STRUCTURE-FUNCTION CORRELATIONS OF FORMYLMETHIONINE TRANSFER RNA*

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Received May 28,1974

SUMMARY : Preliminary structural analysis of formylmethionine tRNA from the blue-green alga, *Anacystis nidulans*, supports previously postulated structure-function relationships of prokaryotic initiator tRNA species. The T_1 ribonuclease digestion of the purified formylmethionine tRNA yields the oligonucleotide TΨCAAAU(5C,U)G consistent with prokaryotic protein initiation by a formylated methionine tRNA species. The oligonucleotide sequences pCG and $CCACCA_{OH}$ indicate that the 5' terminal base is not paired to the 3' extremity. This structural feature shared by the *E.coli* formylmethionine tRNA has been correlated to this tRNA's lack of interaction with the bacterial elongation factor T. The absence of this base pair in spite of a base change during the independent evolution of bacteria and blue-green algae ($C\underline{A}ACCA_{OH}$ in *E.coli* and $C\underline{C}ACCA_{OH}$ in *A. nidulans*) reflects a strong biological advantage to this feature, which is consistent with an essential function.

Initiation of protein synthesis in most prokaryotes and prokaryote-like organelles involves formylmethionyl-tRNA (1). In contrast, polypeptide initiation in the eukaryotic cytoplasm involves a specific methionyl-tRNA, which is not formylated *in vivo* (2). With the exception of higher plants, the eukaryotic initiator can be formylated *in vitro* by the *E.coli* transformylase (3,4). The transformylase of *A.nidulans* is able, however, to partially formylate Met-$tRNA_i^{Met}$ [1] of wheat germ (5).

Structural work on blue-green algae tRNAs in our laboratory was initiated to provide new prokaryotic sequences for comparison with other organisms. The ancient common ancestry of blue-green algae and

* This work was supported by the Medical Research Council of Canada.

1 Abbreviations used are : $tRNA_i^{Met}$, the initiator methionine tRNA from plant cytoplasm; $tRNA_F^{Met}$, the initiator methionine tRNA from prokaryotic organisms; G, guanosine 3'-phosphate; pC, cytosine 5'-3'-diphosphate, A_{OH}, adenosine.

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other organisms guarantees that all common (conserved) macromolecular sequences or structural elements result from strong selective forces acting against divergence. We now report preliminary sequence data on the $tRNA_F^{Met}$ from Anacystis nidulans, which lend credence to two structure-function correlations previously proposed for the prokaryotic initiator species : 1) that the presence of the sequence TΨCA is concomitant with the involvement of formylated methionyl-tRNA in the protein initiation mechanism (6-9) and 2) that the unpaired 5' terminal base is related to the non-recognition of this tRNA by the protein elongation factor T (10).

MATERIALS AND METHODS : Anacystis nidulans strain 626 was obtained from the Algal Culture Collection of the University of Indiana and was cultured in medium C of Kratz and Myers with the exception that the phosphate concentration was reduced to 5×10^{-5} M (11,12). A preculture was transferred to an erlenmeyer flask containing 200 ml of culture medium. A 5 % (v/v) mixture of CO_2 in air was bubbled continuously through the medium, which was placed between two flood lamps. After 12 hr, 10 mCi of carrier-free $[^{32}P]H_3PO_4$ (from New England Nuclear Corporation) was added and the culture was continued for 3 days. The tRNAs were isolated by phenol extraction, chromatography on BD-cellulose and ethanol precipitation (13). The methionine tRNA isoacceptors were prepared by the Tener procedure (14) and separated by chromatography on a RPC-5 column(5). The phenoxyacetylated Met-$tRNA_F^{Met}$ was deacylated by treatment with 1 M Tris-Cl (pH 8.0) at room temperature for 1 hr. The resulting $tRNA_F^{Met}$ was rechromatographed on a RPC-5 column. Procedures used in nucleotide sequencing were those of Sanger and coworkers as detailed by Barrell (15). T_1, T_2 and U_2 RNases were obtained from Sankyo Co. Pancreatic RNase was purchased from Schwartz Bioresearch Inc. Snake venom phosphodiesterase and bacterial alkaline phosphatase were obtained from Worthington Biochemical

Corp. Two-dimensional thin layer chromatography was performed on cellulose plates pretreated with saturated $(NH_4)_2SO_4:H_2O$ (1/9) and developed with 95 % ethanol:H_2O (7/3) in the first dimension (16) and isobutyric acid:0.5 N NH_4OH (5/3) in the second (17).

RESULTS : A purified sample of A.nidulans $tRNA_F^{Met}$ was subjected to a T_1 ribonuclease digestion. Fig. 1 is a diagram of the resulting two-

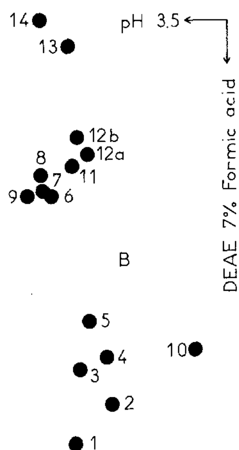

Fig. 1 Diagram of the T_1 ribonuclease digest of A.nidulans $tRNA_F^{Met}$ after two dimensional electrophoresis.

dimensional fingerprint. The spot 9 after treatment with T_2 ribonuclease and electrophoresis on Whatman No. 1 paper in an EDTA buffer gave G and pC. In absence of EDTA, pC produced a long streak typical of nucleoside diphosphate. When spot 9 was previously treated with alkaline phosphatase, only C was obtained. This evidence in addition to the position of the spot in the T_1 fingerprint establishes the sequence as pCG.

The spot 10, which is readily identifiable as the 3' terminal oligonucleotide by its position in the fingerprint, produced 4C and 1A by T_2 ribonuclease digestion. Complete digestion with venom phosphodiesterase, without previous treatment by alkaline phosphatase, gave 2A

and 3C again suggesting the 3' terminal position of this oligonucleotide. The sequence $CCACCA_{OH}$ was established by a U_2 ribonuclease digestion which produced the fragments CCA and CCA_{OH}.

The spot 14 is the only oligonucleotide in the fingerprint containing thymidine. On T_2 ribonuclease digestion, 6C, 3A, 2U, 1T, 1Ψ and 1 G were produced. T and Ψ were identified by their mobility upon two-dimensional thin layer chromatography. This spot was further analyzed by pancreatic ribonuclease which produced AAAU, 6C, 1U, 1Ψ, 1T, and 1G. U_2 ribonuclease degradation gave one minor product TΨC and two major fragments : (5C,2U)G and TΨCA, although the order TΨC has not been rigorously proven. The generation of the oligonucleotides AAAU, TΨCA, and (5C,2U)G by the different ribonucleases establishes the partial sequence TΨCAAAU(5C,U)G. In the pancreatic ribonuclease digest of $tRNA_F^{Met}$, however, the position of the oligonucleotide AAAU in the fingerprint (not shown here) indicates that one of the A's may be methylated.

DISCUSSION : Recently, two structure-function correlations implicating the prokaryotic initiator species have been advanced based on the comparison of known tRNA sequences. Firstly, it was proposed that the sequence TΨCA present in *E.coli* $tRNA_F^{Met}$ is concomitant with the formylmethionyl-tRNA mode of protein initiation (6,8). Support for this hypothesis derives from the absence of this sequence in the eukaryotic initiator which is replaced by AUCG (6,18,19), and the recent evidence that the initiator tRNA of the prokaryote, *S.faecalis*, grown in the absence of folate, is not formylated *in vivo* and does not contain ribothymidine (9). Our finding that the $tRNA_F^{Met}$ of *A.nidulans* contains the sequence TΨCA and is a formylated species (5) is directly pertinent to this correlation, since the sequence of no other prokaryotic initiator species is presently known. The fact that such phylogenetically distant species as blue-green algae and

bacteria show the correlation is strong evidence for the generality of the hypothesis.

Secondly, the exclusive feature of E.coli $tRNA_F^{Met}$ of having a 5' terminal base which cannot be paired to the 3' extremity has been correlated to the non-recognition of this tRNA by the bacterial elongation factor T (10). As supporting evidence, the $tRNA_F^{Met}$ can be recognized by the T factor after being subjected to a bisulfite modification which produces a paired 5' terminal base. The sequences of spots 9 and 10 from the algal $tRNA_F^{Met}$ fingerprint have been established as pCG and $CCACCA_{OH}$, the 5' and 3' extremities respectively. The arrangement of the stem region assuming the cloverleaf conformation is thus, $\begin{matrix} GCp \\ CCACCA_{OH} \end{matrix}$. As in the E.coli $tRNA_F^{Met}$ (20), the 5' terminal base of A.nidulans $tRNA_F^{Met}$ is not paired. This result is in full agreement with and strongly supports the contention that the lack of this base pair is linked to the non-recognition of formylmethionyl-tRNA by T factor. Indeed the most surprising aspect of these results is that, during the independent evolution of E.coli and A.nidulans $tRNA_F^{Met}$, a base change has taken place at the 3' extremity in one of the branches. That this base change did not lead to a paired base indicates a strong selective pressure against base-pairing at this position, and it is feasible that this selection is based on the structural requirement not to be recognized by the elongation factor.

At present, work is continuing on the determination of the total sequence of this algal initiator tRNA. It is already clear from our preliminary results that the A.nidulans $tRNA_F^{Met}$ is very similar to that of E.coli, perhaps more so than other homologous tRNA families.

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