THE PRELIMINARY SEQUENCE OF $tRNA_F^{Met}$ FROM *ANACYSTIS NIDULANS* COMPARED WITH OTHER INITIATOR tRNAs

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1. Introduction

One methionine tRNA species occupies an honored position among tRNAs due to its unique function during the initiation of protein synthesis. This initiator species, which in prokaryotes is generally formylated in vivo [1,2], interacts with proteins and factors showing no affinity for other tRNAs [3]. One might expect that because of these interactions the selective forces on the molecular evolution of this species are also unique. In some respects this hope has been fulfilled in our previous report on the sequence of T_1 RNase-generated fragments of the initiator $tRNA_F^{Met}$ isolated from the blue-green alga, *Anacystis nidulans* [4]. We were able to show that, as was found in the *E. coli* $tRNA_F^{Met}$, the 5′ terminal base was not base paired to the 3′ stem, a feature which now seems to be unique to prokaryotic initiator tRNAs [6–8]. This structural feature is apparently responsible for the non-recognition of the initiator tRNA by the elongation factor T [9] and the peptidyl-tRNA hydrolase [10]. Since bacteria and blue-green algae are only distantly related it could be predicted that any identical structure must be very conservative to evolutionary change.

We now present the preliminary nucleotide sequence of the $tRNA_F^{Met}$ of *Anacystis nidulans* and compare it with other known sequences of initiator tRNAs.

2. Materials and methods

Anacystis strain 625 was obtained from the Algal Culture Collection of the University of Indiana and was cultured in the presence of [^{32}P]phosphate as described previously [4]. The [^{32}P]methionine tRNAs were isolated using the procedure of Gillam et al. [11] and separated by RPC-5 column chromatography [12]. Aminoacylation of algal methionine tRNA was performed by the *E. coli* aminoacyl-tRNA synthetase [12]. Oligonucleotide sequencing was performed using the techniques developed by Sanger and co-workers as detailed by Barrell [13]. 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.

T_1 and pancreatic RNase partial digestion products were deposited as a line on a strip of cellulose acetate and were fractionated by electrophoresis. The nucleotides were then transferred and concentrated onto DEAE-cellulose thin-layer plates, which were developed by homochromatography mixture b [13]. T_2 RNase products of tRNA and oligonucleotides were characterized by two-dimensional thin-layer chromatography on cellulose plates. The solvents were isobutyric acid–0.5 M NH_4OH (5:3, v/v) followed by concentrated HCl–isopropanol–water (15:70:15, v/v/v) [14]. Details of the purification and the structural determination of $tRNA_F^{Met}$ of *Anacystis* will be described elsewhere.

3. Results

The fingerprint of the T_1 RNase digest of *Anacystis* $tRNA_F^{Met}$ is shown in fig.1. The tRNA was isolated by RPC-5 chromatography of the phenoxyacetylated methionyl-tRNA followed by RPC-5 chromatography

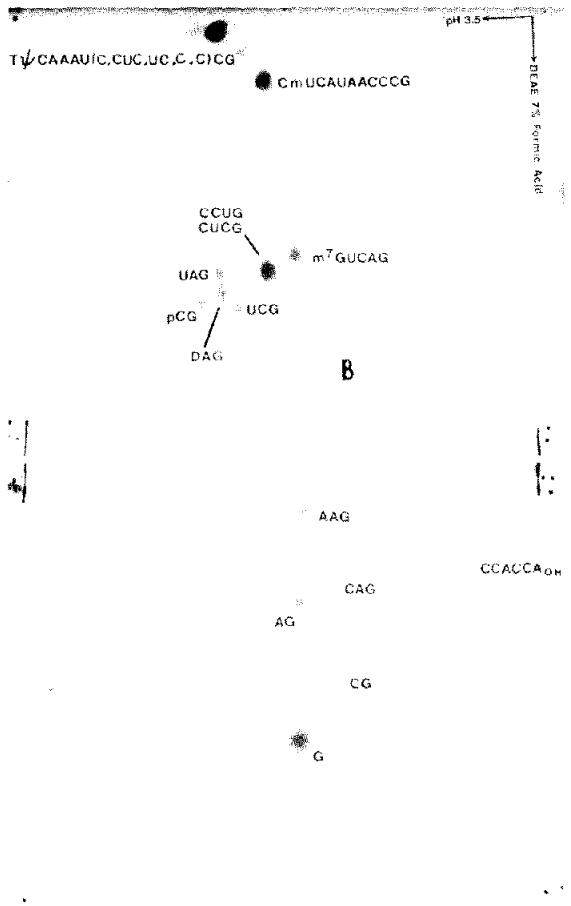

Fig.1. T1 ribonuclease fingerprint of $tRNA_F^{Met}$ of *Anacystis nidulans*.

of the stripped tRNA. This material was judged 95% pure by quantification of the radioactivity that ran in the position of pG and UG, two products known to be absent in the $tRNA_F^{Met}$ sequence. The spot 12b previously reported is now known to be a contaminant and is, therefore, absent in this fingerprint of highly purified tRNA [4]. The exact order of the pyrimidine stretch in the long oligonucleotide containing TψCA has not been rigorously proven by enzymatic digestion. All oligonucleotides of the pancreatic RNase digest have been established however.

Further characterization of the sequence was performed by partial digestion of the tRNA by both T_1 and pancreatic RNases. The structure of large oligonucleotides was determined by subsequent T_1, T_2 and pancreatic RNase analysis. The reconstruction of this data permitted the derivation of the sequence. Unfortunately we were unable to unambiguously establish the positions 11–13, 17–18 and 25–26. The order of these nucleotides were deduced from constraints imposed by the cloverleaf model.

The *Anacystis* $tRNA_F^{Met}$ is 77 nucleotides in length and contains five modified nucleotides: D, T, ψ, m^7G and C_m. Whereas 4-thiouridine occupies position 8 in *E. coli* $tRNA_F^{Met}$ [5], no evidence for this modified nucleoside has been found in *Anacystis* even after isolation of the tRNA in the presence of thiosulfate [15]. *Anacystis* $tRNA_F^{Met}$ is homogenous both with regard to nucleotide sequence and base modification, although the *E. coli* initiator is heterogenous in one position of the sequence [5].

As pointed out in a previous article [4], *Anacystis* $tRNA_F^{Met}$ possesses the two striking features shared by *E. coli* $tRNA_F^{Met}$: the absence of the base pair between the 5′ terminal nucleotide with the 3′ stem and the presence of the subsequence GTψCA, In fig.2 we compare the *Anacystis* sequence with that of *E. coli*. The positions that are different in the two sequences have been inclosed in boxes.

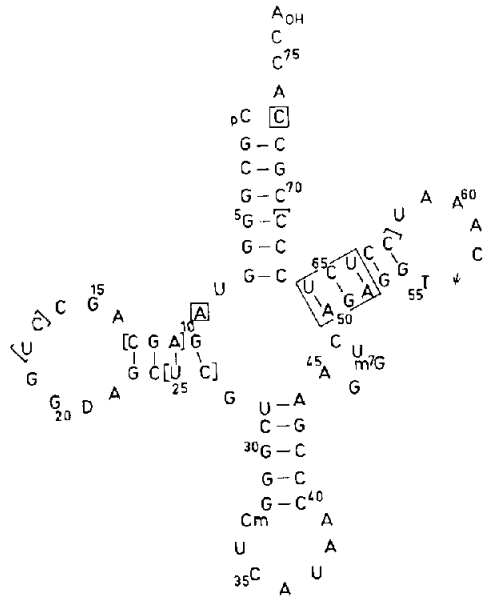

Fig.2. The Preliminary sequence of $tRNA_F^{Met}$ of *Anacystis nidulans*. Positions enclosed in boxes are those that differ from the sequence of *E. coli* $tRNA_F^{Met}$. Nucleotide sequences enclosed in brackets have not been experimentally proven.

4. Discussion

With the algal sequence presented here, three formylmethionine tRNA sequences from widely divergent prokaryotes are now available for comparison [5,8]. The three prokaryotic sequences have a non base-paired 5′ terminal nucleotide. This structural anomaly is likewise present in the initiator tRNA sequences of *mycoplasma* [7] and *S.faecalis* [6], although it is absent in all other known tRNA sequences including eukaryotic initiator sequences. We feel this strengthens our earlier hypothesis on the strict conservation of this structural element [4] because of its involvement is an important function of the molecule [9,10]. In addition prokaryotic initiator sequence comparison indicates that the stem of the TψC loop is most subject to evolutionary change.

In comparing all initiator tRNAs the following remarks can be made: (1) The majority of base changes are in the base-paired regions in spite of the fact that these mutations generally imply a second base change in the opposite strand. (2) Transitional mutations are favored over transversions. In other words, mutations which respect the purine or pyrimidine nature of a given position are more easily fixed. This observation is identical to the case of 5S RNA evolution [16]. (3) The algal sequence has an adenosine in position 9. The A_9 is novel to initiator tRNAs, but is present in non-initiator $tRNA_F^{Met}$ sequences. The presence of A_9 may be partially responsible for the thermal lability of the algal tRNA, since this position is directly involved in at least two tertiary interactions [17,18].

The matrix of sequence differences between each sequence is given in table 1. Thus, the similarity of the three prokaryotic sequences is astonishing (8 and 9/77 differences with *E. coli* and *Bacillus* respectively), especially if one considers fossil data which indicate that a common ancestor is 3 billion years old [21]. It would seem from this data that taxonomically *E. coli* and *Bacillus* are more related to each other than to blue-green algae. This hypothesis is in disagreement with results obtained from the T_1 RNase-generated catalogs of ribosomal RNA showing a closer relationship between algae and *Bacillus* than between *Bacillus* and *E. coli* [22]. It should be noted, however, that both hypotheses assume a relatively constant fixation rate of mutations for each class of RNA.

It is curious that the number of base differences between prokaryotic initiator tRNAs is considerably smaller than the number between eukaryotes. Also the distance between prokaryotic and eukaryotic initiator tRNAs seems inordinate. In fact using a test of similarity that we previously developed [23], the two families are no more related to each other than random tRNA sequences i.e. random sequences respecting constant positions developed from known tRNA sequences.

One possible explanation is that the genesis and early evolution of the eukaryotic cell and its 80S ribosome was concomitant with an accelerated rate of tRNA molecular evolution. It is equally possible that the eukaryotic and prokaryotic families of $tRNA_F^{Met}$ have an independant evolutionary origin.

Finally, the prokaryotic $tRNA_F^{Met}$ sequences show the lowest fixation rate of natural mutations among macromolecules. $tRNA_F^{Met}$ is approximately 5 times more resistant to evolutionary change than eukaryotic histones or ferrodoxins [24]. With regard to this evolutionary conservation, the sequences of initiator tRNAs from chloroplasts, mitochondria and eukary-

Table 1
Sequence differences[a] between initiator tRNAs

Source	*A. nid*	*E. coli*	*B. sub*	Yeast	Rabbit liver
A. nidulans	0	8	9	25	26
E. coli		0	6	21	23
B. subtilis			0	24	22
yeast (19)				0	16
rabbit liver (ZO)					0

[a] Insertions or deletions of one or more nucleotides were counted as one difference.

otic protista would be extremely valuable in the deduction of their evolutionary past.

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