Two Exoribonucleases Act Sequentially to Process Mature 3’-Ends of atp9 mRNAs in Arabidopsis Mitochondria*

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In plant mitochondria, transcription proceeds well beyond the region that will become mature 3’ extremities of mRNAs, and the mechanisms of 3’ maturation are largely unknown. Here, we show the involvement of two exoribonucleases, AtmtPNPase and AtmtRNaseII, in the 3’ processing of atp9 mRNAs in Arabidopsis thaliana mitochondria. Down-regulation of AtmtPNPase results in the accumulation of pretranscripts of several times the size of mature atp9 mRNAs, indicating that 3’ processing of these transcripts is performed mainly exonucleolytically by AtmtPNPase. This enzyme is however not sufficient to completely process atp9 mRNAs, because with down-regulation of another mitochondrial exoribonuclease, AtmtRNaseII, about half of atp9 transcripts exhibit short 3’ nucleotide extensions compared with mature mRNAs. These short extensions can be efficiently removed by AtmtRNaseII in vitro. Taken together, these results show that 3’ processing of atp9 mRNAs in Arabidopsis mitochondria is, at least, a two-step phenomenon. First, AtmtPNPase is involved in removing 3’ extensions that may reach several kilobases. Second, AtmtRNaseII degrades short nucleotidic extensions to generate the mature 3’-ends.

Functional RNAs are generated through a series of complex post-transcriptional processes. The nature of these processes can vary depending on the organism or cellular compartment considered and may include maturation of 5’ and 3’ termini, splicing of introns, capping, editing, base modifications, or addition of nongenomically-encoded nucleotides such as polyadenylation. Processing of mRNAs is well documented for bacterial and eukaryotic nuclear mRNAs (1, 2). Our knowledge of post-transcriptional processes in plant mitochondria is far less well characterized. Two lines of evidence suggest that processing of 3’ extremities occurs for any RNA in plant mitochondria. First, run-on experiments have demonstrated that transcription proceeds beyond the region that corresponds to mature 3’-ends of mRNAs and rRNAs (4). Second, it has been shown using an in vitro transcription system that inverted repeats that terminate some mitochondrial mRNAs are not recognized as transcription termination signals (5). These inverted repeats seem to be essential as processing signals in vitro (5) and in vivo (6, 7). However, the ribonucleases involved in 3’ mRNA maturation processes remain to be identified in plant mitochondria.

Escherichia coli represents one of the model organisms for which the function, mechanism, and regulation of ribonucleases (RNases) are intensively studied (for example, see Refs. 1, 8, and 9). Among the eight characterized exoribonucleases in E. coli, polynucleotide phosphorylase (PNPase) and RNaseII are the main exoribonucleases involved in degrading mRNAs in a 3’ to 5’ direction but participate also in processing events. PNPase-like proteins are also present in human mitochondria (10) and in chloroplasts. In the latter, it is involved both in degradation and maturation of several RNAs (11–14). In yeast mitochondria, the RNA degradosome is composed of two sub-units: a RNA helicase, Suv3p, and an exoribonuclease, Dss1p, belonging to the RNase II family (15). In yeast mutants lacking degradosome components, several molecular phenotypes have been described, including the accumulation of mRNA precursors (Ref. 15 and references therein).

We show here that two exoribonucleases, AtmtPNPase and AtmtRNaseII, are present in Arabidopsis thaliana mitochondria. Down-regulation of AtmtPNPase expression results in the appearance of atp9 pretranscripts that can reach several times the size of the mature mRNA indicating that AtmtPNPase is involved in 3’ maturation processes. However, AtmtPNPase is not sufficient to process all atp9 mRNAs as precursors harboring short nucleotidic extensions accumulate in a mutant affected in the expression of a second mitochondrial ribonuclease, AtmtRNaseII. This enzyme is able to remove these short extensions in vitro. These results show that processing of atp9 mRNAs is an exonucleolytic mechanism and that at least two exoribonucleases act in succession to process the 3’ extremities of atp9 mRNAs in Arabidopsis mitochondria to the mature form.

EXPERIMENTAL PROCEDURES

In Vivo Localization of AtmtPNPase and AtmtRNaseII—Sequences encoding the first 107 and 192 amino acids of AtmtPNPase (CAB43865) and AtmtRNaseII (AAQ62877), respectively, were fused to the egfp gene and cloned in the pCB vector under the control of the CaMV 35 S promoter. Biologic transformation of tobacco leaf cells and confocal

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) Y14685.†
microscope observation were as previously described (16).

Western Blot Analysis—Anti-PNPase antibodies were raised in rabbits against a protein overexpressed in E. coli and corresponding to amino acids 659–991 of AtmtPNPase. Anti-PNPase antibodies were then affinity-purified using the soluble overexpressed protein linked to CNBr-activated agarose beads as described in Ref. 17 and used in Western blot analysis using standard procedures. Subcellular fractionation of Arabidopsis protoplasts were performed as previously described (18).

Transgenic Arabidopsis Plants—cDNAs encoding AtmtPNPase and AtmtRNaseII were obtained by RT-PCR, fused to a sequence encoding the FLAG peptide and the calmodulin binding peptide (CBP) and cloned into the TOPO cloning kit (Invitrogen).

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respectively, to nucleotides 279261–279280 and 278923–278945 for mat-
erate

The region containing the junction of 5′ and 3′ extremities was then amplified by RT-PCR using forward and reverse primers corresponding, respectively, to nucleotides 279261–279280 and 278923–278945 for mature atp9 RNAs or 279331–279354 and 278923–278945 for atp9 pre-transcripts. PCR amplification consisted of 30 cycles of 30 s at 94°C, 30 s at 50°C, 1 min at 72°C. All RT-PCR products were cloned using the TOPO cloning kit (Invitrogen).

Purification of RNaseII Tag and Activity Assay—Wild-type (wt) plants and transgenic Arabidopsis plants expressing RNaseII tagged at its C-terminal extremity with the FLAG peptide and the calmodulin binding peptide (CBP) and cloned into the TOPO cloning kit (Invitrogen) and a reverse primer corresponding to nucleotides 279062–279080. The region containing the junction of 5′ and 3′ extremities was then amplified by RT-PCR using forward and reverse primers corresponding, respectively, to nucleotides 279261–279280 and 278923–278945 for mature atp9 RNAs or 279331–279354 and 278923–278945 for atp9 pre-transcripts. PCR amplification consisted of 30 cycles of 30 s at 94°C, 30 s at 50°C, 1 min at 72°C. All RT-PCR products were cloned using the TOPO cloning kit (Invitrogen).

Mapping of 3′ Extremities of atp9 Transcripts by Circular RT-PCR (cRT-PCR)—All numbering referring to primers given here are as in NC_001284, the accession number of the Arabidopsis complete mitochondrial genome sequence. 5 μg of total or mitochondrial RNA were incubated with 40 units of T4 RNA ligase (New England Biolabs) in the supplied buffer supplemented with 2 units of RNase inhibitor and 1 unit of RQ1 DNase (Promega) in a total volume of 25 μl. After phenol/chloroform extraction and ethanol precipitation, cDNAs were synthesized using SuperscriptTM II RNaseH− reverse transcriptase (Invitrogen) and a reverse primer corresponding to nucleotides 279062–279080.

The deduced protein sequence is predicted to be imported into mitochondria by the following programs: MitoProt II 1.0a and TargetP 1.1. The C-terminal part of the protein has no clear homology with known mitochondrial proteins. Such a gene (At5g14580) encodes a putative protein harboring significant homologies with bacterial and chloroplast PNPase (see below). This gene is expressed in Arabidopsis as shown by the availability of four expressed sequence tags (ESTs) in databases (DBU238738, AV528692, AU229971, BE526020), although its level of expression is relatively low as the corresponding mRNAs could not be detected by Northern blot experiments. cDNAs were amplified by RT-PCR and cloned (GenBankTM accession number: Y14685). Sequence analysis revealed the presence of an open reading frame of 991 codons encoding a putative protein of 108 kDa. The protein sequence from amino acids 58 to 741 of this protein is highly homologous (47% identity, 65% homology) to the conserved domain (CCG1185.1, Pnp) derived from 48 PNPases, mostly of bacterial origin. An alignment of this protein with its bacterial and chloroplast counterparts has been recently presented (20). A typical S1 RNA binding domain is present at amino acids 741–749. The C-terminal part of the protein has no clear homologies with known proteins.

The deduced protein sequence is predicted to be imported into mitochondria by the following programs: MitoProt II 1.0a (score 0.9872), Predotar (score 0.881), PSORT (score 0.800). To experimentally verify these predictions, the first exon encoding 107 amino acids was fused to the eGFP gene expressed transiently in tobacco leaf. Only the lower guard cell of the stomata shown was transfected. a, visible light; b, chloroplasts detected by autofluorescence of chlorophyll have been colored in blue, and fluorescence of the mitochondrial marker is in red. c, fluorescence of PNP-eGFP fusion protein is in green. d, merged images. B, Western blot analysis of subcellular fractions purified from Arabidopsis cell cultures. P, protoplasts; C5, cytosol; Cp, chloroplasts; M, mitochondria. Antibodies directed against the following proteins were used: PNP, AtmtPNPase; TRX3, Arabidopsis cytoplasmic thioredoxin; LCHIII, Chlamydomonas reinhardtii light-harvesting complex II protein; ElaPDH, Zea mays Ela subunit of mitochondrial pyruvate dehydrogenase. AtmtPNPase could be detected in the protoplast fraction upon longer exposure.
consisting of the yeast COXIV mitochondrial precursor fused to the DsRED1 protein (Fig. 1A). Antibodies were also raised against the C-terminal part of the protein (amino acids 659–991) that was overexpressed in E. coli. Western blot analysis of different subcellular fractions extracted from cultured Arabidopsis cells revealed that the protein is present in mitochondria (Fig. 1B). In summary, the nuclear gene At5g14580 encodes a protein targeted to mitochondria that is highly homologous to bacterial and chloroplastic PNPases. Hence, this protein was called AtmtPNPase.

**Down-regulation of AtmtPNPase Expression in Vivo**—To study in planta the effects of disrupting AtmtPNPase gene expression, we first searched for mutants in collections of T-DNA insertion lines of Arabidopsis. We identified such a potential line in the SIGnAL indexed insertion mutant collection (line SALK_051838). Insertion of a T-DNA in the second intron of the AtmtPNPase gene was confirmed by PCR methods, and we obtained several plants heterozygous for the T-DNA insertion. However, no plants homozygous for the T-DNA insertion were obtained in the progeny of these plants, suggesting that the AtmtPNPase gene is essential for viability in Arabidopsis at least at some stages of development. Thus, down-regulation of AtmtPNPase gene expression must be performed by inducing RNA silencing at a late stage of development. Although not controlled, co-suppression is a relatively common phenomenon in plants and consists of the extinction of the expression of an endogenous gene triggered by ectopically expressing this same gene in sense orientation. At this time, we had produced transgenic plants overexpressing a tagged version of AtmtPNPase (PNPase tag) (see details under “Experimental Procedures”) and observed that about 10–15% of T2 plants exhibited a clear morphological phenotype (Fig. 2A). The initial development of these plants was not delayed as compared with that of wild-type plants but after 2–3 weeks development was altered. These plants exhibited round, downward curled leaves and failed to set up new leaves. None of all the plants that presented this phenotype ever recovered and never resumed growth. To assess the expression of the endogenous PNPase as well as the ectopically expressed PNPase tag, Western blot analysis was performed using leaves collected after 6 weeks of development (week 6) from a normal looking transgenic plant (plant 1), from two transgenic plants which exhibited abnormal phenotypes (plants 2 and 3) and from a wt plant (see Fig. 2A).

As shown in Fig. 2B, purified antibodies detect the endogenous PNPase in the wild-type plant, whereas more intense signals could be observed in all three transgenic lines indicating that these plants overexpress PNPase tag. Interestingly, in all three transgenic plants degradation intermediates of PNPase could be detected. We believe that these signals indeed correspond to degradation intermediates as they are detected only in transgenic plants, and thus must originate from the transgene (compare Figs. 1B and 2, B and C). These degradation products were present to a much greater extent in plants 2 and 3 than in plant 1. We repeated the Western blot experiments a week later (week 7), and, whereas expression pattern for the ectopic or endogenous PNPase did not vary in plant 1 or wt, respectively, full-length forms of endogenous or ectopic PNPase could not be detected anymore in plants 2 and 3 (Fig. 2C). Nevertheless, PNPase degradation products were still present in these plants, indicating that PNPase was once expressed but was then turned over. An experiment performed using monoclonal antibodies directed against the E1α subunit of the pyruvate dehydrogenase (E1α-PDH) was used as a loading control for protein deposition between samples (Fig. 2D). No degradation of E1α-PDH was observed in plants 2 and 3. The loss of expression of both forms of PNPase suggested silencing of both genes by co-suppression. This hypothesis was verified at the RNA level by Northern blot experiments. As already mentioned, endogenous AtmtPNPase mRNAs could not be detected in wild-type plants. However, the transgene mRNAs were present at high levels at week 6 in plants 1 and 3 (Fig. 2E). The steady-state level of these mRNAs does not change at week 7 for plant 1 (the normal looking transgenic plant), but only trace amounts of the transgene mRNAs can be detected in plants 2 and 3 at week 7 (Fig. 2F), confirming the idea that co-suppression has been induced in these plants by overexpressing mRNAs encoding the tagged version of AtmtPNPase. The induction of co-suppression is not controlled but is highly reproducible even if this implies that every single plant has to be tested individually. The plants for which no PNPase could be detected by Western blot will now be called PNP− plants for convenience.
although residual expression of PNase might still occur but at levels so low that it could not be detected by antibodies. In any case, our results show that the expression of PNase is at least severely decreased in PN− plants. Transgenic plants still overexpressing PNase tag will be called PN+, but it should be noted that the purified PNase tag did not exhibit any activity under various conditions in vitro, and it is thus possible that the C-terminal extension inactivates this protein (data not shown).

Unprocessed atp9 Transcripts Accumulate in PN− Plants—In order to test a possible involvement of AtmtPNPase in mitochondrial mRNA processing, we compared the expression patterns of the atp9 gene between wild-type, PN+, and PN− plants. The atp9 gene was chosen as it represents a model gene whose expression has been studied in several plant species. However, the following results are not restricted to atp9 transcripts, because identical results were obtained for orf9 RNAs (data not shown). First, we analyzed the expression of the atp9 gene by Northern blot (Fig. 3). Mature atp9 mRNAs of the expected size of about 500 nucleotides were observed in all plants. In addition, a faint signal corresponding to 1.5 kb RNA could also be detected in wild-type plants and may represent a processing intermediate of atp9 pretranscripts with unprocessed 3′-ends. In conclusion, down-regulation of AtmtPNPase in planta results in the accumulation of atp9 transcripts that are processed faithfully at their 5′-ends but not at their 3′-extremities.

Most atp9 Pretranscripts Are Polyadenylated in PN− Plants—In plant mitochondria, mature mRNAs are not constitutively polyadenylated. However, using oligo(dT)-based RT-PCR approaches, we and others (22–25) have shown that mRNAs may be polyadenylated at low levels. The use of the cRT-PCR technique allows in theory the analysis of both the 3′- and 5′-ends of transcripts regardless of the polyadenylation status of the transcripts. In wild-type plants, 33 clones obtained by cRT-PCR (see Fig. 6), no poly(A) tails were detected at the 3′-mature ends of atp9 mRNAs. This result does not imply that atp9 mRNAs cannot be polyadenylated in vivo but merely reflects the fact that the proportion of polyadenylated as compared with non-polyadenylated transcripts is rather low. By contrast, about 80% of atp9 pretranscripts in PN− plants are polyadenylated (Table I). Poly(A) tails vary in size from 5 to 17 nucleotides and were mostly composed of adenosines (Table I).

Polyadenylated atp9 Pretranscripts Exist but Are Rare Abundant in Wild-type Plants—The difficulty to detect atp9 pretranscripts in wild-type plants by Northern blot and cRT-PCR analysis could be caused by their low abundance. Thus, cRT-PCR experiments were performed using mitochondrial RNA purified from Arabidopsis cell cultures rather than total RNA and using two successive PCR amplifications. This resulted in the amplification of a smear containing discrete bands (Fig. 4B). These PCR products were cloned and indeed correspond to atp9 transcripts with correct 5′-extremities and unprocessed 3′-extensions of various lengths (from 42 to 430 nucleotides) that match the mitochondrial genome sequence downstream of the atp9 gene (Table I). The PCR amplification and cloning steps certainly favor the detection of shorter compared with longer transcripts, but these results confirm the Northern analysis, i.e. the large transcripts correspond to atp9 pretranscripts with unprocessed 3′-ends. In conclusion, down-regulation of AtmtPNPase in planta results in the accumulation of atp9 transcripts that are processed faithfully at their 5′-ends but not at their 3′-extremities.

A RNaseII-like Protein Is Present in A. thaliana Mitochondria—Down-regulation of AtmtPNPase results in the accumulation of large pretranscripts that are processed at their 5′-ends but not at their 3′-extremities, indicating that this enzyme is involved in the 3′ maturation of atp9 transcripts. However, these data do not show that AtmtPNPase is sufficient for establishing mature 3′-ends of atp9 mRNAs and, in particular, do not rule out the involvement of a second exoribonuclease. The

![Fig. 3. Down-regulation of AtmtPNPase modifies atp9 mRNA expression pattern.](Image)

![Fig. 4. Amplification by cRT-PCR of atp9 pretranscripts.](Image)
putative protein (AAQ62877) encoded by the nuclear gene At5g02250 is predicted to belong to the RNaseII-like protein family. Comparison of this sequence with the Conserved Domain data base revealed three hits with E-values of 5.10$^{-111}$, 6.10$^{-61}$, and 7.10$^{-12}$, respectively, with the following conserved domains: KOG2102 (subunit Rrp44/Dis3 of the exosomal 3′-5′ exoribonuclease complex), COG0557 (Exoribonuclease R) and pfam00773 (Exoribonuclease II). For instance, the region corresponding to amino acids 119–506 exhibits 37% identity and 55% similarity with RNase II from Synchocystis. The At5g02250 gene is expressed as shown by the availability of several ESTs (AV523171, AV523993, AV529781, AV528791). cDNAs were amplified by RT-PCR, and sequence analysis confirmed the presence of an open reading frame of 803 codons encoding a putative protein of 98.7 kDa as predicted from the genomic sequence except that amino acid 513 is a threonine in our sequence as opposed to an isoleucine in the sequence from the database.

The putative protein is predicted to be imported into mitochondria by the following programs: MitoProt II 1.04a (score 0.9391), Predotar (score 0.928), PSORT (score 0.742). To determine its subcellular location, a fusion protein consisting of the N-terminal 192 amino acids fused to the eGFP was expressed in tobacco leaf cells as described above for AtmtPNPase. The fusion protein co-localized with the mitochondrial marker (Fig. 5A). The presence of AtmtRNaseII in mitochondria is further confirmed by the fact that its lack of expression affects atp9 mRNAs (see below). Because of its sequence homology and mitochondrial location, this protein was named AtmtRNaseII.

**Characterization of rnaII-1 Mutant Plants**—To study the biological role of AtmtRNaseII in planta, we obtained from the SIGnAL indexed insertion mutant collection a line (SALK_044726) harboring a putative T-DNA insertion in the penultimate intron of the AtmtRNaseII gene. We identified heterozygous plants harboring a single T-DNA insertion as determined by Southern blot analysis (data not shown). Seeds from one of these plants were sown on agar plates containing MS salts and 0.5% sucrose. After 3 weeks, plants without apparent morphological phenotype (plants 1–6) and plants severely affected in their development (plants 7 and 8) could clearly be discriminated (Fig. 5B). In addition, an identical phenotype was observed for two other lines exhibiting distinct T-DNA insertions in the AtmtRNaseII gene (lines SALK_138535 and SALK_990294) (data not shown). However, the molecular basis for the morphological phenotype is unknown at present and it will thus not be further detailed here. We then purified separately total RNA and DNA from each plant 1–8, as well as from a wild-type plant as a control. Genotyping by PCR on genomic DNA revealed that both plants 7 and 8 were homozygous for the T-DNA insertion whereas the other plants were either wild-type or heterozygous for the insertion (Fig. 5C). We also checked by PCR using genomic DNA or oligo(dT)-primed cDNA that the gene At5g02260, located 3′ to the AtmtRNaseII gene, is still present and expressed in these plants (data not shown). RT-PCR experiments were then conducted with RNA extracted from plants 1 to 8 to assess the level of expression of the AtmtRNaseII gene. First, as a control, AtSUV3 mRNAs were amplified by RT-PCR. The nuclear gene AtSUV3 (At4g14790) is expressed at low levels and encodes a mitochondrial RNA helicase (26). AtSUV3 mRNAs could be detected by RT-PCR in all plants 1–8 (Fig. 5D). However, AtmtRNaseII mRNAs could be detected in all but plants 7 and 8 (Fig. 5D). Individual analysis of 12 other plants pre-
senting the same phenotype revealed that all these plants were homzygous for the T-DNA insertion that prevented expression of the AtmtRNASelII gene (data not shown). These data show that the AtmtRNASelII gene is not expressed anymore in this T-DNA insertion mutant, which was called rnaII-1.

Accumulation of atp9 mRNA Precursors Exhibiting Short Extensions in rnaII-1 Plants—The 3’ extremities of atp9 mRNAs were then mapped by crT-PCR both in wild-type and rnaII-1 plants (Fig. 6). In wild-type plants, most atp9 3’ extremities (33 of 34 clones) map in a small window of 5 nucleotides (nucleotides 279329–279333 in NC.001284). For 19 of 33 clones, the 3’ extremity was localized at nucleotides 279331, which we will consider as the major mature 3’-end. The remaining clone exhibited a short extension of 6 nucleotides as compared with this mature end, indicating that in wild-type plants, this type of atp9 mRNA precursors exist. However, the ratio of these precursors versus mature RNAs increases dramatically in rnaII-1 plants as they represent almost half of the clones (13 of 28 clones) and exhibit up to 11 nucleotide extension as compared with the mature 3’-end. Thus, atp9 mRNAs are not all processed faithfully in rnaII-1 plants (see “Discussion”).

Purified RNaseII Tag Efficiently Removes atp9 3’ Extensions—Although this molecular phenotype in rnaII-1 plants was well in agreement with the supposed activity of AtmtRNASelII, we could not exclude that the accumulation of these precursors could be caused by an indirect effect of the mutation. Thus, to determine whether AtmtRNASelII has a direct role in the 3’ processing of atp9 mRNAs, we purified a tagged version of this enzyme (RNaseII tag) from transgenic Arabidopsis plants (Fig. 7A). To ensure that the activity observed is caused by the expression of the transgene, a mock purification was also performed using wild-type plants. RNaseII tag was then incubated with RNA substrates corresponding either to the last 67 nucleotides of mature atp9 mRNAs (substrate M) or to these 67 nucleotides plus an extension of 9 nucleotides (M – 9). As shown in Fig. 7B, RNaseII tag cannot degrade the M substrate whereas it removes efficiently the extension from the M – 9 substrate. These data confirm that AtmtRNASelII is responsible for polishing atp9 mRNA 3’ extremities.

**DISCUSSION**

Previous results obtained in vivo and in organello (4) but also in vitro (5) strongly suggested that transcripts of sizes larger than that of mature RNAs exist in plant mitochondria. By using a reverse genetic approach, we characterized such large pre-transcripts for the atp9 gene in planta. Our results strongly suggest that there is no efficient transcription termination mechanism near the regions that would become the mature 3’-ends of atp9 mRNAs. Instead, transcription continues for several kilobases downstream of this gene. Although we cannot exclude that down-regulation of AtmpPNPase could have an adverse effect on a putative transcription termination mechanism, we are not in favor of such a hypothesis as such low abundant, large transcripts exist in wild-type plant mitochondria.

A possible scenario for 3’-end maturation of atp9 transcripts would have been that an endonuclease would efficiently cut near the future 3’ extremities and that the remaining nucleotides would be trimmed by an exonuclease. We did not observe abundant processing intermediates relatively close in size to the mature atp9 mRNAs in PNP plus plants. Thus, the 3’-end processing of atp9 mRNAs seems to be mainly exonucleolytic. Maturation processes may be similar between plant organelles as a PNPase-like protein has also been recently shown to be involved in mRNA maturation in chloroplasts (14). However, we make here two additional observations, the polyadenylation status of pretranscripts and the involvement of a second exoribonuclease in the 3’ maturation process.

First, most atp9 pretranscripts are polyadenylated. The exact ratio of polyadenylated versus non-polyadenylated mature mRNAs is still unknown but it is likely to be less than a few percent considering the results that we and others (Refs. 25 and 27, this study) have obtained while cloning mature 3’-ends of several transcripts. In contrast, most atp9 pretranscripts described in PNP plus plants are polyadenylated. Given the known affinity of bacterial and chloroplastic PNPases for poly(A) tails, it is possible that in mitochondria as well poly(A) tails could be used for recognition of its substrates by AtmpPNPase. Given the reversibility of the chemical reaction carried out by PNPases, the possibility that PNPases could be involved both in synthesis and degradation of poly(A) tails has been reported for poly(A) polymerase 1 (PAP 1)-deficient E. coli mutants and for chloroplasts (20, 28, 29). Nevertheless, in E. coli, polyadenylation is mostly performed by PAPI and in plants where AtcpPNPase has been down-regulated, plastid polyadenylated RNAs can still be detected by oligo(dT)-based RT-PCR experiments, suggesting the existence of a poly(A) polymerase yet to be identified (14). Similarly, the fact that atp9 precursors are polyadenylated in plants for which AtmpPNPase expression is no longer detectable suggests that polyadenylation is not performed, or at least not solely, by AtmpPNPase itself in plant mitochondria.

The second observation we made is that, although AtmpPNPase seems to be involved in eliminating the large 3’ extensions of pretranscripts, not all atp9 mRNAs are faithfully processed in rnaII-1 mutants as about half of the transcripts harbor small nucleotide extensions. Thus, either a putative
RNase able to fully complement AtRNaseII is not expressed in all tissues or a RNase activity that could be AtmtPNPase or another unidentified RNase seems able to perform only inefficiently the accurate 3′ processing of atp9 3′-ends. In wild-type plants, AtmtRNaseII would then perform a type of quality control by polishing the remaining unprocessed RNAs. Obviously, this represent only one of the biological roles of RNaseII as the molecular phenotype observed here is unlikely to be the cause of the strong morphological abnormalities of rnaII-1 plants. Interestingly, the extensions removed by AtmtRNaseII are located downstream of a putative double stem-loop structure present at the 3′-ends of atp9 mRNAs similar to what has been observed for some mitochondrial mRNAs (for instance see Refs. 30–32). These data plus additional experiments with other RNA substrates including polyadenylated ones (data not shown) indicate that AtmtRNaseII is able to digest any unstructured regions of a RNA substrate but is stopped by a secondary structure, similarly to its bacterial counterpart (1).

Although we show here that at least two exoribonucleases act in succession to establish 3′-ends of atp9 mRNAs, the complete mechanism by which 3′-ends are matured is still a puzzling phenomenon. Actually, the putative double stem-loop structure present at the 3′-ends of atp9 mRNAs in Arabidopsis might be an important determinant for the processing of 3′-extremities as shown for pea atp9 mRNAs (5). However, another equally stable structure can be predicted just 30 nucleotides downstream, and this putative structure is not recognized as a processing signal. Somehow there is a mechanism able to discriminate between these secondary structures. In chloroplasts, faithful 3′-end maturation requires RNA-binding proteins that are thought to stop the progression of the PNPase-like protein involved in 3′ extremity processing (11). A similar phenomenon might exist in plant mitochondria although these putative specificity factors remain to be identified.

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