Major Anticodon-binding Region Missing from an Archaeabacterial tRNA Synthetase

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The small size of the archaeabacterial Methanococcus jannaschii tyrosyl-tRNA synthetase may give insights into the historical development of tRNAs and tRNA synthetases. The L-shaped tRNA has two major arms—the acceptor-TψC minihelix with the amino acid attachment site and the anticodon-containing arm. The structural organization of the tRNA synthetases parallels that of tRNAs. The more ancient synthetase domain contains the active site and insertions that interact with the minihelix portion of the tRNA. A second, presumably more recent, domain interacts with the anticodon-containing section of tRNA. The small size of the M. jannaschii enzyme is due to the absence of most of the second domain, including a segment thought to bind to the anticodon. Consistent with the absence of an anticodon-binding motif, a mutation of the central base of the anticodon had a relatively small effect on the aminoacylation efficiency of the M. jannaschii enzyme. In contrast, others showed earlier that the same mutation severely reduced charging by a normal-sized bacterial enzyme that has the aforementioned anticodon-binding motif. However, the M. jannaschii enzyme has a peptide insertion into its catalytic domain. This insertion is shared with all other tyrosyl-tRNA synthetases and is needed for a critical minihelix interaction. We show that the M. jannaschii enzyme is active on minihelix substrates over a wide temperature range and has preserved the same peptide-dependent minihelix specificity seen in other tyrosine enzymes. These findings are consistent with the concept that anticodon interactions of tRNA synthetases were later adaptations to the emerging synthetase-tRNA complex that was originally framed around the minihelix.

The tRNA secondary structure consists of two domains: the acceptor-TψC stem-loop and the anticodon-D stem-loop (Fig. 1) (1–3). This secondary structure forms an L-shaped tertiary fold where one domain (the acceptor-TψC minihelix) is formed by the coaxial stacking of the 5-bp TψC stem onto the 7-bp acceptor helix (1, 4, 5). It contains the amino acid attachment site at the 3′-end. For many aminoacyl-tRNA synthetases (aaRSs), the acceptor-TψC minihelix alone is a substrate for specific aminoacylation where sequence and structural elements in the acceptor helix confer specific recognition by an aaRS (6–14). The second domain contains the anticodon triplet of the genetic code and is formed by the stacking of the anticodon stem onto the D stem. The anticodon domain serves as the template (mRNA) reading head.

Aminoacyl-tRNA synthetases are universal proteins believed to have arisen early during the development of the genetic code. They are organized into two classes designated as class I and class II (15–19). This classification is based on the sequences and structures of their active site domains. Like tRNAs, aaRSs are comprised of two major domains—a conserved class-defining catalytic domain and a second domain that is not conserved even for synthetases in the same class (20–23). These two domains of the synthetase interact with the separate domains of the L-shaped tRNA. The more ancient catalytic domain interacts with the acceptor-TψC minihelix through insertions and variations within its conserved architecture that allow sequence-specific interactions with identity elements in the acceptor helix (21, 24–28). The second, nonconserved synthetase domain provides for interactions with parts of the tRNA distal to the acceptor stem such as the anticodon.

The class-defining active site domain of class I synthetases is typically in the N-terminal half, whereas the second domain makes up the C-terminal half of the protein. The second domain varies significantly in size and structure across the class. The C-domain of the typical class I tyrosyl-tRNA synthetase (TyrRS) extends for approximately 180 amino acids beyond the catalytic domain. Anticodon recognition is important for tRNA Tyr binding and aminoacylation in bacteria and has been shown explicitly to involve the C-domain. For example, a mutation in the anticodon of Escherichia coli tRNA Tyr reduces aminoacylation efficiency by 200-fold (29). Although a co-crystal structure of TyrRS with tRNA Tyr has not been solved, molecular modeling with the known crystal structure of the Bacillus stearothermophilus TyrRS and extensive mutagenesis of the C-domain provided evidence for contacts between the last third of the domain and the anticodon (30–32). The portions of the C-domain needed for these contacts are present in eukaryotic TyrRSs, including the human enzyme, although the sequences diverge widely from their bacterial counterparts.

B. stearothermophilus TyrRS is a dimer of identical 419 amino acid polypeptides (33, 34). Binding of tRNA occurs across the dimer interface, that is, the active site of one subunit binds the acceptor stem although the C-domain of the other interacts with the anticodon (30, 31, 35, 36). This model for tRNA Tyr recognition in B. stearothermophilus can be generalized for bacterial TyrRS because the bacterial TyrRS enzymes are highly homologous. (For example, E. coli and B. stearothermophilus share 52% sequence identity.) Furthermore, residues identified in the B. stearothermophilus enzyme as making anticodon contacts are conserved among bacterial organisms.
At 306 amino acids, the primary sequence of *Methanococcus jannaschii* TyrRS is significantly shorter than its eukaryotic or bacterial counterparts (Fig. 2). The small size of this enzyme is because of the absence of a significant portion of the C-domain. In particular, the region of the *B. stearothermophilus* enzyme that contains anticodon-binding residues is completely missing from the sequence of *M. jannaschii* TyrRS (37). Thus, the enzyme may have no contacts with the anticodon and may represent more closely an early tRNA synthetase that based most of its interactions on the minihelix. Significantly, the *M. jannaschii* enzyme retains a peptide insertion (known as CP1) into the catalytic domain. This insertion is widely conserved across all taxonomic domains. It is needed for a critical sequence-specific minihelix contact (38). To pursue the questions raised by the organization of the sequence of *M. jannaschii* TyrRS, we cloned, expressed, and purified the enzyme and investigated its activity on wild-type and mutant tRNA and minihelix substrates. These substrates were designed to test directly the relative role of the anticodon triplet and the minihelix, and the significance of the large missing piece of the putative anticodon-binding domain of TyrRS.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—A clone of the *M. jannaschii* tyrS gene (AMJAR37) (encodes TyrRS (39)) was obtained from the American Type Culture Collection of The Institute for Genomic Research. A 950-bp *M. jannaschii* tyrS gene (AMJAR37) (encodes TyrRS (39)) was obtained from the plasmid AMJAR37 containing the *tyrS* gene was cloned into the pET-3a-based expression vector pBAS6 (40), and the resulting construction (plasmid pBAS50) was confirmed by DNA sequencing. Plasmid pBAS50 was used for expression of *M. jannaschii* TyrRS in *E. coli*.

*M. jannaschii* TyrRS was purified from *E. coli* strain BL21(DE3) (41). Cultures were grown and harvested as described (40). The cells were resuspended in 10 ml of lysis buffer (20 mM sodium phosphate (pH 7.5), 100 mM NaCl, 10 mM β-mercaptoethanol, and 10% glycerol). Following the addition of 10 μl of a saturated isopropanol solution of phenylmethylsulfonyl fluoride, the cells were lysed by a French press (12,000 p.s.i.). After lysis, 1.2 ml of 2% protamine sulfate (Sigma) was added to precipitate nucleic acids. The lysate was incubated on ice for 15 min with periodic mixing and then centrifuged at 180,000 × g for 10 min. The supernatant was treated with an additional 1.2 ml of 2% protamine sulfate, incubated on ice for 15 min, and subsequently centrifuged at 180,000 × g for 30 min. Host cellular proteins were denatured by heating the supernatant at 85 °C for 20 min and then pelleted by centrifugation (180,000 × g for 30 min), thus leaving *M. jannaschii* TyrRS in the supernatant. The purified enzyme was concentrated with a Centricon (Amicon, Beverly, MA) and stored in 50% glycerol at -20 °C. Enzyme concentrations were determined by the Bradford protein assay (42) using bovine serum albumin as a standard.

**RNA Preparation and Construction of Mutant tRNA**—Crude yeast tRNA was obtained from Roche Molecular Biochemicals and crude *E. coli* tRNA from Sigma. Minihelix RNA substrates were chemically synthesized as described previously (43, 44) and were purified using denaturing electrophoresis (16% polyacrylamide, 8 μm urea) followed by electroelution and ethanol precipitation. The RNA pellet was resuspended in 2 mM Tris-HCl (pH 8.0), 0.2 mM EDTA, and concentration was determined by absorbance at 260 nm (ε260 = 3.2 × 104). *M. jannaschii* tRNA(77) was transcribed in vitro from a linearized plasmid DNA template and processed using the hammerhead ribozyme (45). Specificity of EcoRI-BamHI ribozyme (tRNA(tranzyme) gene under the control of a T7 RNA polymerase promoter was constructed by annealing and ligating together four DNA oligonucleotides. The resulting 150-bp fragment was then ligated into EcoRI-BamHI-digested plasmid pLYSF119 (46) to give plasmid pBS82. This pUC119-based plasmid contains a FokI site at the 3′-end of the tRNA gene that allows the generation (by in vitro run-off transcription (47)) of a transcript having the correct 3′-CCA terminus. The transcript encoded by the tranzyme gene consisted of the hammerhead ribozyme fused to the 5′-end of *M. jannaschii* tRNA(77). The autocatalytic activity of the transcript efficiently cleaved itself to generate a tRNA with the correct 5′-nucleotide. The U36G mutation in *M. jannaschii* tRNA(77) was introduced by overlap extension polymerase chain reaction mutagenesis using the Quickchange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The resulting mutant gene was verified by DNA sequencing.

In vitro transcription reactions were performed as described earlier (40) (with the exceptions that 25 mM MgCl2 was used and 4 μg/ml yeast pyrophosphatase was added) using FokI-linearized plasmid as a template. The transcript was cleaved in cis by the hammerhead ribozyme at 60 °C in a solution of 4 mM Tris-HCl (pH 8.0), 0.2 mM EDTA, and 30 mM MgCl2. To reduce the interaction of cleaved tRNA with uncut transcript, the transcript was taken through 12–14 cycles (0.5 min at 95 °C and 1 min at 60 °C) in a thermocycler (Robocycler Gradient 96, Stratagene, La Jolla, CA). This thermocycling resulted in 80–90% cleavage. The tRNA was isolated from free ribozyme and uncleaved transcript using denaturing electrophoresis (see above for minihelix purification). Concentrations were determined by absorbance at 260 nm (ε260 = 7.0 × 104) and were consistent with those determined by measurements of total amino acid content (1600 pmol/μg). Prior to assay, RNA substrates were heated to 95 °C followed by slow cooling to 55–60 °C at which time MgCl2 was added (2 mM final concentration) with further cooling of the sample to room temperature.

Thermal melting curves for tRNA(77) and minihelix(77) were obtained by monitoring the change in absorbance at 260 nm over a temperature range of 30–95 °C using a Cary 3E UV-visible spectrophotometer equipped with a temperature controller. Melts were performed in 20 mM PIPES buffer (pH 7.5 at room temperature), 20 mM KCl, and 10 mM MgCl2. Where possible, the thermal transition midpoint (Tm) was estimated from the first derivative of the melting profile.

**Size-exclusion Chromatography**—Fast protein liquid chromatography size-exclusion chromatography was performed using a Pharmacia Superose-12 HR 10/30 column equilibrated with 20 mM sodium phosphate (pH 7.5), 100 mM NaCl, 0.1 mM DTT, and 1 mM 4-dithiothreitol. Using a flow rate of 0.4 ml/min, 200 μl of sample (approximately 1 mg/ml concentration) was injected, and eluted protein was subsequently detected by UV absorption measurement (280 nm). The solution molecular size of *M. jannaschii* TyrRS was compared with several standard proteins (66 kDa bovine serum albumin, 45 kDa ovalbumin, 31 kDa carbonic anhydrase, and 14 kDa lysozyme) using a standard curve of log Mm versus retention time on the column.

*Fig. 1.* Sequences and base pairing of *M. jannaschii* tRNA(Tyr) showing the tRNA cloverleaf structure and the formation of the L-shaped tertiary fold together with the minihelix. The sequences of the tRNA and minihelix correspond to the synthetic RNAs studied here. The tRNA has two main domains: the TψC minihelix domain (shaded) and the anticodon domain. Dashed lines show tRNA structural interactions. The positions of the central anticodon mutation in the tRNA and the 1:72 pair mutation in the minihelix are indicated.
Enzyme Assays—Aminoclaylation assays were typically performed at 45 °C in a reaction mixture consisting of 50 mM sodium phosphate (pH 7.5), 1 mM 1,4-dithiothreitol, 10 mM MgCl₂, 3 mM ATP, 100 μM tyrosine, 0.04 μCi/μl [³H]tyrosine, and 5–60 μM tRNA transcript or 100 μM crude tRNA (concentration of tyrosine acceptance was determined to be ~5 μM), or 15–100 μM minihelix. For the analysis of the temperature dependence of aminoclaylation rates, 20 mM PIPES buffer (adjusted to pH 7.5 with HCl) was used rather than sodium phosphate buffer to avoid any precipitation of MgPO₄ at higher temperatures. (The pH of PIPES buffer was measured at room temperature and does not change by more than 0.5 units over the temperature range assayed (48).) Reactions were initiated with the addition of TyrRS (typically 250 nM for tRNA aminoclaylation and 2.5–15 μM enzyme for minihelix aminoclaylation). As described previously (49), aliquots were removed at appropriate time intervals and spotted onto trichloroacetic acid-soaked Whatman (grade 3) filter pads; the pads were washed with 5% trichloroacetic acid and then subjected to liquid scintillation counting.

Kinetic parameters (k_cat and K_m) for aminoclaylation were determined from initial rates with amino acid and ATP at saturating concentration (100 μM and 3 mM, respectively) over a tRNA concentration range of at least 10-fold. (The initial rate of aminoclaylation was unchanged over a tyrosine concentration range of 20–100 μM and an ATP concentration range of 1–3 mM (data not shown).) Initial (linear) rates were measured using a minimum of four time points and were determined two or more times at each substrate concentration. Values of k_cat and K_m were calculated by direct fitting of the Michaelis-Menten equation using nonlinear regression analysis (50) (Microcal Origin, MicroCal Software Inc., Northampton, MA). For the minihelix, k_cat/K_m was estimated from the slope of a double reciprocal plot because k_cat and K_m could not be reliably determined because of limitations on the concentration of the RNA substrate that could be achieved.

RESULTS

Purification and Characterization of M. jannaschii TyrRS—The M. jannaschii TyrRS gene encodes an enzyme with a calculated molecular mass of 35,049 daltons. The gene was cloned into a pET-3a-based vector under the control of the T7 RNA polymerase promoter to provide high levels of expression. Heat purification of the overexpressed enzyme (see “Experimental Procedures”) enabled the isolation of approximately 20 mg of pure enzyme per liter of cell culture. The purified enzyme was subject to SDS-polyacrylamide gel electrophoresis and migrated as a single band corresponding to a molecular mass of approximately 35 kDa (data not shown).

Because the bacterial and human TyrRSs are dimeric, we wanted to determine whether the missing C-terminal segment of M. jannaschii TyrRS affected the quaternary structure. If so, then use of the aforementioned model for the TyrRS-tRNA Tyr complex (based on the B. stearothermophilus and E. coli systems) would be questionable. Using size-exclusion fast protein liquid chromatography, a standard curve of log M_r against retention time was generated with several protein standards (see “Experimental Procedures”). M. jannaschii TyrRS was then passed through the column and its retention time was measured. When its elution position was plotted on a standard curve, M. jannaschii TyrRS clearly fell near bovine serum albumin (66 kDa), thus establishing that the enzyme is dimeric (Fig. 3).

Anticodon Recognition—Given the importance of the anticodon for tRNA recognition by many class I enzymes (51) including TyrRS (29, 52), we were interested in testing the significance of anticodon recognition by the M. jannaschii enzyme. The absence of a major anticodon-binding motif suggested that the anticodon might not be particularly important for charging of M. jannaschii tRNA Tyr. For E. coli TyrRS, a U36G mutation of the central anticodon base of E. coli tRNA Tyr affected both k_cat and K_m parameters to give an overall reduction in k_cat/K_m of more than 200-fold (29). As illustrated in Fig. 2, M. jannaschii TyrRS lacks the anticodon-binding region identified in bacteria. Therefore, wild-type and U36G mutant M. jannaschii synthetases were prepared by in vitro transcription and tested with the M. jannaschii enzyme. We found that the U36G substitution had a small effect on k_cat and no effect on the K_m for tRNA. The overall change in k_cat/K_m was only 6-fold (Table I). Thus, unlike the bacterial enzyme, M. jannaschii TyrRS does not appear to make direct contact with the central base of the anticodon.

Species-specific tRNA Recognition—The CP1 insertion (Fig. 2) in bacterial and eukaryotic TyrRSs is needed for discrimination of the first base pair of the acceptor stem are species-specific determinants for tRNA recognition by many class I enzymes (51, 52). The G1:C72 pair is required for aminoacylation by eukaryotic TyrRSs and a C1:G72 pair is required for aminoacylation by bacterial enzymes. A small segment of 39 amino acids within CP1 is responsible for C1:G72 discrimination. For example, amino acid peptide (between bacterial and human enzymes) is sufficient to switch the specificity of recognition at the 1:72 position (38). As in eukaryotes, M. jannaschii tRNA Tyr and other archaebacterial tyrosine tRNAs have a C1:G72 base pair (53). These base pairs at the beginning of the acceptor stem are species-specific determinants for tRNA Tyr recognition (38, 54, 55). The G1:C72 discrimination. For example, a swap of the 39 amino acid peptide (between bacterial and human enzymes) is sufficient to switch the specificity of recognition at the 1:72 position (38). As in eukaryotes, M. jannaschii tRNA Tyr and other archaebacterial tyrosine tRNAs have a C1:G72 base pair (53). Thus, given the presence of the CP1 insertion in M. jannaschii TyrRS, we thought that it might charge eukaryotic tRNA Tyr because of specificity for a C1:G72 pair.

To test this hypothesis, we compared the aminoacylation of...
Minihelix Aminoacylation—RNA minihelices are substrates for sequence-specific minihelix aminoacylation by many tRNA synthetases including TyrRS (12, 38, 55). To investigate minihelix aminoacylation on the natural tRNA substrate that contains modified bases. For example, the natural yeast tRNA substrate was acylated at a rate about 8-fold higher than that achieved with the transcript of M. jannaschii tRNA (~Tc (data not shown).) The average standard error obtained from non-linear regression is given.

### Table 1

Kinetic parameters for tyrosine aminoacylation of wild-type and anticodon-mutant tRNA substrates by M. jannaschii TyrRS at pH 7.5 and 45 °C

| tRNA            | k_{cat} | K_M | k_{cat}/K_M |
|-----------------|---------|-----|-------------|
| Wild-type tRNA^Tyr | 15 ± 1  | 15 ± 3 | 1.0         |
| U36G tRNA^Tyr    | 2.0 ± 0.2 | 12 ± 4 | 0.17        |

FIG. 3. Plot illustrating the dimeric nature of M. jannaschii TyrRS determined by size-exclusion chromatography. The molecular mass of the M. jannaschii TyrRS monomer is 35 kDa (calculated from its primary sequence). Protein standards are described under “Experimental Procedures.”

FIG. 4. Comparison of aminoacylation of yeast (S. cerevisiae) and bacterial (E. coli) crude tRNA. Yeast tRNA^Tyr has a C1:G72 pair, and bacterial tRNA^Tyr has a G1:C72 pair (53). Activity was assayed at pH 7.5 and 45 °C as described under “Experimental Procedures.”

FIG. 5. Minihelix aminoacylation and specificity for C1:G72 of M. jannaschii TyrRS. Activity was assayed at pH 7.5 and 45 °C as described under “Experimental Procedures.”

eukaryotic (Saccharomyces cerevisiae) and bacterial (E. coli) tRNA by M. jannaschii TyrRS. The M. jannaschii enzyme efficiently aminoacylated yeast tRNA (C1:G72 tRNA^Tyr) but was unable to aminoacylate E. coli tRNA (G1:C72 tRNA^Tyr) with tyrosine (Fig. 4). The same selectivity for yeast tRNA is seen with the Pneumocystis carinii and human TyrRSs (38, 55). In this respect, therefore, the M. jannaschii enzyme behaves like a eukaryotic TyrRS. In addition, the capacity for G:C versus C:G specificity is exactly as expected given the presence of the CP1 insertion in M. jannaschii TyrRS.

Minihelix Aminoacylation—RNA minihelices are substrates for sequence-specific minihelix aminoacylation by many tRNA synthetases including TyrRS (12, 38, 55). To investigate minihelix aminoacylation, an RNA substrate based on the acceptor^Tc/C minihelix of M. jannaschii tRNA^Tyr was synthesized and tested. (As a negative control, a 16-nucleotide single-stranded RNA that included the GACCA sequence at the 3′-end of minihelix^Tyr was not aminoacylated by the enzyme (data not shown).) The acceptor^Tc/C minihelix was aminoacylated with tyrosine by M. jannaschii TyrRS (Fig. 5). Aminoacylation of the minihelix at 45 °C was reduced relative to the full-length tRNA by about 3 orders of magnitude (k_{cat}/K_M = 10 s^{-1} M^{-1} versus 10^4 s^{-1} M^{-1} (data not shown)) compared with the tRNA transcript. The difference in rates is much less at 85 °C (see below). (When acceptor stem substrates for mesophilic class I tRNA synthetases (glutaminyl-, methionyl- and valyl-tRNA synthetases) are compared with the full tRNA, the relative aminoacylation efficiency is reduced as much as 10^5–10^6-fold (8, 56–59).) As observed for eukaryotic TyrRS (38, 55), aminoacylation by the archaeabacterial synthetase was specific for the C1:G72 terminal base pair. Thus, the species-specific aminoacylation of tRNA^Tyr by the M. jannaschii enzyme is consistent with its inability to charge a minihelix with a G1:C72 base pair.

Effect of Temperature on Aminoacylation—M. jannaschii is a thermophilic organism that grows over a broad temperature range with an optimal growth temperature of 85 °C (39). As such, we were curious as to what effect increasing temperature might have on the rate of aminoacylation in vitro. More specifically, we wanted to compare the aminoacylation rate of the M. jannaschii tRNA transcript to that of the minihelix. M. jannaschii tRNA^Tyr is highly G-C-rich and, with the exception of one G-U mismatch, all other pairs in the acceptor^Tc/C minihelix are G-C (Fig. 1). Because of this high G-C content, the acceptor^Tc/C minihelix would be expected to remain double-stranded at high temperatures and, therefore, be in a form suitable for aminoacylation.

We found that the initial rate for aminoacylation of the minihelix increased 13-fold over a temperature range of 45–85 °C (Fig. 6). Thus, the rate was highest at the optimal growth temperature for M. jannaschii (85 °C) (39). (For technical reasons, we were unable to investigate temperatures above 85 °C.) In contrast, whereas the aminoacylation rate for the tRNA...
substrate initially increased with temperature (up to 75 °C), the rate at 85 °C was significantly diminished to a value of about one-fifth the rate at 75 °C. Although the initial rate of aminoacylation of tRNA^Tyr^ is about 400-fold greater than that of the minihelix at 45 °C, the rate of aminoacylation of tRNA is only 20-fold greater at 85 °C. Thus, at higher temperatures, the aminoacylation rate for the minihelix approaches that of the tRNA.

Assuming that the second domain of the tRNA contributes to recognition, our data are consistent with the premise that the tertiary structure of the tRNA is disrupted at 85 °C. This disruption may decrease the rate of aminoacylation of tRNA because contacts with parts of the tRNA outside of the minihelix are lost. However, the rate of aminoacylation of the TöC minihelix is maximal at 85 °C, suggesting that the minihelix remains intact at this temperature. We found that, in the same buffer used for the aminoacylation assays (20 mM PIPES (pH 7.5), 20 mM KCl, and 10 mM MgCl₂), the tertiary structure of the tRNA^Tyr^ transcript melts with a T_m of about 73 °C, whereas the T_m for the minihelix is near 85 °C (data not shown).

**DISCUSSION**

As with the bacterial and eukaryotic TyrRSs, we have shown that specific recognition of the TöC minihelix in the archael synthetase involves the 1:72 acceptor stem base pair (Fig. 5). Recognition of this pair requires the CP1 peptide of about 60 amino acids that is inserted into the Rossman fold of the catalytic domain (38). In contrast to the C-domain, the size of the CP1 insertion is conserved across bacteria, archaea, and eukaryotes. Thus, whereas M. jannaschii TyrRS is missing a critical anticodon-binding piece of the C-domain, the minihelix-recognizing CP1 piece is retained. The conservation of the class-defining, active-site domain of TyrRS, with the CP1 insertion for minihelix recognition, is consistent with the idea that specific recognition of two to four nucleotides in the acceptor stem. The relationship between these sequences/structures in minihelices and specific amino acids constitutes an operational RNA code for amino acids (14, 21, 27, 63). The duplication and recombination of minihelices can lead to the full two-domain tRNA structure, where the anticodon-containing domain originated from the original minihelix (14, 64, 65). In this scenario, the triplets of the code arose from nucleotides associated with the operational RNA code (14, 61, 64–67).

In the high temperature environment of thermophiles such as M. jannaschii, the docking of the minihelix on one subunit may facilitate folding of the second domain of the tRNA so that it can dock with the sister subunit of TyrRS. This minihelix-initiated "induced fit" may explain why the charging rate of the full tRNA at 85 °C is still higher than that of the minihelix alone. In the natural environment, modified bases in M. jannaschii tRNA^Tyr^ may help to stabilize the tRNA structure above 73 °C (which corresponds to the T_m of the transcript).

Structure-specific tRNA binding proteins, such as the recently described Trbp111 (68), may also play a role in stabilizing the L-shape in the high temperature environment of thermophilic organisms.

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