Review

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RNA metabolism in plant mitochondria

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Mitochondria are essential for the eukaryotic cell and are derived from the endosymbiosis of an α-proteobacterial ancestor. Compared to other eukaryotes, RNA metabolism in plant mitochondria is complex and combines bacterial-like traits with novel features that evolved in the host cell. These complex RNA processes are regulated by families of nucleus-encoded RNA-binding proteins. Transcription is particularly relaxed and is initiated from multiple promoters covering the entire genome. The variety of RNA precursors accumulating in mitochondria highlights the importance of post-transcriptional processes to determine the size and abundance of transcripts. Here we review RNA metabolism in plant mitochondria, from RNA transcription to translation, with a special focus on their unique features that are controlled by *trans*-factors.

Plant mitochondria are not simple relics of the past

Mitochondria are considered the powerhouses of the cell that provide the energy necessary for cell activities. Mitochondria are organelles that originated from a free-living oxygen-using prokaryote that is thought to have been taken up as endosymbiont into another prokaryotic host cell. During the symbiont evolution that led to functional mitochondria the majority of its genetic content was transferred to the nucleus of the host cell. Thus, thousands of proteins are nucleus-encoded and imported into plant mitochondria. In fact, plant mitochondria have only retained a small portion of their ancestral genome, which now only encodes for a partial set of the components of their energy-transducing membranes and core components of their gene expression machineries (Box 1). The coevolution between organellar and nuclear genomes led to a progressive loss of autonomy for the organelle; mitochondrial biogenesis today relies on the coordinated expression of the two genomes. The importance of this coordination is illustrated by the fact that both mitochondrion- and nucleus-encoded subunits are required for the assembly of the respiratory chain complexes and the mitochondrial ribosome.

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Plant mitochondria have acquired unique traits that differentiate them from their prokaryotic ancestor and from the mitochondria of other kingdoms. In particular, plant mitochondria employ distinct and complex RNA metabolism mechanisms that combine features retained from their prokaryotic ancestors with novel features that evolved in their eukaryotic host – including RNA transcription, RNA editing, the splicing of group I and group II introns, the maturation of transcript ends, and RNA degradation and translation (Figure 1). In plant mitochondria, post-transcriptional mechanisms play a predominant role in determining gene product abundance. Dysfunction of RNA metabolism in plant mitochondria often leads to severe consequences for plant development and fertility. In the past decade, major advances have unraveled the mechanism of RNA metabolism in plant mitochondria. In particular, the use of genetic tools in model plants such as *Arabidopsis* (*Arabidopsis thaliana*), maize (*Zea mays*), and moss (*Physcomitrella patens*) allowed the identification of many nuclear genes encoding proteinaceous factors that influence various aspects of RNA metabolism in mitochondria. Surprisingly, these studies revealed that most identified protein families were not encoded by bacterial ancestor genes, but were instead recruited by the eukaryotic host cell during a still not understood nuclear/organellar coevolution. Many of these eukaryote-specific factors involved in mitochondrial RNA metabolism belong to the superfamily of helical-repeat proteins ([1] for review) among which pentatricopeptide repeat (PPR) proteins are particularly prevalent in plants (Box 2).

Here we summarize the most up-to-date knowledge on RNA metabolism in plant mitochondria that has emerged from recent work, with a particular emphasis on the nucleus-encoded factors supporting it.

RNA transcription

Mitochondrial transcription has diverged considerably during evolution, coinciding with the extensive structural drift of mitochondrial genomes in different eukaryote clades. Similarly to its bacterial counterpart, mitochondrial transcription in plants often results in polycistronic messengers. Nevertheless, the dynamic rearrangement of the mitochondrial genome in plants has led to a loss of functional transcriptional units. Consequently, the functions of cotranscribed genes are rarely related [2,3]. In addition, cryptic transcripts are generated from intergenic regions as a result of relaxed control of transcription [4]. These regions include open reading frames (ORFs) that are not conserved across species and thus are probably not functionally significant [5,6].

Box 1. Structure and content of plant mitochondrial DNA

The genome in plant mitochondria differs considerably from the one of its bacterial ancestor and has undergone massive loss of genetic content during the functional integration of the symbiont within the host cell. The mitochondrial genome in animals has retained a small size (~15 kb), whereas the plant mitochondrial genome in land plants is relatively large (~150–6 700 kb). Gene density in plant mitochondria is particularly low. For example, the 360 kb mitochondrial genome of the model plant *Arabidopsis* encodes 57 genes covering only about 10% of the genome [2]. These genes encode for some of the components of the respiratory chain and the cytochrome *c* maturation complexes, as well as the translation apparatus, in other words ribosomal proteins, tRNAs, and rRNAs. In addition, the plant mitochondrial genome of higher plants contains many ORFs whose functions and significance cannot be inferred from sequence homology. These ORFs of dubious function would have arisen from rearrangements of the mitochondrial genome that involve recombination between active repeated sequences.

In contrast to animals, where mitochondrial transcription is initiated from 3–4 promoters, numerous promoters drive transcription in plant mitochondria [7]. The distribution of promoters is poorly conserved among plant species and even among ecotypes, thus leading to frequent transcript polymorphisms [8].

In contrast to chloroplasts that encode a bacterial-type RNA polymerase termed PEP (plastid-encoded plastid RNA polymerase) [9], plant mitochondrial transcription exclusively relies on nucleus-encoded phage-type RNA polymerases (NEPs). Three NEPs are present in flowering plants. Among them, RpoTm (phage-type RNA polymerase, mitochondrial) localizes to mitochondria, whereas RpoTmp localizes to both mitochondria and chloroplasts [10]. RpoTm seems to be the RNA polymerase essential for the transcription of most mitochondrial genes, whereas the function of RpoTmp remains largely unclear, although it could be implicated in the transcription of specific mitochondrial genes [11]. The mechanism governing promoter selection and transcription termination in plant mitochondria is not yet understood, but may involve the participation of transcription factors. In animals, proteins of the mTERF (mitochondrial transcription termination factor) family are involved in the termination of mitochondrial transcription [12]. These helical-repeat proteins are also encoded in plant genomes and are targeted to organelles [13,14]. In the green algae Chlamydomonas (*Chlamydomonas reinhardtii*), the mTERF member MOC1 (mterf-like gene of Chlamydomonas 1) binds specifically to a sequence in the mitochondrial genome and acts as a transcription terminator by altering transcription read-through [15]. Another member of the family, SHOT1 (suppressor of hot1-4 1), influences the steady-state level of a subset of mitochondrial RNAs in *Arabidopsis*, although its role in mitochondrial transcription is not clear [16].

Overall, current data imply that plant mitochondrial transcription is a relaxed and loosely controlled process. The size of precursor RNA molecules seems to be defined largely by promoter selection and by the processivity of RNA polymerases.

Definition of RNA 5′ and 3′ ends

After transcription, RNA precursors require to be processed at both the 5′ and 3′ ends. It was proposed that RNA end maturation might be achieved through direct endoribonuclease and/or exoribonucleases activities that would be blocked by stable RNA secondary structures defining mature transcript ends (reviewed in [17]). In favor of this hypothesis, RNA stem-loop folds were found to be involved in RNA processing in plant mitochondria [18,19]. In addition, RNA-binding proteins may serve a similar function as secondary structure in blocking RNA degradation and defining transcripts ends. In *Arabidopsis*, the mitochondrion-localized PPR protein MTSF1 (mitochondrial stability factor 1) binds to *nad4* (NADH dehydrogenase subunit 4) mRNA and defines its 3′ end. Other reported PPR proteins, termed RPF (RNA processing factor), might have similar modes of action in mitochondria [20–23]. A recent model established in chloroplasts further supports the use of RNA-binding proteins for the definition of transcript ends in organelles. In this model, a PPR protein binds to the intergenic region in a polycistronic messenger and impedes the progression of both 5′ to 3′ or 3′ to 5′ exoribonucleases to define transcript ends. Interestingly, a small RNA representing the MTSF1 binding site accumulates *in vivo* in an MTSF1-dependent manner and certainly constitutes the MTSF1 RNA 'footprint' [24]. Thus, the use of protein barriers for RNA decay and for termini definition may be widespread in plant mitochondria. Clues for its prevalence might come from the identification and analysis of small RNAs in mitochondria.

The RNases involved in this process are of prokaryote origin. Two 3′ to 5′ exoribonucleases, RNase II and a polynucleotide phosphorylase (PNPase), were found in plant mitochondria. Their study suggest that PNPase would first remove large 3′ extensions of precursor transcripts and RNase II would subsequently trim short nucleotide extensions to generate mature 3′ ends [25]. A 5′ to 3′ exoribonuclease has not yet been identified in plant mitochondria. Similar to other transcripts, tRNAs are expressed as precursors and undergo both 5′ and 3′ maturation. These steps are catalyzed by two ubiquitous endoribonucleases, RNase P and RNase Z. In *Arabidopsis*, four RNase Z proteins are encoded in the nucleus, among which two are localized to mitochondria [26]. In contrast to prokaryotes, which use ribonucleoprotein RNase P enzymes, plant mitochondria use a protein-only RNase P, termed PRORP (protein-only RNase P) that contains PPR motifs [27,28]. Beyond tRNA maturation, RNase P and RNase Z are also involved in the maturation of mRNAs of tRNA-like structures known as 't-elements' [19].

RNA editing

RNA editing is a process that alters the nucleotide sequence of an RNA molecule after transcription, changing the primary information that was encoded by the gene. The first evidence for messenger RNA being edited in a plant organelle was demonstrated for the first time in the late 1980s [29–31]. With the exception of the marchantiid liverworts, RNA editing in mitochondria and chloroplasts occurs in all land plant groups [32]. RNA editing in both organelles is believed to be evolutionarily related, and indeed displays similar features. In plants, RNA editing mainly consists of C to U or U to C bases conversions. In