

Fate of a Larch Unedited tRNA Precursor Expressed in Potato Mitochondria*

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In higher plant mitochondria, post-transcriptional C to U conversion known as editing mostly affects mRNAs. However, three tRNAs were also shown to be edited. Among them, three editing sites were identified in larch mitochondrial tRNA^{His}. We have previously shown that only the edited version can undergo maturation *in vitro*. In this paper, we introduced via direct DNA uptake the edited or unedited version of larch mitochondrial *trnH* into isolated potato mitochondria and expressed them under the control of potato mitochondrial 18 S rRNA promoter. As expected, the edited form of larch mitochondrial tRNA^{His} precursor was processed in the isolated organelles. By contrast, no mature tRNA^{His} was detected when using the unedited version of *trnH*. However, precursor molecules could be characterized by reverse transcription-PCR. These data demonstrate that the potato mitochondrial editing machinery is not able to recognize these “foreign” editing sites and confirm that these unedited tRNA precursor molecules are not correctly processed *in organello*. As a consequence, the fate of these RNA precursor molecules is likely to be degradation. Indeed, we detected by PCR two 3'-end truncated precursor RNAs. Interestingly, both RNA species exhibit poly(A) tails, a hallmark of degradation in plant mitochondria. Taken together, these data suggest that, in plant mitochondria, a defective unedited RNA precursor that cannot be processed to give a mature stable tRNA, is degraded through a polyadenylation-dependent pathway.

In plant mitochondria, the generation of functional tRNA molecules from larger primary transcripts requires several processing steps including the removal of 5'- and 3'-extensions. The processing of the 5'-end is catalyzed by the endoribonuclease RNase P (1), and the removal of the 3'-extremity is performed by another endonuclease termed RNase Z (2). Unlike in *Escherichia coli*, the CCA end of tRNAs is not encoded by plant mitochondrial tRNA genes and this sequence is subsequently added post-transcriptionally by a tRNA nucleotidyltransferase activity (3). Mitochondrial tRNAs also contain a low number of post-transcriptionally modified nucleotides. Some of these modifications are essential to yield active tRNAs. For instance, initiator tRNA^{Met} has to be formylated (4), and tRNA^{Ile}(LAU) is encoded by a mitochondrial gene possessing a methionine-specific CAU anticodon that will be subsequently

transformed in a L*AU anticodon (L*, derivative of lysidine), (5). Finally, in higher plant mitochondria, although post-transcriptional cytidine to uridine conversion known as editing mostly affects mRNAs, three tRNAs were also shown to be edited. In the case of potato or *Oenothera* mitochondrial tRNA^{Phe} and larch mitochondrial tRNA^{His}, *in vitro* experiments have shown that editing of precursors is a prerequisite for 5' and 3' processing to generate a mature tRNA (6–8).

Although biological systems are accurate enough to end up with a high proportion of functional macromolecules, a small number of them probably escape one or several maturation steps leading to defective products. These defective products will likely not accumulate in the cell, first because of potential deleterious effects. As a consequence, various quality control mechanisms exist to correct or eliminate defective macromolecules. DNA repair mechanisms, tmRNA-directed proteolysis of unfinished protein, or nonsense-mediated mRNA decay represent good examples of such mechanisms. However, despite their importance, RNA quality control of stable RNAs is still largely unknown with only few and recent data available. It was shown in *E. coli* that stable RNA precursors that cannot be converted to their mature forms are polyadenylated and subsequently degraded (9) and that a defective denaturable tRNA^{Trp} does not accumulate to normal levels because its precursor is rapidly degraded (10). In *Saccharomyces cerevisiae*, a polyadenylation-mediated RNA surveillance is involved in rRNA biogenesis (11) and was shown to be implicated in the elimination of aberrant tRNA_i^{Met} (12). In plant mitochondria, the existence of such RNA surveillance process remains to be determined.

A possible way to investigate a putative RNA surveillance mechanism in plant mitochondria is the expression of defective foreign genes *in organello*. Indeed, two *in organello* approaches have been successfully developed for the transformation of isolated plant mitochondria. First, an electroporation-mediated method was described in wheat mitochondria (13). Using this method, splicing and editing of transgene products were studied allowing recognition of cis-elements (14–16). Second, exogenous DNA was incorporated into isolated potato mitochondria via direct DNA uptake (17). The authors showed that the imported DNA was transcribed within the mitochondria but no other molecular approaches were developed to analyze further the potential use of this transformation procedure in studying the multiple steps involved in plant mitochondrial gene expression. In the present paper, we have taken advantage of this new methodology to determine the fate of tRNA precursor molecules that cannot be efficiently converted in their mature forms in plant mitochondria. We introduced via direct DNA uptake the edited or unedited version of larch mitochondrial *trnH* into isolated potato mitochondria and expressed them under the control of potato mitochondrial 18 S rRNA promoter. We first show that upon uptake and expression in isolated potato mitochondria, edited larch mitochondrial tRNA^{His} precursors were faithfully processed in the organelles. By contrast, we present evidence that unedited larch

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Expression and Maturation of a Foreign tRNA Gene in Potato Mitochondria

tRNA^{His} precursor transcripts, although expressed *in organello*, cannot be edited and converted in mature and stable tRNAs. Rather, two 3'-end truncated precursor RNA species exhibiting poly(A) tails, a hallmark of degradation in plant mitochondria, were detected. These results suggest that, in plant mitochondria, a defective unedited RNA precursor that cannot be processed to give a mature stable tRNA, is transiently polyadenylated and subsequently degraded.

MATERIALS AND METHODS

Gene Construct for Mitochondrial DNA Import—Edited or unedited larch mitochondrial tRNA^{His} cDNA sequences with 5'- and 3'-flanking sequences of 21 and 25 nucleotide, respectively (7) were amplified by PCR. Using oligonucleotide-directed mutagenesis (18), these fragments were fused downstream of a 178-nucleotide-long fragment containing the promoter sequence of the potato mitochondrial 18 S rRNA gene ((19) amplified by PCR from potato (*Solanum tuberosum*) mitochondrial DNA (Fig. 1). The resulting PCR products were cloned using the TOPO cloning kit (Invitrogen). Finally, the DNA substrates used for mitochondrial DNA import were PCR-amplified from the two constructs with primers flanking the cloning sites.

Isolation of Potato Mitochondria, Mitochondrial Import Assays, and Mitochondrial Transcription of Imported DNA—Mitochondria were isolated from potato (*S. tuberosum*) tubers by differential centrifugation and purification on Percoll gradient as described in (20). Mitochondrial import of DNA followed by mitochondrial transcription of imported DNA sequences was mostly carried out as described in Ref. 17. Standard mitochondrial DNA import was carried out in 40 mM potassium phosphate and 0.4 M sucrose, pH 7.0 (import buffer). The samples (200 μ l) containing 200 ng of DNA and an amount of purified mitochondria corresponding to 1 mg of proteins were incubated at 25 °C for 30 min under mild shaking. After incubation, mitochondria were washed twice with 1 ml of import buffer and resuspended in 240 μ l of transcription medium (330 mM sucrose, 90 mM KCl, 10 mM MgCl₂, 12 mM Tricine,³ 5 mM KH₂PO₄, 1.2 mM EGTA, 1 mM GTP, 2 mM dithiothreitol, 2 mM ADP, 10 mM sodium succinate, 0.15 mM CTP, 0.15 mM UTP) according to Farre and Araya (13). Upon further incubation for 3 h at 25 °C under mild shaking, mitochondria were pelleted, and nucleic acids were extracted with 150 μ l of 10 mM Tris-HCl, 10 mM MgCl₂, 1% (w/v) SDS, pH 7.5, and 1 volume of phenol. After centrifugation, the nucleic acids recovered in the aqueous phase were purified by gel-filtration through a Sephadex G-50 spin column and submitted to RNase-free DNase I (RQ1 DNase, Promega) digestion according to the recommendations of the manufacturer. After ethanol precipitation, the RNA was used as a template for RT-PCR or circular RT-PCR (cRT-PCR).

Alternatively, unlabeled UTP was replaced by [α -³²P]UTP (800 Ci/mmol) in the above transcription medium (100 μ Ci/240 μ l of medium) to generate radioactive transcripts. Following DNA import and transcription, nucleic acids were extracted as above and unincorporated [α -³²P]UTP was eliminated by gel-filtration through a Sephadex G-50 spin column.

Southern, RT-PCR, and cRT-PCR Analyses—Labeled RNAs were hybridized to Southern blots carrying 1 μ g per lane of PCR-generated DNA corresponding to either larch mitochondrial tRNA^{His} precursor cDNA sequence (see above) or to potato mitochondrial tRNA^{Phe} cDNA precursor sequence (6) Hybridizations were at 65 °C in 2 \times SSC, 0.1% (w/v) SDS.

All primer sequences used in this study are indicated below and their respective location are shown on the appropriate figures.

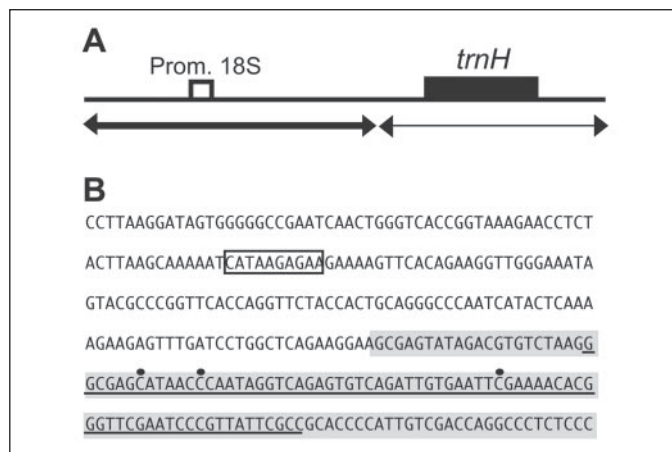


FIGURE 1. Organization of the DNA import substrate. *A*, schematic representation of the construct used for mitochondrial transformation. Larch mitochondrial *trnH* (black box) with its flanking sequences (thin arrow) was fused to the potato mitochondrial 18 S *rrn* promoter sequence (open box, Prom. 18S) with its flanking sequences (thick arrow). This construct was obtained as described under "Materials and Methods" and cloned using a TOPO cloning kit. For mitochondrial DNA import, DNA substrate was PCR-amplified from the construct with primers corresponding to the reverse and forward priming sites present on the TOPO vector. *B*, nucleotide sequence of the construct. The potato mitochondrial 18 S rRNA promoter (19) is boxed. The larch mitochondrial DNA sequence is under a gray background, and *trnH* is underlined. The three editing sites previously characterized (7) are depicted by dots.

For RT-PCR experiments, cDNA were synthesized using SuperscriptTM III (Invitrogen) and the reverse primer P3. PCRs were performed with pairs of primers P3/P4 or P3/P5 using Invitrogen TaqDNA polymerase as follows: 94 °C for 3 min; 30 cycles at 92 °C for 20 s, 50 °C for 20 s, and 72 °C for 30 s.

Circular RT-PCR was used to determine 5'- and 3'-extremities of RNA substrates. DNase-treated RNA extracted from mitochondria was incubated with 40 units of T4 RNA ligase (New England Biolabs) in the supplied buffer supplemented with 2 units of RNase inhibitor and in a total volume of 25 μ l. After phenol extraction and ethanol precipitation, cDNAs were synthesized using ImProm-IITM reverse transcriptase (Promega) in the presence of reverse primer P1. The region containing the junction of 5'- and 3'-extremities was then amplified by PCR using P1 and P2 primers. PCR amplification was performed as follow: 94 °C for 3 min; 30 cycles at 92 °C for 20 s, 50 °C for 20 s, and 72 °C for 30 s. When indicated, prior to circularization by T4 RNA ligase, RNAs were treated with tobacco acid pyrophosphatase (Invitrogen) according to manufacturer's instructions to generate primary transcripts with a 5'-monophosphate extremity.

Primer Sequences—PCR primers used in this study are: P1, TCTGACACTCTGACCTATTG; P2, GAAAACACGGGTTCTGAATCC; P3, GGGAGAGGGCCTGGTCG; P4, CTTAAGCAAAAATCATAAGA; P5, GAAGAAAAGTTTACAGAAGG.

RESULTS

Expression of the Edited Version of Larch *trnH* in Potato Mitochondria—A PCR product containing the edited version of the larch mitochondrial *trnH* sequence under the control of the promoter sequence of the potato mitochondrial 18 S rRNA gene (Fig. 1) was incubated with isolated potato mitochondria in standard DNA import conditions. Following import, mitochondria were further incubated for 3 h in a transcription medium and in the presence of [α -³²P]UTP to yield labeled transcripts. Labeled transcripts were then hybridized to Southern blots carrying the larch *trnH* gene or potato *trnF* gene as internal control. A strong hybridization signal was obtained with the labeled RNAs extracted from mitochondria transformed with the larch *trnH* gene, whereas no signal was

³ The abbreviations used are: Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; L, lysidine; RT, reverse transcription; cRT, circularized reverse transcription.

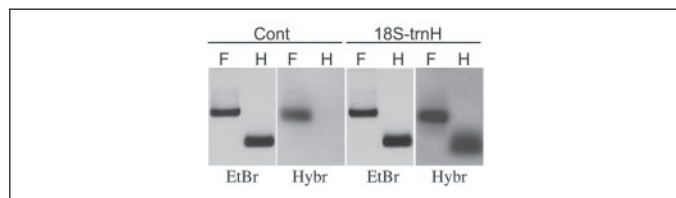


FIGURE 2. Transcription of larch mitochondrial edited *trnH* in potato mitochondria. Potato mitochondria were incubated in the absence (Cont) or presence (*18S-trnH*) of a PCR-amplified DNA fragment containing the edited version of the larch mitochondrial *trnH* sequence under the control of the potato mitochondrial 18 S *rrn* promoter sequence. Following incubation, transcription was run for 3 h in the presence of [α - 32 P]UTP. Mitochondrial nucleic acids were extracted, and identical amounts of radioactive transcripts were hybridized to Southern blots carrying the DNA fragments H or F. PCR-generated DNA fragments H and F correspond to larch mitochondrial tRNA^{His} precursor cDNA sequence (Fig. 1, thin arrow) and to potato mitochondrial tRNA^{Phe} cDNA precursor sequence (6), respectively. *EtBr*, negative images of the ethidium bromide-stained gel of PCR products F and H. *Hybr*, corresponding autoradiograms after hybridization.

observed with RNA from control mitochondria (*i.e.* incubated without larch *trnH*) (Fig. 2). Our results indicated that larch mitochondrial *trnH* incorporated into potato mitochondria via direct DNA uptake was expressed under the control of a heterologous potato mitochondrial 18 S rRNA promoter.

The Edited Version of Larch tRNA^{His} Is Correctly Processed in Potato Mitochondria—The expression of larch *trnH* in isolated potato mitochondria was further confirmed by cRT-PCR. Following DNA uptake, mitochondrial RNAs were extracted, circularized by T4 RNA ligase, and used for cRT-PCR analysis. The location of primer P1, used to initiate cDNA synthesis, and of primer P2, used with P1 for PCR amplification, are shown on Fig. 3A. No RT-PCR product was detected when no exogenous DNA was added during the import step preceding transcription. By contrast, a specific reverse transcriptase-dependent product of about 70 nucleotide was amplified when using RNAs extracted from mitochondria transformed with *trnH* construct (Fig. 3B). The size of this PCR product corresponds to the size of the expected product if the expressed tRNA^{His} was correctly processed *in organello*.

To confirm this hypothesis, the PCR product was cloned and several clones were analyzed by sequencing. Out of 15 sequences, 7 revealed fully processed larch tRNA^{His} (Fig. 4) harboring an extra CCA sequence at the 3'-end and a 5'-extremity containing an extra G residue (called G⁻¹), a characteristic of all tRNA^{His} molecules (21). The presence of these fully processed tRNA molecules indicate correct removal of the 5'- and 3'-extensions on the precursor molecules, thus implying that both potato mitochondrial endonucleases, RNase P at the 5'-end and RNase Z at the 3'-end, were able to recognize, *in organello*, the edited version of this foreign larch mitochondrial tRNA^{His}. Furthermore, the post-transcriptional addition of the 3'-CCA sequence demonstrate a correct recognition by the endogenous mitochondrial tRNA-nucleotidyltransferase.

Four sequences revealed the same 5'-extremities at G⁻¹, but the 3' termini was incomplete: either the CCA (2 clones) or CA (2 clones) sequences were missing. The presence of such 3'-truncated tRNA molecules represents a classical situation, resulting for instance from nucleolytic attacks. Unexpectedly, four other sequences revealed the presence of an extra non-encoded T present either at the 5'- or the 3'-ends of the tRNA. Three of these sequences do not contain the additional CCA 3'-end sequence whereas the fourth one does. In conclusion, these experiments showed that the larch mitochondrial *trnH* precursor transcript expressed in isolated potato mitochondria can be fully processed by removal of the 5'- and 3'-extensions and by 3'-CCA addition.

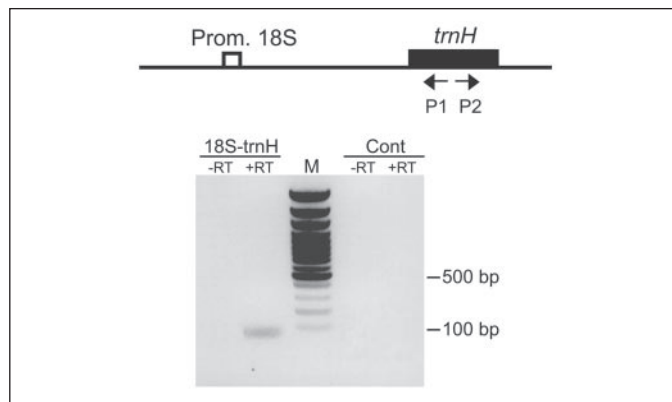


FIGURE 3. Expression and maturation of larch mitochondrial edited *trnH* in potato mitochondria determined by cRT-PCR. Potato mitochondria were incubated in the absence (Cont) or presence (*18S-trnH*) of a PCR-amplified DNA fragment containing the edited larch mitochondrial *trnH* sequence under the control of the potato mitochondrial 18 S *rrn* promoter sequence. Following incubation, transcription was run for 3 h. Mitochondrial nucleic acids were extracted, treated with DNase I, and circularized by T4 RNA ligase. cDNAs synthesis was performed with primer P1 in the presence (+RT) or in the absence (-RT) of reverse transcriptase. A negative image of an ethidium bromide-stained gel of PCR products amplified using primers P1 and P2 is presented. Fragment sizes were determined with a DNA ladder (M).

An Unedited Version of Larch Mitochondrial *trnH* Transcribed in Potato Mitochondria Is Neither Edited nor Correctly Processed—Using direct DNA uptake into plant mitochondria, we tested whether the unedited version of larch mitochondrial tRNA^{His} precursor transcript could be recognized by the editing machinery of potato mitochondria. We thus introduced in potato mitochondria a chimeric construct corresponding to the unedited version of the larch mitochondrial *trnH* sequence under the control of the potato mitochondrial 18 S *rrn* promoter sequence (Fig. 5A). Following transformation, mitochondria were further incubated for 3 h in a transcription medium and total nucleic acids were extracted. As judged by PCR, DNA uptake was efficient (data not shown). However, in contrast to the specific reverse transcriptase-dependent product amplified by cRT-PCR when using the chimeric edited *trnH* construct (Fig. 3), no cRT-PCR product could be obtained in the presence of primers P1 and P2 (data not shown).

Two reasons could explain the absence of an amplification product: either there was no expression of the incorporated DNA in potato mitochondria, or the processing steps leading to a mature and stable tRNA were impossible from the unedited tRNA^{His} primary transcript. To test the first hypothesis, we performed RT-PCR experiments with the following strategy. First-strand cDNA was synthesized in the presence of primer P3. PCR amplification experiments were then conducted using primer P3 in combination with either primer P4, located upstream of the potato mitochondrial 18 S rRNA promoter sequence, or primer P5, located downstream of this promoter. A band of 250 bp was amplified only with the P3-P5 combination (Fig. 5B). Cloning and sequencing of this product confirmed the amplification of the unedited version of larch mitochondrial tRNA^{His} (data not shown). As expected, primer P4 being located upstream of the initiation transcription site, no amplification was observed with the couple of primers P3 and P4. These results indicate that no mature unedited or edited tRNA^{His} can accumulate in potato mitochondria, although the exogenous DNA was expressed. In conclusion, our data demonstrate that the unedited tRNA precursor molecules are not correctly processed *in organello* suggesting that the potato mitochondrial editing machinery is not able to recognize these "foreign" editing sites.

Analysis of the Fate of Larch Mitochondrial Unedited tRNA^{His} Precursor Molecules in Potato Mitochondria—As the unedited tRNA^{His} precursor transcripts are not correctly processed *in organello*, they repre-

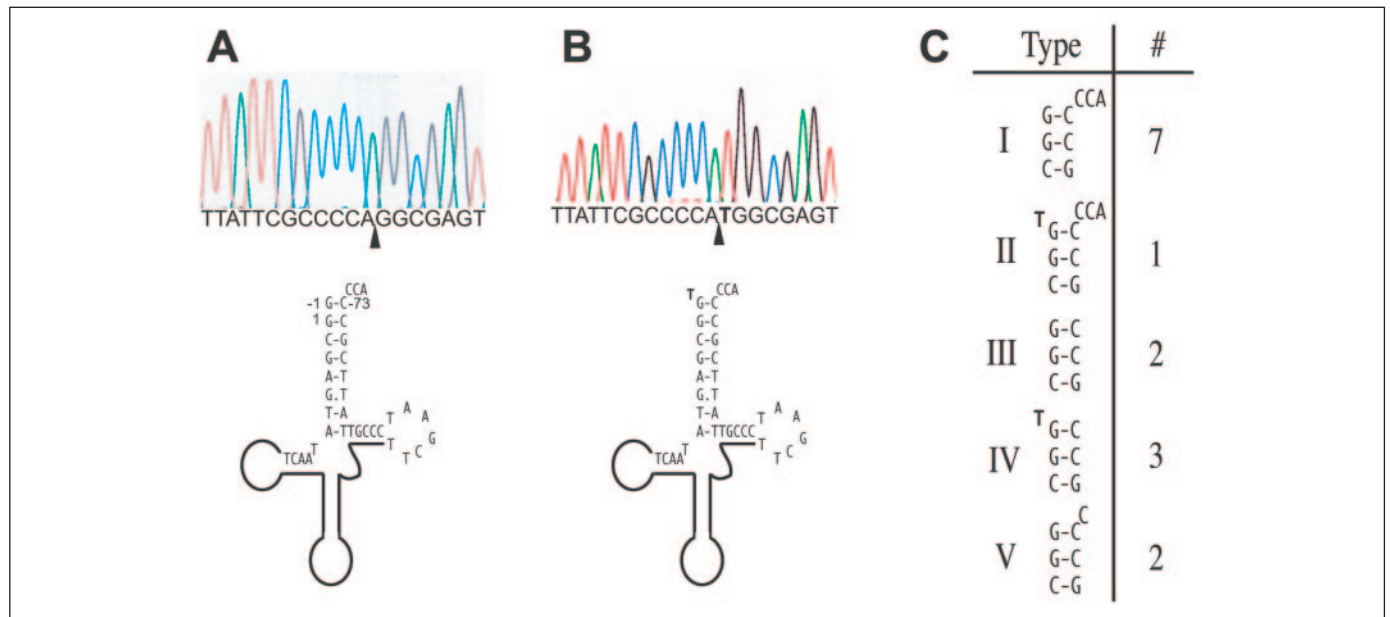


FIGURE 4. Maturation analysis of larch mitochondrial edited tRNA^{His} precursor transcript in potato mitochondria. The 70-bp PCR product shown in Fig. 3 was cloned in a TOPO vector. Fifteen clones were sequenced. DNA sequences of a clone of type I (A) and a clone of type II (B) are presented. Junctions between 5'- and 3'-extremities are indicated by arrowheads. C, summary of the number (#) and the different types of clones obtained. The non-genomically encoded T is written in bold.

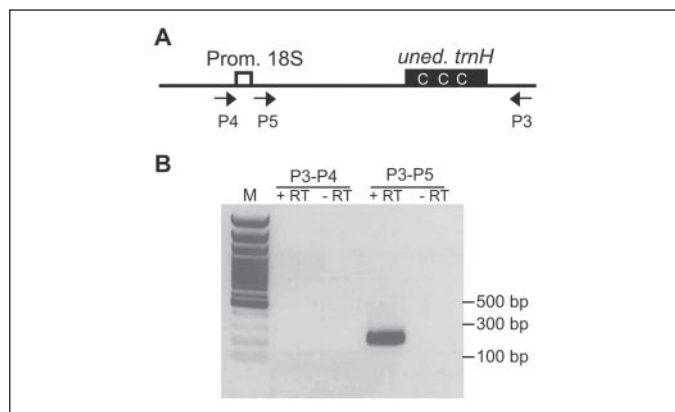


FIGURE 5. Transcription analysis of larch mitochondrial unedited *tmH* in potato mitochondria. A, schematic representation of the construct used for mitochondria transformation. The unedited version of larch mitochondrial *tmH* gene (black box with white Cs) with its flanking sequences was fused to the potato mitochondrial 18 S rRNA promoter sequence (open box, *Prom. 18S*) with its flanking sequences. This construct was obtained as described under "Materials and Methods" and cloned using a TOPO cloning kit. For mitochondrial DNA import, DNA substrate was PCR-amplified from the construct with primers flanking the cloning sites on the TOPO vector. Following incubation of potato mitochondria in the presence of the PCR-amplified DNA fragment, transcription was run for 3 h. Mitochondrial nucleic acids were extracted, treated with DNase I, and analyzed by RT-PCR. The positions of the primers P3, P4, and P5 used for RT-PCR analysis are indicated. B, negative image of an ethidium bromide-stained gel of PCR products amplified using primer P3 in combination with primer P4 or P5 as indicated. The presence (+RT) or absence (-RT) of reverse transcriptase during the cDNA synthesis is indicated. Fragment sizes were determined with a DNA ladder (M).

sent defective RNA molecules. As a consequence, the fate of these RNAs is likely to be degradation. The long precursor molecules starting close to the initiation transcription site were detected by RT-PCR (Fig. 5). However, these RNA species were not observed in cRT-PCR experiments possibly because the presence of a triphosphate at the 5' termini of primary transcripts prevents their circularization by T4 RNA ligase. To convert the 5'-triphosphate of primary transcripts to a monophosphate, total RNAs extracted from potato mitochondria transformed with the chimeric 18 S-unedited *tmH* construct were incubated in the presence of tobacco acid pyrophosphatase prior to their circularization by T4 RNA ligase (Fig. 6A). Circular RT-PCR was then conducted as

described above. Interestingly, one major product of 250 bp and a minor product of 180 bp were amplified using primers P1 and P2 (Fig. 6B). The two PCR products were cloned and sequenced.

Five clones were analyzed from the largest product and were identical (Fig. 6C, open triangles). The 5'-ends of these cDNAs were located a few nucleotides downstream the nonanucleotide motif usually present in dicot promoters and found in potato mitochondrial 18 S rRNA promoter region (Figs. 1 and 6 and Ref. 19). As no cRT-PCR products were obtained in the absence of RNA dephosphorylation, it is likely that this 5'-end corresponds to a true transcription initiation site. Fine-scale mapping of the potato mitochondrial 18 S rRNA primary transcript terminus by primer extension revealed several bands in addition to the bona fide transcription initiation site (19). These bands were attributed to premature stops of the reverse transcriptase. Our results suggest that the GTTCA sequence located a few nucleotides downstream from the nonanucleotide motif and corresponding to the region where the extra bands were observed might also initiate transcription. Our data are well supported by those obtained by Kuhn *et al.* (22). They showed, by an extensive analysis of transcription initiation sites using *in vitro* capping experiments, that most *Arabidopsis thaliana* mitochondrial genes are expressed from multiple promoters and in particular, four transcription initiation sites were identified for *A. thaliana* mitochondrial 18 S *rrn* gene (22). Vector sequences were still present at the 3'-ends of the transcripts (Fig. 6C) implying that they do not represent true biological intermediates. However, it is interesting to note that these transcripts are polyadenylated (Fig. 6C). Very similar results were obtained when analyzing 8 clones from the 180-bp-long PCR product (Fig. 6C, black arrows). All cDNAs showed the same 5' termini. Their 5'-ends exactly correspond to the bona fide transcription initiation site determined by primer extension and capping experiments (19). This result validates the efficient use of an additional dephosphorylation step before ligation for the analysis of primary transcripts that is otherwise not possible when using this 5'- to 3'-end ligated method. The 3' termini of the eight cDNAs were located at the 3'-end of the T-stem of larch tRNA^{His} and short poly(A) tails were observed. Due to the presence of an A in the tRNA sequence, the precise 3' termini could not be determined: either it is after the last T in the T-stem or it is after the following A. For the

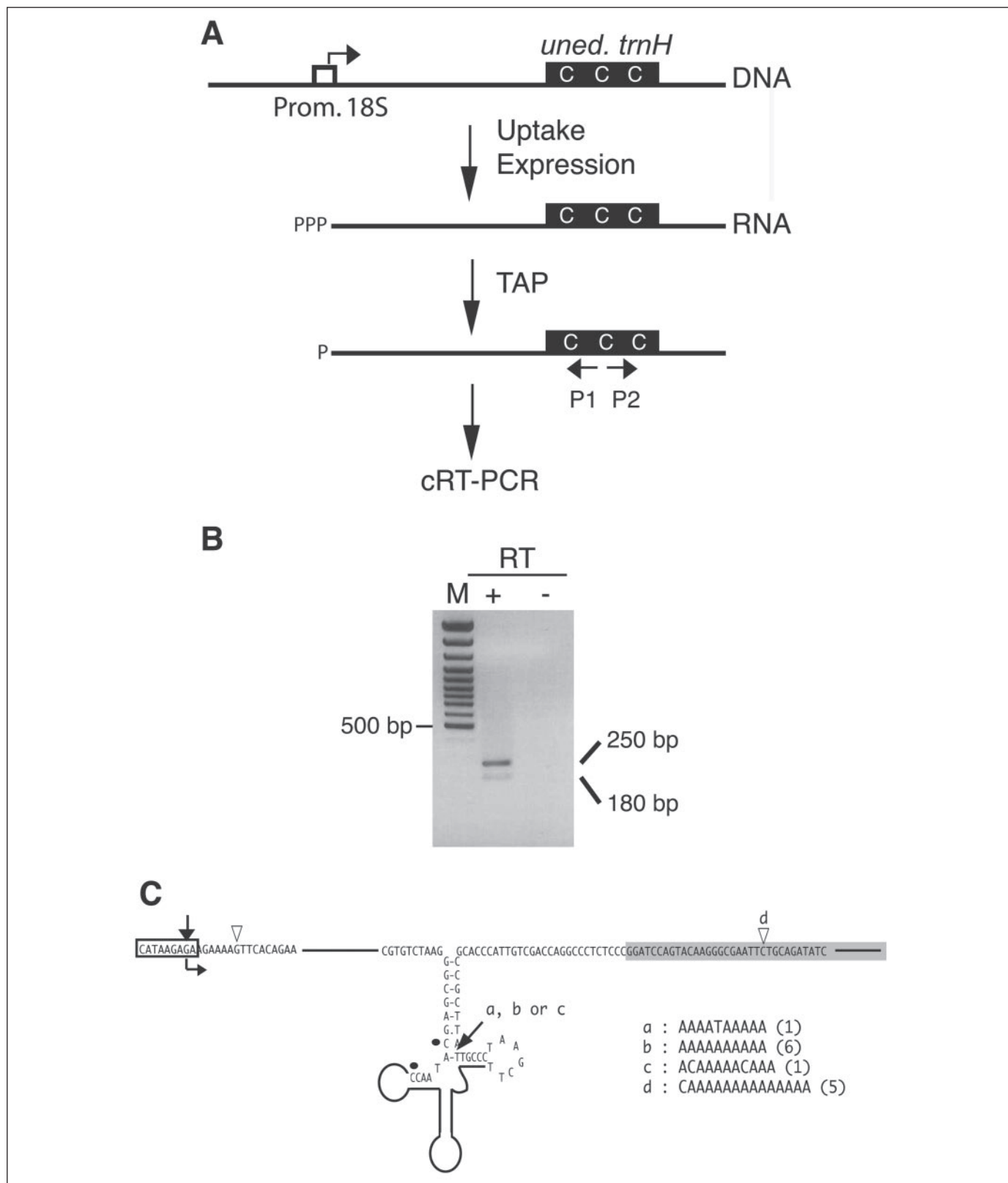


FIGURE 6. Analysis of the fate of the larch mitochondrial unedited *trnH^{His}* precursor transcript in potato mitochondria. *A*, schematic representation of the strategy. Upon DNA uptake into potato mitochondria, larch mitochondrial unedited *trnH* is transcribed from the potato mitochondrial 18 S rRNA promoter sequence (open box), as shown in Fig. 5. Total nucleic acids were extracted and pyrophosphate present on primary transcripts was eliminated by tobacco acid pyrophosphatase. Circularized products are then analyzed by cRT-PCR. *B*, negative image of an ethidium bromide-stained gel of PCR products amplified using primers P1 and P2. The presence (+RT) or absence (-RT) of reverse transcriptase during the cDNA synthesis in the presence of primer P1 is indicated. The lane marked *M* shows the migration of a DNA ladder. The size of PCR products obtained is indicated on the right. *C*, mapped unedited *trnH* transcript termini. The 5' and 3' termini determined by the sequencing analysis of the 180- and 250-bp PCR products are indicated by black arrows and open triangles, respectively. The sequences (a-d) of the non-genomically encoded poly(A) tails mapped at the 3'-extremities are indicated. Due to the presence of an A at the 3' termini of the 180-nucleotide-long transcript, there is an ambiguity with respect to the terminal nucleotide, and the number of As added post-transcriptionally can be plus/minus one. The number of clones analyzed is given in parentheses. Two of the edited sites present on larch mitochondrial *trnH* are indicated by dots. Potato mitochondrial 18 S rRNA promoter sequence is boxed. Primary transcript terminus of the potato mitochondrial 18 S rRNA as identified in (19) is indicated by a curved arrow. TOPO vector sequence is under a gray background.

same reason, the length of the tails was between 9 and 11 nucleotides. Five clones out of eight exhibited homopolymeric stretches of As, whereas we detected one T and two Cs in the poly(A) tails of the two other cDNAs. The presence of poly(A) tails has already been found at the 3'-end of several plant mitochondrial RNAs (23–28), and it has been shown that polyadenylation promotes degradation of RNA molecules in plant mitochondria (29). In conclusion, we identified the presence of two types of transcripts corresponding to unedited tRNA^{His} precursors both harboring poly(A) tails at their 3'-truncated extremities.

DISCUSSION

It is currently impossible to use stable transformation of higher plant mitochondria to study organellar gene expression. However, two *in organello* approaches have been recently developed: electroporation and direct DNA uptake (14, 17). In this report, we have used this direct DNA uptake methodology to transform isolated potato mitochondria with a transgene corresponding to a foreign larch mitochondrial *trnH* under the control of the potato mitochondrial 18 S rRNA promoter region. As larch mitochondrial *trnH* shows little homology with the endogenous “chloroplast-like” *trnH* expressed on the potato mitochondrial genome and contains three editing sites (7), it was an interesting substrate to study the fate of precursor transcripts once expressed in the mitochondria. We first showed that precursor transcripts of the edited larch tRNA^{His} were faithfully processed: the 5'- and 3'-extremities were presumably removed by the RNase P and RNase Z enzymes, and the CCA sequence was added at the 3'-end by the tRNA-nucleotidyltransferase. This is the first report demonstrating that DNA directly incorporated into purified plant mitochondria can be transcribed and subjected to different post-transcriptional steps, leading to a mature and stable RNA. Direct DNA uptake of short DNA fragments into mitochondria is relatively efficient. However, the study of gene expression using this technique still needs to be optimized as, in our hands, expression of the incorporated DNA was not always observed.

Among tRNAs, tRNA^{His} is unique in that it contains an extra base pair in the acceptor stem between G⁻¹ and C₇₃ (Fig. 4). This feature is essential for recognition by histidyl-tRNA synthetase and is conserved between prokaryotes and eukaryotes (30). Whereas the G⁻¹ nucleotide is added post-transcriptionally in eukaryotes (31), it is present in the precursor transcript in prokaryotes and in chloroplasts because RNase P cuts between -2 and -1 rather than between -1 and 1 as in all other precursor tRNAs (32). Both chloroplast-like potato mitochondrial *trnH* and larch mitochondrial *trnH* genes contain a G at the -1 position. When edited larch mitochondrial tRNA^{His} precursor transcripts were processed *in vitro* in the presence of an heterologous potato mitochondrial protein extract, two 5' termini corresponding to G¹ and C² were found by primer extension experiments (7). By contrast, we reported here that about two thirds of the mature RNAs obtained after *in organello* processing corresponded to larch tRNA^{His} ending at G⁻¹. We cannot exclude a two-step process including first a cleavage by RNase P between G⁻¹ and G¹ followed by addition of an extra G as in eukaryotes. However, *in organello* expression conditions are likely to better reflect the *in vivo* environment than *in vitro* experiments. Thus we favor a RNase P cut between -2 and -1 of tRNA^{His} precursors as in prokaryotes and chloroplasts.

When unedited larch mitochondrial tRNA^{His} precursors were expressed in transformed potato mitochondria, no RNA editing at any of the three usual sites was detected. No homologous sequence of larch mitochondrial tRNA^{His} does exist in potato mitochondria (7). The absence of editing modification when transferred in another plant may reflect the absence of the editing trans-factors required for the specific

recognition of sequence determinants present on the tRNA precursor molecule. Accordingly, in higher plant mitochondria as well as in chloroplasts, it has been shown that the editing machinery recognizes cis-elements surrounding the C residues involved in mRNA editing. Editing site selection is sequence specific and likely requires trans-factors (for a review, see Ref. 33). For instance, when the second exon of petunia mitochondrial *coxII-2* gene was transcribed in transgenic tobacco chloroplasts, no RNA editing was observed, demonstrating the presence of editing components that are specific of each organelle (34). Furthermore, transcripts of mitochondrial *Sorghum bicolor atp6-1* gene expressed *in organello* upon introduction via electroporation into isolated maize mitochondria, were not edited (35). Here, we showed that a foreign mitochondrial tRNA precursor was also not edited when incorporated in the mitochondria of another plant species, supporting the involvement of sequence-specific trans-factors for editing not only of mRNAs but also of stable RNAs.

In the absence of editing modification, larch mitochondrial tRNA^{His} precursor molecules remained unprocessed in potato mitochondria and no accumulation of mature tRNA^{His} was observed. These *in organello* data confirmed those obtained *in vitro*; when incubated with a heterologous potato mitochondrial processing extract, only the edited form of larch mitochondrial tRNA^{His} precursors were efficiently processed (7). As a consequence, the unprocessed tRNA precursor molecules are likely to be considered as defective RNAs by the mitochondria and thus are likely to be degraded. Indeed, we detected, two 3'-truncated polyadenylated primary transcripts. The longest product almost corresponded to a full-length primary transcript, whereas the second one ended within the tRNA molecule. Recently, several authors reported that in plant mitochondria polyadenylated mRNAs and rRNAs are the target for rapid exoribonucleolytic degradation (for a review, see Ref. 33). Thus, polyadenylation promotes degradation of RNAs not only in bacteria such as *E. coli* and in chloroplasts but also in plant mitochondria. In plant mitochondria polyadenylation was shown to regulate the steady-state level of transcripts by acting on several aspects of RNA metabolism such as maturation or degradation. This was recently illustrated for *A. thaliana* mitochondrial 18 S rRNA metabolism (27). A link between quality control and polyadenylation was also suggested by the characterization of high levels (80%) of polyadenylated 3'-unprocessed transcripts such as *atp9* and *orfB* (23). However, it is not known whether these long pretranscripts can truly be considered as defective. Here we show that when a defective unedited transcript cannot be processed to give a mature and stable tRNA, 3'-truncated and polyadenylated intermediate products are generated. Taking polyadenylation as a hallmark of degradation, these intermediate products are likely good substrates for the plant mitochondrial degradation machinery. The degradation of an unstable tRNA^{Trp} has been reported to be dependent on polyadenylation of the precursor molecule in *E. coli* (10). This supports the idea that polyadenylation might serve as a signal to trigger RNA degradation when defective RNAs are not correctly and/or efficiently processed and shows that a quality control system is present in eubacteria for eliminating “bad” RNAs. In *S. cerevisiae*, a polyadenylation-mediated RNA surveillance was shown to be implicated in the elimination of aberrant tRNA^{iMet} (12). Removal of yeast polyadenylated rRNAs could also be explained by the existence of a surveillance mechanism (11). Here our data suggest that such an RNA quality control involving polyadenylation also probably exists in plant mitochondria. Additional studies are required to know whether this RNA quality control can be extended to other types of defective plant mitochondrial RNAs and whether the activities involved in this process are similar to those already implicated in mRNA and rRNA degradation.

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