EVIDENCE SUPPORTING A REVISED SEQUENCE FOR YEAST ALANINE tRNA

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Received 25 November 1974

1. Introduction

Yeast alanine tRNA splits into a quarter and a three-quarter molecular fragments [1] when heated in the presence of magnesium ion [2]. We have now investigated the sequence of the quarter molecule from both brewer's yeast $tRNA^{Ala}_{1}$ and baker's yeast $tRNA^{Ala}_{1}$ which was sequenced by Holley et al. [3]. Both these tRNA's, which differ slightly in modification and apparent susceptibility to splitting, give a quarter molecule which extends from the 5′ end into the hU loop. Sequence analyses of these quarters show that neither contains the sequence Py–G–C– and this result requires that two nucleotides be deleted from the earlier sequence for $tRNA^{Ala}_{1}$ (yeast) [3] such that the structure of the 5′ quarter is pG_1–G–G–C–G–U–G–U–m^1G–G–C–G–U–A–G_{15}p. The revised, complete sequence now conforms to the general rule that tRNA's have A_{14} followed by G_{15} or A_{15} [4].

2. Materials and methods

Fractions of baker's yeast tRNA, enriched in $tRNA^{Ala}$ by countercurrent distribution, were a gift from Dr J. T. Madison. Brewer's yeast tRNA was obtained from Boehringer Mannheim GmbH., Mannheim, W. Germany. In each case the major $tRNA^{Ala}_{1}$ was isolated by a three step purification using, in turn, countercurrent distribution [5,6], chromatography on benzoylated DEAE-cellulose [7] and on the reverse-phase system, RPC-5 [8]. Purity and identity of the $tRNA^{Ala}_{1}$ was checked by fractionation of T_1 RNase (EC 3.1.4.8) hydrolysis products on a column of DEAE-cellulose in 7 M urea, pH 8 [9]. The non-enzymatic splitting was carried out by heating 1 to 2 mg/ml tRNA in 0.1 M Tris–HCl pH 9.5, 0.05 M. $MgCl_2$ for 100 min at 50°C. 1/4 and 3/4 fragments were isolated on a column of DEAE-cellulose in 7 M urea at 55°C [2], and then the 1/4 fragments were purified by a second chromatography at pH 3 and room temperature. Pancreatic RNase (EC 3.1.4.22) hydrolyses were performed in 0.05 M triethylamine bicarbonate pH 8, and using 10 μg enzyme for 5 A_{260} units 1/4, 20 μg for 6 units 3/4, and 30 μg for 8 units $tRNA^{Ala}$, with 20 minutes incubation at 37°C. Products were separated by isophoresis on DEAE-cellulose paper in 7% formic acid, recovered, and analysed for nucleotide composition [10,11].

3. Results and discussion

PR (Pancreatic RNase) products from the 1/4 molecules are shown separated in fig. 1a, with the spot identities and yields given in table 1. A dinucleotide is present in the position which is normal for A–Gp (or A–G–cyclic-p). This spot is not present among the PR

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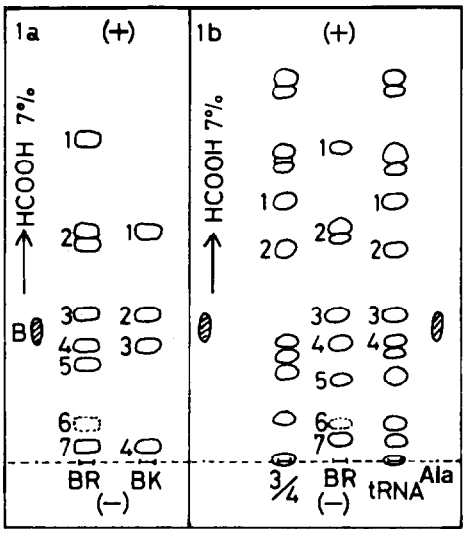

Fig. 1. Separation of Pancreatic RNase (PR) products by ionophoresis on DEAE-cellulose paper in 7% formic acid. (a) 5 A_{260} nm units each of brewer's 1/4 (BR) and baker's 1/4 (BK). (b) 2 A_{260} nm units 1/4, 6 units 3/4 and 8 units $tRNA^{Ala}$ from brewer's yeast. B – marker dye.

Table 1
Yields of PR products from 1/4, 3/4 and whole molecules of brewer's $tRNA^{Ala}$ (yeast); and 1/4 molecule of baker's $tRNA^{Ala}$ (yeast)

(a) Quarters

	Baker's 1/4		Brewer's 1/4	
Oligonucleotide	Spot No.	Yield	Spot No.	Yield
A–Gp (I)	1	0.9	2	0.68
m^1 G–G–Cp	2	0.95	3	0.92
G–Up	3	3.1	4	3.15
pG–G–G–Cp	4	1.0	7	1.0
Cp	–	0	1	0.27
A–G–hUp	–	0	5	0.28
G–G–hUp	–	0	6	0.12

(b) 3/4 and $tRNA^{Ala}$

Oligonucleotide	Spot No.	Yield in 3/4	Yield in $tRNA^{Ala}$
G–Cp	1	1.16	1.21
A–G–Cp	2	1	1
m^1 G–G–Cp	3	0	0.88
G–Up	4	+*	3.78

+* present but not measured.

products from whole $tRNA^{Ala}$ and must arise from the point of non-enzymatic splitting. Furthermore, it can be concluded that the putative A–Gp is at the 3′ end of the 1/4 fragments since they contain the 5′ terminal pG–G–G–Cp. Identification as A–Gp comes from nucleotide composition, the presence of U–A–Gp as the only T_1 product from the 1/4 which contains Ap, and from the assumption that it is an alkaline hydrolysis product with 3′-phosphate or cyclic-phosphate. The complete list of PR products from the baker's 1/4 add up to 15 nucleotides which can be arranged into a sequence pG_1–G–G–C–/G–U–/G–U–/m^1G–G–C_{11}–/G–U–/A–G_{15}p. This is the same as the original one [3] except for the deletion of a G–C– following C_{11}. The complete absence of G–Cp from the PR products of both 1/4 fragments proves the absence of the 'extra' G–C– following a pyrimidine. Small yields of additional PR products are obtained from the brewer's 1/4 and these can be explained by incomplete splitting after G_{15} and splits further along the sequence of the hU loop. The absence of G–Cp from the quarter is confirmed by the presence of one mole of G–Cp, relative to A–G–Cp, in both the 3/4 and whole $tRNA^{Ala}$ from brewer's yeast (table 1b). This result is different from the observation of Holley et al., who found 2 moles and were thus forced to include the 'additional' G–C– in their structure [3]. It seems likely that the extra G–Cp arose from over-exposure to the Pancreatic RNase when hydrolysis was deliberately carried beyond the point where no cyclic phosphates remained.

The T_1 hydrolysis products (table 2) support the above conclusions about the sequence and non-enzymatic splitting. Actual sequences of the longer oligonucleotides from the brewer's $tRNA^{Ala}_I$ (yeast) were assumed to be the same as in baker's by identity of chromatographic position and nucleotide composition. The only observable difference between the $tRNA^{Ala}_I$ species from the two yeasts is that brewer's has the terminal A_{76} and has complete reduction at hU_{47}. In the T_1 hydrolyses of the 1/4 fragments much less enzyme was used with the consequence that there is little splitting after m^1G. The T_1 products from the baker's 1/4 are as expected from the sequence given above, i.e. pGp, Gp, Gp, C–Gp, U–Gp, U–m^1G–Gp, C–Gp, U–A–G_{15}p. In each case we have expressed yields relative to a molar yield of T–C–Gp or U–Gp and we find mole ratios of the principal dinucleotides

Table 2
Yields of T_1 products from 1/4, 3/4 and whole molecules of brewer's $tRNA^{Ala}$ (yeast); and 1/4 molecule of $tRNA^{Ala}$ (yeast)

Oligonucleotide	Brewer's			Baker's
	$tRNA^{Ala}$	3/4	1/4	1/4
C-m_2^2 Gp›	+	+	0	0
U–m^1 Gp›	+	0	0.55	0
U–m^1G–Gp	0	0	0.45	0.60
Gp	+	+	2.75	1.9
U–Gp	0.95	0	1.0	1.0
C–Gp	3.15	1.15	2.1	2.1
A–Gp	2.1	1.95	0	0
hU–C–Gp	0.9	0.75	0.30	0
hU–A–Gp	0.9	0.95	0	0
U–A–Gp	0.95	0	1.0	0.75
pGp	0.9	0	0.85	0.75
C–m^1I–Ψ–Gp	0.9	+	0	0
T–Ψ–C–Gp	1.0	1.0	0	0
U–C–C–A–C–C–A_{OH}	0.9	+	0	0
A–C–U–C–Gp	1.0	+	0	0
hU–C–U–C–C–Gp	1.1	+	0	0
A–U–U–C–C–Gp	1.1	+	0	0
C–U–C–C–C–U–U–Ip (›)	1.05	+	0	0

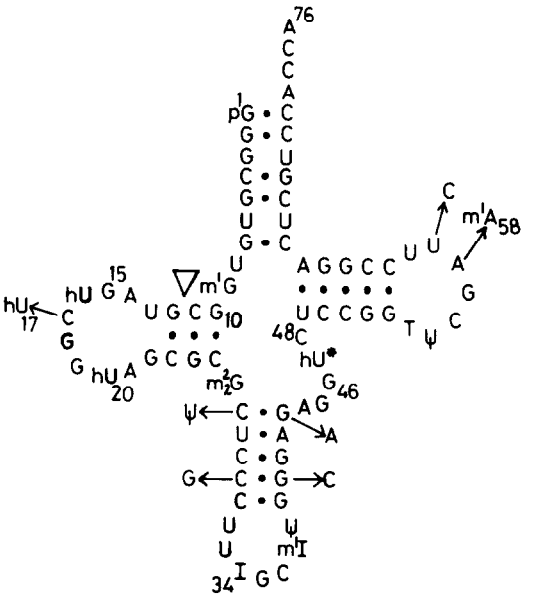

Fig. 2. Sequence of $tRNA^{Ala}$ (yeast) arranged into a cloverleaf structure. Arrows point to the substitution found in $tRNA^{Ala}$ (*T. utilis*). hU* indicates some U in baker's yeast. ∇ shows point where the additional G–C– has been deleted.

consistent with the revised sequence. Again we find a sequence in the brewer's 1/4 in addition to those in baker's 1/4 – 0.3 mol hU–C–Gp– which places a secondary split at or beyond G_{18}. The 3/4 shows a corresponding reduction in hU–C–Gp but not much reduction in hU–A–Gp, which is indicative that the secondary split is before hU_{20}. However the presence of G–G–hUp in the PR products shows a small amount of splitting after hU_{20}. These secondary splits probably occur in the non-enzymatic splitting of baker's $tRNA^{Ala}$ (yeast) since two 3/4 bands were observed in polyacrylamide gel electrophoresis [1].

In fig.2 the revised sequence, including G_{46} proposed by Merrill [12], is shown as a cloverleaf secondary structure. Following the deletion between C_{11} and G_{12}, the sequence is more like that for $tRNA^{Ala}$ (*Torulopsis utilis*) which was determined by Takemura et al. [13]. The positions where *T. utilis* differs from yeast are substitutions at C_{17} by hU, U_{59} by C, two base pairs in the anticodon stem as indicated in the figure, and by modification of A_{58} to m^1A. The rather unusual acceptor stem with G + U and U + U' pairs is conserved in these fungi.

Acknowledgements

We thank Dr J. T. Madison for providing the baker's yeast tRNA and are grateful to Mme Schlegel for carrying out the countercurrent distributions. Our thanks also to Professor J. P. Ebel, Dr J. Bonnet, Dr G. Keith and Dr J. Weissenbach for helpful discussions. One of us (J.R.P.) received an EMBO Short-term Fellowship to visit Strasbourg and was supported in part by USPHS Research Grant No. GM 10791. He thanks Professors J. P. Ebel, R. Markham and E. B. Keller for their helpful support.

References

[1] Penswick, J. R., in preparation.
[2] Wintermeyer, W. and Zachau, H. G. (1973) Biochim. Biophys. Acta 229, 82–90.
[3] Holley, R. W., Apgar, J., Everett, G. A., Madison, J. T., Marquissee, M., Merrill, S. H., Penswick, J. R. and Zamir, A. (1965) Science 147, 1462–1465.
[4] Dirheimer, G., Ebel, J. P., Bonnet, J., Gangloff, J., Keith, G., Krebs, B., Küntzel, B., Roy, A., Weissenbach, J. and Werner, C. (1972) Biochimie 54, 127–144.

[5] Holley, R. W., Apgar, J., Everett, G. A., Madison, J. T., Merrill, S. H. and Zamir, A. (1963) Cold Spring Harbor Symp. Quant. Biol. 28, 117–121.
[6] Dirheimer, G. and Ebel, J. P. (1967) Bull. Soc. Chim. Biol. 49, 1679–1687.
[7] Gillam, I., Millward, S., Blew, D., von Tigerstrom, M., Wimmer, E. and Tener, G. M. (1967) Biochemistry 6, 3043–3056.
[8] Pearson, R. L., Weiss, J. F. and Kelmers, A. D. (1971) Biochim. Biophys. Acta 228, 770–774.
[9] Penswick, J. R. and Holley, R. W. (1965) Proc. U.S. Natl. Acad. Sci. 53, 543–546.
[10] Sanger, F., Brownlee, G. G. and Barell, B. G. (1965) J. Mol. Biol. 13, 373–398.
[11] Gangloff, J., Keith, G. and Dirheimer, G. (1970) Bull. Soc. Chim. Biol. 52, 125–133.
[12] Merrill, C. R. (1968) Biopolymers 6, 1729–1731.
[13] Takemura, S., Ogawa, K. and Nakazawa, K. (1972) FEBS Lett. 25, 29–32.