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The Pentratricopeptide Repeat Protein Pigment-Defective Mutant2 is Involved in the Regulation of Chloroplast Development and Chloroplast Gene Expression in Arabidopsis

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The development of functional chloroplasts, which is assisted by a series of nuclear-encoded auxiliary protein factors, is essential for plant autotrophic growth and development. To understand the molecular mechanisms underlying chloroplast development, we isolated and characterized a pigment-defective mutant, *pdm2*, and its corresponding variegated RNA interference (RNAi) lines in Arabidopsis. Sequence analysis revealed that PDM2 encodes a pentatricopeptide repeat protein that belongs to the P subgroup. Confocal microscopic analysis and immunoblotting of the chloroplast protein fraction showed that PDM2 was located in the stroma. In RNAi plants, protein-related photosynthesis was severely compromised. Furthermore, analysis of the transcript profile of chloroplast genes revealed that plastid-encoded polymerase-dependent transcript levels were markedly reduced, while nuclear-encoded polymerase-dependent transcript levels were increased, in RNAi plants. In addition, PDM2 affects plastid RNA editing efficiency in most editing sites, apparently by directly interacting with multiple organellar RNA editing factor 2 (MORF2) and MORF9. Thus, our results demonstrate that PDM2 is probably involved in the regulation of plastid gene expression required for normal chloroplast development.

Keywords: Arabidopsis • Chloroplast • Development • Pentatricopeptide repeat protein • Pigment-Defective Mutant2.

Abbreviations: GFP, green fluorescent protein; MORF, multiple organellar RNA editing factor; NEP, nuclear-encoded RNA polymerase; PEP, plastid-encoded RNA polymerase; PPR, pentatricopeptide repeat; RNAi, RNA interference; PDM2, Pigment-Defective Mutant2; RT–PCR, reverse transcription–PCR.

Introduction

In higher plants, chloroplasts develop from proplastids. During the process of chloroplast biogenesis, the enlarged proplastids respond to light, and then the invaginated inner envelope of proplastids enfolds to form more vesicles that are linked and merged together. Subsequently, these vesicles fuse into a lamellar structure that can be complemented by smaller, disc-shaped structures that develop into grana stacks. Meanwhile, the developing chloroplast forms a typical lens shape. Finally, the mature chloroplast containing a well-developed thylakoid membrane is formed (López-Juez 2007, Jarvis and López-Juez 2013, Osteryoung and Pyke 2014).

Chloroplast development is tightly linked to embryogenesis (Kobayashi et al. 2007). Accompanied by the accumulation of proteins and lipids required for photosynthesis, Chl is synthesized in the heart-stage embryos; photosynthesis in chloroplasts subsequently offers energy for embryo development (Kobayashi et al. 2007). As the critical event in embryogenesis, the synthesis of some compounds such as amino acids, lipids and phytohormones also occurs in chloroplasts (Ruuska et al. 2004). Genetic screens in Arabidopsis have demonstrated that impairment of plastid functions can perturb embryogenesis and even result in embryo lethality (Bryant et al. 2011, Muralla et al. 2011, Lloyd and Meinke 2012).

Indeed, among the 400 Arabidopsis genes so far identified as essential for embryo development, about 30% encode chloroplast-localized proteins (see the SeedGenes Project database, http://www.seedgenes.org/). Several of these genes which encode chloroplast proteins which belong to the pentatricopeptide repeat (PPR) protein family have been reported (Myouga et al. 2010). The PPR proteins are characterized by the presence of a degenerate 35 amino acid repeat sequence that is found extensively in higher plants (Barkan and Small 2014). The PPR protein family is classified into two subfamilies: P and PLS. The PLS subfamily is further classified into PLS, E and DYW subclasses (Lurin et al. 2004, Barkan and Small 2014). *PPR* genes constitute a large multigene family in higher plants, such as Arabidopsis and rice, and most members of the PPR family are predicted to localize to either mitochondria or chloroplasts (Lurin et al. 2004). Genetic studies on *PPR* genes reveal that most proteins belonging to the PPR family are involved in editing, splicing and regulating the stability of various organellar transcripts (Schmitz-Linneweber and Small 2008, Barkan and Small 2014).

During embryogenesis and chloroplast development of vascular plants, coupled plastid gene expression is a modulated

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