THE SEQUENCES OF NUCLEOTIDES IN $tRNA^{Arg}_{III}$ FROM BREWER'S YEAST

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1. Introduction

Three major species of $tRNA^{Arg}$ are found when brewer's yeast tRNA is fractionated by countercurrent distribution [1]. We called $tRNA^{Arg}_{III}$ the arginine acceptor tRNA having the highest solubility in the organic phase of the solvent system used in the countercurrent distribution [2]. It was further purified [3] by a chromatography on DEAE-cellulose at 65° according to Bergquist et al. [4] followed by chromatography on hydroxyapatite according to one of us [5]. This last column fractionation could be replaced by a reversed phase chromatography derived from the method described by Kelmers et al. [6, 7]. The yield of the reversed phase chromatography step was higher than the hydroxyapatite one as will be described elsewhere [8]. Here we present the nucleotide sequence of a pool of pure $tRNA^{Arg}_{III}$ in which two sequence variants were detected.

2. Experimental

The purified $tRNA^{Arg}_{III}$ were completely digested with T_1 or pancreatic ribonuclease. The mono- and oligonucleotides obtained after these hydrolyses were separated by chromatography on DEAE-cellulose [9] followed, after desalting [10, 11], by monodimensional high voltage electrophoresis on DEAE-cellulose paper [12–14].

The structure of these oligonucleotides was determined by methods previously published [13]. A total of 15 partial pancreatic and T_1 ribonuclease digestion products, isolated by DEAE-cellulose column chromatography, were degraded by the same methods. A specific cleavage into two halves of the molecules of $tRNA^{Arg}_{III}$ at the level of the anticodon could be obtained by a partial hydrolysis with pancreatic ribonuclease in the presence of Mg^{2+} and at 0° [15]. The structure of all these oligonucleotides obtained by partial hydrolysis permitted to build up the primary structure of $tRNAs^{Arg}_{III}$.

3. Results and discussion

The results of the analyses showed that $tRNA^{Arg}_{III}$ was a mixture of at least two arginine-tRNAs differing in two nucleotides: position 6 from the 5′terminal end where a C is replaced by a U in 30% of the molecules and position 72, before the CCA 3′terminal end, where a G is replaced by a U in 30% of the molecules. The sequences containing 75 nucleotides are shown in fig. 1. They may easily be folded into a typical planar cloverleaf with an aminoacyl stem 7 basepairs long, two 5 base paired stems for the TΨC and the anticodon arms and a 4 base pair stem for the dihydrouracil arm. The TΨC and the anticodon loops contain 7 residues as in other known tRNA structures. In the hU loop we have 7 nucleotides.

The $tRNAs^{Arg}_{III}$ have a pG at the 5′terminal end and a G–C–C–A or a U–C–C–A sequence at the 3′terminal end. The first nucleotide after the amino-acid stem, position 8 from the 5′terminal end, is a U or a s^4U in all sequenced tRNAs with one exception: $tRNA^{His}$ which has also a s^4U in the first position afte the amino acid stem but this position is the 9th from the 5′terminal end [16]. The $tRNAs^{Arg}_{III}$ have a U in position 8 from the 5′terminal end.

The sequence m^1G–m^2G in position 9 and 10 has been found previously in $tRNA^{Tyr}$ from *Torulopsis utilis* [17] and in $tRNA^{Trp}$ from *Saccharomyces cerevisiae* [18].

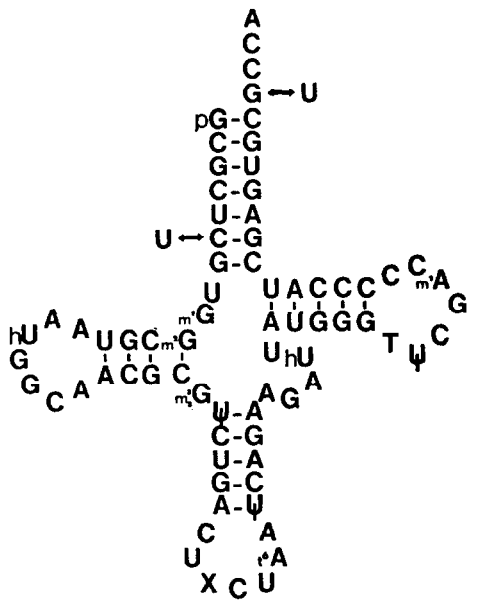

Fig. 1. Clover-leaf model of the nucleotide sequence of brewer's yeast $tRNA^{Arg}_{III}$ species. Standard abbreviations are used for the common nucleosides. Other abbreviations are: m^2_2G, 2-dimethylguanosine; m^1G, 1-methylguanosine; m^2G, 2-methylguanosine; Ψ, pseudouridine; hU, dihydrouridine; m^1A, 1-methyladenosine; T, ribosylthymine; t^6A, *N*-[9-(β-D-ribofuranosyl)-purin-6-yl carbamoyl] threonine.

As in seven other sequenced tRNAs (for a general review of tRNAs primary structures see [14] a sequence A–A–hU is found in $tRNAs^{Arg}_{III}$ which could take the tertiary structure proposed by Levitt [19] with a base pair between A_{17} and U_{47}.

All sequenced tRNAs concerned with protein biosynthesis have a sequence G–G or Gm–G in positions corresponding to position 17 and 18 in $tRNAs^{Arg}_{III}$ which follows also this general law. The sequence Gm–G is not followed by a hU contrary to all sequenced tRNAs of yeast except $tRNA^{Phe}$ [20]. The $tRNAs^{Arg}_{III}$ have a m^2_2G in position 25. The extra arm has a sequence A–G–A–hU like $tRNA^{Ile}$ of Torula yeast [21] and $tRNA^{Tyr}$ of baker's yeast [22] but contrary to these tRNAs this sequence is followed by a U in place of m^5C.

The sequence G–T–Ψ–C has been found in all sequenced tRNAs concerned with protein biosynthesis. In $tRNAs^{Arg}_{III}$ it is followed by a G and a m^1A.

The anticodon of $tRNAs^{Arg}_{III}$ is X–C–U where X is an unknown nucleotide. Its UV absorption spectra looks like the spectra of Up with a slight shift at acidic and alkaline pH. Its maxima of absorption are 266 nm at pH 1 and 268 nm at pH 13 whereas Up has its maxima at 262 nm and 261 nm. The sequence X–C is not resistant to pancreatic RNase, Xp behaves like Ψp in thin-layer cellulose chromatography with the solvent propanol/NH_4OH/H_2O, 60:30:10 (v/v) [23]. It has the same R_f as Up with the solvent isopropanol/HCl H_2O, 68:17.6:14.4 (v/v) [24]. The anticodon X–C–U could give base pairs with 2 of the 6 codons of arginine A–G–A or A–G–G but we have not yet tested its coding properties.

Finally the anticodon is followed by a nucleotide which behaves acidic at neutral pH. It gave an Ap after alkaline hydrolysis. Its structure has been investigated after a $T_1 + T_2$ [25] ribonuclease hydrolysis of the trinucleotide A^*–A–Ψ obtained by pancreatic RNase hydrolysis of the $tRNAs^{Arg}_{III}$ (A^* is the unknown nucleotide). By bidimensional chromatography with the solvents of Krebs and Hems [26] and Wyatt [24] it separates from Ap. Its spectrum is exactly like the one of *N*-[9(β-D-ribofuranosyl)-purin-6-yl carbamoyl] threonine [27] Torula yeast $tRNA^{Ile}$ [28] and baker's yeast $tRNA^{Lys}$ [29] have been shown to have this nucleotide at a similar place in the sequence and it has been suggested [27, 30] that a similar nucleotide will be found adjacent to all anticodons in tRNAs responding to codon which begin with an A. The determination of the primary structure of $tRNA^{Arg}_{III}$ improves this hypothesis.

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References

[1] G. Dirheimer et J.P. Ebel, Bull. Soc. Chim. Biol. 49 (1967) 1679.
[2] R.W. Holley, J. Apgar, G.A. Everett, J.T. Madison, S.H.

Merrill and A. Zamir, Cold Spring Harbor, Symp. Quant. Biol. 28 (1963) 117.
[3] B. Kuntzel and G. Dirheimer, Nature 219 (1968) 720.
[4] P.L. Bergquist, B.C. Baguley, J.M. Robertson and R.K. Ralph, Biochim. Biophys. Acta 108 (1965) 531.
[5] G. Dirheimer, Bull. Soc. Chim. Biol. 50 (1968) 1221.
[6] A.D. Kelmers, G.D. Novelli and M.P. Stulberg, J. Biol. Chem. 240 (1965) 3979.
[7] G. Dirheimer and B. Kuntzel, Bull. Soc. Pharm. Strasbourg 9 (1966) 97.
[8] B. Kuntzel, J. Weissenbach and G. Dirheimer, Biochimie, in preparation.
[9] J.T. Madison, G.A. Everett and H. Kung, J. Biol. Chem. 246 (1967) 1318.
[10] B.C. Baguley and M. Staehelin, Biochemistry 7 (1968) 45.
[11] J. Gangloff, G. Keith and G. Dirheimer, Bull. Soc. Chim. Biol. 52 (1970) 125.
[12] F. Sanger, G.G. Brownlee and B.G. Barrell, J. Mol. Biol. 13 (1965) 373.
[13] J. Gangloff, G. Keith, J.P. Ebel and G. Dirheimer, Biochim. Biophys. Acta 259 (1972) 198.
[14] G. Dirheimer, J.P. Ebel, J. Bonnet, J. Gangloff, G. Keith, B. Krebs, B. Kuntzel, A. Roy, J. Weissenbach and C. Werner, Biochimie 54 (1972) 127.
[15] B. Kuntzel, J. Weissenbach and G. Dirheimer, Biochim. Biophys. Acta (1972) in press.
[16] C.E. Singer and G.R. Smith, J. Biol. Chem. (1972) in press.
[17] S. Hashimoto, M. Miyasaki and S. Takemura, J. Biochem. Japan 65 (1969) 659.
[18] G. Keith, A. Roy, J.P. Ebel and G. Dirheimer, FEBS Letters 17 (1971) 306.
[19] M. Levitt, Nature 224 (1969) 759.
[20] U.L. Raj Bhandary, S.H. Chang, A. Stuart, R.D. Faulkner, R.M. Hoskinson and H.G. Khorana, Proc. Natl. Acad. Sci. U.S. 57 (1967) 751.
[21] S. Takemura, M. Murakami and M. Miyazaki, J. Biochem. Japan 65 (1969) 489.
[22] J.T. Madison, G.A. Everett and H. Kung, Science 153 (1966) 531.
[23] C.S. Hanes and F.A. Isherwood, Nature 164 (1949) 1107.
[24] G.R. Wyatt, Biochem. J. 48 (1951) 584.
[25] M. Hiramaru, T. Uschida and F. Egami, Anal. Biochem. 17 (1966) 135.
[26] H.A. Krebs and R. Hems, Biochim. Biophys. Acta 12 (1953) 172.
[27] Y. Hamada, K. Murao, M. Saneyoshi and S. Nishimura, Biochem. Biophys. Res. Commun. 37 (1969) 990.
[28] S. Takemura, M. Murakami and M. Miyazaki, J. Biochem. Japan 65 (1969) 553.
[29] J.T. Madison, S.J. Bogulawski and G.H. Teetor, Science 176 (1972) 687.
[30] A. Peterkofsky and C. Jesensky, Biochemistry 8 (1969) 3798.