Functional Compensation of a Recognition-Defective Transfer RNA by a Distal Base Pair Substitution†

Ya-Ming Hou‡ and Paul Schimmel*

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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ABSTRACT: A single G3:U70 base pair in the acceptor helix is the major determinant of alanine acceptance in alanine transfer RNAs. Transfer of this base pair into other transfer RNAs confers alanine acceptance. A G3:C70 substitution eliminates alanine acceptance in vivo and in vitro. In this work, a population of mutagenized G3:C70 alanine tRNA amber suppressors was subjected to a selection for mutations that compensate for the inactivating G3:C70 substitution. No compensatory mutations located in the acceptor helix were obtained. Instead, a U27:U43 substitution that replaced the wild-type C27:G43 in the anticodon stem created a U27:U43/G3:C70 mutant alanine tRNA that inserts alanine at amber codons in vivo. The U27:U43 substitution is at a location where previous footprinting work established an RNA–protein contact. Thus, this mutation may act by functionally coupling a distal part of the tRNA structure to the active site.

The specific interaction between a tRNA and its cognate aminoacyl tRNA synthetase results in the correct charging of an amino acid to the 3′ end of the tRNA. This interaction is an essential step in the decoding of genetic information. The crystal structures of two tRNA–synthetase complexes, one for *Escherichia coli* glutamine tRNA (Rould et al., 1989, 1991) and the other for yeast aspartyl tRNA (Ruff et al., 1991), have been recently solved. In each case, the enzyme makes specific contacts with nucleotides in the tRNA that are located in the D stem, the anticodon loop, and at the end of the acceptor helix, including the discriminator base (N73). Among all of these contacts, however, biochemical analyses suggest that only a subset of the nucleotides that are in contact with the enzyme determine the specificity of aminoacylation (Putz et al., 1991; Jahn et al., 1991).

The 20 aminoacyl tRNA synthetases vary in their sizes and quaternary structures (Schimmel & Soll, 1979; Schimmel, 1987). In *E. coli*, the monomeric cysteine tRNA synthetase of 461 amino acids forms a relatively small complex with its cognate tRNA with an approximately 2:1 mass ratio of protein to RNA (Hou et al., 1991; Eriani et al., 1991). Because synthetase polypeptides can have up to 4 times the mass of tRNAs, the wide range of protein–RNA contacts, as seen in the crystal structures of glutamine and aspartyl complexes, may be general. Here we used *E. coli* alanine tRNA to explore the possibility whether mutations of nucleotides extraneous to the major determinant of tRNA identity and that are even dispensible for aminoacylation can compensate for mutations at the major determinant.

Our studies with the alanine system established that the major determinant for the specificity of aminoacylation is a single G3:U70 base pair in the acceptor stem (Hou & Schimmel, 1988). Substitutions of G3:U70 eliminate aminoacylation with alanine, even with substrate levels of enzyme (Park et al., 1989). Introduction of G3:U70 into the sequence frameworks of *E. coli* $tRNA^{Cys}$, $tRNA^{Phe}$, $tRNA^{Tyr}$, and $tRNA^{Lys}$ confers alanine acceptance (Hou & Schimmel, 1988, 1989; McClain & Foss, 1988; McClain et al., 1988). Further, the G3:U70 base pair enables short RNA helices that are based on the seven base pairs of the acceptor stems of $tRNA^{Ala}$ and $tRNA^{Tyr}$ to be efficiently aminoacylated with alanine (Francklyn & Schimmel, 1989). Thus, the anticodon stem–loop region of $tRNA^{Ala}$ is dispensible for aminoacylation. However, RNA footprint studies show that the alanine enzyme makes contact with the anticodon stem of $tRNA^{Ala}$ (Park & Schimmel, 1988). This raises the question of whether a dispensible region that makes contact with the enzyme can be recruited to compensate for a mutation in the essential acceptor stem of $tRNA^{Ala}$.

Our approach to this question was to construct an amber suppressor alanine tRNA (by changing the wild-type anticodon GGC to the amber-reading anticodon CUA) in which the G3:U70 base pair in the acceptor stem has been altered. In the wobble pairing of G3:U70, the exocyclic 2-amino group of G3 protrudes into the minor groove of the helix and the 4-carbonyl group of U70 lies in the major groove (C. d. I. Santos, C. Francklyn, P. Schimmel, and D. Patel, unpublished results). Recent evidence suggests that the unpaired exocyclic 2-amino group of G3 is essential for the interaction with alanine tRNA synthetase that produces an active complex (Musier-Forsyth et al., 1991). A G3:C70 mutation apparently blocks the essential exocyclic 2-amino group of G3 by formation of the Watson–Crick base-pairing configuration. In the present study, we used as the starting material the G3:C70 $tRNA^{Ala/CUA}$ into which random nucleotide substitutions throughout the sequence framework were introduced (Hou & Schimmel, 1992). The use of the amber suppressor form of alanine tRNA provided a genetic system in which mutants that compensated for the defective G3:C70 could be selected by their ability to insert alanine at an amber codon.

MATERIALS AND METHODS

The gene for G3:C70 $tRNA^{Ala/CUA}$ was synthesized in two degenerate DNA oligonucleotides (Applied Biosystems 380B DNA synthesizer) as previously described, where permutations of bases in the tRNA gene were included at each step of the synthesis such that 90% of the wild-type nucleotide and 3.3% each of the other three nucleotides were incorporated (Hou & Schimmel, 1992). After synthesis, 10 pmol of the double-stranded DNA containing the tRNA gene were digested with

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‡ Present address: Centre National de la Recherche, Institut de Biologie Moleculaire et Cellulaire, 15 rue Rene Descarte, 67084 Strasbourg, France.

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*Eco*RI and *Pst*I restriction enzymes and were ligated into plasmid pGFIB-I that had been cleaved at the *Eco*RI and *Pst*I restriction sites. Plasmid pGFIB-I contains an *lpp* promoter on the 5′ side of the *Eco*RI restriction site and an rrnC terminator sequence on the 3′ side of the *Pst*I restriction site (Masson & Miller, 1986). *E. coli* XAC-I cells [F′ lacI $lacZ_{am}$ $proB^+$/F-δ(lacproB), nalA, rif, $ArgE_{am}$, ara] which carry this plasmid, into which a synthetic tRNA gene was inserted between the *Eco*RI and *Pst*I restriction sites, have been shown to produce the plasmid-encoded tRNA that functions in protein synthesis (Normanly et al., 1986a,b). The ligated DNA was introduced into *E. coli* strain XAC-I by transformation (Kleina et al., 1990). The ampicillin-resistant colonies were selected on LB plates containing x-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranose) at 40 μg/mL. Functional tRNA mutants conferred a blue-colored phenotype to the colony whereas nonfunctional mutants appeared white (Hou & Schimmel, 1992). From positive clones, plasmid DNAs were prepared and were introduced into *E. coli* strain *trpA*(UAG234) [F′*trpA*(UAG234)/metB glyV55 Δ(tonB-*trpA*B17] (Murgola & Hijazi, 1983). From these transformants, individual colonies were tested on minimal media with a suboptimal concentration of tryptophan (0.2 μg/mL) to screen for mutants that were able to insert alanine at the *trpA*(UAG234) amber codon.

Total *E. coli* tRNA (from 250-mL cells) was prepared by phenol extraction, followed by a 1-mL DEAE (diethylamino)-ethyl-cellulose column in 300 mM NaCl, 100 mM Tris-HCl (pH 7.4), and 1 mM EDTA. The tRNAs were eluted within 2 vol of column buffer in the presence of 1 M NaCl, while rRNAs were eluted much later. Total tRNAs were then fractionated by a 12% polyacrylamide gel under native conditions (400 v, Tris–borate–EDTA buffer). The plasmid-encoded suppressor tRNA was identified as the overproduced species after staining the gel with ethidium bromide. The suppressor tRNA was then eluted from the gel, and concentrated by ethanol precipitation. The concentration of the tRNA was determined by A_{260} absorbance (1 o.d. unit = 40 μg/mL of tRNA).

RESULTS

Random Nucleotide Substitutions in G3:C70 tRNA$^{Ala/CUA}$. In order to sample the widest variety of possible tRNA mutants, we decided to allow random permutation of nucleotides throughout the sequence framework of G3:C70 tRNA$^{Ala/CUA}$, including those for the conserved and semiconserved nucleotides. While studies of yeast tRNA^{Phe} have shown that the conserved and semiconserved nucleotides do not participate directly in sequence-specific interactions with the synthetase (Sampson et al., 1990), they may contribute to the affinity of tRNA for the enzyme. In this study, random nucleotide substitutions were introduced by synthesizing two degenerate deoxyoligonucleotides that collectively encode the gene for G3:C70 tRNA$^{Ala/CUA}$. Of the 76 nucleotides, however, 10 were exempt from mutations (Figure 1). These were the CCA terminal sequence for amino acid attachment, C70, and six nucleotides (^{33}UCUAAA^{38}) in the anticodon loop that encompassed the CUA anticodon sequence. The rest of the nucleotides contained a 10% frequency of substitutions such that on average a mutant tRNA would have 6.6 nucleotide substitutions (10% × 66*nt* = 6.6*nt*).

In a previous study, a subset of the mutants were introduced into *E. coli* strain XAC-I by transformation. Sequence analysis of 82 transformants showed that the average number of mutations in a tRNA was 7.2 and that nucleotide

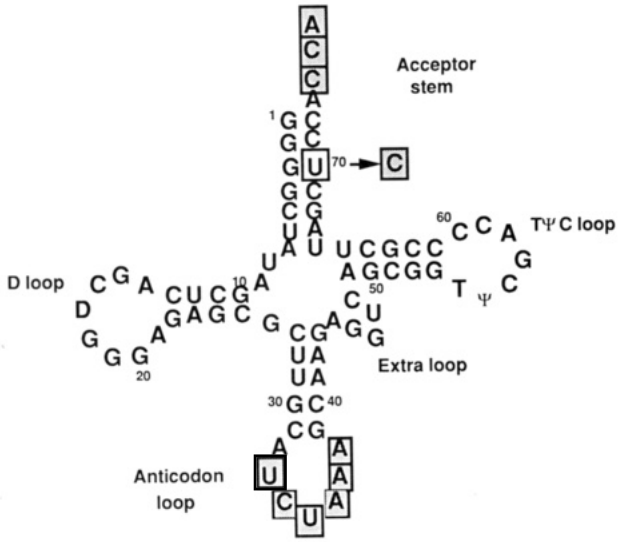
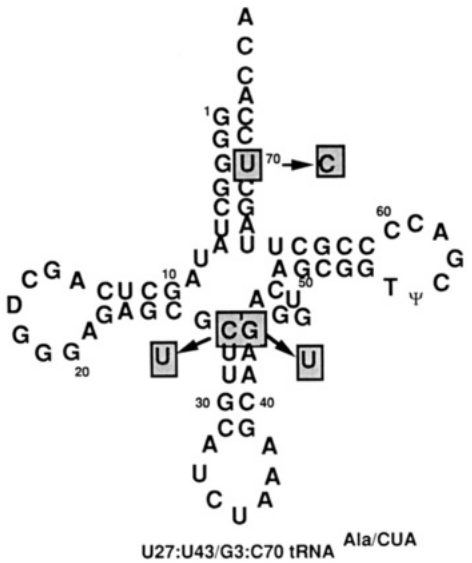


FIGURE 1: Sequence and cloverleaf structure of *E. coli* G3:C70 tRNA$^{Ala/CUA}$ that served as the starting material in this study. The sequence was derived from *E. coli* tRNA$^{Ala/GGC}$ by changing the anticodon sequence from GGC to CUA and by changing the nucleotide at position 70 from U to C. One additional change in the anticodon loop (U38 to A38) was introduced in order to have a sequence context for obtaining a better efficiency of suppression at an amber codon (Raftery & Yarus, 1987). Except for the 10 nucleotides that are boxed and shaded, the remaining 66 nucleotides contained a 10% frequency of mutations during the synthesis of gene for G3:C70 tRNA$^{Ala/CUA}$ (Hou & Schimmel, 1992).

substitutions were randomly distributed throughout the sequence framework of G3:C70 tRNA$^{Ala/CUA}$ (Hou & Schimmel, 1992). In XAC-I, an amber mutation was created in the lacI-Z fusion gene, which can be suppressed by the insertion of any of the 20 amino acids (Kleina et al., 1990). In the presence of x-gal, mutant suppressor tRNAs that suppress an amber codon were easily differentiated from those that were nonfunctional. With this system, we found that less than 1% of the 10^5 tRNA mutants which were tested were functional suppressor tRNAs. Thus, starting from G3:C70 tRNA$^{Ala/CUA}$, which lacks the major determinant for its synthetase, we have generated some tRNA mutants that were recognized by at least one aminoacyl tRNA synthetase so as to suppress an amber mutation. It was from among this limited population that we sought for a variant of G3:C70 tRNA$^{Ala/CUA}$ that was aminoacylated with alanine.

Screening of Mutants That Are Aminoacylated with Alanine. To screen for functional tRNA mutants that were specifically aminoacylated with alanine, we used *E. coli* strain *trpA*(UAG234). The amber mutation in *trpA*(UAG234), located in the gene for the α-subunit of tryptophan synthetase, can be suppressed by insertion of glycine or alanine (Murgola & Hijazi, 1983). In the absence of suppression, *trpA*-(UAG234) has a Trp^- phenotype and fails to grow on minimal medium with suboptimal supplement of tryptophan. Suppression at *trpA*(UAG234) confers Trp^+ and provides a sensitive screen for functional tRNA mutants that were aminoacylated with alanine (or glycine).

We took the functional tRNA mutants from the previous study, which totaled approximately 250 colonies from a population of 10^5 transformants. In the previous study, 82 of the functional mutants were sequenced, and we showed that 15 contained sequence variants while 67 were of the starting G3:C70 tRNA$^{Ala/CUA}$ sequence (Hou & Schimmel, 1992). We prepared plasmid DNAs from the 15 variants and individually tested them for their ability to suppress the amber codon at *trpA*(UAG234). Of the remaining 168 or so



Suppression Phenotype on Minimal Media			
Supplement of tryptophan	0	0.2 μg/ml	20 μg/ml
$tRNA^{Ala/CUA}$	+	+	+
G3:C70 $tRNA^{Ala/CUA}$	-	-	+
U28:U43/G3:C70 $tRNA^{Ala/CUA}$	-	+	+

FIGURE 2: Nucleotide sequence of U27:U43/G3:C70 $tRNA^{Ala/CUA}$ that compensates for the defect at G3:C70 in alanine tRNA. Arrows indicate the substitutions at positions 27:43 from C:G to U:U and at 70 from U to C. Suppression phenotypes at *trpA*(UAG234) for the wild-type $tRNA^{Ala/CUA}$, the G3:C70 variant, and the U27:U43/G3:C70 variant are shown at the bottom. The tRNA suppressors were tested in minimal media (M9) with 0, 0.2 μg/mL (suboptimal), or 20 μg/mL of tryptophan supplements. Growth of cells harboring respective suppressor tRNAs was scored after 2 days at 37 °C for suppression (+) or nonsuppression (–).

functional mutants that were not analyzed for their sequences, we estimated that about 82% would be of the starting tRNA sequence and only about 30 would have sequence variations. We grouped these 168 mutants in 10 pools and tested the pooled suppressor tRNAs for suppression of *trpA*(UAG234). (We tested about 72 transformants from each pool so that, on a statistical basis, all of the approximately 30 sequence variants would likely be present.) None of these pooled mutants suppressed *trpA*(UAG234).

Thus, of about 250 functional variants that were derived from 10^5 transformants, we obtained one that gave a Trp^+ phenotype at a suboptimal concentration of the tryptophan supplement (Figure 2). This tRNA suppressor [AlaGC8, see Hou and Schimmel (1992)] contains two nucleotide substitutions in the anticodon stem that replace the wild-type C27:G43 base pair with a U27:U43 mismatch. The G3:C70 base pair in the acceptor helix remained unaltered. To determine the amino acid specificity of U27:U43/G3:C70 $tRNA^{Ala/CUA}$ in vivo, we used this tRNA to suppress an amber-containing fol_{am} gene (encoding for dihydrofolate reductase) whose gene product, after suppression of the amber codon, can be subjected to Edman degradation to establish the amino acid that is inserted at the amber codon (Normanly et al., 1986a,b). Protein sequence analysis confirmed that U27:U43/G3:C70 $tRNA^{Ala/CUA}$ inserted alanine at an amber codon, which accounted for the Trp^+ phenotype of *trpA*(UAG234) (Figure 3). In addition, U27:U43/G3:C70 $tRNA^{Ala/CUA}$ also inserted tyrosine. With an accounting for the "carry over" of alanine from position 9 to 10 in the Edman cycles, we estimated that

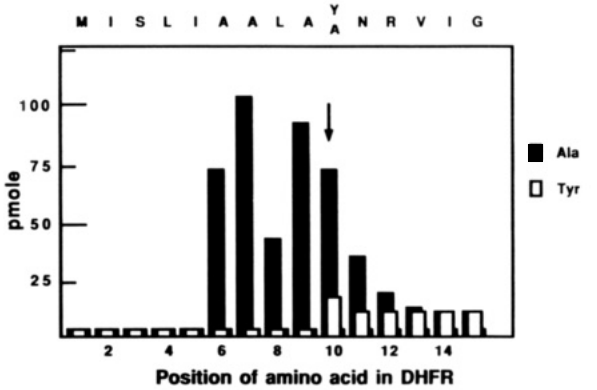


FIGURE 3: Amino acid sequence analysis of the first 15 residues of dihydrofolate reductase (DHFR), encoded by the fol_{am} gene whose amber codon at position 10 was suppressed by U27:U43/G3:C70 $tRNA^{Ala/CUA}$. The picomolar yields of alanine (black box) and tyrosine (open box) are plotted versus the residue number. The sequence of the gene product is given across the top of the diagram.

the amount of tyrosine represents 30% of the total amino acids at position 10.

The suppression efficiency of U27:U43/G3:C70 $tRNA^{Ala/CUA}$ on the XAC-I/lacI-Z_{am} amber mutation was 4.85% relative to that of $tRNA^{Ala/CUA}$. Thus, despite the elimination of the major determinant and the introduction of a U:U mismatch in the anticodon stem that may affect the tRNA structure, U27:U43/G3:C70 $tRNA^{Ala/CUA}$ was a functional tRNA in protein synthesis that was recognized by alanine tRNA synthetase and was accepted by the ribosomal translation machinery.

Aminoacylation of U28:U43/G3:C70 $tRNA^{Ala/CUA}$ with Alanine in Vitro. Although U27:U43/G3:C70 $tRNA^{Ala/CUA}$ contains a mismatch in the anticodon stem, it is stably synthesized in vivo and at a level comparable to the wild-type $tRNA^{Ala}$ (data not shown). This allows isolation of the mutant tRNA from *E. coli* for the purpose of in vitro characterization. Figure 4 shows the initial rate of aminoacylation of U27:U43/G3:C70 $tRNA^{Ala/CUA}$ with alanine. Compared to the rate of the wild-type $tRNA^{Ala}$, the rate of aminoacylation of U27:U43/G3:C70 $tRNA^{Ala/CUA}$ is reduced by 83-fold. [As expected, the plateau of aminoacylation is also reduced because of the slow velocity (Dietrich et al., 1976).] Nonetheless, this slow rate of aminoacylation is significant because under the same conditions with G3:C70 $tRNA^{Ala/CUA}$, no aminoacylation with alanine was detected. We also determined the plateau of aminoacylation (with elevated concentrations of alanine tRNA synthetase) for wild-type $tRNA^{Ala}$, the G3:C70 $tRNA^{Ala/CUA}$ starting material, and U28:U43/G3:C70 $tRNA^{Ala/CUA}$. While G3:C70 $tRNA^{Ala/CUA}$ is not a substrate for the alanine enzyme, U27:U43/G3:C70 $tRNA^{Ala/CUA}$ is aminoacylated to 0.19% of the level of the wild-type $tRNA^{Ala}$ (data not shown).

The results demonstrate that introduction of the U27:U43 substitutions into the sequence framework of G3:C70 in an alanine tRNA restored aminoacylation to an otherwise recognition-defective tRNA substrate. This result suggests that the second-site revertant established a productive complex with alanine tRNA synthetase that compensated for the defect at the 3:70 position. Although the initial rate of aminoacylation and the level of total charging for the second-site revertant were less than 2 and 0.2% of the wild-type alanine tRNA, respectively, the mutation at position 27:43 conferred a functional phenotype in vivo and enabled the tRNA to participate in protein synthesis.

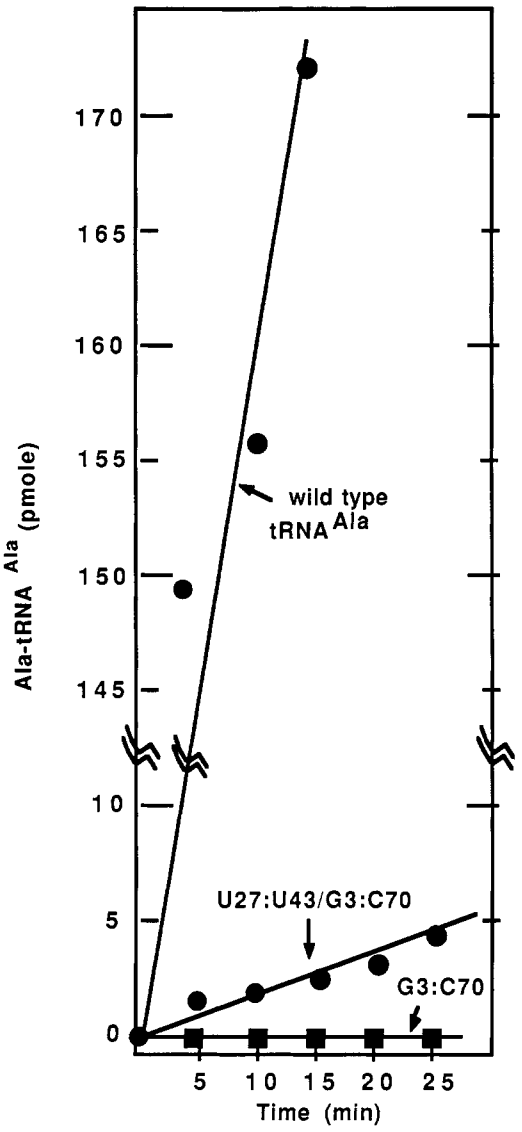


FIGURE 4: Initial rate of aminoacylation with alanine for the wild-type $tRNA^{Ala}$, and the G3:C70 and the U27:U43/G3:C70 variants of $tRNA^{Ala/CUA}$. Assays were performed at 37 °C in 10 mM $MgCl_2$, 5 mM DTT (dithiothreitol), 4 mM ATP, 40 mM sodium phosphate (pH 7.5), 20 μM alanine, 2.4 μM [2,3-^{3}H]alanine, and 100 nM purified alanine tRNA synthetase. Each amber suppressor variant was gel purified and was added at a final concentration of 2.5 μM. Aliquots of the reaction were spotted on a Whatman filter paper and were TCA precipitated as described (Schreier & Schimmel, 1972). The wild-type $tRNA^{Ala}$ was purchased from Subriden RNA (Rolling Bay, WA). Previous study has shown that $tRNA^{Ala}$ and amber suppressor $tRNA^{Ala/CUA}$ have virtually identical kinetic parameters for aminoacylation with alanine (Park et al., 1989).

DISCUSSION

The wild-type base pair at position 27:43 is at a site where previous footprint analysis had established an RNA–protein contact (Park & Schimmel, 1988). Specifically, alanine tRNA synthetase protects the phosphodiester backbone of $tRNA^{Ala}$ from nuclease digestion at sites along the 3′ side of the acceptor helix, and along both the 5′ and 3′ sides of the anticodon stem. An enhanced cleavage at position 27 was observed in the presence of the enzyme. This was interpreted to be a consequence of the physical contact between the protein and tRNA that leads to a conformational change in the RNA structure. Our studies show that alteration of the nucleotides at position 27:43 from C:G to U:U enabled alanine tRNA synthetase to recognize the G3:C70 variant of alanine tRNA that lacks the major determinant for aminoacylation. This suggests that, while the G3:U70 base pair in the acceptor helix constitutes the active site of the protein–RNA interaction, the nucleotides at base pair 27:43 can provide a contact with the alanine enzyme that is functionally coupled to the active site. Thus, when the major contact at the active site is eliminated, it is possible for the alanine enzyme to alter the interaction at position 27:43 in order to reestablish aminoacylation with the tRNA.

The mechanism by which U27:U43 restored aminoacylation is unknown. We cannot rule out the possibility that the U27:U43 substitution recruits a new modification or alters an existing one, in $tRNA^{Ala/CUA}$. However, it is unlikely that a new modification would occur in the acceptor helix, where the enzyme makes contacts for recognition of wild-type $tRNA^{Ala}$, because this part of the tRNA is generally unmodified among *E. coli* tRNAs (Sprinzl et al., 1989).

In principle, the two uracils can exist as an unpaired structural motif that serves as a signal for alanine tRNA synthetase. Additional studies suggest that this is unlikely because another mismatched base pair, such as U27:C43, did not confer aminoacylation (unpublished data). Alternatively, the ribose–phosphate backbone at the two uracils can shift so as to allow two hydrogen bonds between the bases that involve the N3 and 2-carbonyl group of the uracil. The molecular structure of a U:U pairing would therefore be distinct from that of the wild-type C:G pairing and could provide a "new" contact site for recognition by the alanine enzyme. Because the U27:U43 substitution is at the joint between the D- and anticodon stem, this substitution may also allow sufficient flexibility in the tRNA structure to enable the alanine enzyme to make a weak but functional contact with the Watson–Crick paired 2-NH_2 group of G at the critical 3:70 position.

The nucleotides at positions 27:43 are in the anticodon stem, which is distal in the acceptor helix. For aminoacylation with alanine, the interaction between the enzyme and the seven base pairs of the acceptor helix (microhelix^{Ala}) accounts for more than 65% of the free energy of activation (Francklyn et al., 1992). The functional coupling between the acceptor helix and the anticodon stem implies a communication between these two domains of the tRNA structure that is activated upon binding of the enzyme. The anticodon and the acceptor helix are two common locations for determinants of the specificity of aminoacylation (Schimmel, 1989; Normanly & Abelson, 1989; Schulman, 1991). Recent studies have shown that even for systems in which the synthetases use the anticodon sequence as a major determinant for aminoacylation specificity, [e.g., the methionine tRNA (Martinis & Schimmel, 1992) and valine tRNA (Frugier et al., 1992)], the sequence elements in the acceptor helix are sufficient to confer specific aminoacylation. The compensatory mutation isolated in these studies may reflect a relationship between the tRNA acceptor helix and the anticodon stem domain, which was developed during the evolution of the genetic code.

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REFERENCES

Dietrich, A., Kern, D., Bonnet, J., Giege, R., & Ebel, J.-P. (1976) *Eur. J. Biochem. 70*, 147–158.

Eriani, G., Dirheimer, G., & Gangloff, J. (1991) *Nucleic Acids Res. 19*, 265–269.

Francklyn, C., & Schimmel, P. (1989) *Nature 337*, 478–481.

Francklyn, C., Shi, J.-P., & Schimmel, P. (1992) *Science 255*, 1121–1125.
Frugier, M., Florentz, C., & Giege, R. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 3990–3994.
Hou, Y.-M., & Schimmel, P. (1988) *Nature 333*, 140–144.
Hou, Y.-M., & Schimmel, P. (1989) *Biochemistry 28*, 4942–4947.
Hou, Y.-M., & Schimmel, P. (1992) *Biochemistry 31*, 4157–4160.
Hou, Y.-M., Shiba, K., Mottes, C., & Schimmel, P. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 976–980.
Jahn, M., Rogers, M. J., & Soll, D. (1991) *Nature 352*, 258–260.
Kleina, L. G., Masson, J.-M., Normanly, J., Abelson, J., & Miller, J. H. (1990) *J. Mol. Biol. 213*, 705–717.
Martinis, S. A., & Schimmel, P. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 65–69.
Masson, J. M., & Miller, J. H. (1986) *Gene 47*, 179–183.
McClain, W. H., & Foss, K. (1988) *Science 240*, 793–796.
McClain, W. H., Chen, Y.-M., Foss, K., & Schneider, J. (1988) *Science 242*, 1681–1684.
Murgola, E. J., & Hijazi, K. A. (1983) *MGG, Mol. Gen. Genet. 191*, 132–137.
Musier-Forsyth, K., Usamn, N., Scaringe, S., Doudna, J., Green, R., & Schimmel, P. (1991) *Science 253*, 784–786.
Normanly, J., & Abelson, J. (1989) *Annu. Rev. Biochem. 58*, 1029–1049.
Normanly, J., Odgen, R. C., Horvath, S. J., & Abelson, J. (1986a) *Nature 321*, 213–219.
Normanly, J., Masson, J.-M., Kleina, L., Abelson, J., & Miller, J. H. (1986b) *Proc. Natl. Acad. Sci. U.S.A. 83*, 6548–6552.
Park, S.-J., & Schimmel, P. (1988) *J. Biol. Chem. 263*, 16527–16530.
Park, S. J., Hou, Y.-M., & Schimmel, P. (1989) *Biochemistry 28*, 2740–2746.
Putz, J., Puglishi, J. D., Florentz, C., & Giege, G. (1991) *Science 252*, 1696–1699.
Raftery, L. A., & Yarus, M. (1987) *EMBO J. 6*, 1949–1506.
Rould, M. A., Perona, J. J., Soll, D., & Steitz, T. A. (1989) *Science 246*, 1135–1142.
Rould, M. A., Perona, J. J., & Steitz, T. A. (1991) *Nature 352*, 213–218.
Ruff, M., Krishnaswamy, S., Boeglin, M., Poterszman, A., Mitshler, A., Podjarny, A., Rees, B., Thierry, J. C., & Moras, D. (1991) *Science 252*, 1682–1689.
Sampson, J. R., DiRenzo, A. B., Behlen, L. S., & Uhlenbeck, O. C. (1990) *Biochemistry 29*, 2523–2532.
Sanger, F., Nicklen, S., & Coulson, A. R. (1979) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.
Schimmel, P. (1987) *Annu. Rev. Biochem. 56*, 125–158.
Schimmel, P. (1989) *Biochemistry 28*, 2727–2759.
Schimmel, P. R., & Soll, D. (1979) *Annu. Rev. Biochem. 48*, 601–648.
Schreier, A. A., & Schimmel, P. (1972) *Biochemistry 11*, 1582–1589.
Schulman, L. H. (1991) *Prog. Nucleic Acid Res. Mol. Biol. 41*, 23–87.
Sprinzl, M., Hartmann, T., Weber, J., Blank, J., & Zeidler, R. (1989) *Nucleic Acids Res. 17*, r1–r172.

Registry No. Alanine tRNA synthetase, 9031-71-4; uracil, 66-22-8; cytosine, 71-30-7; guanine, 73-40-5.