NUCLEOTIDE SEQUENCE OF ALANINE tRNA I FROM *TORULOPSIS UTILIS*

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1. Introduction

Nucleotide sequences of *Torulopsis utilis* $tRNA_1^{Val}$*, $tRNA^{Ile}$ and $tRNA^{Tyr}$ (all the major molecular species) have been determined in our laboratory [1–3]. *T.utilis* is often called torula yeast but belongs to a taxonomically different "class" from baker's or brewer's yeasts (*Saccharomyces cerevisiae*); the former is classified into *Fungi imperfecti* and the latter into *Ascomycetes.* Such a difference of species reflects in the primary structures of tRNA's, and thus *T.utilis* $tRNA^{Tyr}$ and $tRNA_1^{Val}$ differ in several nucleotides from the corresponding *S.cerevisiae* tRNA's [3,4]. However these and several other tRNA's from *T.utilis* so far assayed have been fully aminoacylated with the corresponding aminoacyl-tRNA synthetases from baker's yeast. Sequence homology between *T.utilis* and *E.coli* $tRNA's^{Ile}$ [2,5] is less than that between the above *T.utilis* and *S.cerevisiae* tRNA's, and approximately half of either $tRNA^{Ile}$ can be aminoacylated with the heterologous isoleucyl-tRNA synthetase**. A comparison of the sequences of tRNA's of different species but of identical amino acid specificity, and kinetics of the heterologous aminoacylation will offer useful information on the aminoacyl-tRNA synthetase recognition sites in tRNA. Alanine tRNA of *T.utilis* can be also fully acylated with *S.cerevisiae* alanyl-tRNA synthetase. We would like to report the established sequence of the major species of *T.utilis* alanine tRNA ($tRNA_1^{Ala}$) and structural differences between *T.utilis* and *S.cerevisiae* $tRNA's^{Ala}$ [6,7].

* Abbreviations used are: $tRNA^{Val}$, valine transfer RNA; Standard abbreviations are used for the major nucleosides; m^1A, 1-methyladenosine; m^1G, 1-methylguanosine; m_2^2G N^2-dimethylguanosine; hU, 5,6-dihydrouridine; ψ, pseudouridine; I, inosine; m^1I, 1-methylinosine; T, ribothymidine.

** The heterologous charging has been assayed by exchanging *E.coli* and *T.utilis* $tRNA's^{Ile}$ between Dr.M.Yarus and us.

2. Materials and methods

From a crude tRNA mixture of *T.utilis* supplied by Jūjō Paper Co.Ltd., $tRNA_1^{Ala}$ was isolated by successive chromatography on DEAE-Sephadex A-25 columns with a linear gradient of ammonium sulfate, with a linear gradient of KCl in 1 M phosphate and in 0.5 M borate [8]. The purified tRNA accepted 1.68 nmoles of alanine per A_{260} unit (=ca. 1.8 nmole of tRNA.

The purified tRNA was completely digested with pancreatic ribonuclease and with ribonuclease T_1. The end-products were isolated and sequenced by column chromatographic procedures as used for analysis of fragments of $tRNA_1^{Val}$, $tRNA^{Ile}$ and $tRNA^{Tyr}$ [9–11]. Generally the purified fragments obtained from pancreatic RNAase digest were digested with RNAase T_1, and those from RNAase T_1 digest were with pancreatic RNAase. Snake venom phosphodiesterase, *E.coli* alkaline phosphatase, RNAase T_2 and RNAase U_2 were also used. Some nucleosides and nucleotides including minors were isolated by paper chromatography. The nucleosides and nucleotides were identified by their spectra at pH's 2 and 12 and by their paper chromatographic behavior.

The RNAase T_1 end-products were assigned to either of the 3′- or 5′-half molecules prepared by splitting the anticodon linkages I–G–C with RNAase T_1 at 0° in the presence of magnesium.

Large oligonucleotides were obtained by partial digestion of the tRNA with RNAase T_1 at 0° in the absence of magnesium, and by chromatography on DEAE-cellulose columns in 7 M urea at pH 7.6 and further at pH 3.5. Sequences of the isolated frag-

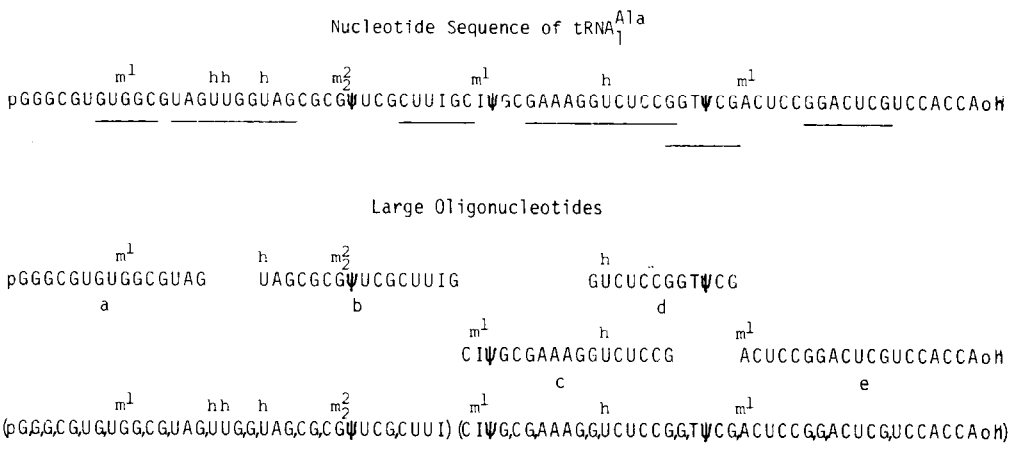

Fig. 1. Nucleotide sequences of oligonucleotides obtained by partial digestion of tRNA$_1^{Ala}$ with RNAase T_1. The lines under the sequence show sequences obtained by overlaps of the endproducts of RNAase T_1 and pancreatic RNAase digestions. Fragments from RNAase T_1 digestion of the 5′- and 3′-half molecules are shown in the parentheses.

ments were determined by their complete RNAase T_1 or pancreatic RNAase digestions as in [4,12]. For determination of the 5′-terminal sequence of fragment c, it was labeled with ^{32}P by polynucleotide kinase and [γ-^{32}P] ATP, and then digested with RNAase T_1. Some of the identified large fragments necessary for establishing the total sequence are shown in fig.1.

3. Results and dsicussion

By overlapping of the fragments from the complete and partial RNAase digestions, the overall primary structure of tRNA$_1^{Ala}$ was derived and arranged in the cloverleaf pattern shown in fig.2.

This tRNA is composed of 76 nucleotide residues including 13 modified nucleotides, and has IGC as the anticodon. The position adjacent to the 3′-side of the anticodon is occupied by 1-methylinosine. The amino acid stem of this tRNA contains only six base pairs which is usually seven. All of these structural features are identical with those of *S.cerevisiae* tRNA^{Ala}.

Although the sequence of tRNA$_1^{Ala}$ from *T.utilis* is similar to *S.cerevisiae* tRNA^{Ala}, eight nucleotide substitutions and a dinucleotide deletion can be seen in the *T.utilis* tRNA. Fig.3 shows the cloverleaf structure of *S.cerevisiae* tRNA^{Ala} which was first determined by Holley et al.[6] and revised later by Merril [7].

Our sequence lacks C–G which is present at positions 13 and 14 from the 5′-end of their sequence. Thus the first base pair from the dihydrouridine loop in our sequence is U·G instead of C·G, and the loop sustained by this stem contains eight nucleotides.

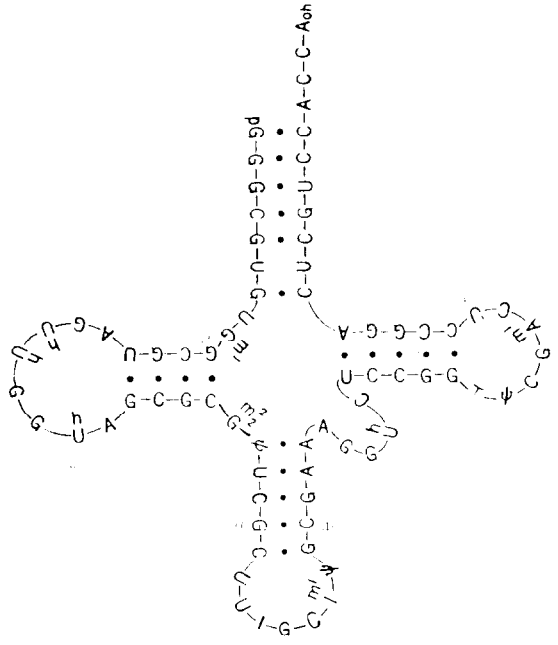

Fig. 2. Cloverleaf pattern of the nucleotide sequence of tRNA$_1^{Ala}$ from *T.utilis*.

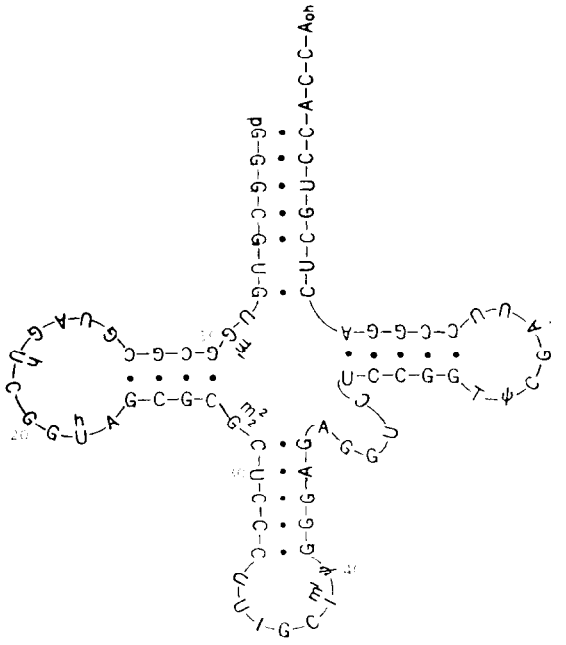

Fig. 3. Cloverleaf pattern of the nucleotide sequence of $tRNA^{Ala}$ from baker's yeast determined by Holley [6] and revised by Merril [7].

Alternatively the dihydrouridine stem and loop consist of three base pairs and ten nucleotides, respectively. Our sequence has hU, hU, m^1A and C at positions 17, 47, 58 and 59, respectively, while theirs has C, U, A and U at the corresponding positions on the cloverleaf pattern. Our sequence also has $\psi_{27}\cdot A_{43}$ and $C_{30}\cdot G_{40}$ pairs in the anticodon stem, instead of $C_{29}\cdot G_{45}$ and $G_{32}\cdot C_{42}$ pairs at the corresponding positions. The $m_2^2G-\psi$ sequence present at positions 26 and 27 of our sequence has been also found in *T.utilis* $tRNA^{Ile}$ and $tRNA^{Tyr}$ [2,3], but not in *S.cerevisiae* tRNA's of known sequence. Thus our sequence is very different from theirs in the anticodon stem; two pairs out of five are different. The TψC and dihydrouridine loops are also not very similar. However, the stems bearing both terminal strands and sustaining TψC loop, and the anticodon loop are identical.

Since $tRNA^{Ala}$ from *T.utilis* can be fully charged with alanine by the *S.cerevisiae* synthetase, the different bases or regions between the two tRNA's^{Ala} may not function as the synthetase recognition sites. We can compare sequences of some other tRNA's which are chargeable heterologously; e.g., *T.utilis* and *S.cerevisiae* tRNA's$_1^{Val}$ [4,13,14] and tRNA's^{Tyr} [3,15], *S.cerevisiae* and wheat germ tRNA's^{Phe} [16,17], *E.coli* and *S.cerevisiae* tRNA's^{Trp} [18,19], etc. It appears that the synthetase recognition sites are different as the specificity of tRNA differs. For further details, many other tRNA's of various species should be sequenced.

Acknowledgements

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