The nucleotide sequence of a cytoplasmic tRNA^{Phe} from *Scenedesmus obliquus* and comparison with a tRNA^{Tyr} species

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The nucleotide sequence of a cytoplasmic tRNA^{Phe} from the eukaryotic green alga *Scenedesmus obliquus* was determined as: pG-G-C-U-U-G-A-U-A-m²G-C-U-C-A-G-C-D-Gm-G-G-A-G-A-G-C-m²G-Ψ-Ψ-A-G-A-Cm-U-G-A-A-m¹G-A-Ψ-C-U-A-C-A-G-m⁷G-N^{*}-m⁵C-C-C-C-A-G-T-Ψ-C-G-m¹A-U-m⁵C-Cm-U-G-G-G-U-C-A-G-G-C-C-A-C-C-A-OH. The structure has some notable features. Unlike other tRNA^{Phe} species from plant sources, it has an unmodified G as the first residue of the anticodon and m¹G rather than a Y derivative as the residue following the anticodon. The sequence m⁵C₍₆₀₎–Cm₍₆₁₎ is unique to this tRNA. The sequence of *S. obliquus* tRNA^{Phe} shows close homology with *S. obliquus* tRNA^{Tyr}.

INTRODUCTION

Although many new tRNA sequences are deduced by the determination of gene sequences, the sequencing of the tRNAs themselves uniquely provides information about one of the most interesting features of the tRNAs, namely their modified nucleosides. tRNA^{Phe} species have a particularly interesting pattern of modification at residue 37. The true eubacteria follow Nishimura's rule [1] and have ms²i⁶A or related modifications, as do those from the higher-plant chloroplasts [2]. The higher-plant major cytoplasmic species, mammalian and yeast tRNAs have a derivative of Y at this position [2]. Just a few species, e.g. *Drosophila melanogaster*, *Bombyx mori*, mouse neuroblastoma, *Mycoplasma* sp. and yeast mitochondria, have m¹G₃₇ [2].

Although considerable knowledge has been obtained on the structure of tRNAs from the higher plants, little work has been done on tRNAs from the lower plant kingdom. We have extended our studies on the structures of tRNAs from the eukaryotic unicellular green alga *Scenedesmus obliquus* to that of the cytoplasmic tRNA^{Phe}. We have been able to compare its structure and modification patterns with tRNA^{Phe} species from other sources, in particular the higher plants. Not only does *S. obliquus* tRNA^{Phe} have some interesting and unique features, it also shows close sequence homology with *S. obliquus* cytoplasmic tRNA^{Tyr} [3].

MATERIALS AND METHODS

Chemicals, enzymes, radiochemicals and chromatographic materials were obtained or prepared as described previously [3, 4]. Total crude tRNA was isolated from *S. obliquus* by the method of Kirby [5], and the tRNA^{Phe} was purified as described elsewhere [6]. It was characterized by aminoacylation studies and by hybridization of [³²P]tRNA^{Phe} to *S. obliquus* DNA fractionated by CsCl-density-gradient centrifugation [7].

The primary sequence was deduced by using a combination of ³²P-post-labelling techniques as described previously [4]. These were the 'limited hydrolysis' method

[8], 'direct read-off' gels [9] and 'mobility shift' analysis [10]. The 5'- and 3'-terminal bases were determined as described previously [11] and modified nucleosides were identified by the methods of Nishimura [1] and Silberklang *et al.* [10].

RESULTS AND DISCUSSION

Structure of *S. obliquus* cytoplasmic tRNA^{Phe}

The sequence of *S. obliquus* cytoplasmic tRNA^{Phe} was determined by using post-labelling techniques. Each residue was identified unambiguously by at least two methods or experiments. The structure deduced is shown in clover-leaf form in Fig. 1.

This tRNA^{Phe} from a green alga shows slightly more overall sequence homology with mammalian tRNA^{Phe} species (78%) than with the higher-plant cytoplasmic tRNA^{Phe} species (72%). This is quite surprising, since the major tRNA^{Phe} species from different higher plants are completely homologous [2]. The homology with a prokaryotic blue-green-algal tRNA^{Phe} is only 63%. Also, whereas the higher-plant tRNA^{Phe} species have only six base-pairs in the amino acid stem, *S. obliquus* tRNA^{Phe} has the full complement of seven base-pairs. In contrast, however, with all other tRNA^{Phe} species, *S. obliquus* tRNA^{Phe} has only four base-pairs in the anticodon stem.

Like other cytoplasmic tRNA^{Phe} species, *S. obliquus* tRNA^{Phe} has a large number of modified nucleosides, including m²G, m³G, m⁵C, m¹A, which are found only in eukaryotic tRNAs. However, m⁵C is absent from all higher-plant tRNA^{Phe} species, whereas two are present in *S. obliquus* tRNA^{Phe}. Also, unlike all the other eukaryotic tRNA^{Phe} species, *S. obliquus* tRNA^{Phe} has an unmodified G as the first base of the anticodon rather than Gm. *S. obliquus* tRNA^{Phe} can be added to the few tRNA^{Phe} species that have m¹G as the nucleoside following the anticodon (see Fig. 2), and again in this respect it is different from plant tRNA^{Phe} species, which have Y in this position.

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Fig. 1. Clover-leaf structure of *S. obliquus* cytoplasmic tRNA^{Phe}

The unique features of this tRNA are m⁵C₍₆₀₎ and Cm₍₆₁₎ (see Fig. 2). No other tRNAs reported to date have any modification at all at these positions. *S. obliquus* cytoplasmic tRNA^{Met}, however, has an m⁵C residue present in the T-stem that has been assigned tentatively to position 63 [12].

The unidentified nucleotide present at position 47 has also been found at this position in other *S. obliquus* cytoplasmic tRNAs (namely tRNA^{Met} [12] and tRNA^{Tyr} [3]). The chromatographic properties of this nucleotide are unlike those of any of the known modified nucleotides.

Comparison of *S. obliquus* tRNA^{Phe} and tRNA^{Tyr}

Comparison of the sequences of *S. obliquus* tRNA^{Phe} and tRNA^{Tyr} shows that they have 70% homology. Common features of the sequence are shown in Fig. 3. Similar homologies are found also between tRNA^{Phe} and

Fig. 3. Common features of the sequences of *S. obliquus* tRNA^{Phe} and tRNA^{Tyr}

□, Residues at these positions are not common to the two tRNAs.

tRNA^{Tyr} from *Drosophila melanogaster* (E. Kubli, personal communication) and *Torulopsis utilis* [13]. Although the two tRNAs from *S. obliquus* are similar, as might be expected, neither is charged by the non-cognate amino acid *in vitro*.

Hallick *et al.* [14] have noted previously that in *Euglena gracilis* chloroplasts some different amino acid-accepting tRNAs have high homology. Also, in *Escherichia coli* it has been shown [15] that certain pairs of different amino acid-accepting tRNAs have greater than 66% homology. From these observations it has been proposed that in chloroplasts and in bacteria the tRNA genes have evolved from one or a small group of ancestral genes. The results described here suggest that in eukaryotes also similar evolutionary processes may have occurred.

Fig. 2. Identification of modified nucleosides at positions 37, 60 and 61 of *S. obliquus* tRNA^{Phe}

5'-labelled mononucleotides, obtained from the 'limited hydrolysis' method [8], were subjected to two-dimensional t.l.c. on cellulose: first dimension, isobutyric acid/conc. NH₃/water (66:1:33, by vol.); second dimension, 0.1 M-sodium phosphate buffer (pH 6.8)/(NH₄)₂SO₄/propan-1-ol (50:30:1, v/w/v).

For *S. obliquus* tRNA^{Phe} and tRNA^{Tyr} the sequences flanking the anticodon are the same (except for methylation of C₍₃₂₎), and in the lower part of the anticodon arm the only difference in sequence is the middle base of the anticodon itself. The fundamental recognition between the tRNA and mRNA is at the anticodon/codon level, but it has been proposed [16] that other features of the tRNA must be involved in order to maintain the high fidelity of translation, and in some instances residues surrounding the anticodon have been implicated [17, 18]. Clearly this cannot be the case here. Interestingly Nishikawa *et al.* [13] have shown recently that a modified *T. utilis* tRNA^{Tyr} having the anticodon GAA can be aminoacylated by phenylalanine as well as tyrosine. Also, this modified tRNA is as active as native tRNA^{Phe} in polymerizing phenylalanine in response to poly(U). It is likely therefore that for tRNA^{Phe} and tRNA^{Tyr} the anticodon sequence alone distinguishes the correct codon.

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