THE NUCLEOTIDE SEQUENCES OF TWO TRYPTOPHANE-tRNAs FROM BREWER'S YEAST

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$tRNA^{Trp}$ from brewer's yeast was isolated by countercurrent distribution followed by column chromatography on BD-cellulose as described elsewhere [1]. The purified tRNA was completely digested with T_1 or pancreatic ribonuclease. The mono- and oligonucleotides obtained after these hydrolyses were separated by chromatography on DEAE-cellulose [2] followed after desalting [3] by fractionation of the isopliths by monodimensional high voltage electrophoresis on DEAE-cellulose paper [4]. The sequences were determined with the methods described elsewhere [4].

In order to overlap the fragments, many large oligonucleotides were prepared by partial digestion of the tRNA with pancreatic ribonuclease in the presence of Mg^{2+} and at 0° [5]. After a phenol extraction the oligonucleotide mixture was heated 3 min at 80° in the presence of EDTA 0.01 M and chromatographed on a DEAE-cellulose column at pH 7.5 [5]. The oligonucleotides obtained were further purified by chromatography on DEAE-cellulose at pH 3 [5]. The sequences of the isolated large fragments were determined from their enzymatic degradation products by the methods described elsewhere [5].

The results of the analyses showed that the $tRNA^{Trp}$ we isolated was a mixture of two tryptophane-tRNAs differing in only one nucleotide: $tRNA_{II}^{Trp}$ having a U in place of a ψ in $tRNA_{I}^{Trp}$.

The information gathered from the complete and limited enzymatic digestion products was sufficient to construct the complete sequence which has been arranged in the cloverleaf pattern characteristic of the tRNAs (fig. 1).

The two $tRNA^{Trp}$ are composed of 75 nucleotide residues including 17 ($tRNA_{I}^{Trp}$) or 16 ($tRNA_{II}^{Trp}$) minor nucleotides. They have a pGp at the 5′ terminal end and a sequence ACCA at the 3′ terminal end. T occupies position 23 from the 3′ terminal end in these tRNAs as well as in all other tRNAs of known structure. Like all sequenced tRNAs which have a U or a derivative of U in position 8 from the 5′ terminal end, the tryptophane tRNAs have a U in this position.

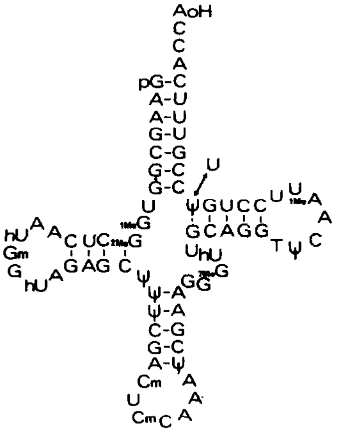

Fig. 1. Clover-leaf model of the nucleotide sequence of two brewer's yeast $tRNA^{Trp}$ species. Standard abbreviations are used for the common nucleosides. Other abbreviations are: Gm, 2′-O-methylguanosine; Cm, 2′-O-methylcytidine; 7 MeG, 7-methylguanosine; 1 MeG, 1-methylguanosine; 2 MeG, 2-methylguanosine; ψ, pseudouridine; hU, dihydrouridine; 1 MeA, 1-methyladenosine; T, ribosylthymine.

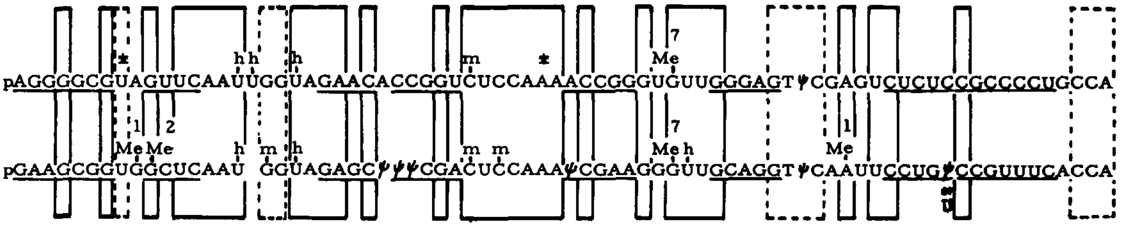

Fig. 2. Comparison of the nucleotide sequences of tryptophane tRNAs from *E. coli* (above) and brewer's yeast (below). Sequences in the boxes are identical in the $tRNA^{Trp}$ of *E. coli* and yeast without being common to all tRNAs. Sequences in dotted boxes are common to all tRNAs. Underlined sequences are paired.

The sequence 1 MeG-2 MeG-C in position 9 to 11 has been found previously only in $tRNA^{Tyr}$ of *Torulopsis utilis* [6] and in $tRNA^{Arg}_{III}$ of brewer's yeast [7]. As in five other sequenced tRNAs ($tRNA^{Asp}$ (yeast) [8, 9], $tRNA^{Leu}$ (*E. coli*) [10, 11], $tRNA^{Trp}$ (*E. coli*) [12], $tRNA^{Arg}_{III}$ (yeast) [7]), a sequence AAhU is found in $tRNA^{Trp}$ (yeast). This tRNA could take the tertiary structure proposed by Levitt [13] with a base pair between A_{15} and U_{47}.

All sequenced tRNAs have a sequence G–G or Gm–G in positions corresponding to position 17 and 18 of $tRNA^{Trp}$ which follows also this general law. The sequence GmG is followed by a hU as in all yeast tRNAs of known structure except $tRNA^{Phe}$ [14].

The tryptophane tRNAs have a nucleotide ψ in position 25. This is an exception because all sequenced tRNAs have a purine nucleotide in this position between the two stems. This ψ is followed by two other ψ. The sequence ψ–ψ–ψ has not yet been found in other tRNAs.

The extra arm has a sequence 7 MeG–hU which has been found in $tRNA^{Val}$ from yeast [15] and in wheat germ $tRNA^{Phe}$ [16].

The sequence GTψC has been found in all sequenced tRNAs. In $tRNA^{Trp}$ this sequence is followed by A as in five tRNAs of known structure.

The stem bearing the 3′ and 5′ terminal sequences has seven base pairs. There are five base pairs in the stems sustaining the TψC and the anticodon loops, which is a general feature. The stem sustaining the hU loop has four base pairs.

The anticodon and the TψC loops contain 7 nucleotides as in all sequenced tRNAs. The hU loop is exceptionally short: it is only 7 nucleotides long. The extra loop has 5 nucleotides.

Tryptophane is the only aminoacid having only one codon: UGG. Only one sequence in $tRNA^{Trp}$ can correspond to it: CmCA. This triplet is localized in the anticodon loop and its nucleotides are respectively the third, fourth and fifth base in the loop. It is the first tRNA where the first nucleotide of the anticodon is a 2′-O-methylcytidine. As in all known cases, this anticodon is preceded by uridine. It is followed by A as in four sequenced tRNAs. The anticodon triplet is highly susceptible to pancreatic ribonuclease attack at the level of C.

Finally it is interesting to compare the structures of the tryptophane tRNAs of yeast and *E. coli* [12] since both are charged by the tryptophane tRNA synthetase from yeast and *E. coli* [17]. Fig. 2 shows that these sequences are very different in the stem bearing the 5′ and 3′ ends. On the contrary the anticodon loops are identical except for the replacement of one of the two C in the anticodon by a Cm. The hU loop and the stem sustaining this loop also present a high degree of analogy since among 15 nucleotides 13 are identical, if a supplementary hU in the $tRNA^{Trp}$ of *E. coli* is not taken into account. At last the primary structure of the extra arm is also very similar, since 4 nucleotides among 5 are similar. However the TψC loops and the stems sustaining them have only few analogies except for the GTψC sequence common to all sequenced tRNAs.

It can be seen that among the tRNAs accepting the same aminoacid, but originating from different microorganisms, the $tRNA^{Trp}$ from *E. coli* and yeast are those presenting the highest amount of common sequences. Taking into account the high degree of similitude of the tryptophane tRNAs and the existence of only one codon for Trp, it is tempting to suggest that these tRNAs have only undergone a slight evolution.

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