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The nucleotide sequence of tRNA tyrosine from the fission yeast Schizosaccharomyces pombe

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ABSTRACT

The sequence of tRNA tyrosine from the fission yeast Schizosaccharomyces pombe is pCUCCUGAU$^{m^1}$GGUGψAGDDGGDDAUCACACor(ψ)CCGGCUGψA$^{1^6}$AACCGGUUG$^{m^7}$GU$^{m^3}$C GCUAGTψCG$^{m^1}$AUUCUGGCUCAGGAGACCAOH. This sequence differs in 30 nucleotides from the tRNA-Tyr sequence of the budding yeast Saccharomyces cerevisiae. It has a unique anticodon stem of only four GC base pairs. The normal fifth pair position of nucleotide 28-44 is occupied by a C-U and in 20% of the tRNA-Tyr molecules it is ψ-U. This unusual feature and its implications are considered in the discussion.

INTRODUCTION

tRNA-Tyr from the fission yeast Schizosaccharomyces pombe is one of the tRNAs altered in strains carrying the anti-suppressor mutation sin-1 (Janner et al., in preparation). Since knowledge of the primary structure of tRNA-Tyr is important in elucidating the biochemical basis of this anti-suppressor mutation, I have determined its nucleotide sequence.

MATERIALS AND METHODS

Schizosaccharomyces pombe (L972h$^-$) (1) were grown overnight with 1 mCi [^{32}P]orthophosphate and 0.04 mg catalase per ml of yeast extract medium from which the phosphate had been removed (2). The cells were phenolized, the RNA precipitated with ethanol and separated by stepwise elution from a BD-cellulose column (3). The RNA eluting with 1 M NaCl, 20% ethanol was applied to a RPC-5 column (4). tRNA-Tyr elutes toward the end of the 150 ml gradient (0.45 M - 0.9 M NaCl). The RNA from the fractions containing tRNA-Tyr was separated by two-dimensional gel electrophoresis (5). After elution (6) from the gel the RNA was subjected to the standard sequence analysis (7). RNase digestion products were separated by cellulose acetate electrophoresis followed by homochromatography on PEI thin layer plates. The oligonucleo-

tides were further analyzed by digestion with RNAse T_1 or pancreatic RNase, digestion with RNase T_2, RNase U_2, Physarum RNase (8), partial digestion with spleen exonuclease or treatment by water-soluble carbodiimide followed by pancreatic RNase. Modified nucleotides were analyzed after T_2 RNase digestion by chromatography on cellulose thin layer plates (9).

Pure tRNA-Tyr was labelled at the 5' end by reaction with [^{32}P] gamma ATP and T4 polynucleotide kinase (10). Several end-labelled fragments of tRNA-Tyr were obtained and analyzed by one-dimensional (11) or two-dimensional (12) gel electrophoresis. In addition to the standard nucleases (7), RNase from Physarum (8) and treatment with KOH (12) or formamide (13) was also used.

RESULTS

Uniformly [^{32}P]orthophosphate labelled tRNA-Tyr from Schizosaccharomyces pombe has been purified by stepwise elution from BD-cellulose followed by chromatography on RPC-5. After two-dimensional gel electrophoresis, four major tRNAs are found. To identify which of the four tRNAs is tRNA-Tyr I used the observation (R. Fluri, personal communication) that the aminoacylated tRNA-Tyr elutes at a higher salt concentration from RPC-5 than the unacylated tRNA-Tyr. The amino acid specificity of the three other tRNAs has not been determined. Data from Janner et al. (in preparation) suggest that two of the three are probably tRNA-Ser and tRNA-Trp.

Pancreatic RNase digestion products

After digestion of uniformly labelled tRNA-Tyr with pancreatic RNase seventeen fragments were found:

Fragment p'3 is a mixture of two oligonucleotides, one of them is identical with fragment p11b. That the same oligonucleotide found in two different locations on the fingerprint is due to the fact that ^{m1}A is converted during the course of tRNA isolation to ^{m6}A (14). The final analysis of the modification on thin layer chromatography shows for both oligonucleotides the secondary altered modification ^{m6}A. Initially one of them must still have contained the unaltered ^{m1}A and therefore separated from the oligonucleotide containing ^{16}A. Fragment p11 is also a mixture of two oligonucleotides. If tRNA-Tyr is split at ^{7m}G (15) and the two tRNA fragments analyzed separately, fragment p11 from the 3' side of the tRNA-Tyr molecule contains AGT-, whereas the 5' side contains GAU-.

TABLE I. Pancreatic RNase End Products

Fragment No.	Sequence	Molar Yields	
		Measured	From Final Sequence
p1	C-, ^{m5}C-	12.0	11
p2	U-, ψ-, D-	6.0	11
p3	G^{m7}GU-, G^{m1}AU-	0.3	2
p4	AU-	1.1	1
p5	AC-	2.0	2
p6	GU-	1.8	2
p7	^{m1}GGU-	1.1	1
p8	GGD-	0.8	1
p9	GGU-	1.0	1
p10	AGD-	0.8	1
p11	AGT-, GAU-	2.2	2
p11b	G^{m1}AU-	0.4	1
p12	GC-	1.1	1
p13	A^{i6}AAC-	1.0	1
p14	GGC-	2.2	2
p15	-C-	1.2	1
p16	AGGAGAC-	0.9	1

T_1 RNase digestion products

After digestion of uniformly labelled tRNA-Tyr with RNase T_1, eighteen fragments were found.

Fragments t6 and t6b were in some fingerprints separated into two distinct oligonucleotides. Fragment t8: Treatment with water soluble carbodiimide followed by pancreatic RNase and the analysis with spleen exonuclease gave the sequence ^{m1}AUUCUG-. RNase T_2 digestion of total tRNA-Tyr gave ^{m1}A- as modified nucleotide. The conversion (14) to ^{m6}A- therefore must occur during the isolation and analysis of the fragment t8. Fragment t11b: This fragment was found in variable amounts. The sequence around ^{m7}G- has also been determined from the analysis of 5' [^{32}P]-labelled tRNA-Tyr by acrylamide gel electrophoresis. Fragment t13 and fragment t14: RNase U_2 and partial digestion with spleen exonuclease showed that both oligonucleotides have the same sequence with the exception that a C- is replaced by a ψ- in fragment t14.

TABLE II. T1 RNase End Products

Fragment No.	Sequence	Molar Yields	
		Measured	From Final Sequence
t1	G-	6.0	1
t2	AG-	1.0	1
t3	UG-	1.5	1
t3b	AU^{m1}G-	0.6	1
t4	DDG-	0.4	1
t5	UUG-	1.1	1
t6	ΨAG-	1.0	1
t6b	CUG-	1.2	1
t7	TΨCG-	0.8	1
t8	^{m1}AUUCUG-	0.8	1
t9	ACCAOH	0.9	1
t10	CUAG-	0.9	1
t11	CUCAG-	1.2	1
t11b	^{m7}GU^{m5}CG-	0.5	1
t12	Ψ$_A$^{16}AACCG-	0.9	1
t13	DDAUCACACCCG-	0.5	1
t14	DDAUCACAΨCCG-	0.1	
t15	-CUCCUG-	1.0	1

Fragment t14 is found in an amount 20% of fragment t13.

Arrangement of oligonucleotides to give the final sequence

Electrophoresis of partially cleaved [^{32}P]-end labelled tRNA-Tyr on polyacrylamide gels (11, 12, 13) has been used to order the oligonucleotides to give the final sequence. Since the initial sequencing data indicated that no base pairing occurred between position 28 (C- or Ψ-) and position 44 (U-) (Figure 6), 5' [^{32}P]-labelled tRNA-Tyr was analyzed after formamide fragmentation (13) by two-dimensional acrylamide gel electrophoresis (12). In the region of the anticodon stem two different rows of partial tRNA-Tyr molecules run side by side due to the different electrophoretic behavior of C- compared with Ψ-. The double spots start with molecules a little bit longer than the position of Ψ- and with the shift of Ψ- to A- (position 28 to 27) no further double spots can be seen.

The results are summarized in Figure 1.

DISCUSSION

The sequence of tRNA-Tyr from S. pombe shows a unique anticodon stem of only four base pairs. This is the first tRNA sequenced (16) not having five base pairs in the anticodon stem.

The anticodon stem of tRNA-Tyr of S. pombe has four GC pairs with a total of 12 hydrogen bonds; this is not especially low and does agree with the numbers of hydrogen bonding interactions found in other tRNAs.

In other tRNAs (16) there are an average of 13.1 ± 1.0 hydrogen bonds per anticodon stem; the other four sequenced tRNA-Tyr have 13 (E. coli), 14 (B. stearothermophilus), 12 (S. cerevisiae) and 12 (T. utilis) hydrogen bonds in the anticodon stem.

The CU "pair" or the ΨU "pair" probably will not disturb the RNA backbone of the anticodon stem, especially since the region involved is stabilized through tertiary interactions (17). This base "pair" might still contribute to base stacking. Biophysical measurements could be used to analyze the stability of the tRNA-Tyr from S. pombe in anticodon-anticodon interactions and compare it with the published measurements (18).

Since the nucleotide C- in position 28 is replaced in 20% of the molecules with Ψ-, it is most likely that there probably are at least two different

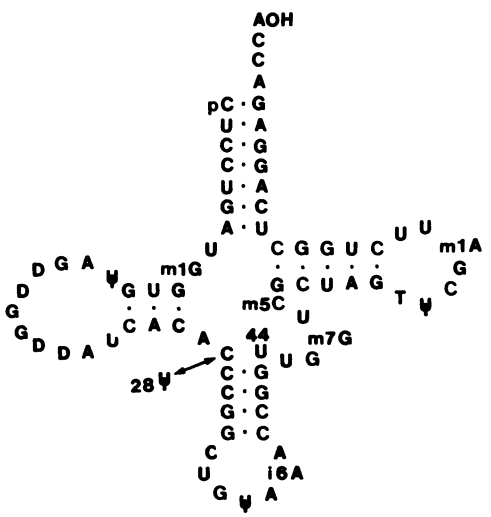

Figure 1. Sequence of tRNA-Tyr from Schizosaccharomyces pombe.

genes for tRNA-Tyr in S. pombe.

A mutation in position 28 to A- or G- (or a mutation in position 44 to G-) would restore base pairing and make the anticodon stem more stable, but such molecules were not found. One must assume that this mutation would impair the biological function of the tRNA-Tyr, e.g., the interaction with the aminoacyl tRNA synthetase could, for example, be affected. Since the tyrosyl tRNA synthetase from S. pombe has been purified to near homogeneity (Fluri, manuscript in preparation), it will be possible to analyze the interaction of tRNA-Tyr from S. pombe with its cognate synthetase and with synthetases from other organisms with this question in mind.

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REFERENCES

1. Gutz, H., Heslot, H., Leupold, U. and Loprieno, N. (1974) in Handbook of Genetics, King, R.C., Ed., Vol. 1, pp. 395-446. Plenum Press, New York
2. Rubin, G.M. (1974) J. Biol. Chem. 248, 3860-3875
3. Roy, K.L., Bloom, A. and Söll, D. (1971) in Proc. Nucleic Acids Res., Cantoni, G.L. and Davies, D.R., Eds., Vol. 2, pp. 524-541. Harper & Row, New York
4. Pearson, R.L., Weiss, J.F. and Kelmers, A.D. (1971) Biochim. Biophys. Acta 228, 770-774
5. Vögeli, G., Stewart, T.S., McCutchan, T. and Söll, D. (1977) J. Biol. Chem. 252, 2311-2318
6. Maxam, A.M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560-564
7. Barrell, B.G. (1971) in Proc. Nucleic Acids Res., Cantoni, G.L. and Davies, D.R., Eds., Vol. 2, pp. 751-779, Harper & Row, New York
8. Braun, R. and Behrens, K. (1969) Biochim. Biophys. Acta 195, 87-98
9. Nishimura, S. (1972) Progr. Nucl. Acid Res. Molec. Biol. 12, 50-86
10. Silberklang, M., Prochiantz, A., Haenni, A.L. and RajBhandary, U.L. (1977) Eur. J. Biochem. 72, 465-478

11. Donis-Keller, H., Maxam, A.M. and Gilbert, W. (1977) Nucleic Acids Res. 4, 2527-2538
12. Lockard, R.E., Alzner-Deweerd, B., Heckman, J.E., MacGee, J., Tabor, M.W. and RajBhandary, U.L. (1978) Nucleic Acids Res. 5, 37-56
13 Simoncsits, A., Brownlee, G.G., Brown, R.S., Rubin, J.R. and Guilley, H. (1977) Nature 269, 833-836
14. Macon, J.B. and Wolfenden, R. (1968) Biochemistry 7, 3453-3458
15. Simsek, M., Petrissant, G. and RajBhandary, U.L. (1973) Proc. Natl. Acad. Sci. USA 70, 2600-2604
16. Sprinzl, M., Gruter, F. and Gauss, D.H. (1978) Nucleic Acids Res. 5, r15-r27
17. Rich, A. and RajBhandary, U.L. (1976) Ann. Rev. Biochem. 45, 805-860
18. Grosjean, H.J., DeHenau, S. and Crothers, D.M. (1978) Proc. Natl. Acad. Sci. USA 75, 610-614