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Differential import of nuclear-encoded tRNA^{Gly} isoacceptors into *Solanum tuberosum* mitochondria

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ABSTRACT

In potato (*Solanum tuberosum*) mitochondria, about two-thirds of the tRNAs are encoded by the mitochondrial genome and one-third is imported from the cytosol. In the case of tRNA^{Gly} isoacceptors, a mitochondrial-encoded tRNA$^{Gly}_{(GCC)}$ was found in potato mitochondria, but this is likely to be insufficient to decode the four GGN glycine codons. In this work, we identified a cytosolic tRNA$^{Gly}_{(UCC)}$, which was found to be present in *S.tuberosum* mitochondria. The cytosolic tRNA$^{Gly}_{(CCC)}$ was also present in mitochondria, but to a lesser extent. By contrast, the cytosolic tRNA$^{Gly}_{(GCC)}$ could not be detected in mitochondria. This selective import of tRNA^{Gly} isoacceptors into *S.tuberosum* mitochondria raises further questions about the mechanism underlying the specificity of the import process.

INTRODUCTION

In plant cells, translation occurs in three compartments: the cytosol, the mitochondria and the chloroplasts, and all tRNAs necessary for the transfer of the 20 amino acids to the elongating polypeptide chains have to be present in these three compartments. Despite its large size, the plant mitochondrial genome lacks a number of tRNA genes, and some of the tRNAs involved in translation of mitochondrial mRNAs are nuclear-encoded and imported from the cytosol (1,2). The number of imported tRNAs and their identities vary from one plant species to another. In *Marchantia polymorpha*, almost all the tRNA genes needed are present in the mitochondrial genome (3). In *Arabidopsis thaliana*, about one-third of the genes are missing (4) and the corresponding tRNAs have to be imported from the cytosol into the mitochondria. Concerning the mitochondrial-encoded tRNAs, these are of two types: the 'authentic' mitochondrial-encoded tRNAs called 'native' tRNAs, and the 'chloroplast-like' tRNAs whose genes originate from chloroplasts and were inserted into the mitochondrial genome during evolution.

Most of the time, simple sequence comparisons allow us to determine to which category a tRNA belongs (5,6). Nuclear-encoded tRNAs are usually very different from mitochondrial- and chloroplast-encoded tRNAs (<60% identity). Mitochondrial 'native' tRNAs show 70–75% identity with chloroplast and prokaryotic tRNAs. Mitochondrial 'chloroplast-like' tRNAs are identical or nearly identical to their chloroplast counterparts (95–100% identity). Whereas isoacceptor tRNA sequences may vary to a large extent from one cellular compartment to another, there is an impressive conservation of the tRNA sequences with a given genetic origin (nuclear, plastidic or mitochondrial) among higher plants.

The number of mitochondrial tRNA^{Gly} genes and the genetic origin of mitochondrial tRNAs^{Gly} change from one plant to another. Two genes coding for native tRNA^{Gly} isoacceptors (with GCC and UCC anticodons, respectively) have been identified in the mitochondrial genome of the bryophyte *M.polymorpha* (3). Only a native tRNA$^{Gly}_{(GCC)}$ gene was found in the mitochondrial genome of the dicotyledon *A.thaliana* (4). In wheat (monocotyledon), maize (monocotyledon) and larch (gymnosperm), a nuclear-encoded tRNA$^{Gly}_{(GCC)}$ was detected in mitochondria (7,8). In the dicotyledon *Solanum tuberosum* (potato), only a mitochondrial-encoded tRNA$^{Gly}_{(GCC)}$ has been identified so far in mitochondria (1). According to the 'two out of three' and the 'wobble' translation rules (9), at least one other tRNA^{Gly}, in addition to the already known organelle- or nuclear-encoded tRNA$^{Gly}_{(GCC)}$, would be necessary in the mitochondria of higher plants to decode the four GGN glycine codons. Here we show that in *S.tuberosum* mitochondria, the needs for glycine codon decoding are likely to be fulfilled thanks to a selective import of cytosolic tRNA$^{Gly}_{(UCC)}$ and tRNA$^{Gly}_{(CCC)}$, whereas cytosolic tRNA$^{Gly}_{(GCC)}$, which has a mitochondrial-encoded counterpart, is not imported.

MATERIALS AND METHODS

Isolation of mitochondria

In order to obtain potato mitochondria free from cytosolic nucleic acid contaminants, the crude mitochondrial pellet (10) was purified by centrifugation successively on a continuous Percoll/PVP gradient [0.3 M sucrose, 10 mM potassium phosphate pH 7.5, 1 mM EDTA, 0.1% (w/v) BSA, 0–30% (v/v) Percoll, 0–10% (w/v) polyvinylpyrrolidone K25 (PVP)] and on a 13.5/21/45% (v/v) discontinuous Percoll gradient (11). Purification on two gradients reduced cytosolic contamination of mitochondrial tRNAs 5–10 times compared with purification on a single continuous Percoll/PVP gradient.

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Transfer RNA extraction

Total and mitochondrial tRNAs were extracted from bean (*Phaseolus vulgaris*) hypocotyls or potato (*S.tuberosum*) tubers according to Maréchal-Drouard *et al.* (12). The proportion of mitochondrial tRNAs in total tRNA preparations from such plant material does not usually exceed 0.5% (13).

Northern blot analysis of tRNAs

Transfer RNAs were separated on a 15% (w/v) polyacrylamide gel, electro-transferred onto Hybond-N nylon membranes (Amersham) and hybridized to oligonucleotide probes at 55°C (for cytosolic $tRNA^{Gly}$ probes except AM40 and AM42), 45°C (for non-cytosolic $tRNA^{Gly}$ probes) or 40°C (for AM40 and AM42) in 6× SSC, 0.5% (w/v) SDS. Washes were performed at the hybridization temperature in 2× SSC, 0.1% SDS. The oligonucleotides listed below were used as probes:

A.thaliana cytosolic 5S RNA (accession no. M65137), 5′-GGAGGTCACCCATCCTAGTACTAC-3′; *A.thaliana* cytosolic $tRNA^{Lys}_{(CUU)}$ (accession no. U67679), 5′-CGCCCACCGTGG-GGCTCGAACCC-3′; *P.vulgaris* cytosolic $tRNA^{Leu}_{(C*AA)}$ (6), 5′-TGTCAGAAGTGGGATTTGAACCCA-3′; *A.thaliana* cytosolic $tRNA^{Glu}_{(UUC)}$ (accession no. AC000106), 5′-CTCCT-GGGTGAAAGCCAGATA-3′; *Lupinus luteus* mitochondrial $tRNA^{Gly}_{(GCC)}$ (6), AM15 5′-AGCGGAAGGAGGGACTT-GAACCCTCA-3′; *A.thaliana* cytosolic $tRNA^{Gly}_{(GCC)}$ (accession no. AB010700), AM23 5′-TGCACCAGCCGGGAATC-GAAC-3′, AM40 5′-GCAGGGTACTATTCT-3′; *A.thaliana* cytosolic $tRNA^{Gly}_{(CCC)}$ (accession no. AC005309), AM29 5′-TGCGCATCCAGGGAATCGAAC-3′, AM42 5′-GGAGGG-TACTATGAT-3′; *Rattus norvegicus* cytosolic $tRNA^{Gly}_{(UCC)}$ (accession no. K03130), AM27 5′-TGCGTTGGCCGGGAATT-GAACCCGGGG-3′; *P.vulgaris* cytosolic $tRNA^{Gly}_{(UCC)}$ (Fig. 2; accession no. AJ012213), AM30 5′-TTGCTTGGUAGGCAAT-TATCC-3′, AM31 5′-CCGGGTTCGACTCCCGG-3′, AM32 5′-GCGTCTGTAGTCCAACGGTTAG-3′, AM33 5′-TGCGTCT-GCCGGGAGTCGAAC-3′.

Transfer RNA purification and sequencing

Separation of tRNAs by 2D-polyacrylamide gel electrophoresis (2D-PAGE) and dot blotting were performed according to Maréchal-Drouard *et al.* (12). Aminoacylations were assayed with 10^{-4} M [^{3}H]glycine and with either a bean cytosolic enzymatic extract or a potato mitochondrial enzymatic extract (12).

Determination of the tRNA sequence was performed using the technique of Stanley and Vassilenko (14). For analysis by homochromatography (15), the tRNA was 3′ end-labeled with [^{32}P]pCp (16) and statistically hydrolyzed by heating in water. Reverse transcription, tRNA circularization with T4 RNA ligase, and PCR were performed according to Yokobori and Pääbo (17).

RESULTS

Northern blot analysis revealed that some cytosolic $tRNA^{Gly}$ probes hybridized to *S.tuberosum* mitochondrial tRNAs

As mentioned above, only a native $tRNA^{Gly}_{(GCC)}$ has been identified so far in mitochondria of *S.tuberosum* (1), although at least one additional $tRNA^{Gly}$ isoacceptor would be necessary to decode the four glycine codons in mitochondria. The hypothesis of an import of some nuclear-encoded $tRNAs^{Gly}$ into the mitochondria of *S.tuberosum* was therefore emitted. To test this hypothesis, total and mitochondrial tRNAs from *S.tuberosum* were run on 15% polyacrylamide gels and northern blot hybridizations were performed. Oligonucleotides complementary to different higher plant tRNA sequences were used as probes, taking advantage of the fact that only very few differences are observed from one higher plant to another in the sequence of tRNAs having the same genetic origin, that is nuclear-encoded tRNAs, mitochondrial 'native' tRNAs, or chloroplast-encoded tRNAs and mitochondrial-encoded 'chloroplast-like' tRNAs. Transfer $RNA^{Leu}_{(C*AA)}$, which is a known imported tRNA (1), was taken as an import reference. Cytosolic 5S RNA and cytosolic $tRNA^{Lys}_{(CUU)}$ probes allowed the evaluation of cytosolic contamination. Cytosolic $tRNA^{Gly}_{(GCC)}$ and $tRNA^{Gly}_{(CCC)}$ probes (AM23 and AM29, respectively) were derived from *A.thaliana* sequences. For cytosolic $tRNA^{Gly}_{(UCC)}$, no sequence had at that time been identified in plants, and an oligonucleotide (AM27) complementary to the rat (*R.norvegicus*) cytosolic $tRNA^{Gly}_{(UCC)}$ was used as a probe. Until now, no eukaryotic cytosolic $tRNA^{Gly}_{(ACC)}$ has ever been identified.

As expected, the mitochondrial $tRNA^{Gly}_{(GCC)}$ probe hybridized with *S.tuberosum* mitochondrial tRNAs (1), and the three cytosolic $tRNA^{Gly}$ probes gave a signal with *S.tuberosum* total tRNAs (Fig. 1A). Hybridizations of cytosolic $tRNA^{Gly}$ probes with mitochondrial tRNAs showed different results, depending on the probe. Indeed, no cytosolic $tRNA^{Gly}_{(GCC)}$ could be detected in mitochondria, but a strong signal was obtained with the rat cytosolic $tRNA^{Gly}_{(UCC)}$ probe (AM27) and a weaker one with the cytosolic $tRNA^{Gly}_{(CCC)}$ probe (AM29) (Fig. 1A). The tRNA hybridizing with AM27 was assumed to be a nuclear-encoded $tRNA^{Gly}_{(UCC)}$, but this had to be confirmed (see below).

These results suggested that cytosolic $tRNA^{Gly}_{(UCC)}$ and $tRNA^{Gly}_{(CCC)}$, but not cytosolic $tRNA^{Gly}_{(GCC)}$, were imported into *S.tuberosum* mitochondria, and that the level of import was dependent on the tRNA (Fig. 1A): strong import of the tRNA hybridizing with AM27, little import of $tRNA^{Gly}_{(CCC)}$, and no detectable import of $tRNA^{Gly}_{(GCC)}$.

Isolation and sequencing of a new plant cytosolic $tRNA^{Gly}$, with a UCC anticodon

In order to unambiguously identify the tRNA hybridizing with AM27, bean total tRNAs were separated by 2D-PAGE. Bean was preferred to potato for preparing total plant tRNA because of much higher yields. The material in each spot resolved by 2D-PAGE was eluted, hybridized with AM27 and tested by aminoacylation with [^{3}H]glycine. The positive samples, which corresponded to confluent spots on the gel, were pooled and further purified by another 2D-PAGE, followed by a 15% polyacrylamide denaturing gel. Only one tRNA band yielding a hybridization signal with AM27 was thus obtained.

This purified tRNA was directly sequenced according to Stanley and Vassilenko (14). Nucleotides from positions 13 to 65 could be identified by this method (Fig. 2), yielding a partial sequence corresponding to a $tRNA^{Gly}$ with an NCC anticodon. A few bases were modified: dihydrouridine was found at positions 20 and 20a, pseudouridine at position 55, and methyladenosine at position 58. An unmodified U was present at position 54 instead of the usual T_{54}. An unknown modified nucleotide was found at the wobble position in the anticodon. Analysis by 2D-thin layer chromatography (1) (data not shown) suggested that this

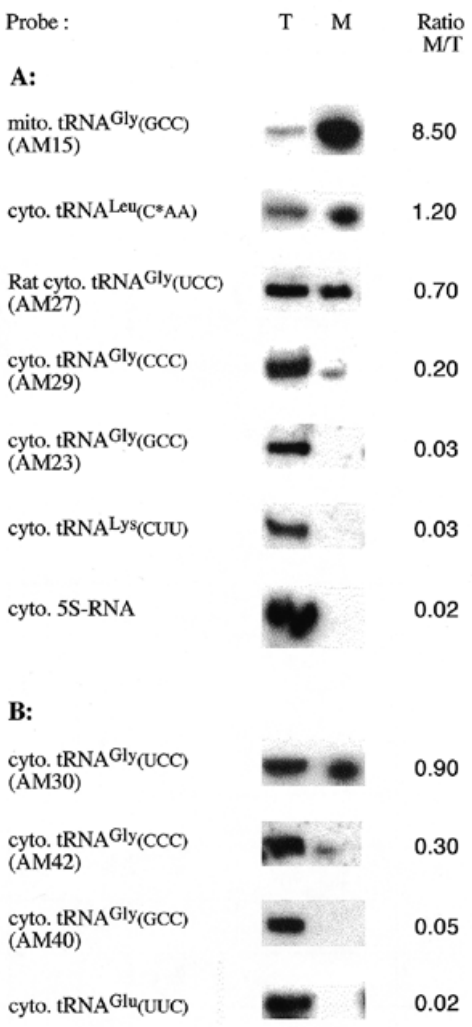


Figure 1. Northern blots with *S.tuberosum* total (T) and mitochondrial (M) tRNAs. The oligonucleotide probes are given in Materials and Methods; mito. and cyto. indicate mitochondrial and cytosolic, respectively. Hybridization signals were quantified, and the ratio between the mitochondrial (M) signal and the total (T) signal is indicated. (**A**) The hybridizations obtained with the probes used as a first approach. (**B**) The results with the probes designed after analysis of the *S.tuberosum* tRNA^{Gly} sequences.

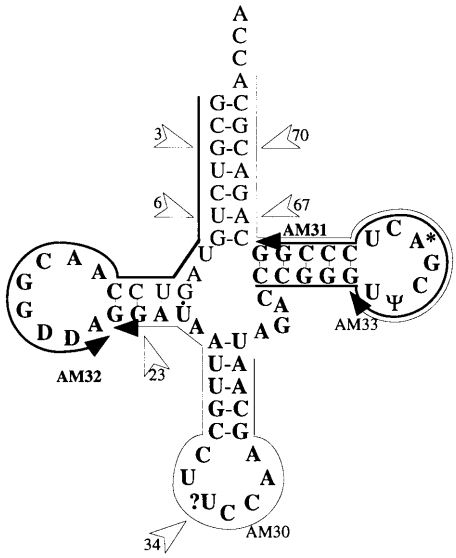


Figure 2. Secondary structure of *P.vulgaris* tRNA$^{Gly}_{(UCC)}$. The nucleotides in bold correspond to those identified by direct sequencing of the tRNA. Oligonucleotides AM30–AM33 are indicated around the sequence. Open arrows point to the positions detailed in Table 1. D, dihydrouridine; Ψ, pseudouridine; A*, methyladenosine; ?U, unknown modified uridine.

nucleotide, which was highly hydrophilic, was a modified U (G.Keith, personal communication).

In order to determine the whole sequence, RNA circularization was performed on the purified tRNA. This ligation product was used in a reverse transcription reaction with AM30 as a primer (Fig. 2). A PCR reaction was performed with the reverse transcription product in the presence of oligonucleotides AM30 and AM31 (Fig. 2). Analysis of the PCR product allowed the determination of the sequence of the 5′ and 3′ ends of the tRNA.

To confirm the data obtained by direct sequencing with the Stanley and Vassilenko method, a PCR reaction was performed with oligonucleotides AM32 and AM33 (Fig. 2), using as a template the product of a reverse transcription performed with the purified bean tRNA^{Gly}. This confirmed that the new tRNA^{Gly} had a UCC anticodon, and the obtained sequence corresponded exactly to that established by direct sequencing. The complete bean tRNA$^{Gly}_{(UCC)}$ sequence can be found under DDBJ/EMBL/GenBank accession no. AJ012213.

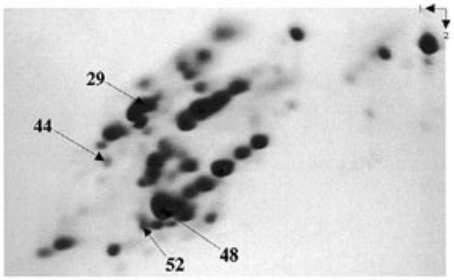
Before using this new plant tRNA$^{Gly}_{(UCC)}$ sequence for further confirmation of the mitochondrial tRNA^{Gly} pattern in potato, we verified that there were no differences between the potato and the bean tRNA$^{Gly}_{(UCC)}$ sequences. A PCR reaction was therefore performed with oligonucleotides AM32 and AM33 and with *S.tuberosum* total DNA as a template. The partial sequence thus obtained (nucleotides 22–53) was identical to that of the bean tRNA$^{Gly}_{(UCC)}$.

More recently, two sequences were obtained from the *A.thaliana* genome sequencing program (accession nos AB12245 and AC005315), which were identical to the *P.vulgaris* AJ012213 sequence. The *P.vulgaris* or *A.thaliana* tRNA$^{Gly}_{(UCC)}$ sequence presented 71% identity with the *A.thaliana* tRNA$^{Gly}_{(GCC)}$ sequence (accession no. AB010700), and 58% identity with the *A.thaliana* tRNA$^{Gly}_{(CCC)}$ sequence (accession no. AC005309).

Determination of partial cytosolic tRNA$^{Gly}_{(GCC)}$ and tRNA$^{Gly}_{(CCC)}$ sequences in *S.tuberosum*

Still to prevent misinterpretation of the northern blots, partial sequences of the *S.tuberosum* cytosolic tRNA$^{Gly}_{(GCC)}$ and tRNA$^{Gly}_{(CCC)}$ were determined. This was performed by PCR reactions with *S.tuberosum* total DNA as a template, and oligonucleotides specific for the 5′ and 3′ regions of either tRNA$^{Gly}_{(GCC)}$ or tRNA$^{Gly}_{(CCC)}$.

The cytosolic tRNA$^{Gly}_{(GCC)}$ sequence was previously determined in *A.thaliana* (accession no. AB010700), *L.luteus* (accession no. Z49255), *Oriza sativa* (accession no. X14039), *Eleusine coracana* (accession no. U02636), *Sorghum bicolor* (accession no. X0695) and *Triticum aestivum* (accession no. M28427). Exactly the same sequence was found in all cases. The partial *S.tuberosum*



probe	AM15	AM29	AM30
spot n°	29	44	48; 52

Figure 3. *Solanum tuberosum* mitochondrial tRNAs fractionated by 2D-PAGE as described in (1). The gel was stained with methylene blue. Arrows indicate the spots hybridizing with tRNA^{Gly} probes as listed below the picture.

tRNA$^{Gly}_{(GCC)}$ sequence we obtained (nucleotides 23–52) was also identical to these previously established sequences.

The *A.thaliana* tRNA$^{Gly}_{(CCC)}$ sequence was the only plant tRNA$^{Gly}_{(CCC)}$ sequence known so far. In the partial *S.tuberosum* tRNA$^{Gly}_{(CCC)}$ sequence we obtained (nucleotides 22–52), only one divergence was found compared to the *A.thaliana* sequence: a G at position 50 of the tRNA instead of an A in *A.thaliana*.

Further northern blot analyses supported an import of cytosolic tRNA$^{Gly}_{(UCC)}$ and cytosolic tRNA$^{Gly}_{(CCC)}$ into *S.tuberosum* mitochondria

To confirm the first observations (Fig. 1A), further northern blot hybridizations were performed with *S.tuberosum* total and mitochondrial tRNAs, using cytosolic tRNA^{Gly} probes which took into account the above sequence studies and which were 100% identical to *S.tuberosum* cytosolic tRNA^{Gly} sequences: oligonucleotides AM30 for tRNA$^{Gly}_{(UCC)}$, AM40 for RNA$^{Gly}_{(GCC)}$, and AM42 for tRNA$^{Gly}_{(CCC)}$ (Fig. 1B). As already shown in Figure 1A, the probes specific for cytosolic tRNA$^{Gly}_{(UCC)}$ and tRNA$^{Gly}_{(CCC)}$ hybridized to mitochondrial tRNAs, and no signal was obtained in the mitochondrial tRNA lane with the probe specific for cytosolic tRNA$^{Gly}_{(GCC)}$. Because *A.thaliana* cytosolic tRNA$^{Glu}_{(UUC)}$ presented strong similarities with *P.vulgaris* or *A.thaliana* cytosolic tRNA$^{Gly}_{(UCC)}$, a futher control was performed with a cytosolic tRNA$^{Glu}_{(UUC)}$ probe, which showed that tRNA$^{Glu}_{(UUC)}$ was only located in the cytosol (Fig. 1B). Altogether, these results further supported the idea that cytosolic tRNA$^{Gly}_{(UCC)}$ and tRNA$^{Gly}_{(CCC)}$ were both imported into *S.tuberosum* mitochondria, whereas cytosolic tRNA$^{Gly}_{(GCC)}$ was not.

Isolation and identification of the mitochondrial tRNAs hybridizing with the cytosolic tRNA$^{Gly}_{(UCC)}$ and tRNA$^{Gly}_{(CCC)}$ probes: confirmation of an import of some nuclear-encoded tRNAs^{Gly} into mitochondria

To definitely identify the mitochondrial tRNAs hybridizing with the cytosolic tRNA$^{Gly}_{(UCC)}$ and tRNA$^{Gly}_{(CCC)}$ probes, *S.tuberosum* mitochondrial tRNAs fractionated by 2D-PAGE (1) (Fig. 3) were hybridized with different tRNA^{Gly} probes. As already shown, the tRNA eluted from spot 29 hybridized with the mitochondrial tRNA$^{Gly}_{(GCC)}$ probe (oligonucleotide AM15) (1). The material from the minor spot 44 hybridized with a cytosolic tRNA$^{Gly}_{(CCC)}$ probe (oligonucleotide AM29), whereas spots 48 and 52 contained a tRNA hybridizing with a cytosolic tRNA$^{Gly}_{(UCC)}$ probe (oligonucleotide AM30). As a complementary control, the same probes were also hybridized to Southern blots of restriction endonuclease-digested *S.tuberosum* mitochondrial DNA (data not shown). As expected, the mitochondrial tRNA$^{Gly}_{(GCC)}$ probe hybridized to the mitochondrial DNA, but no signal was detected with the tRNA$^{Gly}_{(UCC)}$ and the tRNA$^{Gly}_{(CCC)}$ probes, confirming that the mitochondrial tRNAs hybridizing with these probes had to be imported into mitochondria.

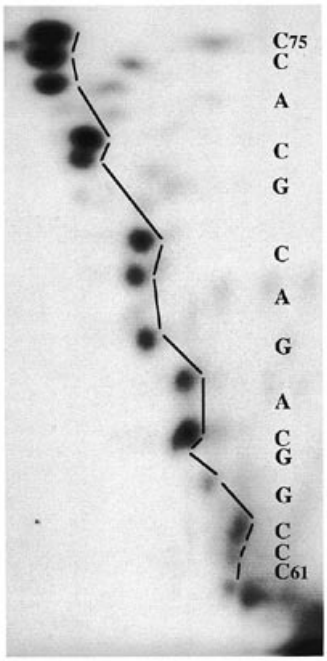


Figure 4. Homochromatography 3′-end sequencing of the major tRNA present in spot 48 of the 2D-PAGE fractionation of *S.tuberosum* mitochondrial tRNAs shown in Figure 3. The obtained sequence is identical to that of the *P.vulgaris* cytosolic tRNA$^{Gly}_{(UCC)}$ from positions 61 to 75 (see Fig. 2).

Aminoacylation with [^{3}H]glycine was obtained in the presence of a *S.tuberosum* mitochondrial enzymatic extract with the tRNAs eluted from the four spots mentioned above (29, 44, 48 and 52). This showed that each of these four spots contained a tRNA^{Gly}. Finally, spot 48, which is supposed to correspond to the cytosolic tRNA$^{Gly}_{(UCC)}$, appeared to contain several tRNAs which migrated as three bands on a 15% polyacrylamide gel. The tRNA eluted from the major band was 3′ end-labeled with [^{32}P]pCp, statistically hydrolyzed in water, and analyzed by homochromatography (Fig. 4). The obtained 3′ end sequence, from nucleotides 61 to 75, was 100% identical to the previously determined sequence of the cytosolic tRNA$^{Gly}_{(UCC)}$, thus confirming that the major tRNA species in spot 48 corresponded to this nuclear-encoded tRNA. Spot 44 was a minor spot and contained little material, so that a similar sequencing experiment could not be performed in this case. Altogether, these results confirmed the presence of the cytosolic tRNA$^{Gly}_{(UCC)}$ and tRNA$^{Gly}_{(CCC)}$ in *S.tuberosum* mitochondria.

DISCUSSION

In this study, we show that nuclear-encoded and mitochondrial-encoded tRNAs^{Gly} coexist in *S.tuberosum* mitochondria. A similar result was obtained with *P.vulgaris*: northern blots were prepared with *P.vulgaris* total and mitochondrial tRNAs, and hybridization allowed the detection of both the mitochondrial-encoded tRNA$^{Gly}_{(GCC)}$ and the nuclear-encoded tRNA$^{Gly}_{(UCC)}$ in the mitochondrial tRNA population (data not shown). Coexistence of imported and organelle-encoded isoacceptors in mitochondria has been previously implied for tRNA^{Ile} in higher plants (18), and for tRNA^{Ile}, tRNA^{Thr} and tRNa^{Val} in *M.polymorpha* (19). In some of these cases, tRNA isoacceptors might be redundant for decoding certain codons. Taking into account the 'two out of three' and the 'wobble' translation rules, two tRNA^{Gly} isoacceptors, with UCC and GCC anticodons, respectively, would a priori be sufficient to decode the four GGN glycine codons. Indeed, both tRNA$^{Gly}_{(UCC)}$ and tRNA$^{Gly}_{(GCC)}$ genes were found in *M.polymorpha*, *Pinus thunbergiana*, *Nicotiana tabacum* or *O.sativa* chloroplast genomes and in *M.polymorpha* mitochondrial genome [refer to The Organelle Genome Database (GOBASE); http://megasun.bch.umontreal.ca/gobase]. Translation rules in non-plant mitochondria allow an unmodified U at the first position of the anticodon to pair with all four bases at the third position of the codon for sets of four synonymous codons (9). This is the case for non-plant mitochondrial tRNAs^{Gly}: only a tRNA$^{Gly}_{(UCC)}$ gene is found in the mitochondrial genome of *Caenorhabditis elegans*, *Homo sapiens* or *Saccharomyces cerevisiae* (refer to GOBASE), and there is no mitochondrial import of cytosolic tRNAs^{Gly} in these organisms. According to our results, the situation is likely to be different in *S.tuberosum* mitochondria, where the organelle-encoded tRNA$^{Gly}_{(GCC)}$ probably decodes the GGC and GGU codons. Direct sequencing showed that, at least in bean, the U at the first position of the anticodon in the cytosolic tRNA$^{Gly}_{(UCC)}$ is modified, which may limit the decoding capacity of this tRNA to the GGA codon. In that case, the presence of the tRNA$^{Gly}_{(CCC)}$ may also be necessary in mitochondria to read the GGG codons. Our observations fit this analysis, as cytosolic tRNA$^{Gly}_{(UCC)}$ and tRNA$^{Gly}_{(CCC)}$ were found to be present in *S.tuberosum* mitochondria. It therefore seems likely that the set of tRNAs^{Gly} we identified fulfills the requirements for glycine codon recognition in *S.tuberosum* mitochondria and that no decoding overlap occurs.

Although one cannot rule out a difference in the stability of these isoacceptors in the organelles, we bring here strong evidence for a selective import of only two out of the three cytosolic tRNAs^{Gly} into *S.tuberosum* mitochondria. This is the first clearly documented case of a selective import of cytosolic isoacceptors into plant mitochondria, and only very few examples are known in other organisms: in yeast only one out of two cytosolic tRNAs^{Lys} is imported into mitochondria (20,21), and selective import of cytosolic tRNA^{Gln} isoacceptors into mitochondria has been observed in the protozoans *Tetrahymena thermophila* (22) and *Leishmania tarentolae* (23).

Two different mechanisms have been proposed for mitochondrial tRNA import. The first one is a direct import of the tRNAs through the mitochondrial membranes, and in *Leishmania tropica* the UGGYAGAG sequence present in the D-loop of the tRNAs was proposed to be essential and sufficient for *in vitro* import (24). The second mechanism is a co-import of the tRNAs with protein factors, including the corresponding aminoacyl-tRNA synthetases: in yeast, co-import of the cytosolic tRNA$^{Lys}_{(CUU)}$ with the precursor of the mitochondrial lysyl-tRNA synthetase was proposed (25). In plants, little is known so far about the tRNA import mechanism, but it was shown that a point mutation in a normally imported tRNA, tRNA^{Ala}, blocked both the aminoacylation of this tRNA by alanyl-tRNA synthetase and its import into mitochondria (26). In *S.tuberosum*, preliminary studies showed that a mitochondrial enzymatic extract was able to aminoacylate both the mitochondrial-encoded tRNA$^{Gly}_{(GCC)}$ and the three cytosolic tRNAs^{Gly}, including the non-imported cytosolic tRNA$^{Gly}_{(GCC)}$. This suggests that recognition of the tRNAs^{Gly} aminoacylation identity is not sufficient to promote specific import. Therefore, if co-import of tRNAs^{Gly} with a mitochondrial glycyl-tRNA synthetase occurs, this may require another factor for specificity, to explain the presence or absence of the different cytosolic tRNA^{Gly} isoacceptors in mitochondria.

Whatever the mechanism of import, the sequence of the tRNA was found to be essential. In *T.thermophila*, substitution of a unique nucleotide in the anticodon abolished the import of a normally imported tRNA^{Gln}, or permitted the import of a normally non-imported tRNA^{Gln} (27). In *L.tarentolae*, the replacement of the D-stem of a non-imported tRNA^{Gln} by the D-stem of an imported tRNA (tRNA^{Ile}) allowed the import of the mutated tRNA^{Gln} (23). In yeast, *in vitro* and *in vivo* import of mutated tRNAs^{Lys} underlined the role of the anticodon region and of the acceptor stem in import (28). In transgenic tobacco, the substitution of U_{70} into C_{70} in tRNA^{Ala} abolished the import of this tRNA (26).

Comparison of the cytosolic tRNA^{Gly} sequences should point out the differences between the non-imported tRNA$^{Gly}_{(GCC)}$ isoacceptor and the imported tRNA^{Gly} isoacceptors [tRNA-$^{Gly}_{(UCC)}$ and tRNA$^{Gly}_{(CCC)}$]. As a first attempt to run this kind of alignment, we took into account the partial *S.tuberosum* tRNA^{Gly} sequences we established and, betting on the very high conservation of the cytosolic tRNA sequences from one plant to the other, we used the known *A.thaliana* tRNA^{Gly} sequences for the missing 5′ and 3′ end regions. On such a basis, the tRNA$^{Gly}_{(GCC)}$ sequence presents 71% identity with the tRNA$^{Gly}_{(UCC)}$ sequence and 83% identity with the tRNA$^{Gly}_{(CCC)}$ sequence. Only six nucleotides in tRNA$^{Gly}_{(GCC)}$ are changed both in tRNA$^{Gly}_{(UCC)}$ and in tRNA$^{Gly}_{(CCC)}$ (Table 1). Four of these are located in the acceptor stem. The A_3-U_{70} base-pair should be pointed out, as the C_3-G_{70} pair is an identity element for aminoacylation in yeast tRNAs^{Gly} (29). In the D-stem, A_{23}, which theoretically pairs with C_{12}, should somehow destabilize the tRNA$^{Gly}_{(GCC)}$ structure. The last difference is the wobble position in the anticodon. Some of these six positions may be essential to avoid or to promote import into *S.tuberosum* mitochondria. Expression of chimeric tRNAs^{Gly} in transgenic potato upon exchange of these nucleotides would be an approach to analyzing the sequence dependence of the import process and trying to understand why the cytosolic tRNA$^{Gly}_{(GCC)}$ is not imported. However, with a broader view the problem looks obviously more complex. Although the sequence of the cytosolic tRNA$^{Gly}_{(GCC)}$ is likely to be the same in the two plant species, this tRNA is not imported into the mitochondria of the dicotyledonous species *S.tuberosum* and is imported into the mitochondria of the monocotyledonous species *Triticum aestivum* (7). Such variations illustrate the flexibility of the import process.

Table 1. Comparison of plant cytosolic $tRNA^{Gly}$ sequences

Nucleotides in $tRNA^{Gly}_{(GCC)}$	Nucleotides in $tRNA^{Gly}_{(UCC)}$ and $tRNA^{Gly}_{(CCC)}$
A_3-U_{70}	G_3-C_{70}
A_6-U_{67}	U_6-A_{67}
A_{23}	G_{23}: in $tRNA^{Gly}_{(UCC)}$
(C_{12}-A_{23})	(C_{12}-G_{23})
	C_{23}: in $tRNA^{Gly}_{(CCC)}$
	(G_{12}-C_{23})
G_{34}	U_{34}: in $tRNA^{Gly}_{(UCC)}$
	C_{34}: in $tRNA^{Gly}_{(CCC)}$

Only six nucleotides in $tRNA^{Gly}_{(GCC)}$ are changed both in $tRNA^{Gly}_{(UCC)}$ and $tRNA^{Gly}_{(CCC)}$. The positions of these nucleotides are also indicated in Figure 2.

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