each smear and covered with a cover slip. Slides were placed in airtight chambers containing a tissue saturated with 2 ml of the hybridization mixture and incubated at 37°C for 5 to 16 hours. Cover slips were removed by immersion in five times SET, and the slides were immediately washed three times in 0.2 times SET at 37°C for 10 minutes each time. Slides were then dried in air in the dark and viewed immediately, or stored in the dark at 4°C until they were evaluated.
8. Samples were mounted in Citifluor (Citifluor, Ltd., London) and viewed under oil immersion with a Neofluor 100 times objective on a Zeiss Photomicroscope III fitted with an epifluorescence condenser, a mercury lamp, and Zeiss filter sets #48-77-09 and #48-77-15. Photomicrographs were taken with Kodachrome or Fujichrome 400 ASA color film.
9. S. W. Skinner, *Genetics* **109**, 745 (1985); A. M. Huger, S. W. Skinner, J. H. Werren, *J. Invert. Pathol.* **46**, 272 (1985); J. H. Werren, S. W. Skinner, A. M. Huger, *Science* **231**, 990 (1986).
10. S. Skinner, S. Turner, E. DeLong, in preparation. The 21-nucleotide probe that distinguishes sonkiller from *P. vulgaris* is complementary to *E. coli* 16S rRNA nucleotides 59 to 79 and has four mismatches with the corresponding *P. vulgaris* sequence.
11. H. Bremer and P. P. Dennis, in Escherichia coli *and* Salmonella typhimurium *Cellular and Molecular Biology*, F. C. Neidhardt *et al.*, Eds. (American Society for Microbiology, Washington, DC, 1987), pp. 1527–1542.
12. *Escherichia coli* B (ATCC 11303) was grown at 37°C in a salts medium (42 m*M* Na_2HPO_4, 22 m*M* KH_2PO_4, 9.5 m*M* NaCl, 19 m*M* NH_4Cl, 2 m*M* $MgSO_4$, 0.1 m*M* $CaCl_2$) supplemented with one of the following: 0.2% acetate, 0.2% pyruvate, 0.2% glucose or 0.2% glucose containing 0.2% casein hydrolysate. Cells at the fastest growth rate were cultured in 1% tryptone, 0.5% yeast extract, 0.2% glucose, 5.8 m*M* NaCl. Growth was assessed by measurements of optical density at 450 nm. Cells were harvested at an optical density of about 0.6 for RNA analysis and hybridization experiments.
13. E. F. DeLong, unpublished data.
14. E. Ohtsuka, S. Matsuki, M. Ikehara, T. Takahashi, K. Matshubara, *J. Biol. Chem.* **260**, 2605 (1985).
15. R. S. Hanson and J. A. Phillips, in *Manual of Methods for General Bacteriology*, P. Gerhardt *et al.*, Eds. (American Society for Microbiology, Washington, DC, 1981), pp. 328–364.
16. We thank L. Washington for expert assistance in oligonucleotide synthesis and P. Rouvière for supplying methanogen cultures. Supported by Office of Naval Research contract N14-87-K-0813 and NIH grant GM34527 (N.R.P.).

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Nucleotides in Yeast $tRNA^{Phe}$ Required for the Specific Recognition by Its Cognate Synthetase

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An analysis of the aminoacylation kinetics of unmodified yeast $tRNA^{Phe}$ mutants revealed that five single-stranded nucleotides are important for its recognition by yeast phenylalanyl-tRNA synthetase, provided they were positioned correctly in a properly folded tRNA structure. When four other tRNAs were changed to have these five nucleotides, they became near-normal substrates for the enzyme.

THE ACCURATE INCORPORATION OF amino acids into proteins depends on the correct aminoacylation of each tRNA by its cognate aminoacyl-tRNA synthetase. How each synthetase recognizes its set of iso-acceptor tRNAs among all of the tRNAs in the cell remains unknown. We have used a biochemical approach to identify nucleotides in yeast $tRNA^{Phe}$ (Fig. 1A) that are required for its specific recognition and subsequent aminoacylation by yeast Phe-tRNA synthetase (FRS). Anticodon loop replacement experiments established that substitution of any one of the anticodon nucleotides G_{34}, A_{35}, or A_{36} resulted in a 3- to 12-fold reduction of the rate of aminoacylation with purified FRS (*1*). When yeast $tRNA^{Tyr}$ was modified to have a Phe anticodon by changing $\psi_{35} \rightarrow A_{35}$, it became a much better substrate for misacylation by FRS, yet still aminoacylated poorly when compared to $tRNA^{Phe}$ (*2*). These data suggested that although FRS, like many other synthetases (*3*), requires the anticodon for the specific recognition of $tRNA^{Phe}$, other features in $tRNA^{Phe}$ must contribute as well. A method that allows substitution of nucleotides elsewhere in the $tRNA^{Phe}$ sequence involves in vitro transcription by T7 RNA polymerase (*4*). Although the wild-type $tRNA^{Phe}$ transcript lacked all of the modified nucleotides normally found in yeast $tRNA^{Phe}$, it was a good substrate for FRS, thus allowing extensive structure-func-

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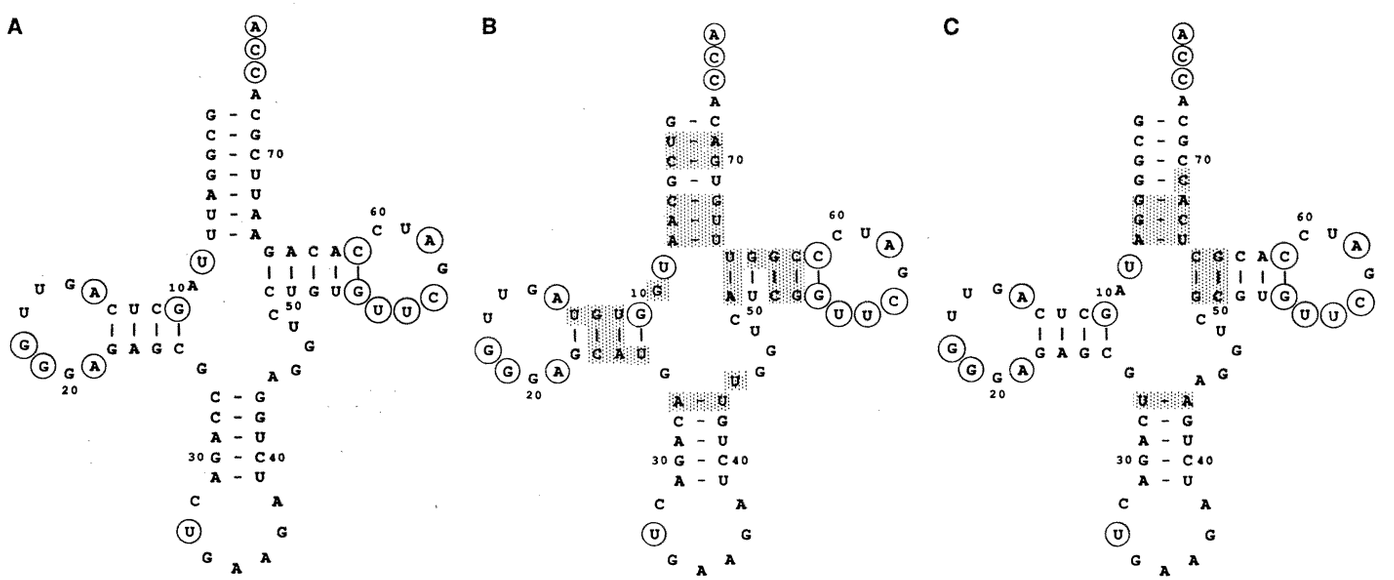

Fig. 1. Three tRNAs [(**A**) yeast $tRNA^{Phe}$, (**B**) *S. pombe* $tRNA^{Phe}$, and (**C**) wheat germ $tRNA^{Phe}$] that are active substrates for yeast FRS. The circled nucleotides are conserved in all cytoplasmic yeast tRNAs and the nucleotides in *S. pombe* $tRNA^{Phe}$ and wheat germ $tRNA^{Phe}$ that differ from yeast $tRNA^{Phe}$ are shaded. *S. pombe* $tRNA^{Phe}$ aminoacylates with the same k_{cat} and K_m as yeast $tRNA^{Phe}$ (*7*), whereas wheat germ $tRNA^{Phe}$ has the same k_{cat} and 1.3-fold lower K_m (*6*).

tion studies. Substitution of G_{20} or A_{73} with a U reduced the rate of aminoacylation by 12-fold (*4, 5*). In contrast, substitution of four other single-stranded residues (U_{16}, U_{17}, U_{59}, and C_{60}) resulted in less than a twofold reduction in the rate of aminoacylation. These results suggested that although G_{20} and A_{73} are also potential recognition nucleotides, not all residues are important for the recognition process.

The phenylalanyl-tRNAs from both wheat germ and *Schizosacchromyces pombe* are aminoacylated by yeast FRS with kinetics virtually identical to that of yeast $tRNA^{Phe}$ (*6, 7*). These two tRNAs contain G_{20}, G_{34}, A_{35}, A_{36}, and A_{73}, but differ from the yeast $tRNA^{Phe}$ sequence at 13 and 27 positions, respectively (Fig. 1). Most of these differences involve base pairs in the acceptor and TψCG stems, suggesting that FRS does not make nucleotide-specific contacts with these helices. In addition, *S. pombe* $tRNA^{Phe}$ has six substitutions in the D stem that are believed to form tertiary interactions structurally similar to those in yeast $tRNA^{Phe}$ (*8*). Thus, the nucleotides involved in tertiary interactions may only be required for the stabilization of the folded structure and not involved in FRS recognition. This is supported by aminoacylation kinetic data for mutations in the nine tertiary interactions in the yeast $tRNA^{Phe}$ transcript (*5, 9, 10*). In general, mutations that disrupted tertiary interactions aminoacylated poorly, whereas mutations that resulted in structurally similar tertiary interactions aminoacylated well. We therefore propose that the five single-stranded nucleotides G_{20}, G_{34}, A_{35}, A_{36}, and A_{73} constitute most if not all of the nucleotides required for recognition by FRS, provided that they are positioned as dictated by

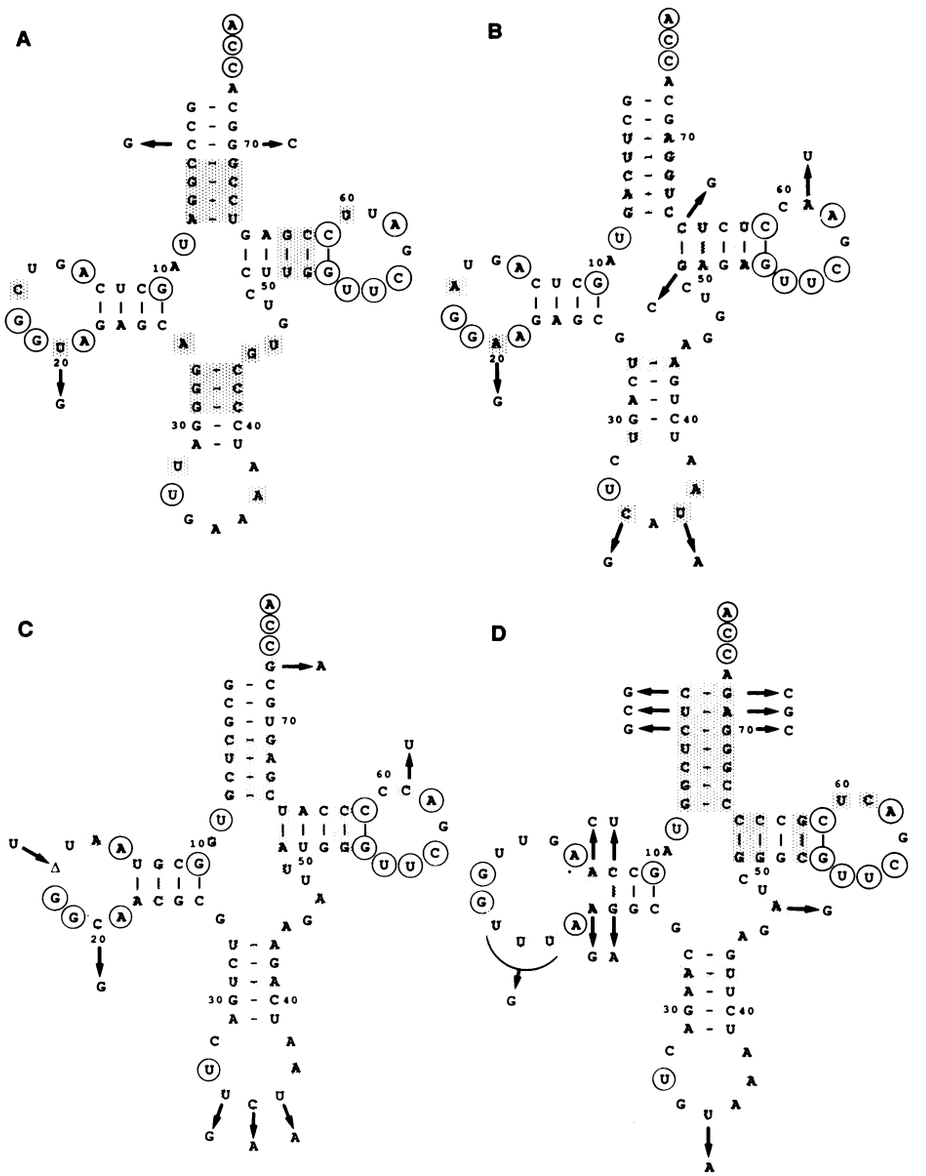

Fig. 2. Alteration of four tRNAs to be substrates for FRS. The sequences of unmodified (**A**) *E. coli* $tRNA^{Phe}$ (*23*), (**B**) yeast $tRNA^{MetM}$ (*24*), (**C**) $tRNA^{Arg}$ (*25*), and (**D**) $tRNA^{Tyr}$ (*26*) are given with the conserved nucleotides circled and the residues that differ from $tRNA^{Phe}$ shaded. Δ indicates the absence of a nucleotide. The nucleotide substitutions used to change each tRNA into a substrate for FRS are indicated by arrows. The C_3-$G_{70} \rightarrow G_3$-C_{70} change in *E. coli* $tRNA^{Phe}$, the G_{49}-$C_{65} \rightarrow C_{49}$-$G_{65}$ and A_{59} changes in $tRNA^{Met \rightarrow Phe}$, the $C_{59} \rightarrow U_{59}$ change in $tRNA^{Arg \rightarrow Phe}$, and the U_2-$A_{71} \rightarrow C_2$-G_{71} and $C_3 \rightarrow G_{70} \rightarrow G_3$-$C_{70}$ changes in $tRNA^{Tyr \rightarrow Phe}$ occur at positions that vary between the tRNAs in Fig. 1 and thus are not expected to contribute to recognition by FRS. Each mutant tRNA gene was constructed from six synthetic DNA oligonucleotides between an upstream T7 promoter and downstream Bst NI restriction site and inserted into a plasmid. The tRNA transcripts were synthesized and purified as previously described (*4*).

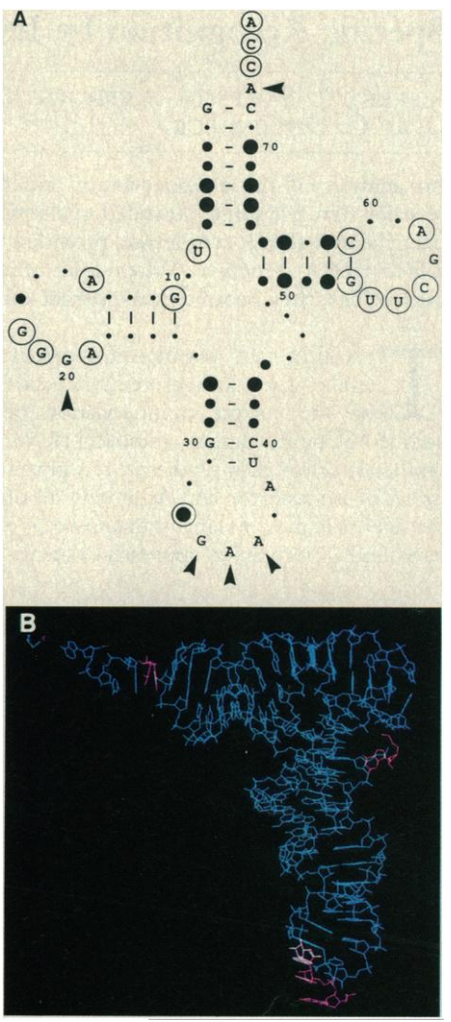

Fig. 3. Sequence requirement for FRS recognition. (**A**) The yeast $tRNA^{Phe}$ sequence with circles around nucleotides present in all tRNAs, arrows indicating the five proposed recognition nucleotides and small, medium, and large dots indicating positions where the nucleotide had been changed to one, two, or three other nucleotides, respectively, without altering the activity with FRS. (**B**) The structure of yeast $tRNA^{Phe}$ (*27*) with the five proposed recognition nucleotides indicated in red.

the $tRNA^{Phe}$ secondary and tertiary structure. We tested this model by attempting to improve the misacylation of four other tRNAs with FRS by making the necessary nucleotide changes predicted by the model.

Escherichia coli $tRNA^{Phe}$ is a rather poor substrate for FRS, showing a ratio of the rate constant to the Michaelis constant (k_{cat}/K_m) 30-fold less than that of yeast $tRNA^{Phe}$ (*6*). According to our model, this reduced rate of aminoacylation should be the result of U_{20} in *E. coli* $tRNA^{Phe}$ instead of the required G_{20}. Thus, we synthesized an *E. coli* $tRNA^{Phe}$ transcript and a mutant having a G at position 20. Both molecules also contained the C_3-$G_{70} \rightarrow G_3$-C_{70} change to ensure that the nucleotides 1 to 5 of the tRNA gene are consistent with an active T7 promoter (Fig. 2A). Consistent with the data on the fully modified tRNAs, the *E. coli* $tRNA^{Phe}$ transcript showed a 24-fold lower k_{cat}/K_m than the yeast $tRNA^{Phe}$ transcript (Table 1). As predicted by the model, the $U_{20} \rightarrow G_{20}$ substitution in the *E. coli* $tRNA^{Phe}$ transcript substantially improved its ability to aminoacylate, resulting in a k_{cat}/K_m only twofold lower than that of the yeast $tRNA^{Phe}$ transcript. This $G_{20} \rightarrow U_{20}$ mutation reduced the k_{cat}/K_m by nearly the same amount in both tRNA sequence backgrounds (Table 1), supporting our earlier conclusion that G_{20} is one of the FRS recognition nucleotides.

Since FRS only encounters yeast tRNAs in vivo, it would be of greater interest to change other yeast tRNAs into substrates for FRS. The yeast elongator $tRNA^{Met}$ is a good candidate because the k_{cat}/K_m for the misacylation of this tRNA by FRS has been measured to be about 0.5 percent of yeast $tRNA^{Phe}$ (*11*). Our model suggests that only three nucleotide substitutions, $A_{20} \rightarrow G_{20}$, $C_{34} \rightarrow G_{34}$, and $U_{36} \rightarrow A_{36}$, would be required to change $tRNA^{Met}$ into an active substrate for FRS. Thus, we synthesized a $tRNA^{Met \rightarrow Phe}$ that incorporated these three changes as well as the nonessential $A_{59} \rightarrow U_{59}$ substitution, to create the proper Pb^{2+} cleavage domain for structural analysis, and a G_{49}-$C_{65} \rightarrow C_{49}$-$G_{65}$, to destroy an internal Bst NI restriction site (Fig. 2B). The $tRNA^{Met \rightarrow Phe}$ exhibits relatively normal aminoacylation kinetics with FRS (Table 2), supporting the importance of G_{20}, G_{34}, and A_{36} for the specific recognition by FRS.

Inspection of the yeast $tRNA^{Arg}$ sequence indicated that all five of the essential nucleotides needed to be changed to convert this tRNA into a substrate for FRS. To ensure that the structure of this tRNA would be similar to $tRNA^{Phe}$, U_{17} was inserted to give the correct number of nucleotides in the D loop. We constructed $tRNA^{Arg \rightarrow Phe}$ with these six substitutions as well as the nonessential $C_{59} \rightarrow U_{59}$ for structural analysis by Pb^{2+} cleavage (Fig. 2C). Again supporting the model, $tRNA^{Arg \rightarrow Phe}$ exhibited a k_{cat}/K_m similar to $tRNA^{Phe}$ (Table 2). In addition, a mutant $tRNA^{Arg + Phe}$ that retained the G at position 73 showed a fivefold lower k_{cat}/K_m as compared with $tRNA^{Arg \rightarrow Phe}$, indicating that A_{73} is an important recognition nucleotide in this tRNA background.

Finally, yeast $tRNA^{Tyr}$ (Fig. 2D) contains three of the five essential single-stranded nucleotides, but has only three base pairs in the D stem and a larger D loop than $tRNA^{Phe}$. The k_{cat}/K_m for misacylation of the fully modified $tRNA^{Tyr}$ with FRS is about 10^{-4} lower than $tRNA^{Phe}$ (*2*). Introducing a Phe anticodon into $tRNA^{Tyr}$ by substituting $U_{35} \rightarrow A_{35}$ improved k_{cat}/K_m only 15-fold, despite the fact that the tRNA now contained four of the five essential single-stranded nucleotides (*2*). To convert $tRNA^{Tyr}$ into an efficient substrate for FRS, the two missing essential nucleotides must be supplied and the overall structure altered to be more similar to $tRNA^{Phe}$. Thus, $tRNA^{Tyr \rightarrow Phe}$ was designed by replacing the three uridines in the D loop with a single G_{20} and substituting U_{35} with A_{35}. To maintain the base triples found in $tRNA^{Phe}$, the D stem was closed with a C_{13}-G_{22}-G_{46}, and U_{12}-A_{23} was introduced to combine with A_9. Finally the first three base pairs in the acceptor stem were changed to make them consistent with an active T7 promoter. The resulting $tRNA^{Tyr \rightarrow Phe}$ showed a k_{cat}/K_m 1.5-fold greater than that of the $tRNA^{Phe}$ transcript (Table 2). However, it is likely that not all 13 of the changes we made were necessary to convert $tRNA^{Tyr}$ into an efficient substrate for FRS. Our recent data showing that $tRNA^{Phe}$ mutants having U_{13}-A_{22}-A_{46} and C_{12}-G_{23}-G_9 are fully active (*5*) suggest that only $A_9 \rightarrow G_9$ and $A_{13} \rightarrow U_{13}$ would have been sufficient to make $tRNA^{Tyr}$ structurally similar to $tRNA^{Phe}$. In addition, *S. pombe* $tRNA^{Phe}$ differs from yeast $tRNA^{Phe}$ at base pairs 2 to 71 and 3 to 70, suggesting that these pairs are not specifically recognized by FRS. Although no conclusion can be made for G_1-C_{72}, these data indicate that only four changes (U_{20}, U_{20a}, $U_{20b} \rightarrow G_{20}$; $A_9 \rightarrow G_9$; $A_{13} \rightarrow U_{13}$; and $U_{35} \rightarrow A_{35}$) may be sufficient to convert yeast $tRNA^{Tyr}$ into an active substrate for FRS.

The availability of four more active substrates for FRS makes it possible to eliminate a substantial number of additional nucleotides as potential recognition sites for FRS. For example, the A_{29}-U_{41} base pair in yeast $tRNA^{Phe}$ is a G_{29}-C_{41} in *E. coli* $tRNA^{Phe}$ ($U_{20} \rightarrow G_{20}$) and a U_{29}-A_{41} in $tRNA^{Arg \rightarrow Phe}$. In addition, $tRNA^{Arg \rightarrow Phe}$ differs from $tRNA^{Phe}$ at six positions that

Table 1. Aminoacylation of the wild-type and mutant yeast and *E. coli* $tRNA^{Phe}$ transcripts. The final aminoacylation levels for each tRNA were determined in a 60-μl reaction mixture containing 30 m*M* Hepes-KOH (*p*H 7.45), 4 m*M* dithiothreitol, 25m*M* KCl, 15m*M* $MgCl_2$, 10 μ*M* [^{3}H]phenylalanine, 2 m*M* adenosine triphosphate (ATP), 0.4 μ*M* tRNA, and 6.0 U/ml (40 n*M*) FRS. Each tRNA was renatured in 10 m*M* tris-HCl, and 1 m*M* ETDA (*p*H 7.6) by heating to 80°C and slow cooling to 25°C before the addition of the reaction mixture. At 15-s intervals, 7-μl aliquots were spotted on Whatman 3-mm paper and treated as previously described (*5*). The aminoacylation kinetics were performed in the same buffer by using six tRNA concentrations at a final FRS concentration of 0.1 U/ml (0.8 n*M*). The apparent K_m and k_{cat} values were calculated from an Eadie-Hofstee analysis of the initial rates with the use of a least squares analysis. The values of k_{cat}/K_m for two or three separate determinations were within ±10%. A_{260}, absorbance at 260 nm.

tRNA	pmol/A_{260}	K_m (n*M*)	k_{cat} (min^{-1})	K_{cat}/K_m (relative)
Yeast Phe	1400	350	160	(1.0)
Yeast Phe (U_{20})	1300	2100	80	0.083
E. coli Phe	1300	1800	35	0.042
E. coli Phe (G_{20})	1400	420	100	0.52

Table 2. Aminoacylation of tRNA transcripts. The final aminoacylation levels and kinetics for each tRNA were performed as described in Table 1. All tRNA transcripts were renatured before the addition to the aminoacylation reaction mixture as described above, with the exception of $tRNA^{Tyr \rightarrow Phe}$, which contained 15 m*M* $MgCl_2$ in the renaturation buffer.

tRNA	pmol/A_{260}	K_m (n*M*)	k_{cat} (min^{-1})	k_{cat}/K_m (relative)
Yeast Phe	1400	340	160	(1.0)
Yeast Met → Phe	1400	410	130	0.68
Yeast Arg → Phe	1400	380	110	0.64
Yeast Met → Phe (G_{73})	1200	1000	60	0.13
Yeast Tyr → Phe	1400	360	250	1.5

are involved in three separate tertiary interactions. Of the 76 nucleotides in $tRNA^{Phe}$, 16 can be eliminated from consideration because they are conserved in all cytoplasmic yeast tRNAs. Of the remaining 60 nucleotides, 25 have been changed to at least two other nucleotides and an additional 23 to one other nucleotide without altering the aminoacylation kinetics (Fig. 3A). Although this suggests that these 48 residues are not required for FRS recognition, the possibility remains that an essential functional group could be conserved. For example, position 15 can be an A or a G, but neither pyrimidine has been tested, leaving open the possibility of a contact with the purine N-7. Of the remaining 12 nucleotides, 5 have been identified as recognition nucleotides and the remainder have not yet been tested. Thus, a limited number of additional nucleotides for FRS recognition could still emerge.

The evidence indicates that the five purine residues in $tRNA^{Phe}$, G_{20}, G_{34}, A_{35}, A_{36}, and A_{73} are important components of the FRS recognition site. Since all five of these nucleotides are single stranded it is unlikely that the reduction in aminoacylation rate for mutations at these positions is caused by a large change in the folding of the molecule. Furthermore, in two cases presented here, the removal of one of the essential nucleotides in a totally different tRNA background resulted in a similar reduction in k_{cat}/K_m as was observed with $tRNA^{Phe}$. Finally, when a different tRNA acquired all five of these nucleotides, it inevitably improved the ability of the tRNA to be misacylated by FRS with nearly normal kinetics.

Are only five nucleotides required for FRS to successfully discriminate $tRNA^{Phe}$ from other tRNAs in yeast? A survey of all the available yeast tRNA sequences reveals that many share one or more of the essential nucleotides with $tRNA^{Phe}$. Although G_{20} is unique to $tRNA^{Phe}$, each of the three anticodon nucleotides are present in about 25% of yeast tRNAs and A_{73} is present in about 60%. Although only yeast $tRNA^{Phe}$ has all five of the essential nucleotides and none has four, there are a substantial number of yeast tRNAs that have two or even three of the five essential nucleotides. It is therefore important to understand why these yeast tRNA are not misacylated by FRS in vivo.

If we assume that each of the essential nucleotides contributes to the k_{cat}/K_m independently, as was found with yeast Tyr-tRNA synthetase (*12*), then it is possible to estimate the k_{cat}/K_m of misacylation for other yeast tRNAs based on the data obtained from $tRNA^{Phe}$ mutations. For example, a $tRNA^{Phe}$ with recognition nucleotides changed to those of $tRNA^{Met}$ (A_{20}, C_{34}, A_{35}, U_{36}, and A_{73}) would be expected to have a k_{cat}/K_m reduced by a value equal to the product of the amounts that the three individual mutations reduce k_{cat}/K_m. Such a calculation predicts that $tRNA^{Met}$ should misacylate with a k_{cat}/K_m about 300-fold less than $tRNA^{Phe}$, which agrees well with the experimental 200-fold decrease (*11*). The other eight known yeast tRNAs that share two of the five discriminator nucleotides are calculated to have k_{cat}/K_m reduced by two to three orders of magnitude depending on the position and type of substitutions. While such discrimination observed in an in vitro misacylation experiment with purified tRNA and synthetase is much less than is observed in vivo, the presence of the competing homologous tRNA synthetases would be expected to greatly decrease misacylation by FRS.

The discrimination by FRS against yeast tRNAs that share three of the five essential nucleotides with $tRNA^{Phe}$ is illustrated by yeast $tRNA^{Tyr}$. Although $tRNA^{Tyr}$ has G_{34}, A_{36}, and A_{73}, the k_{cat}/K_m of misacylation for this tRNA by FRS is nearly 9000-fold lower than $tRNA^{Phe}$ (*4*). If only the five essential nucleotides contributed to k_{cat}/K_m, we would predict that $tRNA^{Tyr}$ should have a k_{cat}/K_m only 1/100 that of $tRNA^{Phe}$. This discrepancy probably arises because the different $tRNA^{Tyr}$ structure excludes it from the tRNA binding site of FRS despite the presence of three recognition nucleotides. A similar argument could explain the discrimination of yeast $tRNA^{Leu}$ by FRS, which also has three of the essential nucleotides. Thus the combination of the tRNA tertiary structure and the five sequence-specific contacts should be sufficient to ensure specific recognition of the cognate $tRNA^{Phe}$.

The five nucleotides used for discrimination by FRS are well suited for ensuring a specific RNA-protein interaction. Since all five are single-stranded and on the surface of the molecule, their functional groups are available for specific contact by the protein (Fig. 3B). That the five nucleotides are located very far apart from one another in the tRNA tertiary structure implies that the protein contacts the entire surface of the RNA molecule. Footprinting studies of the tRNA-FRS complex by ethylnitrosourea support this view (*13*).

Potential recognition nucleotides have been identified in several other synthetase-tRNA interactions. Similar biochemical experiments have established that the anticodon nucleotides in *E. coli* $tRNA^{Met}$ and $tRNA^{Val}$ are the major determinants for recognition by their cognate synthetases (*14, 15*). The alteration of amber suppression specificity has been used to identify nucleotides important for the in vivo identity of *E. coli* $tRNA^{Gln}$ (*16, 17*), $tRNA^{Ser}$ (*18*), $tRNA^{Phe}$ (*19*), and $tRNA^{Ala}$ (*20, 21*). However, it is presently difficult to relate these in vivo results with the biochemical data, since full activity and complete accuracy of aminoacylation are not needed for suppression. In fact, the yeast $tRNA^{Phe}$ amber and ochre suppressors are quite efficient in vivo (*22*) even though G_{34} and A_{35} have been mutated. When an *E. coli* $tRNA^{Cys}$ suppressor was successfully altered to a $tRNA^{Ala}$ suppressor by transplanting a single G-U pair, the resulting tRNA was not fully active when assayed with purified Ala-tRNA synthetase, suggesting that additional elements in $tRNA^{Ala}$ contribute to its recognition (*21*). A combination of genetic and biochemical experiments need to be performed to identify all the nucleotides in the tRNA necessary to define its specific interaction with its synthetase.

REFERENCES AND NOTES

1. A. G. Bruce and O C. Uhlenbeck, *Biochemistry* **21**, 3921 (1982).
2. L. Bare and O. C. Uhlenbeck, *ibid.* **24**, 2354 (1985).
3. L. L. Kisselev, *Prog. Nucleic Acid Res. Mol. Biol.* **32**, 237 (1985).
4. J. R. Sampson and O. C. Uhlenbeck, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1033 (1988).
5. J. R. Sampson, A. DiRenzo, L. Behlen, O. C. Uhlenbeck, unpublished results.
6. B. Roe, M. Sirover, B. Dudock, *Biochemistry* **12**, 4146 (1973).
7. T. McCutchan, S. Silverman, J. Kohlig, D. Söll, *ibid.* **17**, 1622 (1978).
8. S. H. Kim, *Prog. Nucleic Acid Res. Mol. Biol.* **17**, 181 (1976).
9. O. C. Uhlenbeck, H. Wu, J. R. Sampson, in *Molecular Biology of RNA, New Perspectives*, M. Inouye and B. S. Dudock, Eds. (Academic Press, San Diego, CA, 1987), pp. 285–294.
10. J. R. Sampson *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **52**, 267 (1987).
11. H. Feldman and H. G. Zachau, *Hoppe-Seylers Z. Physiol. Chem.* **358**, 891 (1977).
12. L. A. Bare and O. C. Uhlenbeck, *Biochemistry* **25**, 5825 (1986).
13. P. Romby *et al.*, *J. Mol. Biol.* **184**, 455 (1985).
14. L. Schulman and H. Pelka, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6755 (1983).
15. ———, *Science* **242**, 765 (1988).
16. Y. Shimura *et al.*, *FEBS Lett.* **22**, 144 (1972).
17. M. L. Hooper, R. L. Russell, J. D. Smith, *ibid.*, p. 149.
18. J. Normanly, R. C. Ogden, S. J. Horvath, J. Abelson, *Nature* **321**, 213 (1986).
19. W. H. McClain and K. Foss, *J. Mol. Biol.* **202**, 697 (1988).
20. ———, *Science* **240**, 793 (1988).
21. Y.-M. Hou and P. Schimmel, *Nature* **333**, 140 (1988).
22. J.-M. Masson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6815 (1987).
23. B. G. Barrell and F. Sanger, *FEBS Lett.* **3**, 275 (1969).
24. H. Gruhl and H. Feldman, *ibid.* **57**, 146 (1975).
25. B. Kuntzel, J. Weissenback, G. Dirheimer, *ibid.* **25**, 189 (1972).
26. J. T. Madison, G. A. Everett, H. Kung, *Science* **153**, 531 (1966).
27. S. H. Kim *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3711 (1974).

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