Wheat mitochondrial DNA encodes a eubacteria-like initiator methionine transfer RNA

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An initiator methionine transfer RNA ($tRNA_f^{Met}$) gene has been identified by sequence analysis of a cloned *Sal*I restriction fragment of wheat mitochondrial DNA. The 3'-end of this gene, which does not encode the CCA terminus of the mature tRNA, is separated by only one basepair from the 5'-end of the 18 S ribosomal RNA gene, suggesting that the two genes are part of the same transcriptional unit. The wheat mitochondrial $tRNA_f^{Met}$ sequence, the first from plant mitochondria, displays strong structural affinity with eubacterial/chloroplast $tRNA_f^{Met}$ sequences.

Initiator methionine tRNA *Wheat mitochondria* *Endosymbiont hypothesis*

1. INTRODUCTION

Mitochondrial transfer RNAs (tRNAs), especially those of mammals, display many deviations from the general structural pattern followed by both bacterial and eukaryotic (cytoplasmic) tRNAs (reviewed in [1,2]). In certain cases, these structural peculiarities have been correlated with an altered and/or expanded codon recognition pattern, so that mitochondria have provided the first exceptions to the 'universal' genetic code [3–6]. The divergent sequences of fungal and particularly animal mitochondrial tRNAs, which appear to reflect an accelerated mutation rate in these mitochondrial systems (see [2]), have so far made it impossible to draw any firm conclusions about their evolutionary origin [7]. Here, we discuss the structural features of an initiator methionine tRNA ($tRNA_f^{Met}$) from the mitochondrial of wheat, *Triticum aestivum*, as deduced from the sequence of its gene. This is the first mitochondrial tRNA sequence to be reported for higher plants. In contrast to mitochondria tRNA sequences from animal and fungal mitochondria, it displays strong structural affinity with eubacterial/chloroplast $tRNA_f^{Met}$ sequences.

2. MATERIALS AND METHODS

Clones of pBR322 carrying *Sal*I restriction fragments of wheat mtDNA were kindly supplied by F. Quetier and B. Lejeune (Orsay). Sequence analysis of the 6.2 kilobasepair (kbp) *Sal*I fragment S19 (no. 19 of [8]), contained in plasmid pTam-S19, will be described in detail elsewhere (in preparation). Briefly, insert was separated from vector by hydrolysis of pTam-S19 with either *Sal*I or *Sal*I + *Xho*I followed by electrophoresis of the resulting fragments in a 1% low melting point agarose gel. Individual purified DNA fragments were subjected to primary digestion with one or more restriction endonucleases, and the resulting subfragments were treated with bacterial alkaline phosphatase, 5'-end-labelled using [γ-^{32}P]ATP and T4 polynucleotide kinase, and digested with additional restiction endonuclease(s) to produce secondary subfragments labelled at only one end. After recovery, these secondary fragments were sequenced using the chemical modification approach [9], with several of the procedures adapted from [9,10] but with some novel reactions and conditions (unpublished).

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3. RESULTS AND DISCUSSION

3.1. Localization of the $tRNA_f^{Met}$ gene in wheat mtDNA

It was inferred from Southern hybridization studies that *Sal*I fragment S19 contains closely-linked 18 S and 5 S rRNA genes, as well as tRNA gene(s) [8]. Sequence analysis has confirmed these conclusions and shown that the 3′-end of the 18 S gene is 114 bp from the 5′-end of the 5 S rRNA gene, while the 5′-end of the 18 S gene is separated by only 1 bp from the 3′-end of a $tRNA_f^{Met}$ gene (fig.1). All three RNAs are encoded by the same DNA strand. The exact 5′-end of the 18 S gene has been determined by sequence analysis of 5′-end-labelled 18 S rRNA (submitted). The $tRNA^{Met}$ gene (so designated on the basis of a CAU anticodon in the derived tRNA sequence) was the only tRNA coding sequence detected on either strand within the leftward 3.6 kbp of S19. Data establishing the primary structure (supplied to reviewers but not shown) were obtained by sequencing in both directions from the lone *Pst*I site located within the $tRNA_f^{Met}$ gene, as well as from a *Bst*NI cleavage site located within the 18 S rRNA gene, 27 bp from its 5′-end (fig.1). The 5′-end of the $tRNA_f^{Met}$ gene is located 750 bp from the leftward end of S19; its 3′-end does not encode the CCA terminus of the mature tRNA. Restriction fragments of S19 containing the *Pst*I site selectively hybridize with a wheat mitochondrial [^{32}P]tRNA probe, indicating that the gene is transcribed (J. McIntosh, unpublished).

As discussed in [11], the 18 S–5 S rRNA coding unit in S19 (fig.1) is also present in three other *Sal*I fragments (S21, S5,6a, S5,6b) of wheat mtDNA, with different arrays of flanking sequences in each case. Each of these 18 S–5 S coding units appears to contain a $tRNA_f^{Met}$ gene, as judged by the presence of the *Pst*I site that occurs within this gene, and by Southern hydribization experiments. We note that a *Pst*I site also appears to be close to the 5′-end of the mitochondrial 18 S rRNA gene in maize [12], suggesting that the same $tRNA_f^{Met}$–18 S rRNA–5 S rRNA gene arrangement as shown in fig.1 also occurs in maize mtDNA.

3.2. Mode of expression of the wheat mitochondrial $tRNA_f^{Met}$ gene

The intimate physical linkage of wheat mitochondrial 18 S and $tRNA_f^{Met}$ genes resembles the situation in the closely-packed mammalian mitochondrial genome [13–15]. It is, in fact, a rather surprising finding given the potential spaciousness of plant mtDNA [16]. Since only a single bp separates the end of the $tRNA_f^{Met}$ gene from the beginning of the 18 S rRNA gene in wheat mtDNA, the two genes are almost certainly co-transcribed from the same promoter, in which case the $tRNA_f^{Met}$ sequence may serve as a signal for the nucleolytic processing of the primary transcript, as postulated for the mammalian mitochondrial genome [17]. Co-transcription of the two genes also provides a simple mechanism for coordinating the production of mitochondrial initiator tRNA and ribosomes.

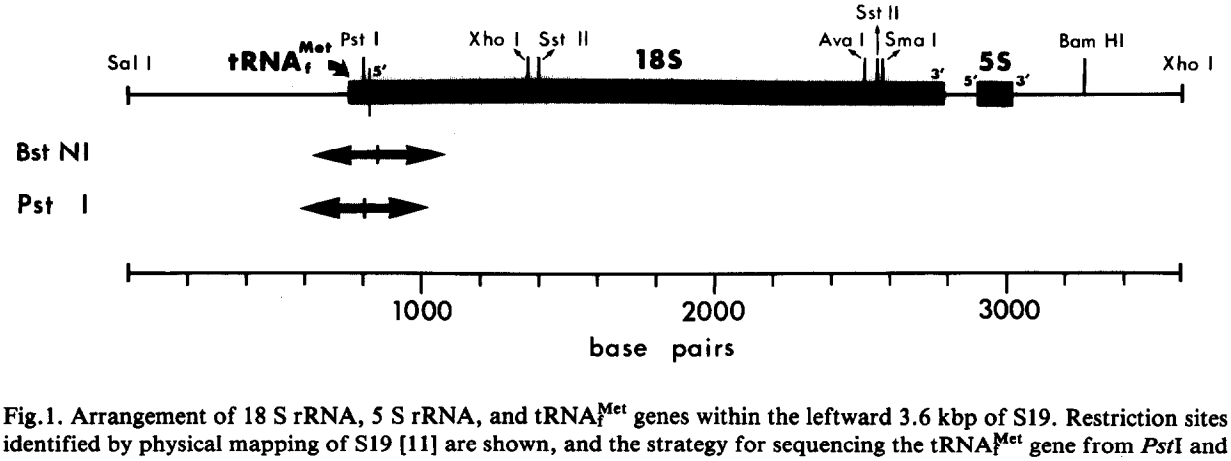

Fig.1. Arrangement of 18 S rRNA, 5 S rRNA, and $tRNA_f^{Met}$ genes within the leftward 3.6 kbp of S19. Restriction sites identified by physical mapping of S19 [11] are shown, and the strategy for sequencing the $tRNA_f^{Met}$ gene from *Pst*I and *Bst*NI sites is indicated.

3.3. Secondary structure of wheat mitochondrial $tRNA_f^{Met}$

The wheat mitochondrial $tRNA_f^{Met}$ sequence can be folded into a typical cloverleaf secondary structure (fig.2) that contains virtually all of the characteristic features of the generalized tRNA model [18]. A singular departure from this model, the A_{11}–U_{25} pair, also occurs at the same position in the secondary structure of eubacterial and chloroplast, but not eukaryotic (cytoplasmic), initiator $tRNAs^{Met}$ [19]. The presence of GUUC at positions 54–57 and an unpaired 5′-terminal residue additionally characterize the wheat mitochondrial sequence as a eubacterial type $tRNA_f^{Met}$, as does the fact that it displays considerably more sequence identity with the initiator $tRNA^{Met}$ of *Escherichia coli* (23 differences) than with the elongator $tRNA^{Met}$ from the same organism (33 differences) (fig.3, table 1). However, some structural features that eubacterial and chloroplast initiators share in common [19] are not present in wheat mitochondrial $tRNA_f^{Met}$ (fig.2). Deviations from the eubacterial/chloroplast pattern are also observed at all of these positions in each of the three known fungal (*Aspergillus nidulans* [20], *Neurospora crassa* [21], *Saccharomyces cerevisiae* [22]) mitochondrial $tRNA_f^{Met}$ sequences (cf. fig.3), except that the fourth residue from the 3′-end (position 74 in fig.2) is A in the *A. nidulans* and *S. cerevisiae* mitochondrial sequences, as in the eubacterial and chloroplast initiators. None of the organelle initiators (chloroplast included) has three consecutive G–C pairs proximal to the loop in the anticodon stem, a feature in eubacterial and eukaryotic initiators that may contribute to a specialized conformation in the anticodon loop of these particular tRNAs [23].

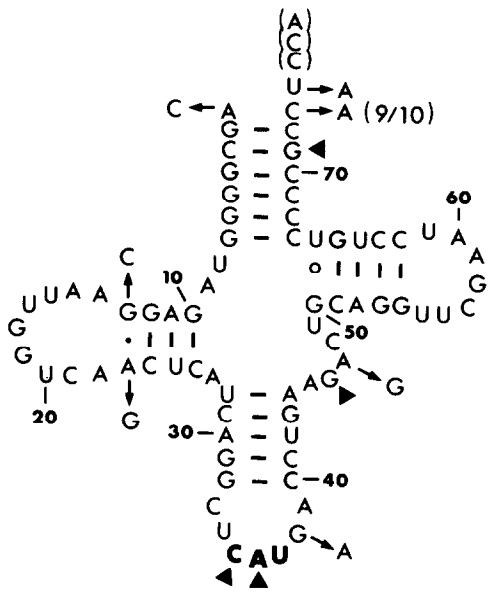

Fig.2. Potential secondary structure of wheat mitochondrial $tRNA_f^{Met}$, deduced from the gene sequence. The CCA terminus (bracketed residues) of the mature tRNA is not encoded. The anticodon is marked in bold letters. Arrows indicate positions at which the mitochondrial sequence differs from the pattern characteristic of eubacterial and chloroplast $tRNA_f^{Met}$ (see [19]). Closed triangles denote residues whose chemical modification in *E. coli* $tRNA_f^{Met}$ abolishes amino acid acceptor activity [27].

The presence of a formylatable initiator $tRNA^{Met}$ in plant mitochondria is expected from [24–26], who demonstrated that all three of the mitochondria-specific $tRNAs^{Met}$ in bean (*Phaseolus vulgaris*) can be charged by *E. coli* Met-tRNA synthetase, and that two of these can be formylated by either the *E. coli* or a bean mitochondrial transformylase. Residues in *E. coli* $tRNA_f^{Met}$ that have been implicated in the synthetase recognition site [27] are all conserved in the wheat mitochondrial $tRNA_f^{Met}$ sequence (fig.2; closed triangles). Although the residue in the fourth position from the 3′-terminus (position 74, the 'discriminator' position) is U rather than A in the mitochondrial sequence, recent work [28] indicates that this residue is not in fact critically important in the specificity of aminoacylation in *E. coli* $tRNA_f^{Met}$. Based on the secondary structure deduced here for wheat mitochondrial $tRNA_f^{Met}$, as well as that of a $tRNA^{Pro}$ whose gene is present in a different part of S19 (unpublished), it appears that plant mitochondrial tRNAs will turn out to be considerably more conservative in structure than other mitochondrial tRNAs studied to date. They may well display more conventional decoding properties, although there is some evidence of altered codon recognition even in plant mitochondria [29].

3.4. Evolutionary origin of wheat mitochondrial $tRNA_f^{Met}$

Comparison of the primary sequence of the wheat mitochondria $tRNA_f^{Met}$ with other initiator $tRNA^{Met}$ sequences (fig.3, table 1) strengthens the

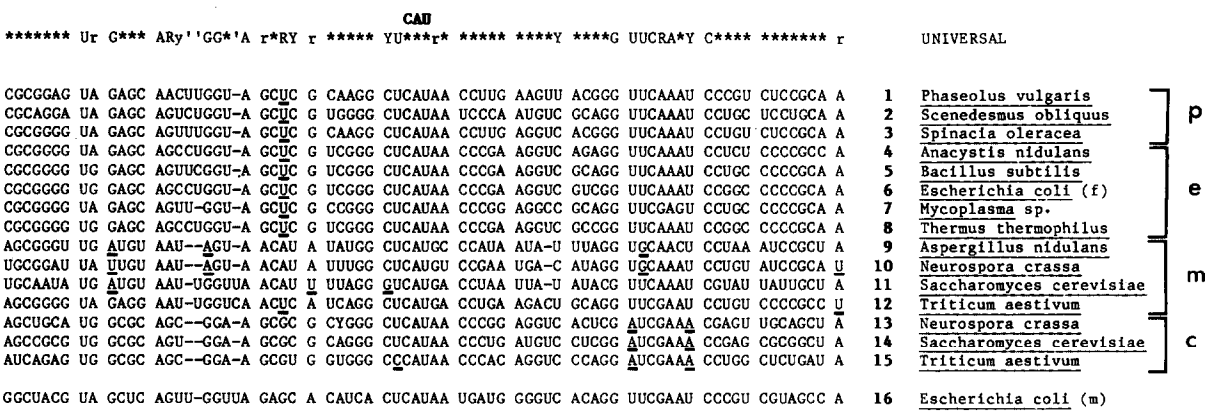

Fig.3. Alignment of initiator $tRNA^{Met}$ sequences from plastids (p), eubacteria (e), mitochondria (m), and eukaryotic cytoplasm (c). The alignment is based on that of [30] and [31], which list and give primary references for all the sequences except that of wheat (*Triticum aestivum*) mitochondrial $tRNA_f^{Met}$. The top line shows variable and conserved residues in the generalized tRNA structure [18]: *, variable residue; R and r, invariant and semi-invariant purine, respectively; Y and y, invariant and semi-invariant pyrimidine, respectively; ', position not occupied in some sequences (–). The anticodon sequence is indicated in bold letters. Y27 in the *N. crassa* cytoplasmic sequence [13] is an unidentified pyrimidine. Also listed for comparison is the sequence of the elongator $tRNA^{Met}$ of *E. coli* [16].

conclusion that the mitochondrial sequence is eubacterial in nature. Pairwise differences range from 17–23 (mean 20.2) with five eubacterial sequences and from 21–26 (mean 23.3) with three chloroplast sequences, as opposed to 35–36 (mean 35.8) with three cytoplasmic initiator sequences, including that of wheat. By comparison, bean chloroplast $tRNA_f^{Met}$ differs in 17–18 (mean 17.8)

Table 1

Difference matrix of initiator methionine tRNA sequences

	Sequence		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*Phaseolus vulgaris*	(p)	–	22	6	18	18	18	18	17	34	32	37	23	28	27	30	30
2	*Scenedesmus obliquus*	(p)	22	–	17	14	11	13	15	12	35	34	35	26	28	29	28	34
3	*Spinacia oleracea*	(p)	6	17	–	13	12	15	12	14	33	30	37	21	28	24	28	26
4	*Anacystis nidulans*	(e)	18	14	13	–	10	9	14	9	35	32	39	21	27.5	27	26	31
5	*Bacillus subtilis*	(e)	18	11	12	10	–	6	7	5	32	31	37	18	27.5	25	25	31
6	*Escherichia coli*	(e)	18	13	15	9	6	–	12	1	34	33	40	23	27.5	23	27	35
7	*Mycoplasma* sp.	(e)	18	15	12	14	7	12	–	11	35	33	41	17	26.5	24	27	28
8	*Thermus thermophilus* 1	(e)	17	12	14	9	5	1	11	–	34	35	41	22	26.5	24	26	34
9	*Aspergillus nidulans*	(m)	34	35	33	35	32	34	35	34	–	16	25	27	40	34	38	44
10	*Neurospora crassa*	(m)	32	34	30	32	31	33	33	35	16	–	26	26	41.5	41	37	41
11	*Saccharomyces cerevisiae*	(m)	37	35	37	39	37	40	41	41	25	26	–	31	38.5	41	41	42
12	*Triticum aestivum*	(m)	23	26	21	21	18	23	17	22	27	26	31	–	36.5	35	36	33
13	*Neurospora crassa*	(c)	28	28	28	27.5	27.5	27.5	26.5	26.5	40	41.5	38.5	36.5	–	16	21.5	29
14	*Saccharomyces cerevisiae*	(c)	27	29	24	27	25	23	24	24	34	41	41	35	16	–	18	29
15	*Triticum aestivum*	(c)	30	28	28	26	25	27	27	26	38	37	41	36	21.5	18	–	36

The table lists the number of differences in pairwise comparisons between the indicated sequences, based on the alignment of fig. 2 (a total of 75 positions): p, plastid; e, eubacterial; m, mitochondrial; c, cytoplasmic. In comparisons with the *N. crassa* cytoplasmic sequence [13], which contains an unidentified pyrimidine at position 27, a difference of 0.5 was counted if other sequences had either C or U at the same position. Also listed are values for pairwise comparisons with the elongator $tRNA^{Met}$ of *E. coli* [16].

positions from the same five eubacterial sequences and in 27–30 (mean 28.3) positions from the eukaryotic initiator sequences, while the corresponding values are 31–41 (mean 35.5) and 37–41 (mean 39.1), respectively, for the three fungal mitochondrial $tRNAs_f^{Met}$. On this basis, wheat mitochondrial $tRNA_f^{Met}$ clearly groups with the eubacterial/chloroplast initiators, whereas the three fungal mitochondrial $tRNAs_f^{Met}$ do not. Although the pattern observed here will have to be confirmed by determination of additional plant mitochondrial tRNA sequences, our results provide the strongest indication yet of a eubacterial evolutionary origin for any mitochondrial tRNA gene. It is interesting that the three fungal mitochondrial initiator sequences are closer to the wheat mitochondrial sequence (26–31 differences) than they are to any of the five eubacterial sequences (31–41 differences) or the three chloroplast sequences (30–37 differences). Plant mitochondrial tRNAs may therefore provide an important link between tRNAs of eubacteria and fungal mitochondria in the construction of a mitochondrial phylogenetic tree.

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