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Biochimica et Biophysica Acta 1779 (2008) 566–573
Biochimica et Biophysica Acta BBA
www.elsevier.com/locate/bbagrm

Review

Coping with cryptic and defective transcripts in plant mitochondria

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Received 7 November 2007; accepted 5 February 2008
Available online 15 February 2008

Abstract

Plant mitochondria are particularly prone to the production of both defective and cryptic transcripts as a result of the complex organisation and mode of expression of their genome. Cryptic transcripts are generated from intergenic regions due to a relaxed control of transcription. Certain intergenic regions are transcribed at higher rates than genuine genes and therefore, cryptic transcripts are abundantly produced in plant mitochondria. In addition, primary transcripts from genuine genes must go through complex post-transcriptional processes such as C to U editing and *cis* or *trans* splicing of group II introns. These post-transcriptional processes are rather inefficient and as a result, defective transcripts are constantly produced in plant mitochondria. In this review, we will describe the nature of cryptic and defective transcripts as well as their fate in plant mitochondria. Although RNA surveillance is crucial to establishing the final transcriptome by degrading cryptic transcripts, plant mitochondria are able to tolerate a surprising high level of defective transcripts.

Keywords: Plant mitochondria; Polyadenylation; RNA degradation; PNPase

1. Introduction

Mitochondria are vital organelles for the majority of eukaryotes and are best known for their production of chemical energy via oxidative phosphorylation. Mitochondria are actually involved in several other essential processes such as programmed cell death, ion homeostasis, amino acid metabolism or cofactor biosynthesis. In plants, mitochondria host particular pathways including steps of biotin and folate biosynthesis and photorespiration [1–3]. Another peculiarity of plant mitochondria is also that NAD(P)H oxidation can be uncoupled from proton pumping by alternative oxidases and NAD(P)H dehydrogenases [4,5].

Besides these metabolic particularities, plant mitochondria are characterized by unique genome structure and expression. First, their genomes are extremely large as compared to other mitochondria. While human and yeast mitochondrial genomes are about 16 kb and 86 kb, respectively, plant mitochondrial genomes are often larger than 300 kb. This size increase is not correlated with a larger coding capacity but is rather due to the presence of large intergenic regions. Second, plant mitochondrial genomes have a complex structure and form multiple subgenomic molecules that recombine at high frequencies [6]. Third, gene expression in plant mitochondria requires several intricate post-transcriptional processes, such as *cis*- and *trans*-splicing or C to U editing. Editing affects several hundred of nucleotides and is required to "correct" most mRNA sequences. Editing results in a better evolutionary conservation of protein sequences deduced from edited mRNA than from genomic sequences [7–9].

Plant mitochondrial genes encode 3 rRNAs, 15 to 20 tRNAs and about 30 proteins, which are subunits of respiratory chain complexes, cytochrome *c*-type maturation or ribosomal proteins. Thus, all mitochondrial genes are either directly or indirectly required for oxidative phosphorylation. Mutations that affect mitochondrial gene expression can be lethal, or result in severe growth or developmental phenotypes such as sterility or homeotic conversion of floral whorls [10]. Mitochondrial mutations can also result in defective chloroplast biogenesis. Proper expression of the mitochondrial genome is therefore essential for plant growth and development. Yet, plant mitochondria have

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doi:10.1016/j.bbagrm.2008.02.004

to face a permanent threat: they have to cope with a massive and continuous production of cryptic and defective transcripts. Cryptic transcripts are produced from regions that presumably do not contain genetic information or from the opposite DNA strand of genuine genes. Defective transcripts are encoded by true genes but have failed to properly complete the series of complex post-transcriptional processes necessary to produce a functional RNA. How does the system ensure that this production of spurious transcripts has no deleterious consequences on mitochondrial function and therefore on cell viability? In this review, we define the causes for the abundant production of aberrant transcripts in plant mitochondria and describe their molecular features. We also discuss on how these transcripts are dealt with to allow proper function of plant mitochondria.

2. Production of cryptic transcripts in plant mitochondria

One of the most peculiar features of plant mitochondria is the structure and organization of their genome. The size of mitochondrial genomes is extremely variable between plant species and can range from 220–600 kbp in most species to 2.3 Mbp in melon [11]. However, the gene content is relatively conserved between species and is rather limited, less than 60 genes. The gene density in plant mitochondrial genomes is therefore low as compared to humans or animals. Most of the size increase in plant mitochondrial genomes is a consequence of an accumulation of introns and intergenic regions. Intergenic regions are partly composed of nuclear and chloroplastic sequences but the majority of the intergenic regions in plant mitochondria is of unknown genetic origin. An additional reason for the size increase of plant mitochondrial genomes is the frequent duplication of parts of the genome. For instance, duplicated regions constitute about 23% and 26% of the maize and rice mitochondrial genome, respectively [12,13].

Presence of large intergenic sequences in the plant mitochondrial genomes would be of little consequence if transcription were tightly controlled and limited to bona fide genes. However, a large variety of cryptic transcripts are produced from intergenic regions. The first evidence of this phenomenon came from a study in maize that showed that transcription activity of 21 kbp around *rrn* genes [14]. Since then, progress has been made to understand this relaxed control of transcription in plant mitochondria. Mitochondrial genes are transcribed by nuclear-encoded T7 phage-type RNA polymerases ([15] and references therein). These enzymes are capable of promoter recognition, initiation, and elongation on their own but require auxiliary factors to recognize all transcription initiation sites in vivo [15]. Promoter motifs were identified by a combination of sequence analysis and functional studies using in vitro transcription systems (reviewed in [16]). Following the sequencing of the *Arabidopsis* mitochondrial genome, it became apparent that potential promoters exist in intergenic regions [17]. In addition, a number of genuine genes do not show the typical promoter consensus motifs raising the possibility that different sequences can initiate transcription (e.g. [18–20]). In fact, transcription is actually initiated from a variety of sequences and at multiple sites for a given gene [20,21]. This multiplicity of promoter sequences also results in the frequent initiation of transcription in intergenic regions or on the opposite strand of genuine genes. Indeed, synthesis of antisense RNA was demonstrated for the four *Arabidopsis* genes that were investigated to date (atp9, nad4, nad5 and nad7) [22]. Although this study has not yet been extended to complete mitochondrial genomes, antisense RNA are likely to be common in plant mitochondria.

To make things worse, no efficient transcription termination mechanism seems to exist in plant mitochondria. Therefore, once initiated, transcription gives rise to RNAs with variable sizes and sometimes to extremely large transcripts. For instance, primary transcripts of *atp9* and *atp8* can reach more than ten times the size of the mature mRNA [23]. Thus, the lack of transcription termination mechanisms contributes significantly to the transcription of intergenic regions.

Cryptic transcripts are also produced as a consequence of recombination events that can result in the insertion of pieces of functional genes or regulatory regions into a new genetic context. For instance, a sequence of about 230 nt containing the efficient promoter driving the expression of the 26S rRNA was duplicated in a region devoid of functional genes in *Arabidopsis thaliana* [24]. This duplicated promoter retains its original activity and induces transcription of the downstream region [22].

The combination of large intergenic regions with the duplication of promoters by recombination, the relaxed promoter specificty, and the absence of termination mechanisms account

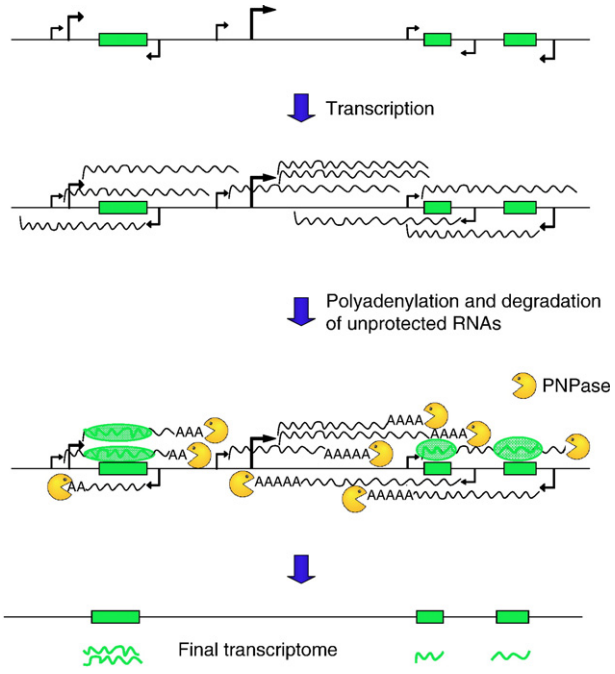

Fig. 1. RNA surveillance in plant mitochondria. Diagram showing part of the mitochondrial genome with genes shown by green rectangles, promoters by bent arrows, transcribed RNAs by black undulate lines. Cryptic transcripts result from transcription initiated in intergenic regions, or antisense to genuine genes, as well as lack of transcription termination. Transcripts are polyadenylated by a yet unidentified poly(A) polymerase and degraded by PNPase unless stabilized by specific stability factors. RNAs constituting the final mitochondrial transcriptome are in green.