Volume 9 Number 17 1981 Nucleic Acids Research

The nucleotide sequence of the $tRNA_M^{Met}$ from the archaebacterium Thermoplasma acidophilum

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Received 3 July 1981

ABSTRACT

Using in vitro labelling techniques, a $tRNA_M^{Met}$ from Thermoplasma acidophilum, a member of the Archaebacteriae, has been shown to have the sequence: pGCCGGG $Gs^4UGGCUCANCUGGAGGAGC\,m_2^2GCCGGAC_mUCAUt^6AAUCCGGAGGUCUCGGG$ $\Psi\Psi C_mGAUCCCCGAUCCCGGCACCA_{OH}$. Despite the small genome size of this non-parasitic organism, eight modified nucleosides are present, one of which is typically eubacterial, one of which is typically eukaryotic and some of which appear to be unique to the archaebacteria. There is no close sequence homology between this tRNA and that of any other methionine tRNA so far sequenced (<70%) but it has almost 90% homology with the nucleotide sequence proposed by Eigen and Oswatitsch for the ancestral quasi-species.[1]

INTRODUCTION

Thermoplasma acidophilum, currently classified in a genus of uncertain taxonomic position in the class of Mollicutes (the Mycoplasma),[2] has recently been shown to possess characteristics expected for a member of the kingdom Archaebacteriae[3] and is thus probably not even a member of the True-Bacteria (Eubacteria). T.acidophilum is the smallest non-parasitic organism known; it has a genome size of 1.0×10^9 daltons. We here describe the determination of the nucleotide sequence of the $tRNA_M^{Met}$ of T.acidophilum, this is the first sequence of any species of tRNA from an archaebacterium to be published although several tRNA sequences from some species of Halobacter and Sulpholobus acidocaldarius are available (C.R. Woese, U.L. RajBhandary and S. Nishimura, unpublished results).

MATERIALS AND METHODS

T. acidophilum cells were provided by Dr. D.G. Searcy and the $tRNA_M^{Met}$ was purified[4] and characterised[5] as previously described. Enzymes, radioactive materials, chemicals and most techniques were all as previously described.[6,7]

Sequence data was obtained (1) by analysis of the products obtained by complete T_1 - RNase digestion of the tRNA followed by 5'-labelling.[7] (2) by the use of 5'-^{32}P *in vitro* end-labelled tRNA by (a) nuclease P_1 treatment followed by electrophoresis and homochromatography[7] (b) rapid gel sequencing methods using RNase T_1 and U_2 to locate the purines.[8] The individual pyrimidines could not be resolved satisfactorily (3) by the method of Stanley and Vassilenko[9] as modified by Tanaka *et al*.[10] (4) by determination of the nucleotide composition of the tRNA as previously described.[6]

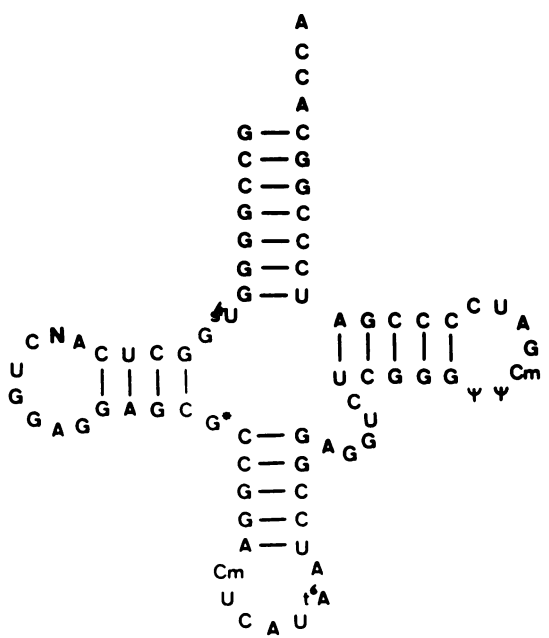
RESULTS

1. Nucleotide composition. Two-dimensional TLC of the [^{32}P]Np spots resulting from a T_2-RNase digest of the tRNA followed by incubation with polynucleotide kinase in the presence of γ-[^{32}P]ATP, showed that besides the four major nucleoside diphosphates; pΨp, pm_2^2Gp, pt^6Ap, pC_mpGp, pC_mpUp and one unknown nucleoside diphosphate pNp were present. Treatment of the mixture with P_1 - nuclease revealed the presence of the four major nucleotides and pΨ, pm_2^2G, pt^6A, pC_m and pN. The positive identification of these modified nucleoside derivatives together with the evidence for the existence of s^4U-8, the establishment of which are crucial from a phylogenetic standpoint, is reported elsewhere.[5] Other than the fact that N-15 is likely to bear a positive charge, we can provide no further evidence for its structure.

2. Total sequence. The total sequence of this methionine tRNA is shown in the figure and this unequivocal structure was obtained and confirmed, by a combination of all the techniques described above.

DISCUSSION

It should be emphasised that this tRNA is recognised by the *E. coli* methionyl-tRNA synthetase as are all the initiator tRNAs and the *E. coli* elongator, $tRNA_M^{Met}$. This now means that apart from the few "invariant" nucleotides which are present in almost all tRNAs and the presence of the anticodon CAU, the only element of primary sequence which all these tRNAs have in common is the pair C-3 : G-70. This makes it extremely doubtful that it is any feature of the primary sequence which dictates directly tRNA - synthetase recognition. It is somewhat surprising that this tRNA should be recognised at all by this enzyme as it is not usual to obtain efficient heterologous charging of tRNAs, other than initiator tRNAs, between species which are phylogenetically very distant. Here however, under normal charging conditions, it has proved possible



to get the tRNA fully charged. There is also another methionyl tRNA in T. acidophilum which, unlike this tRNA described here, can be formylated in an E. coli system and is thus presumably the initiator tRNA (Y. Kuchino and S. Nishimura, personal communication). This tRNA has less than 65% homology with the sequence described here which is thus presumably the elongator tRNA used for putting methionine into internal positions in polypeptides.

The sequence 53-56 of G$\Psi\Psi$C$_m$, although coming from the same DNA sequence as that giving rise to the G$T\Psi$C sequence normally found, is modified in a way which is typical for an archaebacterium. The unknown residue N-15 has almost certainly been located in an identical position in several tRNA species from the archaebacterium, Halobacter volcanii (R. Gupta, personal communication). As a modification in this position is unknown in any other tRNA species so far reported, it would seem likely that this modification is also typical of an archaebacterium.

Of the other modifications identified, C$_m$-32 and t^{6}A-37 are found in both eubacterial and eukaryotic tRNAs whereas s^{4}U-8 is a typical eubacterial modification and m$_2^2$G-26 is a typical eukaryotic modification.[11]

Only a few $tRNA_M^{Met}$ sequences have been reported and their homology is not too high. Thus it is difficult to compare this sequence with others to obtain phylogenetic data as none has a homology of greater than 70% with the sequence described here. Recently however, Eigen and Winkler-Oswatitsch[1] have proposed a nucleotide sequence for a tRNA representing the ancestral quasi-species. It is interesting that the $tRNA_M^{Met}$ from T. acidophilum differs in only 9 residues from this sequence and thus shows considerably more homology to this sequence than to any other sequence of an existing tRNA. Thus perhaps T. acidophilum is phylogenetically a very old organism.

ACKNOWLEDGEMENTS

We are grateful to Dr. D.G. Searcy for providing T. acidophilum cells and to Mr. J. Simmons for technical assistance. We also thank Professor K. Kleppe for a gift of polynucleotide kinase and Professor G.G. Brownlee for a gift of t^6Ap. This work was supported by a grant from the Science Research Council, M.W.K. is a recipient of a CASE award with the P.H.L.S. Mycoplasma Reference Laboratory and we are very grateful to Dr. R.H. Leach for his advice and encouragement.

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