NUCLEOTIDE SEQUENCE OF $tRNA^{Cys}$ FROM BAKER'S YEAST

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1. Introduction

Cysteine tRNA has been the subject of very few published investigations. Zekavat et al. [1] have described a method for the purification of $tRNA^{Cys}$ and James et al. [2] have discussed aspects of charging $tRNA^{Cys}$ with cysteine. The only information on the nucleotide sequence has been a report on the presence of a cytokinin active nucleotide, probably isopentenyladenylic acid [3].

We wish now to describe briefly our work which leads to a sequence for a $tRNA^{Cys}$ from baker's yeast.

2. Experimental

The isolation of $tRNA^{Cys}$ from baker's yeast tRNA by reversed phase chromatography has already been briefly reported [4]. Half molecules were prepared by incubation with ribonuclease T 1 at 2°C for 45 min in 0.1 M Tris–chloride buffer pH 7.5 with 0.02 M magnesium chloride followed by chromatography on DEAE cellulose in 7 M urea at pH 7.5 and then by chromatography on a column of P100 polyacrylamide beads at pH 7.4.

The oligonucleotides produced by complete ribonuclease T1 or pancreatic ribonuclease digestion were separated by chromatography on DEAE cellulose in 7 M urea at pH 7.5 and then in the case of complex mixtures, on DEAE cellulose in 7 M urea with 0.02 M formic acid at pH 3.8 using linear gradients of sodium chloride.

The sequences of the isolated oligonucleotides were determined by conventional methods by digestion with the complementary nuclease or by partial digestion with snake venom or spleen phosphodiesterase. Nucleotide compositions were determined as previously described [5].

A number of longer oligonucleotides isolated from partial digestion experiments have provided sufficient overlaps to permit us to propose the linear arrangement of nucleotides as shown in fig. 1.

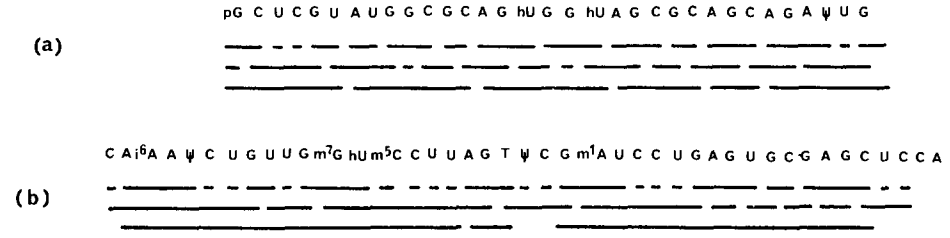

Fig. 1. Nucleotide sequences of (a) the terminal half, and (b) the acceptor half of $tRNA^{Cys}$. In each case the first two interrupted lines show the fragments obtained by pancreatic ribonuclease and ribonuclease T1 digestion respectively and the third, some of the fragments resulting from partial degradation of the whole molecule.

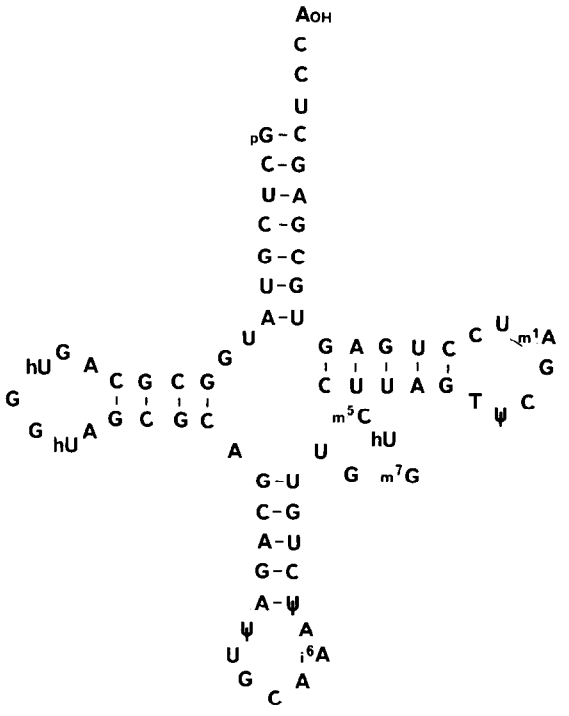

Fig. 2. The conventional clover-leaf arrangement of the nucleotide sequence of baker's yeast $tRNA^{Cys}$.

3. Results and discussion

The molecule is composed of 75 nucleotides and can be written in the conventional clover-leaf pattern (fig. 2). As isolated the tRNA lacks the adenosine and one cytidylic acid residue from the terminal CCA. The position preceding this triplet is occupied by U instead of the frequently found purine residue. Among other examples with UCCA at the 3′ terminus are $tRNA^{Gly}_3$ from *E. coli* [6], $tRNA^{Lys}$ from haploid yeast [7], $tRNA^{Gly}$ from T4 infected *E. coli* [8], $tRNA^{Arg}_3$ from yeast [9] and $tRNA^{Gly}_1$ from staphylococcus species [10].

$tRNA^{Cys}$ is similar to the last two RNAs in having 7 nucleotides in the dihydro U loop and the sequence is the same as that reported for yeast $tRNA^{Gly}_1$ [11]. Other members of this group with a small dihydro U loop are yeast $tRNA^{Trp}$ [12] and $tRNA^{Met}_f$ from a number of species [13–15].

$tRNA^{Met}_f$ is similar to $tRNA^{Cys}$ in the occurrence in the fifth loop of the sequence m^7G–hU–m^5C at positions 31 to 29 from the 3′ terminus. The same sequence is shown by yeast $tRNA^{Lys}_2$ [16] but in $tRNA^{Phe}$ from rabbit liver [17,18], $tRNA^{Phe}$ from yeast [19], and $tRNA^{Val}_2$ from brewer's yeast [20,21] the m^5C is found at position 28.

There are two codon possibilities for cysteine, UGU and UGC; the sequence shown here contains the anticodon GCA corresponding to the latter alternative. The anticodon is followed by isopentenyladenylic acid as predicted for all tRNAs that recognise codons beginning with U [3].

A full account of the isolation and purification of $tRNA^{Cys}$ and of the sequence analysis will be published later.

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