# STUDIES ON POLYNUCLEOTIDES, LXVIII.* THE PRIMARY STRUCTURE OF YEAST PHENYLALANINE TRANSFER RNA

By U. L. RajBhandary, S. H. Chang, A. Stuart, R. D. Faulkner, R. M. Hoskinson, and H. G. Khorana

INSTITUTE FOR ENZYME RESEARCH, UNIVERSITY OF WISCONSIN, MADISON

Following the pioneering work of Holley and co-workers[1] on yeast alanine tRNA,[2] nucleotide sequences of two similar serine tRNA's[3] and of one tyrosine tRNA[4] from yeast have been reported. The present report contains a brief account of the work leading to the elucidation of the nucleotide sequence of yeast phe-tRNA.

*Purification and Nucleotide Composition.*—The method used for the purification of phe-tRNA was that of countercurrent distribution as described previously.[5] This method gives a major and a minor peak; the two peaks differ most probably only in the nature of the 3'-end group, the major peak having lost the 3'-terminal adenosine residue.[6] The structural work reported herein has all been on the major peak which contains cytidine at 3'-terminus. The purity of RNA used was estimated to be higher than 90 per cent.

Phe-tRNA contains a total of 76 nucleotide units, including the terminal A required for amino acid acceptance. Fourteen nucleosides of minor bases[7] are present, 13 of which are as follows, their number/mole of tRNA being shown in parentheses: T(1), $\psi$(2), DiHU(2), MeC(2), 2'OMeC(1), MeA(1), 2MeG(1), DiMeG(1), 2'OMeG(1), and 7MeG(1). The 14th nucleoside, which is still unidentified, is strongly fluorescent under ultraviolet light and is simply designated as Y. Of the identified minor nucleosides, 2'OMeC and 7MeG have been isolated from a pure tRNA for the first time.

*Products of Digestion with Pancreatic and $T_1$-RNases.*—The structural work followed the general principles used by previous workers.[1, 3, 4] Thus, the first phase consisted of the characterization of the total products obtained on complete degradation with the two RNases, and the second phase involved the isolation and sequential analysis of larger fragments obtained by partial enzymic degradation until the overlaps permitted the derivation of a unique sequence. Methods used for the separation of the number of large oligonucleotide products formed involved column chromatography using diethylaminoethyl cellulose in the presence of 7 $M$ urea.[8] Table 1 lists the products of complete digestion of phe-tRNA with pancreatic RNase. Identification of the 5'-terminal sequence as pG-C- supports earlier conclusions arrived at using end-group labeling techniques.[9] Analyses of most of the fragments were possible by the usual methods.[10] The octanucleotide G-G-G-A-G-A-G-C- was characterized by the application of a technique developed for end-group labeling of 2',3'-diol residues in RNA.[6, 11] Sequence analysis of the hexanucleotide 2'OMeG-A-A-Y-A-$\psi$- was more difficult and involved the use of micrococcal nuclease and $T_2$-RNase.

Table 2 lists the products obtained by complete degradation with $T_1$-RNase. As is seen, the only product not bearing a 3'-phosphate end group was the oligonucleotide C-A-C-COH, and this clearly represented the sequence at 3'-terminus of the RNA. This result is in agreement with conclusions drawn previously from end-

| TABLE 1 | | TABLE 2 | |
|---|---|---|---|
| PRODUCTS FORMED BY COMPLETE DEGRADATION OF PHE-tRNA WITH PANCREATIC RNASE | | PRODUCTS OBTAINED BY COMPLETE DIGESTION OF PHE-tRNA WITH $T_1$-RNASE | |
| COH | G-U- | 4G- | T-$\psi$-C-G- |
| DiHU-cyclic-p | pG-C- | pG- | C-C-A-G- |
| 7C- | A-2MeG-C- | C-DiMeG-cyclic-p | C-U-C-A-G- |
| 2MeC- | G-MeA-U- | C-G- | A-U-U-U-A-2MeG- |
| 6U- | A-G-DiHU- | | cyclic-p |
| $\psi$- | G-G-A-U- | U-G- | 7MeG-U-C-MeC-U-G- |
| 2A-C- | A-G-A-A-U- | 3A-G- | A-A-U-U-C-G- |
| DiMeG-C- | A-G-A-2'OMeC-U- | DiHU-DiHU-G- | MeA-U-C-C-A-C-A-G- |
| G-C- | 2'OMeG-A-A-Y-A-$\psi$- | C-A-C-COH | A-2'OMeC-U-2'OMeG- |
| G-T- | G-(G-, A-G-)7MeG-U-* | | A-A-Y-A-$\psi$-MeC-U- |
| G-G-G-A-G-A-G-C- | | | G- |

\* Subsequently shown to be G-G-A-G-7MeG-U-.

group labeling methods.[9, 12]    The tetranucleotide T-$\psi$-C-G- proposed[13] to be common to tRNA's is also present in this tRNA.    The largest oligonucleotide (Table 2), which is a dodecanucleotide, contains a total of five minor bases.

The information contained in the products formed by pancreatic and $T_1$-RNases is mutually consistent.    The overlap of minor bases or unique sequences present in the two sets of fragments allows the deduction of longer sequences (Table 3).

*Longer fragments produces by partial degradation with $T_1$- and pancreatic RNases:* Partial degradation with $T_1$-RNase was used for the most part, and among the longer fragments thus produced only those which are essential for the derivation of phe-tRNA structure are shown in Figure 1 (fragments (*a*) to (*e*) and (*g*) to (*i*)). The information thus obtained left only two alternative structures for phe-tRNA. On partial degradation with pancreatic RNase, one of the fragments produced was (*f*) (Fig. 1) and this contained the decisive information.    Sequences of fragments (*a*) to (*i*) were deduced as follows:

*Fragment (a):*    Complete digestion with $T_1$-RNase yielded pG-, C-G-, G-, and A-U-U-U-A·2MeG-cyclic-p.    This oligonucleotide containing pG- group must represent the 5'-terminal sequence of phe-tRNA, and since pancreatic RNase produces pG-C-(Table 1), the dinucleotide C-G- is placed adjacent to pG.    The mononucleo-

## TABLE 3

NUCLEOTIDE SEQUENCES DEDUCED TO BE PRESENT IN PHE-tRNA FROM OVERLAPS EVIDENT IN FRAGMENTS OF TABLES 1 AND 2

Pyrimidine-G-T-$\psi$-C-G-

Pyrimidine-A-G-DiHU-DiHU-G-

Pyrimidine-A-G-A-A-U-U-C-G-

Pyrimidine-G-G-A-U-U-U-A-2MeG-C-

Pyrimidine-G-G-A-G-7MeG-U-C-MeC-U-G-

Pyrimidine-A-G-A-2'OMeC-U-2'OMeG-A-A-Y-A-$\psi$-MeC-U-G-

Pyrimidine-G-MeA-U-C-C-A-C-A-G-

Underlined sequences are fragments produced by the action of pancreatic RNase on phe-tRNA.  Dashed lines above sequences indicate fragments obtained by the action of $T_1$-RNase on phe-tRNA.

STRUCTURE OF PHENYLALANINE S-RNA

LARGE OLIGONUCLEOTIDE FRAGMENTS

FIG. 1.—The structure of yeast phe-tRNA is shown at the top. Large oligonucleotides crucial for elucidation of structure are shown below.

tide G- cannot be at the 3'-end of this fragment since no oligonucleotide containing the sequence A-2MeG-G- was isolated from phe-tRNA. Furthermore pancreatic RNase on intact RNA yielded only the tetranucleotide G-G-A-U- and no trinucleotide G-A-U-; hence G- must be placed between pG-C-G- and A-U-U-U-A-2MeG.

*Fragment* (*d*): Treatment with phosphomonoesterase followed by complete digestion with $T_1$-RNase yielded the following products: 2G-, C-G-, C-U-C-A-GOH, and A-U-U-U-A-2MeG-. The oligonucleotide C-U-C-A-GOH lacking a 3'-phosphate group must represent the 3'-terminus of fragment (*d*), and since the rest of the products are accounted for in fragment (*a*), fragment (*d*) must contain fragment (*a*) and C-U-C-A-GOH at the 3'-terminal. This arrangement is consistent with the production of A-2MeG-C- as a product of pancreatic RNase on phe-tRNA (Table 1).

*Fragment* (*e*): The products of complete $T_1$-RNase digestion were C-DiMeG-cyclic-p and C-C-A-G-. Since no A-G-C- was obtained by the action of pancreatic RNase on the intact RNA, C-DiMeG-cyclic-p can only be placed at the left end of C-C-A-G-in fragment (*e*). This sequence is further supported by the products formed (2C-, DiMeG-C-, and A-G-) by the action of pancreatic RNase on this fragment.

*Fragment* (*b*): On complete digestion with $T_1$-RNase, it produced DiHU-DiHU-G-,2G, 2A-G-, C-DiMeG-cyclic-p, C-C-A-G-, and A-2'OMeC-U-2'OMeG-A-A-Y-A-ψ-MeC-U-G-. The considerations for the ordering of these products are as follows: (*i*) The 2G- and the 2A-G- must be part of the oligonucleotide pyrimidine-G-G-G-A-G-A-G-C-, since pancreatic RNase on the intact fragment yielded the octanucleotide G-G-G-A-G-A-G-C-. (*ii*) The isolation of fragment (*e*) above requires that the products, C-DiMeG-cyclic-p and C-C-A-G- be connected to give C-DiMeG-C-C-A-G-. (*iii*) In the intact phe-tRNA, both DiHU-DiHU-G- and A-2'OMeC-U-2'OMeG-A-A-Y-A-ψ-MeC-U-G- are preceded by a pyrimidine -A-G- sequence since pancreatic RNase on the RNA yielded A-G-DiHU- and A-G-A-2'OMeC-U-. Further, since only one pyrimidine -A-G- sequence is present in fragment (*b*), either DiHU-DiHU-G- or A-2'OMeC-U-2'OMeG-A-A-Y-A-ψ-MeC-U-G- must represent the left end of fragment (*b*). (*iv*) Fragment (*b*) cannot contain the sequence pyridimidine -G-C-. This was inferred from the finding that there is only one such sequence in intact phe-tRNA (formation of only one mole of G-C- on degradation with pancreatic RNase, Table 1) and that the sequence C-G-C is known to be present in fragment (*c*) (see below). The absence of pyrimidine -G-C-sequence in fragment (*b*) was further shown by the absence of any G-C- among the products of pancreatic RNase digestion of this fragment. The number of possible structures for fragment (*b*) can thus be limited to only two, one as depicted in Figure 1 and the other as A-2'OMeC-U-2'OMeG-A-A-Y-A-ψ-MeC-U-G-G-G-A-G-A-G-C-DiMeG-C-C-A-G-DiHU-DiHU-G- in which the positions of DiHU-DiHU-G- and the dodecanucleotide are interchanged. A decision in favor of the structure shown in Figure 1 was made from data obtained on degradation of this fragment with pancreatic RNase. Thus, A-G-A-2'OMeC-U- and not A-2'OMeC-U- was obtained, indicating the presence of a pyrimidine-A-G-A-2'OMeC-U- sequence in this fragment. Further, no A-G-DiHU- was produced, indicating the absence of a pyrimidine -A-G-DiHU- sequence.

*Fragment* (*h*): This gave, on $T_1$-RNase degradation, all of the products obtained

from fragment (b) in addition to a pentanucleotide C-U-C-A-G-.  This fragment, as discussed above, also cannot contain a pyrimidine -G-C- sequence and hence C-U-C-A-G- cannot be placed on the right end of fragment (b).  The only sequence possible for this fragment is as shown in Figure 1 and this is consistent with the production of A-G-DiHU- on complete digestion of the RNA with pancreatic RNase.

*Fragment (f)*:  This fragment, which was located among the products of controlled degradation of phe-tRNA with pancreatic RNase, was further degraded with the same enzyme.  The products were G-G-A-G-7MeG-U-, C-, MeC-, U-, and G-U-.  The presence of 7MeG-U-C- MeC-U-G- among the oligonucleotides isolated by the action of $T_1$-RNase on the intact fragment establishes its sequence as shown in Figure 1.

*Fragment (c)*:  The products of $T_1$-RNase on this fragment were identified as A-A-U-U-C-G- and C-A-C-COH, and since C-A-C-COH is known to be the 3′-terminal sequence of the RNA, the hexanucleotide A-A-U-U-C-G- must be placed to the left of C-A-C-COH.

*Fragment (g)*:  This on degradation with $T_1$-RNase produced, in addition to the products given by fragment (c), the octanucleotide MeA-U-C-C-A-C-A-G- and, by the same reasoning as above, this octanucleotide must be placed on the left end of fragment (c) as shown in Figure 1.

*Fragment (i)*:  Digestion with $T_1$-RNase yielded the following oligonucleotides in addition to those contained in fragment (g):  A-G-, 7MeG-U-C-MeC-U-G, U-G-, and T-$\psi$-C-G-.  As shown above in Table 1, the sequences A-G- and 7MeG-U- are present in the hexanucleotide G-G-A-G-7MeG-U-, a product of pancreatic RNase degradation of phe-tRNA.  Since only a part of this sequence is present among the products obtained from fragment (i), i.e., no G- is produced on degradation of fragment (i) with $T_1$-RNase, cleavage of phe-tRNA by $T_1$-RNase to produce fragment (i) must have occurred within the above hexanucleotide and, consequently the dinucleotide A-G-must represent the left end of fragment (i).  Further, from the data given in Table 3, the sequence of fragment (i) can be represented as A-G-7MeG-U-C-MeC-U-G-(U-G-,T-$\psi$-C-G-) followed on the right by fragment (g).  The relative order of U-G- and T-$\psi$-C-G- was not known at this stage.  The structure for fragment (i) as shown in Figure 1 was, however, settled by the identification of fragment (f) described above.

*Derivation of the total sequence for phe-tRNA*:  The overlap represented by the unique sequence C-U-C-A-G- in fragments (d) and (h) of Figure 1 allows joining of these two large fragments with C-U-C-A-G- as the connective link between them and provides the sequence of 42 nucleotide units from the 5′-terminal end of phe-tRNA as follows:  pG-C-G-G-A-U-U-U-A-2MeG-C-U-C-A-G-DiHU-DiHU-G-G-G-A-G-A-G-C-DiMeG-C-C-A-G-A-2′OMeC-U-2′OMeG-A-A-Y-A-$\psi$-MeC-U-G- (sequence A).

Similarly, a combination of the information contained in fragments (f) and (i) results in the following nucleotide sequence for the 3′-terminal end of the RNA: G-G-A-G-7MeG-U-C-MeC-U-G-U-G-T-$\psi$-C-G-MeA-U-C-C-A-C-A-G-A-A-U-U-C-G-C-A-C-COH (sequence B).  Other fragments isolated in large amounts and which essentially confirm this sequence as a part of phe-tRNA are: (i) A-G-7MeG-U-C-MeC-U-G-U-G-T-$\psi$-C-G-MeA-U-C-C-A-C-A-G-A-A-U-U-C-G- and (ii) 7MeG-U-C-MeC-U-G-U-G-T-$\psi$-C-G-MeA-U-C-C-A-C-A-G-A-A-U-U-C-G-C-A-C-COH.

The two large sequences A and B derived from the 5'- and 3'-terminal of the RNA account for all of the products listed in Tables 1 and 2, and these two sequences can be linked together through the common G- residue present at the 3'-terminal of sequence A and at the 5'terminal of sequence B to yield the total nucleotide sequence shown in Figure 1 in which the 3'-terminal A normally missing in the isolated RNA is included.

*Discussion.—General structural features:* Perhaps the most significant feature of the sequence now deduced for phe-tRNA is that it can adopt a secondary structure (Fig. 2) very similar to those previously proposed for the four yeast tRNA's whose sequences are known. Some of the key structural features of this cloverleaf model common to all the five tRNA's are as follows: (1) The sequences at the ends are complementary with the base-pairing starting between the fifth base of the acceptor end and the first base of the 5'-terminus. The complementary region so formed comprises seven base-pairs, including a G:U base-pair. A minor exception to this rule is found in alanine tRNA, in which one U:U combination is found inside this region. (2) Three major loops containing runs of unpaired nucleotide residues are present in the following order from the 5'- to the 3'- terminal of the RNA's: a loop containing DiHU residues, a loop containing the anticodon, and a loop containing the common sequence T-ψ-C-. (3) Both the presumed anticodon loop and the loop containing the sequence T-ψ-C- can consist of seven bases and each of these loops is sustained by five base-pairs. (4) The size of the loop containing DiHU varies in the different tRNA's but in all cases this loop, besides DiHU units, contains mainly purine bases. There are additional common features in the primary structures of the different tRNA's. Thus (1) no odd bases are found close to the acceptor end; (2) T is uniformly the 23rd base from the acceptor end; (3) DiMeG, is, invariably, the ninth base from the first letter corresponding to the presumed anticodon;[14] and (4) besides the terminal C-C-AOH, the following sequences are common: e.g., A-G-DiHU-, G-C-DiMeG-, G-T-ψ-C-. Furthermore, a comparison of the sequences between phenylalanine and tyrosine RNA shows that in both cases, 2MeG and two DiHU residues occupy positions 10, 16, and 17, respectively, from the 5'-terminal and the minor base MeA follows T-ψ-C-G-.

Some support for a cloverleaf type of arrangement has been recently provided by optical rotatory dispersion measurements on alanine and tyrosine RNA's,[15] though alternative proposals have been made by several authors.[16–18] In view of the fact that all the five tRNA's of known primary structure can be represented by an essentially identical secondary structure of the cloverleaf type, it would be difficult to dismiss this model. It is recalled that at an earlier phase of structural work on phe-tRNA when the available data could have been accommodated into an enormously large number of different sequences, it was possible to deduce an essentially correct sequence for the
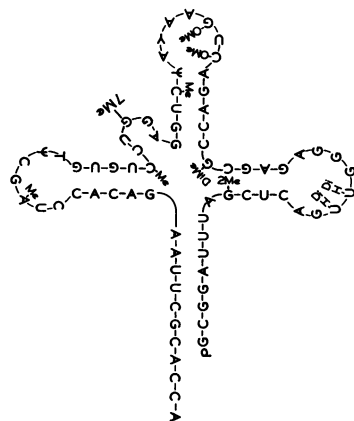


Fig. 2.—Possible secondary structure of phe-tRNA in the cloverleaf type of arrangement.

RNA from a careful scrutiny of the structural similarities of the known tRNA's.[6]

*The anticodon:*   Assuming (see below) that an anticodon consists of three contiguous bases, there are only two possible trinucleotide sequences in this RNA which can represent the anticodon for the two phenylalanine codons. (It is assumed that the unidentified base Y cannot base-pair with U or C.) These are the trinucleotide 2'OMeG-A-A which is present in a loop and the trinucleotide G-A-A- which is present near the acceptor end, no A-A-A sequence being present in this RNA. As concluded above, there is only one species of phe-tRNA in yeast, and the binding experiments by Söll *et al.*[19] have shown that this purified species can be recognized by both U-U-U and U-U-C. Furthermore, in incorporation experiments, in the presence of either poly U or poly r-UUC, transfer of phenylalanine from purified phe-tRNA into polyphenylalanine occurs with equal efficiency.[20] For recognition of both U-U-U and U-U-C by one species of tRNA, the anticodon sequence G-A-A or 2'OMeG-A-A would be consistent with the Wobble hypothesis.[21] It is further noted that there is no A-A-G- sequence in this RNA so that codon-anticodon base-pairing must be antiparallel. Of the two possible anticodon sequences listed above, 2'OMeG-A-A is preferred to the G-A-A for the following reasons: (1) In alanine, serine, and tyrosine RNA's, the presumed anticodon sequence is the most susceptible site of enzymatic attack; [3, 4, 22] under similar or even more drastic conditions for enzymatic degradation, phe-tRNA was resistant to the action of $T_1$-RNase at 0°C in the presence of magnesium ions. This would be expected if the anticodon contained a 2'OMeG-A-A rather than a G-A-A sequence. (2) The location of 2'OMeG-A-A in phe-tRNA is very similar to the relative locations of presumed anticodons in the serine, alanine, and tyrosine tRNA's. Thus, 2'OMeG-A-A is also present in a loop, the anticodon sequence representing the third, fourth, and fifth bases in this loop. In all cases the anticodon is preceded by U and followed by a minor base, and the minor base DiMeG is the ninth base from the first letter of the anticodons. (3) Finally, the over-all arrangement of nucleotide residues around the probable anticodon sequences between serine, tyrosine, and phe-tRNA's are very similar. Thus in serine RNA, the anticodon I-G-A is preceded by A-G-A-ψ-U- and followed by -iPA-A-ψ-C-U-, in tyrosine RNA the anticodon G-ψ-A is preceded by A-G-A-C-U- and followed by -DiMeA-A-ψ-C-U-, and in phenylalanine RNA the 2'OMeG-A-A is preceded by A-G-A-2'OMeC-U- and followed by -Y-A-ψ-5MeC-U-.

The experiments of Chapeville *et al.*[23] and Weisblum *et al.*[24] demonstrated the correctness of the adapter function of tRNA in protein synthesis. The concept that the different codons on messenger RNA are actually recognized by the tRNA's by base-pairing of the three nucleotide units of the codons with three contiguous bases on the tRNA's is now supported by the known structures of the five tRNA's. All of the five tRNA's can adopt an essentially identical cloverleaf structure and, in this form, every one of them turns out to have at essentially the same place and in identical environment a group of three nucleotides which can in fact base-pair with the established codons for the amino acid to which the tRNA corresponds. This evidence appears to be especially strong when one considers that the ribosomal surface on which amino acid condensations occur must require all tRNA's to adhere to certain rigidly defined physical dimensions.

*Summary.*—The total primary sequence of yeast phe-tRNA has been elucidated (Fig. 1). This tRNA can adopt a secondary structure of the cloverleaf type similar to other tRNA's of known structure (Fig. 2). The most probable anticodon sequence in this tRNA is 2'OMeG-A-A.

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[2] Abbreviations used are: tRNA, transfer ribonucleic acid; phe, phenylalanine; p or - indicate a phosphate group; p on the left indicates a 5'-phosphate residue, p on the right, a 3'-phosphate group; OH is used to indicate a free 2',3'-hydroxyl end group; A is adenosine; MeA, 1-methyl adenosine; C, cytidine; MeC, 5-methylcytidine; 2'OMeC, 2'O-methylcytidine; U, uridine; DiHU, 5,6-dihydrouridine; $\psi$, pseudouridine; T, ribothymidine; G, guanosine; 2MeG, N²-methylguanosine; DiMeG, N²-dimethylguanosine, 7MeG, N⁷-methylguanosine; 2'OMeG 2'-O-methylguanosine; iPA, isopentenyl adenosine; RNase, ribonuclease; Poly U, polyuridylic acid.

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