for specific recognition by GatCAB.

3.2 U49 in $tRNA^{Asp}$ is a major antideterminant for GatCAB recognition

To reveal a more detailed picture of Asp-$tRNA^{Asn}$ recognition by GatCAB, fifteen $tRNA^{Asn}$ mutants were generated based on the sequence differences between *M. thermautotrophicus* $tRNA^{Asp}$ and $tRNA^{Asn}$ (Fig. 2A). After aspartylation their substrate qualities were determined in initial velocity experiments with GatCAB (Table 1). Most mutations did not significantly impact the ability of GatCAB to use the tRNA variants as substrates. Confirming our anticodon swap experiment (Fig. 2B), point mutations in the anticodon loop did not appreciably alter the ability of GatCAB to productively bind to the tRNA. Two tRNA mutants could not be transamidated; they had replacements of the A49-U65 base pair with a U49-G65 wobble pair (present in *M. thermautotrophicus* $tRNA^{Asp}$) or with a U49-A65 pair. Base pair changes in this position (to C49-G65 or G49-U65) led to tRNAs that were much less affected in their ability to be substrates for GatCAB. Therefore we conclude that U49 may act as an antideterminant for GatCAB recognition of $tRNA^{Asp}$. Three other $tRNA^{Asn}$ mutants in our collection showed marked intermediate effects on transamidation; position 9 located between the acceptor and D-stems, position 47 in the variable loop and position 56 in the T-loop. Nucleotides in these positions should contribute to the overall tertiary structure of the tRNA.

A comparison of position 49 in archaeal $tRNA^{Asn}$ and $tRNA^{Asp}$ species makes it apparent that U49 is not conserved as an anti-determinant (Table 2). In most archaea encoding a GatCAB position 49 differs in $tRNA^{Asp}$ and $tRNA^{Asn}$. When purine at position 49 occurs in $tRNA^{Asn}$, $tRNA^{Asp}$ has a pyrmidine or vice versa; this suggests that position 49 may still serve as anti-determinant for GatCAB recognition in many organisms (Table 2). *Nanoarchaeum equitans* and *Methanopyrus kandleri* are notable exceptions. Position 49 is invariant between $tRNA^{Asp}$ and $tRNA^{Asn}$ in archaea with an AsnRS (Table 2).

3.3 Asp-AdT active across Domains of Life

In bacteria that use the two-step transamidation pathway $tRNA^{Asn}$ and $tRNA^{Gln}$ have a U1-A72 base pair, while a G1-C72 pair is common in $tRNA^{Asp}$ and $tRNA^{Glu}$; these are important identity elements for the bacterial GatCAB (Fig. 3A) [16]. Mutating the U1-A72 pair to G1-C72 in *S. aureus* $tRNA^{Gln}$ significantly decreased the ability of GatCAB to recognize the tRNA [11]. Similar results were obtained for the *N. meningitidis* GatCAB and $tRNA^{Asn}$ [16]. In the case of GatDE, the archaeal Glu-AdT, the first base pair of the acceptor stem is also important to achieve productive tRNA binding [15]. The archaeal $tRNA^{Asn}$ and $tRNA^{Asp}$ species contain a G1-C72 base pair (Fig. 2A); therefore this position in the tRNA is not used for discriminating $tRNA^{Asn}$ from $tRNA^{Asp}$ by the archaeal GatCAB. Since we are also working with the purified bacterial GatCAB enzyme from *Helicobacter pylori*, we compared the ability of this bacterial AdT with that of the archaeal *M. thermautotrophicus* enzyme to use either archaeal Asp-$tRNA^{Asn}$ or *H. pylori* Asp-$tRNA^{Asn}$ as a substrate. The results show (Fig. 3B) that the archaeal

enzyme could transamidate the heterologous bacterial Asp-tRNA^{Asn} whereas the bacterial enzyme could only transamidate its own Asp-tRNA^{Asn}. A similar finding with the *N. meningitidis* and *Methanoscarcina bakeri* enzymes was very recently reported [16].

4. Discussion

Studies of specific tRNA recognition by aminoacyl-tRNA synthetases by genetic, biochemical and structural techniques [20] have led to a detailed understanding of the set of discrete tRNA identity elements that distinguish an isoacceptor group from all the other tRNA species [21]. Such investigations are now underway to characterize the enzymes that form aa-tRNA by tRNA-dependent transamidation (*e.g.*, [11,15,16]). Therefore it is appropriate to ask whether the tRNA recognition process of tRNA-dependent amidotransferases is the same as that for aaRSs. In theory, these processes could operate differently. While the aaRS selects its cognate tRNA from amongst a large number of cellular tRNAs, *M. thermautotrophicus* GatCAB, for example, may first recognize the Asp moiety on the 3′ end of the aa-tRNA, narrowing the pool to Asp-tRNA^{Asn} and Asp-tRNA^{Asp}. In a second step only the rejection of the perfect cognate aa-tRNA (Asp-tRNA^{Asp}) is required. Following this reasoning we synthesized 'transplantation' tRNA mutants (Fig. 2).

It is more difficult to establish a complete list of tRNA identity elements for such enzymes, as their substrates need to be synthesized by another enzyme, an aaRS. The generation of substrates for *M. thermautotrophicus* GatCAB, a collection of mischarged Asp-tRNA^{Asn} species, therefore relies on at least partial preservation of the tRNA^{Asn} identity elements used by AspRS. Indeed, many tRNA^{Asn} mutants that were constructed in this study could not be aspartylated satisfactorily; however a sufficient number was obtained for our studies (Table 1). Although some of the tRNA^{Asx} mutants used in this study (including the D-loop and T-arm swap mutants) could be aspartylated only to half the level obtained with the wild-type tRNA^{Asn}, we do not believe that inhibition by uncharged tRNA played a significant effect in our transamidation results. When we added a 64-fold excess of uncharged tRNA^{Asn}, we were unable to compete GatCAB away from Asp-tRNA^{Asn}. We were able to show that the D-loop and the T-stem/loop of tRNA^{Asn} are important for GatCAB recognition. Since no tRNA^{Asn} mutants with single base changes in the D-loop could satisfactorily be aspartylated, it is currently unclear, if size of the D-loop or specific bases are important for GatCAB recognition. However, the data clearly show that a U49 in the T-arm (as found in *M. thermautotrophicus* tRNA^{Asp}) is sufficient for GatCAB rejection (Table 2). Given the tRNA sequences of archaeal tRNA^{Asp} species (Table 2), position 49 is probably used as an anti-determinant by many archaeal GatCAB enzymes to discriminate against Asp-tRNA^{Asp}. The importance of the variable loop for GatCAB recognition is also evident from the significant activity loss observed in the domain-swap mutant (Fig. 2B) and in the U47 → Δ47 tRNA^{Asn} (Table 1). This region was also predicted to be important based on detailed tRNA sequence comparisons [16]. In *N. equitans* and *M. kandleri*, whose tRNA^{Asp} and tRNA^{Asn} species are invariant at position 49, discrimination against Asp-tRNA^{Asp} is probably accomplished either by use of the D and variable loops alone or by recognition of additional elements in the tRNAs.

In bacteria, a single AdT, GatCAB, exists to transamidate Asp-tRNA^{Asn} and Glu-tRNA^{Gln} [7]. It is not too surprising then that the major recognition elements, the U1-A72 pair and the smaller D-loop, are conserved between bacterial tRNA^{Gln} and tRNA^{Asn} species [11,16]. In archaea, two AdTs exist, GatDE and GatCAB [3]. The former is solely a Glu-AdT discriminating Glu-tRNA^{Gln} from Glu-tRNA^{Glu}, Asp-tRNA^{Asp}, and Asp-tRNA^{Asn} [3]. One of the major elements GatDE recognizes in tRNA^{Gln} is the A1-U72 pair in the acceptor stem [15]. The other three tRNA species mentioned have a G-C pair at that position. It appears, one of the ways archaea may have evolved an AdT specific for Glu-tRNA^{Gln}, was to encode tRNA^{Asn} with the G1-C72 pair found in tRNA^{Glu} and tRNA^{Asp} making Asp-tRNA^{Asn} a poorer

substrate for GatDE. Evolution of the specific Glu-AdT, GatDE, in archaea, therefore, enacted a selective pressure on the archaeal GatCAB, forcing its coevolution with $tRNA^{Asn}$ in order to maintain its Asp-AdT function. This same pressure may have been responsible for the evolution of new antideterminants, such as position 49, in $tRNA^{Asp}$.

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References

1. Ibba M, Söll D. Aminoacyl-tRNA synthesis. Annu. Rev. Biochem 2000;69:617–50. [PubMed: 10966471]
2. Curnow AW, Ibba M, Söll D. tRNA-dependent asparagine formation. Nature 1996;382:589–590. [PubMed: 8757127]
3. Tumbula DL, Becker HD, Chang WZ, Söll D. Domain-specific recruitment of amide amino acids for protein synthesis. Nature 2000;407:106–110. [PubMed: 10993083]
4. Curnow AW, Tumbula DL, Pelaschier JT, Min B, Söll D. Glutamyl-$tRNA^{Gln}$ amidotransferase in Deinococcus radiodurans may be confined to asparagine biosynthesis. Proc. Natl. Acad. Sci. U. S. A 1998;95:12838–12843. [PubMed: 9789001]
5. Becker HD, et al. The heterotrimeric Thermus thermophilus Asp-$tRNA^{Asn}$ amidotransferase can also generate Gln-$tRNA^{Gln}$. FEBS Lett 2000;476:140–144. [PubMed: 10913601]
6. Becker HD, Roy H, Moulinier L, Mazauric MH, Keith G, Kern D. Thermus thermophilus contains an eubacterial and an archaebacterial aspartyl-tRNA synthetase. Biochemistry 2000;39:3216–3230. [PubMed: 10727213]
7. Raczniak G, Becker H, Min B, Söll D. A single amidotransferase forms asparaginyl-tRNA and glutaminyl-tRNA in Chlamydia trachomatis. J. Biol. Chem 2001;276:45862–45867. [PubMed: 11585842]
8. Akochy PM, Bernard D, Roy PH, Lapointe J. Direct glutaminyl-tRNA biosynthesis and indirect asparaginyl-tRNA biosynthesis in Pseudomonas aeruginosa PAO1. J. Bacteriol 2004;186:767–76. [PubMed: 14729703]
9. Wilcox M, Nirenberg M. Transfer RNA as a cofactor coupling amino acid synthesis with that of protein. Proc. Natl. Acad. Sci. U. S. A 1968;61:229–236. [PubMed: 4972364]
10. Curnow AW, Hong K, Yuan R, Kim S, Martins O, Winkler W, Henkin TM, Söll D. Glu-$tRNA^{Gln}$ amidotransferase: a novel heterotrimeric enzyme required for correct decoding of glutamine codons during translation. Proc. Natl. Acad. Sci. U. S. A 1997;94:11819–11826. [PubMed: 9342321]
11. Nakamura A, Yao M, Chimnaronk S, Sakai N, Tanaka I. Ammonia channel couples glutaminase with transamidase reactions in GatCAB. Science 2006;312:1954–8. [PubMed: 16809541]
12. Harpel MR, et al. Mutagenesis and mechanism-based inhibition of Streptococcus pyogenes Glu-$tRNA^{Gln}$ amidotransferase implicate a serine-based glutaminase site. Biochemistry 2002;41:6398–6407. [PubMed: 12009902]
13. Feng L, Sheppard K, Tumbula-Hansen D, Söll D. Gln-$tRNA^{Gln}$ formation from Glu-$tRNA^{Gln}$ requires cooperation of an asparaginase and a Glu-$tRNA^{Gln}$ kinase. J. Biol. Chem 2005;280:8150–5. [PubMed: 15611111]
14. Wilcox M. Gamma-glutamyl phosphate attached to glutamine-specific tRNA. A precursor of glutaminyl-tRNA in Bacillus subtilis. Eur. J. Biochem 1969;11:405–412. [PubMed: 4983848]
15. Oshikane H, et al. Structural basis of RNA-dependent recruitment of glutamine to the genetic code. Science 2006;312:1950–4. [PubMed: 16809540]
16. Bailly M, Giannouli S, Blaise M, Stathopoulos C, Kern D, Becker HD. A single tRNA base pair mediates bacterial tRNA-dependent biosynthesis of asparagine. Nucleic Acids Res 2006;34:6083–6094. [PubMed: 17074748]

17. Tumbula-Hansen D, Feng L, Toogood H, Stetter KO, Söll D. Evolutionary divergence of the archaeal aspartyl-tRNA synthetases into discriminating and nondiscriminating forms. J. Biol. Chem 2002;277:37184–90. [PubMed: 12149259]

18. Milligan JF, Groebe DR, Witherell GW, Uhlenbeck OC. Oligoribonucleotide synthesis using T7 RNA polymerase and synthetic DNA templates. Nucleic Acids Res 1987;15:8783–98. [PubMed: 3684574]

19. Knowlton RG, Yarus M. Discrimination between aminoacyl groups on su^+ 7 tRNA by elongation factor Tu. J. Mol. Biol 1980;139:721–32. [PubMed: 6157832]

20. Ibba, M.; Francklyn, CS.; Cusack, S. The Aminoacyl-tRNA Synthetases, Landes Bioscience. Georgetown, Texas: 2005.

21. Giege R, Sissler M, Florentz C. Universal rules and idiosyncratic features in tRNA identity. Nucleic Acids Res 1998;26:5017–35. [PubMed: 9801296]

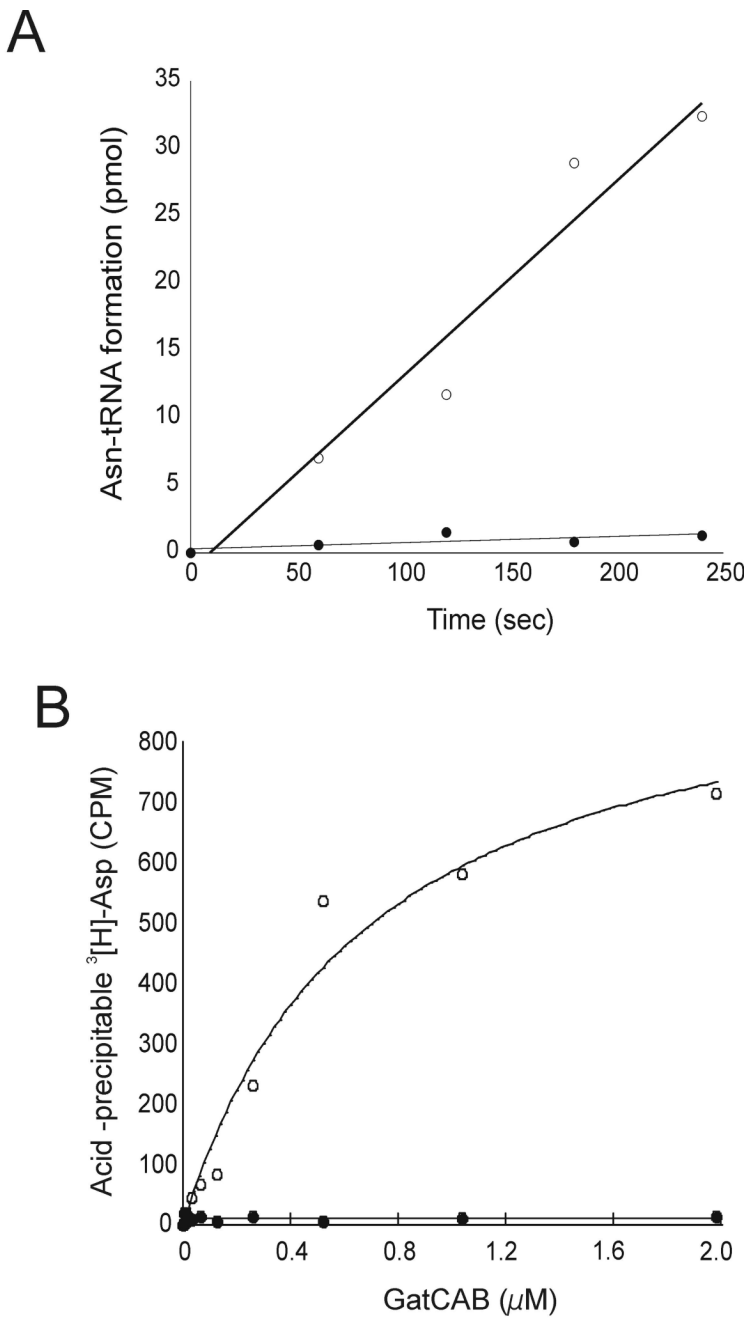

Figure 1.

Recognition of Asp-tRNA^{Asn} and Asp-tRNA^{Asp} by GatCAB. (A) Time course of transamidation of Asp-tRNA^{Asn} (o) and Asp-tRNA^{Asp} (●). [^{14}C]Asp-tRNA^{Asn} or [^{14}C]Asp-tRNA^{Asp} (0.5 μM) were incubated with *M. thermautotrophicus* GatCAB (10 nM) at 37°C. (B) RNase A protection analysis of GatCAB:Asp-tRNA complexes. [^{3}H]Asp-tRNA^{Asn} (o) (10 nM) and [^{3}H]Asp-tRNA^{Asp} (●) (10 nM) were mixed with 0-2000 nM of GatCAB (A) and treated with RNase mix (10μg) for 30 seconds at 4°C. The residual radioactivity on the filter after TCA washing was determined as described in Materials and Methods.

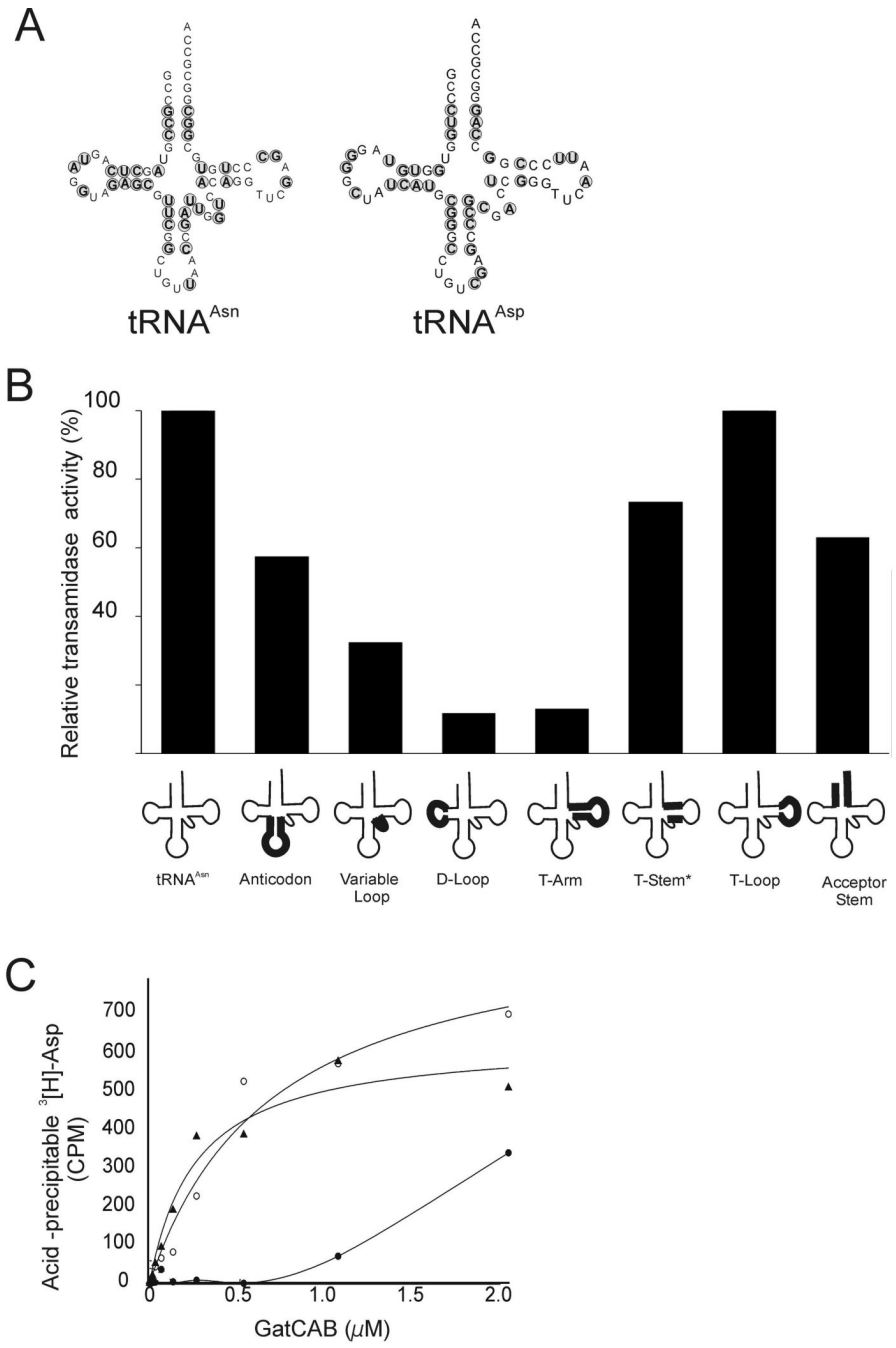

Figure 2.

Effect of domain swap tRNA^{Asn} mutants on transamidation activity and binding to GatCAB. (A) Clover-leaf representation of *M. thermautotrophicus* tRNA^{Asn} and tRNA^{Asp}. Sequence differences between the two tRNAs are circled. (B) Relative transamidation activities of various Asp-tRNA^{Asx} mutants and wild-type Asp-tRNA^{Asn}. Transamidation activity was measured as described in Materials and Methods with 1 μM wild-type Asp-tRNA^{Asn} or mutant Asp-tRNA^{Asx} and relative activity was expressed as ratio of conversion rate of wild-type Asp-tRNA^{Asn} to Asn-tRNA^{Asn}. The T-stem* swap did not include the first base pair of the stem. (C) RNase A protection analysis of GatCAB:Asp-tRNA complexes. Wild type Asp-

$tRNA^{Asn}$ (o) and swap $tRNA^{Asn}$ mutant with T-arm (●) or anticodon arm (▲) portion of $tRNA^{Asp}$. The curves were fitted from the experimental points.

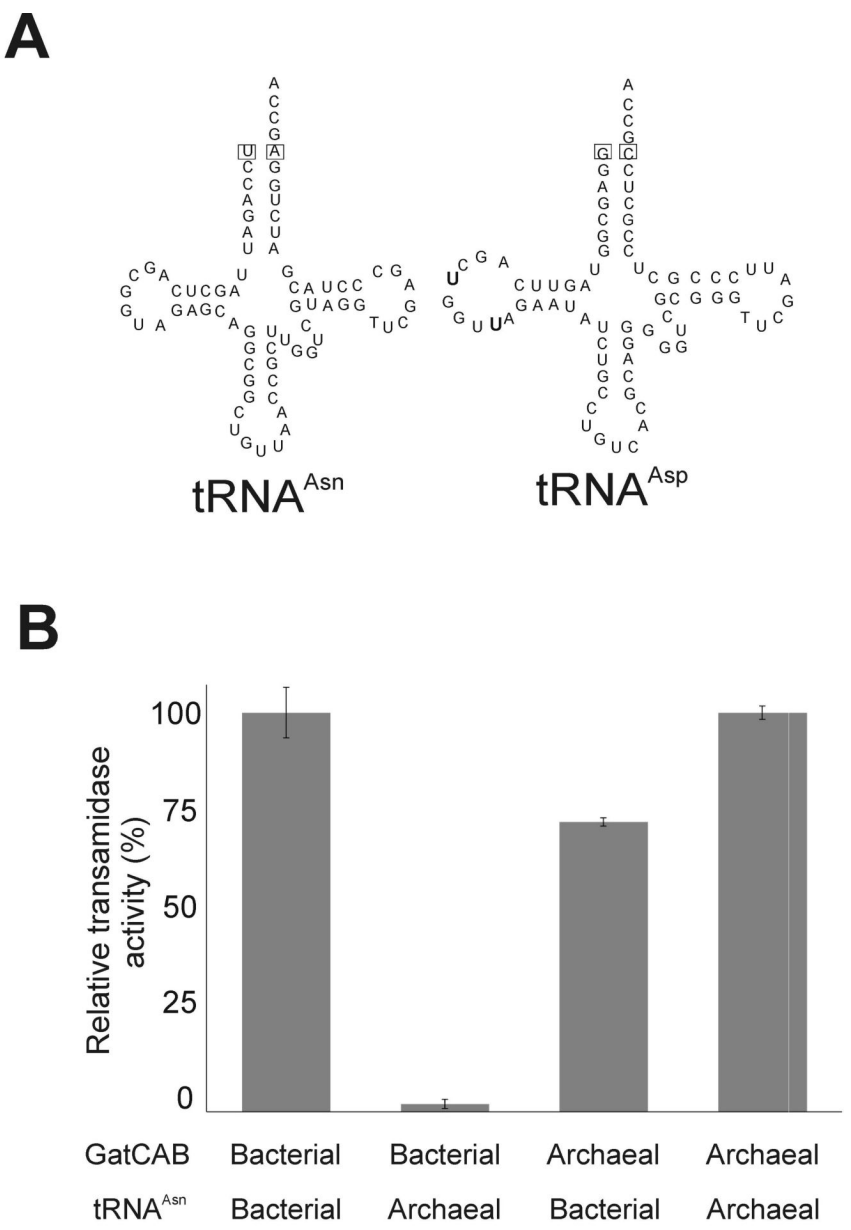

Figure 3.

Transamidation by *H. pylori* or *M. thermautotrophicus* GatCAB with homologous and heterologous Asp-tRNA^{Asn}. (A) Clover-leaf presentation of *H. pylori* tRNA^{Asn} and tRNA^{Asp} species. The important identity elements [16] are boxed. The two additional U residues in the D-loop are shown in bold. (B) Transamidation of *M. thermautotrophicus* and *H. pylori* Asp-tRNA^{Asn} by a bacterial (*H. pylori*) or archaeal (*M. thermautotrophicus*) GatCAB. Reactions were carried out over 5 minutes with 10 nM enzyme. Activities are plotted relative to the homologous reaction; e.g., the activity of the *H. pylori* GatCAB with the *M. thermautotrophicus* Asp-tRNA^{Asn} was relative to the activity of the *H. pylori* with its own Asp-tRNA^{Asn}.

Asp-AdT		AsnRS	
Species		Species	
tRNA	49/65	tRNA	49/65
A.pernix		*P. furiosus*	
Asn	GU	Asn	CG
Asp	CG	Asp	CG
A.fulgidus		*P. horikoshii*	
Asn	GC	Asn	CG
Asp	UG	Asp	CG
H. salinarum		*P. aerophilum*	
Asn	CG	Asn	CG
Asp	GC	Asp	CG
M. jannaschii		*P. abyssi*	
Asn	GC	Asn	CG
Asp	UG	Asp	CG
M. thermautotrophicus		*T. kodakaraensis*	
Asn	AU	Asn	CG
Asp	UG	Asp	CG
S. solfataricus		*T. acidophilum*	
Asn	GC	Asn	AU
Asp	CG	Asp	AU
S. tokodaii		*T. volcanium*	
Asn	GC	Asn	AU
Asp	CG	Asp	AU
S. acidocaldarius			
Asn	GC		
Asp	CG		
H. marismortui			
Asn	CG		
Asp	GC		
M. acetivorans			
Asn	GC		
Asn	GC		
Asp	UG		
M. maripaludis			
Asn	GC		
Asp	UG		
M. bakeri fusaro			
Asn	GC		
Asp	CG		
M. kandleri			
Asn	CG		
Asp	CG		
N. equitans			
Asn	CG		
Asp	CG		

[a]Species were divided by whether their genomes encode GatCAB or AsnRS. Pyrimidines are shaded grey with black lettering while purines are shaded black with white lettering.

Table 2.
Alignment of the archaeal $tRNA^{Asn}$ and $tRNA^{Asp}$ isoacceptors at positions 49 and 65 in the T-stem.[a]

Table 1

GatCAB activity with Asp-tRNA^{Asn} mutants[a]

Base change	Region in tRNA	Relative Transamidase Activity (%)
Wild-type tRNA^{Asn}		100.0
A9 → G9	D-stem	29.8
G10·C25 →A10·U25		98.7
C11·G24 →U11·A24		91.3
G13·C22 →U13·U22		70.9
U27·U43 →C27·G43	Anticodon stem	55.4
U36→A36	Anticodon loop	68.9
U36→C36		98.7
A37→G37		66.5
U47→Δ47	Variable loop	40.1
A49·U65→U49·G65	T-stem	< 0.5
A49·U65→C49·G65		62.8
A49·U65→G49·U65		49.9
A49·U65→U49·A65		< 0.5
G56→A56	T-loop	38.1
C5·G68→U5·A68	Acceptor stem	51.1
Wild-type tRNA^{Asp}		< 0.5

[a] [^{14}C]Asp-tRNA (0.5 μM) was incubated with *M. thermautotrophicus* GatCAB (10 nM) at 37°C. The enzyme activity with each variant was expressed as relative percentage of conversion rate for wild type Asp-tRNA^{Asn} to Asn-tRNA^{Asn}.