The nucleotide sequence of the major glutamate transfer RNA from Schizosaccharomyces pombe

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ABSTRACT

The nucleotide sequence of glutamate $tRNA_1$ from *Schizosaccharomyces pombe* was determined to be pU-C-C-G-U-U-G-U-m^1G-G-U-C-C-A-A-C-G-G-C-D-A-G-G-A-U-U-C-G-U-C-G-C-U-U*-U-C-A-C-C-G-A-C-G-G-G-A-G-m^5C-G-G-G-T-Ψ-C-G-A-C-U-C-C-C-G-C-A-A-C-G-G-A-G-C-C-A_{OH}. The sequence differs markedly from that of *S. cerevisiae* $tRNA^{Glu}$. *S. pombe* glutamate $tRNA_1$ can be aminoacylated by the homologous glutaminyl-tRNA synthetase as well as by the corresponding enzyme from *S. cerevisiae*.

INTRODUCTION

Transfer RNA biosynthesis is a complex process involving the transcription of tRNA genes into precursor RNA molecules which are subsequently modified to form minor nucleotides at specific sites and from which excess nucleotides are trimmed off at both ends to produce mature-sized tRNA (1). The recent finding of intervening sequences in yeast tRNA genes absent in the corresponding mature tRNA (2,3) added yet another facet to the intricate series of enzymatic events (4,5) culminating in the formation of mature tRNA.

A current research interest in this laboratory is the transcription and processing of yeast tRNA genes (6). The sequence analysis of the mature tRNA species is a prerequisite to the investigation of the related precursor tRNA molecules. The knowledge of additional tRNA sequences of *S. pombe* may shed more light on the considerable sequence diversity of its tRNAs with those from *Saccharomyces cerevisiae*. Therefore we undertook to purify the major $tRNA^{Glu}$ and to determine its nucleotide sequence.

MATERIALS AND METHODS

General. Most of the materials, chemicals and enzymes, and methods used

in this work were described previously (7,8).

Preparation of tRNA. Uniformly labeled (^{32}P)tRNA was extracted from *S. pombe* grown in the low phosphate medium EMM1 (9) containing carrier-free (^{32}P)orthophosphate. The labeled unfractionated tRNA obtained was subjected to tRNA-anticodon affinity chromatography (10); $tRNA^{Phe}$, which has an anticodon complementary to that of $tRNA^{Glu}$, was affixed to the column support. Passage of the labeled unfractionated tRNA through such a column caused the (^{32}P)$tRNA^{Glu}$ to be retained, which was subsequently eluted. The eluted tRNA was further purified by two-dimensional polyacrylamide gel electrophoresis (11). Two $tRNA^{Glu}$ isoacceptors were obtained by this procedure; Figure 1 shows a representative autoradiogram of the tRNA species after separation by two-dimensional gel electrophoresis. The nucleotide sequence of the faster-moving of the two isoacceptors ($tRNA_1^{Glu}$ in Figure 1) is presented in this paper.

The corresponding unlabeled isoacceptor was obtained from *S. pombe* grown in a 0.5% yeast extract-3% glucose medium to late logarithmic phase and purified analogous to the procedures described earlier (12) by chromatography on benzoylated DEAE cellulose, Sepharose 4B, and RPC-5, followed by two-dimensional polyacrylamide gel electrophoresis. In contrast to the affinity column chromatography used for separating the radioactive tRNA, the successive chromatographic procedures used to purify the non-radioactive tRNA led only to $tRNA_1^{Glu}$, the major isoacceptor. This tRNA could be charged to 1500 pmoles/A_{260} unit at the end of the RPC-5 separation and to 1300 pmoles/A_{260} unit at the end of the two-dimensional electrophoresis. The gel-purified $tRNA_1^{Glu}$ was found to be free of contaminants on fingerprinting; the de-

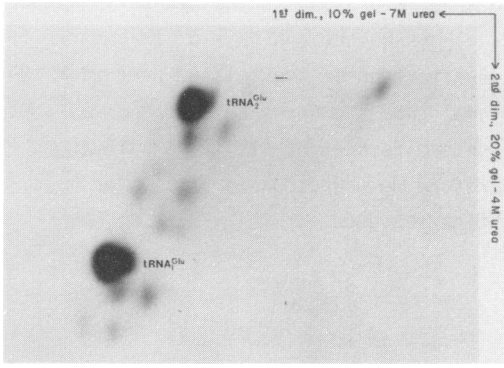


Figure 1.
Autoradiogram of (^{32}P)tRNA species (from the anticodon affinity column) separated by two-dimensional polyacrylamide gel electrophoresis.

crease in its charging capacity is probably related to changes in tRNA conformation as a result of electrophoresis in a urea gel.

Sequence Analysis of Glutamate tRNA. The nucleotide sequence was elucidated by using both uniformly *in vivo* labeled radioactive tRNA and non-radioactive tRNA post-labeled *in vitro* (7,8).

Complete RNase A or RNase T1 digests of *in vivo* labeled $tRNA_1^{Glu}$ were analyzed by standard methods (13). The sequences of the longer and more complicated oligonucleotides were confirmed by using non-radioactive $tRNA_1^{Glu}$. The latter was sequenced by the procedures described (7,8).

Modified Nucleosides. They were identified in RNase T2 hydrolysates of oligonucleotides derived from uniformly labeled tRNA after two-dimensional chromatography (11). In addition, unlabeled $tRNA_1^{Glu}$ was digested to nucleosides by P1 RNase and alkaline phosphatase digestion. The resulting mixtures were analyzed by HPLC chromatography (14).

Aminoacylation of *S. pombe* $tRNA_1^{Glu}$. Crude aminoacyl-tRNA synthetase preparations were obtained from *S. pombe* as well as *S. cerevisiae* by homogenizing the cells in 0.15 M potassium phosphate (pH 7.5) - 0.01 M $MgCl_2$ - 0.02 M 2-mercaptoethanol - 10% glycerol (v/v), followed by successive centrifugations at 30,000 g and 100,000 g. The final supernatant was diluted to contain 0.075 M potassium phosphate (pH 7.5) and passed through a DEAE cellulose column to remove endogenous tRNA; the proteins were then eluted with 0.1 - 0.3 M potassium phosphate. The active fractions were dialyzed against 0.01 M Tris-HCl (pH 7.5) - 0.01 M $MgCl_2$ - 0.02 M 2-mercaptoethanol - 50% glycerol (v/v), and used in studying the aminoacylation kinetics of *S. pombe* $tRNA^{Glu}$ as follows. The reaction mixtures (0.1 ml) contained 50 mM potassium cacodylate (pH 7.5), 10 mM $MgCl_2$ - 60 mM KCl, 2 mM ATP, 2 mM CTP, 0.1 mM (^{14}C)glutamate (296 mCi/mmole), 33 µg of crude *S. pombe* or *S. cerevisiae* synthetase proteins, and 5 different concentrations of purified *S. pombe* $tRNA^{Glu}$ ranging from 2×10^{-7} M to 1.5×10^{-6} M. Incubations were carried out at 25°C. Aliquots (20 µl) of the reaction mixture were withdrawn for determination of the extent of aminoacylation at 5 different time intervals ranging from 1 to 20 minutes. From the V_0 (initial velocity) of aminoacylation obtained at the various tRNA concentrations, the K_m was calculated using the Eadie-Hofstee plot.

RESULTS

The nucleotide sequence determination of the oligonucleotides from

complete T1 or pancreatic RNase digests was performed with uniformly labeled (^{32}P)tRNA^{Glu} and the results confirmed by fingerprints of post-labeled non-radioactive tRNA fragments. Overlapping of the RNase T1 fragments was carried out by sequencing gel or partial P1 nuclease digestion analysis of 5'-end or 3'-end labeled intact tRNA.

Pancreatic RNase Digestion Products. Uniformly labeled (^{32}P)tRNA^{Glu} was digested with pancreatic RNase, and the products were separated by electrophoresis on cellulose acetate at pH 3.5 in the first dimension and homochromatography on PEI plates in the second dimension. Figure 2 shows the resulting fingerprint. Fifteen oligonucleotide fragments were found; their molar yields were as indicated in Table I. Analysis of these pan-

TABLE I

Pancreatic RNase End Products

Fragment Number	Sequence	Molar Yields	
		Measured	From Final Sequence
p1	G-G-G-G-T	0.2	1
p2	G-G-G-A-G-C-	0.6	1
p3	G-G-A-G-C-	0.6	1
p4	A-G-G-A-U-	0.6	1
p5	pU-	1.0	1
p6	G-G-C-	1.1	1
p7	G*-G-U-	1.1	1
p8	G-A-C-	2.1	2
p9	U*-U-	1.5	1
p10	A-A-C-	2.1	2
p11	G-C-	3.3	2
p12	G-U-	3.9	3
p13	A-C-	0.9	1
p14	U- + D- + Ψ-	6.0	6
p15	C-	11.0	14
p16	A_{OH}		1

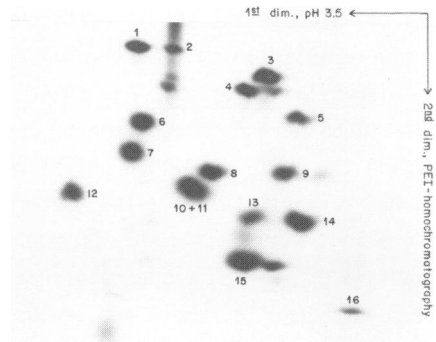


Figure 2

Fingerprint of a complete RNase A digest of uniformly labeled (^{32}P)tRNA^{Glu}.

creatic RNase fragments by further digestion with T1 RNase and exhaustive digestion with T2 RNase, and the deduction of their nucleotide sequence are shown in Table II. Most of the fragments could be characterized by the combined results of T1 RNase and T2 RNase digestions, with the exception of fragments p2, p3, p4, and p7. For these four oligonucleotides, the following additional analyses were performed.

Sequences of Fragments p2, p3, and p4. Each of these fragments was analyzed by partial digestion of the uniformly labeled (^{32}P)oligonucleotide with spleen phosphodiesterase and subsequent two-dimensional homochromatog-

raphy on DEAE-cellulose thin layer plates. The sequence was deduced by the characteristic mobility shifts generated by successive removals of individual nucleotides from the particular oligonucleotide fragment. In each case, the sequence assignment was confirmed using pancreatic digests of the non-radioactive tRNA, by partial snake venom phosphodiesterase digestion of the corresponding fragment which had been post-labeled at the 5'-end with ^{32}P.

Sequence of Fragment p7. Digestion of the uniformly labeled fragment p7 with T1 RNase yielded U and a second product with the electrophoretic mobility of a dinucleotide upon electrophoresis at pH 3.5. On T2 RNase digestion, the same fragment yielded G, U, and a third nucleotide with chromatographic behavior compatible with m^1G (designated G* in Table II). When the corresponding 5'-end labeled fragment was subjected to complete P1 nuclease digestion, the resulting 5'-end nucleotide was found to be modified. Hence fragment p7 must be the G*-G-U. This sequence was confirmed by partial digestion of the 5'-end labeled fragment with snake venom phosphodiesterase followed by two-dimensional homochromatography.

TABLE II

Analysis of Pancreatic RNase End Products

Fragment Number	T2 RNase Products					T1 RNase Products	Conclusion
	C-	A-	G-	U-	Others		
p1			4.2		T-(1.0)	G-(4.4),T-(1.0)	G-G-G-G-T-
p2	1.0	1.2	3.4			C-(1.0),A-G-(1.2),G-(2.6)	G-G-G-A-G-C-[b]
p3	1.0	0.8	2.6			C-(1.0),A-G-(1.2),G-(2.3)	G-G-A-G-C-[b]
p4		2.1	1.8	1.0		A-G-(1.1),G-(1.3),A-U-(1.0)	A-G-G-A-U-[b]
p5					pU	pU-	pU-
p6	1.0		2.1			G-(2.0),C-(1.0)	G-G-C-
p7			0.9	1.0	G*-(0.8)	G*-G-(1.0),U-(1.0)	G*-G-U-[b]
p8	1.0	1.1	0.8			A-C-(1.0),G-(0.8)	G-A-C-
p9				1.0	U*-(0.8)	U*-U-	U*-U-
p10	1.0	2.1				A-A-C-	A-A-C-
p11	1.0		1.1			G-(0.9),C-(1.0)	G-C-
p12			0.6	1.0		G-(0.9),U-(1.0)	G-U-
p13	1.0	1.0				A-C-	A-C-
p14				+[a]	D-,ψ-		U- + D- + ψ-
p15	+						C-
p16							A_{OH}

[a]This material was identified as a mixture of U-,D-, and ψ- by thin layer chromatography.
[b]Sequence determined by further analyses as described in text.

T1 RNase Digestion Products. Digestion of the uniformly labeled (^{32}P)-$tRNA^{Glu}$ with T1 RNase gave the standard two-dimensional fingerprint shown in Figure 3. Sixteen fragments were found; their experimentally determined molar yields are shown in Table III. Analysis of the T1 RNase digestion pro-

ducts and their sequences are shown in Table IV. Only six fragments (t1, t9, t12, t13, t14, and t15) could be unambiguously sequenced by the combined results of additional pancreatic and T2 RNase digestions. The remaining fragments of the T1 RNase digest were further analyzed as follows.

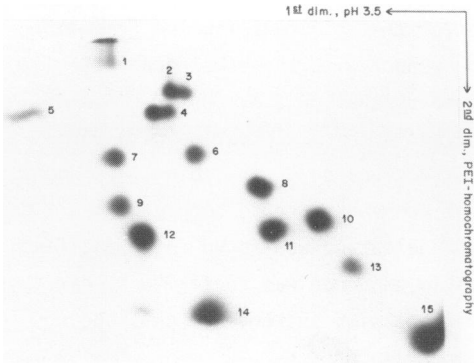


Figure 3

Fingerprint of a complete RNase T1 digest of uniformly labeled (^{32}P)tRNA^{Glu}.

TABLE III

T1 RNase End Products

Fragment Number	Sequence	Molar Yields	
		Measured	From Final Sequence
t1	pU-C-C-G-	1.0	1
t2	C-U-U$^{\Psi}$-U-C-A-C-C-G-	0.3	1
t3	A-C-U-C-C-C-C-G-	0.8	1
t4	U-C-C-A-A-C-G-	0.4	1
t5	C-A-A-C-G-	0.4	1
t6	A-U-U-C-G-	1.0	1
t7	T-ψ-C-G-	1.0	1
t8	C-D-A-G-	0.9	1
t9	A-C-G-	0.8	1
t10	U-G$^{\Psi}$-G-	1.0	1
t11	U-C-G-	1.0	1
t12	U-U-G-	1.0	1
t13	A-G-	0.9	2
t14	C-G-	2.3	1
t15	G-	6.7	8
t16	C-C-A$_{OH}$	0.7	1

TABLE IV

Analysis of T1 RNase End Products

Fragment Number	T2 RNase Products					Pancreatic RNase Products	Conclusion
	C-	A-	G-	U-	Others		
t1	2.1		1.0		pU-(1.0)	G-(1.0),C-(2.0),pU-(1.0)	pU-C-C-G-
t2	3.6	1.0	1.0	1.3	U$^{\Psi}$-(0.5)	A-C-(0.9),G-(1.0),C-(3.3), U-(1.3),U$^{\Psi}$-U-(0.6)	C-U-U$^{\Psi}$-U-C-A-C-C-G-[a]
t3	5.2	1.0	1.0	1.2		A-C-(1.1),G-(1.0),C-(4.0),U-(1.1)	A-C-U-C-C-C-C-G-[a]
t4	3.4	2.2	1.0	1.2		A-A-C-(1.2),G-(1.0),C-(2.2), U-(1.1)	U-C-C-A-A-C-G-[a]
t5	2.2	2.1	1.0			A-A-C-(1.2),G-(1.0),C-(1.0)	C-A-A-C-G-[a]
t6	1.3	1.0	1.0	2.2		A-U-(1.0),G-(1.0),C-(1.1),U-(0.9)	A-U-U-C-G-[a]
t7	1.4		1.0		T-(1.1), ψ-(1.0)	G-(1.0),C-(1.0),T-(1.0),ψ-(0.9)	T-ψ-C-G-[a]
t8	1.1	1.1	1.0		D-(0.9)	A-G-(1.0),C-(0.9),D-(0.9)	C-D-A-G-[a]
t9	1.2	1.2	1.0			A-C-(1.1),G-(1.0)	A-C-G-
t10			1.0	1.0	G$^{\Psi}$-(0.8)	G$^{\Psi}$-G-(0.9),U-(1.0)	U-G$^{\Psi}$-G-[a]
t11	1.2		1.0	1.2		G-(1.0),C-(0.9),U-(0.8)	U-C-G-[a]
t12			1.0	2.4		G-(1.0),U-(1.8)	U-U-G-
t13		1.3	1.0			A-G-	A-G-
t14	0.9		1.0			G-(1.0),C-(0.9)	C-G-
t15			+			G-	G-
t16							C-C-A$_{OH}$[a]

[a]Sequence determined by further analyses as described in text.

Sequence of Fragment t2. T2 RNase digestion of the uniformly labeled fragment t2 indicated that its composition was (C_4,A,U_2,S)G. Partial snake venom phosphodiesterase digestion of the corresponding 5'-end labeled oligonucleotide yielded the sequence C-U-U*-U-C-A-C-C-G. The autoradiogram obtained from the two-dimensional homochromatography of the partial digest (Figure 4) is of interest. As the snake venom phosphodiesterase removed consecutive nucleotides from the fragment C-U-U*-U-C-A-C-C-G beginning from the 3'-end, the successive spots on the autoradiogram representing C-U-U*-U-C-A-C-C-G, C-U-U*-U-C-A-C-C, C-U-U*-U-C-A-C, C-U-U*-U-C-A, C-U-U*-U-C, C-U-U*-U, and C-U-U* were found to be double, suggesting that each of these

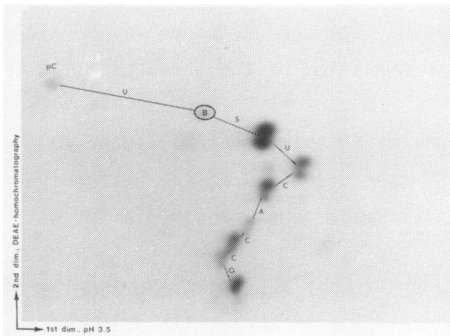


Figure 4

Autoradiogram of partial digestion of 5'-end ^{32}P-labeled fragment t2 with snake venom phosphodiesterase. B denotes the position of the xylene cyanol FF.

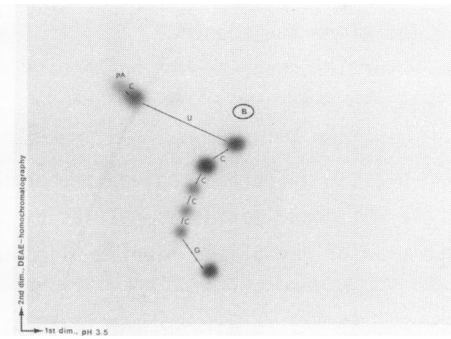


Figure 5

Autoradiogram of partial digest of 5'-end ^{32}P-labeled fragment t3 with snake venom phosphodiesterase. B denotes the position of the xylene cyanol FF.

oligonucleotides existed in two forms: containing a thiolated or a non-thiolated thiouridine derivative. Only the two uppermost spots representing C-U and C were single, as expected. The autoradiogram also indicates a difficulty of the snake venom phosphodiester to digest past the modified nucleotide U*; consistent with this interpretation, the spot representing C-U-U* was extremely dark as a result of accumulation of this partial digestion product, whereas the spots representing C-U and C were very faint by comparison.

Sequence of Fragment t3. Pancreatic RNase digestion of the uniformly labeled fragment t3 gave rise to (C_4,AC,U)G. Partial spleen phosphodiester-

ase digestion of the same fragment indicated that the first three nucleotides were A-C-U. Hence the sequence of the fragment is A-C-U-C-C-C-C-G. The same sequence was obtained by partial snake venom phosphodiesterase digestion of the 5'-end labeled oligonucleotide and two-dimensional homochromatography (Figure 5).

Sequence of Fragment t4. The sequence of this fragment was determined to be U-C-C-A-A-C-G by partial snake venom phosphodiesterase digestion of the 5'-end labeled oligonucleotide.

Sequence of Fragment t5. T2 RNase digestion of the uniformly labeled fragment t5 indicated that its composition was (C_2,A_2)G. Pancreatic RNase digestion of the same fragment gave rise to (C,A-A-C)G. Partial spleen phosphodiesterase digestion of the fragment showed the leading nucleotide to be C. Therefore the sequence of t5 is C-A-A-C-G. The same sequence was obtained by partial snake venom phosphodiesterase digestion of the 5'-end labeled oligonucleotide.

Sequence of Fragment t6. The sequence of this fragment was found to be A-U-U-C-G by partial spleen phosphodiesterase digestion of the uniformly labeled oligonucleotide as well as by partial snake venom phosphodiesterase digestion of the 5'-end labeled oligonucleotide.

Sequence of Fragment t7. T2 RNase digestion of the uniformly labeled fragment t7 and thin layer chromatography yielded the composition (T,Ψ,C)G. The nucleotide sequence was unambiguously determined by partial snake venom phosphodiesterase digestion of the 5'-end labeled fragment to be T-Ψ-C-G.

Sequence of Fragment t8. The composition of this fragment was shown by T2 RNase digestion of the uniformly labeled oligonucleotide and thin layer chromatography to be (C,A,D)G. The nucleotide sequence of C-D-A-G was established by partial snake venom phosphodiesterase digestion of the 5'-end labeled fragment.

Sequence of Fragment t10. Pancreatic RNase digestion of the uniformly labeled fragment t10 yielded U and a dinucleotide with the same electrophoretic mobility as the dinucleotide found in fragment p7. On T2 RNase digestion of fragment t10, the products were U, G, and a G* with chromatographic behavior identical to that found in fragment p7. These data plus the information on fragment p7 established the sequence of t10 as U-G*-G.

Sequence of Fragment t11. The sequence of U-C-G was established by partial spleen phosphodiesterase digestion of the uniformly labeled fragment t11 as well as by partial snake venom phosphodiesterase digestion of the corresponding 5'-end labeled oligonucleotide.

Sequence of Fragment t16. The sequence of this fragment was shown to be C-C-A_{OH} by partial snake venom phosphodiesterase digestion of the 5'-end labeled oligonucleotide.

Overlapping of Oligonucleotide Fragments and Determination of the Total Sequence. Partial P1 nuclease digestion of 5'-end and 3'-end ^{32}P-labeled intact $tRNA_1^{Glu}$ coupled with two-dimensional homochromatography was used to establish the sequence of approximately 20 nucleotides at the 5'- and 3'-terminus of the tRNA molecule. An example of the use of this procedure analyzing the 5'-terminal sequence of $tRNA^{Glu}$ is shown in Figure 6. Further overlaps were established by sequencing gels performed on 5'-end as well as 3'-end labeled $tRNA^{Glu}$ as is shown in Fig. 7. The information obtained from the partial P1 digests and a number of sequencing gels permits the oligonucleotide fragments found in the fingerprints to be ordered in a unique sequence, shown in the cloverleaf form in Figure 8.

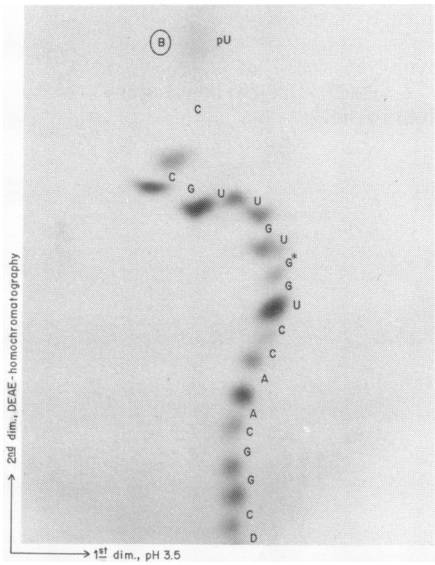


Figure 6

Autoradiogram of a P1 nuclease partial digest of 5'-end ^{32}P-labeled intact $tRNA^{Glu}$. B denotes the position of the xylene cyanol FF.

Characterization of Modified Nucleotides. T2 RNase digestion of the uniformly labeled (^{32}P)oligonucleotides obtained from fingerprinting followed by thin layer chromatography led to identification of the following modified nucleotides: 1 mole of G* (present in fragments t10 and p6); 1 mole of D (present in fragments t8 and p14); 1 mole of U* (present in fragments t2 and p9); 1 mole of T (present in fragments t7 and p1); 1 mole of Ψ (present in fragments t7 and p14).

The above results were confirmed by high-pressure liquid chromatography analysis of the nucleosides derived from non-radioactive $tRNA^{Glu}$ (14). By this procedure, the G* was identified as m^1G and U* was identified as 2-thiouridine-5-acetic acid methyl ester.

Aminoacylation of *S. pombe* $tRNA_1^{Glu}$. The purified *S. pombe* $tRNA_1^{Glu}$ could be aminoacylated by the crude synthetases from *S. pombe* as well as from *S.*

cerevisiae. The K_m for the *S. pombe* synthetase was 1.9 x 10^{-7} M and that for *S. cerevisiae* 2.0 x 10^{-7} M. The V_{max} for *S. pombe* was 0.98 pmole/min while that for *S. cerevisiae* synthetase was 0.26 pmole/min for a similar amount of crude synthetase proteins (33 µg each).

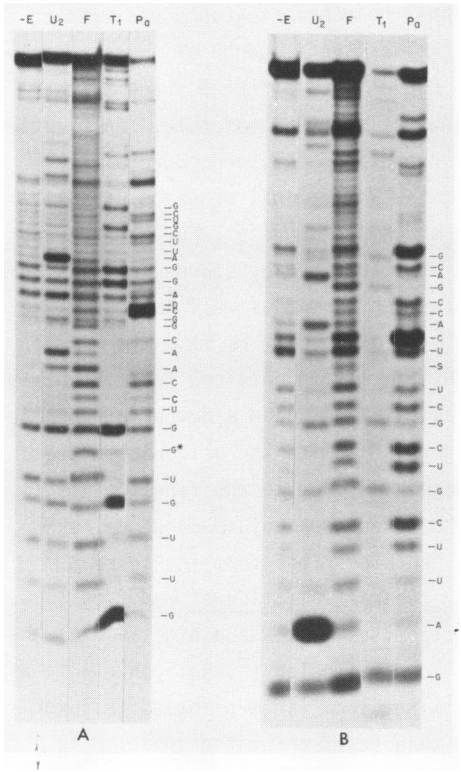
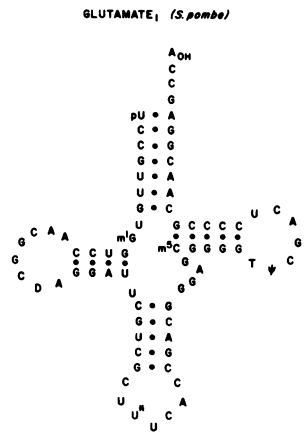


Figure 7.

Sequencing gel of 5'-end ^{32}P-labeled intact $tRNA^{Glu}$. -E, incubation without enzymes; U_2, partial digest of U2 RNase; F, partial digest of formamide ("ladder"); T1, partial digest of T1 RNase; Pa, partial digest of pancreatic RNase. Panel A is from the 5'-end of the tRNA molecule and begins with the nucleotide G at position 4. Panel B is from the region some distance away from the 5'-end of the tRNA molecule and begins with the nucleotide G at position 23.

Figure 8

Cloverleaf model of *S. pombe* $tRNA_1^{Glu}$. U* is 5-methyloxycarbonylmethyl-2-thiouridine.



DISCUSSION

The nucleotide sequence of *S. pombe* $tRNA_1^{Glu}$ differs at 18 positions from that of *S. cerevisiae* $tRNA^{Glu}$ (15) (Figure 8). The anticodon loops in the two tRNAs are identical, as are the D-loop and the T-Ψ-C-G loop and its stem. We tested the recognition of *S. pombe* $tRNA^{Glu}$ by *S. pombe* synthetase as compared to *S. cerevisiae*

synthetase, and found that the K_m values with the two crude enzyme preparations were identical. This parallels the findings made with $tRNA^{Phe}$ from *S. pombe* that lacks the specific sequences that had been proposed as recognition site for *S. cerevisiae* phenylalanyl-tRNA synthetase (16).

As documented by Figure 1, passage of the uniformly labeled unfractionated *S. pombe* tRNA through a $tRNA^{Phe}$-affinity column led to the isolation of two isoacceptors of $tRNA^{Glu}$. The retention of both isoacceptors by the affinity column implies that their anticodons are similar or identical. Preliminary sequencing studies indicate that this in fact is the case. Interesting is that the nucleotide sequence of the minor $tRNA_2^{Glu}$ differs even more drastically from the one found in *S. cerevisiae* (17). The nucleotide sequences of lysine tRNAs from *Drosophila* and rabbit (as an example) are identical despite the evolutionary distance between these organisms (8). It is puzzling to observe the diversity of tRNA sequences in two yeast species placed into the same family by some taxonomists.

ACKNOWLEDGEMENTS

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REFERENCES

1. Altman, S. (1978) In: Transfer RNA (S. Altman, ed.) MIT Press, Cambridge, p.48-77.
2. Goodman, H.M., Olson, M.V. and Hall, B.D. (1977) Proc. Natl. Acad. Sci. 74, 5453-5457.
3. Valenzuela, P., Venegas, A., Weinberg, F., Bishop, R. and Rutter, W.J. (1978) Proc. Natl. Acad. Sci. 75, 190-194.
4. Knapp, G., Beckmann, J.S., Johnson, P.F., Fuhrman, S.A. and Abelson, J. (1978) Cell 14, 221-236.
5. O'Farrell, P.Z., Cordell, B., Valenzuela, P., Rutter, W.J. and Goodman, H.M. (1978) Nature 274, 438-445.
6. Ogden, R., Beckmann, J.S., Abelson, J., Kang, H.S., Söll, D. and Schmidt, O. (1979) submitted to Cell.
7. Silverman, S., Heckmann, J.S., Cowling, G.J., Delaney, A.D., Dunn, R.J., Gillam, I.C., Tener, G.M., Söll, D. and RajBhandary, U.L. (1979) Nucleic Acids Res. 6, 421-433.
8. Silverman, S., Gillam, I.C., Tener, G.M. and Söll, D. (1979) Nucleic Acids Res. 6, 435-442.

9. Mitchison, J.M. (1970) Methods Cell. Physiol. 4, 131-165.
10. Grosjean, H., Takada, C. and Petre, J. (1973) Biochem. Biophys. Res. Commun. 53, 882-893.
11. Ohashi, K., Harada, F., Ohashi, Z., Nishimura, S., Stewart, T.S., Vögeli, G., McCutchan, T. and Söll, D. (1976) Nucleic Acids Res. 3, 3369-3375.
12. Kohli, J., Kwong, T., Altruda, F. and Söll, D. (1979) J. Biol. Chem. in press.
13. Brownlee, G.G. (1972) Determination of Sequences in RNA. American Elsevier Publishing Co., Inc., New York.
14. Gehrke, C.W., Kuo, K.C., Davis, G.E., Suits, R.D., Waalkes, T.P. and Borek, E. (1978) J. Chromatog. 150, 455-476.
15. Kobayshi, T., Irie, T., Yoshida, M., Takeishi, K. and Ukita, T. (1974) Biochim. Biophys. Acta 336, 168-181.
16. McCutchan, T., Silverman, S., Kohli, J. and Söll, D. (1978) Biochemistry 17, 1622-1628.
17. Wong, T.W. and McCutchan, T., unpublished results.