

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 (tRNA₁^{Met} = tRNA_f^{Met}) has been shown to function as an initiator tRNA [8], while the two others (tRNA₂^{Met} and tRNA₃^{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 tRNA₃^{Met} (brewer's yeast).

We want to report here on the primary structure of tRNA₃^{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 mammalian sources. Some interesting features were found in tRNA₃^{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 [³²P]phosphate as described in [11]. One procedure employed in purification of tRNA₃^{Met} was essentially similar to that used in the isolation of non-labeled tRNA₃^{Met} from brewer's yeast [8]. A second, simplified procedure to isolate labeled tRNA₃^{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 T₁ were resolved by homochromatography [13] and by two-dimensional gel electrophoresis [14].

3. Results and discussion

3.1. Primary structure of tRNA₃^{Met}

The primary structure of tRNA₃^{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 by the ³²P-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

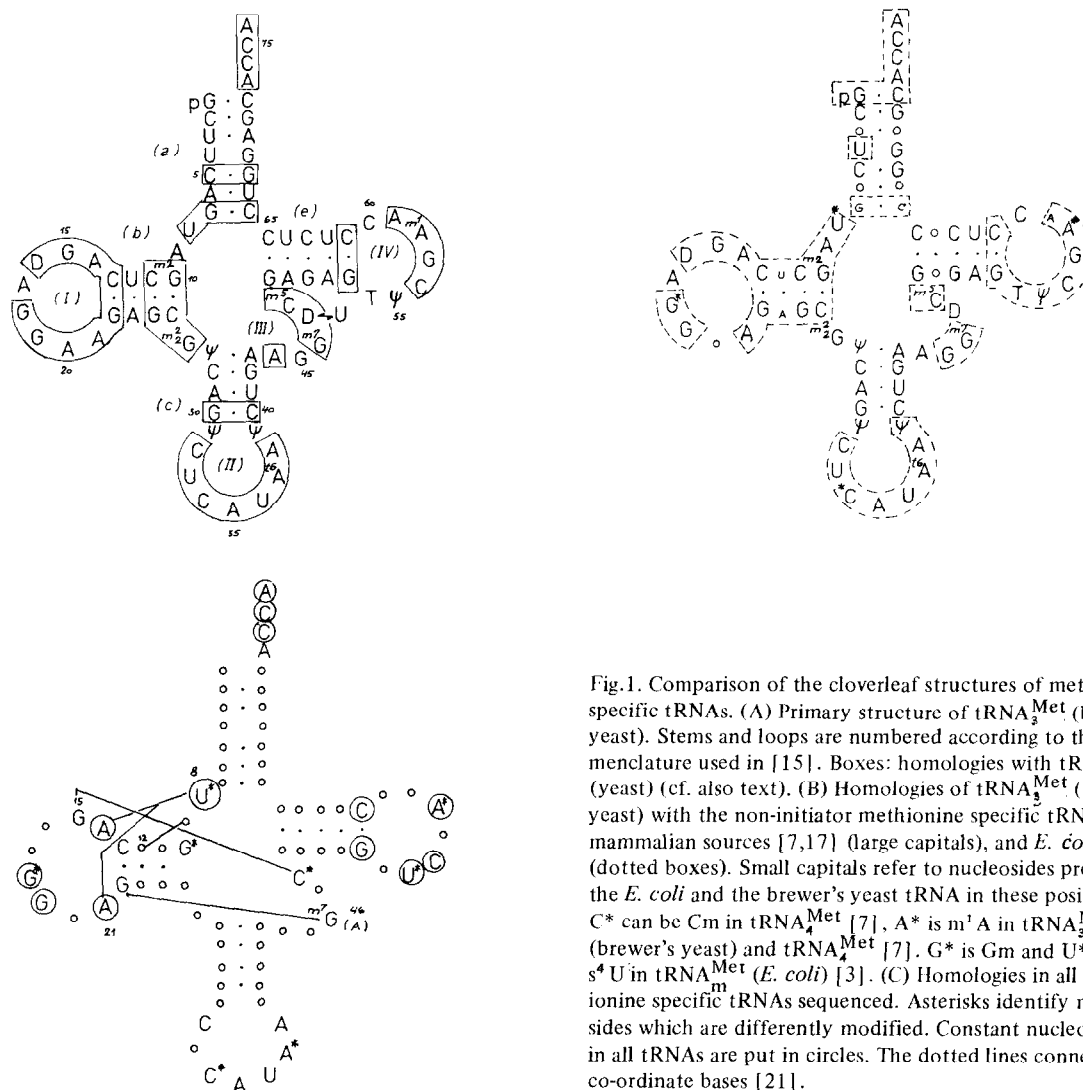


Fig.1. Comparison of the cloverleaf structures of methionine specific tRNAs. (A) Primary structure of tRNA_{Met}^b (brewer's yeast). Stems and loops are numbered according to the nomenclature used in [15]. Boxes: homologies with tRNA_{Met}^f (yeast) (cf. also text). (B) Homologies of tRNA_{Met}^b (brewer's 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_{Met}^f [7], A* is m¹A in tRNA_{Met}^f (brewer's yeast) and tRNA_{Met}^f [7]. G* is Gm and U* is s⁴U in tRNA_{Met}^m (*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 T₁. The details of sequencing will be fully documented in a further publication.

tRNA_{Met}^b (brewer's yeast) is 76 nucleotides in length, possessing 13 modified nucleosides (4 ψ, 2 D, T, m¹A, m²G, m²Gp, m⁷G, m⁵C, and t⁶A). 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 m²Gpψp (positions 26 and 27) were found to be modified to only 50%, the rest was GpUp.

3.2. Comparison of tRNA_{Met}^b sequences from yeast

The sequence of tRNA_{Met}^b (brewer's yeast) is largely different from that of the initiating tRNA_{Met}^b (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_{Met}^b and tRNA_{Met}^f 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 -m⁷G-D-m⁵C- in the extra loop (III), the last four nucleotides at the acceptor end, and the positions of m²G and m²G. 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 tRNA₃^{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 tRNA₃^{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 m⁷G.

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, m⁷G46 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 m¹G9, G12, C23 in the initiator tRNAs, and A9, G12, C23 (?) in tRNA₄^{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^{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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