LPA66 Is Required for Editing psbF Chloroplast Transcripts in Arabidopsis\footnote{[W]}[OA]

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To gain insight into the molecular mechanism of RNA editing, we have characterized the low psii accumulation\textsuperscript{66} (lpa66) Arabidopsis (Arabidopsis thaliana) mutant, which displays a high chlorophyll fluorescence phenotype. Its perturbed chlorophyll fluorescence is reflected in reduced levels of photosystem II (PSII) proteins. In vivo protein labeling showed that synthesis rates of the PSII reaction center protein D1/D2 were lower, and turnover rates of PSII core proteins higher, than in wild-type counterparts. The assembly of newly synthesized proteins into PSII occurs in the lpa66 mutant but with reduced efficiency compared with the wild type. LPA66 encodes a chloroplast protein of the pentatricopeptide repeat family. In lpa66 mutants, editing of psbF that converts serine to phenylalanine is specifically impaired. Thus, LPA66 is specifically required for editing the psbF transcripts in Arabidopsis, and the amino acid alteration due to lack of editing strongly affects the efficiency of the assembly of PSII complexes.

PSII is a large pigment-protein complex found in the membranes of chloroplasts, containing more than 20 subunits, which catalyzes light-driven water oxidation and the reduction of plastoquinone concomitant with oxygen evolution. Some PSI proteins are encoded by the nuclear genome and others by the chloroplast genome in higher plants (Wollman et al., 1999; Nelson and Yocum, 2006). Thus, coordinated regulation of nuclear and chloroplast gene expression is essential for the biogenesis and assembly of photosynthetically competent protein complexes (Goldschmidt-Clermont, 1998). Chloroplast gene expression is regulated mainly at posttranscriptional levels by various mechanisms (Deng and Gruissem, 1987), and a number of nucleus-encoded factors have been shown to be involved in RNA splicing, processing, editing, degradation, and translation in plants (Barkan and Goldschmidt-Clermont, 2000; Zerges, 2000; Choquet and Wollman, 2002; Manuell et al., 2004; Marin-Navarro et al., 2007). Among them, a PPR superfamily has received much attention recently, because of its members’ involvement in posttranscriptional regulation of gene expression in plastids (Shikanai, 2006; Andrés et al., 2007; Delannoy et al., 2007). In Chlamydomonas reinhardtii, MCA1, the only PPR protein from this organism characterized to date, is involved in the regulation of petA gene expression by interacting with the first 21 nucleotides of the 5' untranslated region of its transcripts, thereby protecting them from 5'-3' degradation (Loiselay et al., 2008). In Arabidopsis (Arabidopsis thaliana), numerous PPR proteins have been shown to play various roles, including the following. CRR2 and HCF152 have been shown to be involved in cleavage and splicing of their specific mRNA targets, respectively (Hashimoto et al., 2003; Meierhoff et al., 2003; Nakamura et al., 2003). PGR3 is involved in the stabilization of petL RNA operons and the translation of petL (Yamazaki et al., 2004). CRR4, CRR21, CRR22, CRR28, CLB19, Y51, and RARE1 have been shown to be specifically required for editing their corresponding RNA targets (Kotera et al., 2005; Okuda et al., 2006, 2007, 2009; Chatteigner-Boutin et al., 2008; Robbins et al., 2009; Zhou et al., 2009). In addition, DG1 and pTAC2 have been shown to be involved in the regulation of plastid-encoded RNA polymerase-dependent transcript accumulation (Pfalz et al., 2005; Chi et al., 2008). PPRs have also been found to regulate plastid gene expression in other organisms, such as ZmCRP1, ZmPPR2, ZmPPR4, and ZmPPR5 in maize (Zea mays; Fisk et al., 1999; Williams and Barkan, 2003; Schmitz-Linneweber et al., 2006; Beick et al., 2008), OsPPR1 in rice (Oryza sativa; Gothandam et al., 2005), and PPR531-11 in the moss Physcomitrella patens (Hattori et al., 2007).

PPR proteins, which constitute one of the largest families of proteins in plants, are defined by the tandem array of PPR motifs with a highly degenerate

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unit consisting of 35 amino acids (Lurin et al., 2004). There are about 100 members of the family in *P. patens* and over 450 members in both Arabidopsis and rice (Lurin et al., 2004; Merchant et al., 2007; O’Toole et al., 2008). The plant PPR protein family can be divided into two subfamilies on the basis of their motif content and organization, the P subgroup and combinatorial and modular proteins (PCMP) subfamilies (Lurin et al., 2004; Rivals et al., 2006). In proteins of the P subgroup, such as HCF152 and PGR3 in Arabidopsis, the 35 amino acid repeats are organized as tandem repeats. Members of the PCMP subfamily (also referred to as the PLS subgroup) contain PPR-like motifs with either short (PPR-like S) or longer (PPR-like L) repeats. This subfamily is specific to land plants and is subdivided into three subgroups according to their C-terminal contents: PLS, E, and DYW proteins (Lurin et al., 2004; Rivals et al., 2006). In Arabidopsis, the PCMP subfamily contains about 200 members, including the recently characterized CRR4, CRR21, CRR22, CRR28, CLB19, YS1, MEF1, and RARE1 (Kotera et al., 2005; Okuda et al., 2007, 2009; Chateigner-Boutin et al., 2008; Robbins et al., 2009; Zehrmann et al., 2009; Zhou et al., 2009). CRR4, CRR21, and CLB19 belong to the E subgroup and share a common E domain required for editing the target site, but not for target specificity, which suggests that physical recruitment of the editing machinery may involve non-PPR domains (Okuda et al., 2007). The other five members belong to the DYW subgroup, which additionally contains the DYW domain besides the E domain.

RNA editing is a posttranscriptional process that, in plants, alters specific C nucleotides to U (Maier et al., 1996; Brennicke et al., 1999; Bock, 2000; Shikanai, 2006) or occasionally U to C in bryophytes and ferns (Kugita et al., 2003; Wolf et al., 2004). Such processes have been observed in both plastids and mitochondria of the land plants examined except the Marchantiaidea, but not in any of the investigated algae and cyanobacteria (Schmitz-Linneweber et al., 2004). Usually, RNA editing results in the modification of the encoded amino acid sequences (Bock et al., 1994; Sasaki et al., 2001; Okuda et al., 2007; Chateigner-Boutin et al., 2008) or the generation of either a translational start codon (Hoch et al., 1991; Kotera et al., 2005) or stop codon (Wintz and Hanson, 1991). There are about 20 to 50 known editing sites in plastid transcripts and more than 400 known sites in mitochondrial transcripts in the angiosperms that have been studied (Giege and Brennicke, 1999; Handa, 2003; Sugiuira, 2008). In some ferns, there may be hundreds of editing sites in plastid transcripts (Wolf et al., 2004), while in the moss *Physcomitrella*, there are probably less than 10 sites in mitochondrial transcripts (Terasawa et al., 2007). In total, 34 editing sites have been identified to date in Arabidopsis chloroplast transcripts (Chateigner-Boutin and Small, 2007), almost half of which are in subunits of NDH transcripts ndhB, ndhF, ndhD, and ndhG, while there are only three known sites in the transcripts encoding the PSIIs proteins *psbE*, *psbF*, and *psbZ* (Chateigner-Boutin and Small, 2007). Several mutants deficient in specific RNA editing sites in plastid transcripts have been identified, and the genes responsible for them have been characterized (Bock et al., 1994; Kotera et al., 2005; Okuda et al., 2007, 2009; Chateigner-Boutin et al., 2008; Zhou et al., 2009). Here, we report the identification of a high chlorophyll fluorescence Arabidopsis mutant, *low psii accumulation66* (*lpa66*), with reduced levels of PSII. We present evidence that *LPA66* is specifically involved in editing *psbF* and that the amino acid alteration due to lack of editing is responsible for the perturbation of efficient assembly of PSII complexes in the mutant.

**RESULTS**

**PSII Activity Was Impaired in the lpa66-1 Mutant**

The *lpa66-1* mutant was isolated by screening for mutants from the Scheible and Somerville T-DNA Arabidopsis lines (Weigel et al., 2000) with a high chlorophyll fluorescence phenotype (Meurer et al., 1996; Peng et al., 2006; Ma et al., 2007). The mutant plants showed reduced growth, and the leaves appeared pale green under optimal growth conditions (Supplemental Fig. S1). The ratio of variable fluorescence to maximum fluorescence (Fv/Fm), reflecting the maximum potential capacity of the photochemical reactions of PSII (Krause and Weis, 1991), was significantly lower in the mutant (0.40 ± 0.02) than in wild-type plants (0.83 ± 0.02; Fig. 1A), indicating that PSI functions were perturbed in the mutant. However, P700 could be oxidized in the mutant plants (Fig. 1B), suggesting that PSI was functional, as reportedly observed in both *lpa1* and *lpa2* mutants (Peng et al., 2006; Ma et al., 2007).

**Molecular Cloning of the LPA66 Gene**

Genetic analysis showed that the *lpa66-1* mutation was recessive and that the *lpa66-1* mutant phenotype did not cosegregate with the phosphinotrin resistance marker, indicating that the mutated *LPA66* gene is not tagged by the T-DNA (data not shown). Map-based cloning of the *lpa66-1* mutant based on simple sequence length polymorphism molecular markers revealed a nucleotide substitution in the gene *At5g48910* (Fig. 2A), which led to an amino acid change of Gly to Arg (Fig. 2B). Reverse transcription (RT)-PCR analysis showed that the abundance of *At5g48910* transcripts in the *lpa66-1* mutant was comparable to that in wild-type plants (Fig. 2C). An independent T-DNA insertion line carrying a T-DNA insertion at nucleotide position 1,110 bp of the *At5g48910* gene relative to the ATG codon from the Arabidopsis Biological Resource Center (ABRC) was designated *lpa66-2*. The phenotype of *lpa66-2* mutants was pale green and indistinguishable from that of *lpa66-1* (Supplemental Fig. S1). No expression of *At5g48910* in the *lpa66-2* mutant was detected by RT-PCR analysis (Fig. 2C), indicating that it is a null mutant. Therefore, attention was mainly...
focused in the analyses reported here on the \emph{lpa66-1} mutant, and the discussed results apply solely to this mutant, unless otherwise specified.

To confirm that the phenotype of the \emph{lpa66} mutant was due to the mutation in \emph{At5g48910}, a complementation experiment was carried out using wild-type genomic \emph{At5g48910} DNA. Eight successfully complemented transgenic plants had similar growth rates and chlorophyll fluorescence induction kinetics (Fig. 1; Supplemental Fig. S1). Thus, the inactivation of the \emph{At5g48910} gene is responsible for the \emph{lpa66} mutant phenotype.

\textbf{LPA66 Encodes a Chloroplast PPR Protein with an E Motif and a DYW Domain}

The \emph{LPA66 (At5g48910)} gene is not disrupted by any introns and encodes a protein of 646 amino acids. The N-terminal 53 amino acids are predicted to be a chloroplast transit peptide by the programs TargetP 1.1 and ChloroP 1.1 (Fig. 3A). To determine the subcellular localization of the LPA66 protein, a fragment of the 257 N-terminal amino acids of LPA66 was fused to the N terminus of the synthetic GFP (sGFP) with a S65T mutation. The LPA66-GFP fusion proteins were transiently expressed in protoplasts under the control of the cauliflower mosaic virus 35S promoter, and GFP fluorescence was found to be exclusively colocalized with the chloroplastic chlorophyll, in accordance with results obtained when the GFP was fused to the transit peptide of the small subunit of ribulose bisphosphate carboxylase (Lee et al., 2002b). When GFP was fused to the targeting signals of the fibrillarin and FRO1 proteins from Arabidopsis (Pih et al., 2000; Lee et al., 2002a), GFP signals were found to be located specifically in the nucleus and mitochondria, respectively (Fig. 3B). Thus, these results indicate that \emph{LPA66} is localized to the chloroplast.

BLAST searches revealed that LPA66 is a member of the PPR protein family. It contains 11 PPR and PPR-like motifs followed by an E motif and a DYW domain in its C terminus (Figs. 2B and 3A). Thus, according to the classification of PPR proteins (Lurin et al., 2004; Rivals et al., 2006), LPA66 is a DYW protein of the PLS (PCMP) subfamily. Genomic database searches and protein sequence alignments revealed that it shares significant sequence identity with an unknown protein in grapevine (\emph{Vitis vinifera}; 66% identity, 81% similar-
ity; Fig. 3A). However, our efforts to identify potential LPA66 orthologs in *P. patens*, spinach (*Spinacia oleracea*), tobacco (*Nicotiana tabacum*), rice, wheat (*Triticum aestivum*), and maize, based on BLAST search of genome or related ESTs, were unsuccessful (data not shown).

**Editing of psbF mRNA Transcripts Is Impaired in lpa66**

To assess the possibility that the defective PSII function in *lpa66* mutants is due to a defect at the RNA transcript level, we compared the abundance and patterns of chloroplast mRNA transcripts in the mutants and wild-type counterparts by RNA gel-blot hybridization analysis. Our results showed that there were similar amounts of *psbA* and *psbC* transcripts (encoding D1 and CP43 proteins of PSII, respectively) in the mutant and wild-type plants (Fig. 4A). In addition, similar abundance and patterns of PSII transcripts, including *psbD/C* (encoding D2 and CP43 proteins, respectively), *psbEFLJ* (encoding the α- and β-subunits of cytochrome *b*559, PsbL, and PsbJ proteins, respectively), and *psbKI* (encoding the PsbK and PsbI proteins, respectively), were observed in the *lpa66* mutant and wild-type plants (Fig. 4A). There were also no significant differences in levels of *psaA* and *petA* transcripts (encoding PSI subunit PsaA and cytochrome *b*6f subunit cytochrome *f*, respectively) between the mutant and wild-type plants (Fig. 4A).

Several PPR proteins of the PLS family have been shown to be involved in RNA editing (Kotera et al., 2005; Okuda et al., 2007, 2009; Chateigner-Boutin et al., 2008; Zhou et al., 2009). Therefore, we examined the possibility that LPA66 has such a role by directly sequencing RT-PCR products encompassing the 34 editing sites identified to date in Arabidopsis chloroplast transcripts, in both mutant and wild-type plants (Fig. 4A). A. In the wild-type plants, nucleotide 77C was edited to 77U in *psbF* transcripts, which introduces a conserved Phe protein.
Figure 4. RNA transcripts and editing analysis in lpa66 mutants. A, RNA gel blot hybridization with total RNA from leaves of wild-type (WT) and lpa66-1 mutant plants. Ten micrograms of total leaf RNA per well from 5-week-old wild-type and lpa66-1 plants was loaded per well. The probes for the genes psbA, psbB, psbC, psbD, psbEFLJ, psbKI, psaA, and petA are indicated to the left. rRNA was visualized by staining with ethidium bromide as an equal loading control. B, Analysis of RNA editing of psbF and psbE transcripts. RT-PCR products containing the psbF and psbE editing sites (asterisks) were directly sequenced. C and D,
Reduced Levels of PSII Proteins in lpa66

To assess the possibility that impairment of the PSII function might be reflected at the level of plastid plant proteins, immunoblot analysis was performed with specific antibodies against the subunits of photosynthetic protein complexes using total protein extraction prepared from the leaves of mutant and wild-type plants. The protein contents of the chloroplast-encoded PSII subunits D1, D2, cytochrome b$_{559}$, CP47, and CP43 were found to be reduced to approximately 25% of wild-type levels. The levels of nucleus-encoded PSII proteins, the 33-kD protein of the oxygen-evolving complex, and LHCl were slightly reduced in the mutants (Fig. 5A). The contents of PSI reaction center Psaa/A/B proteins were also slightly reduced compared with wild-type levels (Fig. 5A), but the levels of cytochrome f of the cytochrome b$_{f}$ complex and the CFb-subunit of the ATP synthase were slightly increased (Fig. 5A).

In further analyses, the composition of photosynthetic protein complexes in the thylakoid membranes of mutant and wild-type plants was analyzed by blue-native (BN)-PAGE electrophoresis (Schagger et al., 1994; Guo et al., 2005), which resolved six major bands corresponding to PSII supercomplexes (band I), monomeric PSI and dimeric PSII (band II), monomeric PSI (band III), CP43-free PSII (band IV), trimeric LHCl/PSI reaction center (band V), and monomeric LHClI (band VI; Guo et al., 2005). As shown in Figure 5B, the amounts of chlorophyll-containing protein complexes (labeled I and II) were reduced in the lpa66 mutant compared with that in wild-type plants when thylakoid membranes containing equal amounts of chlorophyll were compared. Further analyses of the two-dimensional SDS-urea-PAGE gels after Coomassie Brilliant Blue staining showed that the PSI core proteins (D1/D2/CP47/CP43) in PSI supercomplexes, dimers, and monomers were significantly reduced in the mutant (Fig. 5C). In addition, there was more LHClI accumulated in band V in the mutant than in wild-type plants.

Protein Synthesis Rates of D1 and D2 Were Reduced in lpa66

Reduced levels of PSII complexes may be due to either reduced rates of protein synthesis or increased degradation of PSII subunits. The possible effect of the mutation on the protein synthesis capacity of chloroplasts, therefore, was investigated by analyzing the polysome association patterns of psbA and psbD transcripts after Suc gradient fractionation. The results showed no obvious alterations of polysome association between the mutant and wild-type plants (Fig. 6A). The synthesis and degradation of plastid-encoded thylakoid membrane proteins were further studied in wild-type and mutant leaves by in vivo pulse-chase labeling experiments in the presence of cycloheximide, which inhibits the translation of nucleus-encoded proteins. After a 20-min pulse labeling, the incorporation of [35S]Met into PSII proteins D1 and D2 was dramatically reduced, to about 25% of wild-type levels, while the synthesis rates of other PSII subunits CP43/CP47, PSI reaction center Psaa/A/B proteins, and the α- and β-subunits of the chloroplast ATP synthase (CF1α/β) were comparable to those in their wild-type counterparts (Fig. 6B). As shown in Figure 6C, the turnover rates of core PSI proteins CP47, CP43, D1, and D2 were increased in the lpa66 mutant during the chase with unlabeled Met after pulse labeling for 20 min.

Assembly of PSII Complexes Was Impaired in lpa66

In the wild-type plants, PSII assembly is very efficient (Fig. 6D). After a 20-min pulse labeling, most of the radioactivity detected in PSII components was incorporated in PSII protein complexes, and no visible radioactivity was detected in unassembled proteins. In lpa66 the PSII protein complexes were clearly labeled; however, there was a considerable amount of radioactivity in unassembled proteins after pulse labeling for 20 min. These results indicate that the assembly of PSII proteins into PSII complexes was less efficient in lpa66 mutants (Fig. 6D).

DISCUSSION

psbF editing changes a genomically encoded Ser codon into a Phe codon, which is evolutionarily con-
served, in the psbF-encoded protein (β-subunit of cytochrome b\textsubscript{559}) of many photosynthetically active organisms but not in wild-type tobacco plants, in which the correct Phe codon is already specified at the DNA level (Fig. 4, C and D). Previous studies have shown that replacement of part of the tobacco psbF gene with the homologous region from spinach results in the production of unedited psbF transcripts, suggesting that there is a trans-factor for the psbF site in spinach and presumably many other plants (Bock et al., 1994). Further analysis showed that psbF editing could be restored by the presence of the spinach nucleus through an interspecific protoplast fusion approach (Bock and Koop, 1997). Thus, it is possible that this trans-specific factor has been lost in tobacco due to the lack of selective pressure following loss of the psbF editing site and that this factor is specific for psbF editing. In this study, we report the identification of a PPR protein, LPA66, which is specifically required for editing psbF transcripts in Arabidopsis.

LPA66 is a DYW-class PPR protein containing single E and DYW domains. Similar PPR proteins have been shown to be involved in RNA editing or RNA cleavage. CRR4 and CRR21 are specifically involved in editing sites ndhD-1 and ndhD-2 (Kotera et al., 2005; Okuda et al., 2007), YS1 is required for editing of rpoB-1 (Zhou et al., 2009), while CLB19 has been demonstrated to be required for the editing of two independent sites in rpoA and clpP transcripts (Chateigner-Boutin et al., 2008). CRR22 is involved in editing of the sites ndhB-7, ndhD-5, and rpoB-3, and CRR28 is required for editing of ndhB-2 and ndhD-3 (Okuda et al., 2009). RARE1 is required for editing of accD1 transcripts (Robbins et al., 2009). MEF1, the first plant mitochondrial editing factor, is involved in editing of three specific sites, rps4-956, nad7-963, and nad2-1160, of mRNAs (Zehrmann et al., 2009). The E motif has been suggested to interact with an editing enzyme catalyzing an alteration of C to U, rather than to be the motif that catalyzes the RNA editing reaction (Okuda et al., 2007). Comparison of the C-terminal regions of LPA66 and these factors revealed that LPA66, like YS1, CRR22, CRR28, MEF1, and RARE1, also contains a DYW domain (Supplemental Fig. S2). The C-terminal DYW domain has been proposed to be an essential (“missing”) catalytic domain for RNA editing activity (Salone et al., 2007), but there is no direct evidence that proteins containing DYW domains have such activity; for example, CRR2 (a PPR protein containing both E and DYW domains) has been reported to be specifi-
cally involved in RNA cleavage but not RNA editing (Hashimoto et al., 2003, Okuda and Shikanai, 2008). It appears that, despite their sequence similarity, the functions of different DYW family members have diverged (Okuda et al., 2009).

psbE and psbF belong to the same operon, psbEFLJ, and there is only a 123-bp distance between the editing sites of the transcripts they encode in Arabidopsis. BLAST searches and sequence analysis detected no obvious contiguous conserved cis-elements surrounding the editing sites in psbE and psbF transcripts (data not shown), implying that these two sites may be independently edited. In both lpa66-1 and lpa66-2 mutants, only the psbF site is unmodified by RNA editing, but it was successfully edited in wild-type plants (Fig. 4B). Accordingly, the 26th amino acid of the β-subunit of cytochrome $b_{559}$ is a hydrophilic Ser in the lpa66 mutants, while it is a hydrophobic Phe in wild-type plants. In cyanobacteria and alga, in which no evidence of RNA editing has been published, the psbF gene encodes a Phe at the corresponding site (Fig. 4, C and D). However, alignment of the predicted sequences of proteins directly translated from the genomes of various higher plant species has shown that this site encodes a Phe in some species (both dicotyledonous and monocotyledonous) and a Ser in others (Fig. 4D). Such distribution of the Phe and Ser codons implies that this editing site existed before the divergence of monocots and dicots. Coupled with the data from tobacco, this suggests a relatively recent loss of the site and the associated editing factor (LPA66) from certain lineages (e.g. tobacco). A protein putatively orthologous to LPA66 is encoded by the genome of grapevine, where a Ser codon is present at the position corresponding to the psbF editing site. This would suggest that in grapevine, this codon is also edited, and that the grapevine protein LOC100261359 is likely to be involved. In contrast, a Phe codon is already present at the position corresponding to the psbF editing site in P. patens, rice, and maize (Fig. 4). Although P. patens, rice, and maize contain many DYW-class PPR proteins with homology to LPA66 (O’Toole et al., 2008), BLAST searches revealed that there are no putative LPA66 orthologs in these species (data not shown). Thus, LPA66 is likely to be a site-specific factor for RNA editing of psbF in the higher plants that require this site to be edited and probably has no other function, as it appears to have been lost in those plants that do not require editing of psbF.

It has been previously demonstrated that cytochrome $b_{559}$ is essential for the stable accumulation of PSII protein complexes. Deletion of the genes encoding the α- and β-subunit, or both, has been shown to abolish PSII activity completely (Pakrasi et al., 1990; Morais et al., 1998; Swiatek et al., 2003), and mutants

![Figure 6](image-url). Polysome association and in vivo labeling of wild-type (WT) and lpa66-1 plants. A, Association of psbA and psbD mRNAs with polysomes. Ten fractions of equal volume were collected from the top to bottom of 15% to 55% Suc gradients, and equal proportions of the RNA purified from each fraction were analyzed by gel-blot hybridization. rRNAs were detected by ethidium bromide staining. The RNA size markers are indicated to the left. B, Pulse labeling of thylakoid membrane proteins. After pulse labeling young Arabidopsis seedlings in the presence of cycloheximide for 20 min, thylakoid membranes were isolated, and the proteins were separated by SDS-urea-PAGE and visualized autoradiographically. C, Pulse and chase labeling of thylakoid membrane proteins. After pulse labeling for 20 min followed by 1-, 2-, or 4-h chases with cold Met, thylakoid membranes were isolated, and the proteins were separated by SDS-urea-PAGE and visualized autoradiographically. D, BN gel analysis of labeled thylakoid membrane protein complexes after pulse labeling. After a 20-min pulse in Arabidopsis young seedlings in the presence of cycloheximide, the thylakoid membranes were isolated and solubilized with dodecyl-β-D-maltoside, then the protein complexes were separated by BN-PAGE and visualized autoradiographically. Bands corresponding to various PSII assembly complexes are indicated to the right.
examined by Pakrasi et al. (1991), in which one of the heme-ligating His residues was mutated, displayed inefficient PSII assembly and impaired PSII activity. Our results show that defective psbF editing results in reduced growth rates and pale green coloration (Supplemental Fig. S1), phenotypic traits that are associated with reduced levels of PSII proteins (Fig. 5). These results are consistent with previous studies on a tobacco mutant in which the conserved Phe was changed to Ser (Bock et al., 1994; Bondarava et al., 2003), and suggest that the amino acid substitution caused by the lack of editing leads to the reduced levels of PSII proteins observed in the mutants.

The detection of similar amounts and patterns of PSII transcripts (Fig. 4A) in the wild-type and mutant plants indicates that the reduced levels of PSII proteins in the latter may be due to posttranscriptional regulation. The decreased synthesis of D1 and D2 proteins in the mutant may be due to the decreased efficiency of PSII assembly. In Chlamydomonas, cytochrome f synthesis has been shown to be regulated by the level of unassembled cytochrome f in the thylakoid membranes, via interactions with the 5′ untranslated region of petA transcripts (Choquet et al., 1998). A similar mechanism, which has been termed “control by epis tally of synthesis” (CES), has been found to influence the biogenesis of photosynthetic protein complexes in Chlamydomonas (Wostrikoff et al., 2004; Minai et al., 2005). Recently, evidence has been presented indicating that the CES mechanism also operates in plant chloroplasts in the regulation of Rubisco large subunit translation in response to its assembly state (Wostrikoff and Stern, 2007). The CES mechanism is crucial for the stoichiometric synthesis and assembly of photosynthetic protein complexes (Choquet and Wollman, 2002; Pogson et al., 2008). Similarly, in Arabidopsis, the presence of unassembled PSII proteins due to the impairment of assembly efficiency in the lpa66 mutant could down-regulate the translation of their corresponding transcripts. It is also interesting that the accumulation of unassembled D1 also leads to the increased turnover rates of D1 in the mutant. Since the accumulation of PSII core protein appears to occur in a coordinated manner, a lesion in one of the main integral subunits of PSII will result in a concomitant decrease of other PSII core proteins (Jensen et al., 1986; de Vitré et al., 1989; Yu and Vermaas, 1990, 1993). In the mutant, the stability of other PSII core proteins was reduced, which may account for the reduced PSII protein levels.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The lpa66-1 mutant was screened from a collection of p5ki015 T-DNA-mutagenized Arabidopsis (Arabidopsis thaliana ecotype Columbia) lines from the ABRC based on the high chlorophyll fluorescence phenotypes using a chlorophyll fluorescence imaging system (Ma et al., 2007). The lpa66-2 mutant (T-DNA insertion line CS813518) was obtained from the ABRC, and the homozygous mutant was verified by PCR using primers LP (5′-GTGAAAAACGTTCGCTCGTACC-3′) and RP (5′-ATGATATCAGGTTATCGCTGAACGG-3′). The T-DNA insertion was confirmed by PCR and sequencing with primers LB (5′-GGCTTTTCAGAAATGGATAATAGCCTTGGTTCC-3′) and RP. Wild-type and homozygous mutant plants were grown in soil under short-day conditions (10 h of light/14 h of dark) with a photon flux density of 120 μmol m⁻² s⁻¹ in a growth chamber at 22°C.

Chlorophyll Fluorescence

Chlorophyll fluorescence was measured with a PAM-2000 portable chlorophyll fluorometer (Walz) connected to leaves, which were dark adapted for 30 min before measurements, by a leaf-clip holder (model 2000-B; Walz). The variables Fv/Fm, Fv/Fo and the Fo/Fm ratio were measured and calculated basically according to Meurer et al. (1996). For measurement of light-induced P700 absorbance changes at 820 nm, the PAM chlorophyll fluorometer was equipped with an ED 800T emitter-detector unit (Walz), and the measurements were performed according to Meurer et al. (1996).

Map-Based Cloning and Complementation

The lpa66-1 mutation was mapped with a series of simple sequence length polymorphism markers based on the polymorphisms between two Arabidopsis ecotypes, Columbia and Landsberg erecta (Lukowitz et al., 2000). The mutant plants (Columbia) were crossed with wild-type Landsberg erecta to generate F1 seeds, which were allowed to self-fertilize to generate a segregation population. Homozygous F2 mutant plants (lpa66/lpa66) were screened based on the high chlorophyll fluorescence phenotype described above. Genomic DNA was extracted from about 1,024 mutant plants and subjected to PCR with specific molecular markers. The mutation was mapped to a 216-kb region between two bacterial artificial chromosomes (MJE7 and K19E20) on chromosome 5. Candidate genes with a predicted chloroplast transit peptide were sequenced and analyzed with genomic DNA from lpa66 and wild-type plants (Columbia). To confirm the mutation, a complementation experiment was performed as follows. A fragment containing the full-length lpa66 coding sequence was amplified with primers 5′-GGCTTTGATCATGAACCCAACACAGAC-3′ and 5′-ATGCTCTGAGTCACAATATCCATAC-3′ and subcloned into the pB121 vector under the control of the cauliflower mosaic virus 35S promoter. The constructs were then transformed into Agrobacterium tumefaciens strain C58 and introduced into the lpa66 mutant plants by a floral dip method (Clough and Bent, 1998). Transgenic plants were grown on Murashige and Skoog medium containing 40 μg mL⁻¹ kanamycin monosulfate. Resistant plants were transferred to soil and grown in a growth chamber to produce seeds. The success of the complementation was confirmed by chlorophyll fluorescence analysis.

Analysis of RNA Editing

Total extracted RNA from leaves of lpa66 mutant and wild-type plants was treated with DNase I and then reverse transcribed with random hexamers (Takara). A series of specific primers (Supplemental Table S1) for the genes encompassing the editing sites in Arabidopsis (Tillich et al., 2005; Chateigner-Boutin and Small, 2007; Okuda et al., 2007) were used to amplify these gene sequences from the cDNA by RT-PCR, and the products were sequenced with primers LB (5′-GGCTTTGATCATGAACCCAACACAGAC-3′) and RP (5′-ATGCTCTGAGTCACAATATCCATAC-3′) and subcloned into the pB121 vector under the control of the cauliflower mosaic virus 35S promoter. The constructs were then transformed into Agrobacterium tumefaciens strain C58 and introduced into the lpa66 mutant plants by a floral dip method (Clough and Bent, 1998). Transgenic plants were grown on Murashige and Skoog medium containing 40 μg mL⁻¹ kanamycin. The editing results were analyzed with genomic DNA from Arabidopsis str.
antibodies, and signals from secondary conjugated antibodies were detected by the enhanced chemiluminescence method.

**Northern-Blot and Polysome Association Analyses**

Total RNA was extracted from fresh leaf tissues using Trizol reagent, and polysomes were isolated from leaf tissues according to Barkan (1988). RNA in each fraction was isolated, separated, and transferred onto nylon membranes (Amersham Pharmacia Biotech), which were probed with 3P-labeled probes prepared according to Peng et al. (2006) and then exposed to x-ray films.

**In Vivo Labeling Assays**

In vivo protein labeling was performed essentially as described previously (Meurer et al., 1998). For pulse labeling, primary leaves from 12- to 15-d-old plants were incubated in 1 μCi mL−1 [35S]Met in the presence of 20 μg mL−1 cycloheximide for 20 min at 25°C after preincubation with cycloheximide for 30 min. Pulse labeling was followed by a chase in the presence of 1 μCi unlabeled Met. After labeling, thylakoid membranes were isolated according to Peng et al. (2006), and the proteins were subjected to SDS-PAGE or BN gel analysis.

**GFP Fusion Constructs for Transient Expression in Protoplasts**

A fragment encoding the N-terminal 1 to 257 amino acids of LPA66 was amplified by RT-PCR with primers 5'-ACGTCGACATCTTTGTTGATTCT-3' and 5'-TCATGGATTCGGAATCGGTAAGGTCAGG-3' and subcloned into the pUC19-35S-GFP vector to generate a fusion protein with the GFP as a reporter in the C terminus. In the N-terminal part (1-202 amino acids) of AtFbr1, the transit peptide (1–81 amino acids) of the small subunit of ribulose bisphosphate carboxylase, and the entire coding region of psbF were amplified (using primer pairs 5'-CACGTCGACAAACCTCAGTCAG-3' and 5'-TCATGGATTCGGAATCGGTAAGGTCAGG-3' and subcloned into the pUC19-35S-GFP vector to generate a fusion protein with the GFP as a reporter in the N terminus. In addition, the N-terminal part (1–257 amino acids) of AtFbr1, the transit peptide (1–81 amino acids) of the small subunit of ribulose bisphosphate carboxylase, and the entire coding region of FRO1 except the termination codon were amplified (using primer pairs 5'-ACGTCGACATCTTTGTTGATTCT-3' and 5'-TCATGGATTCGGAATCGGTAAGGTCAGG-3' and subcloned into the pUC19-35S-GFP vector to generate a fusion protein with the GFP as a reporter in the C terminus. In the N-terminal part (1–257 amino acids) of AtFbr1, the transit peptide (1–81 amino acids) of the small subunit of ribulose bisphosphate carboxylase, and the entire coding region of FRO1 except the termination codon were amplified (using primer pairs 5'-ACGTCGACATCTTTGTTGATTCT-3' and 5'-TCATGGATTCGGAATCGGTAAGGTCAGG-3' and subcloned into the pUC19-35S-GFP vector to generate a fusion protein with the GFP as a reporter in the N terminus.

**Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers LPA66 (A05g48910; NP_197902) and Vitis protein LOC100261339 (XP_002268530).**

**Supplemental Data**

The following materials are available in the online version of this article.

**Supplemental Figure S1.** Photograph showing the phenotypes of 5-week-old wild-type, lpa66-1, lpa66-2, and lpa66-1 mutant complemented with LPA66 cDNA plants.

**Supplemental Figure S2.** Comparison of the predicted E motif of LPA66 with the corresponding regions of CLB19, CRR2, CRR4, CRR21, CRR22, CRR28, YSI1, MEF1, and RARE1.

**Supplemental Table S1.** Primers used for analyzing RNA editing in Arabidopsis chloroplasts.

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**LITERATURE CITED**

Andrés C, Lucin C, Small ID (2007) The multifarious roles of PPR proteins in plant mitochondrial gene expression. Physiol Plant 129: 14–22

Barkan A (1988) Proteins encoded by a complex chloroplast transcription unit are translated from both monocistronic and polycistronic mRNAs. EMBO J 7: 2637–2644

Barkan A, Goldschmidt-Clermont M (2000) Participation of nuclear genes in chloroplast gene expression. Biochimie 82: 559–572

Beick S, Schmitz-Linneweber C, Williams-Carrier R, Jensen B, Barkan A (2008) The pentatricopeptide repeat protein PPRS stabilizes a specific tRNA precursor in maize chloroplasts. Mol Cell Biol 28: 5337–5347

Bock R (2000) Sense from nonsense: how the genetic information of chloroplasts is altered by RNA editing. Biochimie 82: 549–557

Bock R, Koop HU (1997) Extrapolastic site-specific factors mediate RNA editing in chloroplasts. EMBO J 16: 3282–3288

Bock R, Küssel H, Maliga P (1994) Introduction of a heterologous editing site into the tobacco plastid genome: the lack of RNA editing leads to a mutant phenotype. EMBO J 13: 4623–4628

Bonderova N, De Pascalis L, Al-Babili S, Guo J, Zhang Z, Bi Y, Yang W, Xu Y, Zhang L (2003) The complete nucleotide sequence and RNA editing content of the mitochondrial genome of rapeseed (Brassica napus L.): comparative analysis of the mitochondrial genomes of rapeseed and Arabidopsis thaliana. Nucleic Acids Res 31: 5907–5916

Hashimoto M, Endo T, Peltier G, Tasaka M, Shikanai T (2003) A nucleus-encoded factor CRR2, is essential for the expression of chloroplast ndhB in Arabidopsis. Plant J 36: 541–549

Hattori M, Miyake H, Sugita M (2007) A pentatricopeptide repeat protein
is required for RNA processing of clpP pre-mRNA in moss chloroplasts.
J Biol Chem 28: 10773–10782

Hoch B, Maier RM, Appel K, Iglói GL, Kossel H (1991) Editing of a chloroplast mRNA by creation of an initiation codon. Nature 353: 179–180

Jensen KH, Herrin DL, Plumley FG, Schmidt GW (1986) Biogenesis of photosystem II complexes: transcriptional, translational and posttranslational regulation. J Cell Biol 105: 1315–1325

Katera E, Tasaka M, Shikanai T (2005) A pentatricopeptide repeat protein is essential for RNA editing in chloroplasts. Nature 433: 326–330

Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol 42: 313–349

Kugita M, Yamamoto Y, Fujikawa T, Matsumoto T, Yoshinaga K (2003) RNA editing in hornwort chloroplasts makes more than half the genes functional. Nucleic Acids Res 31: 2417–2422

Lee BH, Lee H, Xiong L, Zhu JK (2002a) A mitochondrial complex I defect impairs cold-regulated nuclear gene expression. Plant Cell 14: 1235–1245

Lee KH, Kim DH, Lee SW, Kim ZH, Hwang I (2002b) Molecular cloning and targeting of a fibrillarin homolog from Arabidopsis. Plant Physiol 129: 795–806

Lurin C, André C, Aubourg S, Bellaux M, Bitton E, Bruyère C, Coboche M, Debast C, Gualberto J, Hoffmann B, et al. (2004) Genome-wide analysis of Arabidopsis pentatricopeptide repeat protein repeats reveals their essential role in organelle biogenesis. Plant Cell 16: 2089–2103

Ma J, Peng L, Guo J, Zhu S, Lu Q, C, Zhang L (2007) LPA2 is required for efficient assembly of photosystem II in Arabidopsis thaliana. Plant Cell 19: 1980–1993

Maier RM, Zelis P, Küssel H, Bonnard G, Gualberto JM, Grienberger JM (1996) RNA editing in plant mitochondria and chloroplasts. Plant Mol Biol 32: 343–365

Manuell A, Beligni MV, Yamaguchi K, Mayfield SP (2004) Regulation of chloroplast translation: interactions of RNA elements, RNA-binding proteins and the plastid ribosome. Biochem Soc Trans 32: 601–605

Marín-Navarro J, Manuell AL, Wu J, Mayfield SP (2007) Chloroplast translation regulation. Photosynth Res 94: 359–374

Meierhoff K, Felder S, Nakamura T, Bechtold N, Schuster G (2003) HCF152, an Arabidopsis RNA binding pentatricopeptide repeat protein involved in the processing of plastid polb-polf-polb-polf-polD RNAs. Plant Cell 15: 1480–1495

Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB, Terry A, Salamov A, Fritz-Laylin LK, Marechal-Drouard L, et al. (2007) The Chlamydomonas genome reveals the evolution of key animal and plant functions. Science 318: 245–250

Meurer J, Meierhoff K, Westhoff P (1996) Isolation of high-chlorophyll-fluorescence mutants of Arabidopsis thaliana and their characterisation by spectroscopy, immunoblotting and northern hybridization. Planta 195: 385–396

Meurer J, Plücke H, Kowallik KV, Westhoff P (1998) A nuclear-encoded protein of prokaryotic origin is essential for the stability of photosystem II in Arabidopsis thaliana. EMBO J 17: 5286–5297

Minai I, Wosterikoff K, Wollman FA, Choquet Y (2005) Chloroplast biogenesis of photosystem II cores involves a series of assembly-controlled steps that regulate translation. Plant Cell 17: 159–175

Morais F, Barber J, Nixon PJ (1998) The chloroplast-encoded alpha subunit of cytochrome b-559 is required for assembly of the photosystem two complex in both the light and the dark in Chlamydomonas reinhardtii. J Biol Chem 273: 29315–29320

Nakamura T, Meierhoff K, Westhoff P, Schuster G (2003) RNA-binding properties of HCF152, an Arabidopsis PPR protein involved in the processing of chloroplast RNA. Eur J Biochem 270: 4070–4081

Nelson N, Yocum CF (2006) Structure and function of photosystem I and II. Annu Rev Plant Biol 57: 521–565

Okuda K, Chateigné-Boutin AL, Nakamura T, Delannoy E, Sugita M, Myouga F, Motohashi R, Shinozaki K, Small I, Shikanai T (2009) Pentatricopeptide repeat proteins with the DYW motif have distinct molecular functions in RNA editing and RNA cleavage in Arabidopsis chloroplasts. Plant Cell 21: 146–156

Okuda K, Myouga F, Motohashi R, Shinozaki K, Shikanai T (2007) Conserved domain structure of pentatricopeptide repeat proteins involved in chloroplast RNA editing. Proc Natl Acad Sci USA 104: 8178–8183

Okuda K, Nakamura T, Sugita M, Shimizu T, Shikanai T (2006) A pentatricopeptide repeat protein is a site recognition factor in chloroplast RNA editing. J Biol Chem 281: 37661–37667

Okuda K, Shikanai T (2008) PPR proteins function as a translation factor in chloroplast RNA editing. In: JF Allen, E Gantt, JH Golbeck, B Osmond, eds, Photosynthesis. Energy from the Sun: 14th International Congress on Photosynthesis. Springer, Dordrecht, The Netherlands, pp 1211–1214

O’Toole N, Hiltori M, Andrews C, Iida K, Lurin C, Schmitz-Linneweber C, Sugita M, Small I (2008) On the expansion of the pentatricopeptide repeat gene family in plants. Mol Biol Evol 25: 1120–1128

Pakrasi HB, De Ciepi C, Whitmarsh J (1991) Site directed mutagenesis of the heme axial ligands of cytochrome b559 affects the stability of the photosystem II complex. EMBO J 10: 1619–1627

Pakrasi HB, Nyhus KJ, Granok H (1990) Targeted deletion mutagenesis of the nucleus subunit of cytochrome b-559 protein destabilizes the reaction center of photosystem II. Z Naturforsch C 45: 423–429

Peng L, Ma J, Chi W, Guo J, Zhu S, Lu Q, C, Zhang L (2006) LOW PSI ACCUMULATION is involved in efficient assembly of photosystem II in Arabidopsis thaliana. Plant Cell 18: 955–969

Pfalz J, Liere K, Kandibinder A, Dietz K, Oelmüller R (2005) pTAC2, -6, -8, which are components of the transcriptionally active plastid chromo- some that are required for plastid gene expression. Plant Cell 18: 176–197

Ph KT, Yi MJ, Liang YS, Shin BJ, Cho MJ, Hwang I, Son D (2000) Molecular cloning and targeting of a fibrillarin homolog from Arabidopsis. Plant Physiol 123: 51–58

Pogson BJ, Woo NS, Förster B, Small ID (2008) Plastid signalling to the nucleus and beyond. Trends Plant Sci 13: 602–609

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectrometry. Biochim Biophys Acta 975: 384–394

Rivals E, Bruyère C, Toffano-Nioche C, Lechamy A (2006) Formation of the Arabidopsis pentatricopeptide repeat family. Plant Physiol 141: 825–839

Robbins JC, Heller WP, Hanson MR (2009) A comparative genomics approach identifies a PPR-DYW protein that is essential for C-to-U editing of the Arabidopsis chloroplast accD transcript. RNA (in press)

Salone V, Rudinger M, Polsakiewicz M, Hoffmann B, Groth-Malonek M, Szurek B, Small I, Knoop V, Linur C (2007) An hypothesis on the interaction of the editing enzyme in plant organelles. FEBS Lett 581: 4132–4138

Sasaki Y, Kozaki A, Ohmori A, Iguchi H, Naganoya Y (2001) Chloroplast RNA editing required for functional acetyl-CoA carboxylase in plants. J Biol Chem 276: 9397–9404

Schagger H, Cramer WA, Von Jagow G (1994) Analysis of molecular masses and oligomeric states of protein complexes by blue native electrophoresis and isolation of membrane protein complexes by two-dimensional native electrophoresis. Anal Biochem 217: 220–230

Schmitz-Linneweber C, Herrmann RM, Maier RM (2004) Evolutionary fluctuation of plastid RNA editing. Endocytobiosis Cell Res 15: 246–255

Schmitz-Linneweber C, Williams-Carrier RE, Williams-Voelker PM, Kroeger TS, Vichas A, Barkan A (2006) A pentatricopeptide repeat protein facilitates the trans-splitching of the maize chloroplast rps12 pre-mRNA. Plant Cell 18: 2650–2663

Shikanai T (2006) RNA editing in plant organelles: machinery, physiological function and evolution. Cell Mol Life Sci 63: 698–708

Sugiura M (2008) RNA editing in chloroplasts. Nucleic Acids Mol Biol 28: 123–142

Swiatek M, Regel RE, Meurer J, Winner G, Pakrasi HB, Ohad I, Herrmann RG (2003) Effects of selective inactivation of individual genes for low-molecular-mass subunits on the assembly of photosystem II, as revealed by chloroplast transformation: the pphFL1 operon in Nicotiana tabacum. Mol Gen Genet 266: 699–710

Terasawa K, Odahara M, Kabeya Y, Kikugawa T, Sekine Y, Fujishima M,
Sato N (2007) The mitochondrial genome of the moss Physcomitrella patens sheds new light on mitochondrial evolution in land plants. Mol Biol Evol 24: 699–709

Tillich M, Funk HT, Schmitz-Linneweber C, Poltnigg P, Sabater B, Martin M, Maier RM (2005) Editing of plastid RNA in Arabidopsis thaliana ecotypes. Plant J 43: 708–715

Weigel D, Ahn JH, Blázquez MA, Borevitz JO, Christensen SK, Fankhauser C, Ferrándiz C, Kardailsky I, Malancharuvil EJ, Neff MM, et al (2000) Activation tagging in Arabidopsis. Plant Physiol 122: 1003–1014

Williams PM, Barkan A (2003) A chloroplast-localized PPR protein required for plastid ribosome accumulation. Plant J 36: 675–686

Wintz H, Hanson MR (1991) A termination codon is created by RNA editing in the petunia atp9 transcript. Curr Genet 19: 61–64

Wolf PG, Rowe CA, Hasebe M (2004) High levels of RNA editing in a vascular plant chloroplast genome: analysis of transcripts from the fern Adiantum capillus-veneris. Gene 339: 89–97

Wollman FA, Minai L, Nechushtai R (1999) The biogenesis and assembly of photosynthetic proteins in thylakoid membranes. Biochim Biophys Acta 1411: 21–85

Wostrikoff K, Girad-Bascou J, Wollman FA, Choquet Y (2004) Biogenesis of PSI involves a cascade of translational autoregulation in the chloroplast of Chlamydomonas. EMBO J 23: 2696–2705

Wostrikoff K, Stern D (2007) Rubisco large-subunit translation is auto-regulated in response to its assembly state in tobacco chloroplast. Proc Natl Acad Sci USA 104: 6466–6471

Yamazaki H, Tasaka M, Shikanai T (2004) PPR motifs of the nucleus-encoded factor, PGR3, function in the selective and distinct steps of chloroplast gene expression in Arabidopsis. Plant J 38: 152–163

Yu J, Vermaas W (1990) Transcript levels and synthesis of photosystem II components in cyanobacterial mutants with inactivated photosystem II genes. Plant Cell 2: 315–322

Yu J, Vermaas W (1993) Synthesis and turnover of photosystem II reaction centre polypeptides in cyanobacterial D2 mutants. J Biol Chem 268: 7407–7413

Zehrmann A, Verbitskiy D, van der Merwe JA, Brennicke A, Takenaka M (2009) A DYW domain-containing pentatricopeptide repeat protein is required for RNA editing at multiple sites in mitochondrial of Arabidopsis thaliana. Plant Cell 21: 558–567

Zerges W (2000) Translation in chloroplasts. Biochimie 82: 583–601

Zhang L, Paakkarinen V, van Wijk KJ, Aro EM (1999) Co-translational assembly of the D1 protein into photosystem II. J Biol Chem 274: 16062–16067

Zhou W, Cheng Y, Yap A, Chatteigner-Boutin AL, Delannoy E, Hammani K, Small I, Huang J (2009) The Arabidopsis gene YS1 encoding a DYW protein is required for editing of rpoB transcripts and the rapid development of chloroplasts during early growth. Plant J 58: 82–96
Cai W., Ji D., Peng L., Guo J., Ma J., Zou M., Lu C., and Zhang L. LPA66 Is Required for Editing psbF Chloroplast Transcripts in Arabidopsis.

In Figure 6A, the background of the image of the psbD polysome profile for lpa66 was erased inappropriately. The original data could not be found, thus Figure 6A was reconstituted with the data from a duplicated experiment. The legend was revised accordingly. The original conclusions of this article are not affected by these corrections. The original and corrected versions of this figure are presented with the corrected version of the figure indicated.
Figure 6. Original: Polysome association and in vivo labeling of wild-type (WT) and lpa66-1 plants. A, Association of psbA and psbD mRNAs with polysomes. Ten fractions of equal volume were collected from the top to bottom of 15% to 55% Suc gradients, and equal proportions of the RNA purified from each fraction were analyzed by gel-blot hybridization. rRNAs were detected by ethidium bromide staining. The RNA size markers are indicated to the left. B, Pulse labeling of thylakoid membrane proteins. After pulse labeling young Arabidopsis seedlings in the presence of cycloheximide for 20 min, thylakoid membranes were isolated, and the proteins were separated by SDS-urea-PAGE and visualized autoradiographically. C, Pulse and chase labeling of thylakoid membrane proteins. After pulse labeling for 20 min followed by 1-, 2-, or 4-h chases with cold Met, thylakoid membranes were isolated, and the proteins were separated by SDS-urea-PAGE and visualized autoradiographically. D, BN gel analysis of labeled thylakoid membrane protein complexes after pulse labeling. After a 20-min pulse in Arabidopsis young seedlings in the presence of cycloheximide, the thylakoid membranes were isolated and solubilized with dodecyl-β-D-maltoside, then the protein complexes were separated by BN-PAGE and visualized autoradiographically. Bands corresponding to various PSII assembly complexes are indicated to the right.

The Pea Gene NA Encodes ent-Kaurenoic Acid Oxidase