Transfer RNAs of potato (*Solanum tuberosum*) mitochondria have different genetic origins

Laurence Maréchal-Drouard, Pierre Guillemaut+, Anne Cosset, Michèle Arbogast, Frédérique Weber, Jacques-Henry Weil and André 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

Total transfer RNAs were extracted from highly purified potato mitochondria. From quantitative measurements, the *in vivo* tRNA concentration in mitochondria was estimated to be in the range of 60 μM. Total potato mitochondrial tRNAs were fractionated by two-dimensional polyacrylamide gel electrophoresis. Thirty one individual tRNAs, which could read all sense codons, were identified by aminoacylation, sequencing or hybridization to specific oligonucleotides. The tRNA population that we have characterized comprises 15 typically mitochondrial, 5 'chloroplast-like' and 11 nuclear-encoded species. One $tRNA^{Ala}$, 2 $tRNAs^{Arg}$, 1 $tRNA^{Ile}$, 5 $tRNAs^{Leu}$ and 2 $tRNAs^{Thr}$ were shown to be coded for by nuclear DNA. A second, mitochondrial-encoded, $tRNA^{Ile}$ was also found. Five 'chloroplast-like' tRNAs, $tRNA^{Trp}$, $tRNA^{Asn}$, $tRNA^{His}$, $tRNA^{Ser}$(GGA) and $tRNA^{Met}$m, presumably transcribed from promiscuous chloroplast DNA sequences inserted in the mitochondrial genome, were identified, but, in contrast to wheat (1), potato mitochondria do not seem to contain 'chloroplast-like' $tRNA^{Cys}$ and $tRNA^{Phe}$. The two identified $tRNAs^{Val}$, as well as the $tRNA^{Gly}$, were found to be coded for by the mitochondrial genome, which again contrasts with the situation in wheat, where the mitochondrial genome apparently contains no $tRNA^{Val}$ or $tRNA^{Gly}$ gene (2).

INTRODUCTION

The higher plant mitochondrial genome seems to have undergone important modifications during evolution, not only through sequence divergence, amplification or reduction and rearrangement, but also through the loss, replacement and/or acquisition of genes (3, 4). Despite its very large and complex structure (5), it has now become evident that the mitochondrial DNA of plants does not contain a complete set of tRNA genes (2, 6). We demonstrated previously that bean mitochondria utilize at least 8 tRNAs coded for by the nuclear genome and therefore imported from the cytosol (7). Among these, we identified 4 $tRNAs^{Leu}$ (7–9). Import of $tRNA^{Leu}$ also occurs in potato mitochondria (10). Furthermore, some of the plant mitochondrial tRNA genes are part of promiscuous chloroplast DNA sequences inserted into the mitochondrial genome during evolution (1, 11 and references therein). At least some of these genes seem to be transcribed and to produce mature 'chloroplast-like' tRNAs in bean or wheat mitochondria (1, 12, 13). All these data indicate that, in higher plant mitochondria, tRNAs of different genetic origins may function together in protein biosynthesis.

We decided to study the genetic origin of individual tRNAs in a plant mitochondrial tRNA population, and especially to determine which species, besides $tRNAs^{Leu}$, are nuclear-encoded. We present here the identification of 31 tRNAs, after fractionation of total potato mitochondrial tRNA by two-dimensional polyacrylamide gel electrophoresis. Among these tRNAs, we found 11 nuclear-encoded species and 5 'chloroplast-like' species. This report is the first showing a nuclear origin for higher plant mitochondrial tRNAs specific for alanine, arginine, isoleucine and threonine. Our results also indicate that differences can be found in the genetic origin of individual tRNAs, in particular between monocotyledon and dicotyledon plants.

MATERIALS AND METHODS

Mitochondrial tRNA: purification, fractionation and identification

Highly purified mitochondria were prepared from potato (*Solanum tuberosum*) tubers according to Neuburger *et al.* (14), lyophilized and stored at −20°C. The mitochondrial fraction was assayed for marker enzymes of plastids (phosphorylase, EC 2.4.1.1), peroxisomes (catalase, EC 1.11.1.6) and cytosol (pyrophosphate:fructose 6-P phosphotransferase, EC 2.7.1.90) and found to be essentially free of plastidial, peroxisomal or cytosolic contaminations (14). Total tRNA was extracted as previously described (15) from these mitochondria and subjected to two-dimensional polyacrylamide gel electrophoresis (16). After

* To whom correspondence should be addressed

+ Present address: Institut Universitaire de Technologie Louis Pasteur, 3 rue de l'Argonne, F-67000 Strasbourg, France

staining with methylene blue, mitochondrial tRNA species were identified by i) aminoacylation using a bean mitochondrial enzymatic extract (see below), ii) hybridization using 5'-end labeled oligonucleotides complementary to already known plant mitochondrial tRNA or tRNA gene sequences (9), or iii) direct sequencing of the tRNAs using post-labeling techniques (17) and methods previously described (18), especially 3'-end labeling with [^{32}]pCp in the presence of T4 RNA ligase (15).

Northern Blot Analysis of tRNAs

Five μg of total potato mitochondrial tRNA were fractionated by polyacrylamide gel electrophoresis, transferred on nylon membranes (Hybond-N, Amersham) by electroblotting and hybridized against 5'-end labeled oligonucleotides as already described (10). Total potato germ cytoplasmic and total bean (*Phaseolus vulgaris*) chloroplast tRNAs, prepared as previously described (10, 12, respectively), were also fractionated and transferred on the blots as negative or positive hybridization controls.

Bean mitochondrial enzymatic extract and aminoacylation assays

Bean (*Phaseolus vulgaris*) seedlings were grown, in the dark at 26°C, on moist vermiculite and the hypocotyls were harvested after 5−6 days. One kg of hypocotyls was homogenized during 30 sec at 4°C, using a home-mixer, in 1.5 l of a 50 mM Tris-HCl buffer (pH 7.5) containing 0.6 M mannitol, 1 mM EDTA, 1g/l BSA, 150 mg/l ATP and 2 mM β-mercaptoethanol. After homogenization, the pH was adjusted to 7.0 with a 2 M Tris solution. The homogenate was filtered through four layers of cheesecloth and one layer of nylon net (50 μm mesh size) and centrifuged at 2500×g for 5 min. The supernatant was placed in new centrifuge bottles (330ml/bottle) and 70 ml of a 27% (w/v) sucrose solution containing 0.1 mM EDTA, 50 mM Tris-HCl (pH 7.5), 1g/l bovine serum albumin and 1 mM β-mercaptoethanol, were gently introduced under the supernatant using a syringe. After 15 min of centrifugation at 15 000×g, the pellets, which contain the mitochondria, were resuspended in 3 ml of enzyme buffer (50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10% (v/v) glycerol, 1 mM EDTA, 5 mM β-mercaptoethanol, 10 μg/ml α_2 macroglobulin, 10 μg/ml leupeptin and 0.5 mM phenylmethyl-sulfonylfluoride) and sonicated for 30 sec. The suspension was centrifuged for 20 min at 15 000×g and the supernatant, adjusted to 150 mM NaCl, was passed through a DEAE-cellulose column (1.5×3 cm) equilibrated with enzyme buffer containing 150 mM NaCl. The flow-through was loaded (1ml/column) on Sephadex G75 columns prepared in 10 ml syringes, equilibrated with enzyme buffer and first packed at 1000×g for 5 min at 4°C. After a second centrifugation in the same conditions, the material excluded from the Sephadex was recovered, aliquoted, immediately frozen in liquid nitrogen and stored at −80°C.

Aminoacylation assays were done as previously described (19).

Mitochondrial, nuclear and chloroplast DNA: isolation and hybridization

Mitochondrial, nuclear and chloroplast DNAs were isolated from potato tubers, etiolated germs and green leaves, respectively, according to Kemble (20) and Green *et al.* (8).

To check their genetic origin, mitochondrial tRNAs were labeled at the 3'-end in the presence of T4 RNA ligase (15) and hybridized to nuclear and mitochondrial DNAs on dot blots or Southern blots (7).

Determination of protein amounts in potato mitochondria preparations

Protein amounts were estimated using the method of Bradford (21), after sonication of the mitochondrial suspension and adjustment to a final concentration of 0.4% (w/v) sodium deoxycholate.

RESULTS AND DISCUSSION

Amounts of tRNA, rRNA and protein in potato mitochondria

The amounts of tRNA, rRNA and protein were determined from six different samples of lyophilized mitochondria. As an average, we found about 0.2 mg of protein per mg of lyophilized mitochondria and respectively 3 μg of tRNA and 20 μg of rRNA (18S + 26S) per mg of protein. Considering a matrix volume of 2 μl per mg of protein (22) and a tRNA molecular weight of 25000, the *in vivo* concentration of tRNA in potato mitochondria was estimated to be in the range of 60 μM.

Fractionation of total potato mitochondrial tRNA by two-dimensional polyacrylamide gel electrophoresis

Fig. 1 shows the two-dimensional gel pattern obtained after methylene blue staining, starting with 100 μg of total potato mitochondrial tRNA. Fifty nine spots were resolved by this technique. Taking the staining intensity as an indication of the amount of tRNA, there were 35 major and 10 less intense tRNA spots, whereas 14 spots could be considered as minor. Most of the tRNAs were pure at this stage, only some of them (e.g. spots 4 or 26) yielding two bands after a further purification step on a 15% polyacrylamide denaturing gel. On the other hand, there were several spots for some tRNAs (e.g. $tRNA^{Tyr}$, $tRNA^{Leu}$(NAA), $tRNA^{Met}$f)(see Table I). This supports the view that a tRNA can exist under different forms, due for instance to the presence or absence of some of the post-transcriptional modifications, as already found for bean mitochondrial $tRNAs^{Tyr}$ (23) and bean chloroplast $tRNAs^{Phe}$ (24) ; in both cases, the two tRNA species were identified by two-dimensional polyacrylamide gel electrophoresis and were shown to differ only by their minor nucleotide content.

Identification of the mitochondrial tRNA species fractionated by two-dimensional polyacrylamide gel electrophoresis

The 31 individual tRNAs identified so far by aminoacylation, hybridization with specific oligonucleotides or sequencing are listed in Table I. They account for 41 out of the 59 spots obtained after two-dimensional polyacrylamide gel electrophoresis.

When mitochondrial tRNA or tRNA gene sequences from other plant species were available, we localized the corresponding potato mitochondrial tRNA by hybridization, using as probes specific oligonucleotides (Table II) complementary to these sequences. By this method, 18 mitochondrial-encoded tRNA species were identified. To confirm the results of the oligonucleotide hybridization experiments, some of the tRNAs identified by this method were also characterized either by partial sequencing (e.g. $tRNA^{Cys}$, $tRNA^{Glu}$, $tRNA^{Pro}$, $tRNA^{Ser}$(GGA), $tRNA^{Ser}$(CGU)) or by aminoacylation (e.g. $tRNA^{Phe}$, $tRNA^{Pro}$, $tRNA^{Tyr}$, $tRNA^{Trp}$).

In the case of $tRNA^{Asp}$, located in spot 27, the hybridization signal, as well as the aminoacylation, were weak, suggesting that this tRNA is either present in low amounts in potato mitochondria or unstable. These results are in agreement with the fact that Joyce and Gray (1) did not detect mitochondrial $tRNA^{Asp}$ after two-

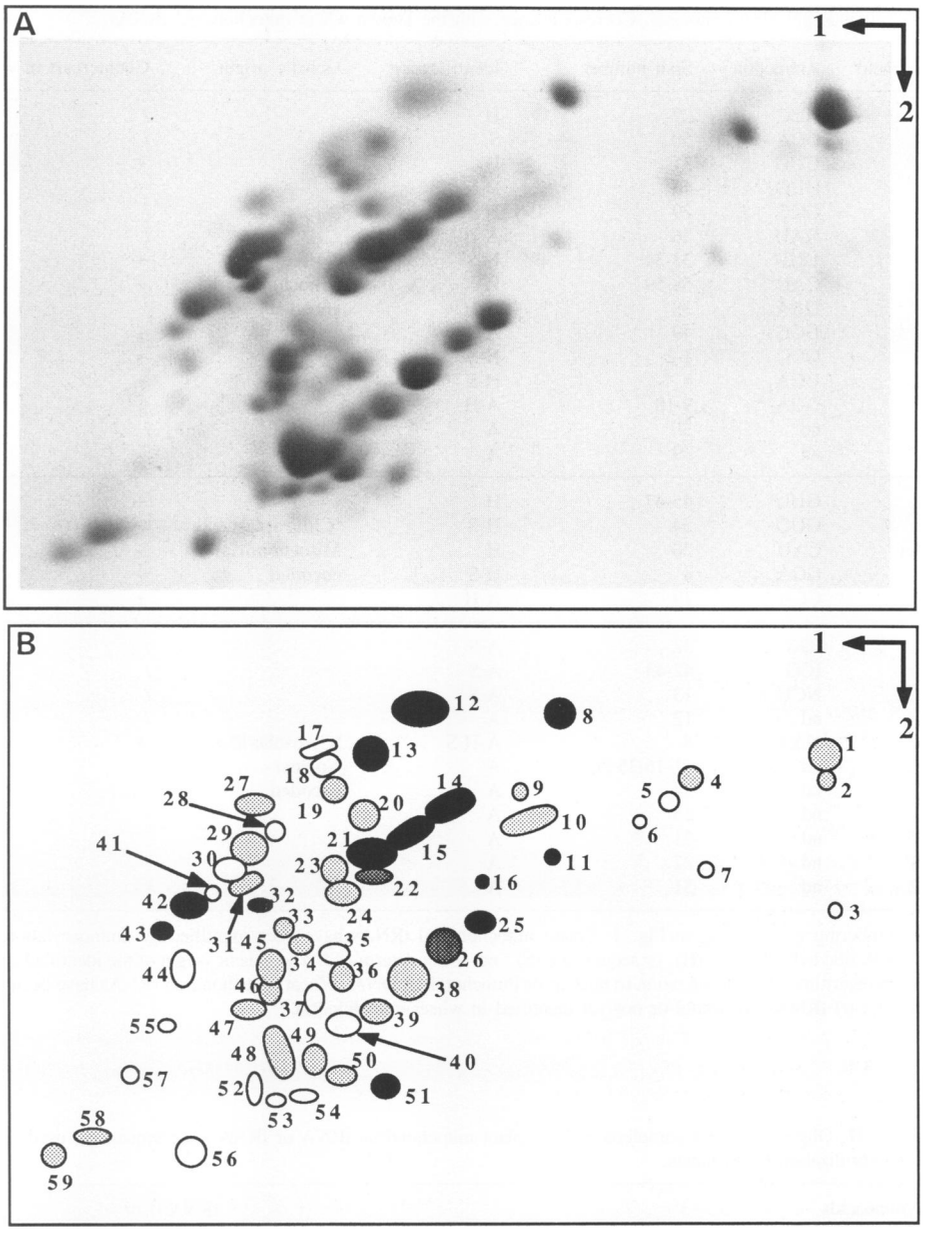

FIGURE 1. Fractionation of potato mitochondrial tRNAs by two-dimensional polyacrylamide gel electrophoresis. A) Pattern after methylene blue staining of the gel; B) Schematic diagram. Spots have been numbered from 1 to 59 and the tRNA species present in 41 of them have been identified (Table I). The arrows show the directions of migration. ▨ mitochondrial-encoded tRNAs; ■ nuclear-encoded tRNAs; ▩ spots containing a mitochondrial- and a nuclear-encoded tRNA; □ tRNAs not yet identified.

dimensional polyacrylamide gel electrophoresis of total wheat mitochondrial tRNA.

Up to now, neither mitochondrial $tRNA^{Ala}$, $tRNA^{Arg}$, $tRNA^{Ile}$ and $tRNA^{Thr}$, nor the corresponding genes, had been identified in higher plant mitochondria. Using aminoacylation assays with a bean mitochondrial enzymatic extract and the respective [^{3}H]-aminoacids, we were able to characterize 1 $tRNA^{Ala}$, 2 $tRNAs^{Arg}$, 2 $tRNAs^{Thr}$ and 2 $tRNAs^{Ile}$. By the same method, we also localized five $tRNA^{Leu}$ species and 2 $tRNAs^{Val}$. All these data are presented in table I.

Surprisingly, the $tRNA^{Ile}$ located in spot 26 hybridized to a 5'-end labeled oligonucleotide complementary to the G46-A60 sequence of a maize $tRNA^{Met}$(CAU) gene (indicated by an asterisk in Table II). This is reminiscent of the $tRNA^{Ile}$(LAU) in *E. coli* (25) and in *Mycoplasma capricosum* (26), which is transcribed from a gene with a methionine specifying anticodon CAU, but is changed into an isoleucine-specific tRNA by a post-transcriptional transformation of the C into lysidine (L), an hypermodified nucleotide. The same phenomenon probably occurs in spinach chloroplasts (27). The study of this potato mitochondrial $tRNA^{Ile}$ and of its gene are in progress and will be described elsewhere.

Genetic origin of potato mitochondrial $tRNA^{Ala}$, $tRNAs^{Arg}$, $tRNA^{Gly}$, $tRNAs^{Leu}$, $tRNAs^{Ile}$, $tRNAs^{Thr}$ and $tRNAs^{Val}$

The genetic origin of the different tRNAs isolated from purified potato mitochondria (see Table I) was determined by

Table I. Identification and genetic origin of 31 potato mitochondrial tRNAs isolated after fractionation by two-dimensional polyacrylamide gel electrophoresis. Correspondence with the known wheat mitochondrial tRNAs.

Aminoacid	Anticodon	Spot number	Identification	Genetic origin	Counterpart in wheat
Asp	GUC	27	H		/
Cys	GCA	50	S		/
Gln	UUG	31	H		+
Glu	UUC	46	H-S		+
Gly	GCC	29	H		/
Ile1	NAU	26	A-H-S		/
Lys	UUU	33-34	H-S	**Native**	+
fMet	CAU	58-59	H	**Mitochondrial-**	+
Phe	GAA	38	A-H	**encoded**	/
Pro	UGG	49	A-S		+
Ser1	GCU	1-2	H-S		+
Ser2	UGA	4	H-S		+
Tyr	GUA	9-10	A-H		+
Val1	nd	36	A		/
Val2	nd	39	A		/
Asn	GUU	45-47	H		+
His	GUG	24	H-S	**'Chloroplast-like'**	/
mMet	CAU	20	H	**Mitochondrial-**	+
Ser3	GGA	4	H-S	**encoded**	+
Trp	CCA	19	A-H		+
Ala	IGC	32	A-S		/
Arg1	ICG	42-43	A-S		/
Arg2	NCU	13	A-S		/
Ile2	nd	12	A		/
Leu1	NAA	8	A-H-S	**'Cytosolic-like'**	+
Leu2	nd	11-16-25-26	A	**Nuclear-**	/
Leu3	nd	14	A	**encoded**	/
Leu4	nd	15	A		/
Leu5	nd	21	A		/
Thr1	nd	22	A		/
Thr2	nd	51	A		/

Spot numbering is according to Fig. 1. Potato mitochondrial tRNAs have been identified by aminoacylation (A), oligonucleotide hybridization (H), or sequencing (S) ; nd = not determined. The genetic origin of the identified species has been determined by hybridization to nuclear or mitochondrial DNA. Wheat mitochondrial tRNAs have been taken from (1) ; (/) tRNAs not found or not yet identified in wheat mitochondria.

Table II. Oligonucleotides complementary to plant mitochondrial tRNA or tRNA gene sequences, used for hybridization experiments.

Aminoacids	5'→3' oligonucléotides	tRNA(t) or tRNA gene(g)
Asn ^{cp}	5'-CCAATCGGTTAACAGCCGAC-3'	maize (g)
Asp	5'-CTTCTGGCGTGACAGACCAG-3'	bean (t)
Cys	5'-GATACATTTCCATTATGTTACCTAGCC-3'	tomato (g)
Cys ^{cp}	5'-CCTGCCTTACCGCTTGGCCATGCCGCC-3'	maize (g)
Gln	5'-GGAGTAAAAGGATTCGAACCTTTG-3'	wheat (g)
Glu	5'-CATCCCTTACGGGAATCGAACCCGTGT-3'	soybean (g)
Gly	5'-AGCGGAAGGAGGGACTTGAACCCTCA-3'	lupine (g)
His ^{cp}	5'-GCGCATGGTGGATTCACAATCCAC-3'	maize (g)
Lys	5'-TGGGTATAGCAGGACTTGAACCTGCG-3'	maize (g)
mMet ^{cp}	5'-TACCTACTATTGGATTTGAACCAATG-3'	bean (t)
mMet*	5'-TGGGCTTAGTAGGGCTCGAACCTACAA-3'	maize (g)
fMet	5'-GGCGGGGGCAGGATTCGAACCTGCAAT-3'	bean (t)
Phe	5'-TTGCTCTACCAGCTGAGCTACCTAAAC-3'	bean (t)
Phe ^{cp}	5'-TGCCAGGAACCAGATTTGAACTGGTG-3'	wheat (g)
Pro	5'-GCGCTGACCAGACTGCGCTACACCTCG-3'	bean (t)
Ser (GCU)	5'-TGCCTTAAGCCACTCAGCCATACCTCC-3'	maize (g)
Ser (UGA)	5'-CGGATGGAGAGGGATTCGAACCCCCG-3'	maize (g)
Ser (GGA)^{cp}	5'-GGTAAACAAAAGCCTACATAGCAGTTC-3'	wheat (g)
Trp ^{cp}	5'-GGCACGCTCGTAGGATTTGAACCTAC-3'	bean (t)
Tyr	5'-CGCTTTTGACCACTCGGCCACTCTCCC-3'	bean (t)

(^{cp}): Complementary sequence to a 'chloroplast-like' tRNA or tRNA gene. (*): Oligonucleotide hybridizing with one of the potato mitochondrial tRNAs^{Ile}.

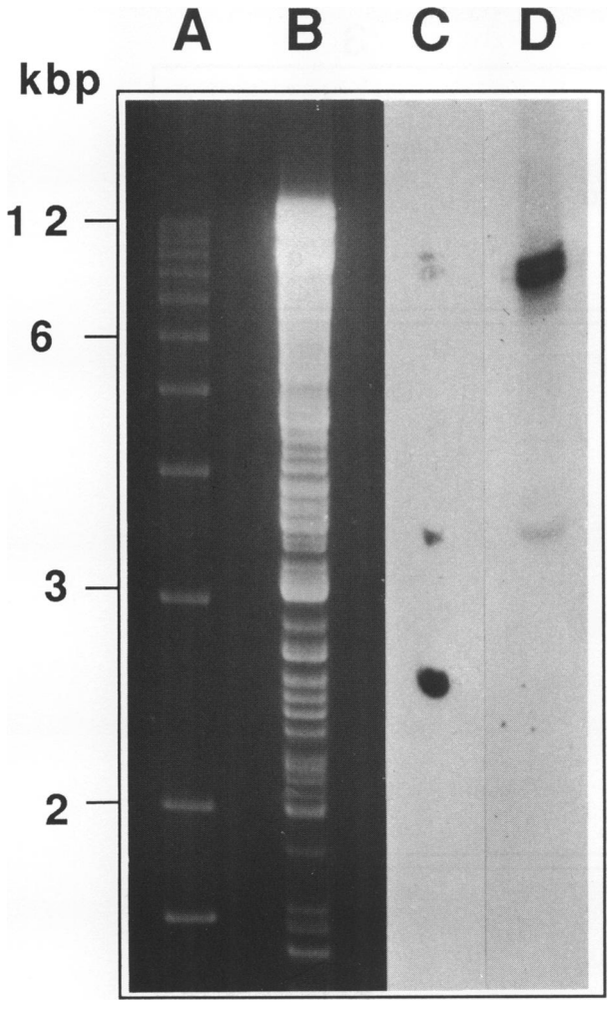

FIGURE 2. Hybridization of [^{32}P]pCp-labeled potato mitochondrial tRNAs^{Val} to BamHI restriction digests of potato mitochondrial DNA. A) 1 kbp DNA ladder (Bethesda Research Laboratories); B) BamHI potato mitochondrial DNA digest after ethidium bromide staining; C) and D) Autoradiograms showing the hybridization of [^{32}P]pCp-labeled tRNA^{Val}1 (spot 36 on the two-dimensional polyacrylamide gel of Fig. 1) and tRNA^{Val}2 (spot 39), respectively to a BamHI potato mitochondrial DNA digest. Numbers refer to the sizes (kbp) of the 1 kbp ladder fragments.

hybridization experiments using nuclear and mitochondrial DNAs.

Using dot blot hybridization, we showed that the tRNA^{Ala}, the two tRNAs^{Arg}, the two tRNAs^{Thr}, one tRNA^{Ile} and the five tRNAs^{Leu} are coded for by the nuclear genome. A similar observation was made previously in the case of bean mitochondrial tRNA^{Leu} species (7–9), but we show here for the first time a nuclear origin for alanine, arginine, threonine and isoleucine-specific mitochondrial tRNA species. These data are essentially in agreement with the results of Joyce *et al.* (2), which suggested that no gene for tRNA^{Ala}, tRNA^{Arg}, tRNA^{Ile}, tRNA^{Thr} and tRNA^{Leu} is present on the wheat mitochondrial genome. However, it should be pointed out that in the case of potato tRNAs^{Ile} one isoacceptor (spot 12 on the two-dimensional polyacrylamide gel) is indeed nuclear-encoded, but the second one (spot 26) is mitochondrial-encoded.

The tRNA^{Gly} and the two tRNAs^{Val} (spots 29, 36 and 39, respectively), hybridized to the mitochondrial genome. This contrasts with the situation in wheat, where 'cytosolic-like' mitochondrial tRNA^{Gly} and tRNA^{Val} have been characterized (1). However, we cannot rule out the possibility that a nuclear-encoded tRNA^{Gly} or tRNA^{Val} exists in our potato mitochondrial tRNA preparations as a minor species that we have not yet been able to identify by aminoacylation.

Southern blot experiments (Fig. 2) showed that one of the two characterized tRNAs^{Val} (spot 36) hybridized strongly to a 2.6 and a 3.5 kbp BamHI fragments of the mitochondrial DNA and more weakly to two BamHI fragments of large size. The second tRNA^{Val} (spot 39) gave the same type of hybridization pattern, but the intensities of the bands were inverted : in this case, the signals with the 2.6 and 3.5 kbp fragments were weak, whereas the two large fragments gave a strong hybridization. This suggests that the two tRNAs^{Val} show sufficient sequence similarity to allow cross-hybridization with the respective genes. On the other hand, the fact that each tRNA^{Val} hybridizes strongly to two different fragments could indicate the presence of repeated sequences, which is a common feature of higher plant mitochondrial genomes (28).

Partial sequencing of mitochondrial tRNA^{Ala} and tRNAs^{Arg}

Anticodon loop sequences of the two tRNAs^{Arg} (spots 13 and 42–43, respectively, on the two-dimensional gels) are shown on Fig. 3. One of these tRNAs (anticodon NCU) corresponds to the codons AGA and AGG. Unless another species exists which escaped detection, the second tRNA^{Arg}, which contains an inosine in the 'wobble' position of the anticodon, should therefore decode the other four arginine codons (CGN). According to the 'wobble' hypothesis (29), an ICG anticodon should not be able to recognize the CGG codon, but one can propose that this tRNA^{Arg}(ICG) could read the four (CGN) codons using a 'two out of three' mechanism (30), as recently shown for the tRNA^{Arg}(ICG) in bean chloroplasts (24). A similar mechanism could also operate in the case of the single tRNA^{Ala} that we identified in potato mitochondria (spot 32 on the two-dimensional gels). In this case, the IGC anticodon found by partial sequencing of the tRNA (Fig. 3) has to decode the four (GCN) codons, although the recognition of GCG is not in agreement with the 'wobble' hypothesis. If a 'two out of three' mechanism can operate in potato mitochondria, the tRNA^{Ala} and the two tRNAs^{Arg} reported here would be sufficient to allow reading of all alanine and arginine codons used for mitochondrial protein synthesis.

'Chloroplast-like' tRNAs in potato mitochondria

Among the tRNAs which have been shown to hybridize to the mitochondrial genome, 13 are 'native' tRNAs, according to the endosymbiotic hypothesis, and correspond to the 'native' mitochondrial tRNAs described so far in higher plants, whereas 5 are 'chloroplast-like' mitochondrial tRNAs. These species are presumably transcribed from tRNA genes present on promiscuous chloroplast DNA sequences (which have been inserted into mitochondrial DNA) and processed in order to produce mature functional tRNAs (1, 12 and 13). It is quite intriguing that chloroplast tRNA genes were inserted into the mitochondrial genome during evolution and that at least some of them are expressed to produce tRNAs participating in mitochondrial protein synthesis. It is interesting to see whether the same tRNAs are concerned by this phenomenon in different plant species. So far, 'chloroplast-like' tRNAs or tRNA genes specific for asparagine, cysteine, histidine, methionine (elongator),

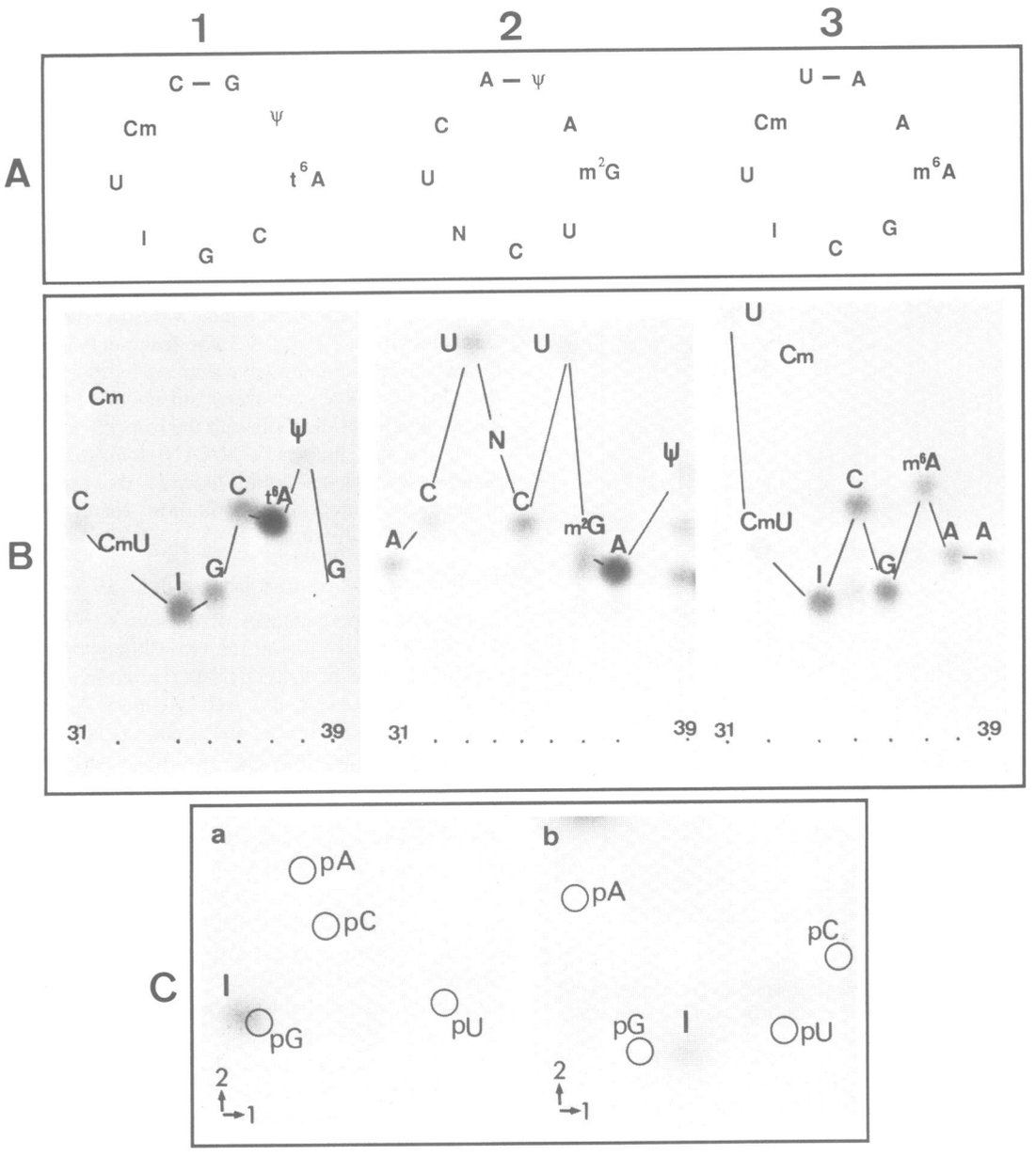

FIGURE 3. Nucleotide sequences of potato mitochondrial $tRNA^{Ala}$(IGC) (1), $tRNA^{Arg}$(NCU) (2) and $tRNA^{Arg}$(ICG) (3) from position 31 to 39. A) Schematic diagram of the anticodon loop nucleotide sequences; B) Sequence analysis from position 31 to position 39, using the technique of Stanley and Vassilenko (35); C) Two-dimensional thin layer chromatographic analysis of the inosine nucleotide present at position 34 in both potato mitochondrial $tRNA^{Ala}$(IGC) and $tRNA^{Arg}$(ICG); solvent common to (a) and (b) for the first dimension: isobutyric acid/water/25 per cent ammonia (66/33/1); second dimension solvents: (a) 2-propanol/37 per cent 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).

phenylalanine, serine (anticodon GGA) and tryptophane have been described in various plant mitochondria (31). Among the potato mitochondrial tRNAs that we have identified, $tRNA^{Asn}$, $tRNA^{His}$, $tRNA^{Met}$m, $tRNA^{Ser}$(GGA) and $tRNA^{Trp}$ are of the chloroplast type (Table I), but not $tRNA^{Cys}$ and $tRNA^{Phe}$. To check whether the 'chloroplast-like' $tRNA^{Cys}$ and $tRNA^{Phe}$ could be present in potato mitochondria only as minor species undetectable by staining, total potato mitochondrial tRNA fractionated by polyacrylamide gel electrophoresis was transferred onto nylon membrane and hybridized against 5'-end labeled oligonucleotides (Table II) complementary to sequences of the maize mitochondrial $tRNA^{Cys}$ or wheat mitochondrial $tRNA^{Phe}$ of the chloroplast type. Total potato cytosolic and chloroplast tRNAs were also fractionated and transferred onto the blots. No signal was obtained in the case of mitochondrial or cytosolic tRNAs, whereas chloroplast tRNA gave an hybridization signal, indicating that, in potato, $tRNA^{Cys}$ and $tRNA^{Phe}$ species homologous to the maize and wheat probes are indeed present only in chloroplasts.

Southern Blot experiments (Fig. 4) using the same probes showed that indeed no 'chloroplast-like' $tRNA^{Phe}$ gene seems to be present on the potato mitochondrial genome. However, the 5'-end labeled oligonucleotide specific for the 'chloroplast-like' $tRNA^{Cys}$ hybridized to a 3.5 kbp BamHI fragment of the mitochondrial DNA. Potato chloroplast DNA was used as a positive control in the same hybridization experiments. In this

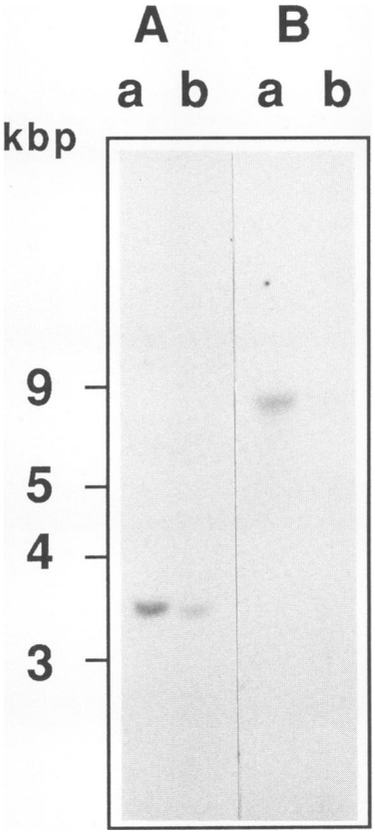

FIGURE 4. Hybridization of [^{32}P]-labeled oligonucleotides specific for 'chloroplast-like' tRNA^{Cys} (A) and tRNA^{Phe} (B) to BamHI restriction digests of potato chloroplast DNA a) or mitochondrial DNA b). Numbers refer to the sizes (kbp) of the 1 kbp ladder fragments. Oligonucleotide sequences are presented in Table II.

case, the 'chloroplast-like' tRNA^{Cys} and tRNA^{Phe}-specific oligonucleotides hybridized to a 3.5 kbp BamHI fragment and a 9 kbp BamHI fragment, respectively. These results suggest that a 'chloroplast-like' tRNA^{Cys} gene (or pseudogene) is present on the potato mitochondrial genome, but, as the corresponding tRNA could not be detected, this gene is probably not expressed.

It should be mentioned at this stage that we have identified 'native' mitochondrial tRNA^{Cys} and tRNA^{Phe} in potato (located in spots 50 and 38, respectively, on the two-dimensional polyacrylamide gels). Bean mitochondrial tRNA^{Phe} is also of 'native' type (15) and the gene coding for a 'native' tRNA^{Cys} was described in tomato mitochondria (32). In contrast, only the 'chloroplast-like' tRNA^{Cys} and tRNA^{Phe} genes seem to be present and expressed in wheat mitochondria (1), whereas the 'native' tRNA^{Phe} apparently remains as a pseudogene (33). Similarly, a potentially functional 'chloroplast-like' tRNA^{Cys} gene has been found in the maize mitochondrial genome (33).

CONCLUSION

We report here the identification of 31 tRNAs expressed in potato mitochondria, including 8 species which had not been characterized so far in higher plant mitochondria. Among the latter are 6 nuclear-encoded tRNAs (1 tRNA^{Ala}, 2 tRNAs^{Arg}, 1 tRNA^{Ile} and 2 tRNAs^{Thr}), which, together with the 5 tRNAs^{Leu}, leads to a total number of 11 nuclear-encoded tRNAs in potato mitochondria. This is more than the minimal number of 8 nuclear-encoded mitochondrial tRNAs estimated in the case of bean by differential hybrid selection (7). Import of tRNAs from the cytosol is therefore an important and general phenomenon in higher plant mitochondria. Two features of this phenomenon can already be underlined. First, we demonstrate here in the case of potato tRNAs^{Ile} that an imported and a mitochondrial-encoded species specific for the same aminoacid can be present together in mitochondria to decode the synonymous codons. The second feature is that differences in the imported tRNA species appear between the dicotyledon and monocotyledon plants studied. In potato mitochondria, we found a mitochondrial-encoded tRNA^{Gly}(GCC) similar to a lupin mitochondrial tRNA^{Gly} gene (34), whereas no tRNA^{Gly} gene could be identified in the whole wheat mitochondrial genome (2) and whereas wheat mitochondria seem to contain a 'cytosolic-like' tRNA^{Gly} with the same anticodon (1). Similarly, we identified two mitochondrial-encoded potato tRNAs^{Val}, whereas no tRNA^{Val} gene seems to be present in wheat mitochondrial DNA (2) and whereas a 'cytosolic-like' tRNA^{Val} has been found in wheat mitochondria (1).

Differences in the distribution and expression of tRNA genes located in promiscuous chloroplast DNA fragments inserted into the mitochondrial genome also exist between dicotyledon and monocotyledon plants. Typically mitochondrial tRNA^{Cys} and tRNA^{Phe} appear to function in mitochondria of dicotyledons, as shown here for potato, whereas in the case of monocotyledons such as wheat or maize the 'chloroplast-like' species are expressed. As it seems to be completely absent in potato mitochondria, the 'chloroplast-like' tRNA^{Phe} gene could have been inserted into the mitochondrial genome after the divergence between dicotyledons and monocotyledons. Mutations could then have inactivated the 'native' mitochondrial tRNA^{Phe} gene, so that only the 'chloroplast-like' tRNA would be present in mitochondria of monocotyledons.

The tRNA population that we have characterized so far in potato mitochondria (Table I) comprises 15 typically mitochondrial, 5 'chloroplast-like' and 11 nuclear-encoded species. Although these different genetic origins of the mitochondrial tRNAs involve important constraints, the fact that these tRNA species really function in mitochondrial protein synthesis is supported by several arguments : i) they are processed to their mature size, contain modified nucleotides and are good substrates for the corresponding aminoacyl-tRNA synthetases, as well as for tRNA nucleotidyltransferase and ii) as the nuclear-encoded or 'chloroplast-like' tRNAs do not have a 'native' counterpart, there is no redundancy, so that all species are necessary (and sufficient) to read the codons in mitochondrial protein synthesis.

One of the most intriguing problems is to understand how some of the tRNAs transcribed in the nucleus are selected to be imported into the mitochondria and by which mechanisms these polynucleotides are able to cross the double mitochondrial envelope.

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