A nuclear-encoded potato (*Solanum tuberosum*) mitochondrial $tRNA^{Leu}$ and its cytosolic counterpart have identical nucleotide sequences

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Sequencing of potato mitochondrial (mt) $tRNA^{Leu}$(NAA) and of its cytosolic (cyt) counterpart revealed that these tRNAs are identical, except for a post-transcriptional modification: a Gm is present at position 18 in mt $tRNA^{Leu}$, instead of a G in cyt $tRNA^{Leu}$. Hybridization studies have shown that potato mt $tRNA^{Leu}$(NAA) has a nuclear origin and must therefore be imported from the cytosol.

Evolution; Plant mitochondria; Mitochondrial $tRNA^{Leu}$ structure; (*Solanum tuberosum*)

1. INTRODUCTION

Among the mitochondrial (mt) tRNAs coded for by the plant mt genome, one can define two categories, showing either a moderate (70–75%) or a high (95–100%) degree of sequence homology with the chloroplastic (cp) counterparts. The first class corresponds to the 'native' tRNAs according to the endosymbiotic hypothesis, the second class contains tRNAs transcribed from genes present on promiscuous cp DNA sequences inserted into the plant mt genome during evolution [1]. Moreover, it has now become evident that, despite its large size (200–2400 kbp) [2], plant mt DNA does not contain a complete set of tRNA genes [3,4]. We demonstrated previously that bean mitochondria utilize tRNAs, especially $tRNAs^{Leu}$, coded for by the nuclear genome and therefore imported from the cytosol [5]. The present work shows that this tRNA import also occurs in other plant species. We present here evidence for the existence, in potato, of a mt and a cytosolic (cyt) $tRNA^{Leu}$(NAA) with identical sequences, except for one post-transcriptional modification. We also show that potato mt and cyt $tRNAs^{Leu}$(NAA) are highly homologous to bean mt and cyt $tRNAs^{Leu}$(NAA). Furthermore, we demonstrate by hybridization that potato mt $tRNA^{Leu}$(NAA) is nuclear-encoded.

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2. MATERIALS AND METHODS

Highly purified mitochondria were prepared from potato (*Solanum tuberosum*) tubers according to Neuburger et al. [6]. Assay of various markers (catalase, glucose-6-phosphate dehydrogenase and carotenoids) showed that sedimentation of potato tuber mitochondria through two successive continuous Percoll gradients completely eliminates plastid and peroxysomal contaminations. Total tRNA was extracted from these mitochondria using the methods described for bean mt tRNA preparation [7]. Total cytoplasmic tRNA was prepared from etiolated potato germs using the same methods. We have shown previously that the proportion of mt tRNAs in such cytoplasmic preparations does not exceed 0.5% [8] and can therefore not interfere with the analysis of cytosolic tRNA species. Total mt and cytoplasmic tRNAs were subjected to two-dimensional polyacrylamide gel electrophoresis [9] and, after staining with methylene blue, mt and cyt $tRNA^{Leu}$ species were identified by aminoacylation using a crude bean cytoplasmic extract, as described previously [7]. The potato $tRNAs^{Leu}$ corresponding to bean mt and cyt $tRNA^{Leu}$(NAA) were identified by hybridization using a 5'-end-labeled oligonucleotide complementary to the bean $tRNA^{Leu}$(NAA) A_{35}-m^5C_{48} sequence. One mt and one cyt $tRNA^{Leu}$ showed a positive signal and these tRNAs were further purified by polyacrylamide gel electrophoresis under denaturing conditions [7].

Sequences of $tRNAs^{Leu}$ were determined using post-labeling techniques [10] and methods described previously [11]. For read-off sequencing gels, $tRNAs^{Leu}$ were first labeled at the 3'-end with [^{32}P]pCp in the presence of T_4 RNA ligase [12].

To check for possible cyt tRNAs contaminations in our mt tRNAs preparations, total potato mt tRNA was fractionated by polyacrylamide gel electrophoresis under denaturing conditions [7] and transferred on nylon membranes (Hybond-N, Amersham) by electroblotting (15 min at 150 mA and then 30 min at 500 mA) in 0.25 × TAE buffer [13]. The blots were hybridized against a 5'-end-labeled oligonucleotide complementary to the plant cyt $tRNA^{Phe}$ A_9-A_{36} sequence [14]. Total potato cytoplasmic tRNA was also fractionated and transferred on the blots as a positive hybridization control.

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Potato nuclear (nu) and mt DNA were prepared according to Green et al. [15] and Kemble [16], respectively.

3. RESULTS AND DISCUSSION

To study mt tRNAs, and especially to characterize nuclear-encoded tRNAs imported from the cytosol into the mitochondria, great care has to be taken to eliminate any cyt contamination from the mt tRNA preparations. Using a crude bean cytoplasmic extract [7] which aminoacylates only cyt $tRNA^{Phe}$ but not mt $tRNA^{Phe}$ [17], we did not detect any cyt $tRNA^{Phe}$ in our mt tRNA preparations. Furthermore, when total mt and cytoplasmic tRNAs, fractionated in parallel on a polyacrylamide gel and transferred on a nylon membrane, were hybridized with a 5′-end-labeled oligonucleotide complementary to a sequence conserved in different plant cyt $tRNAs^{Phe}$, a band lit up only with total cytoplasmic tRNA and not with total mt tRNA. These results demonstrate the absence of cyt tRNA contaminations in our potato mitochondria preparations.

After fractionation of total potato mt or cytoplasmic tRNA by two-dimensional polyacrylamide gel electrophoresis, only one mt and one cyt $tRNA^{Leu}$ isoacceptor gave a positive hybridization with an oligonucleotide specific for bean mt and cyt $tRNA^{Leu}$(NAA). The nucleotide sequences of these potato mt and cyt $tRNAs^{Leu}$ were determined and their structures are presented in fig.1. The two tRNAs are 87 nucleotides long and have a long variable region of 15 nucleotides. The sequences are identical, except for a post-transcriptional modification: in the mt $tRNA^{Leu}$, a Gm residue is present at position 18, whereas in the cyt $tRNA^{Leu}$, the G residue at position 18 is not modified. As reported previously, the presence of a Gm at position 18 in the mt species constitutes also the only difference between the bean mt $tRNA^{Leu}$ isoacceptors sequenced so far and their cyt counterparts [5,7,18]. The anticodon (NAA) contains an unknown modified nucleotide (N) in the wobble position. The N_{34}-A_{35} phosphodiester bond is resistant to RNase T_1, RNase A and *Physarum polycephalum* RNase digestion, but is cut by *Bacillus cereus* RNase, suggesting that this modified nucleotide is a derivative of C. Furthermore, the mobility of this nucleotide in two different thin-layer chromatography systems (fig.2) is identical to that found for the corresponding nucleotide of bean mt and cyt $tRNA^{Leu}$(NAA) (unpublished data) and a C is encoded for this position in the bean $tRNA^{Leu}$(NAA) nu gene sequenced previously [7].

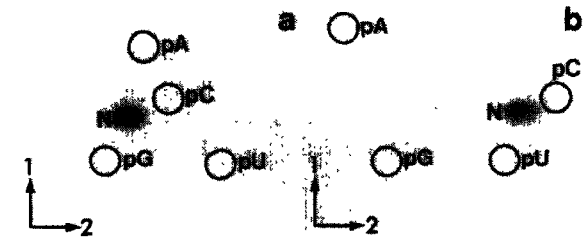

Fig.2. Two-dimensional thin-layer chromatography analysis of the unidentified nucleotide present at position 34 in potato mt and cyt $tRNA^{Leu}$(NAA). The nucleotide was labeled at the 5′-end after hydrolysis of the tRNA. Solvent common to (a) and (b) for the first dimension: isobutyric acid/water/25% ammonia (66:33:1). Second dimension solvents: (a) 2-propanol/37% hydrochloric acid/water (68:17.6:14.4) and (b) 1-propanol/sodium phosphate 0.1 M (pH 6.8)/ammonium sulfate (1 ml:50 ml:30 g).

Potato mt and cyt $tRNAs^{Leu}$(NAA) show only one difference with their bean counterparts: a U is found at position 47 in the case of potato, whereas an A is present at the same position in bean (fig.3). It should be pointed out that the sequence of a lupin cyt $tRNA^{Leu}$ with an (UAA) anticodon [19] is also very similar to that of bean or potato $tRNA^{Leu}$(NAA): this tRNA has a m^7G at position 10 instead of a G in the case of bean or potato, a U at position 34 instead of N (derivative of C) and 3 other differences in the variable region (fig.3).

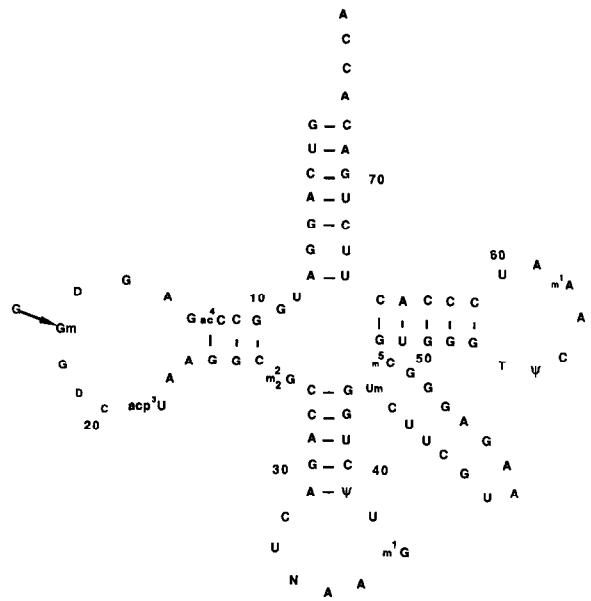

Fig.1. Nucleotide sequence of potato mt and cyt $tRNAs^{Leu}$(NAA). The cloverleaf represents the structure of the mt $tRNA^{Leu}$. The arrow indicates the only difference (G instead of Gm) found between the two species. N_{34} = unidentified modified nucleotide.

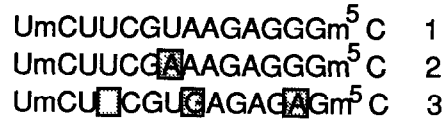

Fig.3. Nucleotide sequence of potato $tRNA^{Leu}$(NAA) (1), bean $tRNA^{Leu}$(NAA) (2) and lupin $tRNA^{Leu}$(UAA) (3) from position 44 to 48 in the variable region. Gray boxes show the differences, taking potato $tRNA^{Leu}$(NAA) as a reference.

Considering that potato mt and cyt tRNAs^{Leu}(NAA) are identical apart from a single methylation, we checked by hybridization experiments the origin of the mt tRNA^{Leu}(NAA) species. No signal could be obtained by hybridizing 3′-end-labeled mt tRNA^{Leu}(NAA) to potato mt DNA, neither after dot blot nor after Southern blot, whereas a positive hybridization was found with nu DNA. Thus, we can conclude that potato mt tRNA^{Leu}(NAA) is nuclear-encoded and therefore has to be imported from the cytosol into the mitochondria; similar results were obtained for the 4 bean mt tRNA^{Leu} isoacceptors studied so far and especially for bean mt tRNA^{Leu}(NAA) [5]. A 'cytosolic-like' tRNA^{Leu}(CAA) seems also to be present in wheat mitochondria [1] and this tRNA should be nuclear-encoded, as no tRNA^{Leu} gene has been found in the whole wheat mt genome [3].

The results presented here support the idea that the import of tRNAs from the cytosol into the mitochondria could be a general phenomenon in higher plants. However, it remains to be determined whether the same set of tRNAs is concerned in all plants, or whether there are differences in the tRNA species imported, in particular between monocotyledon and dicotyledon plants. It can already be pointed out that the sequence of a mt gene coding for a tRNA^{Gly} has been reported for lupin [20], whereas no tRNA^{Gly} gene could be identified in the wheat mt genome [3] and wheat mitochondria seem to contain a 'cytosolic-like' tRNA^{Gly} [1]. We are currently identifying all potato mt tRNAs and determining their respective origin (nuclear, 'chloroplast-like' or typically mitochondrial). The mechanism of tRNA import into plant mitochondria is also studied in vitro and in vivo.

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