THE PRIMARY STRUCTURE OF A NON-INITIATING METHIONINE SPECIFIC tRNA FROM BREWER'S YEAST

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1. Introduction

Methionine specific tRNAs, because of a number of unique features due to their role in protein biosynthesis, are a most interesting family of isoacceptors. In any organism so far investigated, at least two species of $tRNA^{Met}$ are present, the structures of which have been adapted for their participation in peptide chain initiation ($tRNA_f^{Met}$) and peptide chain elongation ($tRNA_m^{Met}$); for a recent review see [1].

The primary structures of a variety of methionine specific tRNAs ($tRNA_f^{Met}$ [2] and $tRNA_m^{Met}$ [3] from *E. coli*, $tRNA_f^{Met}$ from baker's yeast [4], $tRNA_f^{Met}$ [5,6] and one $tRNA_m^{Met}$ [7] from a mammalian source) have been elucidated. The information obtained has been discussed with regard to structure–function relationship, e.g. [5,6] and evolutionary aspects e.g. [6]. We reported earlier [8], that brewer's yeast contains three major species of $tRNA^{Met}$, one of which ($tRNA_1^{Met} = tRNA_f^{Met}$) has been shown to function as an initiator tRNA [8], while the two others ($tRNA_2^{Met}$ and $tRNA_3^{Met}$) are involved in peptide chain elongation. In contrast, baker's yeast [9,10] and a haploid yeast strain [8] have only one non-initiator tRNA, which seems to correspond to $tRNA_3^{Met}$ (brewer's yeast).

We want to report here on the primary structure of $tRNA_3^{Met}$ (brewer's yeast) which can be considered to be a 'missing link' for structural comparison within the $tRNA^{Met}$ species from *E. coli*, yeast, and mamalian sources. Some interesting features were found in $tRNA_3^{Met}$ (brewer's yeast) that will be briefly discussed with respect to the following topics: (i) sequence homologies in $tRNAs^{Met}$, (ii) synthetase recognition, (iii) conservation of structural features in $tRNAs^{Met}$.

2. Experimental

S. cerevisiae, strain C 836, was cultured in a minimal medium and labeled with [^{32}P]phosphate as described in [11]. One procedure employed in purification of $tRNA_3^{Met}$ was essentially similar to that used in the isolation of non-labeled $tRNA_3^{Met}$ from brewer's yeast [8]. A second, simplified procedure to isolate labeled $tRNA_3^{Met}$ was worked out concomitantly with the structural work reported here and will be detailed in a subsequent publication. In general, the methods for determining the nucleotide sequence were the standard procedures as described in [12] and [13]. Oligonucleotides from partial digestions with RNase T_1 were resolved by homochromatography [13] and by two-dimensional gel electrophoresis [14].

3. Results and discussion

3.1. *Primary structure of $tRNA_3^{Met}$*

The primary structure of $tRNA_3^{Met}$ (brewer's yeast), drawn as a clover-leaf, is shown in fig.1(A). The nucleotide sequence was derived by some analyses on non-labeled tRNA, and mainly be the ^{32}P-fingerprinting method [12]. Complete overlaps in the nucleotide sequence could be deduced from the oligonucleotides of digestions with RNase T1 and RNase A, respectively, and from 18 products (out of 29 analyzed) of

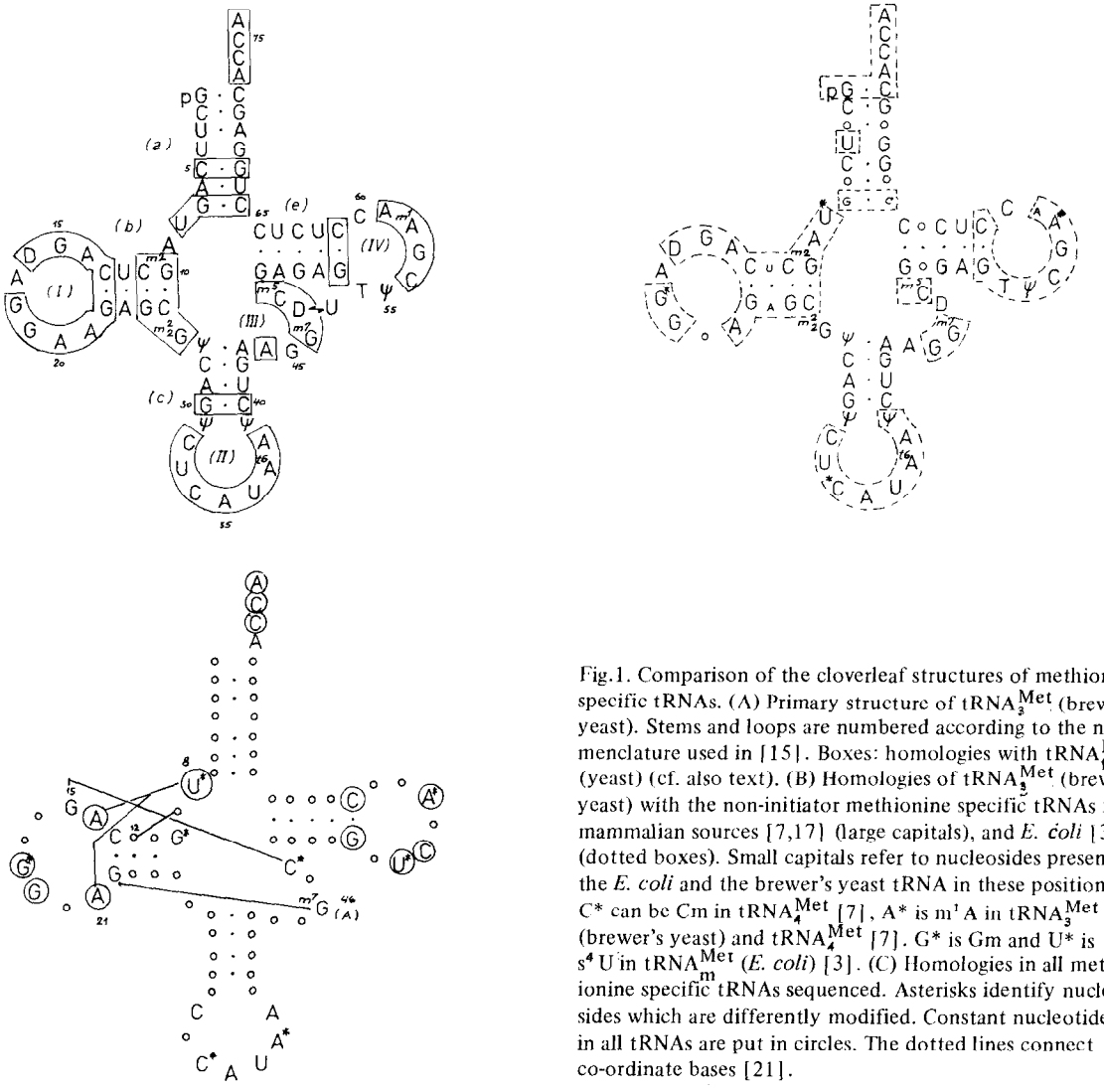

Fig.1. Comparison of the cloverleaf structures of methionine specific tRNAs. (A) Primary structure of $tRNA_3^{Met}$ (brewer's yeast). Stems and loops are numbered according to the nomenclature used in [15]. Boxes: homologies with $tRNA_f^{Met}$ (yeast) (cf. also text). (B) Homologies of $tRNA_3^{Met}$ (brewer's yeast) with the non-initiator methionine specific tRNAs from mammalian sources [7,17] (large capitals), and *E. coli* [3] (dotted boxes). Small capitals refer to nucleosides present in the *E. coli* and the brewer's yeast tRNA in these positions; C* can be Cm in $tRNA_4^{Met}$ [7], A* is m^1A in $tRNA_3^{Met}$ (brewer's yeast) and $tRNA_4^{Met}$ [7]. G* is Gm and U* is s^4U in $tRNA_m^{Met}$ (*E. coli*) [3]. (C) Homologies in all methionine specific tRNAs sequenced. Asterisks identify nucleosides which are differently modified. Constant nucleotides in all tRNAs are put in circles. The dotted lines connect co-ordinate bases [21].

partial digestions with RNase T_1. The details of sequencing will be fully documented in a further publication.

$tRNA_3^{Met}$ (brewer's yeast) is 76 nucleotides in length, possessing 13 modified nucleosides (4 ψ, 2 D, T, m^1A, m^2G, m_2^2G, m^7G, m^5C, and t^6A). In the sequence analyses most of these modifications were found to be complete except the following: D in position 47 was only present in 75%, the rest was found to be U; the bases in $m_2^2Gp\psi p$ (positions 26 and 27) were found to be modified to only 50%, the rest was GpUp.

3.2. *Comparison of $tRNA^{Met}$ sequences from yeast*

The sequence of $tRNA_3^{Met}$ (brewer's yeast) is largely different from that of the initiating $tRNA^{Met}$ (brewer's yeast), which was found to have the same nucleotide sequence [16] as that from baker's yeast [4] (fig.1(A)). Sequence homologies in $tRNA_3^{Met}$ and $tRNA_f^{Met}$ are present in only 42 positions, these including a number of nucleotides in the D-loop (I) and in the D-stem (b), the sequence -m^7G-D-m^5C- in the extra loop (III), the last four nucleotides at the acceptor end, and the positions of m^2G and m_2^2G. Many of these similarities, however, reside in positions in which certain nucleo-

sides were found to be constant in all tRNAs. Also, $tRNA_f^{Met}$ and $tRNA_m^{Met}$ *(E. coli)* were found to have no greater similarities with each other in sequence than with other tRNA sequences from the same organism [3]. The most obvious dissimilarities between $tRNA_3^{Met}$ and $tRNA_f^{Met}$ (in brackets) are: the D-loop (I) contains 8 (7) nucleotides, there is no unusual dinucleotide (G*pA*p) [4] in stem (e), loop (IV) has the 'normal' construction TpψpCpGp (ApUpCpGp), there are two pseudouridine nucleotides in opposite positions at the base of stem (c).

3.3. *Comparison of sequences of non-initiator $tRNA^{Met}$ species from different organisms*

Fig.1(B) shows the sequence homologies between $tRNA_3^{Met}$ (brewer's yeast), the $tRNA_m^{Met}$ species of *E. coli* [3] and $tRNA_m^{Met}$ species from two mammalian sources [7,17]. The latter two tRNAs seem to have identical sequences [7] like the initiator tRNAs of mammalian origin [6]. It is obvious that the sequence variations between *E. coli* and yeast $tRNAs_m^{Met}$ are greater than between yeast and mammalian $tRNAs_m^{Met}$. In the first case, the following similarities are found: the end of the acceptor stem (a), the complete D-stem (b), some of the positions within the D-loop (I) (although $tRNA_m^{Met}$ *(E. coli)* possesses 9 nucleotides in this loop instead of 8 in eukaryotic $tRNAs_m^{Met}$), the anticodon loop (II), and most of the TψC-loop (IV).

The eukaryotic $tRNAs_m^{Met}$ display great similarities: the anticodon arms and the extra loops (III) are identical, only one position (20) is differing in the D-loop (I) and one (59) in the TψC-loop (IV). Altogether 5 base pairs are different in the acceptor stem (a), in the D-stem (b), and in the TψC-stem (e). A unique feature is the occurrence of two pseudouridine nucleotides in opposite positions at the base of the anticodon stem (c), which so far were only found in the eukaryotic non-initiator $tRNAs^{Met}$ [7,17].

3.4. *Recognition sites and evolutionary aspects*

It has been shown that *E. coli* methionyl tRNA synthetase can charge not only the homologous $tRNAs^{Met}$, but also the $tRNA^{Met}$ species from yeast e.g. [8–10] and eukaryotic initiator tRNAs [6,7,18, 19], exceptions being the non-initiating $tRNAs^{Met}$ from reticulocytes [18], wheat germ [19], and from rabbit liver, e.g. [17]. Extended studies on heterologous charging of $tRNAs^{Met}$ with methionine specific synthetases from eukaryotic sources have not been reported.

However, from a comparison of the primary structures of all $tRNAs^{Met}$ so far known (fig.1C) it becomes apparent, that these do not exhibit common features except the following: (i) the constant nucleotides found in similar positions in all tRNAs, (ii) nearly the whole anticodon loop, (iii) the fourth nucleotide from the 3′-end (being Ap), (iv) two base pairs (including the Levitt base pair [20]), and m^7G.

It therefore seems to be justified to conclude that in the case of the methionine specific tRNAs from a number of different organisms charged with *E. coli* methionyl-tRNA-synthetase no homologous sequences (except part of the anticodon loop and the ApCpCpA-end) could be involved in recognition.

It is also obvious from this comparison (fig.1C), that all $tRNAs^{Met}$ belong to the class I type of tRNAs [20] with variations of ± 1 nucleotide in the D-loop (I) and that two out of the three postulated base triples [21], namely C13, G22, m^7G46 and U8,A14, A21 remain constant in this group of tRNAs, while the third possible base triple could vary: A9,U12,A23 ($tRNAs_m^{Met}$ in *E. coli* and yeast), G9,G12,C23 or m^1G9, G12,C23 in the initiator tRNAs, and A9,G12,C23 (?) in $tRNA_4^{Met}$ (myeloma). Co-ordinate basis of this type, however, are not unique to methionine specific tRNAs, but are similarly found in many other specific tRNAs of the class I type in different organisms [21, 22]. It therefore remains to be seen, whether variations of such elements determining the tertiary structure of tRNA could contribute to specificity in recognition processes.

From the comparison of the $tRNA_f^{Met}$ and $tRNA_m^{Met}$ sequences, (especially those from yeast) it is also impossible to detail which features specify whether a certain $tRNA^{Met}$ will function in initiation or elongation of protein synthesis.

It has been suggested [7] that the different sequences of loop (I) and loop (IV) as well as those of the stems (a), (c) and (e) in the mammalian type $tRNAs^{Met}$ may be important in discriminating their functions. This observation also holds largely for the $tRNAs^{Met}$ from brewer's yeast (see section 3.2. and fig.1A). In this context, it is again to be noted that the anticodon arm and loop (IV) seem to have been conserved in the eukaryotic non-initiator tRNAs (fig.1B), so that

these two regions might play an important role in specifying the function of these tRNAs in peptide chain elongation.

There is further indication that certain features of the tRNA sequences in eukaryotes have been stabilized during evolution. This has been emphasized especially for the initiator tRNAs [5,6]. A similar situation appears to exist for the non-initiator $tRNAs^{Met}$.

It would be interesting to include in such comparisons also the primary structures of the iso-accepting non-initiator $tRNAs^{Met}$ from eucaryotes [7,8]. However, the sequences have not yet been determined, and it is also unknown why these multiple tRNA species do occur.

It has not escaped our attention that $tRNA_3^{Met}$ (brewer's yeast) contains the nucleotides found in equivalent positions in those tRNAs that are substrates for homologous or heterologous charging with phenylalanine by phenylalanyl tRNA synthetase from yeast [23].

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