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The nucleotide sequence of Scenedesmus obliquus chloroplast $tRNA_f^{Met}$

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ABSTRACT

The chloroplast initiator $tRNA_f^{Met}$ from the green alga Scenedesmus obliquus has been purified and its sequence shown to be p C-G-C-A-G-G-A-U-A-G-A-G-C-A-G-U-C-U- Gm -G-D-A-G-C-U-C- m_2^2G -Ψ-G-G-G-G-C-U-C-A-U-A-A-Ψ-C-C-C-A-A-U- m^7G -D-C-G-C-A-G-G-T-Ψ-C-A-A-A-U-C-C-U-G-C-U-C-C-U-G-C-A-A-C-C-A-OH. This structure is prokaryotic in character and displays close homologies with a blue green algal initiator $tRNA_f^{Met}$ and bean chloroplast initiator $tRNA_f^{Met}$.

INTRODUCTION

The protein biosynthetic machineries of organelles have more features in common with prokaryotic than with eukaryotic systems [1, 2]. The chloroplast initiator $tRNA_f^{Met}$ of a higher plant (bean) has been shown to be highly homologous with prokaryotic initiator tRNAs [3]. However, the mitochondrial initiator tRNAs of N.crassa [4] and yeast [5] are neither prokaryotic nor eukaryotic in character. We have isolated the chloroplast initiator $tRNA_f^{Met}$ of Scenedesmus obliquus, a unicellular green alga which is an example of a lower plant. The sequence of this tRNA possesses many of the structural characteristics of prokaryotic initiator tRNAs, notwithstanding the presence of the modified nucleoside m_2^2G at position 26 which has not been found in any prokaryotic tRNA. S.obliquus chloroplast $tRNA_f^{Met}$ also has an anticodon stem which differs from all other initiator tRNAs (both prokaryotic and eukaryotic) in that it lacks the G - C base-pair immediately adjacent to the anticodon loop. It has been suggested that G - C base-pairing in the anticodon stem confers a unique anticodon loop conformation on initiator tRNAs [6].

MATERIALS AND METHODS

Chemicals, enzymes, radiochemicals and chromatographic materials were

obtained or prepared as described previously [7]. Total crude tRNA was isolated from S.obliquus by the method of Osterman et al [8]. Chloroplast $tRNA_f^{Met}$ was purified by successive steps of column chromatography on BD - cellulose, arginine-agarose and two stages of RPC-5, followed by one dimensional 15% polyacrylamide gel electrophoresis. The tRNA was characterised by aminoacylation and formylation studies and also by hybridisation to S.obliquus chloroplast DNA (J.M.McCoy, J.A.Pryke and D.S. Jones, manuscript in preparation).

The sequence was deduced using a combination of techniques, including; mobility shift analysis [9] of formamide digested 5'-[^{32}P]-labelled tRNA, direct read-off gels [9], and the limited hydrolysis method [10] as described previously [7]. Modified nucleosides were identified by the tritium derivative method [11] and by the results of the limited hydrolysis procedure. The 5'- and 3'- terminal nucleotides were characterised after total digestion of 5'-[^{32}P]-labelled tRNA with nuclease P_1 and 3'-[^{32}P]-labelled tRNA [12] with RNase T_2.

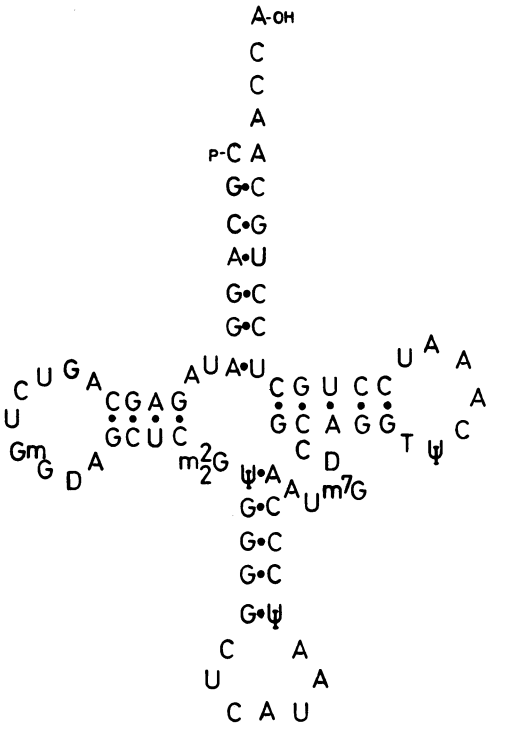
RESULTS

Nucleoside Composition

Two dimensional t.l.c. of [^{3}H]-labelled nucleoside trialcohols showed the presence of the following modified nucleosides in the molar ratios indicated: D, 1.2; T, 0.8; ψ, 1.2; m^7G, 0.9; m_2^2G, 0.8. These, and also Gm, were the modified nucleosides detected during limited hydrolysis sequential analysis. However, 2 residues of D and 3 residues of ψ were found by this latter method.

Nucleotide Sequence

Direct read-off gels enabled most of the sequence from residues 2 - 75 to be deduced. However in several regions it was not possible to distinguish C's from U's, and modifications were not indicated. Mobility shift analysis performed on 5'-[^{32}P]-$tRNA_f^{Met}$ partially digested with formamide (100°C, 30 min) confirmed the sequence of residues 2 - 14. The limited hydrolysis sequencing method gave the sequence of residues 1 - 72, except for residues 2, 3 and 19 (residue 18 is Gm). The positions and identities of the modified nucleosides could also be deduced by this method. Total digestion of 5'-[^{32}P]-tRNA with nuclease P_1 released [^{32}P]pC, and total digestion using RNase T_2 of tRNA labelled at the 3'-end with [^{32}P]pCp [12] released [^{32}P]Ap. The nucleotide sequence of S.obliquus chloroplast $tRNA_f^{Met}$ deduced from these data is shown in clover-leaf form.



Scenedesmus obliquus CHLOROPLAST $tRNA_f^{Met}$

DISCUSSION

S.obliquus chloroplast $tRNA_f^{Met}$ can be regarded as being generally prokaryotic in character, although it does share some common features with both prokaryotic and eukaryotic initiator tRNAs. Several characteristics found in prokaryotic initiators, which are absent in their eukaryotic counterparts, are found in S.obliquus chloroplast $tRNA_f^{Met}$. These include C_1 (not base-paired), the G_6 - C_{67} and A_{11} - U_{24} base pairs, D_{20}, unmodified A_{37}, U_{60} and -GTψC- in loop IV. These features are also found in bean chloroplast $tRNA_f^{Met}$ [3], except for the G_6 - C_{67} base pair which in bean is A_6 - U_{67}. In terms of general sequence homology S.obliquus chloroplast $tRNA_f^{Met}$ is more homologous with E.coli $tRNA_f^{Met}$ (81%) than with S.obliquus cytoplasmic $tRNA_i^{Met}$ (64%).

The general level of base modification in S.obliquus chloroplast $tRNA_f^{Met}$ is low, there being just six types of modified nucleoside and only nine

modifications in all. Gm_{19}, D_{20} and T_{54} are found in prokaryotic, but not eukaryotic initiators. m^7G_{46} and ψ_{55} are found in both classes and D at position 47 has been found previously in eukaryotic, but not prokaryotic initiators. Many modifications common in eukaryotic initiators are not found in S.obliquus chloroplast $tRNA_f^{Met}$. These include; m^1G_9, m^2G_{10}, t^6A_{37}, m^5C_{48} and m^1A_{58}.

The modified $m_2^2G_{26}$ is interesting. This modification has been found only at position 26 and only in eukaryotic tRNAs. Although it is present in N.crassa mitochondrial $tRNA^{Tyr}$ and yeast mitochondrial $tRNA^{Phe}$ [4] it has not been found in any other chloroplast tRNA, including S.obliquus chloroplast $tRNA_m^{Met}$ (J.M.McCoy and D.S.Jones, unpublished results). The modification is present in S.obliquus cytoplasmic $tRNA_m^{Met}$ (J.M.McCoy and D.S.Jones, unpublished results) but does not occur in S.obliquus cytoplasmic $tRNA_i^{Met}$, although it is commonly found in other eukaryotic cytoplasmic initiator tRNAs. The apparently random selection of tRNAs for this modified nucleoside makes it very difficult to suggest either a functional role or a structural determinant which specifies the modification.

Several tRNAs, including $tRNA_m^{Met}$ species, have ψ at positions 27 and/or 39, but this is the first time that these modifications have been found in an initiator tRNA.

It has been suggested [6] that initiator tRNAs have a unique anticodon loop conformation, and that this conformation may be due to the presence of three G - C base-pairs found adjacent to the anticodon loops of all initiator tRNAs. Recently some supporting evidence for this view has been provided by an analysis of E.coli $tRNA_f^{Met}$ tertiary structure [13]. The conformation of the anticodon loop of this molecule was found to be different from that of yeast $tRNA^{Phe}$.

G_{31} - ψ_{39} of S.obliquus chloroplast $tRNA_f^{Met}$ lies immediately next to the anticodon loop in the position which would be expected [6] to be occupied by a G_{31} - C_{39} base-pair. The change of C_{39} to ψ_{39} would weaken the base pairing in the anticodon stem and therefore possibly influence the anticodon loop conformation. Interestingly bean chloroplast [3], N.crassa mitochondrial [4] and yeast mitochondrial [5] initiator tRNA species all have only two G - C base-pairs adjacent to their anticodon loops, and not three as expected [6].

The sequence homology between Anacystis nidulans (blue green algal) $tRNA_f^{Met}$ and either S.obliquus chloroplast $tRNA_f^{Met}$ (82%) or bean

chloroplast $tRNA_f^{Met}$ (78%) is higher than the homology between the two chloroplast $tRNA_f^{Met}$ species (71%). This could reflect evolutionary divergence. According to the endosymbiont hypothesis [14] blue green algae are direct descendants of the organisms which formed the chloroplast symbiosis in precambrian times. The sequences of blue green algal initiator $tRNA_f^{Met}$ species might therefore be expected to show homologies with the initiator tRNAs of chloroplasts. This is now found to be the case. Moreover it might be supposed that plants lower down the evolutionary tree would have chloroplast tRNAs which would be more homologous to A.nidulans. The sequences of S.obliquus and bean chloroplast initiator tRNAs support this view and suggest that S.obliquus might be considered to be the more "primitive" of the two.

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