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Nucleotide Sequence of Phenylalanine Transfer RNA from *Schizosaccharomyces pombe*: Implications for Transfer RNA Recognition by Yeast Phenylalanyl-tRNA Synthetase†

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ABSTRACT: The nucleotide sequence of *Schizosaccharomyces pombe* $tRNA^{Phe}$ was determined to be pG-U-C-G-C-A-A-U-G**-G*-U-G-ψ-A-G-D-D-G-G-G-A-G-C-A-ψ-G*-A-C-A-G-A-Cm-U-Gm-A-A-Y-A-ψ-m^5C-U-G-U-U-G-m^7G-U*-C-A-U-C-G-G-T-ψ-C-G-A-U-C-C-C-G-G-U-U-U-G-U-G-A-C-A-C-C-A_{OH}. This sequence differs from that of *S. cerevisiae* $tRNA^{Phe}$ in 27 nucleotides. *Saccharomyces cerevisiae* phenylalanyl-tRNA synthetase aminoacylates both the homologous $tRNA^{Phe}$ and *S. pombe* $tRNA^{Phe}$; the reactions have similar K_m and V_{max} values. However, the nucleotide sequence in the D stem is different in the two tRNAs. This region was proposed by Roe, B., et al. [(1973) *Biochemistry 12,* 4146-4154] to be the major recognition site for yeast phenylalanyl-tRNA synthetase, but the present results cast doubt on the validity of this hypothesis.

Mischarging, the aminoacylation of tRNA with the wrong amino acid, has often been observed in in vitro reactions, especially when aminoacyl-tRNA synthetases and tRNA from different sources are used (Jacobson, 1971). Dudock et al. (1970) and Taglang et al. (1970) discovered that yeast phenylalanyl-tRNA synthetase will charge *E. coli* $tRNA^{Val}$ with phenylalanine. The ability of this enzyme to charge many cognate and noncognate tRNA species has been used by Dudock in an approach to define the nucleotide sequence responsible for proper recognition by yeast phenylalanyl-tRNA synthetase. Eleven pure tRNA species could be aminoacylated by this enzyme (Roe et al., 1973). A comparison of their nucleotide sequences revealed some common structural features (the same base found in the same position in the different

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tRNAs). From such a composite (see Figure 7A), Roe et al. (1973) proposed that nine nucleotides in the D stem and the fourth nucleotide from the 3′ end comprise the specific recognition sequence for this enzyme. In addition, they showed that all the tRNAs which had a V_{max} similar to that of yeast $tRNA^{Phe}$ in the aminoacylation reaction contain eight nucleotides in the D loop and have a modified guanosine (m^2G) in position 10. These two latter features were thought to be important in determining reaction velocity.

The results of some recent experiments cast doubt on the validity of this appealing hypothesis. Sequence analysis of yeast $tRNA^{Met}$ revealed that this tRNA contained all the nucleotides of the phenylalanyl-tRNA synthetase "recognition site" (Gruhl & Feldmann, 1976). However, the hypothesis requires that other yeast tRNA do not contain these sequences. Under standard aminoacylation conditions only a very low degree of mischarging of yeast $tRNA^{Met}$ could be obtained (Feldmann & Zachau, 1977); this level could be considerably raised when special aminoacylation conditions (Roe et al., 1973) were used. In another experiment, Yaneva & Beltchev (1976) removed four nucleotides from the 3′ terminus of yeast $tRNA^{Lys}$. This tRNA still contains the nine nucleotides in the D stem which are the proposed recognition sequence. Although an equally truncated yeast $tRNA^{Phe}$ still forms a complex with yeast phenylalanyl-tRNA synthetase (Hörz & Zachau, 1973), the modified $tRNA^{Lys}$ neither binds to the enzyme nor inhibits the aminoacylation of yeast $tRNA^{Phe}$ (Yaneva & Beltchev, 1976).

In the course of our studies on tRNA precursors in yeast we determined the nucleotide sequence of *S. pombe* $tRNA^{Phe}$. Although this tRNA does not contain the yeast phenylalanyl-tRNA synthetase "recognition" sequences, it is aminoacylated efficiently by this enzyme. Thus the validity of a recognition site concept based on comparison of cloverleaf structures is questionable.

Materials and Methods

General. Pancreatic RNase, snake venom phosphodiesterase, and *E. coli* alkaline phosphatase (BAPF) were obtained from Worthington Biochemical Corp. T1 and T2 RNase (Sankyo) were purchased from Calbiochem. U2 RNase was a gift of Dr. H. Okazaki of the Sankyo Co., Tokyo. PEI (polyethyleneiminecellulose) plates (Polygram Cell 300 PEI) were a product of Macherey-Nagel. XR-5, NS-5T x-ray films were from Eastman Kodak; Whatman DEAE-cellulose (DE81) paper and Whatman No. 1 chromatography paper were from Fisher Scientific. Cellogel was a product of Colab Inc. Pure *S. cerevisiae* (baker's yeast) phenylalanyl-tRNA synthetase was a kind gift of Dr. S. Hecht.

Preparation of tRNA. Unlabeled $tRNA^{Phe}$ from *S. pombe* (grown in 0.05% yeast extract (Difco)–3% glucose medium to late logarithmic phase) was purified by chromatography on benzoylated DEAE-cellulose, followed by tRNA-anticodon affinity chromatography (Grosjean et al., 1973) on polyacrylamide-bound *E. coli* $tRNA^{Glu}$ to a specific activity of 1550 pmol/A_{260} unit. Uniformly ^{32}P-labeled tRNA was prepared from *S. pombe* grown on EMM1 medium (Mitchison, 1970). tRNA-anticodon affinity chromatography of this tRNA followed by two-dimensional polyacrylamide gel electrophoresis (Ohashi et al., 1976) resulted in pure [^{32}P]$tRNA^{Phe}$. Figure 1 shows a representative autoradiogram of the tRNA species after separation by two-dimensional gel electrophoresis.

S. cerevisiae (brewer's yeast) $tRNA^{Phe}$ was prepared by chromatography on benzoylated DEAE-cellulose (Gillam et al., 1967) and Sepharose 4B (Holmes et al., 1975) to a purity of 1400 pmol/A_{260} unit.

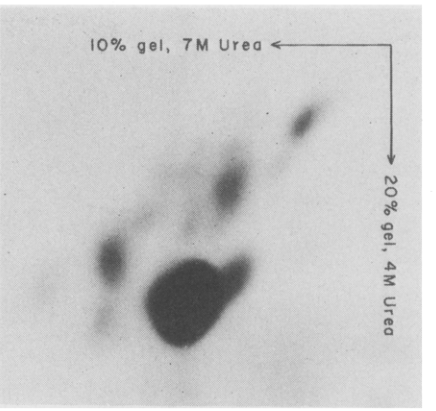

FIGURE 1: Autoradiogram of [^{32}P]tRNA species (from the anticodon affinity column) separated by two-dimensional polyacrylamide gel electrophoresis.

Sequencing Techniques. Standard procedures (Barrell, 1971) were used for enzyme degradation and fingerprinting of [^{32}P]tRNA and also for the identification of the oligonucleotides resulting from the primary enzymatic digestion. Larger oligonucleotide fragments derived by chemical cleavage at m^7G (Simsek et al., 1973) or D (Beltchev & Grunberg-Manago, 1970) were separated by polyacrylamide gel electrophoresis on 15% gels containing 6 M urea (Simsek et al., 1973). The reaction conditions for the cleavage reactions of small amounts (<0.5/A_{260} unit) of radioactive tRNA or of oligonucleotides were as follows.

Y-Base Split. The radioactive RNA was dissolved in 0.05 M sodium citrate, pH 2.9 (6 μL), and incubated for 3 h at 37 °C. After addition of 1 M aniline hydrochloride, pH 5.0 (3 μL), the incubation was continued for 4 h. Then the RNA was precipitated with ethanol and used for further analysis.

m^7G Split. The radioactive RNA was incubated at room temperature for 15 min in 0.05 M sodium hydroxide and 0.1 mM EDTA. Then 0.17 M acetic acid (5 μL) and 1 M aniline hydrochloride, pH 5.0 (7 μL), were added and the reaction was allowed to continue for 4 h at 37 °C. The RNA was ethanol precipitated before further analysis.

D Split. The radioactive RNA was taken up in a solution of 0.001 M lead acetate–0.1 M Tris-Cl (pH 7.2)–2 M sodium chloride (previously boiled to solubilize all components) and incubated for 4 h at 37 °C. The RNA was precipitated before analysis.

Sequencing of oligonucleotides resulting from a complete T1 RNase digest of unlabeled *S. pombe* $tRNA^{Phe}$ was performed as described by Heckman et al. (1978). For partial digests with P1 nuclease the intact tRNA is labeled at either end and the digestion products are separated by two-dimensional homochromatography. For sequencing gels the tRNA is labeled in a similar manner (Silberklang et al., 1977). After phosphatase treatment tRNA was treated with T4 polynucleotide kinase and [γ-^{32}P]ATP to label the 5′ end (Heckman et al., 1977). tRNA-nucleotidyl transferase and [α-^{32}P]ATP were used to label the 3′ end of a tRNA preparation which had been previously subjected to gentle snake venom phosphodiesterase treatment (Heckman et al., 1977).

Characterization of Modified Nucleotides. All oligonucleotide fragments were digested exhaustively with T2 RNase. The hydrolysates were subjected to thin-layer chromatography (on cellulose) in the following two systems: (solvent I) isobutyric acid–0.5 M ammonium hydroxide (5:3, v/v); (solvent II) *tert*-butyl alcohol–concentrated hydrochloric acid–water (70:15:15). The fragments were detected by autoradiography (for ^{32}P-labeled oligonucleotides) or by UV absorbance (cold

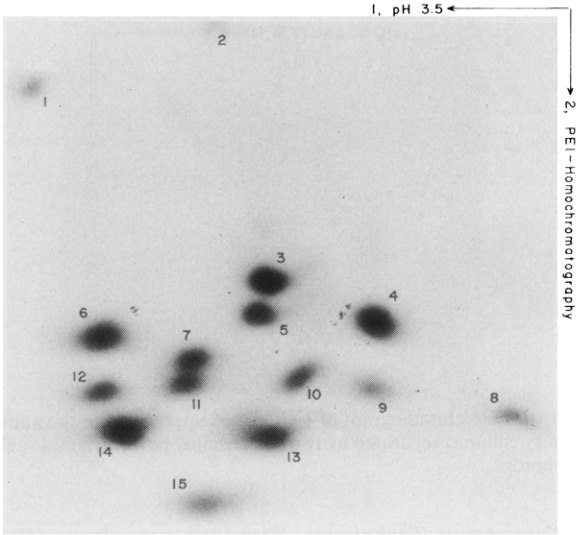

FIGURE 2: Fingerprint of complete RNase A digest of phenylalanine-tRNA.

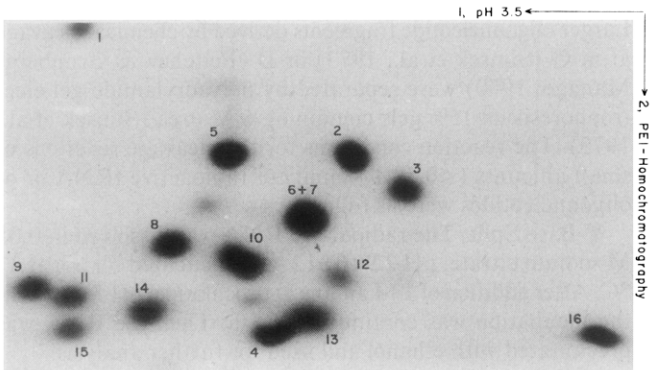

FIGURE 3: Fingerprint of complete T1 RNase digest of phenylalanine-tRNA.

oligonucleotides). In the latter case they were eluted and their spectra determined.

Aminoacylation of tRNA. The kinetics of aminoacylation were studied as follows. The reaction mixtures (0.125 mL) contained 50 mM Tris-Cl (pH 7.6), 20 mM magnesium chloride, 2.5 mM sodium ATP, 0.5 mM sodium EDTA, 50 μg/mL bovine serum albumin, 0.04 mM [^{14}C]phenylalanine (485 Ci/mol), 3 μg of pure yeast phenylalanyl-tRNA synthetase, and six different concentrations of *S. pombe* or *S. cerevisiae* tRNA^{Phe} ranging from 5.7×10^{-8} to 1.4×10^{-6} M. Incubation was carried out at 25 °C for 2 min. All reactions were performed in triplicate and the values averaged.

Results

Most of the nucleotide sequence determination was carried out with ^{32}P-labeled tRNA^{Phe}. However, for determining the order of a few T1 RNase oligonucleotides in the final sequence, the unlabeled tRNA^{Phe} was also sequenced. Since its sequence was the same as that of [^{32}P]tRNA^{Phe} as determined by comparison of T1 RNase fingerprints, we shall present only the relevant evidence.

Pancreatic RNase Digestion Products. The ^{32}P-labeled tRNA^{Phe} was digested with pancreatic RNase and the fragments separated by electrophoresis and homochromatography on polyethyleneimine plates. Figure 2 shows the resulting fingerprint. Fifteen fragments were found. The experimentally determined molar yields for each fragment are shown in Table I. Analysis of the pancreatic RNase end products and their

TABLE I: Pancreatic RNase End Products.

Fragment no.	Sequence	Molar yields: Measured	Molar yields: From final sequence
p1	pG-U-	0.6	1
p2	G-G-G-A-G-C-	0.5	1
p3	A-G-A-Cm-U-	1.1	1
p4	G-A-C- + G*-A-C-	2.0	2
p5	Gm-A-A-Y-A-ψ-	0.6	1
p6	G-G-T- + G-G-U-	1.9	2
p7	G-A-U-	1.0	1
p8	A-C-	1.0	1
p9	G-C-	0.9	1
p10	A-A-U-	0.9	1
p11	A-G-D-	1.1	1
p12	G-m^{7}G-U*- + G**-G*-U-	1.1	2
p13	A-U- + A-ψ-	2.6	2
p14	G-U- + G-ψ- (2:1)	3.1	3
p15	U- + ψ- + D-	4.2	6
p16	C- + m^{5}C-	*a*	9

[a] Fragment p16 ran off the homochromatography plate.

deduced sequences are shown in Table II. With the exception of fragments p2 and p5, all oligonucleotide sequences could be elucidated by the combined results of T2 and T1 RNase digestions. For these two fragments additional enzymatic analyses are described in Table V.

Sequence of Fragment p2. The results in Table V show that A-G-C- is a product in a partial spleen exonuclease digestion of the oligonucleotide; thus its sequence is G-G-G-A-G-C-.

Sequence of Fragment p5. U2 RNase analysis of fragment t5 (Table V) yielded Cm-U-Gm-A-. Therefore Gm-A- must be the 5′ end of oligonucleotide p5. Treatment of p5 with acid leads to cleavage at Y (Thiebe & Zachau, 1968) and resulted in the fragments Gm-A-A- and pA-ψ. Thus the sequence is Gm-A-A-Y-A-ψ.

T1 RNase Digestion Products. Digestion of *S. pombe* tRNA^{Phe} with T1 RNase gave the standard two-dimensional fingerprint shown in Figure 3. Sixteen fragments were found. The experimentally determined molar yields of each fragment are shown in Table III.

Analysis of the T1 RNase end products and their sequences are shown in Table IV. Only eight of the sixteen fragments could be unambiguously sequenced by the combined results of pancreatic and T2 RNase digestions.

Sequence of Fragment t2. U2 RNase treatment (Table V) identified Ap as 5′-terminal nucleotide. Thus the sequence is A-U-C-C-G-.

Sequence of Fragment t4. Since C-A- is a product of the U2 RNase digest (Table V) of this fragment, its sequence is C-A-A-U-G**-G*-.

Sequence of Fragment t5. Since U-G- and C- were obtained by treatment of t5 with water soluble carbodiimide followed by pancreatic RNase (Table V), the sequence of the U2 RNase fragment must be ψ-C-U-G-. The other U2 RNase fragments are Cm-U-Gm-A- and Y-A-. Since pancreatic RNase treatment of fragment t5 gives A-Cm-U- and Gm-A-A-Y-A-ψ- (confirmed by analysis of fragment p5 above), the sequence must be A-Cm-U-Gm-A-A-Y-A-ψ-C-U-G-.

Sequence of Fragment t6. The results of U2 RNase digest (Table V) confirm the sequence C-A-ψ-G* + C-A-ψ-G-.

Sequence of Fragment t7. Treatment of this oligonucleotide with water soluble carbodiimide followed by pancreatic RNase gave m^{7}G-U*-C-, A-U-C-, and G-. Combined with the results

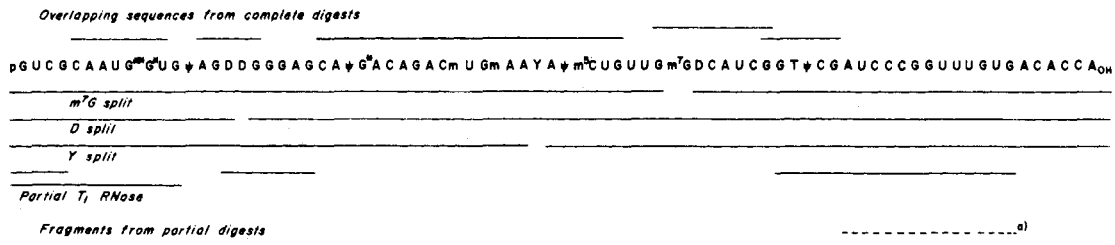

FIGURE 4: Summary of overlapping fragments. (a) The T1 RNase fragments in the indicated region could not be ordered by the data from partial digest of [^{32}P]tRNA^{Phe}.

TABLE II: Analysis of Pancreatic RNase End Products.

Fragment no.	T2 RNase products A-	C-	G-	U-	Other	T1 RNase products	Conclusion
p1				1	pG-(0.9)	pG-(1.0), U-(1)	pG-U-
p2	0.8	1	3.9			A-G-(1.0), G-(3.0), C-(1)	G-G-G-A-G-C-[a]
p3						A-G-(0.7), A-Cm-U-(1)	A-G-A-Cm-U-
p4	1.2	1	1.0		G*-(0.3)	A-C-(1), G- + G*-(1.3)	G-A-C- + G*-A-C-
p5	1.9				Y-(1.0) Gm-A-(1.0) ψ (1)	Gm-A-A-Y-A-ψ-	Gm-A-A-Y-A-ψ-[a]
p6			4.3	1	T-(0.7)	G-(2.1), T- + U- (1)	G-G-T- + G-G-U-
p7	1.0		1.0	1		G-(1.0), A-U-(1)	G-A-U-
p8	1.2	1				A-C-	A-C-
p9		1	0.9			G-(1.0), C-(1)	G-C-
p10	2.0			1		A-A-U-	A-A-U-
p11	1.0		1.1		U*-(1)	A-G-(1.0), D-(1)	A-G-D-
p12			0.5	2	m^{7}G- + G*- + G**-(2.5)	m^{7}G-U-(1), G**-G*-(0.8), G-(1.3), U-(1)	G-m^{7}G-U- + G**-G*-U-
p13	2.0			1	ψ-(1)	A-U- + A-ψ-	A-U- + A-ψ-
p14			1.4	1	ψ-(0.5)	G-U- + G-ψ-	G-U- + G-ψ-
p15					ψ-,D-		U- + ψ- + D-

[a] Sequences determined from data in Table V.

TABLE III: T1 RNase End Products.

Fragment no.	Sequence	Molar yields Measured	From final sequence
t1	pG-	1.0	1
t2	A-U-C-C-C-G-	1.0	1
t3	A-C-A-G-	0.8	1
t4	C-A-A-U-G**-G*-	0.8	1
t5	A-Cm-U-Gm-A-A-Y-A-ψ-C-U-G-	0.5	1
t6	C-A-ψ-G + C-A-ψ-G*	1.2	1
t7	m^{7}G-U*-C-A-U-C-G-	0.6	1
t8	T-ψ-C-G-	1.2	1
t9	U-U-U-G-	0.7	1
t10	ψ-A-G- + U-C-G-	2.2	2
t11	U-U-G-	1.0	1
t12	A-G-	0.9	1
t13	G-	5.1	4
t14	U-G-	2.2	2
t15	D-D-G-	1.0	1
t16	A-C-A-C-C-A$_{OH}$	0.7	1

of the U2 and pancreatic RNase digestions the sequence of this fragment must be m^{7}G-U*-C-A-U-C-G-.

Sequence of Fragment t8. Partial venom diesterase digest of ^{32}P-5′-end-labeled fragment (from kinase sequencing) showed -C-G- as the 3′ terminus (data not shown). Thus the sequence is T-ψ-C-G-.

Sequence of Fragment t10. We could conclude that t10 was a mixture of ψ-A-G- and U-C-G- since fragments U-C- and G- and ψ-A-G- were found after carbodiimide/pancreatic RNase treatment.

Large Overlapping Fragments and Determination of Total Sequence. Large oligonucleotide fragments of [^{32}P]tRNA^{Phe} were obtained by chemical cleavage at D, chemical cleavage at Y, chemical cleavage at m^{7}G, and by partial digestion with T1 RNase. The products were fractionated by two-dimensional polyacrylamide gel electrophoresis. The oligonucleotides were eluted from the gel; one-half of every product was digested completely with T1 RNase and the other half with pancreatic RNase. The resulting products were fractionated by homochromatography and identified by base analysis and further enzymatic digestion. The results are summarized in Figure 4. The order of four T1 RNase end products (see dashed line in Figure 4) could not be unambiguously determined from the data at hand. Therefore we incorporated [α-^{32}P]ATP into the 3′-terminal position of unlabeled tRNA^{Phe} by reaction with tRNA nucleotidyl transferase (Heckman et al., 1977). Two-dimensional electrophoresis/homochromatography of partial P1 RNase digestion products of this labeled tRNA permitted us to obtain the sequence of the 14 nucleotides at the 3′ end (Figure 5). These results were confirmed by analysis on rapid sequencing gels of 5′-labeled tRNA partially digested with T1, T2, and U2 RNases (data not shown). This was sufficient for deducing the total nucleotide sequence, which is presented in Figure 6 in the familiar cloverleaf form.

Characterization of Modified Nucleotides. The modified nucleotides ψp, Cmp, Gmp, m^{5}Cp, m^{7}Gp, Dp, and Tp were found in the tRNA and identified by their chromatographic properties on cellulose thin-layer plates. The nucleotide in position 47 is a modified uridine as judged by its electrophoretic mobility; some Dp is also found in this position. Positions 10 and 26 are occupied by G*p, an unidentified G derivative with chromatographic properties like m^{2}Gp. Position 9 contains a

TABLE IV: Analysis of T1 RNase End Products.

Fragment no.	T2 RNase products A-	C-	G-	U-	Other	Pancreatic RNase products	Conclusions
t1					pG-	pG-	pG-
t2	0.8	3.0	1	0.9		A-U-(1.1), C-(3.5), G-(1)	A-U-C-C-C-G-
t3	2.1	1.2	1			A-C-(1.0), A-G-(1)	A-C-A-G-
t4	2.2	1.1	0.8		G**(0.8), G*(1)	A-A-U-(1),G**-G*- + G- + C-(1)	C-A-A-U-G**-G*-[a]
t5	2.5	0.9	1	0.8	CmU-(1.0), GmA-(0.7), ψ-(0.8), Y-(0.5)	A-Cm-U-(1.1), Gm-A-A-Y-A-ψ-(1.0), C-(1.0), U-(0.7), G-(1)	A-Cm-U-Gm-A-A-Y-A-ψ-C-U-G-[a]
t6	1.1	1.0	0.7		ψ(1.0), G*(0.3)	A-ψ-(0.8), C(1.3), G + G*(1)	C-A-ψ-G-[a] + C-A-ψ-G*-[a]
t7						m^7G-U*-(0.7), A-U-(1.0), C-(0.9), G-(1)	m^7G-U*-C-A-U-C-G-[a]
t8		1.0	1		ψ-(1.0) T-(0.9)	G-(1), C-(1.0), T- + ψ-(1.7)	T-ψ-C-G-[a]
t9			1	2.9		U-(2.8), G-(1)	U-U-U-G-
t10	1.0	1.3	2	0.9	ψ-(1.1)	A-G-(1), G-(1), C-(1.0) U- + ψ-(1.9)	ψ-A-G-[a] + U-C-G-[a]
t11			1	1.8		U-(2.0), G-(1)	U-U-G-
t12	1.0		1			A-G-	A-G-
t13			+				G-
t14			1	1.0		U-(1.0), G-(1)	U-G-
t15			1		D-(1.9)		D-D-G-
t16	1.9	3				A-C-(2.1), C-(1)	A-C-A-C-C-A_{OH}[a]

[a] Sequence determined from data in Table V and as described in text.

TABLE V: Further Analysis of Some Pancreatic or T1 RNase End Products.

Treatment	Fragment no.	Products
U2 RNase	t2	A-, U-C-C-G-
	t4	C-A-, A-, U-G**-G*-
	t5	Cm-U-Gm-A-, Y-A-, ψ-C-U-G-, A-
	t6	C-A-, ψ-G- + ψ-G*-
	t7	m^7G-U*-C-A- + U-C-G-
	t16	A-, C-A-, C-C-A_{OH}
Water soluble carbodiimide followed by pancreatic RNase[a]	t10	ψ-A-G-, U-C-, G-
	t7	m^7G-U*-C-, A-U-C-, G-
	t5	U-G-
Partial digestion with spleen exonuclease	p2	A-G-C-
Y split	p5	Gm-A-A- + pA-ψ-

[a] Only products relevant for sequence determination are listed.

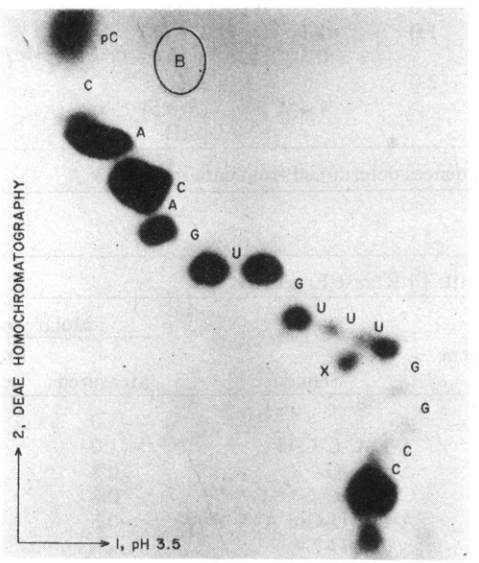

FIGURE 5: Autoradiogram of partial digestion of 3′-^{32}P-labeled *S. pombe* $tRNA^{Phe}$ with nuclease P1. For details see Materials and Methods. B denotes the position of the xylene cyanol FF. Fragment X is probably a cleavage product (having a 3′-phosphate) by a contaminant endonuclease; it appeared in widely varying amounts in different digestions.

different unidentified G derivative, G**p.

Aminoacylation of S. pombe and S. cerevisiae $tRNA^{Phe}$ with Pure S. cerevisiae Phenylalanyl-tRNA Synthetase. Since the *S. pombe* $tRNA^{Phe}$ was so different from that of *S. cerevisiae* and did not contain some of the sequences of the proposed recognition site (Roe et al., 1973) of yeast phenylalanyl-tRNA synthetase, we studied the aminoacylation of the two tRNAs by this enzyme. The kinetic parameters of the aminoacylation reaction by pure *S. cerevisiae* phenylalanyl-tRNA synthetase were determined by conventional Lineweaver–Burke plots. Least-squares analysis of data points for both tRNAs yielded correlation coefficients of 0.99. The K_m values of both tRNAs are the same (3.9×10^{-7} M); the V_{max} value of the *S. pombe* tRNA was slightly higher (125 pmol min^{-1} mg^{-1}) than that of the homologous tRNA (100 pmol min^{-1} mg^{-1}). In an additional test (data not shown) we ascertained that the enzyme was able to misacylate *E. coli* $tRNA_1^{Val}$ with phenylalanine; this indicated that our enzyme had the misacylation characteristics previously described (Roe et al., 1973).

Discussion

The nucleotide sequence of *S. pombe* $tRNA^{Phe}$ is quite different from that of *S. cerevisiae*. We did not expect this for two yeast species since there is very great sequence conservation of tRNA species (e.g., $tRNA^{Phe}$) among different mammalian sources and tissues (Sprinzl, 1978).

The major finding of this paper is the demonstration that yeast phenylalanyl-tRNA synthetase efficiently charges $tRNA^{Phe}$ from a different yeast species, although this tRNA lacks parts of the nucleotide sequences of the proposed "recognition" site, which have been observed so far in all tRNAs found to be substrates for this enzyme (Figure 7A). Roe et al.

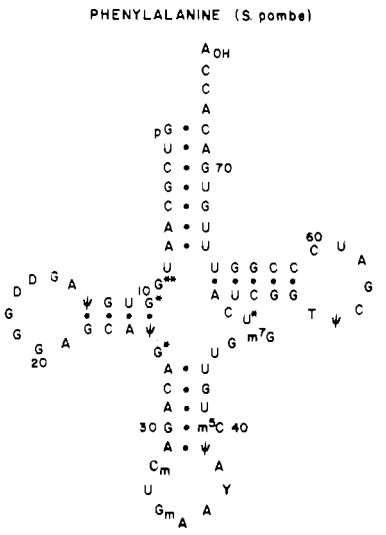

FIGURE 6: Cloverleaf model of *S. pombe* $tRNA^{Phe}$.

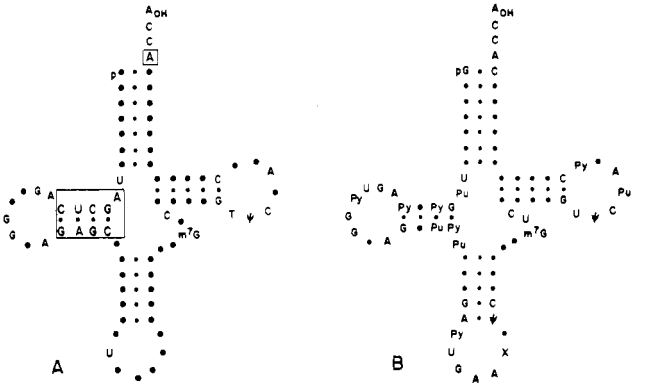

FIGURE 7: (A) Nucleotides proposed by Roe et al. (1973) to be involved in *S. cerevisae* phenylalanyl-tRNA synthetase recognition. (B) Composite of the nucleotide sequences of $tRNA^{Phe}$ from *S. pombe, E. coli, S. cerevisiae,* wheat germ, pea, calf liver, rabbit liver, *B. stearothermophilus, Mycoplasma,* human placenta, *Torulopsis utilis, Euglena gracilis* chloroplast, *Phaseolus vulgaris* chloroplast, and *B. subtilis* (see Sprinzl, 1978). The unmodified parent nucleotide is indicated if the various tRNAs contain different modifications of the parent nucleotide. X denotes a purine or modified purine.

(1973) examined the kinetic differences of these tRNAs in the aminoacylation reaction. The K_m values differed only by a factor of 10 when the 11 tRNA species were compared, while the V_{max} values differed by a factor of 170, being much lower in the mischarging reactions. The *S. pombe* $tRNA^{Phe}$ is aminoacylated with a slightly higher V_{max} than the homologous tRNA and, as such, belongs to the "fast" class (Roe et al., 1973). It shares several features with other fast class tRNAs: the size of the D loop (8 nucleotides) and the presence of a modified guanosine in position 10. In addition, the *S. pombe* $tRNA^{Phe}$ contains three other nucleotides, G_{15}, m^7G_{46} and C_{48}, common to the "fast" and "intermediate" class of tRNAs aminoacylated with yeast phenylalanyl-tRNA synthetase (Roe et al., 1973). These nucleotides are involved in base pairing in the tertiary structure of yeast $tRNA^{Phe}$ as shown by x-ray crystallography (Kim, 1976). The relevance of the tertiary structure (as determined by x-ray crystallography) to the aminoacylation reaction is not clear, since examination of the sequence of *S. pombe* $tRNA^{Phe}$ shows some of the tertiary interactions in this molecule to be different from those in *S. cerevisiae* $tRNA^{Phe}$ (Kim, 1976).

Incorporating this new sequence information into the formalistic concept of Dudock (Roe et al., 1973), the nucleotide sequences shown in Figure 7B are the ones recognized by yeast phenylalanyl-tRNA synthetase. However, the accumulated data (e.g., Feldmann & Zachau, 1977) make it clear that such a concept, based on the consideration of similarities in the two-dimensional cloverleaf structure, cannot be correct. Among the many known tRNA sequences there are examples of greater sequence homology between tRNAs specific for different amino acids than between isoacceptor RNA species, e.g., *E. coli* $tRNA^{Met}$ and $tRNA^{Val}$ (Sprinzl, 1978). It is clear that the correct tertiary structure plays an important role in the aminoacylation process. A point in support of this notion is the fact that 3′-terminal half molecule fragments of tRNA can be aminoacylated somewhat specifically when they are brought into the proper conformation (Wübbeler et al., 1975). Slight disturbances of the native tertiary structure of tRNA (e.g., caused by organic solvents in the reaction medium) do not abolish charging, but lead to a relaxation of specificity. Thus, yeast phenylalanyl-tRNA synthetase aminoacylates practically all *E. coli* tRNA species under these conditions (Kern et al., 1972). Based on the available evidence a general proposal of how tRNA is recognized by the cognate aminoacyl-tRNA synthetase was recently put forward (Rich & Schimmel, 1977). Briefly, this model proposes large domains of interaction between the enzyme and the tRNA, involving its acceptor stem, its D stem and its anticodon. Within these regions of interaction it may be very difficult to distinguish clearly between specific recognition sites and contact sites of lesser specificity. The observed differences in V_{max} in the misacylations may be due to small changes in a few specific interactions, possibly the result of a single base change. Thus, consideration of cloverleaf structures alone in deriving models of tRNA recognition is not enough. It is disappointing to see that after so many studies aimed at a solution of the recognition problem (Rich & Schimmel, 1977; Schimmel, 1977) the final answer is still elusive.

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Mitochondrial Transfer RNAs in Yeast: Identification of Isoaccepting Transfer RNAs†

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ABSTRACT: To delineate the total number of tRNAs encoded by yeast mitochondrial DNA (mtDNA), we have examined mitochondrial tRNA preparations for the presence of heterogenic isoaccepting tRNAs. Analyses of ^{3}H-labeled aminoacylated mitochondrial tRNAs by reversed-phase column chromatography (RPC-5) coupled with hybridization to mtDNA detected only one major mitochondrially coded tRNA for alanine, arginine, aspartic acid, glycine, histidine, isoleucine, leucine, lysine, proline, serine, and tryptophan. Some of these profiles also contained one or more minor peaks that may represent small amounts of heterogenic isoacceptors, but their low concentrations prevented their characterization. Cysteinyl-, methionyl-, phenylalanyl-, threonyl-, tyrosyl-, and valyl-tRNAs separated into multiple species upon RPC-5 chromatography. Two cysteinyl-, two methionyl-, two phenylalanyl-, two threonyl-, four tyrosyl-, and two valyl-tRNA species hybridized to mtDNA. The hybridization of the phenylalanyl- and the valyl-tRNA species was not additive, indicating that their sequences are similar, if not identical, and suggesting that they may be transcribed from the same genes. The two methionyl-, and two threonyl-, and probably the two cysteinyl-tRNAs are transcribed from separate genes, since their hybridizations to mtDNA are additive. That the methionyl-tRNAs are transcribed from separate genes was further confirmed through deletion mapping experiments which showed that the genes coding for these tRNAs are at different locations on the mtDNA. The transcriptional relationship of the four tyrosyl-tRNAs was not established. There is at least a cistron coding for tRNAs corresponding to each of the common amino acids except asparagine and several amino acids (methionine, threonine, and cysteine) are accepted by more than one transcriptionally distinct tRNA. Thus, a minimum of 22 tRNA cistrons have been identified.

The mitochondrion contains its own protein-synthetic system, which is distinct from that found in the cytoplasm and presumably serves to translate mitochondrially coded components of the respiratory system (for review, see Locker & Rabinowitz, 1978). The mitochondrial protein-synthetic system is of dual origin, since most, if not all, of the ribosomal proteins are encoded by nuclear genes whereas rRNA is specified by the organelle genome. Although it has been established that mtDNA codes for some tRNAs, it is not yet known whether the mitochondrial protein-synthetic system is autonomous with respect to its tRNA complement or whether nuclear-coded tRNAs must be imported to supply a complete set of tRNAs for organelle protein synthesis, as has been suggested to be the case for *Tetrahymena* by Chiu et al. (1975).

Estimates of the number of tRNA cistrons in mtDNA have varied with experimental procedure. Early estimates based on saturation-hybridization experiments with bulk labeled 4S RNA indicated the presence of 12–15 tRNA cistrons in HeLa cells and *Xenopus* (Aloni & Attardi, 1971; Dawid & Chase, 1972) to 20–25 in yeast (Reijnders & Borst, 1972; Schneller et al., 1975a,b). Though these estimates raised the possibility that mtDNA may not code for tRNAs for all the 20 common amino acids, more recent experiments using hybridization of [^{3}H]aminoacyl-tRNAs have demonstrated the presence of mt-coded tRNAs in HeLa mitochondria corresponding to 16 amino acids (Lynch & Attardi, 1976) and in yeast corresponding to 19 amino acids (Martin et al., 1977). Since the

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