A potato mitochondrial isoleucine tRNA is coded for by a mitochondrial gene possessing a methionine anticodon

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ABSTRACT

The nucleotide sequence of a potato mitochondrial tRNA^{Ile} has been determined. This tRNA has a lysidine-like minor nucleotide in the first position of the anticodon (position 34). The corresponding mitochondrial gene has been cloned and sequenced. This gene has a CAT anticodon which corresponds to methionine. The C residue in the anticodon must therefore be post-transcriptionally modified. The mature tRNA has isoleucine-accepting activity but no methionine-accepting activity. This is the first report showing that in plant mitochondria a post-transcriptional modification could change the amino-acid specificity of a tRNA, as compared to that of the corresponding gene.

INTRODUCTION

It has now become evident that plant mitochondrial (mt) DNA, despite its large size (200–2400 kbp) (1), does not contain a complete set of tRNA genes. For instance, only about 15 tRNA genes seem to be present in the mt genome of wheat (2). We demonstrated recently that bean and potato mitochondria use tRNAs coded for by the nuclear genome and imported from the cytosol (3–5). In maize the tRNA genes present on the mt DNA comprise, in addition to the initiator tRNA^{Met} (tRNA^{Met}f) gene, two other distinct genes encoding a tRNA with a methionine-specific CAU anticodon (6, 7). One of these two tRNA(CAU) genes is highly homologous (96%) to chloroplast (cp) elongator tRNAs^{Met} (tRNAs^{Met}e) or tRNA^{Met}e genes and is in fact part of a promiscuous cp DNA sequence that has been inserted into the plant mt genome during evolution (7, 8). This 'chloroplast-like' tRNA^{Met} gene was shown to be transcribed in maize mitochondria (7) and the corresponding bean mt tRNA^{Met}e has been sequenced (9). The second putative maize mt tRNA^{Met}(CAU) gene (6) has only 42% sequence homology with the first one and is also transcribed (7). A similar situation is found in wheat mitochondria, where the 'chloroplast-like' tRNA^{Met}e(CAU) and the second putative tRNA^{Met}(CAU) genes are both expressed in addition to the tRNA^{Met}f (10). As the number of tRNA genes present in plant mt genomes seems to be limited, it is quite surprising to find two putative mt tRNA^{Met}e genes. On the other hand, this is reminiscent of the situation in *Escherichia coli* (11) and in *Mycoplasma capricolum* (12), where a tRNA transcribed from a gene having a methionine-specific anticodon CAT is changed into an isoleucine-specific tRNA by a post-transcriptional transformation of C into lysidine (L), an hypermodified nucleotide. The same phenomenon probably also occurs in spinach chloroplasts (13). It has also been shown that in bean mitochondria only two tRNAs, the tRNA^{Met}f and the 'chloroplast-like' tRNA^{Met}e, have methionine-accepting activity (9). Furthermore, we recently identified (5), in potato mitochondria, an isoleucine-accepting tRNA hybridizing to an oligonucleotide complementary to the G46-A74 sequence of the maize tRNA^{Met}(CAU) gene.

We report here that this isoleucine-accepting tRNA contains a lysidine-like minor nucleotide at the first position of the anticodon. The nucleotide sequence of the corresponding potato mt gene has been determined : it contains a C residue at the first position of the anticodon and codes therefore for a methionine-specific CAU anticodon. This tRNA gene shows only one nucleotide difference with the second putative maize mt tRNA^{Met}e(CAU) gene studied by Parks *et al.* (6). These results show that, in plant mitochondria also, a post-transcriptional modification could change the amino-acid specificity of a tRNA, as compared to that of the corresponding gene.

MATERIALS AND METHODS

Purification of potato mt-encoded tRNA^{Ile} and tRNA^{Met}f, extraction of potato mt DNA, preparation of bean mt enzymatic extract and aminoacylation assays were performed according to Maréchal-Drouard et al. (5). Sequence of potato mt tRNA^{Ile} was determined as previously described (4). Southern blot hybridization was performed under conditions previously described (3). For cloning of the potato mt tRNA^{Ile} gene, a PstI mt DNA library in the vector Bluescribe m13+ (Vector Cloning Systems) was constructed using standard recombinant techniques (14). This bank was screened using as a probe an oligonucleotide complementary to the G46-A74 sequence of the maize mt tRNA(CAU) gene (6). DNA sequencing was performed by the dideoxy-chain termination method (15, 16) using specific synthetic oligonucleotides as primers.

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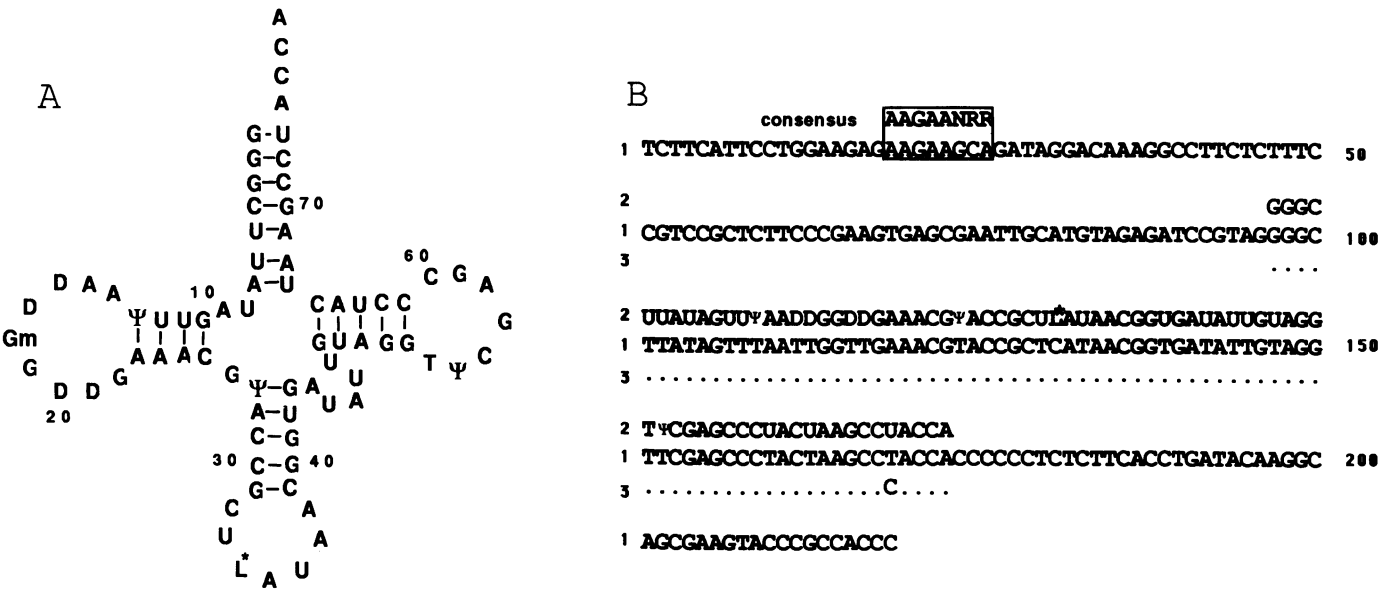

Figure 1. A) Nucleotide sequence of potato mt tRNA^{Ile}(L*AU). L* = Lysidine-like hypermodified nucleotide. B) Nucleotide sequence of the potato mt gene coding for tRNA^{Ile}(L*AU) and of its flanking regions (Lane 1). The sequences of the potato mt tRNA^{Ile}(L*AU) (Lane 2) and of the maize mt tRNA(CAU) gene (Lane 3) were aligned with the sequence of the potato mt gene. Homologies between the potato and the maize genes are represented by dots.

RESULTS AND DISCUSSION

When an oligonucleotide complementary to the G46-A74 sequence of the putative second maize mt tRNA^{Met}(CAU) gene was used as a probe to screen potato mt tRNAs after fractionation by two-dimensional polyacrylamide gel electrophoresis, one tRNA gave a positive signal (5). The nucleotide sequence of this tRNA was determined and is shown on Fig. 1A. It is 77 nucleotides long and contains 10 modified nucleotides, corresponding to a percentage of 11.6% : 4 D (positions 16, 17, 20 and 20a), 3 Ψ (positions 13, 27 and 55), a Gm (position 18), a T (position 54) and a hypermodified nucleotide at the 'wobble' position of the anticodon (position 34). Such a rather low percentage of modified nucleotides is shared by all mt tRNAs, regardless of their origin. By this criterion, this tRNA is more related to prokaryotic tRNAs than to eukaryotic ones (17). The mobility, in two thin-layer chromatography systems (Fig. 2), of the hypermodified nucleotide found in the 'wobble' position 34 differs from that of the modified nucleotides commonly reported in tRNAs and corresponds to the chromatographic behaviour of a lysidine-like (L*) residue present, in addition to the lysidine residue (L), in *E. coli* tRNA^{Ile}(LAU) (11 and Yokoyama, S. and Osawa, S., personnal communication). The modified nucleotide N found at position 34 in spinach cp tRNA^{Ile}(NAU) also shows a very similar chromatographic mobility in the solvent system b described in Fig. 2 (13).

We have shown previously that this potato mt tRNA(L*AU) is aminoacylated with isoleucine (5). Further aminoacylation kinetic studies (Fig. 3) provided evidence that this tRNA can indeed be charged with isoleucine and does not have methionine-accepting activity. Control experiments showed that in the same conditions potato mt tRNA^{Met}f was efficiently charged with methionine.

As already mentioned, in *E. coli* the lysidine and lysidine-like residues in the first position of the anticodon derive from a C encoded by the tRNA gene (11). On the other hand, the potato mt tRNA^{Ile}(L*AU) has been characterized using as a probe an oligonucleotide complementary to part of a maize mt tRNA(CAU) gene. This suggested that potato mt tRNA^{Ile}(L*AU) could also be first synthesized with a C at the 'wobble' position of the

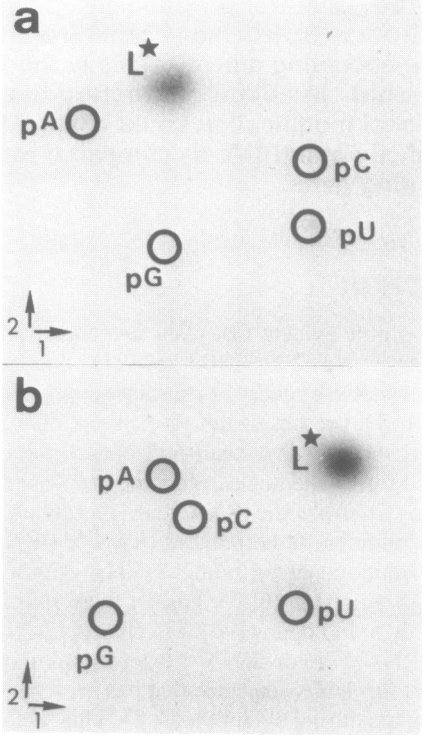

Figure 2. Two-dimensional thin-layer chromatography analysis of the lysidine-like hypermodified nucleotide present at position 34 in potato mt tRNA^{Ile}(L*AU). The nucleotide was labeled at the 5'-end after hydrolysis of the tRNA. Solvent common to (a) and (b) for the first dimension : isobutyric acid/water/25% ammonia (66:33:1). Second dimension solvents : (a) 2-propanol/37% hydrochloric acid/water (68:17.6:14.1) and (b) 1-propanol/sodium phosphate 0.1 M (pH 6.8)/ammonium sulfate (1 ml:50 ml:30g).

Table I. Comparison between potato mitochondrial $tRNA^{Ile}$(L*AU) and isoleucine or methionine tRNAs (t) or tRNA genes (g) from other species.

	Amino-acid specificity	Anticodon	% homology with potato mitochondrial $tRNA^{Ile}$(L*AU)
Zea mays mt (g)	Met (†)	CAU	98.7
Lupinus luteus cyto (t)	Met e	CmAU	59.2
Bean mt (t)	Met e	CAU	42.1
Spinacia oleracera cp (t)	Met e	CAU	40.0
Zea mays mt (g)	Met e	CAU	37.1
Bacillus subtilis (t)	Ile	NAU	69.7
Mycoplasma mycoides (t)	Ile	GAU	61.8
Halobacterium volcanii (t)	Ile	NAU	60.5
Spinacia oleracera cp (t)	Ile	NAU	59.2
Saccharomyces cerevisiae (t)	Ile	GAU	59.2
Yeast mt (g)	Ile	UAU	59.2
Spiroplasma meliforma (t)	Ile	NAU	56.6
Lupinus luteus cyto (t)	Ile	IAU	55.5
Phage T4 (t)	Ile	NAU	54.0
Escherichia coli (t)	Ile	LAU	53.9
Human mt (g)	Ile	GAU	40.8

In the case of tRNA genes (g), the amino-acid specificity was established according to the anticodon specified by the gene ; (†) : this maize mt $tRNA^{Met}$ gene could code for a $tRNA^{Ile}$ (see text). mt = mitochondrial, cyto = cytosolic, cp = chloroplast.

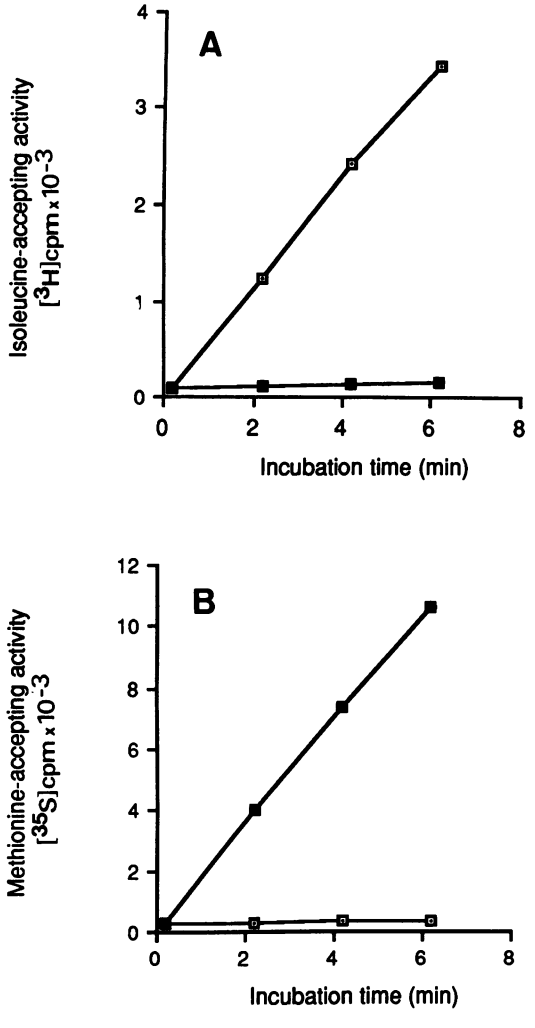

Figure 3. Time course of isoleucylation (A) and methionylation (B) of potato mt $tRNA^{Ile}$(L*AU) (⊡) and $tRNA^{Met}$f(CAU) (■) in the presence of a bean mt enzymatic extract.

anticodon and prompted us to clone and sequence the corresponding gene(s).

The oligonucleotide complementary to the maize mt tRNA(CAU) gene was use to probe Southern blots. This oligonucleotide hybridized to only one fragment in BamHI, EcoRI, HindIII, PstI, SalI or XhoI restriction digests of potato mt DNA (data not shown). It could therefore be assumed that there is only one copy of the gene. The PstI digest of potato mt DNA was cloned in Bluescribe m13+ and the library was screened with the same probe. Recombinant clones carrying the 1.2 kbp PstI fragment hybridizing to the probe were isolated and the sequence of the tRNA gene, as well as of its flanking regions, was determined (Fig. 1B). Like all plant mt tRNA genes studied so far, this gene does not contain an intervening sequence. The coding sequence is colinear with that of the $tRNA^{Ile}$(L*AU), but indeed it contains a CAT in the position of the anticodon. In the tRNA, the C residue in the anticodon must therefore be post-transcriptionally modified into a lysidine-like residue. In the case of *E. coli* $tRNA^{Ile}$(LAU), it has been shown that the single post-transcriptional modification of C into L in the anticodon switches the codon recognition and amino-acid specificity of the tRNA from methionine to isoleucine (11). The fact that the mature potato mt $tRNA^{Ile}$(L*AU) shows isoleucine-accepting activity but no methionine-accepting activity suggests that, also in this case, the modification of the C into a lysidine-like residue changes the amino-acid specificity of the tRNA, as compared to that of the corresponding gene.

All plant mt and nuclear tRNA genes sequenced so far do not code for the 3'-terminal CCA triplet and only a few cp tRNA genes have been reported to encode this triplet (18). This triplet is present in some bacterial tRNA genes, for instance in *Bacillus subtilis*, and in a few cyanobacterial tRNA genes (18). In the case of *E. coli*, this triplet is always encoded by the tRNA genes (19). The potato mt gene coding for $tRNA^{Ile}$(L*AU) contains a 3'-CCA sequence (Fig. 1B) and is therefore potentially able to code for the complete tRNA.

The potato mt $tRNA^{Ile}$(L*AU) gene shows only one nucleotide difference with the maize mt tRNA(CAU) gene (6)

from which we have derived the probe used in this work (Fig. 1B) : a T present at position 73 in the potato gene is replaced by a C in maize, suggesting that this maize mt tRNA(CAU) gene could also code for an isoleucine tRNA. From an evolutionary point of view, potato mt $tRNA^{Ile}$(L*AU) does not show a high sequence homology with either plant cytosolic, cp or mt $tRNAs^{Met}e$, or cp or prokaryotic $tRNAs^{Ile}$ containing lysidine or other modified nucleotides at the first position of the anticodon (Table I). If one postulates that all these $tRNAs^{Ile}$ derive from a common ancestor, one has to assume that they have rapidly diverged during evolution.

Analysis of the 5'-flanking region revealed a purine-rich motif 67 nucleotides upstream of the potato mt $tRNA^{Ile}$ gene (Fig. 1B). Such a purine-rich motif has also been found at about 50 to 65 nucleotides upstream of a number of plant mt tRNA genes (2, 7) and a derived consensus sequence AAGAANRR was proposed (2), which is likely to act as a promoter sequence in higher plant mitochondria. The AAGAAGCA motif found upstream of the potato $tRNA^{Ile}$ gene almost perfectly fits this consensus sequence (Fig. 1B).

Like *E. coli* and *Mycoplasma capricolum* $tRNAs^{Ile}$(LAU) (11, 12, 20, 21), or bacteriophage T4 and spinach cp $tRNAs^{Ile}$(NAU) (22, 13), where N is an unidentified modified nucleoside which is coded for as a C in the corresponding gene (23–25), potato mt $tRNA^{Ile}$(L*AU) very likely recognizes only the AUA codon, suggesting that the second isoleucine tRNA found in potato mitochondria (5) is probably used to decode the AUU and AUC codons. On the other hand, the recognition of AUA as an isoleucine codon in mitochondria seems to rely on the existence of a $tRNA^{Ile}$ having a hypermodified derivative of C (*C), such as lysidine, in the 'wobble' position of the anticodon. It has been proposed that the reassignment of the AUA codon from isoleucine to methionine in mitochondria of yeast, nematodes, flies and vertebrates could have occurred because of the elimination of these $tRNAs^{Ile}$(C*AU) during evolution (26).

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