The Yeast Phenylalanyl-Transfer RNA Synthetase Recognition Site: The Region Adjacent to the Dihydrouridine Loop

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ABSTRACT Purified yeast phenylalanyl-tRNA synthetase can aminoacylate (yeast) $tRNA^{Phe}$, (wheat) $tRNA^{Phe}$, and (*Escherichia coli*) $tRNA_1^{Val}$ (1, 2). We now report that this synthetase can also aminoacylate (*E. coli*) $tRNA^{Phe}$ and (*E. coli*) $tRNA_1^{Ala}$. Highly purified (*E. coli*) $tRNA^{Phe}$ is heterologously aminoacylated to approximately 90% of the extent achieved with the homologous enzyme (crude *E. coli* phenylalanyl-tRNA synthetase). Pure (*E. coli*) $tRNA_1^{Ala}$ (the major species) is heterologously aminoacylated to 70% of the extent achieved with the homologous synthetase (crude *E. coli* alanyl-tRNA synthetase).

(*E. coli*) $tRNA^{Phe}$ is the fourth purified transfer RNA of known sequence to be shown to be an acceptable substrate for purified yeast phenylalanyl-tRNA synthetase. A comparison of these sequences shows that only one region is extremely similar in all four tRNAs. This region is located adjacent to the dihydrouridine loop, and consists of the nucleotides 3′—CUCGA—5′. We conclude that this is the

$$3'—CUCGA—5'$$
$$\quad\ |\ |\ |\ |$$
$$5'—GAGC—3'$$

synthetase recognition site for yeast phenylalanyl-tRNA synthetase.

This conclusion is further supported by partial fragment analysis of (*E. coli*) $tRNA_1^{Ala}$.

Recent progress in the elucidation of sequences of tRNAs has not been accompanied by a significant enhancement of our understanding of the functions of these molecules. For example, the aminoacyl-tRNA synthetase recognition site cannot be unambiguously identified despite much effort. One approach to this problem is to compare the structures of several tRNAs, all of which are aminoacylated by a single synthetase. The region that is very similar or identical would then be a most likely candidate for the synthetase recognition site.

Purified (200-fold) yeast phenylalanyl-tRNA synthetase can aminoacylate (yeast) $tRNA^{Phe}$, (wheat) $tRNA^{Phe}$, and (*Escherichia coli*) $tRNA_1^{Val}$ (the major species of *E. coli* valine tRNA) (1, 2). All three tRNAs were obtained in a purity of at least 90% and all three are aminoacylated essentially completely. A comparison of their structures showed that the dihydrouridine regions (consisting of the dihydrouridine loop and adjacent double-stranded stem) are very similar in all three molecules (1).

We have previously reported that at least two other *E. coli* tRNAs (in addition to (*E. coli*) $tRNA_1^{Val}$) can be aminoacylated by yeast phenylalanyl-tRNA synthetase (1). We have now isolated and identified these two tRNAs as (*E. coli*) $tRNA^{Phe}$ and (*E. coli*) $tRNA_1^{Ala}$.

Highly purified (*E. coli*) $tRNA^{Phe}$ was obtained by chromatography of crude *E. coli* B tRNA on benzoylated DEAE-cellulose (BD-cellulose) at neutral and acidic pH. The final purity was at least 90%, based upon acceptor activity. This purified (*E. coli*) $tRNA^{Phe}$ was aminoacylated with yeast phenylalanyl-tRNA synthetase to about 90% of the extent achieved with homologous enzyme, as shown in Table 1. The heterologous aminoacylation was highly dependent upon enzyme concentration and considerably more enzyme was needed for heterologous than for homologous aminoacylation.

TABLE 1. *Aminoacylation of purified E. coli B tRNA^{Phe} with [^{14}C]phenylalanine*

Enzyme	Radioactivity (cpm)
Crude *E. coli* tRNA synthetase	1155
Purified yeast phenylalanyl-tRNA synthetase	1026

Aminoacylation was performed in a total volume of 1 ml containing 50.0 mM Tris(pH 7.6); 0.5 mM EDTA; 20.0 mM $MgCl_2$; 2.5 mM potassium ATP; 0.05 mM [^{14}C]phenylalanine (10 Ci/mol); 0.06 A_{260} units of 90% pure *E. coli* $tRNA^{Phe}$; and a saturating amount of either crude *E. coli* tRNA synthetase or purified yeast phenylalanyl-tRNA synthetase prepared as described (1). The heterologous reaction was very sensitive to magnesium and synthetase concentrations. Incubation was for 10 min at 30°C. The reaction was stopped by the addition of cold 10% trichloroacetic acid and the mixture was immediately filtered through a Millipore membrane filter, which was then washed with 5% trichloroacetic acid, dried, and counted in Omnifluor–toluene (New England Nuclear Corp.).

TABLE 2. *Aminoacylation of pure E. coli B tRNA$_1^{Ala}$*

Enzyme and amino acid	Radioactivity (cpm)
Crude *E. coli* tRNA synthetase	
[^{14}C]alanine	980
[^{14}C]phenylalanine	0
Purified yeast phenylalanyl-tRNA synthetase	
[^{14}C]phenylalanine	700

Aminoacylation was performed in a total volume of 1 ml containing 50.0 mM potassium cacodylate (pH 5.8); 20.0 mM $MgCl_2$; 2.5 mM potassium ATP; 0.05 mM [^{14}C]amino acid (10 Ci/mol); 0.058 A_{260} units of tRNA; and a saturating amount of crude *E. coli* tRNA synthetase or purified yeast phenylalanyl-tRNA synthetase. Incubation was for 60 min at 30°C. The reaction was stopped by the addition of cold 10% trichloroacetic acid, and the solution was filtered, washed, dried, and counted as described in Table 1.
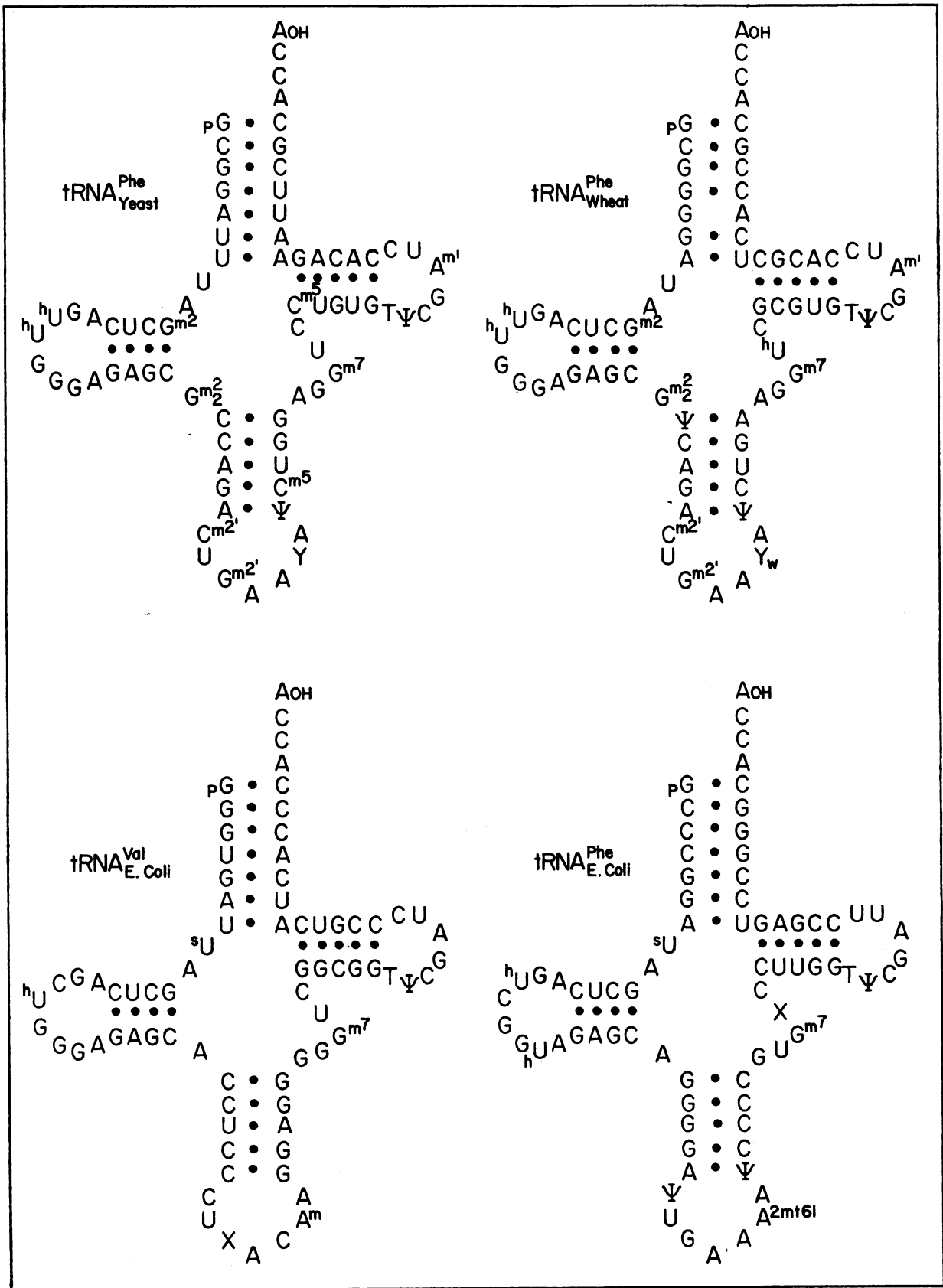
FIG. 1.   The primary structure of the four tRNAs that are aminoacylated by yeast phenylalanyl-tRNA synthetase.

Highly purified (*E. coli*) tRNA₁^{Ala}, the major species of *E. coli* alanine tRNA, was obtained by chromatography of crude *E. coli* B tRNA on benzoylated DEAE-cellulose at neutral and acidic pH, and by chromatography on DEAE-Sephadex at neutral pH. The final purity was essentially 100%, based on acceptor activity. This pure (*E. coli*) tRNA₁^{Ala} was aminoacylated with yeast phenylalanyl-tRNA synthetase to about 70% of the extent achieved with homologous synthetase, as shown in Table 2.

(*E. coli*) tRNA^{Phe} is the fourth purified tRNA of known structure that has been shown to be an acceptable substrate for yeast phenylalanyl-tRNA synthetase. The primary sequences of these four tRNAs, (*E. coli*) tRNA^{Phe} (3), (*E. coli*) tRNA₁^{Val} (4), (yeast) tRNA^{Phe} (5), and (wheat) tRNA^{Phe} (6), are shown in Fig. 1. A composite structure based upon these four tRNAs is shown in Fig. 2. Those nucleotides that are uniquely present in the four tRNAs of Fig. 1 are shown in the composite tRNA in boldface. It is apparent that only one extensive region is very similar in all four tRNAs. This region consists of the nucleotides from adenosine at position 9 to cytidine at position 13, and from guanosine at position 22 to cytidine at position 25, and comprises the complete double-stranded region adjacent to the dihydrouridine loop. Since no other extensive region of similarity exists, we conclude that this is the yeast phenylalanyl-tRNA synthetase recognition site. This conclusion agrees with the results obtained from chemical and enzymatic modifications of tRNAs that are substrates for yeast phenylalanyl-tRNA synthetase. For example, the composite tRNA (Fig. 2) shows that the dihydrouridine loop, itself, should be relatively unimportant for synthetase recognition. Igo-Kemenes and Zachau (7) found no loss of acceptor activity after reacting the dihydrouridine residues of (yeast) tRNA^{Phe} with sodium borohydride (all of the dihydrouridine residues of this tRNA are located in the dihydrouridine loop). More recently Schmidt, Buchardt, and Reid (8) observed the retention of acceptor activity of (yeast) tRNA^{Phe} after enzymatic cleavage of the dihydrouridine loop. In addition, the composite tRNA (Fig. 2) shows that the anticodon loop is unimportant for synthetase recognition. Philippsen, Thiebe, Wintermeyer, and Zachau (9) found that (yeast) tRNA^{Phe} retains acceptor activity after being chemically cleaved at the anticodon loop.

Interestingly, all four tRNAs of known sequence that are acceptable substrates for yeast phenylalanyl-tRNA synthetase are exactly the same size, 76 nucleotides. This raises the possibility that size, as well as nucleotide sequence, are necessary conditions for aminoacylation by this synthetase. Also, the role of the other boldface nucleotides found scattered throughout the composite tRNA (Fig. 2) deserves further study. Most are not likely to participate in the recognition site since they are present in the same position in the great majority of tRNAs, but are not found in every tRNA. Adenosine at position 73 is an example of such a nucleotide. However, certain of the boldface nucleotides are distinctly not in this category. 7-Methyl guanosine is found relatively infrequently in tRNA and yet is present, in the same site, in all four tRNAs that are acceptable substrates for yeast phenylalanyl-tRNA synthetase. These points will be clarified as the sequences of other tRNAs aminoacylated by yeast phenylalanyl-tRNA synthetase become known.

(*E. coli*) tRNA₁^{Ala} is also an acceptable substrate for yeast phenylalanyl-tRNA synthetase and, therefore, would be
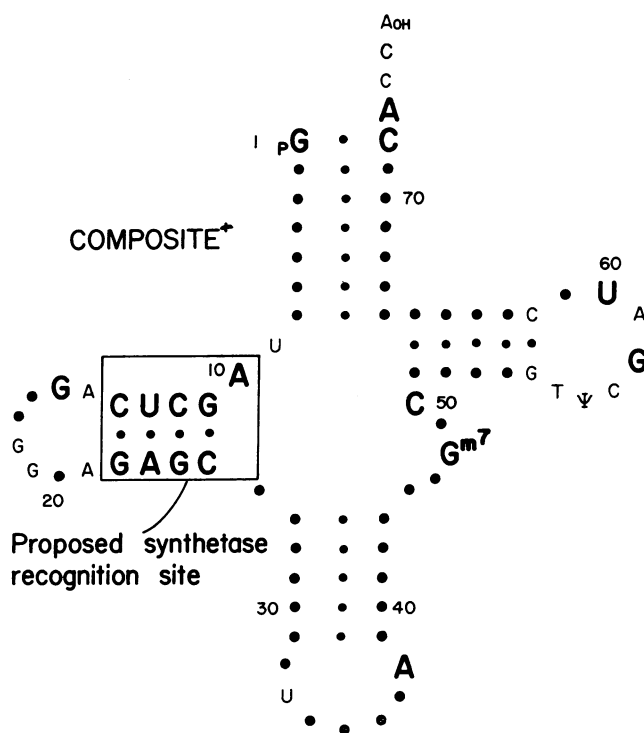

FIG. 2. Composite tRNA. Those nucleotides that are not the same in the four tRNAs of Fig. 1 are shown in the composite with a dot. Those nucleotides that are found in the same position in all tRNAs are shown in the composite in light type. Those nucleotides that are uniquely common to the four tRNAs of Fig. 1 are shown in **boldface**.

+ The central nucleotide of the anticodon of the four tRNAs of Fig. 1 is adenosine. This has been eliminated from the composite tRNA since, based on the genetic code, it could not be present in that position in any species of alanine tRNA.

expected to contain the synthetase recognition site shown in the composite tRNA (Fig. 2). Based upon the four tRNAs of Fig. 1, we would expect to obtain, from a complete pancreatic RNase digestion of (*E. coli*) tRNA₁^{Ala}, either the octanucleotide G—G—G—A—G—A—G—C— or the pentanucleotide A—G—A—G—C—. The isolation of either oligonucleotide fragment would suggest that the dihydrouridine region of (*E. coli*) tRNA₁^{Ala} is indeed similar to that of the other four tRNAs of Fig. 1. This exact octanucleotide, G—G—G—A—G—A—G—C—, has been found by Alvino, Remington, and Ingram (10) in a complete pancreatic RNase digest of (*E. coli* B) tRNA₁^{Ala}. This result further supports the proposed synthetase recognition site; sequence analysis of this tRNA is underway to confirm the location of this octanucleotide in the dihydrouridine region of the molecule.

Although the four tRNAs of Fig. 1 are aminoacylated by a single synthetase, they are not all aminoacylated at the same rate. We interpret this to mean that base modifications and nucleotides located outside of the proposed synthetase recognition site can play a secondary role in modulating the aminoacylation reaction. Finally, we wish to stress that the information presented here applies only to the yeast phenylalanyl-tRNA synthetase recognition site and not necessarily to any other aminoacyl-tRNA synthetase recognition site. It is becoming increasingly apparent that there is no universal tRNA site that is recognized by all synthetases. Detailed information

will have to be obtained for several synthetases before even the most modest generalizations can be attempted.

In summary, we have found that four purified tRNAs of known sequence are recognized by a single synthetase. Only one region of these four tRNAs is uniquely similar. This is a region, comprising nine nucleotides, adjacent to the dihydrouridine loop. We propose that this is the yeast phenylalanyl-tRNA synthetase recognition site.

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