Nucleotide Sequence of Phenylalanine Transfer Ribonucleic Acid from Pea (*Pisum sativum,* Alaska)†

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ABSTRACT: Phenylalanine transfer ribonucleic acid from peas (*Pisum sativum,* Alaska) was completely digested with beef pancreatic ribonuclease (RNase I) and with ribonuclease T1. The resulting oligonucleotides were compared with those from the corresponding hydrolyses of phenylalanine transfer ribonucleic acid from wheat germ. The structures of both ribonucleic acids appeared to be identical. This report is the first to show that identical structures for the same specific acceptor transfer ribonucleic acid are present in two different plant species.

The nucleotide sequences of $tRNA^{Phe}$ (yeast) (RajBhandary et al., 1967), $tRNA^{Phe}$ (*E. coli*) (Barrell and Sanger, 1969), and $tRNA^{Phe}$ (wheat germ) (Dudock et al., 1969) have all been determined and found to differ from each other. We have compared the sequence of $tRNA^{Phe}$ (wheat germ) (Dudock et al., 1969), the structure of which is shown in Figure 1, with that of $tRNA^{Phe}$ (peas) to see how similar this tRNA is in these higher plants, a monocotyledon and a dicotyledon.

Materials and Methods

The various materials and compounds used in this study were obtained commercially as follows: peas (*Pisum sativum,* Alaska), untreated, from W. Atlee Burpee Co.[1] (Philadelphia, Pa.); raw wheat germ from Sigma Chemical Co.; cellulose-coated thin-layer plates (Avicel) from Analtech, Inc. (Wilmington, Del.); pancreatic RNase I, alkaline phosphatase, and snake venom phosphodiesterase from Worthington Biochemical Corp.; polynucleotide phosphorylase from P-L Biochemicals, Inc.; ribonuclease T1 and T2 from Calbiochem; benzoylated DEAE-cellulose from Schwarz/Mann; DEAE-cellulose from Schleicher and Schuell. In earlier experiments, benzoylated DEAE-cellulose was prepared according to the method of Gillam et al. (1967) from DEAE-cellulose. All enzymes were used without further purification.

$tRNA^{Phe}$ Purification. Peas (4.6 kg) were soaked in frequently changed aerated-deionized water for 20 h in the dark. Crude pea tRNA was prepared by a modification of the bulk isolation procedure of Holley (1968) for the extraction of tRNA from yeast. Crude wheat germ tRNA was prepared from 2.3-kg lots by further modifications of the same procedure. Unlike the preparation of tRNA from yeast in which yeast was merely suspended in a phenol–water mixture and allowed to settle, it was necessary with both peas and wheat germ to homogenize in a Waring blendor in the presence of phenol and water. The homogenates were then centrifuged and the supernatant from peas, unlike that from yeast, was extracted with half a volume of diethyl ether after the second phenol extraction. This additional step clarified the supernatant and increased the extinction coefficient of the final tRNA product about fourfold. Further modifications in the preparation of tRNA from wheat germ were adapted from Glitz and Dekker (1963). These included the precipitation of high molecular weight RNA in the cold in the presence of 2.5 to 3 M NaCl and gradient elution of the crude tRNA from DEAE-cellulose. One gram of crude tRNA was obtained from 4.6 kg of peas, 4 g from 4.6 kg of wheat germ.

Crude tRNA from both sources was fractionated by countercurrent distribution in the ammonium sulfate system of Kirby (1960) as described by Doctor (1967). The tubes containing the phenylalanine acceptor activity were extracted twice with diethyl ether and diluted with water to reduce the ammonium sulfate concentration prior to adsorption on benzoylated DEAE-cellulose columns (Gillam et al., 1967). The purified tRNA from the benzoylated DEAE-cellulose columns was assayed directly for acceptor activity as described previously (Madison et al., 1967). Since the phenylalanine-tRNA synthetase isolated from yeast aminoacylated $tRNA^{Phe}$ isolated from yeast, peas, and wheat germ equally well, yeast enzyme was used in all assays.

The purified $tRNA^{Phe}$ was precipitated directly with two volumes of 95% ethanol and collected by centrifugation. Since only about half of the RNA was recovered, the supernatant was shaken with an equal volume of ether in a separatory funnel. The small aqueous layer produced was separated, mixed with two volumes of 95% ethanol in an Erlenmeyer flask, and allowed to stand overnight at room temperature. Sodium chloride, which was precipitated in addition to $tRNA^{Phe}$, adhered to the bottom and sides of the flask. The flocculent suspension of $tRNA^{Phe}$ was decanted and centrifuged. The $tRNA^{Phe}$ was dissolved in water and reprecipitated with ethanol to remove residual sodium chloride.

Analyses. Methods for nuclease digestions, isolation of oligonucleotides, and analyses for nucleotide composition have been described (Madison et al., 1967, 1974).

For a summary of ultraviolet spectra and extinction coefficients of the nucleosides, see Hall (1971). Additional millimolar extinction coefficients used at 260 nm and pH 7

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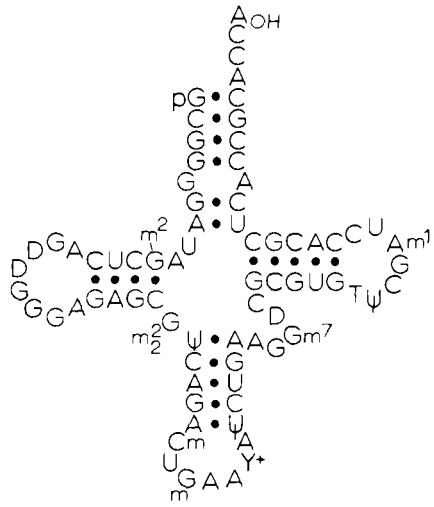

FIGURE 1: Cloverleaf structure presented by Dudock et al. (1969) for tRNA^{Phe} isolated from wheat germ.

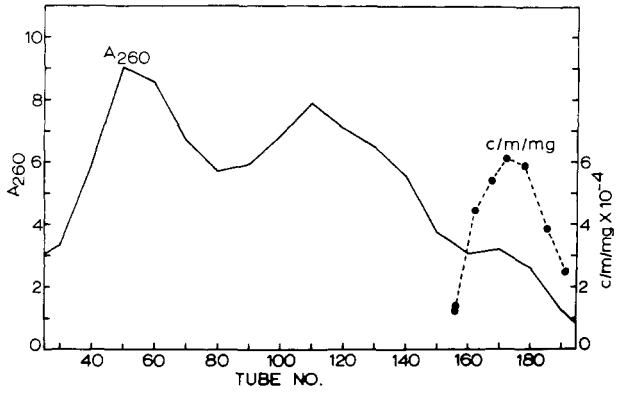

FIGURE 2: Countercurrent distribution pattern of 6 g of pea tRNA in the ammonium sulfate system showing the peak for phenylalanine acceptor activity.

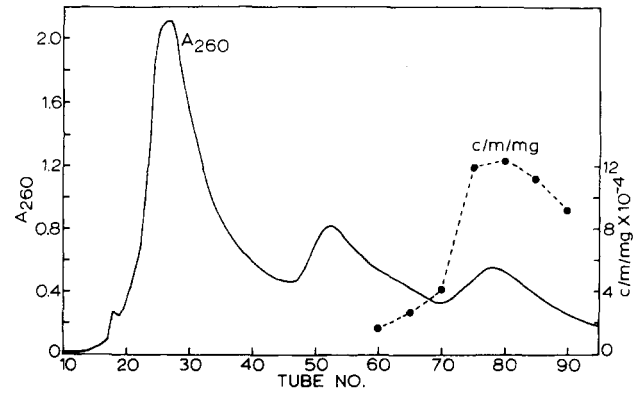

FIGURE 3: Chromatography of tRNA^{Phe} on a 2 × 70 cm column of benzoylated DEAE-cellulose with a gradient of 0.7–1.5 M NaCl in 0.01 M $MgCl_2$, 0.02 M potassium acetate, pH 5.0. Each tube contained 10 ml.

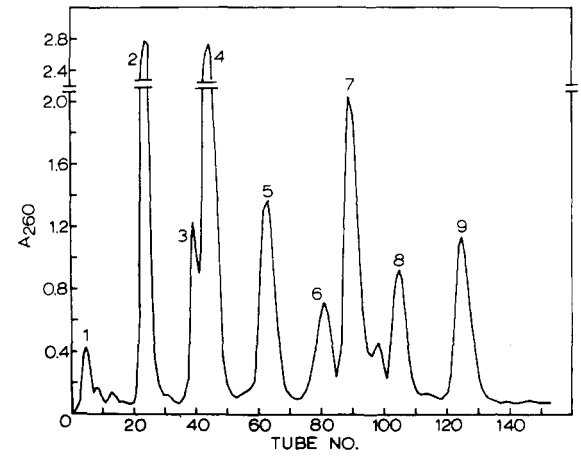

FIGURE 4: Chromatography of products from the digestion of purified tRNA^{Phe} with RNase I on a 0.35 × 25 cm column of DEAE-cellulose. Each tube contained 1.3 ml.

were 5 for Y^+ and 10 for m^7G^* (Katz and Dudock, 1969).[2]

Thin-Layer Chromatography. All separations were carried out on cellulose plates with the following systems, singly, or in two-dimensional combinations: A, 2-propanol–concentrated ammonium hydroxide–water, 65:10:25 v/v/v; B, 2-propanol–concentrated hydrochloric acid–water, 680:176:144 v/v/v; C, isobutyric acid (600 ml) and water (300 ml), adjusted to pH 3.65 with ammonium hydroxide and diluted to 960 ml; D, 0.3% formic acid–*tert*-butyl alcohol, 1:1 v/v, adjusted to pH 4.8 with concentrated ammonium hydroxide.

Results

A typical countercurrent distribution pattern is shown in Figure 2. In the best countercurrent distributions, tRNA^{Phe} was enriched 30-fold. Further purification with a benzoylated DEAE-cellulose column (Figure 3) increased the purity of the tRNA^{Phe} an additional two- to threefold.

The products of complete digestion of tRNA^{Phe} with RNase I were fractionated by chromatography on DEAE-cellulose in 7 M urea at pH 7 (Figure 4). The structures of the products of RNase I digestion are reported in Table I along with a summary of the procedures used to establish the sequences. The *letters* indicate the procedures used, and the *numbers* indicate the molar ratios of the various fragments produced. Most of the peaks from Figure 4 were separable into pure oligonucleotides by chromatography on DEAE-cellulose in 7 M urea containing 0.1 M formic acid.

Pea tRNA^{Phe} was always found to terminate with 3′-adenosine. Adenosine was isolated from peak 1 (Table I); further evidence was the occurrence of 3′-terminal adenosine in a fragment found in peak 6 (Table II) after digestion of tRNA^{Phe} with RNase T1.

The mononucleotide peak was difficult to analyze directly because of losses in the desalting procedure, particularly of ψp and of Dp, which were present in small amounts compared with the amounts of Cp and Up. The values reported in Table I for the mononucleotides are consistent with those reported by Katz and Dudock (1969).

Since some m^1Ap was converted to m^6Ap during incubation with RNase I at pH 7.8, the amount of G-m^1A-Up present in peak 3 varied from one analysis to another. Because of the positive charge on m^1Ap, the trinucleotide G-m^1A-Up was eluted from DEAE-cellulose in 7 M urea with $m^2{}_2$G-ψp both at pH 7 and in the presence of 0.1 M formic acid. The two components of peak 3 were separated by two-dimensional thin-layer chromatography with systems C and D. Hydrolysis of each component with KOH followed by

[2]Abbreviations used: Y^+ is the fluorescent nucleoside isolated from tRNA^{Phe} from peas; D is 5,6-dihydrouridine; m (with no superscript) is 2′-*O*-methyl; >p is 2′:3′-phosphate (cyclic); and m^7G^* is the conversion product obtained from m^7G under alkaline conditions (Katz and Dudock, 1969).

Table I: Products of Ribonuclease I Digestion of $tRNA^{Phe}$.

Peak No. (Figure 4)	mol/mol of tRNA	Product	Basis for Structural Assignment[a]
1	(1)	A_{OH}	D, B: A_{OH} C-A-C-C-A_{OH} found in RNase T1 digest
2	15.3	Cp, Up, ψp, Dp	D, B: Cp, 11.0; Up, 4.0; ψp, 0.4; Dp (1.0)[b]
3	1.1	m^2_2G-ψp	L, D, A, D, B: m^2_2Gp, 1.0; ψp, 0.8
	0.6	G-m^1A-Up	L, D, A, D, B: Gp, 1.0; m^6Ap, 1.0; Up, 1.2
4	2.8	A-Cp	L, A, D, B: Ap, 1.0; Cp, 1.1
	3.2	G-Cp	L, A, D, B: Gp, 1.0; Cp, 1.0
	1.7	G-Up	L, A, D, B: Gp, 3.0; Up, 2.2; Tp, 1.0
	0.8	G-Tp	
5	1.1	A-m^2G-Cp	L, A, D, B: Ap, 0.9; m^2Gp, 1.0; Cp, 1.0 F, K, A, D, B: A-m^2Gp, 1.1; Cp, 1.0
	1.0	A-G-Dp	L, D, A, D, B, C: Ap, 1.0; Gp, 1.1; Dp, 0.9 F, K, C: A-Gp, 1.0; Dp, 0.8
	0.3	G-m^6A-Up	L, D, A, D, B: Gp, 1.0; m^6Ap, 1.0; Up, 1.2
6	1.0	pG-Cp	L, A, D, B: pGp, 1.0; Cp, 0.9; Up, 0.1; Gp, 0.1; Ap, 0.1
7	1.0	A-G-A-Cm-Up	L, A, D, B: Gp, 0.8; Ap, 2.0; Cm-Up, 0.7 L, F, K: A-Gp, 1.0; A-Cm-Up, 1.0
	0.8	G-A-A-G-m^7G-Dp	L, A, D, B: Gp, 2.0; Ap, 2.0; m^7G-Dp destroyed L, F, D: Gp, 2.0; A-A-Gp, 0.8; m^7G-Dp, 1.0
	0.8	Gm-A-A-Y^+-A-ψp	L, A, D, B: Ap, 2.0; ψp, 0.8; Gm-Ap, 1.0; Y^+ destroyed G, D, B: Ap, 2.0; ψp, 0.9; Y^+p, 1.0; Gm-Ap, 1.0
8	0.5–0.7	G-G-G-G-A-Up	L, A, D, B: Gp, 4.0; Ap, 1.0; Up, 0.7 F, K, D: Gp, 4.0; A-Up, 0.9
9	0.9	G-G-G-A-G-A-G-Cp	A, D, B: Gp, 5.1; Ap, 2.0; Cp, 0.7 F, K or D: A-Gp, 2.0; Gp, 3.5; Cp, 1.0

[a] Methods used for the sequence assignment: A, alkaline hydrolysis; B, ultraviolet spectra at pH 2, 7, and 13; C, decrease in A_{230} in 0.1 N KOH (Batt et al., 1954); D, two-dimensional thin-layer chromatography; E, RNase I digestion; F, RNase T1 digestion; G, RNase T2 digestion; I, alkaline phosphatase followed by partial snake venom phosphodiesterase digestion; J, alkaline phosphatase followed by complete polynucleotide phosphorylase digestion; K, chromatography on DEAE-cellulose in 7 M urea (pH 7.0); L, chromatography on DEAE-cellulose in 7 M urea (0.1 M formic acid). [b] Dp was not determined but its presence was inferred from the analysis of D-D-Gp from peak 6, Table II.

thin-layer chromatography of the products gave the composition reported in Table I.

Peak 4 was either separated as indicated, or the entire dinucleotide fraction was separated on a 7 M urea–0.1 M formic acid column. Although G-Up was not separated from G-Tp, their identities were easily deduced following alkaline hydrolysis.

Peak 5 was separated into two peaks on a 7 M urea–0.1 M formic acid column. The first peak contained A-m^2G-Cp; the second contained A-G-Dp plus some G-m^6A-Up. The G-m^1A-Up had been partially converted to G-m^6A-Up, which was eluted as a trinucleotide. The two trinucleotides in the second peak were separated by two-dimensional thin-layer chromatography. The structures of the trinucleotides A-m^2G-Cp and A-G-Dp were defined by hydrolysis with RNase T1.

Peak 6 contained pG-Cp as its main component. Chromatography on DEAE-cellulose columns in 7 M urea–0.1 M formic acid yielded one major peak plus at least three smaller peaks. Although the major peak was slightly contaminated, its predominant constituents, after hydrolysis with KOH, were pGp and Cp.

Peak 7 consisted of the pentanucleotide A-G-A-Cm-Up plus two hexanucleotides. Each of the hexanucleotides contained an unusual nucleotide bearing a positive charge at pH 7 which caused these hexanucleotides to be eluted with the pentanucleotide fraction. These three oligonucleotides were successfully separated on a 7 M urea–0.1 M formic acid column. Hydrolysis of A-G-A-Cm-Up with RNase T1 followed by alkaline hydrolysis of the resulting dinucleotide A-Gp and the trinucleotide A-Cm-Up were useful for defining the structure of this pentanucleotide. Snake venom phosphodiesterase digestion of Cm-Up after removal of the 3′-phosphate produced Cm_{OH} and pU.

Alkaline hydrolysis of G-A-A-G-m^7G-Dp yielded equimolar amounts of Ap and Gp. The destruction of Dp during alkaline hydrolysis accounted for the absence of a pyrimidine. The unusual nucleotide m^7Gp was also destroyed by alkaline hydrolysis. Since Dp does not absorb in the same ultraviolet range as m^7Gp, it was possible to obtain the spectra of m^7Gp from the dinucleotide m^7G-Dp. After Dp was determined by the decrease in A_{230} in 0.1 N KOH (Batt et al., 1954), the spectra for m^7G* were found. The maxima and minima obtained from the spectra of m^7G*p at pH 2 and 12 were in agreement with the values reported by Katz and Dudock (1969). Since there was only one A-A-Gp sequence found, and it was found as a trinucleotide in the RNase T1 digest, Gp must have been to the left of A-A-Gp.

The final component in peak 7 yielded two Ap's, ψp and Gm-Ap on complete alkaline hydrolysis. The nucleotide Y^+p was destroyed by these conditions. There was no bond in this hexanucleotide susceptible to RNase T1. Hydrolysis with RNase T2 yielded two Ap's, ψp, Gm-Ap, and Y^+p.

The ultraviolet spectra of Y^+ closely resembled that for Y (wheat germ) (Katz and Dudock, 1969), and that for Y (calf liver) (Blobstein et al., 1973). For Y^+p obtained from peas by hydrolysis of the hexanucleotide with RNase T2 we obtained maxima at 232 and 277 nm and a minimum at 255 nm at pH 2. At pH 12 we obtained maxima at 239 and 300 nm with a minimum at 280 nm. Yoshikami and Keller (1971) compared Y (wheat germ) with Y (yeast) and with Y (beef liver) and reported that Y (wheat germ) was indistinguishable from Y (beef liver). Feinberg et al. (1974) reported that Y (lupine) is identical with Y (wheat germ) and with Y from four liver sources. The Y bases or nucleosides isolated from these various sources were characterized as

having a peroxy group in the side chain. Blobstein et al. (1973) have inferred that the peroxy base is present in all higher species tested. We therefore assumed that Y^+ also contained the peroxy base.

The free base Y^+ was excised from the hexanucleotide as described by Thiebe and Zachau (1968). When chromatographed in system C a fluorescent spot was obtained which had the same R_f as that reported by Yoshikami and Keller (1971) for Y excised from wheat germ tRNA^{Phe}. The hexanucleotide with Y^+ excised was then split into Gm-A-Ap and pA-ψp by chemical means as described by Philippsen et al. (1968).

Gm and pA were identified after complete digestion of Gm-A with snake venom phosphodiesterase. The occurrence of all of these components in this hexanucleotide plus the occurrence of this hexanucleotide in the dodecanucleotide (peak 16 of Table II) after RNase T1 digestion of tRNA^{Phe} led us to conclude that the structure of this hexanucleotide was the same as that reported by Katz and Dudock (1969). This hexanucleotide has also been found in tRNA^{Phe} isolated from yeast (RajBhandary et al., 1968a).

The sequence of the hexanucleotide G-G-G-G-A-Up (peak 8) was the most difficult to define. Katz and Dudock (1969) found that this hexanucleotide was eluted from DEAE-cellulose after the octanucleotide. Madison et al. (1974) reported a similar experience with another hexanucleotide containing four consecutive guanylic acid residues. However, under the conditions of our study, the bulk of this hexanucleotide isolated from tRNA^{Phe} from both peas and wheat germ was eluted between the pentanucleotides and the octanucleotide. Only a small portion could ever be eluted after the octanucleotide. We were working with considerably smaller amounts of tRNA^{Phe} than Katz and Dudock, so it is possible that this could explain the different results. Aggregation of the oligonucleotides containing four consecutive guanylic acid residues may be enhanced by higher concentration.

The main hexanucleotide fraction was usually contaminated with the hexanucleotide Gm-A-A-Y^+-A-ψp and a lesser amount of the hexanucleotide G-A-A-G-m^{7}G-Dp. As indicated previously, each of these two hexanucleotides contained an unusual nucleotide, positively charged at pH 7, which caused the respective hexanucleotide to be eluted as a pentanucleotide. However, when some Y^+p and m^{7}Gp underwent transformations and lost their positive charges, the respective oligonucleotides were eluted as hexanucleotides. The detection of either or both of these after thin-layer chromatography was simplified since both Y^+p and m^{7}Gp fluoresce under ultraviolet light in the absence of HCl. Other guanylic acid residues fluoresce only in the presence of HCl. The major contaminant of peak 8 was Gm-A-A-Y^+-A-ψp, which was separated by thin-layer chromatography in system C. It moved in a large band with an R_f of about 0.4 exhibiting the fluorescence characteristic of Y^+. In some samples, another component with an intermediate R_f in system C had the fluorescence of m^{7}Gp. Alkaline hydrolysis of the material remaining at the origin (usually about 50% of peak 8) gave products consistent with the indicated structure G-G-G-G-A-Up. Hydrolysis of this oligonucleotide with RNase T1 produced four Gp's and one A-Up which was sufficient evidence to establish the structure.

Alkaline hydrolysis of peak 9 yielded five Gp's, two Ap's, and one Cp. Ribonuclease T1 hydrolysis products of this octanucleotide included three Gp's, two A-Gp's, and one Cp. We concluded that this octanucleotide was the same as that reported by Katz and Dudock (1969).

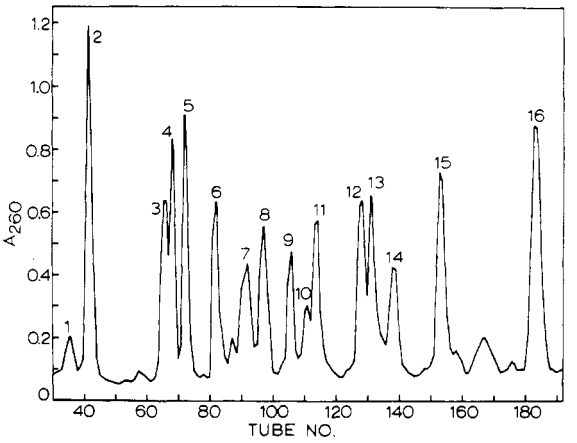

FIGURE 5: Chromatography of products from the digestion of tRNA^{Phe} with RNase T1 on a 0.35 × 120 cm column of DEAE-cellulose. Each tube contained 1.3 ml.

tRNA^{Phe} was also completely digested with RNase T1, and the products were fractionated by chromatography on DEAE-cellulose in 7 M urea at pH 7 (Figure 5). We obtained better separation of the oligonucleotides at pH 7 than at pH 8.1 (Katz and Dudock, 1969). Those oligonucleotides not separated under these conditions were either separated on DEAE-cellulose columns in 7 M urea–0.1 M formic acid or by two-dimensional thin-layer chromatography with systems C and D.

The structures of the products of RNase T1 digestion are listed in Table II with a summary of the data used to support the sequences shown. The code to the letters indicating the procedures used is the same as that in Table I.

Since m$^2_2$G-ψp is very slowly hydrolyzed by RNase T1, the molar ratio of peak 1 was less than 1; the remainder of C-m$^2_2$Gp was found in peak 14 as part of a hexanucleotide. The composition of peak 1 was determined by alkaline hydrolysis.

Identification of peaks 2 through 5 presented no problems.

Peak 6 was separated into two components on a DEAE-cellulose column in 7 M urea–0.1 M formic acid. The structure of D-D-Gp was determined as summarized in Table II. Any questions as to the order of C-A-C-C-A_{OH} were dispelled by the facts that hydrolysis of this peak with RNase I produced A-Cp and that all tRNA molecules end in C-C-A_{OH}. The finding of A_{OH} plus A-Cp and the absence of G-A-Cp in any of the products of RNase I digestion defined the structure of this pentanucleotide.

Peak 7 contained the unusual nucleotide m^{7}Gp as well as Dp. RNase I hydrolysis of this peak produced a dinucleotide which was separated by thin-layer chromatography and exhibited the fluorescence of m^{7}Gp. This spot had the absorption characteristics of m^{7}G*p and also contained Dp. Since m^{7}Gp occurred in the RNase I products (Table I, peak 7) in a hexanucleotide from which m^{7}G-Dp was produced by RNase T1 hydrolysis, the additional Cp and Gp produced by the action of RNase I on peak 7 could only be accounted for by the order indicated in Table II.

Peak 8 was hydrolyzed with alkali and its components were identified as summarized in Table II.

Since G-Tp was found in the products of RNase I digestion, and the 3′-terminus after RNase T1 digestion must be Gp, we concluded that peak 9 must have the sequence pre-

Table II: Products of Ribonuclease T1 Digestion of $tRNA^{Phe}$.

Peak No. (Figure 5)	mol/mol of tRNA	Product	Basis for Structural Assignment[a]
1	0.4	C-m^2_2G>p	A, D, B: m^2_2Gp, 0.9; Cp, 1.0
2	6.9	Gp	A, D, B: Gp
3	2.8	C-Gp	A, D, B: Cp, 1.1; Gp, 1.0
4	1.6	U-Gp	A, D, B: Up, 1.1; Gp, 1.0
5	2.4	A-Gp	A, D, B: Ap, 1.1; Gp, 1.0
6	0.6	C-A-C-C-A_{OH}	L, A, D, B: Cp, 3.0; Ap, 1.1; A, 1.0 E, K: Cp, 1.9; A_{OH}, 1.0; A-Cp, 0.5
	0.8	D-D-Gp	L, C, B: Dp, 1.6; Gp, 1.0
7	1.2	m^7G-D-C-Gp	L, E, D: Cp, 1.1; Gp, 1.0; m^7G*-Dp, 0.8 C: Dp, 0.7
8	1.0	A-A-Gp	L, A, D, B: Ap, 2.1; Gp, 1.0; pGp, 0.9
	1.0	pGp	
9	0.9	T-ψ-C-Gp	L, A, D, B: Tp, 1.0; ψp, 0.9; Cp, 1.0; Gp, 1.0
10	0.4	ψ-C-A-Gp	L, A, D, B: ψp, 0.4; Cp, 1.2; Ap, 0.9; Gp, 1.0; Up, 0.6
11	1.1	A-U-A-m^2Gp	L, A, D, B: Ap, 2.1; Up, 1.0; m^2Gp, 0.9 E, K or D: A-Up, 1.0; A-m^2Gp, 1.0
12	1.0	C-U-C-A-Gp	A, D, B: Cp, 1.6; Up, 1.1; Ap, 1.0; Gp, 1.0 E, D, B: Cp, 2.0; Up, 1.0; A-Gp, 1.0 J, K: C-U-C_{OH} detected
13	0.8	m^1A-U-C-C-A-C-Gp	A, D, B: m^6Ap, 0.9; Up, 0.9; Cp, 2.0; Ap, 0.8; Gp, 1.0 E, D, B: Cp, 2.5; Gp, 1.0; m^1A-Up, 0.5; Up, 0.6; A-Cp, 1.0 I, A, D, B: m^1A-U-C-C_{OH} and m^1A-U-C-C-A_{OH} detected
14	0.6	C-m^2_2G-ψ-C-A-Gp	E, K, D, B: Cp, 2.4; m^2_2G-ψp, 1.5; A-Gp, 1.0 I, A, D, B: C-m^2_2G-ψ-C_{OH} detected
15	0.9	C-U-C-A-C-C-Gp	A, D, B: Cp, 3.7; Up, 1.4; Ap, 1.1; Gp, 1.0 E, D: Cp, 2.9; Gp, 0.8; Up, 1.1; A-Cp, 1.0 J, K, A, D, B: C-U-C_{OH} and C-U-C-A_{OH} detected
16	0.6	A-Cm-U-Gm-A-A-Y^+-A-ψ-C-U-Gp	A, D, B: Ap, 2.8; Cp, 1.3; Up, 1.7; ψp, 0.5; Gp, 1.0; Gm-Ap, 0.7; Cm-Up, 0.8 E, D or K: Cp, 1.0; Up, 1.1; Cp, 0.8; A-Cm-Up, 1.0; Gm-A-A-Y^+-A-ψp, 1.0

[a] See footnote *a*, Table I.

sented in Table II, a sequence common to practically all transfer ribonucleic acids.

Peak 10 was the second product from partial hydrolysis of peak 14 by RNase T1. Identification of the alkaline hydrolysis products of peak 10 plus a complete analysis of peak 14 established the identity of this tetranucleotide.

Peak 11 was identified after alkaline hydrolysis and by hydrolysis with RNase I which produced the dinucleotides A-Up and A-m^2Gp.

Peak 12 on alkaline hydrolysis yielded two Cp's, Up, Ap, and Gp. Hydrolysis with RNase I yielded two Cp's, Up, and the dinucleotide A-Gp. Polynucleotide phosphorylase experiments produced (C-,U-)C_{OH}. Also, hydrolysis of $tRNA^{Phe}$ with RNase I produced but one G-Up residue which was accounted for in the G-U-G-T sequence (Figure 1). We therefore concluded that the structure of peak 12 was C-U-C-A-Gp.

The 5′-terminus of peak 13 was established as m^1Ap when the trinucleotide G-m^1A-Up was found in the products of complete hydrolysis of $tRNA^{Phe}$ with RNase I. The products from the hydrolysis of peak 13 with RNase I and those from partial digestion with snake venom phosphodiesterase, m^1A-U-C-C_{OH} and m^1A-U-C-C-A_{OH} completed the information necessary to determine the structure of peak 13.

The fact that peak 14 was only partially split by RNase T1 to give C-m^2_2G>p and ψ-C-A-Gp simplified the determination of the structure of this peak. RNase I hydrolysis of peak 14 produced the dinucleotides m^2_2G-ψp and A-Gp plus two Cp's. Partial degradation with snake venom phosphodiesterase produced C-m^2_2G-ψ-C_{OH} which confirmed the structure shown.

The alkaline hydrolysis products of peak 15 indicated that it contained four Cp's, Up, Ap, and Gp. RNase I hydrolysis of peak 15 produced one dinucleotide, A-Cp; the remaining products were three Cp's, Up, and Gp. Two products, (C-,U-)C_{OH} and (C-,U-)C-A_{OH}, isolated after digestion with alkaline phosphatase and polynucleotide phosphorylase were sufficient evidence to define the structure of peak 15 as presented in Table II. The reasoning that (C-, U-)C_{OH} is equivalent to C-U-C_{OH} which was used for sequence determination of peak 12 can be applied to this same sequence in peak 15.

In $tRNA^{Phe}$ from peas, as from wheat (Figure 1), the A in the sequence C-U-C-A-C-C-G was positioned opposite G. Thus the A opposite a G has been preserved, suggesting that this unusual combination is important and not simply a random mutation.

Alkaline hydrolysis of peak 16 yielded three Ap's, Up, Cp, ψp, Gp, Gm-Ap, and Cm-Up. The presence of a breakdown or conversion product of Y^+p was noted but not quantitated. The above products were consistent with those found after alkaline hydrolysis of the dodecanucleotide from $tRNA^{Phe}$ (wheat) (Katz and Dudock, 1969).

The products after hydrolysis of peak 16 with RNase I were also consistent with those, Cp, Up, Gp, A-Cm-Up, and Gm-A-A-Y^+-A-ψp, reported by Katz and Dudock (1969). Since the sequence A-Cm-Up had been found in the pentanucleotide A-G-A-Cm-Up from the RNase I digestion of $tRNA^{Phe}$ (Table I, peak 7), the 5′-terminus must be A-Cm-Up. The occurrence of the Y^+ containing hexanucleotide in this dodecanucleotide, plus the fact that the 3′-terminus was Gp, led us to conclude that the structure of peak 16 (Table II) was the same as that of the dodecanucleotide reported by Katz and Dudock (1969). A closely similar sequence of nucleotides was found by RajBhandary et al.

(1968b) in tRNA^{Phe} (yeast). The only difference was the presence of m^{5}Cp in place of Cp near the 3′-terminus.

Discussion

The products of hydrolysis of tRNA^{Phe} from peas by RNase I and by RNase T1 were isolated and compared with the corresponding products found by Katz and Dudock (1969) in tRNA^{Phe} isolated from wheat germ. This was the first time that identical products have been shown to be formed by nuclease digestion of tRNA's specific for a particular amino acid from two different plant species. Vanderhoef et al. (1972) found only minor differences in the relative rates of aminoacylation of leucine and of tyrosine tRNA's from higher plants. These findings also suggest that tRNA's specific for the same amino acid have similar structures in higher plants.

Piper and Clark (1974) and Simsek et al. (1974) have shown the structures of tRNA$_f^{Met}$ from three mammalian sources, mouse myeloma, rabbit liver, and sheep mammary gland, to be identical.

Although we did not carry out any partial nuclease digestions of tRNA^{Phe}, we do not believe that the oligonucleotides produced by both RNase I and by RNase T1 could have been derived from a structure different from the one reported for tRNA^{Phe} (wheat germ) by Dudock et al. (1969). We therefore concluded that the nucleotide sequences of tRNA^{Phe} from peas and from wheat are identical.

Our results infer that not only are the nucleotide sequences of the DNA that make up the genes for tRNA^{Phe} identical in peas and wheat germ, but that the same modifying enzymes are also found in both species.

The preservation of G opposite A in the acceptor stem in plant tRNA^{Phe} and of the G-U base pair in tRNA$_f^{Met}$ from three mammalian species suggest that these variations from Watson-Crick base pairing play an important role in some function of these tRNA's.

Added in Proof

Keith, et al. (1974) have determined the structure of tRNA^{Phe} from calf liver and from rabbit liver. These two structures are identical with each other but different from the structures previously discussed in this paper.

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