# THE NUCLEOTIDE SEQUENCES OF CYTOPLASMIC METHIONINE AND VALINE tRNAS FROM MOUSE MYELOMA CELLS

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## 1. Introduction

The primary structure of the cytoplasmic initiator tRNA ($tRNA_f^{Met}$) of mammalian cells has recently been determined [1–4]. This tRNA has a structure specially adapted for its role of providing the N-terminal methionyl residue of newly synthesised polypeptides. $tRNA_f^{Met}$ is also discriminated against during the elongation phase of protein synthesis since Met-$tRNA_f^{Met}$ has no appreciable ability to donate methionine for polypeptide chain elongation. For comparison purposes it would be interesting to determine the primary structure of a $tRNA^{Met}$ which functions in protein elongation in mammalian cells, but which is totally inoperative as an initiator tRNA. We report here the nucleotide sequence of one such mammalian $tRNA^{Met}$ species. This tRNA was purified from mouse myeloma cells and it corresponds to the species which has been designated $tRNA_4^{met}$ [5,6] on the profile of the RPC-5 column chromatographic separation of the isoaccepting methionine tRNAs of mammalian cells. In addition we also report the primary structure of the major valine tRNA of mouse myeloma cells, since the structure of this tRNA was found unexpectedly to throw some light upon present knowledge concerning the structure–function relationship of the mammalian methionine tRNAs. Details of the sequencing of mouse myeloma $tRNA_4^{Met}$ and $tRNA^{Val}$ will be published elsewhere. As well as the nucleotide sequences discussed in this paper, the sequences of two other mammalian tRNAs, a rat liver $tRNA^{Ser}$ [7] and a rabbit liver $tRNA^{Phe}$ [8] have previously been reported.

## 2. Experimental

The post-microsomal supernatant fraction of mouse P3K plasmacytoma cells cultured in medium containing [$^{32}$P] phosphate [9] was a gift from Drs N. J. Cowan, T. Harrison, G. G. Brownlee and C. Milstein. The purification of $tRNA_4^{Met}$ and $tRNA^{Val}$ from this fraction was according to a procedure essentially similar to that used in the isolation of $tRNA_f^{Met}$ from the same source [4], as will be detailed in a subsequent communication. The procedures employed in the derivation of the nucleotide sequences of these two tRNAs were as described in [4, 10].

## 3. Results and discussion

Fig. 1(A) shows the nucleotide sequence of mouse myeloma $tRNA_4^{Met}$ drawn as a cloverleaf. There is very little structural resemblance between this tRNA and the corresponding $tRNA^{Met}$ which functions in protein elongation in *E. coli* [11] except within loop II, the anticodon loop. The primary structure of $tRNA_4^{Met}$ exhibits two unusual features. Not only is this the first tRNA which has been found to possess the minor nucleoside $m^2$ G within stem (a) of its cloverleaf, but in addition if possesses two pseudouridine nucleosides in opposite positions at the base of stem (c) (Fig. 1(A)). It is improbable that these nucleosides base-pair in the secondary structure of $tRNA_4^{Met}$, and this tRNA species therefore probably has a stem (c) comprised of 4 and not 5 base-pairs. A stem (c) of five base-pairs has



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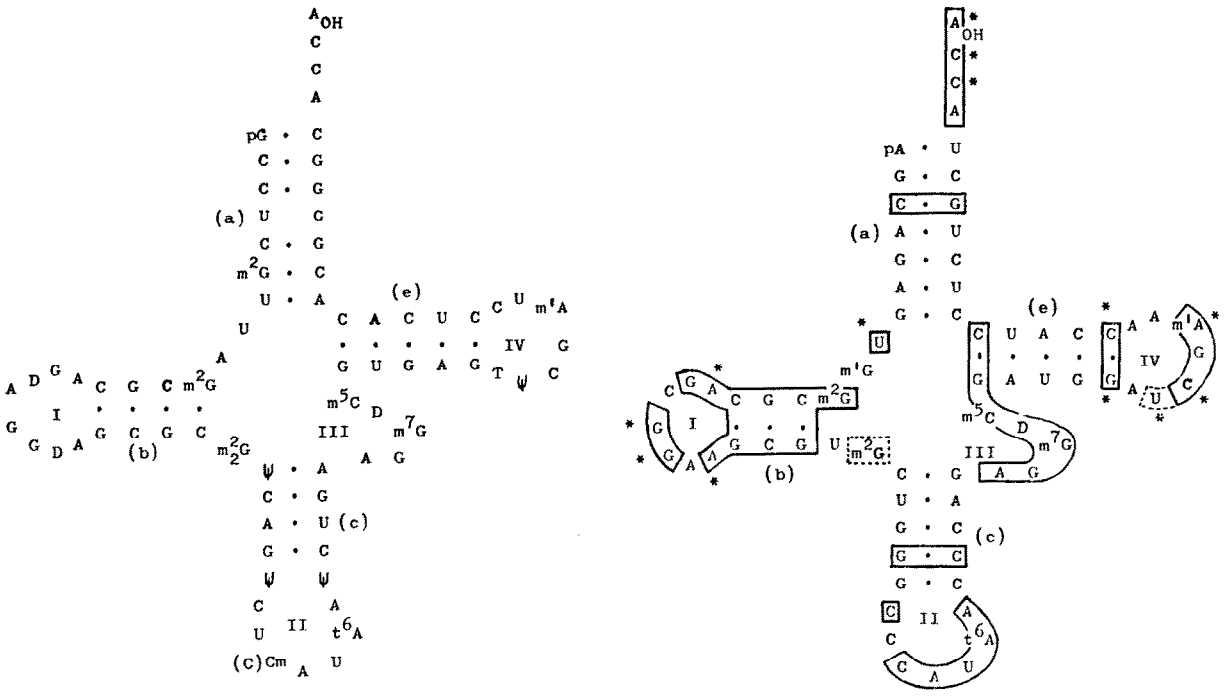

Fig. 1. Comparison of the cloverleaf structures of mammalian methionine tRNAs. A) $tRNA_4^{Met}$ (Mouse myeloma). The cytidine within the anticodon of this tRNA was found to incompletely methylated to Cm. B) $tRNA_f^{Met}$ (mouse myeloma and rabbit liver). Full boxes: regions common to the primary structures of $tRNA_4^{Met}$ and $tRNA_f^{Met}$. Dotted boxes: nucleotides which differ only with respect to base modification between $tRNA_4^{Met}$ and $tRNA_f^{Met}$. The nucleotides identified by an asterisk are those which are conserved in all eukaryotic tRNA of known primary structures. The base-paired stems of the cloverleaf are referred to in the text by the letters (a), (b), (c) and (e) and the non base-paired loops by the numbers I to IV, as indicated. Only standard Watson–Crick base-pairs of the cloverleaf are represented by dots.

always hitherto been found in tRNA structures [12], and it would therefore be of interest to investigate the effect of this feature upon the anticodon loop conformation of $tRNA_4^{Met}$.

Mouse myeloma $tRNA_4^{met}$ is 76 nucleotides in length, one nucleotide longer than the homologous $tRNA_f^{Met}$ [1–4] the sequence of which is illustrated in fig. 1(B). Also $tRNA_4^{Met}$ possesses 15 modified nucleosides whilst mammalian $tRNA_f^{Met}$ has only 8. Sequence analysis indicated that all the nucleoside modifications of $tRNA_4^{Met}$ were complete but for the methylation of the cytidine within the anticodon. This was only found to be 60–80% complete.

The sequences which are shared by mammalian $tRNA_f^{Met}$ and $tRNA_4^{Met}$ are shown in fig. 1(B). The two tRNA species compared here respectively function in the initiation or the elongation of mammalian protein synthesis. Certain of the homologous structural features of fig. 1(B) are common to all tRNAs of known primary structure [12]. Such constant features of tRNA architecture, however, do not include the substantial regions of stem (b), loop II and loop III conserved between the structures of mammalian $tRNA_f^{Met}$ and $tRNA_4^{Met}$. Thus this comparison of mammalian $tRNA^{Met}$ sequences indicates that stem (b), loop II and loop III of the tRNA cloverleaf do not directly specify whether a certain $tRNA^{Met}$ will function in the initiation of the elongation of protein synthesis in mammalian cells. In parts of loops I and IV as well as within stems (a), (c) and (e) (Fig. 1(B)) the $tRNA_f^{Met}$ and $tRNA_4^{Met}$ sequences display little similarity. These regions are probably important in determining the initiation or elongation function of a mammalian methionine tRNA. It is to be noted that the unusual loop IV sequence, –A–U–C–G–m′A–A–A–, of eukaryotic initiator tRNAs [1–4] was not found in this methionine tRNA which functions in protein elon-

gation in a mammalian cell type. This is consistent with the proposed importance of this loop structure in the structure-function relationship of mammalian initiator tRNA [1–4].

After we had determined the $tRNA_4^{Met}$ sequence we learned that the same sequence can be postulated from a knowledge of the complete RNase digestion products of a methionine tRNA from rabbit liver [13]. Evidently $tRNA_4^{Met}$ has the same nucleotide sequence both in mouse myeloma cells and in rabbit liver. The $tRNA_f^{Met}$ species of these two sources are already known to have identical structures [3].

Fig. 2 illustrates the primary structure of the major valine tRNA of mouse mycloma cells. This molecule is

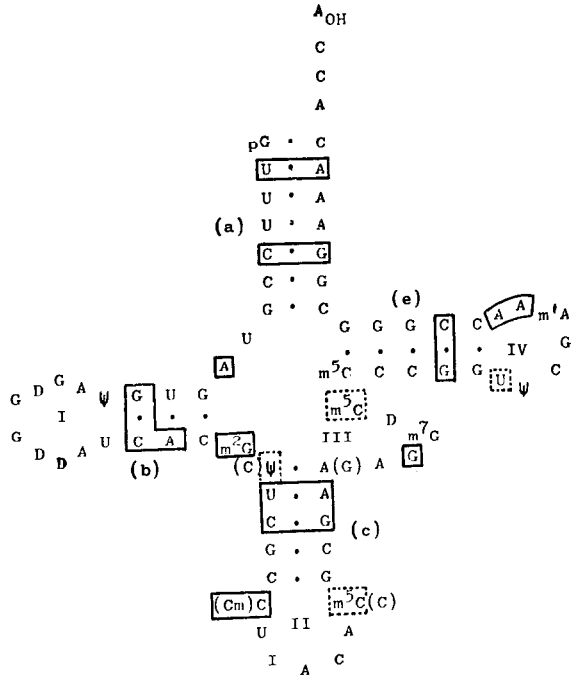

Fig. 2. The cloverleaf structure of the major $tRNA^{Val}$ of mouse myeloma cells. The two methylations within loop II of this tRNA were both found to be incomplete and in each case the nucleoside found in the minor proportion is given in the adjacent parentheses. This major $tRNA^{Val}$ form was found to occur as two nucleoside sequences. In the least abundant sequence a C·G. base-pair replaces the A·ψ base-pair of stem (c), as indicated. Full boxes: regions of $tRNA^{Val}$ structure which are not common to this tRNA and the $tRNA_I^{Val}$ of *S. cerevisiae*. Dotted boxes: nucleotides which differ only in base modification between $tRNA^{Val}$ (mouse myeloma) and $tRNA_I^{Val}$ (*S. cerevisiae*).

also 76 nucleotides in length and is one nucleotide shorter than the equivalent yeast $tRNA_I^{Val}$ [14–16] since there is the deletion of a single nucleotide whitin loop I of yeast $tRNA_I^{Val}$ in the mammalian structure. Fig. 2 also shows the considerable nucleotide sequence homology between $tRNA_I^{Val}$ of *Saccharomyces cerevisiae* [14,15] and mouse myeloma $tRNA^{Val}$. Both eukaryotic valine tRNAs, however, bear very little resemblance in structure to any of the isoaccepting valine tRNAs of *E. coli* [17,18].

Like the homologous $tRNA_4^{Met}$, mouse myeloma $tRNA^{Val}$ also exhibits some unusual features in its primary structure. Its loop IV sequence, –U–ψ–C–G–m′A–A–A–, is unique amongst known tRNA primary structures [12] and, except for the first two nucleotides, resembles the –A–U–C–G–m′A–A–A– loop IV sequence of eukaryotic initiator tRNA species [1–4]. The mammalian $tRNA^{Val}$ also possesses the minor nucleoside $m^2G$ at the position between the (b) and (c) stems of the cloverleaf (fig. 2), whereas practically all other eukaryotic tRNAs have $m_2^2G$, A or ψ at this point in their sequences [12]. Mammalian $tRNA_f^{Met}$ [2–4] is the only other tRNA which has been found to possess $m^2G$ at this position in the cloverleaf. Also noteworthy in the mouse myeloma $tRNA^{Val}$ structure is the loop III sequence, –A–G–$m^7$G–D–$m^5$C–. This sequence was also found to be conserved between the $tRNA_f^{Met}$ and $tRNA_4^{Met}$ species of the same cells (fig. 1(B)). Thus even though this loop structure is found in methionine tRNAs of very different function, it is also to be found in mammalian $tRNA^{Val}$ and is not therefore unique to the methionine tRNAs of mammalian cells. However, this loop III sequence might still be important in tRNA recognition by a mammalian methionyl–tRNA synthetase since the $tRNA^{Val}$ described above can be mischarged with methionine by a crude activating enzyme fraction from mouse cells (P.W.P., manuscript in preparation).

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