Suppressor mutations in *Escherichia coli* methionyl-tRNA formyltransferase: Role of a 16-amino acid insertion module in initiator tRNA recognition

(protein synthesis initiation/tRNA–protein interactions/genetic suppression)

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ABSTRACT The specific formylation of initiator methionyl-tRNA (Met-tRNA) by methionyl-tRNA formyltransferase (MTF; EC 2.1.2.9) is important for the initiation of protein synthesis in eubacteria and in eukaryotic organelles. The determinants for formylation in the tRNA are clustered mostly in the acceptor stem. As part of studies on the molecular mechanism of recognition of the initiator tRNA by MTF, we report here on the isolation and characterization of suppressor mutations in *Escherichia coli* MTF, which compensate for the formylation defect of a mutant initiator tRNA, lacking a critical determinant in the acceptor stem. We show that the suppressor mutant in MTF has a glycine-41 to arginine change within a 16-amino acid insertion found in MTF from many sources. A mutant with glycine-41 changed to lysine also acts as a suppressor, whereas mutants with changes to aspartic acid, glutamine, and leucine do not. The kinetic parameters of the mutant with glycine-41 changed to lysine also acts as a suppressor mutant in MTF that come close to the 3′ end of the initiator tRNA, showed recently that the 3′ end could be crosslinked to a single lysine residue, Lys-206,† of MTF (10). The crystal structure of MTF has been determined recently (11). The enzyme consists of two domains connected by a linker region. The N-terminal domain is strikingly homologous to the anticodon binding domain of *E. coli* lysyl-tRNA synthetase and to the oligonucleotide or oligosaccharide binding domain of other proteins and has been shown to bind tRNA on its own (11). This paper was submitted directly (Track II) to the Proceedings office. Abbreviations: MTF, methionyl-tRNA formyltransferase; GARF, glycineamidase ribonucleotide formyltransferase; CAT, chloramphenicol acetyltransferase; GlnRS, glutaminyl-tRNA synthetase.

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‡ The amino acid numbering of MTF is different from that used previously by us (10). It has been changed to conform with the numbering system used by Schmitt et al. (11) in their description of the crystal structure of MTF. This numbering system (11) is based on the sequence of the protein, which like most proteins lacks the N-terminal methionine encoded in the gene.

The *E. coli* GARG also contains a loop between the second β-strand and the second α-helix. However, the loop contains only 3 amino acids instead of the 16 in the *E. coli* and other MTFs shown on Fig. 2. Yeast mitochondrial MTF has a longer insertion of 37 amino acids.
specific mutagenesis without changing the coded sequence, Leu-80–Gln-81. The fnt gene was then amplified by PCR with the plasmid pACDFMT as template and cloned into the BamHI and BglII sites of pOE16 (resistant to ampicillin, Qiagen, Chatsworth, CA) to generate pOE16 FMTp. The derived amino acid sequence of the recombinant fusion protein is MRGS(\textsuperscript{16}SESL---\textsuperscript{19}NRLV\textsuperscript{34})RSHHHHHHH in which the amino acid sequence within the parentheses corresponds to MTF.

**Mutagenesis of the fnt Gene.** Cells were grown at 37°C in 2× YT medium; the concentrations of antibiotics when used were: ampicillin (Amp), 100 μg/ml; tetracycline (Tet), 15 μg/ml; and chloramphenicol (Cam), 100 μg/ml. N-Methyl-\textsuperscript{14}N-nitro-N-nitosoguanidine was used to treat E. coli DH5\textsuperscript{a} carrying the plasmid pACDFMT as described (21, 22), and the plasmid DNA was isolated from the mutagenized culture to yield a random pool of mutants (23). Suppressor mutations in the fnt gene were identified from this pool of mutants by transforming E. coli CA274 carrying the pRSVCATam1.2.5\textsuperscript{t}rnfM/UM35A36/G72G73/GlnRS plasmid. The transformants were grown for 4 hr in the presence of ampicillin and tetracycline and spread on plates containing (in addition) chloramphenicol. Plasmids were isolated from 20 chloramphenicol-resistant colonies, and the pACD plasmids containing the fnt gene and conferring resistance to chloramphenicol were isolated. The mutation in these was localized to an \textsuperscript{16}1-kbp BsHII fragment bearing the fnt gene (including 100 bp upstream but lacking the sequences coding for the C-terminal 20 amino acids) by subcloning and identified by DNA sequencing.

**Site-Specific Mutagenesis of the Gene for MTF and Puriﬁcation of Mutant MTF Proteins.** Mutations at position 41 to a K, L, Q, or D were generated by QuikChange mutagenesis with Pfu DNA polymerase (Stratagene) by using the plasmids pACDFMT or pOE16 FMTp. E. coli JM109 was used as host to express the MTF–His\textsubscript{6} fusion proteins. The cultures (500 ml) were grown for 4 hr at 37°C in the presence of ampicillin, induced by the addition of isopropyl \textsubscript{14}D-thiogalactoside (0.5 mM, final), and the incubation was continued for 2 more hr. Cells were harvested by centrifugation and resuspended in 20 ml of buffer 1 (20 mM Tris-HCl, pH 8.0/100 mM NaCl), 1 ml of lysozyme (2 mg/ml in buffer 1), and 0.5 ml of 100 mM phenylmethylsulfonyl fluoride. The suspension was incubated at room temperature for 20 min and freeze thawed once; 250 μl of DNase I (2.5 mg/ml in 100 mM MgCl\textsubscript{2}) was added and incubated for a further 20 min. The lysate was clarified by ultracentrifugation (100,000 \times g) and applied onto a Talon Sepharose \textsubscript{Co\textsuperscript{2+}} metal affinity resin column (24) (CLONTECH, 5.0-ml bed volume) preequilibrated with buffer 1. The column was sequentially washed with buffer 1 (50.0 ml) and buffer 1 + 10 mM imidazole-HCl, pH 8.0 (50.0 ml). The bound protein was eluted with buffer 1 + 100 mM imidazole-HCl, pH 8.0, and dialyzed against 20 mM imidazole-HCl, pH 7.5, 150 mM KCl, 50% glycerol, 10 mM 2-mercaptoethanol. Protein was estimated by the dye binding assay (25) with IgG as standard. Recovery of the protein ranged from 10 to 20 mg.

The purity of MTF was monitored by SDS/polyacrylamide gel electrophoresis.

**Measurement of Kinetic Parameters in Formylation of tRNA.** The substrate tRNAs (tRNA\textsubscript{A\textsuperscript{net}} and the U35A36, U35A36/G72G73 mutants) were expressed and purified by gel electrophoresis (8, 26). The assay for formylation used a two-step reaction. tRNA was first quantitatively aminocylated by using either \textsuperscript{35}S-methionine and methionyl-tRNA synthetase or \textsuperscript{3}H-glutamine and GlnRS and then formylated with \textsuperscript{15}N-formyltetrahydrofolate and purified MTF. For the aminocytlation step, the incubation mixture (10 μl) contained 20 mM imidazole-HCl, pH 7.5, 0.1 mM Na\textsubscript{2}EDTA, 2 mM ATP,
RESULTS

Isolation of Suppressor Mutations in MTF. The strategy for isolation of suppressor mutations in MTF is based on the use of the CATam1.2.5 gene carrying the UAG initiation codon as a reporter gene in cells also carrying a mutant initiator tRNA, which can read UAG as an initiation codon but which is inactive in initiation because it is defective in formylation (Fig. 3 Left). The mutant initiator tRNA used for this purpose contains the U35A36 anticodon sequence mutation and the G72G73 mutation in the acceptor stem and the discriminator base, which makes it an extremely poor substrate for MTF (Fig. 4). This mutant tRNA is essentially inactive in initiation even in cells overproducing MTF (20). Because of the anticodon sequence change, the mutant tRNA is aminoacylated with glutamine instead of methionine (28). To ensure that the mutant tRNA was maximally aminoacylated with glutamine, E. coli GlnRS was overproduced by including the gene for GlnRS (29) on the same plasmid as the CATam1.2.5 gene and the mutant tRNA gene (Fig. 3 Left).

E. coli DH5α F’ cells carrying the gene for MTF in the pACDFMT plasmid (Fig. 3 Right) were mutagenized with N-methyl-N’-nitro-N-nitrosoguanidine, and plasmid DNA was isolated from the mutagenized culture. The pool of mutagenized pACDFMT plasmid DNA was then used to transform E. coli CA274 carrying the pRSVCAm1.2.5 plasmid (Fig. 3 Left), and the transformants were selected for growth on plates containing ampicillin, tetracycline, and chloramphenicol. Twenty chloramphenicol-resistant colonies were obtained on plates containing ampicillin, tetracycline, and chloramphenicol. The pool of mutants isolated from the mutagenized culture. The pool of mutants carrying the pRSVCAm1.2.5 plasmid showed that four of these conferred chloramphenicol resistance on the transformants. All four contained the same mutation in the MTF gene (details under Materials and Methods), a GGA → AGA change resulting in a Gly-41 to Arg-41 change in MTF. It is not known whether these mutants are siblings or independent. The G-C to A-T transition mutation in the MTF gene is consistent with the known preference of N-methyl-N’-nitro-N-nitrosoguanidine-induced mutations (21).

The chloramphenicol resistance phenotype of E. coli CA274 carrying the pRSVCAm1.2.5 and the mutant pACDFMT plasmids is also reflected in the increase in CAT activity in extracts of these cells. CAT activity in extracts of four independent transformants carrying the mutant MTF gene on plasmids increased by factors of 5.5–8.8 compared with those carrying the wild-type MTF on plasmids (data not shown).

Allele Specificity of Suppression. The G41R suppressor mutant of MTF did not compensate for the formylation defect of another mutant tRNA, which is also inactive in initiation because it is an extremely poor substrate for formylation. The mutant (Mi:2/5) tRNA, derived from E. coli elongator methionine tRNA (30, 31), has the features in the acceptor stem important for formylation but is an extremely poor substrate for formylation because it is aminoacylated with lysine.

Generation of Gly-41 to Asp, Gln, Leu, and Lys Mutants in MTF by Site-Specific Mutagenesis. To understand the mechanism by which the G41R mutant compensates for the formylation defect of the U35A36/G72G73 mutant initiator tRNA, other mutants were generated at this site by site-specific mutagenesis. The mutant MTF genes were introduced into E. coli CA274 carrying the pRSVCAm1.2.5 plasmid, and the transformants were screened for phenotypic resistance to chloramphenicol. The G41K mutant also rescued the formylation defect of the U35A36/G72G73 tRNA, whereas the G41D, G41L, and G41Q mutants did not. The G41D, G41L, and G41Q mutant MTFs were stably overproduced in E. coli CA274 to the same levels as wild-type MTF. These and other results (described below on the purified proteins) indicate that the presence of a positive charge instead of Gly at position 41 is necessary for suppression of the formylation defect of the U35A36/G72G73 mutant initiator tRNA.

Purification and Catalytic Properties of the Mutant Enzymes. For detailed analysis of the properties of the mutant enzymes, it is necessary to obtain them in homogenous form and free of any contamination from wild-type MTF. This was achieved by expressing the wild-type and the mutant MTFs as His-tagged proteins and purifying them by chromatography on Co2+ chelate columns (24). The proteins thus obtained are...
The gel was stained with Coomassie blue R-250.

wild-type (WT) and the G41R and G41K suppressor mutants of MTF.

fragment of MTF.

U35A36 mutant tRNA behaved similarly to the wild-type anticodon sequence mutation or because of a preference for tRNA could be because of their preference for the U35A36 mutants of MTF on the U35A36

ator tRNA was also used as a substrate in these studies to

activity of wild-type MTF (data not shown).

negligible effect—at the most a 1.5- to 2-fold effect—on the wild-type initiator tRNA. Addition of the His-tag had a

surement of catalytic properties, we studied the effect of

Materials and Methods

acids at the N terminus and eight at the C terminus (see

analysis of the fragment (data not shown).

Fig. 6 compares the relative activities in formylation of the wild-type and mutant initiator tRNAs by the wild-type and the mutant enzymes. The kinetic parameters in formylation of the wild-type and mutant MTFs and

wild-type MTF and the G41R and G41K suppressor mutants of MTF on the wild-type and U35A36 mutant initiator tRNAs. The U35A36 mutant initiator tRNA was also used as a substrate in these studies to examine whether any increased activity of the suppressor mutants of MTF on the U35A36/G72G73 mutant initiator tRNA could be because of their preference for the U35A36 anticodon sequence mutation or because of a preference for glutamine over methionine attached to the tRNA. The U35A36 mutant tRNA behaved similarly to the wild-type initiator tRNA in these assays and differently from the

G41K mutants compensate for the strong formylation defect of the U35A36/G72G73 tRNA, the compensation is only

Table 1.

Table 1. Steady-state kinetic parameters in formylation using wild-type and mutant MTFs and wild-type and mutant initiator tRNAs

<table>
<thead>
<tr>
<th>tRNA used</th>
<th>Enzyme</th>
<th>$K_m$, µM</th>
<th>$k_{cat}$, s$^{-1}$</th>
<th>$k_{cat}/K_m$, µM$^{-1}$s$^{-1}$</th>
<th>Relative ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>WT</td>
<td>0.56 ± 0.14</td>
<td>41.52 ± 13.85</td>
<td>72.48</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>G41R</td>
<td>0.66 ± 0.16</td>
<td>11.40 ± 0.159</td>
<td>18.49</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>G41K</td>
<td>1.02 ± 0.03</td>
<td>14.60 ± 0.053</td>
<td>14.28</td>
<td>0.20</td>
</tr>
<tr>
<td>U35A36</td>
<td>WT</td>
<td>2.90 ± 1.35</td>
<td>8.85 ± 2.29</td>
<td>3.13</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>G41R</td>
<td>2.30 ± 1.12</td>
<td>2.78 ± 0.94</td>
<td>1.21</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>G41K</td>
<td>2.01 ± 0.50</td>
<td>2.95 ± 0.56</td>
<td>1.64</td>
<td>0.52</td>
</tr>
<tr>
<td>U35A36/G72G73</td>
<td>WT</td>
<td>5.70 ± 0.89</td>
<td>(1.48 ± 0.41) × 10$^{-3}$</td>
<td>2.67 × 10$^{-4}$</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>G41R</td>
<td>5.27 ± 1.18</td>
<td>(3.45 ± 0.71) × 10$^{-2}$</td>
<td>6.93 × 10$^{-3}$</td>
<td>25.95</td>
</tr>
<tr>
<td></td>
<td>G41K</td>
<td>5.19 ± 0.07</td>
<td>(3.71 ± 0.28) × 10$^{-2}$</td>
<td>7.14 × 10$^{-3}$</td>
<td>26.69</td>
</tr>
</tbody>
</table>

Kinetic parameters were measured by using Lineweaver–Burk and Eadie–Holstee or Hanes–Woolf plots. These gave basically the same numbers. The kinetic parameters listed are the average of four to six separate measurements. The tRNA concentrations used were 0.2–2 µM for wild-type (WT) tRNA and 0.5–6 µM for the U35A36 and the U35A36/G72G73 mutant tRNAs. The enzyme concentrations used varied from 0.025 to 0.106 nM for the wild-type tRNA, 0.34 to 0.65 nM for the U35A36 tRNA, and 0.12 to 1.55 µM for the U35A36/G72G73 tRNA. For calculation of relative ratio of $k_{cat}/K_m$, the $k_{cat}/K_m$ of wild-type MTF for each tRNA is taken as 1.
The determinants for the specific recognition of the initiator tRNA by MTF are clustered mostly at the end of the acceptor stem (6–8). In attempts to identify the amino acid residues of MTF that interact with these determinants, we have isolated and identified suppressor mutations in MTF that compensate for the formylation defect of the U35A36/G72G73 mutant initiator tRNA. Suppression is allele-specific and requires the presence of a positively charged amino acid at position 41. The G41R and G41K suppressor mutants are 26- to 27-fold more active on the U35A36/G72G73 mutant initiator tRNA than the wild-type MTF. In contrast, these enzymes are 3- to 5-fold less active than the wild-type enzyme on the wild-type initiator tRNA. Besides providing direct in vitro evidence for the phenotypic suppression seen in vivo, these results show that the phenotypic suppression is not because of a general nonspecific increase in activity of the suppressor mutants. Also, the finding that the suppressor mutants are only 3- to 5-fold less active than the wild-type enzyme on the wild-type tRNA suggests that the suppression observed is not because of a disruption of normal tRNA recognition leading to a general loss in selectivity of the enzyme for the tRNA (33).

The mutant tRNA used for selection of the suppressor mutations in MTF has changes in the anticodon sequence (U35A36) and in the acceptor stem (G72G73). Whereas the G72G73 mutation is undoubtedly responsible for the formylation defect of the tRNA, the U35A36 mutation alone makes the tRNA a poor substrate (approximately 20-fold, Table 1) for MTF. Therefore, it was possible that the suppressor mutants compensate for the effect of the U35A36 mutation or for the fact that this mutant tRNA is aminoacylated with glutamine. Our finding that the U35A36 mutant tRNA, also aminoacylated with glutamine, behaves identically to the wild-type tRNA in its properties as a substrate for the wild-type and the G41R and G41K suppressor mutants of MTF (Fig. 6, A and C) rules out this possibility and shows that the suppressor mutations compensate specifically for the strong negative effect of the G72G73 mutation in the acceptor stem of the tRNA on formylation. A direct test of this with just the G72G73 mutant tRNA as a substrate is, unfortunately, not possible because this tRNA is an extremely poor substrate for methionyl-tRNA synthetase and cannot be aminoacylated with methionine to any appreciable extent (8).

The chemical nature of the interaction between Arg-41 or Lys-41 of MTF and the acceptor stem of the mutant tRNA is not known. The U35A36/G72G73 mutant initiator tRNA is defective in formylation because it is lacking one of the most critical determinants for formylation. Extensive mutagenic studies on the tRNA have shown that nucleotides 1 and 72 at the end of the acceptor stem must be unpaired for formylation. The nature of the bases 1 and 72 themselves appears not to be important (7, 8, 34). The three-dimensional structure of the wild-type initiator tRNA (35) and comparative NMR structural analyses of acceptor stem microhelices corresponding to tRNAs, which are either substrates for MTF or not (36), suggest that MTF prefers a tRNA structure in which bases 1 and 72 are unpaired and the 3' end of the tRNA is folded back toward the 5' end of the tRNA. The U35A36/G72G73 mutant initiator tRNA has a C1-G72 base pair. In addition, it has G73 in the discriminator position, which makes it an even worse substrate for MTF, presumably because the mutant tRNA adopts a structure quite different from that required for formylation (37). Therefore, suppression of the formylation defect of the U35A36/G72G73 mutant tRNA by the Arg-41 and Lys-41 mutant enzymes requires that these enzymes facilitate melting of the C1-G72 base pair and/or compensate for the effect of G73 so that the amino acid attached to the 3' end of the tRNA can fit into the catalytic pocket of MTF.

Further evidence for the role of Arg-41 mutant enzyme in facilitating melting of the C1-G72 base pair comes from the finding that the Arg-41 enzyme also suppresses the formylation defect of the U35A36/G72 mutant initiator tRNA (data not shown). This mutant tRNA is a poor substrate for formylation only because it has a C1-G72 base pair at the end of the acceptor stem.

The approximately 26- to 27-fold increase in \( k_{cat}/K_m \) (this difference is exclusively because of the effect on \( k_{cat} \)) of the G41R and G41K enzymes over the wild-type enzyme with the U35A36/G72G73 mutant initiator tRNA as substrate (Table 1) corresponds to a stabilization of the transition state by 2 kcal/mol (1 kcal = 4.18 kJ) \( \Delta G = RT\ln(\frac{k_{cat}}{K_m}) \) (wild type/\( \frac{k_{cat}}{K_m} \)mutant)) (38). This means that the interaction of the mutant enzymes with the U35A36/G72G73 mutant initiator tRNA in the transition state is worth 2 kcal/mol more than that of the wild-type MTF. This difference is higher than the energy of one uncharged hydrogen bond (39). The finding that the Gln-41 mutant is inactive as a suppressor is consistent with this. It is possible that the G41R and G41K enzymes help disrupt the C1-G72 base pair and increase \( k_{cat} \) by forming a hydrogen bond or ionic bond with one or more of the phosphates at the end of the acceptor stem. Additionally, the suppressor mutants could stabilize the transition state by interacting directly with G72 or G73 through hydrogen bond formation with O1' and/or N3' of the guanine residues.

Because a single mutation within the 16-amino acid insertion in MTF suppresses the very strong negative effect of the G72G73 mutation on the tRNA, this region of MTF most likely interacts directly with some of the critical determinants for formylation in the acceptor stem of the tRNA (23, 40). Although this conclusion is based on the isolation and analysis of suppressor mutations at a single position within the 16-amino acid insertion, several other observations from our laboratory and from that of Blanquet and co-workers (see below) support this conclusion. (i) The 16-amino acid insertion is found in all of the MTFs of known sequence, including that in yeast mitochondria, where the insertion sequence is longer. The sequence within the 16 amino acids is quite basic and has several highly conserved residues (Fig. 2). (ii) Mutations within this region greatly affect the activity of the mutant enzymes. For example, mutations of Arg-42, the conserved amino acid adjacent to the site of suppressor mutation (Fig. 2), to Leu or Ala lowers \( k_{cat}/K_m \) of the enzyme by more than 1000-fold (V.R.G., S.G., and U.L.R., unpublished observations; Y. Mechlam, M. Panvert, E. Schmitt, and S. Blanquet, personal communication). (iii) The crystal structure of MTF shows that the 16-amino acid insertion loop is proximal to the catalytic site of MTF and is in a position to interact with the acceptor stem of the tRNA (11). (iv) The loop is mostly unstructured and uniquely susceptible to cleavage by proteases, and the cleavage product is inactive in formylation (D. Mangbro, S.G., and U.L.R., unpublished results; ref. 11; also see Fig. 5). However, in the presence of fMet-tRNA, the product of MTF, the loop is protected against proteolytic cleavage (11). It is possible that in the presence of the cognate tRNA, the mobile loop adopts a more rigid structure similar to the situation in E. coli tyrosyl-tRNA synthetase, in which two mobile loops come together in the transition state during tyrosyl-AMP formation in an induced-fit mechanism (41). The conclusion that the amino acids in the 16-amino acid insertion loop of MTF interact specifically with determinants for formylation in the acceptor stem of the initiator tRNA does not mean that this is the only region of MTF that contacts the determinants in the acceptor stem. MTF also contains a C-terminal extension over GARF (Fig. 1). This C-terminal...
extension can bind to tRNA on its own, and crosslinking experiments have shown that Lys-206, which precedes this part of MTF comes close to the 3'-terminal A of the tRNA (10, 11). It is possible that the C-terminal extension also interacts with the determinants in the acceptor stem. However, binding of the C-terminal fragment of MTF, which contains several basic and aromatic residues on the surface of the enzyme, to tRNA is nonspecific with respect to the tRNA substrate (11). This, combined with the fact that mutations of most of these basic residues individually have only small effects on enzyme activity (Y.L., S.G., and U.L.R., unpublished results; Y. Mechulam, M. Panvert, E. Schmitt and S. Blanquet, personal communication), suggests that the C-terminal extension may contribute mostly nonspecific binding energy, whereas the amino acid residues in the insertion loop provide the necessary specificity for tRNA.

Finally, comparison of MTF and GARF sequences from a variety of sources suggests that MTF has a modular structure in which polypeptide sequences necessary for tRNA binding and discrimination have been inserted into an ancestral enzyme, which could bind N5'-formyltetrahydrofolate and transfer the formyl group to simple acceptors. In this respect, it is similar to aminoacyl-tRNA synthetases, which also interact with tRNAs in a highly specific manner. In aminoacyl-tRNA synthetases, large polypeptide sequences are thought to have been inserted during evolution into a catalytic core, which had the ability to form aminoacyl-AMP (42–45). Depending on the aminoacyl-tRNA synthetase and source of the enzyme, these polypeptide sequences have been inserted into the N terminus, the C terminus, and/or within the catalytic core. These insertions confer on the aminoacyl-tRNA synthetase the properties of binding and aminoacylation of a specific tRNA, editing of misacylated tRNAs, and oligomerization of the protein. Domains of proteins involved in recognition of the acceptor stem region, the anticodon region, and other parts of the tRNA molecule are thought to have been inserted at different times during evolution of the translational machinery (42). A striking example of tRNA specificity conferred by these insertions comes from the work of Auld and Schimmel (46). These authors have shown that the ability of two closely related aminoacyl-tRNA synthetases such as isoleucyl-tRNA synthetase and methionyl-tRNA synthetase to discriminate among the corresponding tRNA anticodons, which differ by a single nucleotide, resides in a 10-amino acid loop sequence that is part of a larger insertion in these enzymes.

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