A unique system for regulating mitochondrial mRNA poly(A) status and stability in plants

Takashi Hirayama*

Institute of Plant Science and Resources; Okayama University; Okayama, Japan

Poly(A) status is the major determinant of mRNA stability, even in endosymbiotic organelles. Poly(A) specific ribonuclease (PARN) is distributed widely among eukaryotes and has been shown to regulate the poly(A) status of cytoplasmic mRNA in various organisms. Surprisingly, our recent study revealed that PARN also directly regulates poly(A) status of mitochondrial mRNA in Arabidopsis. In this addendum, we discuss whether this mitochondrial function of PARN is common in plants and why PARN has been assigned such a unique function.

Introduction

Eukaryotes have endosymbiotic organelles, namely mitochondria and plastids. The idea that these organelles originated from symbiont bacteria has been widely accepted. During the establishment of endosymbiotic organelles, most genes from the symbiont genome were lost or transferred to the nuclear genome, presumably allowing the host to control the behavior of the endosymbiotic organelle. Despite this, the endosymbiotic organelles retained some genes, and the large protein complexes required for respiration in mitochondria are composed of both nucleus- and organelle-encoded components in most eukaryotes.[1] This necessitates tightly coordinated gene expression between the nuclear and organellar genomes. It has been postulated that signaling from organelles to the nucleus/cytoplasm (retrograde signaling) and that from the nucleus/cytoplasm to organelles (anterograde signaling) is involved in achieving such coordinated gene expression.[2-4] Although neither signaling system has been fully elucidated, they are thought to involve regulation at the levels of transcription, post-transcription, and post-translation, in both the nucleus and organelles. Such regulatory events have been extensively studied for nuclear genome-encoded genes. By contrast, those for plant mitochondrial genes are poorly understood, although there has been progress from recent studies especially on regulation at the post-transcriptional level.[5] The current knowledge strongly implies that plants have evolved a mitochondrial post-transcriptional regulation system that is unique among eukaryotes in spite of the single origin of mitochondria in the Eukaryota, which is supported by phylogenetic and phylogenomic analyses.[6] For example, plants possess a large number of pentatricopeptide repeat (PPR) proteins that are involved in various steps of RNA metabolism such as intron splicing and RNA editing in mitochondria and plastids.[7] Recently, we obtained data offering more evidence for unique mitochondrial RNA regulation in plants. We found that the poly(A) specific ribonuclease (PARN) of *Arabidopsis thaliana* directly regulates the poly(A) status of mitochondrial mRNA.[8] This finding is surprising given that PARN is distributed widely among eukaryotes (other than budding yeast and Drosophila[9]) and has been shown to regulate the poly(A) status of cytoplasmic RNA in various organisms, but never in organelles.[10,11]

Distinct functions of multiple deadenylases of eukaryotes

Poly(A) specific ribonuclease (PARN) is a eukaryotic deadenylase. Eukaryotes usually possess 3 types of deadenylases: the CCR4-NOT complex, the PAN2-PAN3

complex and PARN.[12,13] Recent studies have shown that the PAN2-PAN3 complex in mammalian cells initiates poly(A) tract shortening, which in turn facilitates further poly(A) removal by CCR4-NOT before mRNA degradation takes place.[11,14] The physiological role of PARN in animals seems slightly different from those of the other deadenylases. PARN removes the poly(A) tract of the target mRNA with unique *cis* elements but does not induce mRNA degradation. Instead, the resulting target mRNA lacking poly(A) is sequestered as stable, translationally silent mRNA in protein-mRNA complexes.[11]

Plants also have multiple deadenylases. The Arabidopsis genome is predicted to contain 9 *CCR4* genes, 11 *CAF/NOT/ POP2* genes, and 2 *PARN* genes. Although it is not clear whether these different types of deadenylases have any redundant functions, the results of genetic analyses suggest that they do have distinct functions. For example, the *AtCAF1a*-disrupted mutant shows reduced expression of biotic and abiotic stress-related genes, suggesting that this deadenylase has a pivotal (and non-redundant) role in stress responses in Arabidopsis.[15,16]

PARN in plants

There are 2 genes annotated as PARN-like in Arabidopsis, but one of them, At3g25430, seems unlikely to encode a functional PARN enzyme because it would lack several conserved amino acid residues;[17] this remains to be tested experimentally, however. The other *PARN* gene, At5g55879, has been characterized in detail. This gene was reported to be indispensable for early development of Arabidopsis in 2 independent studies using T-DNA insertion mutants.[17,18] Both claimed that this Arabidopsis PARN was localized in the cytoplasm, consistent with observations in other eukaryotes. We genetically identified this gene as *ABA HYPERSENSITIVE GERMINATION2* (*AHG2*) in a screen for ABA-hypersensitive mutants.[19,20] Interestingly, the *ahg2–1* mutation also causes salicylic acid hypersensitivity.[21] The discrepancy between the phenotypes caused by the null mutations and those caused by *ahg2–1* can be most simply explained by the weakness of the *ahg2–1* mutation, which presumably only reduces the gene expression level.[20] Given the clear phenotype of the mutants, it has been postulated that Arabidopsis PARN has an important and specific role that cannot be compensated for by other deadenylases.

Taking advantage of the viability of the *ahg2–1* mutant, we analyzed Arabidopsis PARN in detail. We found that (1) all of the known *ahg2–1* phenotypes are suppressed by loss-of-function mutations in the *AGS1* gene (At2g17580) encoding a bacterial-type poly(A) polymerase although the *ags1* mutant allele alone does not cause any visible phenotypes, (2) mitochondrial mRNA is hyper-accumulated and abnormally polyadenylated in *ahg2–1*, (3) both AHG2/PARN- and AGS1-GFP fusion proteins localize to the mitochondria in transgenic plants, (4) removal of the putative N-terminal transit peptides of AHG2 or AGS1 reduces their activity *in vivo*.[8] We thus concluded that Arabidopsis PARN, along with AGS1, regulates mitochondrial mRNA poly(A) status.

When we compared the amino acid sequence of Arabidopsis PARN with the predicted sequences of putative homologs from various species in the plant lineage, it became apparent that a non-conserved N-terminal extension sequence was common to PARNs of plants compared to those from animals (**Fig. 1**). Most of the higher plant PARNs were predicted to localize to mitochondria by 3 independent prediction programs, iPSORT, TargetP, and Predotar.[22-24] *Selaginella moellendorffii* (a lycophyte) has several PARN-like proteins. Interestingly, one of them (Selaginella1 in **Fig. 1**) has an N-terminal extension while another (Selaginella2) does not. It is possible that the Selaginella1 PARN can function in mitochondria as well. In addition, the putative

Figure 1. Comparison of the amino acid sequence of the N-terminal portion of poly(A) specific ribonucleases from various organisms. Highly conserved or relatively conserved amino acid residues are shaded in black and gray, respectively. The subcellular localization prediction results (M, mitochondria; C, plastids; –, other) from 3 prediction programs (iP, iPSORT; Tp, TargetP; Pd, Predotar) are summarized to the right of the species names. Accession numbers of PARN proteins: Arabidopsis, NP_175983.51; poplar, XP_002300380.2; soybean1, XP_003532241.1; soybean2, XP_003552994.1; tomato, XP_004244040.1; maize, NP_001169499.1; Brachypodium, XP_003581331.1; rice CAH66773.1; Selaginella1, XP_002969601.1; Selaginella2, XP_002987014.1; Physcomitrella, XP_001765586.1; Volvox, XP_002949475.1; Nannochloropsis, EWM30500.1; Guillardia, XP_005838739.1; zebrafish, AAQ97826.1; Xenopus, AAH73682.1; human, NP_002573.1; mouse, NP_083037.1.

PARN from *Physcomitrella* (a bryophyte) has a 122-aa N-terminal extension that is predicted by TargetP and Predotar to direct the protein to mitochondria. *Volvox* also has a PARN with an N-terminal extension, and the genome database for the cryptophyte *Guillardia theta* contains a predicted PARN-like protein possessing a long N-terminal extension. Although none of the prediction programs supported their mitochondrial localization, there remains a possibility that the PARNs with long N-terminal extensions in these organisms localize to and function in mitochondria. In sum, most plant lineages appear to contain *PARN* genes encoding N-terminal extension sequences, strongly suggesting that PARN functions in mitochondria in these organisms, as we observed in Arabidopsis.

Why is PARN used for mitochondrial post-transcriptional regulation in plants?

These observations raise the question of why plants use PARN for poly(A) regulation of mitochondrial mRNA. Although there is no clear answer at this moment, it could be related to the unique features of plant mitochondria, which have larger genomes and contain more protein-coding genes than do mitochondria from other eukaryotes. Presumably, the existence of another genome-possessing organelle, i.e., the plastid, plays a role in these plant-specific characteristics. In order to harbor 2 different types of symbiont (ancestral mitochondria and plastids), plants had to develop systems for tight and separate regulation of symbiont activities. For this purpose, it is possible that plants adopted PARN as a mitochondrion-specific post-transcriptional regulator controlling mRNA stability. Alternatively, PARN might be required to remove harmful non-coding RNAs that are transcribed from the mitochondrial genome. Plant mitochondrial genome is rearranged frequently by its high DNA recombination activity that is presumably required for maintenance of the genome.[25] But it in turn allows plant mitochondrial genomes to encode many non-functional genes and some intergenic regions of the mitochondrial genome to be transcribed.[26] Some of these transcripts or products could be deleterious to mitochondrial or cellular

functions, eg. a case known as cytoplasmic male sterility. The idea that PARN has been recruited to remove these transcripts receives support from the fact that plants have a tremendous number of pentatricopeptide repeat proteins that are thought to suppress the expression of harmful mitochondrial genes or compensate for mutations occurring in mitochondrial genes at post-transcriptional levels, emphasizing the need for such protective activity.[7]

Another question that arises is why PARN, from among the several types of deadenylases, has been selected for this function. One answer could be that PARN originally was not essential and/or had minor roles in the regulation of cytoplasmic mRNA. This seems plausible as PARN is involved in the poly(A) tail regulation of only a limited number of target mRNAs, and not in general mRNA decay. To date, the physiological importance of PARN has been described only for Xenopus oocytes and neuronal cells, in addition to Arabidopsis embryos. Such dispensability would allow plants to adapt PARN for use in different physiological roles, a kind of neofunctionalization.[27] It should be noted that PARN has been reported to function as a homo-dimer while other deadenylases act as large protein complexes. Therefore, it is possible that a mitochondrial post-translational regulation system involving PARN would be easier for plant cells to regulate than those with other deadenylases.

Conclusions and future perspectives

The AHG2-AGS1 regulatory system is novel and unique to plants. Therefore, the discovery of this system for mitochondrial mRNA poly(A) regulation has opened a new frontier for mitochondrial gene expression research. Mitochondria now attract more attention than ever, with studies suggesting mitochondrial involvement in cellular functions such as stress responses, in addition to respiration and metabolism. So far, the physiological relevance of the AHG2-AGS1 system is unclear. Interestingly, however, *ahg2–1* causes abnormal responses to the stress-related plant hormones ABA and SA, implying that regulation by AHG2 or AGS1 is linked to stress responses. Further analysis of AHG2 and AGS1 should

therefore shed light on the molecular nature of the link between mitochondria and stress responses in plants.

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