# PRIMARY STRUCTURE OF WHEAT GERM PHENYLALANINE TRANSFER RNA*

By Bernard S. Dudock,† Gerson Katz,‡ Ellen K. Taylor, and Robert W. Holley§

SECTION OF BIOCHEMISTRY AND MOLECULAR BIOLOGY, CORNELL UNIVERSITY, ITHACA, NEW YORK

*Communicated January 21, 1969*

*Abstract.*—The complete nucleotide sequence of wheat germ phenylalanine transfer RNA ($tRNA^{Phe}$) is presented. This RNA, which is an acceptable substrate for yeast phenylalanine tRNA synthetase, has a structure very similar to that of yeast $tRNA^{Phe}$. Only 16 of the 76 nucleotides are different, and all but two of the nucleotide changes are located in regions that are double-stranded in the cloverleaf model. The two changes in single-stranded regions involve minor modifications of the same nucleotide. The dihydrouridine loop and its supporting stem are completely free of nucleotide changes.

---

The nucleotide sequences of a number of tRNA's have been elucidated.[1-9] The present paper reports the nucleotide sequence of wheat germ phenylalanine tRNA ($tRNA^{Phe}$). This sequence was determined to provide information on the variability of an amino acid–specific tRNA between widely different biological species Wheat germ $tRNA^{Phe}$ was selected for study because wheat germ is a rich source of crude tRNA,[10] from which $tRNA^{Phe}$ is readily purified, and because the sequence of yeast $tRNA^{Phe}$ was already known.[5] The variations in $tRNA^{Phe}$ structure observed between wheat germ and yeast are discussed with respect to their interaction with yeast phenylalanine-tRNA synthetase.

*Materials and Methods.*—*Isolation of tRNA:* 87 liters of distilled water and 14.2 liters (the contents of six 5-pint jars) of liquefied phenol (88%) were added to a 200-liter polyethylene tank. 50 lb of raw wheat germ (Shiloh Farms, Sherman, N. Y.) was added and stirred until wet (about 2 min). 28.4 liters (the contents of twelve 5-pint jars) of liquefied phenol were added and the mixture was stirred thoroughly for 15 min, then allowed to settle for 24 hr at room temperature. The top phase (about 48 liters) was removed and placed in a number of large pipette jars (14 liters per jar). To each pipette jar was added 300 ml of phenol and sufficient sodium chloride to bring the solution to 2.75 *M*. The solution was thoroughly mixed and then placed in the cold room overnight. The following day the pipette jar was removed from the cold room and the clear aqueous phase was siphoned off and mixed thoroughly with two parts of 95% ethyl alcohol. The solution was allowed to stand at room temperature overnight to precipitate the crude tRNA.

A new aqueous phase (48 liters) was added to the phenol–wheat germ mixture remaining in the large polyethylene tank. This mixture was thoroughly stirred and allowed to settle for 24–48 hr, and the aqueous phase siphoned off and worked up as described above. The phenol–wheat germ mixture remaining in the large polyethylene tank was extracted one more time with an aqueous phase (48 liters). The crude tRNA preparations from the three extractions were combined, dissolved in 0.1 *M* NaCl, 0.01 *M* Tris pH 7.5, and placed on a large DEAE-cellulose column prepared from a size F pipette jar. The column was washed with at least 7 column volumes of 0.30 *M* NaCl, 0.01 *M* Tris pH 7.5 (this washing was discarded), and then eluted with 1.0 *M* NaCl, 0.01 *M* Tris pH 7.5, until the optical density at 260 mμ of the effluent dropped to below 2 OD units/ml.

Two parts of 95% ethyl alcohol were added to the column effluent, and the tRNA was allowed to precipitate overnight at room temperature. The tRNA was centrifuged, washed twice with 95% ethyl alcohol, and dried over $P_2O_5$. The yield was about 20 gm of tRNA per 50 lb of wheat germ.

*Purification of tRNA$^{Phe}$: Benzoylated DEAE-cellulose chromatography:* Highly enriched tRNA$^{Phe}$ was prepared by a two-step chromatographic procedure involving benzoylated DEAE-cellulose (BD-cellulose) followed by reversed-phase (Freon) chromatography. BD-cellulose was prepared by the method of Gillam *et al.*[11] The chromatographic elution profile of wheat germ tRNA on a BD-cellulose column is shown in Figure 1. Since the amount of tRNA chromatographed exceeded the column capacity, most of the tRNA simply ran through; however, the tRNA$^{Phe}$ was quantitatively retained. Most of the retained tRNA was then eluted with low salt, and the tRNA$^{Phe}$ was then eluted with high salt. This simple procedure permitted the 10- to 15-fold purification of relatively large amounts of tRNA$^{Phe}$.

*Reversed-phase (Freon) chromatography:* The elution pattern of wheat germ tRNA on reversed-phase (Freon) column chromatography[12] is shown in Figure 2. Under these conditions, tRNA$^{Phe}$ was eluted later than most other tRNA's and therefore this procedure seemed suitable as a second step in a chromatographic purification scheme of tRNA$^{Phe}$. Figure 3 shows the elution profile of enriched tRNA$^{Phe}$ (from the BD-cellulose column) on reversed-phase (Freon) chromatography.

The purified tRNA$^{Phe}$ obtained by this two-step chromatographic procedure was judged to be at least 90% pure, based on the absence of extraneous oligonucleotides upon complete RNase $T_1$ digestion of tRNA$^{Phe}$.

*Results and Discussion.*—The oligonucleotides produced by enzymatic digestion of tRNA$^{Phe}$ with RNase $T_1$ and pancreatic RNase have been analyzed and their sequences determined.[13]

Partial digestion of tRNA$^{Phe}$ with these nucleases provided overlap sequences

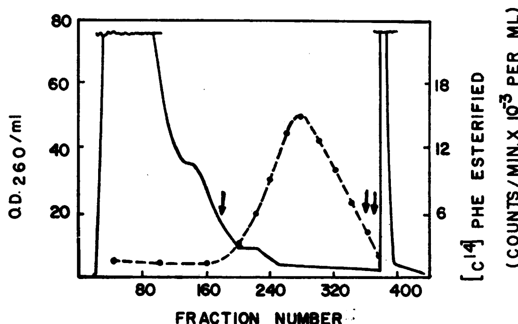


FIG. 1.—Elution pattern of wheat germ tRNA on a BD-cellulose column. 15 gm of tRNA chromatographed on a 5 × 180-cm column. *Gradient I:* 0.5 *M* NaCl, 0.01 *M* $MgCl_2$ to 1.1 *M* NaCl, 0.01 *M* $MgCl_2$, 3,000 ml total volume; *gradient II:* 1.1 *M* NaCl, 0.01 *M* $MgCl_2$ to 2.0 *M* NaCl, 0.01 *M* $MgCl_2$, 3,000 ml total volume; final wash 2.0 *M* NaCl, 0.01 *M* $MgCl_2$, 10% methylcellosolve, total volume, 1,000 ml. Single arrow shows start of second gradient; double arrow shows start of final wash. Fraction size was about 20 ml. $C^{14}$-phenylalanine (10 mc/mmole) was enzymatically esterified to tRNA$^{Phe}$ with a saturating level of a yeast-activating enzyme preparation (soluble fraction of disrupted fresh bakers' yeast purified on DEAE-cellulose and Sephadex G-25 columns). The labeled esterified tRNA was precipitated with trichloroacetic acid, collected on a Millipore membrane filter, and counted in a thin-window gas-flow counter with a counting efficiency of 25%. *Solid line,* optical density; *dashed line,* phenylalanine acceptor activity.

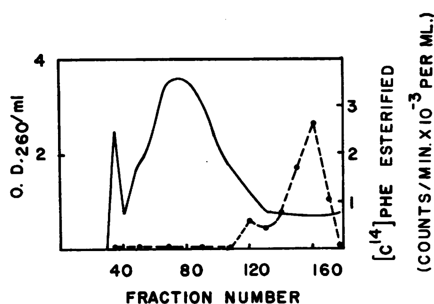


FIG. 2.—Elution pattern of wheat germ tRNA on a reversed-phase (Freon) column. 100 mg of tRNA on a 1 × 120-cm column eluted with a concave gradient of 750 ml of 0.165 *M* NaCl, 0.01 *M* $MgCl_2$, 0.01 *M* NaAc pH 4.5, and 240 ml of 0.34 *M* NaCl, 0.01 *M* $MgCl_2$, 0.01 *M* NaAc pH 4.5. The flow rate was 0.5 ml per minute and the fraction size was 5.3 ml. $tRNA^{Phe}$ was assayed as described in Fig. 1. *Solid line,* optical density; *dashed line,* phenylalanine acceptor activity.

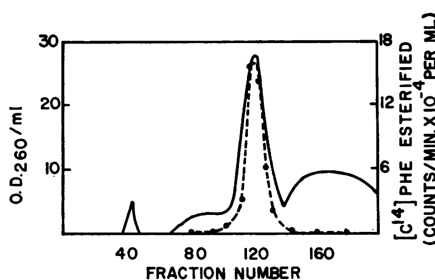


FIG. 3.—Elution pattern of BD-cellulose enriched $tRNA^{Phe}$ on a reversed-phase (Freon) column. 225 mg of tRNA was dissolved in 0.18 *M* NaCl, 0.01 *M* $MgCl_2$, 0.01 *M* NaAc pH 4.5. This was applied to a 1 × 120-cm column which was eluted with a concave gradient composed of 750 ml of 0.21 *M* NaCl, 0.01 *M* $MgCl_2$, 0.01 *M* NaAc pH 4.5, and 240 ml of 0.50 *M* NaCl, 0.01 *M* $MgCl_2$, 0.01 *M* NaAc pH 4.5. The flow rate was 0.5 ml/min and the fraction size was about 5 ml/tube. $tRNA^{Phe}$ was assayed as described in Fig. 1. *Solid line,* optical density; *dashed line,* phenylalanine acceptor activity.

which were pieced together to give the primary structure.[14] Figure 4 shows the sequence of wheat germ $tRNA^{Phe}$ presented with that of yeast $tRNA^{Phe}$ for comparison. The boxed areas in both structures show the regions of dissimilarity.

Both tRNA's contain 76 nucleotides and conform to the cloverleaf model.[1] In a comparison of the two molecules, 16 homologous positions show nucleotide changes. Thirteen of these positions are in double-stranded regions, where one major nucleotide has been replaced by another. The three other changes, two occurring in single-stranded regions and one in a double-stranded region, involve modified forms of the same base.

Seven of the 13 changes in double-stranded regions involve nucleotides in the acceptor stem (at the top in Fig. 4). Four nucleotide changes are located in the stem supporting the TψCG loop and two in the stem supporting the anticodon loop. The nucleotide changes in double-stranded regions allow, in all cases but one, the formation of new hydrogen-bonded base pairs. This constitutes strong additional support for the cloverleaf model, for if base pairing as shown in the cloverleaf model were not a consequence of primary structure, there would be no reason why a mutation in DNA giving rise to one changed base in tRNA should nearly always be accompanied by a second mutation at a different locus.

$tRNA^{Phe}$'s from yeast and wheat germ contain 20 base pairs in double-stranded regions; the two tRNA's differ in six of these base pairs. In both $tRNA^{Phe}$'s there are two opposing residues in the double-stranded region of the stem which cannot form conventional Watson-Crick base pairs. In yeast $tRNA^{Phe}$ these residues are G and U; in wheat germ $tRNA^{Phe}$ they are G and A.

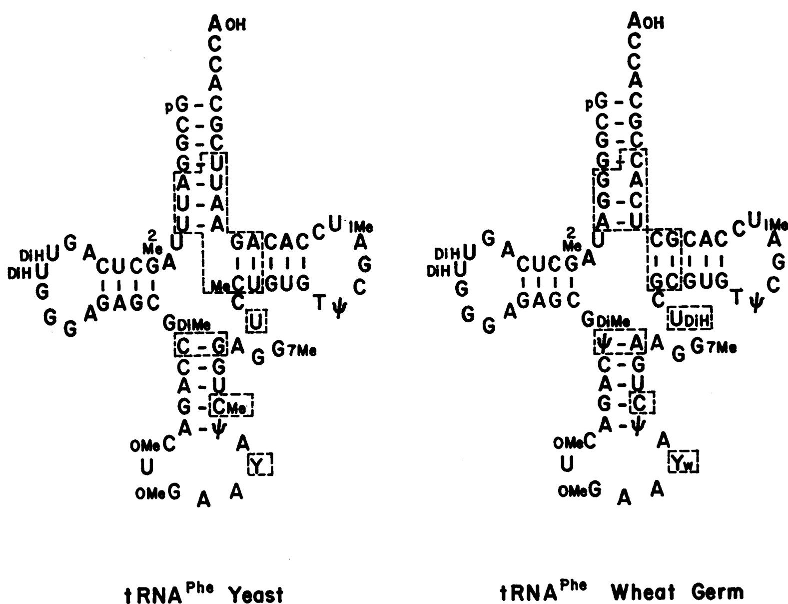


FIG. 4.—A comparison of the structure of yeast and wheat germ tRNA$^{Phe}$. The boxed areas show regions of dissimilarity.

However, the non-hydrogen-bonded nucleotides do not occur in homologous positions in the stems of the two tRNA's. The occurrence of G and A as opposing bases in the stem is so far unique among tRNA structures.

The other base changes involve the modification of nucleotides in homologous positions. Thus wheat germ tRNA$^{Phe}$ contains diHU[18] instead of U as the nucleotide 3′ to 7MeG, and cytidine rather than 5-methylcytidine in the double-stranded stem of the anticodon loop. There is no 5-methylcytidine in wheat germ tRNA$^{Phe}$; however, this minor nucleoside occurs twice in yeast tRNA$^{Phe}$.

Both tRNA's contain an unidentified fluorescent nucleoside[5, 13] in the first position to the right of the anticodon. Ultraviolet spectra of these two residues are quite similar,[5, 13] as are fluorescence spectra of the two tRNA's;[16] however, chromatography of the fluorescent bases after their release from the tRNA's by mild acid hydrolysis[17] indicates a difference between them (Doju Yoshikami, personal communication).

In general, the single-stranded regions of the three major loops are identical in yeast and wheat germ tRNA$^{Phe}$. A segment 19 nucleotides long, including the complete dihydrouridine-containing loop and its supporting stem, is identical in the two tRNA's. Minor bases in this region are diHU, 2MeG, and diMeG. In the anticodon loop, ignoring the minor differences between *Y* and *Yw*, and cytidine and 5-methylcytidine, there is a sequence of 15 identical nucleotides, including the anticodon 2′OMeG-A-A- as well as the minor nucleosides 2′OMeC, 2′OMeG, and $\psi$. The T-$\psi$-C-G-containing loop again includes a long sequence of identical residues, 13 nucleotides in length, which contains the minor bases ribothymidine, $\psi$, and 1-methyladenosine. The only nucleotides in the stem

which are the same are in the three terminal base pairs and in the G-A-C-C-A acceptor end, common to many tRNA's.

Comparison of the sequences of a rat liver $tRNA^{Ser}$ and a yeast $tRNA^{Ser}$ has given results extremely similar to those presented above.[9] Approximately 80 per cent of the residues in the two serine tRNA's are identical and all changes in which major nucleotides replaced one another occurred in double-stranded regions.

Finally, it is extremely interesting to note that the diHU loop with its stem is the only major region in which there are no nucleotide changes either in the two phenylalanine tRNA's or the two serine tRNA's. This suggests the possibility that this region plays a role in synthetase recognition.

* Supported by grant GM-13018-04 from the National Institutes of Health and grant GB-7037 from the National Science Foundation. B. S. D. was a National Institutes of Health postdoctoral fellow, 1966–1968. A preliminary report of these studies was presented at the 52nd Annual Meeting of the Federation of American Societies for Experimental Biology, April 15–20, 1968, and at the Miles Symposium on Transfer RNA, New York, June 7–8, 1968.

† Present address: Biochemistry Section, State University of New York at Stony Brook, Stony Brook, New York. Reprint requests should be sent to B. S. Dudock at this address.

‡ Present address: Department of Biochemistry, Brandeis University, Waltham, Massachusetts.

§ Present address: Salk Institute for Biological Studies, San Diego, California.

[1] Holley, R. W., J. Apgar, G. A. Everett, J. T. Madison, M. Marquisee, S. H. Merrill, J. R. Penswick, and A. Zamir, *Science*, **147**, 1462 (1965).

[2] Zachau, H. G., D. Dutting, and H. Feldmann, *Hoppe-Seyler's Z. Physiol. Chem.*, **347**, 212 (1966).

[3] Madison, J. T., G. A. Everett, and H. Kung, *Science*, **153**, 531 (1966).

[4] Baev, A. A., T. V. Venkstern, A. D. Mirzabekov, A. I. Krutilina, V. A. Axelrod, L. Li, and V. A. Engelhardt, in *Third Symposium, Federation of European Biological Societies*, Warsaw (1966).

[5] RajBhandary, U. L., A. Stuart, R. D. Faulkner, S. H. Chang, and H. G. Khorana, these PROCEEDINGS, **57**, 751 (1967).

[6] Takemura, S., T. Mizutani, and M. Miyazaki, *J. Biochem. Tokyo*, **63**, 274 (1968).

[7] Goodman, H. M., J. Abelson, A. Lardy, S. Brenner, and J. D. Smith, *Nature*, **217**, 1019 (1968).

[8] Dube, S. K., K. A. Marcker, B. F. C. Clark, and S. Cory, *Nature*, **218**, 232 (1968).

[9] Staehelin, M., H. Rogg, B. C. Baguley, T. Ginsberg, and W. Wehrli, *Nature*, **219**, 1363 (1968).

[10] Glitz, D. G., and C. A. Dekker, *Biochemistry*, **2**, 1185 (1963).

[11] Gillam, I., S. Millward, D. Blew, M. Von Tigerstrom, E. Wimmer, and G. M. Tener, *Biochemistry*, **6**, 3043 (1967).

[12] Weiss, J. F., and A. D. Kelmers, *Biochemistry*, **6**, 2507 (1967).

[13] Katz, G., and B. S. Dudock, in press.

[14] Dudock, B. S., and G. Katz, in press.

[15] RajBhandary, U. L., R. D. Faulkner, and A. Stuart, *J. Biol. Chem.*, **243**, 575 (1968).

[16] Yoshikami, D., G. Katz, E. B. Keller, and B. S. Dudock, *Biochim. Biophys. Acta*, **166**, 714 (1968).

[17] Thiebe, R., and H. G. Zachau, *Europ. J. Biochem.*, **5**, 546 (1968).

[18] *Abbreviations:* diHU, 5,6-dihydrouridine; 2′OMeC, 2′-O-methylcytidine; 2′OMeG, 2′-O-methylguanosine; 2MeG, $N^2$-methylguanosine; diMeG, $N^2$-dimethylguanosine; 7MeG, 7-methylguanosine; 1MeA, 1-methyladenosine; 6MeA, 6-methyladenosine; *Yw*, a fluorescent nucleoside of undetermined structure; 1 OD unit is defined as that amount of material per milliliter of solution which produces an absorbance of 1 in a 1-cm light path cell at 260 mμ.