To Thy Proteins Be True: RNA Editing in Plants

BACKGROUND

Plastid and mitochondrial genomes encode for a certain percentage of their own proteins. Which ones and the number of genes are species dependent. The remainder of the proteins are encoded by the nucleus and imported posttranslationally into the organelle. As plastids are derived from a cyanobacterial ancestor, gene expression exhibits a hybrid of prokaryote and eukaryote features, with nuclear-encoded proteins playing a role in gene regulation. One example of this blend is the presence of two RNA polymerases, one of prokaryote origin and the other eukaryote. Unlike the nuclear-encoded genes, the majority of plastid gene expression is regulated posttranscriptionally with exceptional mRNA stability.

Another feature of plastid and mitochondrial posttranscriptional regulation is RNA editing. RNA editing was first identified in the *cox2* mRNA of *Trypanosoma brucei* (Benne et al., 1986) and has since been found to play an important role in organelles of many organisms (Knoop, 2011), especially plants. An exception is *Marchantia polymorpha* (a liverwort), in which there is no evidence of RNA editing (Schmitz-Linneweber et al., 2004), allowing its use as the "baseline" organism for comparing transcripts for evidence of editing. In the terrestrial plants examined, RNA editing occurs in both plastids and mitochondria and typically involves the changing of specific C nucleotides to U (for review, see Shikanai, 2006). Nucleotide editing usually restores a conserved amino acid codon, but it also can create an initiation or stop codon or, alternatively, remove a stop codon. Editing can also occur in introns and untranslated regions, possibly playing a role in increasing transcript stability (for review, see Chateigner-Boutin and Small, 2010).

The exact suite of proteins involved in this process is still being determined, but one protein family, the pentatricopeptide repeat (PPR) family, has been shown to be involved in most studies. PPR proteins are the largest protein family in plants, with more than 450 members in both Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*). As PPR proteins do not have any catalytic sites, they most likely interact with other proteins. PPR proteins are characterized as having a canonical 35-amino acid repeat that can be repeated up to 30 times in the protein. Based on the repeats, PPR proteins have been placed into two major families: P, which do not have any other conserved domains, and PLS, which is further divided into subfamilies based on C-terminal motifs (for review, see Schmitz-Linneweber and Small, 2008). The PLS subfamily appears to be specific to plants. Most of these proteins still have unknown functions, but of those whose roles have been defined, they have been shown to be involved in organellar RNA metabolism. As in yeast and *Neurospora*, plant PPR proteins are RNA binding and are involved in posttranscriptional processes (Lurin et al., 2004), including RNA editing. Why plants edit RNA, as well as how this process occurs at a molecular level, is an open question.

WHAT WAS SHOWN

The Arabidopsis high chlorophyll fluorescence mutant *low psii accumulation66* (*lpa66*), which has impaired PSII functions resulting in the high chlorophyll fluorescence phenotype, was characterized by Cai et al. (2009). Although PSII function is not optimal in *lpa66* mutant plants, PSI appears to be functional. Lower levels of core PSII protein levels, about 25% of the wild type, were found with the *lpa66* mutants. This decrease in protein levels was not due to a decrease in transcript abundance; no change in transcript abundance or patterns between mutant and wild-type plants was observed, indicating the difference is posttranscriptional. No change in polysome association was found, indicating that translation is occurring. *lpa66* has an increase in protein turnover, and assembly of PSII complexes is also not as efficient as the wild type.

Genetic analysis demonstrated that *lpa66*'s high chlorophyll fluorescence phenotype was due to a mutation in *At5g48910*, a chloroplast-targeted protein and a member of the PPR family. In wild-type Arabidopsis, a base change in *psbF*, 77C to 77U, restores a conserved Phe. Direct sequencing of RNA demonstrated this base change did not occur in the mutant plants, leaving a Ser in the cytochrome b_{559} adversely affecting PSII assembly. Other sites were edited as in the wild type, strongly suggesting that the LPA66 protein is responsible for this RNA editing.

THE IMPACT

Despite having a smaller genome, mitochondria have many more editing sites than plastids, 500 versus 34. Although the machinery is most likely similar to plastids, fewer editing factors have been identified in mitochondria. As would be expected with having so many editing sites, the PPR proteins that have been identified are involved in the editing of multiple mitochondrial RNA sites. Sung et al. (2010) were interested in identifying cis-acting elements involved in mitochondrial RNA editing as well as how the editing machinery recognizes sites within the RNA. They identified a PPR protein, SLOW GROWTH1 (SLO1), that appears to be involved in the editing of

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multiple sites in mitochondria, most notably *nad4* and *nad9*, two of the subunits of respiratory complex I (NADH dehydrogenase). In *slo1* mutants, neither of these RNAs is edited, and there is also an increase in the steady-state levels of 56 mitochondrial mRNAs, which would not be related to the lack of editing in *nad4* or *nad9I*. *slo1* plants are also smaller than the wild type, further suggesting that SLO1 has a role beyond editing those two genes and most likely is involved in regulation of mitochondrial growth and development.

Using a reverse genetic screen of T-DNA mutants, Hammani et al. (2009) searched for unedited transcripts of 34 known plastid RNAs that undergo editing, leading to the identification of six PPR proteins that together are responsible for editing nine sites in Arabidopsis plastid RNA. Interestingly, lack of editing in eight of these sites did not lead to an obvious difference in phenotype or growth habit between the mutant and wild-type plants grown under normal growth conditions. This result suggests that, under optimal, nonstressed growth conditions, some editing mutants might go undetected. Further analysis demonstrated that five of these PPR proteins edit multiple sites. Hammani et al. (2009) examined the RNA sequences around the PPR target site to elucidate common RNA sequences. They were able of identify a 15-nucleotide stretch for four out of the five. Analysis of the consensus sequences demonstrated the proteins are able to distinguish between purine and pyrimidine bases and must be able to recognize specific bases in some positions; however, there is no canonical recognition sequence.

CONCLUSION

RNA editing is more than just the correcting of base pairs to restore an RNA sequence; it also plays a role in modulating gene expression. Although the entire suite of proteins involved in the changing of a C to U has yet to be elucidated, the PPR proteins are important players in this process.

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