Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 6776–6780, July 1993
Biochemistry

An unusual RNA tertiary interaction has a role for the specific aminoacylation of a transfer RNA

(cysteinyl-tRNA synthetase/Levitt base pair/chemical probes)

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Communicated by Mildred Cohn, April 26, 1993

ABSTRACT **The nucleotides in a tRNA that specifically interact with the cognate aminoacyl-tRNA synthetase have been found largely located in the helical stems, the anticodon, or the discriminator base, where they vary from one tRNA to another. The conserved and semiconserved nucleotides that are responsible for the tRNA tertiary structure have been shown to have little role in synthetase recognition. Here we report that aminoacylation of *Escherichia coli* $tRNA^{Cys}$ depends on the anticodon, the discriminator base, and a tertiary interaction between the semiconserved nucleotides at positions 15 and 48. While all other tRNAs contain a purine at position 15 and a complementary pyrimidine at position 48 that establish the tertiary interaction known as the Levitt pair, *E. coli* $tRNA^{Cys}$ has guanosine −15 and −48. Replacement of guanosine −15 or −48 with cytidine virtually eliminates aminoacylation. Structural analyses with chemical probes suggest that guanosine −15 and −48 interact through hydrogen bonds between the exocyclic N-2 and ring N-3 to stabilize the joining of the two long helical stems of the tRNA. This tertiary interaction is different from the traditional base pairing scheme in the Levitt pair, where hydrogen bonds would form between N-1 and O-6. Our results provide evidence for a role of RNA tertiary structure in synthetase recognition.**

All transfer RNAs (tRNAs) fold into a cloverleaf structure that consists of four double-helical stems and four single-stranded regions known as the dihydrouridine (D), anticodon, extra (or variable), and TΨC loops, where Ψ is pseudouridine. Within this cloverleaf, a set of conserved and semiconserved nucleotides establish a network of tertiary interactions that fold the cloverleaf into an "L"-shaped tertiary structure. In this structure (Fig. 1), the amino acid acceptor stem stacks directly on the TΨC stem to form one arm of the L structure, while the D stem stacks on the anticodon stem to form the other arm of the L. The two arms are then joined by tertiary interactions between the D and the TΨC loops and between the D and variable loops so that the 3′ CCA sequence and the anticodon are placed at the opposite ends of the L structure (1, 2). The two ends are separated by about 75 Å. Various locations scattered along the inside of the L tertiary structure have recently been implicated as sites for tRNA recognition by aminoacyl-tRNA synthetases (3–6), which are the group of enzymes that catalyze the specific attachment of an amino acid to the CCA end.

The most frequently observed interactions between a tRNA and its cognate synthetase are with the anticodon, the acceptor helix, and the discriminator base (nucleotide 73) adjacent to the CCA end (3–6). In *Escherichia coli*, the alanyl- and histidinyl-tRNA synthetases primarily recognize determinants in the acceptor helix, while the methionyl-, isoleucyl-, and valyl-tRNA synthetases depend largely on the

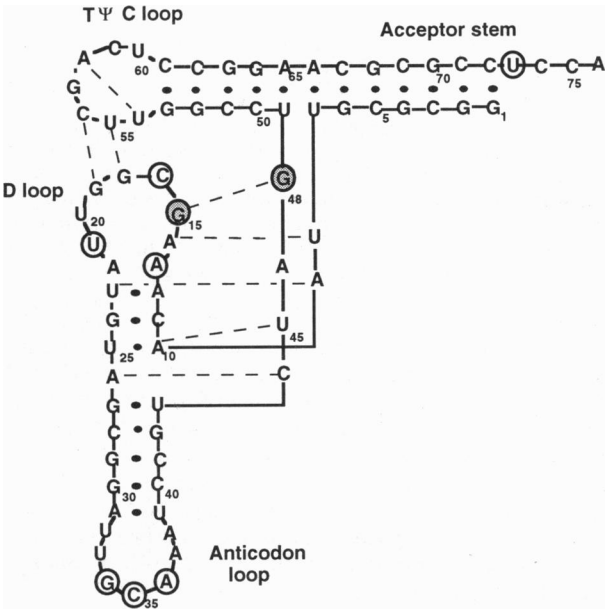

FIG. 1. Nucleotide sequence and the L-shaped presentation of *E. coli* $tRNA^{Cys}$. Backbones are indicated by continuous and solid lines, secondary base pairings are indicated by small dots, and tertiary base pairings are indicated by dashed lines. The locations of nucleotide substitutions that were introduced at the normally semiconserved purine-15 and pyrimidine-48 are indicated by shaded circles, while substitutions introduced at the nonconserved nucleotides are indicated by open circles.

anticodon (6). A large majority of the synthetases interact with a number of nucleotides that include elements in the anticodon and the discriminator base for recognition. Examples of such enzymes are the *E. coli* arginyl-, glutaminyl-, glycyl-, and phenylalaninyl-tRNA synthetases and yeast aspartyl- and phenylalanyl-tRNA synthetases. In the cocrystal of *E. coli* glutaminyl-tRNA synthetase with $tRNA^{Gln}$ and of yeast aspartyl-tRNA synthetase (7, 8), the anticodon and discriminator base are in contact with distinct domains of the protein. Substitutions of the anticodon or the discriminator base decrease the catalytic efficiency of aminoacylation, and transfer of these elements, for example, from yeast $tRNA^{Asp}$ to $tRNA^{Phe}$ confers recognition of the latter by aspartyl-tRNA synthetase (9, 10). The ability to change tRNA identity by switching the anticodon and the discriminator base suggests that the presentation of these nucleotides by the tRNA

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Abbreviations: CMCT, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate; DMS, dimethyl sulfate.

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structure is relatively preserved from one tRNA to another so that they can properly contact the synthetase despite being in a different sequence context.

E. coli $tRNA^{Cys}$ contains a GCA anticodon and a discriminator base, uridine-73 (U73) (11). Previous study shows that introduction of these nucleotides to *E. coli* $tRNA^{Met}$ results in aminoacylation with cysteine *in vivo*, suggesting a role of these nucleotides in recognition by cysteinyl-tRNA synthetase (12). Because aminoacylation *in vivo* does not quantitatively reflect the nature of interaction between a tRNA and its synthetase (13), examination *in vitro* is necessary. However, attempts to aminoacylate tRNA variants that harbor GCA and U73 *in vitro* with cysteine have not been successful (unpublished data). Sequence inspection shows a G15·G48 base composition that is unique to *E. coli* $tRNA^{Cys}$ (11). All other *E. coli* tRNAs contain a G15·C48 or an A15·U48 tertiary interaction that stabilizes the stacking of the D stem with the TΨC stem and keeps the D and variable loops together. This tertiary interaction, also known as the Levitt base pair (14), sits at the top corner of the L structure and contributes to the overall tRNA organization. We explored here whether a possible G15·G48 tertiary interaction in *E. coli* $tRNA^{Cys}$ can influence the recognition by cysteinyl-tRNA synthetase.

In this study, we used chemical mapping and structural modeling to test the wild type and four structural variants at paired positions 15 and 48. We showed that the pairing of G15 and G48 involves two hydrogen bonds between the N-2 and N-3 of guanine bases, instead of the hydrogen bonds between N-1 and O-6 that would mimic a traditional Levitt base pair. We propose that this unusual base pairing is abolished in the defective G15·C48 or C15·G48 mutants but is partially restored in the G15·U48 or U15·G48 variants that can be recognized by cysteinyl-tRNA synthetase. Our results highlight a structural role of G15·G48 in properly presenting the cysteine determinants (the GCA anticodon and U73) to the cysteine enzyme. This structural effect must be considered to confer cysteine aminoacylation *in vitro* in a new sequence context.

MATERIALS AND METHODS

Materials. [γ-^{32}P]ATP (3000 Ci/mmol; 1 Ci = 37 GBq) was purchased from Pharmacia P-L Biochemicals, and [5′-^{32}P]pCp (3000 Ci/mmol) and L-[^{35}S]cysteine (1200 Ci/mmol) were from New England Nuclear. *E. coli* cysteinyl-tRNA synthetase was purified from pYM107 (15), and phage T7 RNA polymerase was purified from pAR1219/BL-21 (16). The following chemical probes were purchased: DMS (dimethyl sulfate), from Fluka; kethoxal, from ICN; and CMCT [1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate], from Aldrich. The wild-type and variant tRNA transcripts of *E. coli* $tRNA^{Cys}$ were synthesized by T7 RNA polymerase from tRNA genes that were constructed in plasmid pTFMa (10).

Aminoacylation with Cysteine. Aminoacylation was assayed at 37°C in 30-μl reaction mixtures with 0.15 μM purified *E. coli* cysteinyl-tRNA synthetase in a buffer containing 20 mM KCl, 10 mM $MgCl_2$, 25 mM dithiothreitol, 2 mM ATP, 20 mM Tris·HCl (pH 7.5), 50 μM cysteine, 0.385 μM [^{35}S]cysteine, and 1–10 μM purified tRNA transcript (in 10 mM Tris·HCl at pH 8.0/1 mM EDTA) that had been previously heated (60°C, 2 min) and reannealed at room temperature. Reactions reached a plateau in 10–15 min, while aliquots of 5 μl were taken every 5 min for 30 min. In each aliquot, the cystinylated tRNA was alkylated by reacting with 0.24 M iodoacetic acid/0.1 M sodium acetate (pH 5.0) in formamide at 37°C for 30 min. A portion of the alkylation reaction (12 μl) was spotted onto a Whatman 3 MM filter pad, precipitated with trichloroacetic acid, and washed as described (15).

End-Labeling of tRNA. Labeling at the 5′ end of the tRNAs with [γ-^{32}P]ATP was achieved with phage T4 polynucleotide kinase on molecules previously dephosphorylated with alkaline phosphatase (Boehringer Mannheim) (17). Labeling at the 3′ end was achieved with [5′-^{32}P]pCp with T4 RNA ligase (New England Biolabs) (17). The labeled tRNAs were purified by electrophoresis on 12% polyacrylamide/8 M urea gels and were eluted by extraction of the gel with 0.125 M NH_4OAc/0.125 mM EDTA/0.025% SDS. Typical labeling yielded 5×10^6 Cerenkov counts per μg of tRNA.

Chemical Modifications. All chemical modifications were carried out at room temperature. Modification with DMS was achieved with 4 μM tRNAs, 30,000 Cerenkov counts of 3′-labeled tRNA in 10 mM $MgCl_2$/50 mM sodium cacodylate, pH 7.2/0.5% DMS for 5 min for the native condition. For the semidenaturing condition, tRNA was heated at 90°C in water for 2 min, cooled on ice for 2 min, equilibrated in 1 mM EDTA/50 mM sodium cacodylate, pH 7.2, and then treated with 0.5% DMS for 2 min. DMS resulted in chain scissions that, after aniline treatment (18), generated labeled tRNA fragments that could be separated by gel electrophoresis and detected by autoradiography.

Modifications with kethoxal and CMCT did not lead to chain scissions and required primer extension to detect the sites of modifications. Modification with kethoxal was achieved with 2.4 μM tRNAs in 50 mM sodium cacodylate, pH 7.2, and 0.02% kethoxal for 10 min with 10 mM $MgCl_2$ for the native condition and with 1 mM EDTA for the semidenaturing condition (previously heated and cooled as above) (17). Conditions for modification with CMCT were the same as in kethoxal except that 10 mg of CMCT per ml in 50 mM sodium borate (pH 8.0) was used (19). The concentrations of kethoxal and CMCT were introduced at a level of less than one modification per molecule (20). After modifications, tRNAs were annealed with a 5′-labeled oligonucleotide that was complementary to the 3′ tRNA sequence from position 61 to 76. The primer was extended by avian myeloblastosis virus reverse transcriptase (Life Sciences), which stopped at the site of modification (17, 19, 20).

RESULTS AND DISCUSSION

Two groups of nucleotide substitutions in *E. coli* $tRNA^{Cys}$ were tested for aminoacylation: those at positions that do not participate in the tertiary folding of the tRNA (the nonstructural mutants) and those at position 15 or 48 (the structural mutants). Aminoacylation was tested with various concentrations of tRNA transcripts that were prepared by the T7 RNA polymerase (see Table 1 for aminoacylation conditions). The plateau of charging for each mutant is given in Table 1 as the fraction of that of the wild-type transcript. Among the nonstructural mutants, the single nucleotide substitution at the discriminator base from U73 to G73 has the largest effect on aminoacylation. This substitution, together with substitutions at positions 35 and 36 of the anticodon, reduced the plateau of aminoacylation to 5%. This is within experimental background of measuring cysteine aminoacylation. Although U21 of $tRNA^{Cys}$ is unique among *E. coli* tRNAs, single nucleotide substitution at this position did not have a major effect on aminoacylation. Among the structural mutants, alteration of G48 or G15 to a cytidine that created a G15·C48 or a C15·G48 mutant also resulted in near background level of plateau charging. Thus, substitutions in the G15·G48 base composition that changed it to a complementary purine·pyrimidine composition have the same deleterious effect on aminoacylation as substitutions of the anticodon and the discriminator base of $tRNA^{Cys}$.

The low level of plateau charging for the G15·C48 and the C15·G48 mutants could reflect parameters other than the forward rate of aminoacylation or reflect a large population

Table 1. Aminoacylation with cysteine of the wild type and variants of *E. coli* $tRNA^{Cys}$ transcript

Transcripts	Relative plateau of aminoacylation, %	Estimate of relative k_{cat}/K_m*
Wild type	100.0	1.0
Nonstructural mutants		
Discriminator base mutant, U73 → G73	9.3	
Anticodon (positions 34–36) mutants		
GCA → GUC	60.0	
GCA → CUA	27.3	
Anticodon and discriminator base mutant		
GCA → GUC and U73 → G73	5.0	
D stem mutant, A13 → U13	100.0	
D loop mutants		
U21 → C21	37.9	
U21 → A21	87.2	
Structural mutants with substitutions at G15 or G48		
G48 → C48 (G15·C48 mutant)	5.0	0.01
G15 → C15 (C15·G48 mutant)	6.2	0.02
G48 → U48 (G15·U48 mutant)	25.8	0.38
G15 → U15 (U15·G48 mutant)	46.2	0.38

Initial rate of aminoacylation was measured with 33 nM cysteinyl-tRNA synthetase (specific activity, 1.8 nmol/min per μg of protein) and 1, 2, 5, 7.5, and 10 μM tRNA transcripts. The K_m of the wild-type tRNA for the enzyme is 5×10^{-6} M.

*The slope of the rates vs. tRNA concentrations gives an estimate of k_{cat}/K_m, which has an accuracy of 30%. Under the same assay conditions, the native *E. coli* $tRNA^{Cys}$ (provided from Subriden, Rolling Bay, Washington) has a plateau of aminoacylation 3 times that of the wild-type transcript and an estimated value of k_{cat}/K_m 4 times that of the wild-type transcript.

of inactive tRNA molecules. It was necessary to investigate these mutants further for the catalytic efficiency of aminoacylation (k_{cat}/K_m). The current method for analyzing aminoacylation relies on trichloroacetic acid precipitation of the aminoacyl-tRNA onto filter pads. However, the active sulfhydryl group of cysteinyl-tRNA generated high background. We developed a modification that involved carboxymethylation of the sulfhydryl groups prior to trichloroacetic acid precipitation. Nonetheless, the unreacted sulfhydryl groups introduced enough noise to the background so that it was difficult to obtain accurate kinetic measurements. We used the initial rate of aminoacylation to estimate k_{cat}/K_m because when the tRNA substrate concentration is below K_m, the initial rate of aminoacylation is proportional to the product of k_{cat}/K_m and the tRNA concentration. We estimate that the k_{cat}/K_m for the G·C and C·G variants at paired positions 15 and 48 are reduced by 2 orders of magnitude relative to the rate of aminoacylation of the wild type (Table 1). On the other hand, the G15·U48 and U15·G48 mutants restored partial aminoacylation with cysteine. We estimate that the catalytic efficiency of these two mutants are reduced by 2.5-fold from that of the wild-type tRNA.

The dependence on the anticodon for cysteine aminoacylation is consistent with the results of *in vivo* studies (12). This synthetase shares common structural motifs with a subclass of synthetases (the class I synthetases) (21) that are known to interact with the anticodon [e.g., the enzymes for methionine, valine (22), and isoleucine (23)]. The U73 discriminator base of $tRNA^{Cys}$ is almost unique among *E. coli* tRNAs (except for the three glycine tRNAs) (11) and in principle could be important for the cysteine enzyme. The dependence on the G15 and G48 residues for cysteine aminoacylation has not been previously observed. Studies on yeast $tRNA^{Phe}$ and $tRNA^{Asp}$ showed that reversal of the nucleotides from purine-15·pyrimidine-48 to pyrimidine-15·purine-48 did not significantly alter the catalytic efficiency of aminoacylation (24, 25). For yeast $tRNA^{Phe}$, substitutions at positions 15 and 48 that were designed to resemble the tertiary interaction of the wild-type base pair, including the introduction of a G15·G48, also had an insignificant effect on aminoacylation (24). These early results established that the nucleotides at positions 15 and 48 primarily contribute to the stability of the tRNA tertiary structure while having little role in synthetase recognition.

Structural modeling showed that G15 and G48 can make a two-hydrogen-bonded base pair with only a small distortion of the sugar-phosphate backbone from that in the normal Levitt base pair. In this pairing scheme, guanines use their N-1 as the hydrogen donor and the exocyclic O-6 as the acceptor (Fig. 2 *Left*). To understand why substitutions of G15 and G48 in $tRNA^{Cys}$ should have a major effect on aminoacylation, we probed to see if these two nucleotides form a tertiary base pair that poses different structural

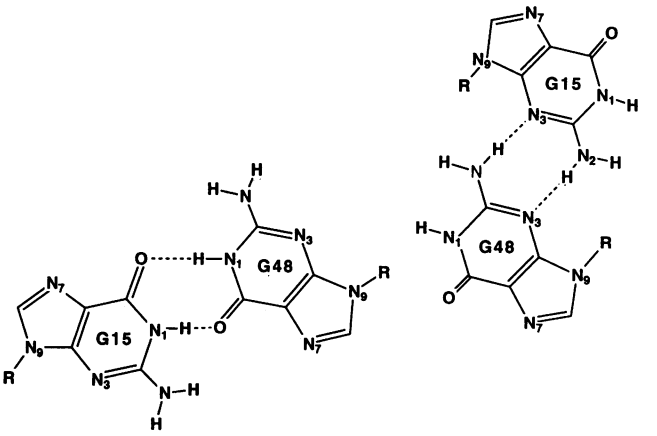

FIG. 2. The proposed tertiary hydrogen bondings between G15 and G48 in *E. coli* $tRNA^{Cys}$. The folding of the tRNA structure orients the glycosidic bonds of G15 and G48 in trans. The proposed base pairing (*Left*) is derived from the Levitt G15·C48 base pair of yeast $tRNA^{Phe}$ by replacing C48 with G48 while maintaining two hydrogen bonds within the base pair. This base pairing retains similar backbone conformation at both positions 15 and 48 as in the other tRNAs so that it is stacked directly on U8·A14 and forms the corner of the tRNA L shape[5,6]. The proposed base pairing (*Right*) contains two hydrogen bonds, but the glycosidic bond of G15 is rearranged relative to that of G48 in *Left*. In the three-dimensional structural modeling, G15 in this rearrangement protrudes away from A14 and away from the corner of the L shape.

constraints than in the normal Levitt pair. DMS was used to test the reactivity of N-7 in G15 and G48 (18, 20). Under both the native (with Mg^{2+}) and the semidenaturing (no Mg^{2+}) conditions, the N-7 of G15 was readily reactive with DMS, whereas the N-7 of G48 was not (not shown). The inaccessibility of G48 to DMS illustrates the compact structural feature of the variable loop. The nucleotides at position 48 in other tRNAs were also protected from chemical probes (17). However, the reactivity of G15 is in contrast to what has been shown for *E. coli* $tRNA^{Thr}$ (17), beef $tRNA^{Trp}$ (26), yeast $tRNA^{Phe}$ (27), and $tRNA^{Asp}$ (27), where purine-15 in a normal Levitt pair is stacked on A14 and is not reactive. Additional model-building suggests an alternative base-pairing scheme between G15 and G48 that maintains the trans base pairing of the sugar phosphate backbone but forms two hydrogen bonds using N-2 hydrogen as the donor and ring N-3 as the acceptor (Fig. 2 *Right*.) This structure places G15 in an outward position away from A14 and provides a rational for the observed reactivity of N-7 of G15 with DMS. G15 and G48 also reacted with kethoxal, whereas the guanosine in a Levitt pair did not (19). Kethoxal condenses the N-1 hydrogen and one of the N-2 hydrogens of guanine to form a five-member-ring adduct (20, 28). The reactivity with kethoxal indicates the presence of a free guanosine N-1 and N-2 and supports the structure in Fig. 2 *Right*.

The four structural mutants at paired positions 15 and 48 were tested with chemical probes to determine if aminoacylation depends on the unusual positioning of G15 in Fig. 2 *Right*. Both the G15·U48 and U15·G48 mutants reacted with kethoxal and with CMCT, which tested a free N-3 of uridine and to a lesser extent N-1 of guanosine (29). Structural modeling suggested that both adopted a backbone conformation at position 15 similar to the wild type (Fig. 3 *a* and *b*). On the other hand, the C15·G48 mutant did not react with kethoxal. This suggested a major rearrangement at positions 15 and 48 to an equivalent of the Levitt pair (Fig. 3*c*). The G15·C48 mutant reacted with kethoxal. Structural modeling suggested two possibilities at positions 15 and 48 (Fig. 3 *d* and *e*). The C48 substitution in this mutant prevented the cysteine enzyme from binding at G15 and the anticodon and seriously decreased the affinity for $tRNA^{Cys}$ (not shown). The loss of affinity implied a structural rearrangement and favored the model that significantly shifted G15 relative to C48 (Fig. 3*e*). Thus, mutants that appear to have rearrangement at position 15 are defective, while those that maintain position 15 retain partial aminoacylation.

The proposed base pairings at positions 15 and 48 of the structural mutants were based on Fig. 2 *Right*, which has only been observed in the crystal structures of deoxyribonucleotides (30, 31). All but the C15·G48 mutant reacted with kethoxal and therefore contained a free N-1 of guanosine. This strengthened the support for Fig. 2 *Right*. If structural modeling of the mutants were based on the Levitt G·G pair in Fig. 2 *Left*, none would have a free guanosyl N-1.

Position 15 sits at the hinge of the right angle formed by the acceptor–TΨC stem and the D–anticodon stem. The protrusion of G15 in the novel base pairing is like pulling the hinge away from the right angle, which can shorten the 75-Å distance between the anticodon and the CCA end. This shortening may be advantageous for cysteinyl-tRNA synthetase, which is the smallest monomeric synthetase in *E. coli* (15, 32) that must contact both the anticodon and the CCA end for aminoacylation. The smaller but dimeric *Bacillus stearothermophilus* tyrosyl-tRNA synthetase achieves contact with both ends by binding $tRNA^{Tyr}$ across the dimer interface (33). Alternatively, the protrusion of G15 may direct specific conformational changes of $tRNA^{Cys}$ after binding to the enzyme. Structural analysis of other tRNAs shows evidence of various conformational changes upon synthetase binding (6–8). While these hypotheses remain to be tested and we cannot exclude the possibility of direct synthetase recognition of G15 and G48, our results suggest that specific features of RNA tertiary structure can assist a synthetase during the transition state of recognition to establish tRNA identity.

We thank Drs. B. Ehresmann, C. Ehresmann, and P. Romby for guidance on the chemical probes; Dr. C. Florentz and the laboratory

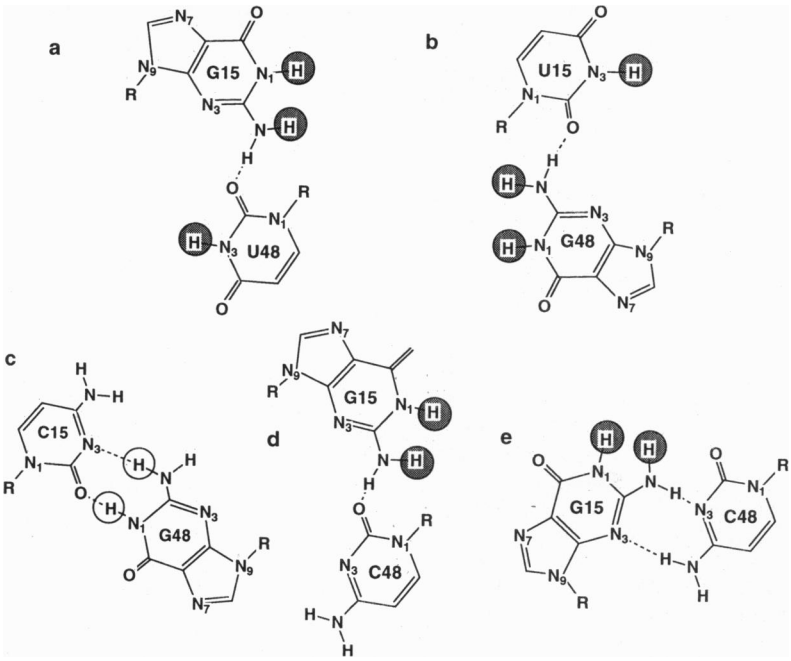

FIG. 3. The proposed base pairings of G15·U48 (*a*), U15·G48 (*b*), and C15·G48 (*c*) and the two possibilities of G15·C48 (*d* and *e*) in the structural variants of *E. coli* $tRNA^{Cys}$ at the G15·G48 base pair. These pairings are derived from Fig. 2 *Right* and are drawn based on the results of reactions of each mutant with kethoxal (test of guanosine N-1 and N-2) and CMCT (test of uridine N-3 and guanosine N-1). The hydrogens of guanosine N-1 and N-2 that were reactive to kethoxal, and those of uridine N-3 that were reactive with CMCT, are shown in shaded circles. The hydrogens that were tested but not reactive to chemical probes are shown in open circles.

of R. Giegé for discussions; Drs. H. Gamper and E. Winter for comments on the manuscript; and M. Wrobleski for preparation of the figures. This work was supported by grants from the Centre National de la Recherche Scientifique, the Human Frontier Science Program, the Université Louis Pasteur, and the National Institutes of Health.

1. Kim, S. H., Suddath, F. L., Quigley, G. J., McPherson, A., Sussman, J. L., Wang, A. H. J., Seeman, N. C. & Rich, A. (1974) *Science* **185,** 435–440.
2. Robertus, J. D., Ladner, J. E., Finch, J. T., Rhodes, D., Brown, R. S., Clark, B. F. C. & Klug, A. (1974) *Nature (London)* **250,** 546–551.
3. Schimmel, P. (1989) *Biochemistry* **28,** 2747–2759.
4. Normanly, J. & Abelson, J. (1989) *Annu. Rev. Biochem.* **58,** 1029–1049.
5. Schulman, L. H. (1991) *Prog. Nucleic Acid Res. Mol. Biol.* **41,** 23–87.
6. Giegé, R., Puglisi, J. D. & Florentz, C. (1993) *Prog. Nucleic Acid Res. Mol. Biol.* **45,** 129–206.
7. Rould, M. A., Perona, J. J., Soll, D. & Steitz, T. A. (1989) *Science* **246,** 1135–1142.
8. Ruff, M., Krishnaswamy, S., Boeglin, M., Poterszman, A., Mitschler, A., Podjarny, A., Rees, B., Thierry, J. C. & Moras, D. (1991) *Science* **252,** 1682–1689.
9. Jahn, M., Rogers, M. J. & Soll, D. (1991) *Nature (London)* **352,** 258–260.
10. Putz, J., Puglisi, J. D., Florentz, C. & Giegé, R. (1991) *Science* **252,** 1696–1699.
11. Sprinzl, M., Hartmann, T., Weber, J., Blank, J. & Zeidler, R. (1991) *Nucleic Acids Res.* **17,** r1–r172.
12. Pallanck, L., Li, S. & Schulman, L. H. (1992) *J. Biol. Chem.* **267,** 7221–7223.
13. Schimmel, P. (1989) *Acc. Chem. Res.* **22,** 232–233.
14. Levitt, M. (1969) *Nature (London)* **224,** 759–763.
15. Hou, Y.-M., Shiba, K., Mottes, C. & Schimmel, P. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 976–980.
16. Grodberg, J. & Dunn, J. J. (1988) *J. Bacteriol.* **170,** 1245–1253.
17. Théobald, A., Springer, M., Grunberg-Manago, M., Ebel, J.-P. & Giegé, R. (1988) *Eur. J. Biochem.* **175,** 511–524.
18. Peattie, D. A. & Gilbert, W. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4679–4782.
19. Wakao, H., Romby, P., Westhof, E., Laalami, S., Grunberg-Manago, M., Ebel, J.-P., Ehresmann, C. & Ehresmann, B. (1989) *J. Biol. Chem.* **264,** 20363–20371.
20. Ehresmann, C., Baudin, P., Mougel, M., Romby, P., Ebel, J.-P. & Ehresmann, B. (1987) *Nucleic Acids Res.* **15,** 9109–9128.
21. Eriani, G., Delarue, M., Poch, O., Gangloff, J. & Moras, D. (1990) *Nature (London)* **347,** 203–206.
22. Schulman, L. H. & Pelka, H. (1988) *Science* **242,** 765–768.
23. Muramatsu, T., Nishikawa, K., Nemoto, F., Kuchino, Y., Nishimura, S., Miyazawa, T. & Yokoyama, S. (1988) *Nature (London)* **336,** 179–181.
24. Sampson, J. R., DiRenzo, A. B., Behlen, L. S. & Uhlenbeck, O. C. (1990) *Biochemistry* **29,** 2523–2532.
25. Giegé, R., Florentz, C., Garcia, A., Grosjean, H., Perret, V., Puglisi, J. D., Théobald-Dietrich, A. & Ebel, J.-P. (1990) *Biochimie* **72,** 453–461.
26. Garret, M., Labouesse, B., Litvak, S., Romby, P., Ebel, J.-P. & Giegé, R. (1984) *Eur. J. Biochem.* **138,** 67–75.
27. Romby, P., Moras, D., Dumas, P., Ebel, J. P. & Giegé, R. (1987) *J. Mol. Biol.* **195,** 193–204.
28. Shapiro, R. & Hachmann, J. (1986) *Biochemistry* **5,** 2799–2807.
29. Van Stolk, B. J. & Noller, H. F. (1984) *J. Mol. Biol.* **180,** 151–177.
30. Wing, R., Drew, H., Takano, T., Broka, C., Tanaka, S., Itakura, K. & Dickerson, R. E. (1980) *Nature (London)* **287,** 755–758.
31. Cruse, W. B. T., Egert, E., Kennard, O., Sala, G. B., Salisbury, S. A. & Viswamitra, M. A. (1983) *Biochemistry* **22,** 1833–1839.
32. Eriani, G., Dirheimer, G. & Gangloff, J. (1991) *Nucleic Acids Res.* **19,** 265–269.
33. Carter, P., Bedouelle, H. & Winter, G. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1189–1192.