Plant mitochondria use two pathways for the biogenesis of tRNA[His]

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ABSTRACT

All tRNA[His] possess an essential extra G_{-1} guanosine residue at their 5' end. In eukaryotes after standard processing by RNase P, G_{-1} is added by a tRNA[His] guanylyl transferase. In prokaryotes, G_{-1} is genome-encoded and retained during maturation. In plant mitochondria, although *trnH* genes possess a G_{-1} we find here that both maturation pathways can be used. Indeed, tRNA[His] with or without a G_{-1} are found in a plant mitochondrial tRNA fraction. Furthermore, a recombinant *Arabidopsis* mitochondrial RNase P can cleave tRNA[His] precursors at both positions G_{+1} and G_{-1}. The G_{-1} is essential for recognition by plant mitochondrial histidyl-tRNA synthetase. Whether, as shown in prokaryotes and eukaryotes, the presence of uncharged tRNA[His] without G_{-1} has a function or not in plant mitochondrial gene regulation is an open question. We find that when a mutated version of a plant mitochondrial *trnH* gene containing no encoded extra G is introduced and expressed into isolated potato mitochondria, mature tRNA[His] with a G_{-1} are recovered. This shows that a previously unreported tRNA[His] guanylyltransferase activity is present in plant mitochondria.

INTRODUCTION

With the exception of a few α-proteobacterial tRNA[His] (1), all known tRNA[His] molecules have an unusual 5'-end consisting of an additional guanylate residue called G_{-1}. The resulting extra base-pair in the acceptor stem is a fundamental determinant for tRNA[His] aminoacylation by histidyl-tRNA synthetases both in prokaryotes and in eukaryotes (2,3).

This extra guanylate residue can be generated via two different processes. In *Escherichia coli* and in chloroplasts, the G_{-1} is genome-encoded and retained during tRNA maturation because of an unusual cleavage of the pre-tRNA[His] at the −1 position by RNase P (4,5). In *Saccharomyces cerevisiae* as well as in *Drosophila melanogaster* the G_{-1} is not genome-encoded and must be post-transcriptionally added at the 5' terminus of the nuclear-encoded tRNA[His] by a specific tRNA[His] guanylyltransferase (6,7). In chicken mitochondria, the extra G is also post-transcriptionally added (8) and a mitochondrial tRNA[His] guanylyltransferase must be present in this organism. Indeed tRNA[His] guanylyltransferases with putative mitochondrial targeting sequences were retrieved from databases by Gu *et al.* (9).

In plants, all mitochondrial-encoded *trnH* genes sequenced so far show a potential extra guanylate residue at their 5' ends. In the liverwort *Marchantia polymorpha* and in larch, a gymnosperm, the 'native' *trnH* gene encodes an extra G at position −1 (10,11). In angiosperms, a 'chloroplast-like' *trnH* gene is expressed on the mitochondrial DNA (i.e. ref. 12). As on plastidial DNA, this mitochondrial gene also potentially encodes a G_{-1}. Thus, it was suggested that, as in chloroplasts, the extra G is mitochondrially encoded, although no experimental evidence was brought so far in plant mitochondria. Here, we provide evidence that, even though plant mitochondrial *trnH* genes potentially encode the G_{-1}, this residue can also be added post-transcriptionally by a guanylyltransferase enzyme. First, we show that plant mitochondrial RNase P cleaves tRNA[His] precursor at both positions −1 and +1 *in vivo* and *in vitro*. Second, using a direct DNA uptake methodology, we show that, during tRNA expression and processing in potato

mitochondria, a guanylate residue is added to a mutated larch mitochondrial tRNA^{His} precursor containing no encoded extra G. Finally, using a biochemical approach, a tRNA^{His}-dependent guanylyltransferase activity was found in potato mitochondria. Allover, our data show that there is an apparent flexibility in the processing of tRNA^{His} in plant mitochondria and strikingly that the two possible routes can be used to generate a functional tRNA for translation.

MATERIALS AND METHODS

In vitro synthesis of tRNA transcripts

Larch mitochondrial pre-tRNA^{His} and mature tRNA^{His} transcripts were synthesized from clones described in (11,13). Oligonucleotide-directed mutagenesis used to generate a larch mitochondrial *trnH* gene having no G$_{-1}$ was performed using the QuickChange^{TM} site-directed mutagenesis kit (Stratagene). Potato tRNA^{His} precursor construct was amplified by polymerase chain reaction (PCR) using relevant pairs of primers and cloned into pGEM-T Easy (Promega). The construct containing *Arabidopsis thaliana* cytosolic *trnA* gene sequence was obtained previously (14). The Riboprobe Kit (Promega) was used to synthesize *in vitro* transcripts in the presence of either Sp6 or T7 polymerases.

Vaccinia guanylyltransferase labeling of potato mitochondrial tRNA fraction and Southern analysis

A potato mitochondrial tRNA fraction prepared as described in ref. (15) was labeled using commercial vaccinia guanylyltransferase in the presence of 50 µCi of [α-^{32}P]GTP (800 Ci/mmol; 10 mCi/ml) according to manufacturer's recommendation (Ambion Inc.). This labeled tRNA fraction was then hybridized to a Southern blot carrying 1 µg per lane of PCR-generated DNA corresponding to either larch mitochondrial tRNA^{His} potato mitochondrial tRNA^{Cys} or tRNA^{His} sequences using conditions described elsewhere (13).

Potato mitochondria isolation, DNA uptake experiment and mitochondrial transcription of imported DNA

Mitochondria were isolated from potato (*Solanum tuberosum*) tubers by differential centrifugation and purification on Percoll gradient as described in ref. (16). As previously shown, the mitochondrial fraction is free of any cytosolic and plastidial contamination. Mitochondrial import of DNA followed by mitochondrial transcription of imported DNA sequences was mostly carried out as described in ref. (13). The gene construct corresponding to the potato mitochondrial 18S rRNA gene promoter sequence fused to larch mitochondrial *trnH* sequence with its 5'- and 3'-flanquing sequences (13) was used as a substrate for oligonucleotide-directed mutagenesis to a generate a construct containing no G$_{-1}$ and G$_{-2}$ upstream of the *trnH* gene. Oligonucleotide-directed mutagenesis was performed using the QuickChange^{TM} site-directed mutagenesis kit (Stratagene).

Circular reverse transcription-PCR

Circular reverse transcription (RT)-PCR (cRT-PCR) was used to determine 5'- and 3'-termini of RNA substrates. DNase-treated RNA extracted from mitochondria was incubated with 40 U of T4 RNA ligase (New England Biolabs) in the supplied buffer supplemented with 2 U of RNase inhibitor and in a total volume of 25 µl. Following circularization, all steps of RT-PCR were carried out essentially as described in ref. (13).

Mitochondrial RNase P activity assays

Arabidopsis thaliana mitochondrial RNase P (called PRORP1) was overexpressed and purified according to ref. (17). Cleavage assays were carried out with 500 ng of pre-tRNA and 100 ng recombinant PRORP1 for 15 min in the reaction buffer previously described (17). The cleavage products were loaded in an 8% (v/v) acryl gel. The band corresponding to the 5'-matured precursors were cut and the matured tRNA was eluted and resuspended in water for subsequent cRT-PCR procedure.

Aminoacylation assays

An enzymatic extract was prepared from highly purified potato mitochondria as already described in ref. (15). Aminoacylation was conducted under optimal conditions in the presence of 8 µg of mitochondrial enzymatic extract, 50 µM [^{3}H]-His (52 Ci/mmol) and 20 µM of tRNA transcript.

Assay for tRNA^{His} guanylyltransferase activity

The potato mitochondrial enzymatic extract prepared for aminoacylation assays was used to test for the presence of a guanylyltransferase activity. Guanylylation assay of 20 µM of tRNA transcript was done in the presence of 8 µg of mitochondrial enzymatic extract and 2.5 µCi of [α-^{32}P]GTP (800 Ci/mmol) in a buffer containing 25 mM HEPES pH 7.3, 10 mM MgCl$_2$, 100 mM KCl, 6 mM adenosine triphosphate (ATP), 3 mM DTT. Upon a 2-h incubation at 37°C, RNAs were phenol-extracted. After centrifugation, the nucleic acids recovered in the aqueous phase were purified by gel-filtration through a Sephadex G-50 spin column and resolved on a 15% (v/v) polyacrylamide gel containing 8 M urea.

Cloning of constructs

The *Arabidopsis* At2g31580 and At2g32320 genes encode a tandem duplication of the *S. cerevisiae* Thg1p homolog [(9) and Supplementary Data Figure SI]. The 5' cDNA copy of each of the *Arabidopsis* genes was amplified by RT-PCR using total *Arabidopsis* RNA as template. PCR products were inserted in pB7FWG2 gateway expression vector according to manufacturer's instruction (Invitrogen).

In vivo localization analysis

Transient expression in *Nicotiana benthamiana* protoplasts and visualization of green fluorescent protein (GFP) fluorescence by confocal mitcroscopy was performed as described in (18).

Miscellaneous

Oligonucleotides were synthesized by Sigma-Aldrich. Oligonucleotide sequences used in this study are available upon request.

RESULTS

Different populations of tRNA^{His} exist in potato mitochondria

The 5′- and 3′-termini of potato mitochondrial tRNA^{His} (Figure 1A) were determined by cRT-PCR. For this purpose, a potato mitochondrial tRNA fraction was first circularized by T4 RNA ligase and then used for RT-PCR analysis. A PCR product of about 70 bp was amplified, cloned and sequenced. Out of 42 sequences (Figure 1B and C), only seven revealed tRNA^{His} sequences without the 3′-CCA triplet. Among them, two possess the extra G at position −1 (type II), whereas for five sequences the G$_{-1}$ is not present (type IV). In 35 clones, the extra 3′-CCA sequence was found and among them, 13 clones possess the G$_{-1}$ (type I), whereas in 22 sequences the G$_{-1}$ is not present (type III). These results show the presence of various 5′ tRNA^{His} maturation intermediates in potato mitochondria with either one or two G at their 5′ends. Interestingly, the presence or not of a G at position −1 seems to be independent from the CCA addition. While the presence of tRNA^{His} molecules containing an extra G was expected as a result of an unusual cleavage of RNase P, the presence of tRNA^{His} molecules showing no extra G at position G$_{-1}$ is more surprising. A capping experiment was performed on a potato mitochondrial tRNA fraction in the presence of vaccinia guanylyltransferase and [α-^{32}P]GTP. The labeled RNA fraction was then probed against larch mitochondrial *trnH* and potato mitochondrial *trnC* and *trnH*. Noteworthy, the 'native' larch mitochondrial *trnH* (11) and the 'chloroplast-like' potato mitochondrial *trnH* sequences (19) differ greatly (28 different nucleotides out of 75). A hybridization signal was obtained only with the potato mitochondrial tRNA^{His} gene, whereas, as expected, no signal was observed with the two other tRNA genes (Figure 1D). As already shown for the chicken mitochondrial tRNA^{His} (8), our results indicate that a proportion of tRNA^{His} molecules present in potato mitochondria is a good substrate for the vaccinia guanylyltransferase and does not likely contain a G$_{-1}$. In agreement with these data, it is worth to mention that, when larch mitochondrial tRNA^{His} precursor was processed *in vitro* using a potato mitochondrial processing extract, two 5′ termini were observed by primer extension (11). At first glance, it can be hypothesized that, in plant mitochondria, the presence of fully processed tRNA^{His} molecules with a G$_{-*1}$ are obtained by an unusual cleavage by RNase P. However, the presence *in vivo* of tRNA^{His} molecules without G$_{-1}$ suggests that the usual cleavage by RNase P can exist. Therefore, the post-transcriptional addition of an extra G by a plant mitochondrial guanylyltransferase to generate a functional tRNA^{His} could also be possible. In agreement with this hypothesis, we showed by using a potato mitochondrial enzymatic extract in an *in vitro* aminoacylation assay that no histidine can be charged on potato mitochondrial tRNA^{His} transcript lacking G$_{-1}$, whereas tRNA^{His} transcript with a G$_{-1}$ is a good substrate for histidylation (Figure 1E). Thus, likewise other organisms (2,3), the additional guanylate residue at position −1 is essential for histidylation of tRNA^{His} in plant mitochondria.
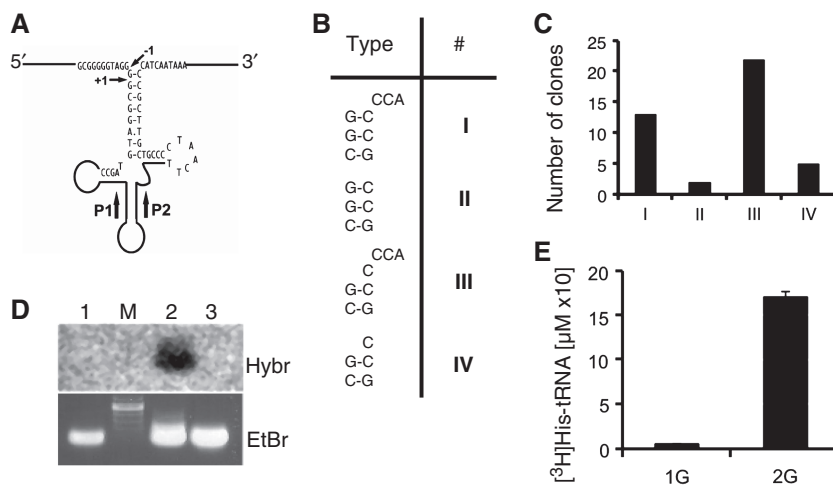

Figure 1. Potato mitochondria contain a heterogeneous population of tRNA^{His} molecules. (**A**) Schematic cloverleaf structure of potato mitochondrial tRNA^{His} precursor molecule (EMBL accession number X93577). The primers P1 and P2 used for cRT-PCR analysis are depicted by black arrows. G$_{+1}$ and G$_{-1}$ are indicated by thin arrows. (**B**) Analysis by cRT-PCR of the 5′ and 3′ ends of the tRNA^{His} population present in a potato mitochondrial tRNA fraction. Four types of extremities (I, II, III and IV) were obtained. (**C**) The number of different clones found for each type of extremities is given. (**D**) [α-^{32}P]GTP labeled potato mitochondrial tRNAs were probed against PCR fragments corresponding to (1) potato mitochondrial tRNA^{Cys}, (2) potato mitochondrial tRNA^{His} and (3) larch mitochondrial tRNA^{His}. Hybr, autoradiogram after hybridization. EtBr, corresponding image of the ethidium bromide-stained gel of PCR products. (**E**) *In vitro* histidylation of potato mitochondrial tRNA^{His} transcripts with or without a G at position −1. The mean value of three independent experiments is presented. Error bars are given. 1G and 2G, potato mitochondrial tRNA^{His} transcripts without or with a G$_{-1}$ respectively.

A guanylyl-transferase activity exists in potato mitochondria

Using direct DNA uptake into potato mitochondria, we investigated the *in organello* maturation of a larch mitochondrial tRNA precursor transcript containing no extra G at position -1 in potato mitochondria. On the larch mitochondrial genome, three G residues are found at positions G_{+1}, G_{-1} and G_{-2} on the *trnH* gene [(11) and Figure 2A]. First, using PCR mutagenesis, G_{-1} and G_{-2} were deleted from the previously used gene construct (13). In this construct, two consecutive A residues precede G_{+1} (Figure 2A). Then, following DNA uptake and expression of this mutated version into isolated potato mitochondria, mitochondrial RNAs were extracted, circularized by T4 RNA ligase, and used for cRT-PCR analysis. A specific reverse transcriptase-dependent product of about 70 nt was amplified (Figure 2B), cloned and sequenced. The size of this PCR product corresponds to the expected size of the product if the precursor RNA expressed by the transgene was correctly processed *in organello*. Out of 22 clones (Supplementary Data Figure S1), six sequences correspond to either processing intermediates or degradation products. Eight sequences showed the presence of the 3'-CCA sequence and terminate at G_{+1} and eight other sequences (see a representative sequence on Figure 2C) showed the presence of the 3'-CCA sequence and an additional G at position -1. As this G

is not gene-encoded, this residue is likely to have been added post-transcriptionally by a potato mitochondrial guanylyl transferase activity consecutively to a normal cleavage by RNase P at G_{+1}.

To confirm this result, we incubated similar amount of potato mitochondrial tRNA^{His} transcript with or without extra G_{-1} or of plant cytosolic tRNA^{Ala} transcript in the presence of $[\alpha\text{-}^{32}P]GTP$ and a potato mitochondrial enzymatic extract. Upon incubation, the corresponding transcripts were resolved on a denaturing polyacrylamide gel. As expected, no radiolabeled guanosine triphosphates (GTP) was incorporated in the tRNA^{Ala} transcript, whereas a strong signal was obtained for the potato mitochondrial tRNA^{His} having no extra G (Figure 3). It must be noted that a weak incorporation of radioactivity was observed for the tRNA^{His} containing a G_{-1}. This is very likely due to a heterogeneous population of tRNA molecules synthesized during *in vitro* transcription with T7 RNA polymerase.

The two A. *thaliana* Thg1p homologs are predominantly localized to the nucleoplasm

A tRNA^{His}-dependent guanylyl transferase activity is present in plant mitochondria. Which component is responsible for this activity? In eubacteria, a tRNA^{His}-dependent guanylyl transferase is not necessary and such an enzyme has not been identified. In eukaryotes, the
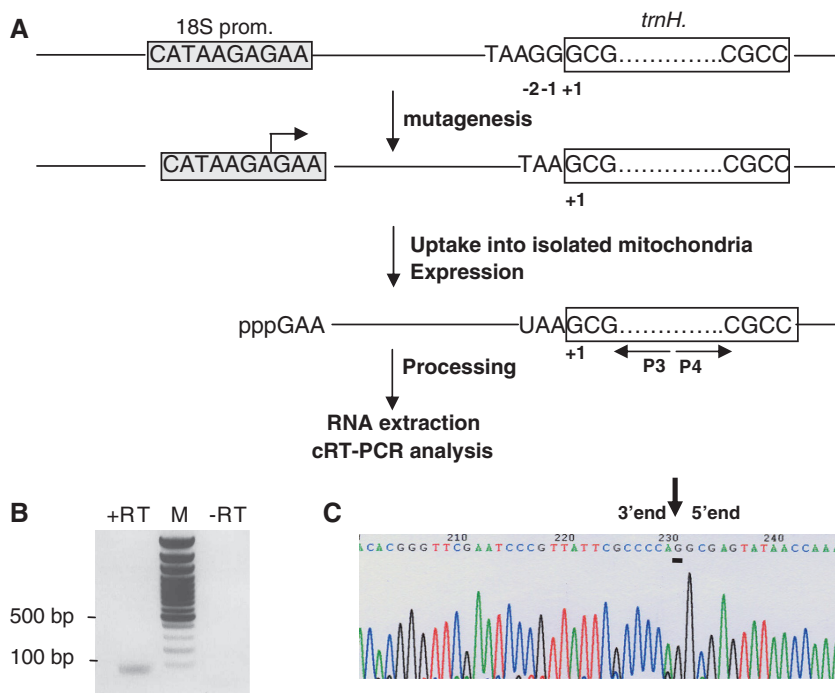

Figure 2. Analysis of the fate of the larch mitochondrial tRNA^{His} precursor transcript having no G_{-1} in potato mitochondria. (**A**) Schematic representation of the strategy. First, G_{-1} and G_{-2} encoded by the larch mitochondrial *trnH* gene are deleted. Then, upon DNA uptake into potato mitochondria, larch mitochondrial tRNA^{His} is transcribed from the potato 18S rRNA promoter sequence (gray box). Total nucleic acids were analyzed by cRT-PCR. (**B**) Image of the ethidium bromide-stained gel of PCR product amplified using primers P3 and P4. The presence (+RT) or absence (–RT) of reverse transcriptase during the cDNA synthesis in the presence of primer P3 is indicated. The lane marked M shows the migration of the DNA ladder. (**C**) The 70-bp PCR product shown in (B) was cloned and 21 clones were sequenced. A sequence showing the junction (black vertical arrow) between 5'- and 3'- termini is presented. This sequence shows that the CCA triplet and the G_{-1} (underlined) have been post-transcriptionally added.

nuclear enzyme responsible for this activity has been well characterized in *S. cerevisiae* and is called Thg1p (7,9,20,21). From the complete *Arabidopsis* nuclear genome, two genes (At2g32320 and At2g31580) that encode Thg1p homologs can be retrieved [(9) and Supplementary Data Figure S2]. The corresponding proteins share 83.3% identity and both correspond to a tandem duplication of two copies of the Thg1p homolog. Although none of them contains any predictable N-terminal mitochondrial targeting sequence, the protein encoded by the At2g31580 gene still presents an N-terminal extension as compared to the second protein. To address the potential involvement of these proteins in mitochondrial tRNA^{His} biogenesis, their subcellular localization was investigated. The 5′ copy of each *Arabidopsis* protein homolog to Thg1p (see Supplementary Figure S2)
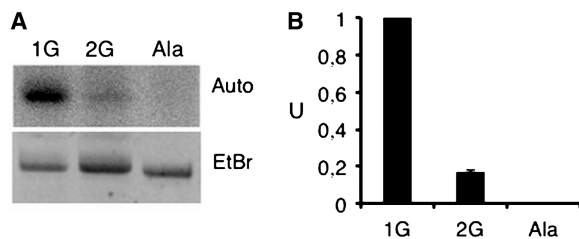
was fused to the GFP and visualized in *N. benthamiana* protoplasts by confocal microscopy. While no GFP fluorescence was observed in chloroplasts and mitochondria, both At2g31580- and At2g31320-GFP proteins were localized in the nucleoplasm (Figure 4). They are thereby not involved in the addition of the G$_{-1}$ on the plant mitochondrial tRNA^{His} but rather implicated in the addition of this residue on nuclear-encoded tRNA^{His}.

The recombinant *Arabidopsis* mitochondrial RNase P, PRORP1, cleaves potato mitochondrial tRNA^{His} precursors at two positions *in vitro*

Our results suggest that, *in vivo*, plant mitochondrial RNase P can cleave tRNA^{His} precursors at two positions, thereby generating tRNA^{His} with or without a G$_{-1}$. In order to verify this hypothesis, *in vitro* cleavage assays were performed with the purified recombinant *Arabidopsis* mitochondrial RNase P called PRORP1 (17) and precursor transcripts of potato mitochondrial tRNA^{His}. In the presence of PRORP1, tRNA^{His} precursor is submitted to an endonucleolytic cleavage and two RNA fragments of 240 and 125 nt length, respectively, are generated (Figure 5A). This is consistent with the expected cleavage at the 5′ end of tRNA^{His} resulting in the release of the 5′-leader sequence (125-nt-long RNA fragment) and a tRNA^{His} with a 3′-trailer (240-nt-long RNA fragment). To precisely map the 5′ endonucleolytic cleavage site, the 5′ matured tRNA precursor fragment was further analyzed by cRT-PCR, using the pair of primers P1 and P2 (Figure 1A). The amplicon obtained is ~240 bp and could correspond to the expected size if the correctly processed 5′ end of the tRNA^{His} has been ligated to the 3′ end of tRNA^{His} precursor. The PCR product was cloned and sequenced. Out of 26 clones, 23 sequences showed that the 3′ terminus of the precursor transcript is
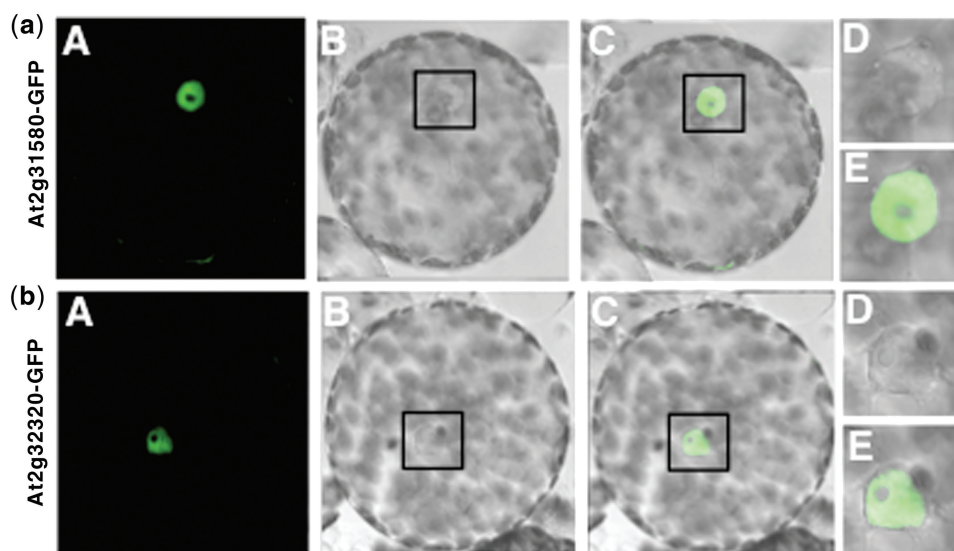

Figure 3. A tRNA^{His}-dependent guanylyl transferase activity is present in potato mitochondria. Larch mitochondrial tRNA^{His} without (1G) or with (2G) a G$_{-1}$ and plant cytosolic tRNA^{Ala} (Ala) transcripts incubated with a potato mitochondrial enzymatic extract in the presence of [α-^{32}P]GTP were analyzed on a polyacrylamide gel electrophoresis. (**A**) EtBr, image of the ethidium bromide-stained gel of RNA transcripts. Auto, autoradiography of the same gel. (**B**) Quantification of the GTP incorporation in the three tRNA transcripts. The amount of labeled tRNA was quantified using the ImageGauge software (Fuji). One unit (U) represents the amount of labeled larch mitochondrial tRNA^{His} transcript without G$_{-1}$ (1G).

Figure 4. Nuclear localization of the two *Arabidopsis* proteins homolog to Thg1p. Half of the coding sequences of At2g31580 (**a**) and At2g32320 (**b**) proteins were cloned in frame with GFP. The resulting fusion proteins were expressed in *N. Benthamiana*. The localization of the two fusion proteins (At2g31580-GFP and At2g31580-GFP) was analyzed in transformed protoplasts by laser confocal microscopy. (A) GFP fluorescence, (B) DIC image and (C) merged image of (A) and (B), (D) and (E) enlarged view of the framed area shown in (B) and (C), respectively.
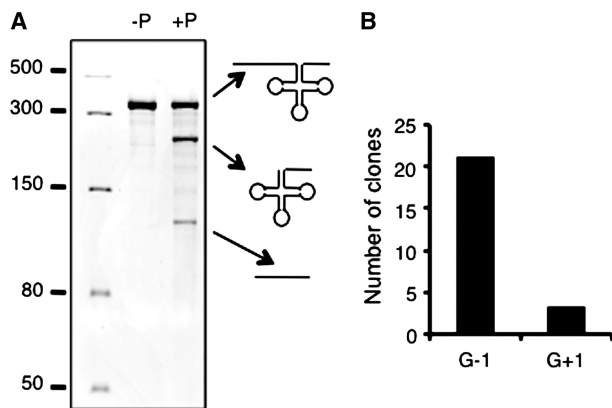
Figure 5. *In vitro* cleavage of potato mitochondrial tRNA^{His} precursor transcripts in the presence of recombinant *Arabidopsis* mitochondrial RNase P, PRORP1. (**A**) The RNA molecules generated upon incubation of a 365-nt-long potato mitochondrial tRNA^{His} precursor transcript incubated (+P) or not (−P) with PRORP1 were analyzed on a polyacrylamide gel. Molecular mass markers given in nucleotides are indicated on the left. (**B**) The circularized 240-nt-long RNA product shown in (A) amplified by RT-PCR using primers P1 and P2 (Figure 1A) was cloned. Clones were sequenced, allowing the analysis of the 5′ extremity. The number of clones corresponding to a cleavage at position G$_{-1}$ or at position G$_{+1}$ is given.

ligated to G$_{-1}$, whereas in three sequences, G$_{-1}$ is missing and G$_{+1}$ is ligated to the 3′-trailer sequence (Figure 5B). This result shows that, *in vitro*, although PRORP1 preferentially cleaves at the unusual cleavage site, this enzyme is also able to cleave at the classical cleavage site.

DISCUSSION

Until now, the biogenesis of plant mitochondrial tRNA^{His} had never been studied. Because plant mitochondrial genomes encode the G$_{-1}$, the direct pathway was thought to occur in these organelles (8). In this work, we report that, although unusual cleavage by RNase P can occur, the second pathway involving an RNase P cleavage between G$_{+1}$ and G$_{-1}$ and a guanylyl transferase activity also exists in plant mitochondria. In some archaea, a G$_{-1}$ is encoded in the genome but a tRNA^{His}-dependent guanylyl transferase is active *in vivo* and the existence of the direct pathway has not been demonstrated so far in these organisms (22). Therefore, the data provided here in plant mitochondria are the first experimental evidence demonstrating that the two routes can coexist for the biogenesis of tRNA^{His} in a same compartment. This observation raises the puzzling questions of how and why the two pathways were maintained during evolution.

Very well conserved homologs of the *S. cerevisiae* guanylyl transferase Thg1p are widely distributed within eukaryotes (9). Among them, N-terminal mitochondrial targeting sequences were identified in metazoan Thg1p homologs. In chicken mitochondria, G$_{-1}$ is not gene-encoded but rather post-transcriptionally added by a guanylyl transferase activity (8). Thus, the eukaryotic enzyme has likely been recruited to function in metazoan mitochondria. Here, a previously unreported guanylyl transferase activity was found in plant mitochondria but

none of the two *Arabidopsis* Thg1p homologs is localized in the organelle. As no other class of tRNA^{His}-dependent guanylyl transferase can be predicted, there is no obvious protein candidate and biochemical and/or genetic approaches will be necessary to identify this new enzyme.

Until recently, the RNase P characterized in most organisms were shown to be ribonucleoproteins composed of a catalytic RNA subunit and several protein subunits (23,24). This ribozyme-type RNase P precisely cleaves tRNA^{His} precursors only at G$_{-1}$ when the extra G is gene-encoded. By contrast, in plant mitochondria, the RNase P activity can be performed by a single protein called PRORP1, which, alone, is able to cleave the canonical structure of tRNAs (17). The question of plant mitochondrial tRNA^{His} precursor processing by a proteinaceous RNase P had not been addressed yet. According to the results obtained here, this proteinaceous RNase P seems to be less efficient than a ribozyme-type RNase P to precisely cleave tRNA^{His} precursors only at G$_{-1}$ when the extra G is gene-encoded and the standard cleavage at G$_{+1}$ is also performed by PRORP1. Consequently, a second factor with a tRNA^{His}-dependent guanylyl transferase activity presumably evolved or was maintained in plant organelles to repair the nonfunctional tRNA^{His} molecules. Due to the existence of the two distinct mechanisms, both plant mitochondrial tRNA^{His} with or without G$_{-1}$ were found *in vivo*, while only tRNA^{His} with G$_{-1}$ can be charged *in vitro*. In yeast when the tRNA^{His} and the histidyl-tRNA synthetase are overexpressed in a mutant strain lacking Thg1p (25), the strain is viable meaning that part of the tRNA^{His} can be charged *in vivo*. Therefore, a small proportion of plant mitochondrial tRNA^{His} without G$_{-1}$ can conceivably be aminoacylated *in vivo*, although most of this tRNA species is very likely present as uncharged within mitochondria. The presence of tRNA^{His} without G$_{-1}$ in the organelle can be the direct consequence of an inefficient repair system to add the extra G. Both in prokaryotes and in eukaryotes, uncharged tRNAs act as effector molecules to regulate gene expression, for example under adverse environmental conditions (e.g. ref. 26). In eukaryotes, under stress conditions, activation of the GCN2 kinase is mediated by uncharged tRNA (27,28) and plays an important role in regulating translation. So far, nothing is known about the presence and hypothetical function of uncharged tRNA in mitochondria or chloroplast. Here, the tRNA^{His} without G$_{-1}$ is detectable in mitochondria under normal growth condition. Whether this uncharged tRNA^{His} has or not a function in the organelle will have to be addressed.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

FUNDING

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