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Editing corrects mispairing in the acceptor stem of bean and potato mitochondrial phenylalanine transfer RNAs

L.Maréchal-Drouard, D.Ramamonjisoa, A.Cosset, J.H.Weil and A.Dietrich
Institut de Biologie Moléculaire des Plantes du CNRS, Université Louis Pasteur, 12 rue du Général Zimmer, F-67084 Strasbourg Cedex, France

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ABSTRACT

Editing is a general event in plant mitochondrial messenger RNAs, but has never been detected in a plant mitochondrial transfer RNA (tRNA). We demonstrate here the occurence of a tRNA editing event in higher plant mitochondria: in both bean and potato, the C encoded at position 4 in the mitochondrial $tRNA^{Phe}$(GAA) gene is converted into a U in the mature tRNA. This nucleotide change corrects the mismatched C_4-A_{69} base-pair which appears when folding the gene sequence into the cloverleaf structure and it is consistent with the fact that C to U transitions constitute the common editing events affecting plant mitochondrial messenger RNAs. The $tRNA^{Phe}$(GAA) gene is located upstream of the single copy $tRNA^{Pro}$(UGG) gene in both the potato and the bean mitochondrial DNAs. The sequences of potato and bean $tRNA^{Pro}$(UGG) genes are colinear with the sequence of the mature bean mitochondrial $tRNA^{Pro}$(UGG), demonstrating that this tRNA is not edited. A single copy $tRNA^{Ser}$(GCU) gene was found upstream of the $tRNA^{Phe}$ gene in the potato mitochondrial DNA. A U_6-U_{67} mismatched base-pair appears in the cloverleaf folding of this gene and is maintained in the mature potato mitochondrial $tRNA^{Ser}$(GCU), which argues in favour of the hypothesis that the editing system of plant mitochondria can only perform C to U or occasionally U to C changes.

INTRODUCTION

Editing is an intriguing post-transcriptional process which changes the primary sequence of RNAs, as compared to that of the corresponding DNA templates and can therefore be regarded as a further step in the regulation of gene expression. So far, RNA editing has mainly been found in mitochondria, and to a lesser extent in chloroplasts. There are only four cases known of editing in transcripts of nuclear genes (1–5). In trypanosome mitochondria, RNA editing involves addition or deletion of uridines (e.g. 6 and references therein), whereas in plant mitochondria and in chloroplasts it usually results in the conversion of cytidines into uridines (for reviews see 7–9).

So far, editing sites have been detected almost exclusively in messenger RNAs. Only one case of ribosomal RNA editing has been reported, namely in *Oenothera* mitochondria (10). Editing was also found in two mammalian cytosolic transfer RNA species, the bovine selenocysteine tRNA and the rat aspartate tRNA (1, 3). More recently, several mitochondrial tRNAs of an amoeboid protozoan (*Acanthamoeba castellanii*) were shown to be edited in the amino acid stem (11), whereas in marsupial mitochondrial $tRNA^{Asp}$ the correct anticodon appears to be generated by editing (12). In *Acanthamoeba castellanii* mitochondria, the changes consist in U to A, U to G or A to G conversions which correct mismatched base pairs (11).

Extensive editing of transcripts from cryptic genes could have been an explanation for the presence, in plant mitochondria, of tRNAs which do not hybridize to mitochondrial DNA and for the inability to identify a complete set of tRNA genes in plant mitochondrial genomes. Several lines of evidence obtained both *in vitro* (13, 14) and *in vivo* (15) have definitely demonstrated that this is not the case and that the tRNA population encoded by the plant mitochondrial genome is complemented by nucleus-encoded tRNA species imported from the cytosol. It has also been established that, due to the occurrence of chloroplast DNA insertions in their genome, plant mitochondria contain 'chloroplast-like' tRNAs (e.g.16). Plant mitochondrial protein synthesis is therefore supported by a non-redundant mosaic of tRNA species of nuclear, chloroplastic and genuine ('native') mitochondrial origins (for a review, see for instance17). Such a complex situation is likely to put important constraints on the sequence divergence of plant mitochondrial tRNAs and the occurrence of tRNA editing in plant mitochondria remains therefore an open question.

By looking for mismatched base-pairs in the secondary structure folding of the sequenced plant mitochondrial tRNAs and tRNA genes, we found one potential editing site: the folded structure of the 'native' *Oenothera* mitochondrial $tRNA^{Phe}$(GAA) gene has a C_4-A_{69} mismatched base pair in the amino acid stem (18), whereas the bean mitochondrial $tRNA^{Phe}$(GAA) contains a normal U_4-A_{69} base-pair, thanks to a unique nucleotide difference with the *Oenothera* gene, namely

a U at position 4 (19). By comparing in two plant species, bean and potato, the sequences of the 'native' mitochondrial tRNA^{Phe}(GAA) genes to those of the corresponding mature tRNAs, we were able to demonstrate for the first time that tRNA editing indeed occurs in plant mitochondria: a C to U change in the mature tRNA^{Phe}(GAA) corrects the mispairing observed when folding the sequence of the gene. However, comparing the sequence of the 'native' potato mitochondrial tRNA^{Ser}(GCU) gene to that of the corresponding mature tRNA, showed that, in contrast to what happens in *Acanthamoeba* mitochondria (11), correction of mispairing in the amino acid stem of tRNAs is not an absolute rule in plant mitochondria.

MATERIALS AND METHODS

Purification of potato and bean mitochondrial tRNAs

Potato mitochondrial tRNA^{Phe}(GAA) and tRNA^{Ser}(GCU) were purified by mono- and two-dimensional polyacrylamide gel electrophoresis according to Maréchal-Drouard et al. (14). Purification of bean mitochondrial tRNA^{Phe}(GAA) was performed as previously described (19).

Cloning and sequencing of potato and bean mitochondrial tRNA genes

Isolation of potato and bean mitochondrial DNAs, as well as 'Southern blot' hybridizations, were performed under conditions previously described (14, 19). To clone the tRNA^{Phe}(GAA) genes, an *Eco*RI library of potato mitochondrial DNA and a *Hind*III library of bean mitochondrial DNA were constructed in the vector Bluescript m13(+) (Vector cloning systems) using standard recombinant techniques (20). These libraries were screened using as a probe an oligodeoxyribonucleotide complementary to the G_1-A_{27} sequence of the bean mitochondrial tRNA^{Phe}(GAA) (19). DNA sequencing was performed by the dideoxyribonucleotide chain termination method.

Transfer RNA sequencing

The sequence of potato mitochondrial tRNA^{Phe}(GAA), as well as the revised sequence of the bean mitochondrial tRNA^{Phe}(GAA) were established as previously described (21).

For the 3'-end sequencing, potato mitochondrial tRNA^{Ser}(GCU) was 3'-end labeled with [^{32}P]pCp (19), submitted to statistical hydrolysis and further analysed by homochromatography (22). The 5'-end sequence of potato mitochondrial tRNA^{Ser}(GCU) was determined by dideoxyribonucleotide chain termination sequencing using reverse transcriptase (23), using as a primer an oligodeoxyribonucleotide complementary to the C_{25}-C_{44} sequence of the corresponding tRNA gene.

Oligodeoxyribonucleotides

The oligodeoxyribonucleotides complementary to the G_1-A_{27} sequence of bean mitochondrial tRNA^{Phe}(GAA), the C_1-C_{25} sequence of bean mitochondrial tRNA^{Pro}(UGG), the G_1-A_{26} sequence of the maize mitochondrial tRNA^{Ser}(GCU) gene and the C_{25}-C_{44} sequence of the potato mitochondrial tRNA^{Ser}(GCU) gene, respectively, were synthesized on an Applied Biosystems DNA synthesizer.

RESULTS

Analysis of potato and bean mitochondrial tRNA^{Phe}(GAA) genes

In the mitochondria of the dicotyledonous plants studied so far, tRNA^{Phe} is a 'native' mitochondrion-encoded species (e.g. 24). This tRNA (anticodon GAA) is typically mitochondrial and the corresponding gene is not present in the nuclear genome (14 and data not shown). To determine the number of tRNA^{Phe}(GAA) gene copies in the bean and potato mitochondrial DNAs, we probed Southern blots with an oligodeoxyribonucleotide complementary to the G_1-A_{27} sequence of bean mitochondrial tRNA^{Phe}(GAA) (19). This oligonucleotide hybridized to only one fragment in different restriction digests (Fig. 1 and data not shown). Using the same probe, clones corresponding to the smallest fragments identified by Southern blot hybridization, 2.8 kbp *Eco*RI for potato and 2.5 kbp *Hind*III for bean (Fig.1), were isolated from an *Eco*RI library of potato mitochondrial DNA and a *Hind*III library of bean mitochondrial DNA, respectively. A 1328 nucleotide sequence from the cloned 2.8 kbp *Eco*RI potato insert and a 750 nucleotide sequence from the 2.5 kbp *Hind*III bean insert were established (EMBL accession numbers X73286 and X73285, respectively) and shown to contain a single tRNA^{Phe}(GAA) gene. Hybridization after further digestion with restriction enzymes or Exonuclease III deletion demonstrated that no tRNA^{Phe}(GAA) gene was present in the two cloned mitochondrial DNA fragments outside of the sequenced regions (data not shown). The 'native' tRNA^{Phe}(GAA) is therefore the product of a unique mitochondrial gene in both potato and bean.

Editing occurs in potato and bean mitochondrial tRNAs^{Phe}(GAA)

In both the potato and the bean mitochondrial tRNA^{Phe}(GAA) genes, a C was found at position 4, which introduces a C_4-A_{69}

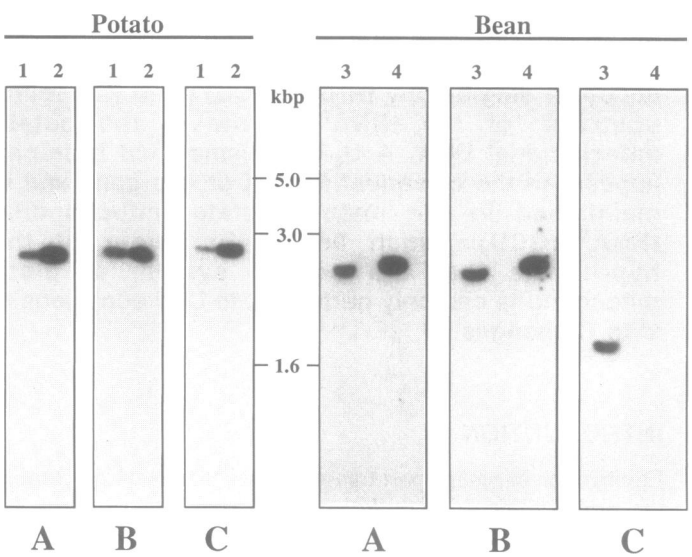

Figure 1. Hybridization of 5' end-labelled oligodeoxyribonucleotides complementary to **A)** the G_1-A_{27} sequence of bean mitochondrial tRNA^{Phe}(GAA) (19), **B)** the C_1-C_{25} sequence of bean mitochondrial tRNA^{Pro}(UGG) (25) and **C)** the G_1-A_{26} sequence of the maize mitochondrial tRNA^{Ser}(GCU) gene (26) with **1)** an *Eco*RI digest of potato mitochondrial DNA, **2)** the cloned 2.8 kbp *Eco*RI potato mitochondrial DNA fragment, **3)** a *Hind*III digest of bean mitochondrial DNA and **4)** the cloned 2.5 kbp *Hind*III bean mitochondrial DNA fragment.

mismatched base pair in the acceptor stem, when folding the DNA sequence in the classical cloverleaf structure (Figs. 2, 3 and 5). Such a mispairing might weaken the stability of the helical structure of the amino acid stem which contains already two G-U base pairs and only two G-C base pairs. Indeed, in bean mitochondria, the mature tRNA^{Phe}(GAA) was shown to contain a U at position 4 (19). We have determined the complete sequence of the potato mitochondrial tRNA^{Phe}(GAA) and a U was found at position 4 also in this tRNA (Figs. 3 and 5), instead of the C present at the same position in the corresponding gene (Figs. 2, 3 and 5). Even after long exposures of the thin layer chromatograms (e.g. Fig. 5C), there was no evidence for the presence of a C (or eventually of a modified nucleotide) at any position from 1 to 9 in the potato mitochondrial tRNA^{Phe}. It should be pointed out that, in both plants, aminoacylation or hybridization to a specific probe always detected a single tRNA^{Phe}(GAA) species upon RPC-5 chromatography and two-dimensional polyacrylamide gel electrophoresis (14, 19), showing that only the tRNA with a U at position 4 is present in significant amounts *in vivo*. These data obtained with two different plant species demonstrate that C to U editing occurs in the 'native' plant mitochondrial tRNA^{Phe}(GAA) to correct a C_4-A_{69} mispairing due to the gene sequence into a normal U_4-A_{69} base-pair. One cannot completely rule out that a tRNA^{Phe}(GAA) gene product with a C at position 4 has escaped detection, but such a tRNA would only represent a minor and/or labile species in potato and bean mitochondria.

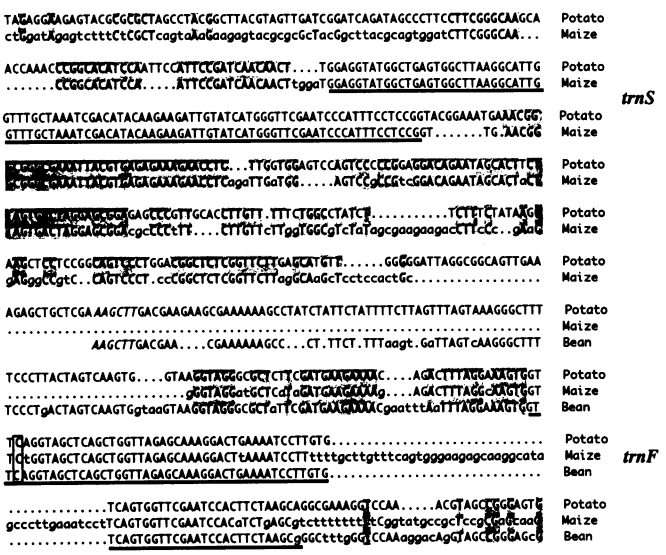

Figure 2. Alignment of the potato mitochondrial DNA region containing the tRNA^{Ser}(GCU) (*trn*S) and tRNA^{Phe}(GAA) (*trn*F) genes (from position 151 to position 831 in the EMBL X73286 file) with the bean mitochondrial DNA region containing the tRNA^{Phe}(GAA) gene (from position 1 to position 235 in the EMBL X73285 file) and with the maize mitochondrial DNA region containing the tRNA^{Ser}(GCU) gene and the tRNA^{Phe} pseudogene (26). The tRNA genes are underlined. The maize tRNA^{Phe} pseudogene contains a 48 bp insertion (26). Dots correspond to the positions of gaps which were inserted to optimize the alignment. Nucleotides in the bean or maize sequence which differ from the potato sequence are written in lower case letters. Sequence homologies in flanking regions are shown with a shaded background. The editing site present in the potato and bean mitochondrial tRNA^{Phe} genes is boxed, together with the C conserved at the same position in the maize mitochondrial tRNA^{Phe} pseudogene.

Except in position 4, the sequence of the potato mitochondrial tRNA^{Phe}(GAA) (Fig. 3) is colinear with that of the corresponding gene (Fig. 2). A further discreapency appears however when aligning the previously determined sequence of the bean mitochondrial tRNA^{Phe}(GAA) (19) with that of the bean mitochondrial tRNA^{Phe}(GAA) gene described in this work (Fig. 2): a U, or a derivative of U, is missing at position 20b in the tRNA, as compared to the gene sequence. We therefore re-examined the sequence of the bean mitochondrial tRNA^{Phe}(GAA) and found that, as in the potato mitochondrial tRNA^{Phe} (Fig. 3), a derivative of acp^{3}U is indeed present at position 20b, which had not been seen previously. Potato and bean mitochondrial tRNAs^{Phe}(GAA) only differ by one nucleotide (the A at position 73 in the potato tRNA is replaced by a G in bean) and these tRNAs are post-transcriptionally modified at six positions (Fig. 3).

Analysis of potato and bean mitochondrial tRNA^{Pro}(UGG) genes

A tRNA^{Pro}(UGG) gene was found located at 248 and 255 nucleotides downstream of the tRNA^{Phe}(GAA) gene in the cloned potato and bean mitochondrial DNA fragments, respectively. Hybridization studies demonstrated that this tRNA^{Pro}(UGG) gene is also present as a single copy in the mitochondrial genome of the two plants (Fig. 1 and data not shown). The potato and bean tRNA^{Pro}(UGG) gene sequences are 100% identical and present all the structural features expected for a normal tRNA (Fig. 4). As they are colinear with the already sequenced bean mitochondrial tRNA^{Pro}(UGG) (25), it can be assumed that no editing occurs in this case.

A mispairing is maintained in the potato mitochondrial tRNA^{Ser}(GCU)

In the cloned 2.8 kbp *Eco*RI fragment of the potato mitochondrial DNA, a tRNA^{Ser}(GCU) gene is located 362 nucleotides upstream of the tRNA^{Phe}(GAA) gene (Fig. 2). This tRNA^{Ser}(GCU) gene is also present as a single copy in the

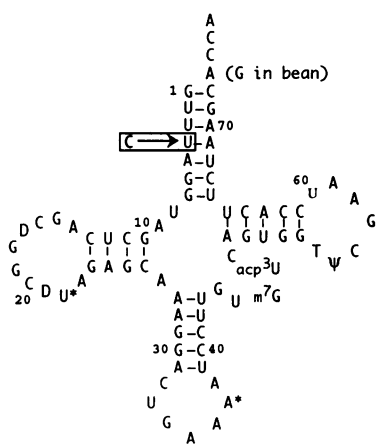

Figure 3. Deduced secondary structure of the 'native' potato mitochondrial tRNA^{Phe}(GAA). Nucleotide numbering is according to Sprinzl *et al.* (31). The C to U change introduced in position 4 by editing is boxed. The single nucleotide difference found in the corresponding bean mitochondrial tRNA^{Phe}(GAA) (position 73) is indicated in parentheses. U*(position 20b): derivative of acp^{3}U; A*(position 37): i^{6}A or ms^{2}i^{6}A.

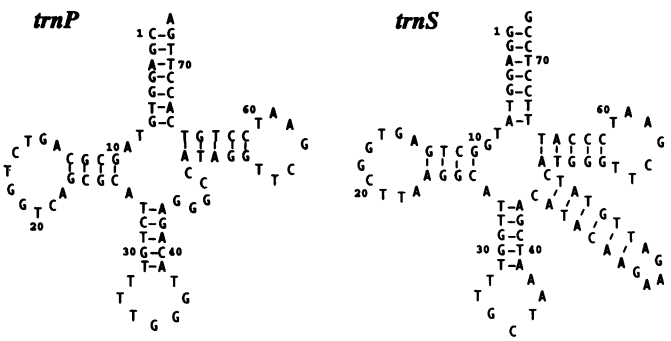

Figure 4. Cloverleaf structure folding of the sequences of potato mitochondrial tRNA^{Ser}(GCU) (*trnS*) and tRNA^{Pro}(UGG) (*trnP*) genes. Nucleotide numbering is according to Sprinzl *et al.* (31).

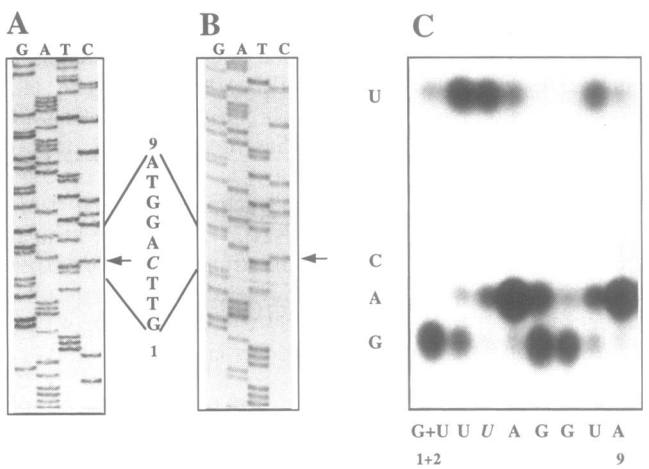

Figure 5. Sequence analysis of the 5′ end of **A)** the potato mitochondrial tRNA^{Phe}(GAA) gene, **B)** the bean mitochondrial tRNA^{Phe}(GAA) gene and **C)** the potato mitochondrial tRNA^{Phe}(GAA). Nucleotides 1 to 9 of the two gene sequences are listed between **A)** and **B)** autoradiograms; the editing site (*C* in position 4) is in italics and indicated by an arrow in the DNA sequencing gels. Transfer RNA sequence analysis was performed using the technique of Stanley and Vassilenko (36). Statistical hydrolysis (0.2 μg of pure tRNA) was performed for 3 min at 85°C in 3 μl of deionized formamide. Nucleotides 1 to 9 of the tRNA sequence are listed below the chromatogram; the edited nucleotide (*U* in position 4) is in italics. The migration of the nucleotides A, G, C and U in the solvent used (2-propanol/37% HCl/$H_2$0, 68/17.6/14.4 by volume) is indicated on the left of the chromatogram. For accurate identification of the residues at positions 1 and 2, the 5′ end-labelled tRNA^{Phe} was digested with either pancreatic RNase or T1 RNase and the 5′ end oligonucleotides were analyzed by electrophoresis on DEAE-cellulose paper (19 and data not shown).

potato mitochondrial genome (Fig. 1 and data not shown) and its sequence is identical to that of the previously described maize and wheat tRNA^{Ser}(GCU) genes (26, 27 and Fig. 2). In all three cases, a T_6-T_{67} mismatched base pair appears in the acceptor stem, when folding the DNA sequence of the tRNA^{Ser}(GCU) gene into the classical cloverleaf structure (Fig. 4). To determine whether editing corrects this mispairing, we isolated and partially sequenced the potato mitochondrial tRNA^{Ser}(GCU). The 5′-end was determined by reverse transcriptase dideoxy-sequencing and the 3′-end by statistical hydrolysis followed by homochromatography. As shown on Fig. 6, the sequence data obtained from the purified tRNA in the acceptor stem all fit the

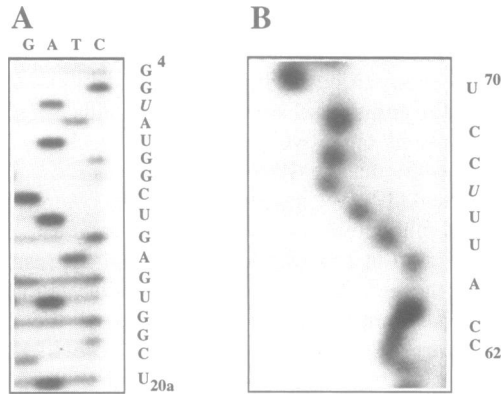

Figure 6. Sequence analysis of **A)** the 5′ end and **B)** the 3′ end of potato mitochondrial tRNA^{Ser}(GCU). The 5′ end sequence was determined by dideoxyribonucleotide chain termination sequencing using reverse transcriptase (23); the nucleotide sequence of the tRNA (which is complementary to the sequence on the gel) is given from position 4 to position 20a; the U_6 involved in the U_6-U_{67} mismatched base-pair mentioned in the text is in italics. For 3′ end sequencing, the labelled tRNA (19) was analysed by homochromatography after statistical hydrolysis in 10 μl water (90 min at 95°C in the presence of 10 μg of carrier tRNA) (22); the nucleotide sequence is given from position 62 to position 70; the U_{67} involved in the U_6-U_{67} mismatched base-pair mentioned in the text is in italics.

corresponding sequences in the gene, demonstrating that a U_6-U_{67} mispairing is indeed maintained in the mature potato mitochondrial tRNA^{Ser}(GCU).

DISCUSSION

The present study gives the first example of an editing site in a plant mitochondrial tRNA: a potential C_4-A_{69} mismatched base pair, which appears in the acceptor stem when folding the sequence of both the potato and the bean mitochondrial 'native' tRNA^{Phe}(GAA) genes into the classical cloverleaf structure, is corrected in the mature tRNAs^{Phe} thanks to a C to U change in position 4. As the unedited form of these tRNAs is not detectable in plants, formation of a normal base pair between nucleotides 4 and 69 might be required to obtain a stable and/or fully active and specific plant 'native' mitochondrial tRNA^{Phe}(GAA). Indeed, although the major sites for recognition by the *E.coli* and the yeast cytosolic phenylalanyl-tRNA synthetases appear to be in the anticodon of tRNAs^{Phe} (for a review see for instance 28), the proper tertiary structure must also be maintained (29). According to footprinting studies (30), the protein contacts the entire surface of the tRNA in the yeast tRNA^{Phe}/phenylalanyl-tRNA synthetase complex, which suggests the existence of additional recognition elements widely separated from the anticodon. Such a distribution is likely to place stringent constraints on the overall structure of the tRNAs to be recognized by phenylalanyl-tRNA synthetase (28). On the other hand, tRNAs recognized by the corresponding aminoacyl-tRNA synthetase primarily outside of the acceptor stem, which seems to be the case for tRNAs^{Phe}, might contain negative identity elements in this helical region near the amino acid attachment site (28). The N_4-N_{69} base pair is often a G-C base pair (31), but it is not conserved among tRNAs^{Phe} and therefore is not by itself a very good candidate for a general positive or negative identity element in these tRNAs. It is also interesting to note that several mature

tRNAs^{Phe} have been described with a mismatched N_4-N_{69} base pair, as for instance the rat liver mitochondrial tRNA^{Phe}(GAA) (32).

The marsupial mitochondrial tRNA^{Asp} is the first example known of a tRNA which clearly appears to be dependent on editing to become fully functional in protein synthesis. This tRNA is encoded in the marsupial mitochondrial genome with a (GCC) anticodon specific for glycine and it acquires its specificity for aspartic acid codons upon a post-transcriptional change of the C in the second position of the anticodon, presumably into an U (12). This situation is to some extent similar to that of the plant 'native' mitochondrial tRNA^{Ile}(L*AU), which is encoded in the mitochondrial genome with a (CAU) anticodon specific for methionine. As it is the case for *E.coli* tRNA^{Ile}(LAU) (33), the plant 'native' mitochondrial tRNA^{Ile} is likely to get the aminoacylation and codon recognition specificity for isoleucine only after a post-transcriptional modification of the C in the first position of the anticodon into a derivative (L*) of lysidine (L, a cytidine with a lysine moiety) (21).

The 'native' tRNA^{Phe}(GAA) described in the present study is probably present only in the mitochondria of dicotyledonous plants, as in the monocotyledonous plants studied so far the corresponding gene has become a pseudogene. In the maize mitochondrial DNA, this pseudogene contains a 48 bp insertion in the variable loop (26) (Fig. 2) and in the wheat mitochondrial genome, in addition to this 48 bp insertion, a second insertion of 64 bp is present in the dihydrouridine stem (27). In the absence of a functional 'native' tRNA^{Phe} gene, a 'chloroplast-like' tRNA^{Phe}(GAA) transcribed from a chloroplast-originating gene integrated in the mitochondrial genome is used in mitochondria of monocotyledonous plants, as shown in the case of wheat (16). The 'chloroplast-like' wheat mitochondrial tRNA^{Phe}(GAA) possesses a classical A-U base pair in position 4–69 (16).

Although the 'native' tRNA^{Phe}(GAA) gene has become a pseudogene in the mitochondria of monocotyledonous plants, the organization of its upstream region in the maize or wheat mitochondrial DNA has remained similar to that of the corresponding sequences in the potato and bean mitochondrial genomes: the 'native' tRNA^{Ser}(GCU) gene described in this work in the case of potato is also present upstream of the wheat and maize tRNA^{Phe} pseudogenes and the region between the tRNA^{Ser} gene and the tRNA^{Phe} gene or pseudogene, as well as the 5′ flanking region of the tRNA^{Ser} gene, appear to be highly conserved between these different plant species (26, 27 and Fig. 2). On the other hand, as the active bean and potato 'native' mitochondrial tRNA^{Phe}(GAA) genes, the maize and wheat tRNA^{Phe} pseudogenes still exhibit a C at position '4'. These data suggest that the regions which contain the 'native' tRNA^{Phe}(GAA) gene or pseudogene in the mitochondrial genomes of monocotyledons and dicotyledons all derive from a common ancestor sequence and that the editing site was present before the divergence between monocotyledonous and dicotyledonous plants. In both the maize and wheat mitochondrial genomes, the tRNA^{Phe} pseudogene is located upstream of the expressed *nad*3 and *rps*12 protein genes, which contain a number of editing sites, and is likely to be co-transcribed with these genes (27, 34). To understand the mechanisms and evolution of editing in plant mitochondria, it would be interesting to know whether, although no functional tRNA^{Phe} can be produced from these pseudogenes, the corresponding sequences are still edited in polycistronic primary transcripts spanning over the *nad*3 and *rps*12 genes.

That a C_4-A_{69} mispairing is corrected in the potato and bean mitochondrial tRNAs^{Phe}(GAA) by a C to U change, and not by a replacement of the A by a G, is consistent with the fact that C to U changes are the common editing events occurring in plant mitochondrial messenger RNAs (e.g. 7–9). Furthermore, that a U_6-U_{67} mispairing is maintained in the mature potato mitochondrial tRNA^{Ser}(GCU) argues in favour of the hypothesis that the editing system of plant mitochondria can only perform pyrimidine changes (usually C to U, occasionally U to C), whereas correcting a U-U mispairing would involve a pyrimidine to purine change (U to A, or U to G). The *Acanthamoeba castellanii* mitochondrial tRNA editing system appears to be very different, as it is able to change a pyrimidine into a purine (U to A or U to G) or a purine into another purine (A to G) (11). Also, in *Acanthamoeba* mitochondria, editing affects one, two or all of the first three nucleotides at the 5′ end of the acceptor stem and always occurs opposite to a pyrimidine (11), whereas the C to U change in bean and potato mitochondrial tRNAs^{Phe}(GAA) takes place at position 4 and opposite to a purine (A). Furthermore, in contrast to the potato mitochondrial tRNA^{Ser}(GCU), all mismatched base-pairs detected in *Acanthamoeba* mitochondrial tRNA genes are corrected, including U-U mispairings (11). Finally, as 8 out of the 10 *Acanthamoeba* mitochondrial tRNAs sequenced so far are likely to be affected, tRNA editing appears to be a quite frequent event in the mitochondria of this amoeboid protozoan. In contrast, the results presented in this work and our search for other potential editing sites in the known tRNA or tRNA gene sequences (e.g. 35) suggest that tRNA editing in plant mitochondria might be restricted to the 'native' tRNA^{Phe}(GAA) of dicotyledonous plants.

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