

Seedling Lethal1, a Pentatricopeptide Repeat Protein Lacking an E/E⁺ or DYW Domain in Arabidopsis, Is Involved in Plastid Gene Expression and Early Chloroplast Development^{1[C][W]}

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Chloroplasts are the site of photosynthesis and the biosynthesis of essential metabolites, including amino acids, fatty acids, and secondary metabolites. It is known that many seedling-lethal mutants are impaired in chloroplast function or development, indicating the development of functional chloroplast is essential for plant growth and development. Here, we isolated a novel transfer DNA insertion mutant, dubbed *sel1* (for *seedling lethal1*), that exhibited a pigment-defective and seedling-lethal phenotype with a disrupted pentatricopeptide repeat (PPR) gene. Sequence analysis revealed that SEL1 is a member of the PLS subgroup, which is lacking known E/E⁺ or DYW domains at the C terminus, in the PLS subfamily of the PPR protein family containing a putative N-terminal transit peptide and 14 putative PPR or PPR-like motifs. Confocal microscopic analysis showed that the SEL1-green fluorescent protein fusion protein is localized in chloroplasts. Transmission electron microscopic analysis revealed that the *sel1* mutant is impaired in the etioplast, as well as in chloroplast development. In *sel1* mutants, plastid-encoded proteins involved in photosynthesis were rarely detected due to the lack of the corresponding transcripts. Furthermore, transcript profiles of plastid genes revealed that, in *sel1* mutants, the transcript levels of plastid-encoded RNA polymerase-dependent genes were greatly reduced, but those of nuclear-encoded RNA polymerase-dependent genes were increased or not changed. Additionally, the RNA editing of two editing sites of the *acetyl-CoA carboxylase beta subunit gene* transcripts in the *sel1* mutant was compromised, though it is not directly connected with the *sel1* mutant phenotype. Our results demonstrate that SEL1 is involved in the regulation of plastid gene expression required for normal chloroplast development.

Chloroplasts have their own genome and gene expression machinery. The expression and regulation of plastid genes are very different from that of nuclear genes, and the coordinated expression of nuclear and plastid genes is important for the development of functional chloroplasts. Plastid genes are generally organized in polycistronic transcription units. Therefore, they are transcribed as long primary transcripts, which are extensively processed into mature transcripts by various posttranscriptional modifications, such as RNA

splicing, RNA editing, RNA cleavage, and RNA stability. These posttranscriptional regulations of plastid gene expression are mediated by many nuclear-encoded proteins (Barkan and Goldschmidt-Clermont, 2000).

Recently, many chloroplast-localized pentatricopeptide repeat (PPR) proteins have emerged as primary nuclear factors that are involved in plastid gene expression and RNA metabolism in higher plants. The PPR proteins are characterized by a tandem array of degenerate 35-amino acid repeats, forming one of the largest protein families that is particularly prevalent in higher plants but not in other eukaryotes (Small and Peeters, 2000; Lurin et al., 2004; Schmitz-Linneweber and Small, 2008). Most of the PPR proteins are predicted to be targeted in the chloroplasts or mitochondria and involved in posttranscriptional regulation of organellar gene expression by interacting with RNA. The PPR protein family is divided into P and PLS subfamilies. The P subfamily usually contains only the classic PPR (P) motifs. By contrast, the PLS subfamily contains repeated blocks of PPR-like (P, L, and S) motifs, is specific to land plants (Lurin et al., 2004), and is also known as the plant combinatorial and modular protein (PCMP) subfamily (Aubourg et al., 2000; Rivals et al., 2006). Based on the presence of different C-terminal motifs, the PLS subfamily is further divided into the PLS, E, E+, and DYW subgroups (Lurin et al., 2004).

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Recent studies have shown that PPR proteins are sequence-specific RNA binding factors recruiting one or more effector proteins to the target RNA and are involved in posttranscriptional regulation of organellar gene expression (Schmitz-Linneweber and Small, 2008). Members of the P subfamily are involved in the transcription of plastid genes (Pfalz et al., 2006), RNA cleavage (Meierhoff et al., 2003; Nakamura et al., 2003), RNA stabilization (Beick et al., 2008; Pfalz et al., 2009), RNA splicing (Schmitz-Linneweber et al., 2006; de Longevialle et al., 2008), and translation (Yamazaki et al., 2004; Schmitz-Linneweber et al., 2005; Cai et al., 2011).

RNA editing is a posttranscriptional process that alters specific cytidine-to-uridine (C-to-U) conversion in the chloroplast and mitochondrial RNA of higher plants (Shikanai, 2006; Bentolila et al., 2008; Stern et al., 2010). In *Arabidopsis* (*Arabidopsis thaliana*), at least 34 sites are known to be edited in chloroplast transcripts (Chateigner-Boutin and Small, 2007), and more than 500 sites are specifically modified in mitochondrial transcripts (Bentolila et al., 2008; Takenaka et al., 2008). Thus far, more than 10 transactors required for the editing of particular C targets in chloroplasts have been identified. They are all members of the E/E+ and DYW subgroups of the PLS subfamily of the PPR protein family. Members of the E/E+ subgroup proteins, CHLORORESPIRATORY REDUCTION4 (CRR4), CRR21, CHLOROPLAST BIOGENESIS19 (CLB19), and ORGANELLAR TRANSCRIPT PROCESSING80 (OTP80), and members of the DYW subgroup proteins, including CRR22, CRR28, YELLOW SEEDLING1 (YS1), EARLY CHLOROPLAST BIOGENESIS2 (*AtECB2*), PROTEIN REQUIRED FOR accD RNA EDITING1 (*AtECB2*), and OTP82, have been shown to be required for the editing of corresponding targets such as *NADH dehydrogenase subunit4* (*ndhB*), *ndhD*, *RNA polymerase alpha subunit* (*rpoA*), *acetyl-CoA carboxylase beta subunit gene* (*accD*), and *ndhG* (Kotera et al., 2005; Okuda et al., 2006, 2007; Chateigner-Boutin et al., 2008; Hammani et al., 2009; Zhou et al., 2009). However, although the *Arabidopsis* genome has six PLS subgroup genes in the PLS subfamily of the PPR protein family (Lurin et al., 2004), the molecular function of members of the PLS subgroup proteins has not yet been reported.

The chloroplasts of higher plants contain two different types of RNA polymerases: nuclear-encoded RNA polymerase (NEP) and plastid-encoded RNA polymerase (PEP). NEP is a bacteriophage-type, single subunit RNA polymerase (Hedtke et al., 1997). PEP is a eubacterial-type, multisubunit RNA polymerase that consists of core subunits encoded by plastid genes (*rpoA*, *rpoB*, *rpoC1*, and *rpoC2*) and σ factors encoded by nuclear genes (Hess and Börner, 1999). It has been suggested that both NEP and PEP are responsible for the transcription of distinct types of plastid genes (Allison et al., 1996; Hajdukiewicz et al., 1997). Class I genes are predominantly transcribed by PEP, class II genes are transcribed by both NEP and PEP, and class III genes are transcribed exclusively by NEP. Whereas NEP transcribes nonphotosynthetic housekeeping

genes, PEP transcribes photosynthesis-related genes (Hajdukiewicz et al., 1997; Swiatecka-Hagenbruch et al., 2007; Liere et al., 2011).

In addition to the core subunits, PEP complex contains many of the regulatory proteins encoded by nuclear genes that are essential for plastid gene expression (Pfalz et al., 2006; Steiner et al., 2011). Most of the loss-of-function mutants for these regulatory proteins show seedling-lethal and albino or pale-green phenotype. Though there have been numerous studies on the function of the regulatory proteins in plastid gene expression, the mechanism of the regulation has not yet been fully understood. Recent maize (*Zea mays*) nucleoid proteome analysis has shown that PEP and its regulatory proteins are found together with other molecules in plastid nucleoids. The nucleoid is a very large complex and composed of plastid DNA, RNA, and various proteins. It is also the site of DNA regulation, transcription, and translation. For example, RNA maturation and ribosome assembly occur cotranscriptionally in association with nucleoids. The function of the nucleoid-enriched proteome is subject to qualitative change from RNA metabolism to translation and homeostasis as proplastids convert to chloroplasts with assistance of regulatory proteins (Majeran et al., 2012).

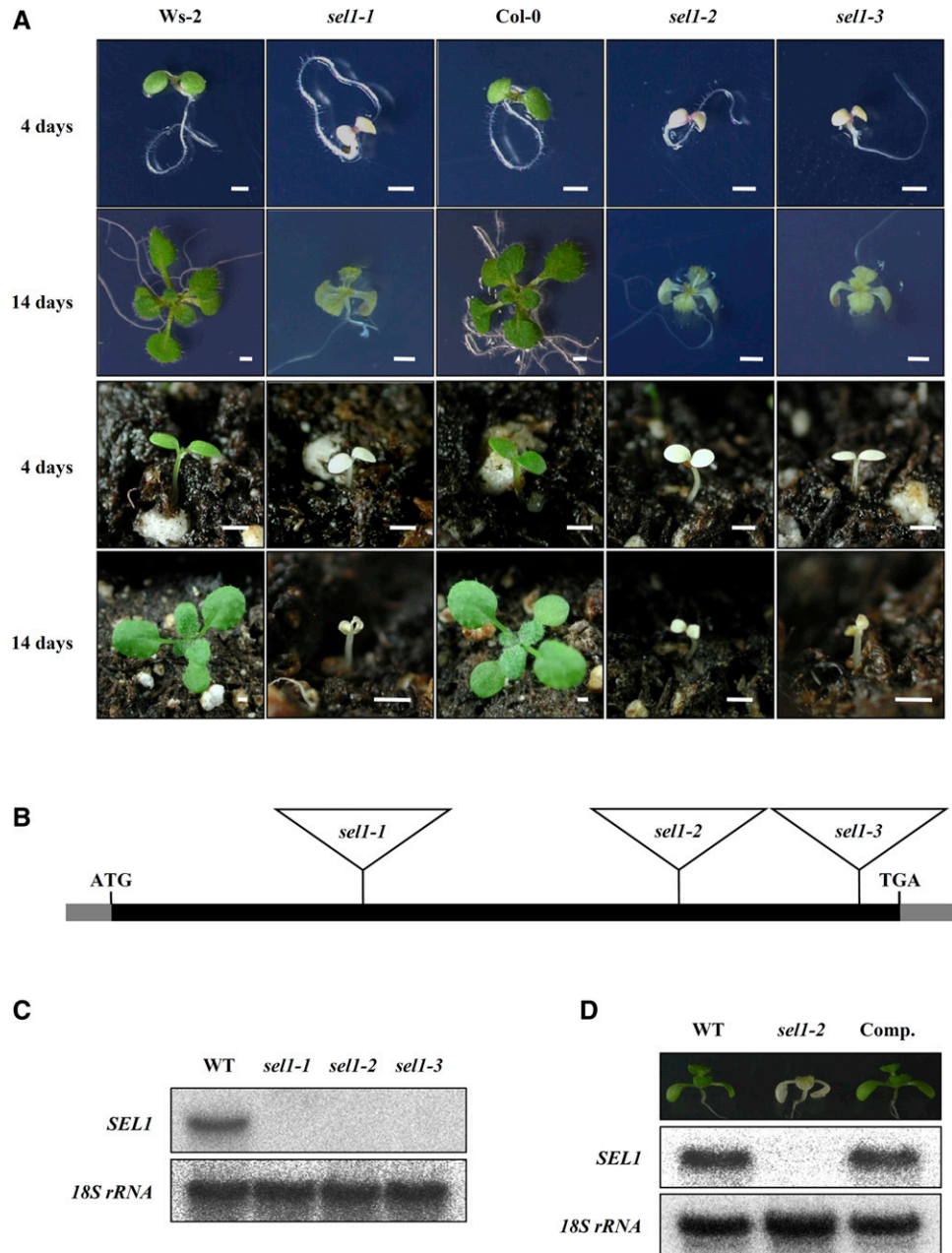
In this study, we report the molecular characterization of *seedling lethal1* (*sel1*) mutant, showing a pigment-defective and seedling-lethal phenotype. *SEL1* encodes a novel chloroplast-localized PPR protein, which is a member of the PLS subgroup in the PLS subfamily of the PPR protein family. The mutation of *SEL1* leads to defects in early chloroplast development. In the *sel1* mutant, the transcription levels of PEP-dependent plastid genes are decreased, but those of NEP-dependent plastid genes are increased or not changed. Therefore, our results suggest that *SEL1* is involved in the regulation of plastid gene expression required for normal chloroplast development.

RESULTS

Isolation and Characterization of *sel1* Mutant

We generated a collection of T-DNA insertion mutants in *Arabidopsis* (ecotype Wassilewskija-2 [Ws-2] background) and isolated several seedling-lethal mutants with pigment-defective phenotypes, such as albino, pale green, and pale yellow. Among these mutants, we characterized a novel pigment-defective and seedling-lethal mutant grown under normal growth conditions. This mutant was named *sel1-1* for the seedling-lethal phenotype. The germinated homozygous *sel1-1* mutant on normal Murashige and Skoog (MS) medium containing Suc developed normally but failed to be pigmented (Fig. 1A). In the absence of Suc, the *sel1-1* mutant was not pigmented and halted at the cotyledon stage (Supplemental Fig. S1). When grown on soil, the *sel1-1* mutant stalled beyond the cotyledon stage and subsequently died (Fig. 1A). Pigment accumulations,

Figure 1. Isolation and characterization of the *sel1* mutants. **A**, Pigment-defective and seedling-lethal phenotypes of the *sel1* mutants. The wild-type (ecotypes Columbia and Ws-2) and *sel1* mutant alleles were grown on MS medium with 1% (w/v) Suc or soil for 4 and 14 d, respectively. Bar = 1 mm. **B**, Gene structure of *SEL1* (At4g18520). The black box represents an exon, and gray boxes represent the 5' and 3' untranslated regions. The positions of the T-DNA insertion in *sel1-1*, *sel1-2* (SAIL_793_F11), and *sel1-3* (SALK_054374) are represented by triangles. T-DNA is not drawn to scale. **C**, Northern-blot analysis of the *SEL1* gene in wild-type (WT) and *sel1* mutant alleles. Twenty micrograms of total RNA was isolated from 7-d-old seedlings grown on MS medium. 18S rRNA was used as a loading control. **D**, Molecular complementation of the *sel1* mutant by *pSEL1::SEL1* genomic DNA. The top row shows the 14-d-old wild type, *sel1-2*, and the complemented line (Comp.). The bottom row shows northern-blot analysis of *SEL1* in the wild type, *sel1-2*, and the complemented line. 18S rRNA was used as a loading control. [See online article for color version of this figure.]



including total chlorophyll and carotenoids, were compared between the wild type and *sel1-1* mutant. The contents of total chlorophylls and carotenoids of the *sel1-1* mutant were about 6 and 10 times lower, respectively, than those of the wild type (Supplemental Fig. S2).

Genetic analysis indicated that the pigment-defective and seedling-lethal phenotype of the *sel1-1* mutant was controlled by a recessive nuclear gene and cosegregated with the phosphinothricin resistance marker. Sequence analysis of the thermal asymmetric interlaced-PCR product revealed that the transfer DNA (T-DNA) was inserted 467 bp downstream from the start codon of the At4g18520 gene (Fig. 1B). Northern-blot analysis confirmed that At4g18520 transcript was detected in the

wild type but not in the *sel1-1* mutant (Fig. 1C). These results indicate that disruption of the At4g18520 gene leads to a pigment-defective and seedling-lethal phenotype of the *sel1-1* mutant. To confirm that the *sel1-1* mutant phenotype was resulted from the T-DNA insertion in the At4g18520 gene, we obtained two independent T-DNA insertion lines for the At4g18520 gene from the SAIL and SALK collections. Genotype and sequence analyses revealed that the position of the T-DNA insertion was 787 bp and 1,747 bp downstream from the start codon of the At4g18520 gene in SAIL_793_F11 (*sel1-2*) and SALK_054374 (*sel1-3*), respectively (Fig. 1B). The phenotype of *sel1-2* and *sel1-3* was identical to that of *sel1-1* (Fig. 1A). Both the *sel1-2*

and *sel1-3* mutants showed no *SEL1* transcripts when using northern-blot analysis (Fig. 1C). Furthermore, we confirmed that the pigment-defective and seedling-lethal phenotype of *sel1* mutants was associated with the T-DNA insertion into At4g18520 by molecular complementation. The genomic DNA of the At4g18520 gene and its putative promoter region was introduced into a heterozygous *sel1-2* mutant. The subsequent genotype, phenotype, and northern-blot analyses confirmed that the introduction of At4g18520 genomic sequence restored the wild-type phenotype in the complemented lines (Fig. 1D). In addition, we generated complemented transgenic plants expressing FLAG-tagged *SEL1* protein under the control of the *SEL1* promoter. FLAG-tagged *SEL1*-complemented plants also grew normally like wild-type plants. Thus, we used the FLAG-tagged *SEL1*-complemented plants for further studies. Taken together, these results revealed that the phenotypes observed in *sel1* mutants are caused by a loss of function of the At4g18520 gene.

***SEL1* Gene Encodes a PPR protein That Is a Member of the PLS Subgroup**

The *SEL1* gene does not have an intron and encodes a putative protein of 617 amino acids with a predicted M_r of 69 kD. Sequence analysis revealed that *SEL1* protein contains 14 PPR or PPR-like (P, L, and S) motifs but does not contain any other known domains (Fig. 2, A and B). The *SEL1* protein is a member of the PLS subfamily and belongs to the PLS subgroup that does not have additional C-terminal domains (Lurin et al., 2004). Furthermore, *SEL1* protein has been classified as AtPCMP A2, a member of the Plant Combinatorial Modular Protein subfamily, and belongs to the PCMP-A subgroup (Aubourg et al., 2000; Rivals et al., 2006). In Arabidopsis, the PLS or PCMP-A subgroup contains six members. Amino acid sequence analysis shows that *SEL1* protein has significant sequence identity with uncharacterized proteins identified in grape (*Vitis vinifera*), rice (*Oryza sativa*), maize, and moss (*Physcomitrella patens*; Fig. 2B).

***SEL1* Is Localized in the Chloroplast**

Many PPR proteins are predicted to be localized in the chloroplasts and mitochondria (Lurin et al., 2004). The ChloroP program predicted that the *SEL1* protein is targeted to the chloroplast, and the N-terminal 19 amino acids of the *SEL1* protein are a putative transit peptide to the chloroplast (Fig. 2B). To determine the subcellular localization of *SEL1* protein, we made transgenic plants expressing full-length *SEL1*-GFP fusion protein under the control of a *Cauliflower mosaic virus* 35S promoter. When protoplasts were isolated and examined by confocal laser scanning microscopy, we observed the green fluorescence derived from the *SEL1*-GFP fusion protein exclusively in the chloroplasts (Fig. 3A). This green fluorescence was colocalized with the chlorophyll

autofluorescence. Thus, this result indicates that *SEL1* protein is localized and functions in the chloroplasts.

To further investigate the *SEL1* protein localization in the chloroplasts, we examined the FLAG-tagged *SEL1* protein in the chloroplast subfractions using western-blot analysis. As shown in Figure 3B, FLAG-tagged *SEL1* protein was detected only in the soluble fraction but not in the membrane fraction of the chloroplasts. Therefore, this result indicates that *SEL1* is a soluble protein located in the chloroplasts.

***SEL1* Protein Is Part of Small and High Molecular Protein Complexes**

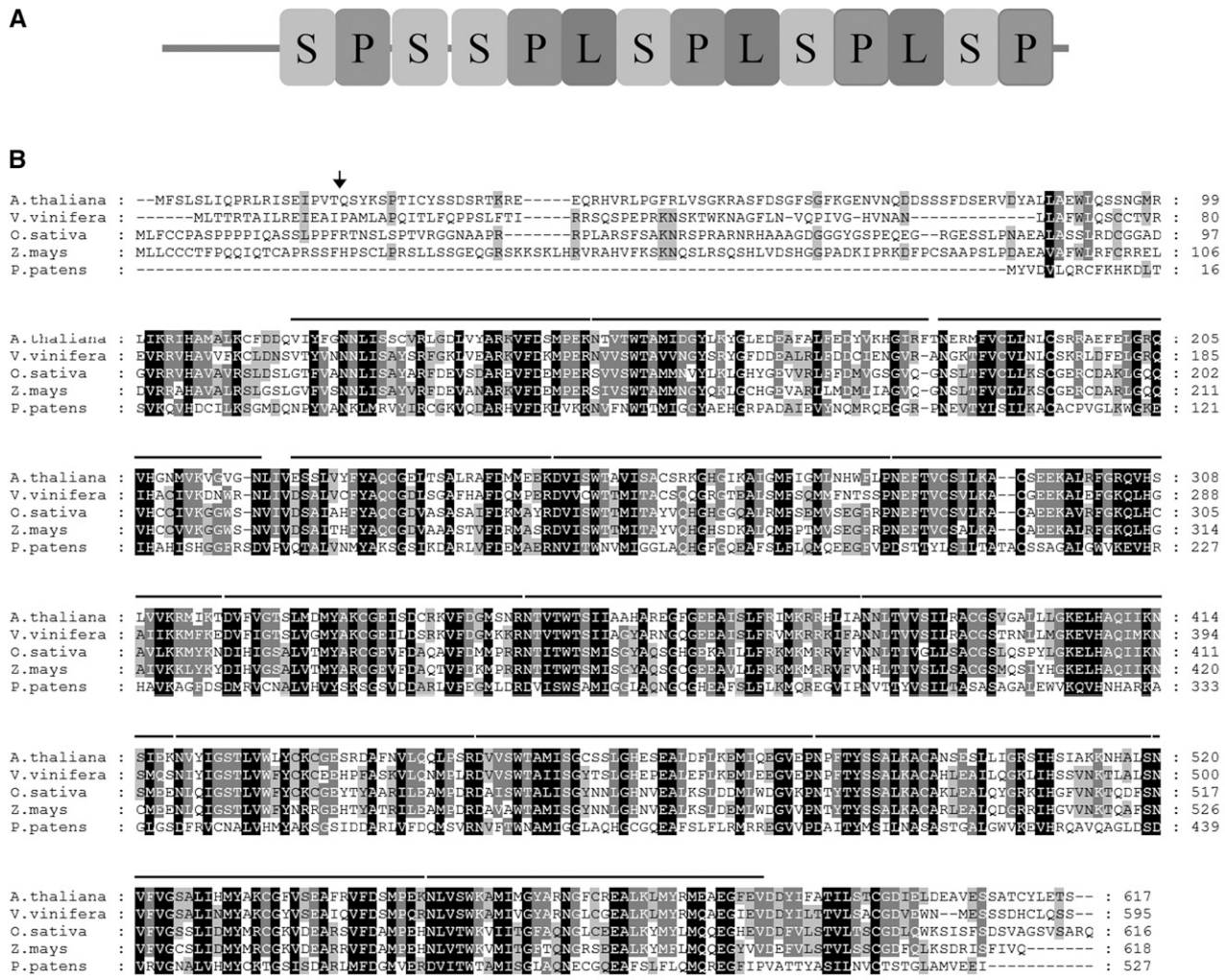
Some PPR proteins are part of a high molecular protein complex that contains RNA molecules (Williams and Barkan, 2003; Schmitz-Linneweber et al., 2006; Beick et al., 2008). To determine whether *SEL1* is a component of the RNA-protein complex in vivo, we examined the position of FLAG-tagged *SEL1* protein in soluble fractions using sedimentation through a Suc gradient and western-blot analysis (Supplemental Fig. S3). FLAG-tagged *SEL1* protein was broadly distributed in Suc gradient fractions and found predominantly in small M_r complexes, which are smaller than Rubisco, although small fractions of FLAG-tagged *SEL1* protein were found in high M_r complexes that are larger than Rubisco. Ribonuclease A treatment prior to sedimentation leads to a reduced level of FLAG-tagged *SEL1* protein in all fractions. These results suggest that *SEL1* might be a part of a protein complex that is associated with RNA.

***SEL1* Is Specifically Expressed in Green Aerial Tissues**

To investigate the expression pattern of the *SEL1* gene in planta, we made transgenic plants expressing the GUS protein under the control of the *SEL1* promoter and examined the GUS expression driven by the *SEL1* promoter (Fig. 4). In germinating seeds and seedlings, GUS staining was observed in cotyledons but not in roots (Fig. 4, B–E). In adult plants, the GUS activity was strongly detected in rosette leaves, cauline leaves, stems, flowers, and siliques (Fig. 4, F–I). In flowers, GUS staining was observed exclusively in green tissues, such as sepals, stamens, and carpels, but not in petals (Fig. 4, G and H). GUS staining was also detected in all of the siliques but not in the stigma (Fig. 4I). Interestingly, GUS staining was also detected in the cotyledons of 3-d-old etiolated seedlings that were grown under dark (Fig. 4A). Therefore, these results indicate that *SEL1* is specifically expressed in green aerial tissues and functions under light as well as dark conditions.

***sel1* Mutants Have Defects Affecting the Development of Chloroplasts and Etioplasts**

Because the *sel1* mutant is pigment defective and *SEL1* protein is localized in the chloroplasts, we examined the chloroplast morphology and ultrastructure in



▼ Predicted cleavage site of transit peptide
 — Predicted PPR motif

Figure 2. Sequence analysis of SEL1. A, Predicted motif structure of SEL1 protein. P or P-L-S block motifs are depicted as boxes with letters as previously proposed (Lurin et al., 2004); P, L, and S designate the PPR, PPR-like S, and PPR-like L motif, respectively. B, Amino acid sequence alignment of SEL1. The amino acid sequence of SEL1 was compared with the homologous proteins in other species using ClustalW 2.0 (Larkin et al., 2007). The putative cleavage site of the transit peptide in SEL1 is shown by an arrow. Lines above the sequences show the predicted P- or PPR-like (S and L) motifs. Black boxes indicate amino acid residues that are greater than 80% conserved, and gray boxes indicate amino acids that are greater than 60% conserved. The amino acid sequences are NP_193587.4 for Arabidopsis, XP_002284293.1 for grape, NP_001173562.1 for rice, ACG29369.1 for maize, and XP_001757146.1 for moss.

sel1 mutant by transmission electron microscopy (TEM). Under light conditions, chloroplasts in the wild type were lens shaped and contained well-organized thylakoid membrane systems composed of stromal and granal thylakoids (Fig. 5A). However, the plastids in the *sel1* mutants were small and abnormally shaped, including round and amoeboid, and did not have thylakoid membranes or any membrane structures but contained a large number of small vesicles (Fig. 5B). In addition, densely stained globule aggregates were frequently observed in *sel1* mutants.

Because SEL1 is expressed in the cotyledons of etiolated seedlings, we also examined the etioplasts ultrastructure. Etioplasts are defined by the presence of the paracrystalline prolamellar body (PLB). Under dark conditions, etioplasts in the wild type showed the typical structures, i.e. spherical or ellipsoidal shape enclosing PLBs and prothylakoid membranes (Fig. 5C). However, plastids in *sel1* mutant did not have well-developed PLBs or prothylakoid membranes (Fig. 5D). Some plastids in *sel1* mutant showed a PLB-like but disorganized structure. Taken together, these results

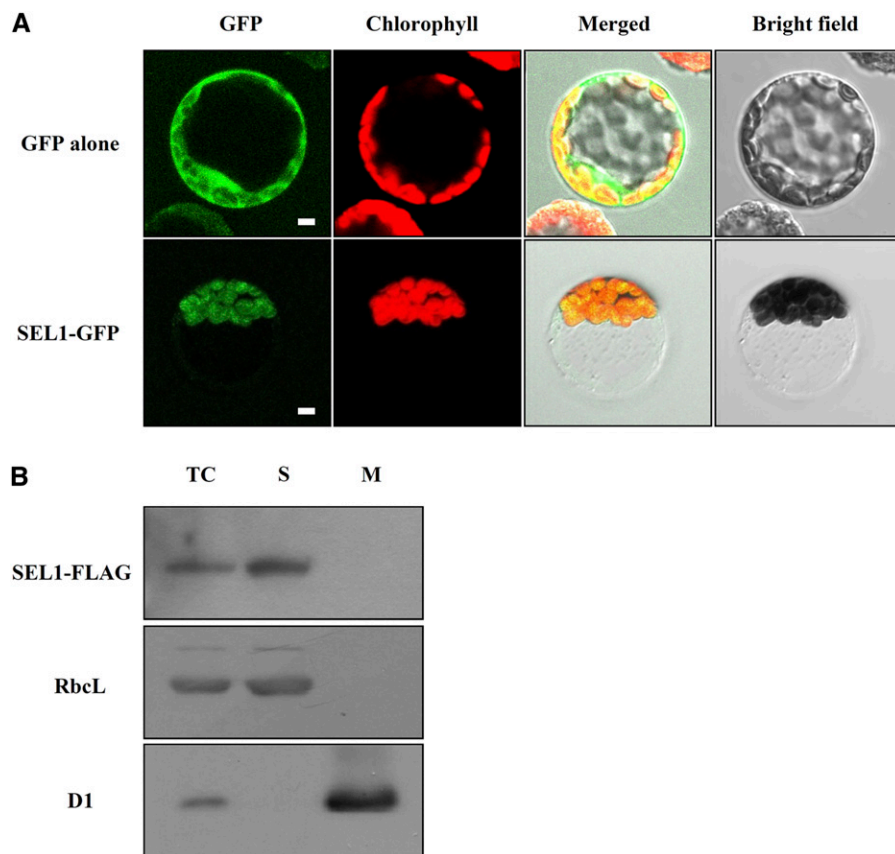


Figure 3. SEL1 is localized in the chloroplasts. A, Subcellular localization of SEL1-GFP. Protoplasts were isolated from *35S::GFP* and *35S::SEL1::GFP* transgenic plants. The fluorescence of GFP and SEL1-GFP fusion protein in protoplasts was observed by confocal laser scanning microscope. Green fluorescence signals, chlorophyll red autofluorescence signals, merged images, and bright-field images are shown. Bar = 5 μ m. B, Western-blot analysis of SEL1-FLAG fusion protein in chloroplast subfractions. Chloroplasts from *pSEL1::SEL1::FLAG* transgenic plants were purified and separated into soluble and membrane fractions by ultracentrifugation. The same proportion of each fraction was separated by SDS-PAGE and transferred to the nitrocellulose membrane. The blot was probed sequentially with antibody to FLAG and specific antibodies to RbcL and D1 as controls for the fractionation of soluble proteins and membrane proteins, respectively. TC, Total chloroplast protein; S, soluble protein; M, membrane protein. [See online article for color version of this figure.]

indicate that the *sel1* mutant is impaired in the development of chloroplast, as well as etioplasts, suggesting that SEL1 functions in the early stage of chloroplast development.

sel1 Mutant Fails to Accumulate Photosynthetic Protein Complexes

To compare the accumulation levels of chloroplast proteins of *sel1* mutant with those of the wild type, protein amounts were evaluated comparably using

western-blot analysis. As shown in Figure 6A, the *sel1* mutant did not have detectable levels of subunits of photosynthetic protein complexes corresponding to the products of the *ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL)*, *PSI P700 chlorophyll a apoprotein A1 (psbA)*, *PSII protein D1 (psbA)*, and *cytochrome f (petA)* genes. However, *ATP synthase CF1 beta subunit (AtpB)* was slightly reduced in the *sel1* mutant when compared with the wild type. The reduction of plastid-encoded proteins could be due to the impaired accumulation of plastid transcripts. To determine whether reduced protein levels in *sel1* mutants result from

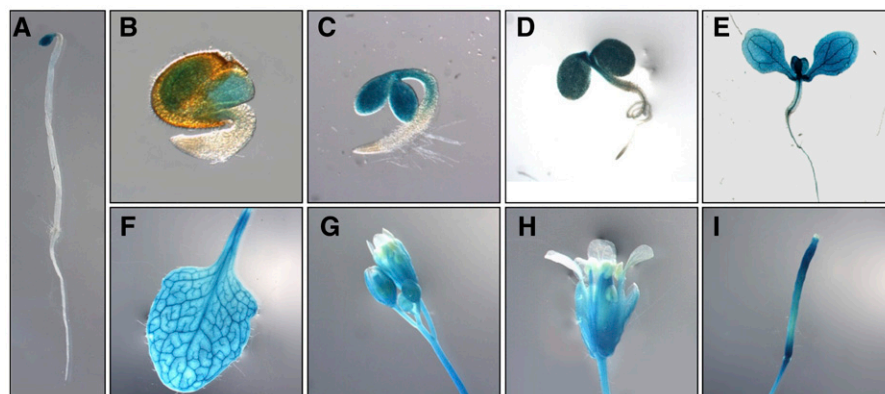
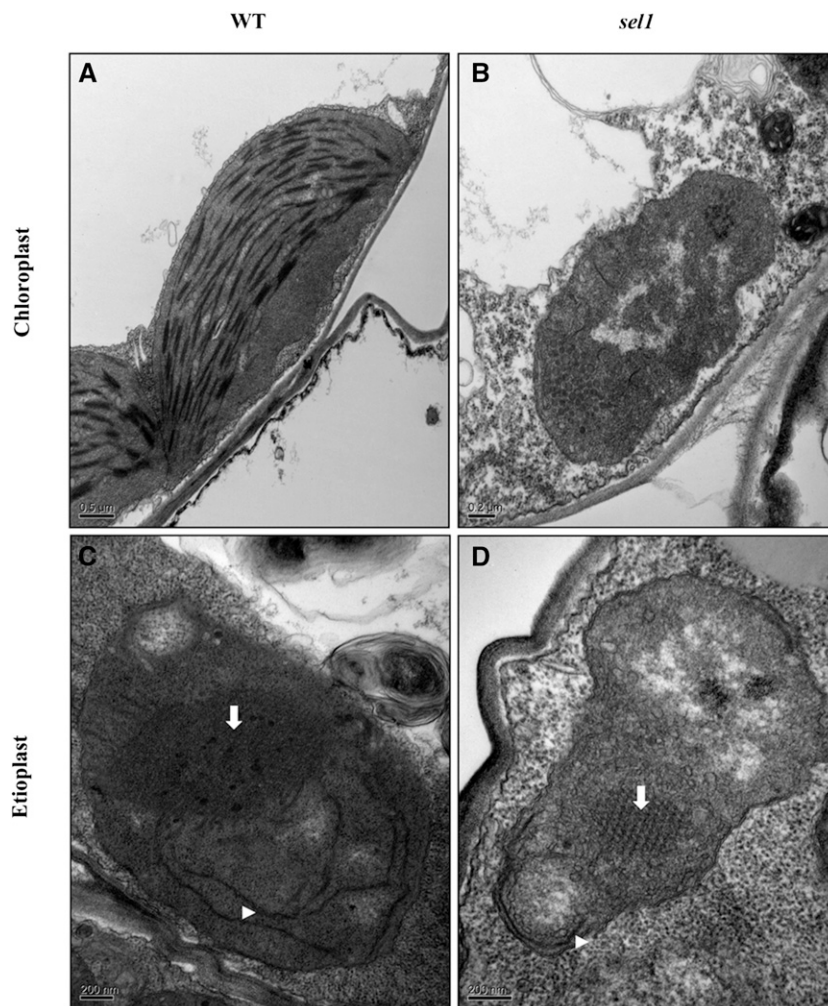


Figure 4. Expression patterns of SEL1. GUS expression was analyzed in *pSEL1::GUS* transgenic plants. A, Three-day-old etiolated seedling. B, Germinating seed. C, Three-day-old seedling. D, Five-day-old seedling. E, Seven-day-old seedling. F, Rosette leaf. G, Flower buds. H, Flower. I, Silique. [See online article for color version of this figure.]

Figure 5. Ultrastructure of chloroplasts and etioplasts in *sel1* mutant. A and B, Chloroplasts are from 7-d-old light-grown cotyledons of the wild type (WT) and *sel1* mutant. C and D, Etioplasts are from 7-d-old dark-grown cotyledons of the wild type and *sel1* mutant. Prolamellar body and prothylakoid are indicated by arrows and triangles, respectively.



transcriptional defects in the expression of the plastid genes, we examined the expressions of corresponding genes encoding subunits of the photosynthetic protein complex in *sel1* mutants by northern-blot analysis (Fig. 6B). Compared with the wild type, the levels of *rbcL*, *psaA*, *psbA*, and *petA* transcripts were not detected in *sel1* mutants. However, transcript pattern of *atpB* gene in *sel1* mutant were different from those in the wild type. While *rbcL*, *psaA*, *psbA*, and *petA* genes belong to class I plastid genes that are transcribed preferentially by PEP, the *atpB* gene is a class II plastid gene, which is transcribed by both NEP and PEP (Hajdukiewicz et al., 1997). In the wild type, there are two major *atpB* transcripts, in which 2.6- and 2-kb transcripts are transcribed by PEP and NEP, respectively (Loschelder et al., 2006; Schweer et al., 2006). However, in the *sel1* mutant, the PEP-dependent transcript is strongly reduced, and the NEP-dependent transcript is not changed. In addition, the two long transcripts, which do not exist in the wild type, are accumulated in the *sel1* mutant (Supplemental Fig. S4). This pattern of *atpB* transcripts in the *sel1* mutant is similar to that observed in PEP-defective mutants such as *clb19* and *sigma factor6* mutants (Ishizaki et al.,

2005; Chateigner-Boutin et al., 2008). This result suggests that the mutation of *SEL1* may affect PEP activity but not NEP activity. Taken together, the deficiencies of the photosynthetic protein complexes in the *sel1* mutant are caused by a defect in the transcription of corresponding plastid genes encoding subunits for photosynthetic protein complexes.

sel1 Mutants Have Global Defects in Plastid Gene Expression

To investigate whether the *SEL1* mutation specifically affects transcription by PEP but not NEP, we examined the transcript abundance of various plastid genes in the *sel1* mutant by quantitative reverse transcription (qRT)-PCR analysis. As shown in Figure 7, there were significant differences in plastid gene expression between the wild type and *sel1* mutant. Compared with the wild type, the expressions of the plastid genes that are transcribed by PEP, including the *psbA*, *psbB*, *psbD*, *psbE*, *petB*, *ndhA*, and *rbcL* genes, were strongly reduced in the *sel1* mutant. In addition, plastid ribosomal RNAs

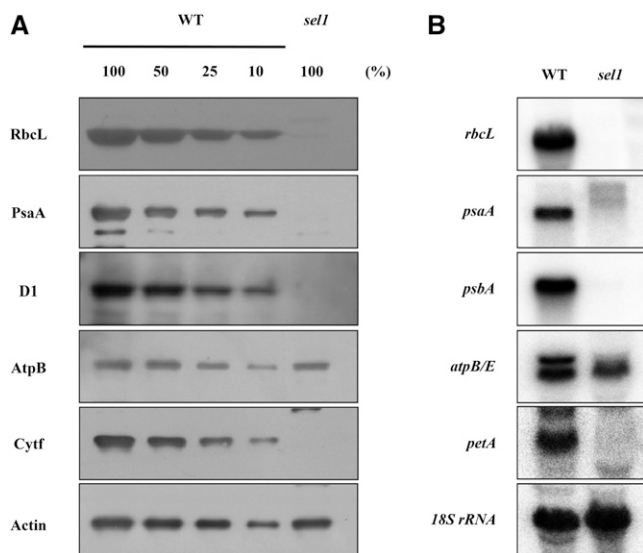


Figure 6. Analysis of photosynthetic protein complexes in the *sel1* mutant. A, Accumulation of representative subunits of photosynthetic protein complexes determined by western-blot analysis with specific antibodies to proteins indicated to the left; RbcL is the large subunit of Rubisco. PsaA and D1 are subunits of PSI and PSII, respectively. AtpB and Cyt f (for cytochrome subunit f) are subunits of ATP synthase and cytochrome b_6/f complex, respectively. Total proteins (10 μ g or the indicated dilution of the wild-type sample) from 7-d-old seedlings were loaded per lane. Actin was used as a loading control. B, Northern-blot analysis for the accumulation of photosynthesis-related transcripts. Five micrograms of total RNA was isolated from 7-d-old seedlings of the wild type (WT) and *sel1* mutant and hybridized with the gene-specific probes indicated. 18S rRNA was used as a loading control.

(rRNAs) genes (*rrn16s* [for 16S ribosomal RNA] and *rrn23s*), which are transcribed by both NEP and PEP, were also significantly reduced in *sel1* mutant. However, transcript levels of the plastid genes, including the *accD*, ribosomal protein L33 (*rpl33*), *rps18*, hypothetical chloroplast open reading frames2, and *rpo* genes, which are transcribed by NEP, were increased or unchanged in *sel1* mutant (Fig. 7). These results indicate that SEL1 is required for proper expression of plastid genes.

sel1 Mutant Is Defective in RNA Editing of *accD* Transcripts

The impairment in RNA editing has been reported in some PPR mutants, such as *clb19*, *ys1*, *atecb2*, and *vanilla cream1* (*vac1*), leading to PEP-defective transcription (Chateigner-Boutin et al., 2008; Yu et al., 2009; Zhou et al., 2009; Tseng et al., 2010). These mutants showed the similar pigment-defective and seedling-lethal phenotype to *sel1* mutants. To examine whether the SEL1 functions in RNA editing, we tested the 34 RNA editing sites of plastid transcripts in the *sel1* mutant using high-resolution melting (HRM) analysis (Chateigner-Boutin and Small, 2007). The *sel1* mutant shows altered editing extent at most of RNA editing sites compared with the

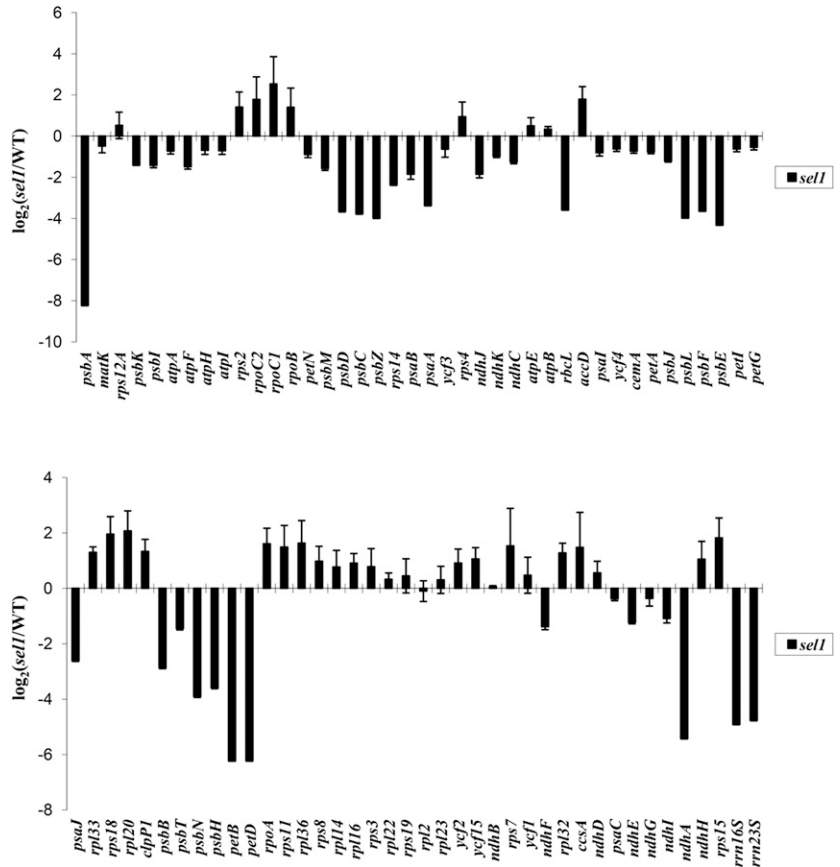
wild type, although they were edited correctly, as in the wild type (Supplemental Fig. S5). Interestingly, among 34 RNA editing sites known to be edited in the wild-type chloroplast, the RNA editing of *accD-2* was severely impaired in the *sel1* mutant, indicating that SEL1, as a member of the PLS subgroup, might be required for RNA editing of *accD-2* site in Arabidopsis. To confirm this defect, we examined the RNA editing of *accD* transcript by direct sequencing of reverse transcription (RT) product and identified defects of RNA editing of *accD-1* as well as *accD-2* in the *sel1* mutant (Fig. 8). The RNA editing of *accD-1* is significantly reduced and that of *accD-2* is completely abolished in the *sel1* mutant. We also evaluated the editing rates of these two editing sites in the wild type and *sel1* mutant. The editing rates of the two sites in the wild type were 100% and 91%, respectively, whereas they were 16% and 0%, respectively, in the *sel1* mutant (Table I). Furthermore, in the *sel1* mutant, RNA editing of some plastid transcripts (*ATP-dependent Clp protease proteolytic subunit*, *rpoA*, *rpoB*, *rpoC1*, and *rps14*) is mildly enhanced relative to the wild type (Supplemental Fig. S6). Taken together, these results suggest that SEL1 might be required for RNA editing of plastid transcripts, including most notably *accD* transcripts.

Plastid Ribosomes Are Functional But Reduced in Amount in the *sel1* Mutant

In mutants with defects in the plastid ribosome, pleiotropic effects in plastid gene expression have been reported, in which they could not translate plastid-encoded proteins, including PEP core subunits (Hess et al., 1993). Therefore, the reduced levels of PEP-dependent transcripts in the *sel1* mutant might be caused by functional defects of the plastid ribosome. To ensure the link of the less PEP activity to *sel1* mutant phenotype, the transcript levels and patterns of plastid rRNAs in *sel1* mutant were investigated using northern-blot analysis (Fig. 9A). Compared with the wild type, the expression levels of all plastid rRNAs were significantly reduced in the *sel1* mutant. However, the transcript patterns of plastid rRNAs in the *sel1* mutant were similar to those of the wild type.

Although the accumulation of plastid rRNAs is strongly reduced, the function of the plastid ribosome may not be impaired in the *sel1* mutant. To test this possibility, we examined plastid rRNAs associated with the polysome in the *sel1* mutant by polysomal RNA gel-blot analysis. As shown in Figure 9B, there was no difference in the distribution of plastid rRNAs between the wild type and *sel1* mutant, although the amount of plastid rRNAs associating with polysomes was reduced in the *sel1* mutant. Because the *sel1* mutant contains significantly fewer plastid rRNAs than the wild type, this is likely a consequence of plastid rRNAs deficiency, which could be either due to a lack of functional PEP activity or to an abnormal rRNA metabolism. Likewise, the majority of the *psaA*, *psbA*, and *rbcL* transcripts were associated with polysomes in the wild type but were not

Figure 7. RNA levels of plastid-encoded genes in *sel1* mutant. The transcript abundance of protein-encoding genes and rRNAs of the plastid genome were measured from the wild type (WT) and *sel1* mutant by quantitative RT-PCR. The graph shows the log₂ ratio of transcript levels in the *sel1* mutant compared with levels in the wild type. The genes are sorted according to physical location on the plastid genome. Error bars indicate sd.



detected in the *sel1* mutant (Supplemental Fig. S7). These results indicate that in *sel1* mutant, the functional plastid ribosomes exist, but the content of functional plastid ribosomes is significantly reduced.

SEL1 Mutation Affects the Transcript Amount But Not the Transcript Pattern of *rpo* Genes

Because the visible and molecular phenotype of *sel1* mutant is similar to those of mutants that have a defect

in PEP function (Ishizaki et al., 2005; Pfalz et al., 2006; Chateigner-Boutin et al., 2008, 2011; Zhou et al., 2009; Gao et al., 2011; Steiner et al., 2011), it is likely that *sel1* mutant is a PEP-defective mutant. PEP is composed of plastid-encoded core subunits and nuclear-encoded σ factors. The core subunits of the PEP are encoded by the plastid *rpoA*, *rpoB*, *rpoC1*, and *rpoC2* genes, which are NEP-dependent genes (class III). In *sel1* mutant, the transcript levels for *rpo* genes showed multiple times greater than those of the wild type (Fig. 7). To probe any

Figure 8. The *sel1* mutant is defective in RNA editing of *accD* transcripts. Sequence analysis for the *accD* transcripts from the wild type (WT) and *sel1* mutant. Nucleotide sequences including the RNA editing sites of *accD-1* and *accD-2* are shown as chromatograms. Editing sites of *accD-1* and *accD-2* are indicated by arrows pointing to the corresponding peaks. [See online article for color version of this figure.]

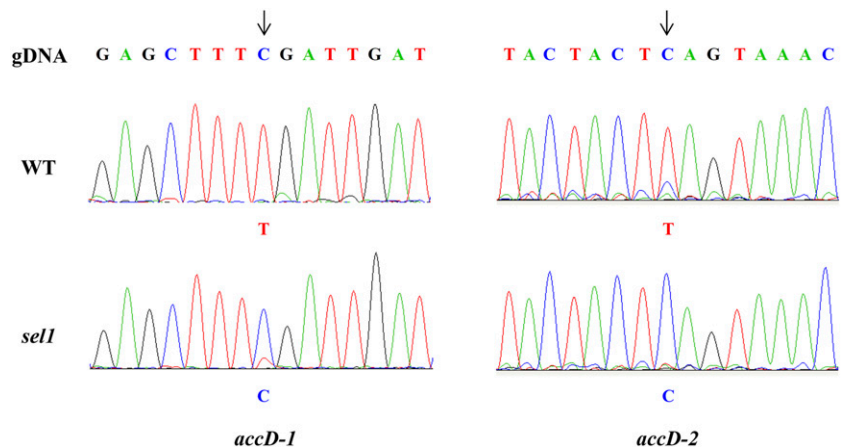


Table 1. RNA editing rate for the editing sites of *accD* transcripts in the wild type and *sel1* mutant

The editing rate was determined by analysis of approximately 25 independent clones from RT-PCR products including *accD-1* and *accD-2* in the wild type and *sel1* mutant.

Genotype	<i>accD-1</i>			<i>accD-2</i>		
	Unedited	Edited	Editing Rate	Unedited	Edited	Editing Rate
Wild type	0	24	100	2	20	91
<i>sel1</i>	21	4	16	25	0	0

possibility of abnormal *rpo* transcripts in *sel1* mutant, the amount and pattern of *rpo* transcripts were compared with those of the wild type using northern-blot analysis. As shown in Figure 10A, *rpoA* is cotranscribed with several ribosomal protein genes as part of *rpl23* operon, whereas *rpoB*, *rpoC1*, and *rpoC2* are cotranscribed as part of *rpoB* operon. In *sel1* mutant, the transcript accumulation for all *rpo* genes were significantly increased compared with the wild type, but the transcript patterns were similar in the wild type and *sel1* mutant (Fig. 10B). This result indicates that *SEL1* mutation causes a significant increase in the amount of *rpo* transcripts with no change of transcript patterns of *rpo* genes. To investigate whether the increased transcripts led to increase the corresponding proteins comparably, RpoB protein in *sel1* mutant was compared with that of the wild type using western-blot analysis. The *sel1* mutant showed similar level of RpoB to the wild type, though the *rpoB*

transcript level of *sel1* mutant is much higher than that of the wild type (Supplemental Fig. S8B). This result suggests that *sel1* mutant may have comparable PEP level to the wild type, but PEP activity of *sel1* mutant is not parallel to the wild type. Taken together, these results suggest that the mutation of *SEL1* disturbed the PEP function, which decreased the overall level of functional rRNAs in plastids. Eventually, the nonoperational transcription and translation in *sel1* mutant impeded the proper chloroplast biogenesis, resulting in seedling-lethal phenotype.

DISCUSSION

SEL1 Gene Is Essential for Early Chloroplast Development

It has been known that a large portion of the seedling-lethal mutants is defective in chloroplast function or

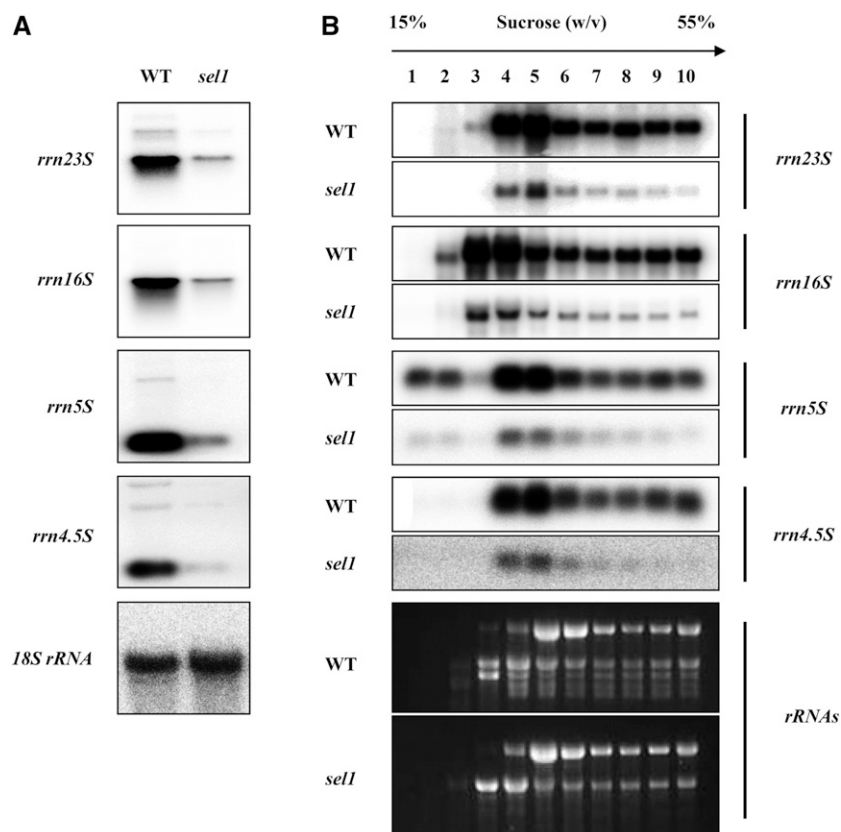
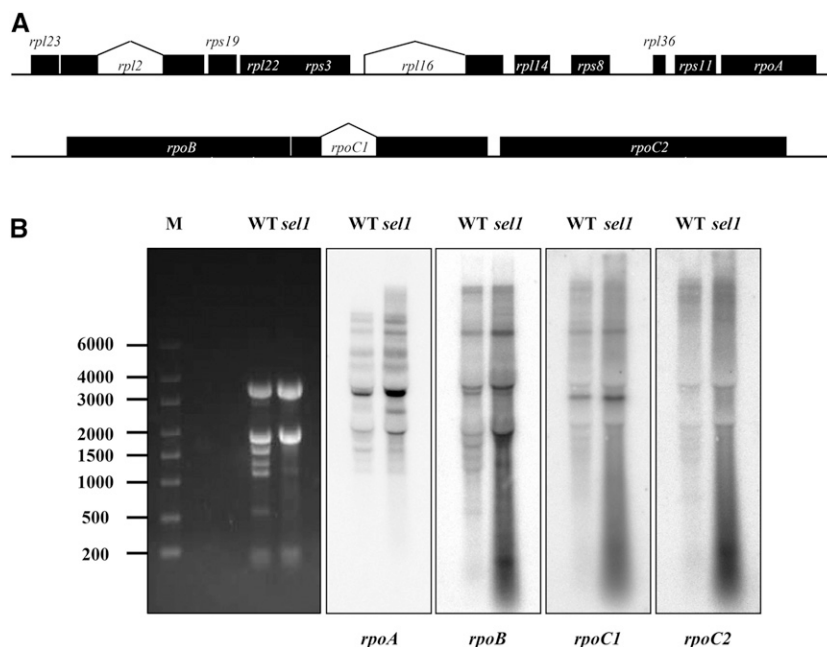


Figure 9. Chloroplast rRNAs are significantly decreased in the *sel1* mutant. A, Northern-blot analysis of chloroplast rRNAs in the wild type (WT) and *sel1* mutant. One microgram of total RNA was isolated from 7-d-old seedlings and analyzed by hybridization to probe for the plastid 23S, 16S, 5S, and 4.5S rRNA. 18S rRNA was used as a loading control. B, Polysome analysis of the wild type and *sel1* mutant. Total polysome isolation and RNA purification from 7-d-old seedlings were performed as described previously (Kwon and Cho, 2008). The ethidium bromide-stained gel represents equal proportional loading of the 10 gradient fractions.

Figure 10. Northern-blot analysis of the *rpo* genes encoding the PEP core subunits in the wild type (WT) and *sel1* mutant. A, Schematic representation of chloroplast *rpl23* and *rpoB* operons. *rpoA* is the last gene of the *rpl23* operon. *rpoB*, *rpoC1*, and *rpoC2* are part of the *rpoB* operon. The bent lines indicate introns. B, Five micrograms of total RNA from 7-d-old seedlings was analyzed by hybridization to the probe for *rpoA*, *rpoB*, *rpoC1*, and *rpoC2*. The size marker (M) is a RNA ladder, and ethidium bromide staining is shown as loading control.



development, suggesting that the development of functional chloroplasts is tightly coordinated with plant growth and development (Meinke et al., 2003, 2008; Waters and Langdale, 2009). The *sel1* mutants show seedling-lethal phenotype accompanied with white cotyledons under autotrophic conditions; however, on Suc-supplemented medium, primary leaves of *sel1* mutants show pale yellow color (Fig. 1A; Supplemental Fig. S1). This ivory phenotype implicates that the plastids are still active and able to synthesize carotenoids (Steiner et al., 2011). In *sel1* mutants, chlorophyll content was strongly reduced, while carotenoids content was moderately reduced (Supplemental Fig. S2). The visible phenotype of *sel1* mutants and complementation analysis with the *SEL1* gene under the control of its own promoter indicate that the disruption of the *SEL1* causes the pigment-defective and seedling-lethal phenotype and that *SEL1* is essential for normal plant growth and development.

Electron microscopy analysis revealed that the pigment-defective and seedling-lethal phenotypes of *sel1* mutants resulted from defects of chloroplast development. In the *sel1* mutant, the ultrastructure of the chloroplasts exhibited abnormal morphology and had no thylakoid membrane structure (Fig. 5B). Interestingly, etioplasts of *sel1* mutants also showed abnormal morphology and failed to develop normal prolamellar body (Fig. 5D). Similar phenotypes were observed in previous studies related to PEP-associated proteins (PAPs) and plastid transcriptionally active chromosome proteins (pTACs; Pfalz et al., 2006; Steiner et al., 2011). Homozygote *pap* or *ptac* knockout mutants, such as *pap5/ptac12* and *pap8/ptac6* (Pfalz et al., 2006), *pap7/ptac14* (Gao et al., 2011; Steiner et al., 2011), *pap4/fad3* and *pap9/fsd2* (Myouga et al., 2008), *pap6/fln1* (Arsova

et al., 2010; Steiner et al., 2011), *pap10/trxz* (Arsova et al., 2010), and *pap11/atmurE* (Garcia et al., 2008) mutants, showed white cotyledon and yellowish primary leaves on Suc-containing medium, while they are lethal when grown with no supply of an exogenous carbon source. Chloroplasts of these mutants do not contain thylakoid membrane structure, which is replaced by oval-shaped vesicles. Therefore, *sel1* mutants are very similar to *pap* or *ptac* mutants regarding chloroplast ultrastructure as well as visible phenotype. These results suggest that *SEL1* plays crucial role in early stage of chloroplast development. In agreement with an essential function in chloroplast development, *SEL1* is localized in chloroplasts (Fig. 3) and the *SEL1* gene is expressed only in green tissues, which was shown in the *SEL1* promoter-GUS transgenic plant analysis (Fig. 4).

SEL1 Is Required for RNA Editing of the *accD* Transcripts

PPR proteins have been shown to be involved in the RNA editing of the chloroplasts or mitochondria (Kotera et al., 2005; Okuda et al., 2006, 2007; Chateigner-Boutin et al., 2008; Cai et al., 2009; Hammani et al., 2009; Kim et al., 2009; Okuda et al., 2009; Robbins et al., 2009; Yu et al., 2009; Zehrmann et al., 2009; Zhou et al., 2009). So far, all of these proteins belong to the E/E+ and DYW subgroup of the PPR protein family. In this study, we found that *sel1* mutant is also defective in RNA editing of plastid transcripts, suggesting that *SEL1* is required for the proper RNA editing process in chloroplast. Interestingly, among 34 known RNA editing sites in chloroplasts (Tillich et al., 2005; Chateigner-Boutin and Small, 2007), the RNA editing of *accD-2* (C1568) sites was completely abolished and that of the *accD-1* (C794) is severely impaired in the *sel1* mutant (Fig. 8;

Supplemental Fig. S5). The RNA editing defects in *sel1* mutant are similar to those in *atecb2* and *vac1* mutants, which are independent mutant alleles of AtECB2/VAC1 encoding a DYW subgroup PPR protein that is required for the editing of *accD* and *ndhF* transcripts (Yu et al., 2009; Tseng et al., 2010). However, it has been reported that the loss of RNA editing at the *accD-1* (C794) site was not associated with a pigment-defective and seedling-lethal phenotype; because *Protein Required for accD RNA Editing1* mutant showed wild type-like phenotype, even the RNA editing of the *accD-1* (C794) site is completely abolished (Robbins et al., 2009). Therefore, the editing defect in the *sel1* mutant is unlikely to be a primary effect by the loss of SEL1 protein. The decreased editing extent is probably caused by the greatly increased level of *accD* transcripts in *sel1* mutant as shown in Figure 7 and Supplemental Figure S8A. Too many transcripts may saturate the editing activity and consequently leads to lower editing extent as shown in previous studies on the relation between the transcript abundance and the level of editing extent (Lu and Hanson, 1992). However, we cannot exclude the possibility that SEL1 is involved in RNA editing process of *accD* transcript, because the editing events on the ATP-dependent *Clp* protease proteolytic subunit, *rpoA*, *rpoB*, *rpoC1*, and *rps14* transcripts are not significantly affected (Supplemental Fig. S5), although they are highly expressed in the *sel1* mutant compared with the wild type (Figs. 7 and 10).

SEL1 Plays an Important Role in the Regulation of Plastid Gene Expression

Analysis of plastid gene expression revealed that, in *sel1* mutants, the transcript levels of PEP-dependent genes were dramatically reduced, whereas those of NEP-dependent genes were increased or unchanged (Fig. 7), suggesting that *sel1* mutant is severely impaired in PEP activity and SEL1 plays an important role in the regulation of plastid gene expression. Thus, it seems likely that the early arrest of chloroplast development in *sel1* mutants is due to the reduced PEP activity. Although the *sel1* mutant shows many similarities in molecular phenotypes, including transcript pattern and accumulation, and expression profiles of plastid genes to other PEP-related mutants (Pfalz et al., 2006; Chateigner-Boutin et al., 2008, 2011; Zhou et al., 2009; Steiner et al., 2011), the molecular function of SEL1 currently remains elusive. However, it is obvious that SEL1 is required for the proper expression of plastid genes. From the recent study, maize ortholog of SEL1 has been identified in nucleoid-enriched protein fractions (Majeran et al., 2012). Thus, it is possible that SEL1 might be involved in the control of PEP activity as a component of nucleoid proteins like pTACs or PAPs, and required for the proper plastid gene expression and normal chloroplast development. In addition to the global reduction of PEP-dependent transcripts, several lines of evidence suggest that *sel1* mutant is defective in

PEP activity. When we investigated the accumulation of plastid-encoded proteins in *sel1* mutants, the accumulation of Rubisco and thylakoid membrane protein complexes, except for ATP synthase, was severely decreased or not detected in the *sel1* mutant due to the lack of corresponding transcripts (Fig. 6A). The amount of PEP-dependent transcript of *atpB* decreased (upper band, approximately 2.6 kb) in the RNA gel blot (Fig. 6B; Supplemental Fig. S4), even though the level of AtpB protein did not change significantly. As seen in Figure 5B, the existence of mitochondria was observed in *sel1* mutant. Thereby, the amount of AtpB protein could be overestimated due to the cross reactivity of antibody used against the mitochondrial ATPase (Kwon and Cho, 2008). The amount of NEP-dependent transcript of *atpB* is not significantly altered and novel 4.8-kb transcript, which is not detected in the wild type, accumulated in *sel1* mutant (Fig. 6B; Supplemental Fig. S4). A similar transcription pattern was also observed in the *sigma factor6* (Loschelder et al., 2006; Schweer et al., 2006) and other PEP-defective mutants, such as *ptac2/ptac6/ptac12/ptac14* (Pfalz et al., 2006; Gao et al., 2011), *clb19* (Chateigner-Boutin et al., 2008), *delayed green1* (Chi et al., 2008), and *ys1* (Zhou et al., 2009).

In terms of the molecular phenotypes, *sel1* mutants show the similarity to those of mutants with defects in plastid ribosome as well (Bang et al., 2012). In ribosome-deficient mutants, the expression of PEP-dependent genes is decreased, whereas that of NEP-dependent genes is increased. In *sel1* mutant, steady-state levels of all plastid rRNAs were significantly decreased (Fig. 9). This may lead to a global defect in the expression of plastid genes due to the lack of functional plastid ribosomes, which causes the level of PEP to be much less than required to synthesize plastid proteins controlled by PEP. In *sel1* mutant, protein levels of AccD and RpoB are not significantly changed, even though their transcripts are highly up-regulated (Figs. 7 and 10B; Supplemental Fig. S8). Thus, reduced translation of *accD* and *rpoB* could be a consequence of the decreased level of plastid rRNAs. Global defects in plastid gene expression in *sel1* mutants are similar to those of plastid ribosome-deficient mutants, suggesting that the SEL1 may be involved in the regulation of plastid ribosome assembly or function. So, the loss of SEL1 leads to overall declines of all PEP-dependent transcriptions caused by the reduced translation of PEP. However, we could not rule out the possibility that the reduction in the plastid ribosome is also caused by defects of PEP activity.

In *sel1* mutant, the expression of PEP-dependent genes is decreased, whereas that of NEP-dependent genes is increased. This increase in NEP-dependent transcripts in *sel1* can be indirect and possibly explained by a recent study on the division of labor between PEP and NEP during plastid development and in mature chloroplasts using the primary transcriptome analysis in barley (*Hordeum vulgare*; Zhelyazkova et al., 2012). In this study, authors found that the extremely high number of promoters, at least 222 NEP promoters, can be used in white

leaves in the absence of PEP and that NEP is able to generate virtually the same mRNAs as PEP in several genes, suggesting that activation of transcription by NEP can be a compensatory mechanism for the absence of PEP in higher plants.

In this work, we report a novel pigment-defective and seedling-lethal mutant, *sel1* mutant, that disrupted genes encoding a member of the PLS subgroup of the PPR protein family. Our results demonstrate that SEL1 is required for the proper plastid gene expression and thus essential for normal chloroplast development. To discover the more detailed molecular function of SEL1, the identification of target RNA and interacting proteins of SEL1 in the future should be helpful to provide the insight into its function in plastid gene expression and chloroplast development.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Seeds were surface sterilized and incubated at 4°C for 3 d to synchronize germination and plated on MS medium (Duchefa Biochemie) containing 1% (w/v) Suc and 0.7% (w/v) agar in a growth chamber at 22°C under 16-h-light/8-h-dark condition. Plants were grown in soil (Sunshine Mix #1; SunGro) in a growth room at 22°C under continuous light. The *sel1-1* mutant was isolated from T-DNA insertion mutant lines, which are generated in *Ws-2* background with simple T-DNA vector pDAP101 (Sessions et al., 2002), whereas seeds of *sel1-2* (SAIL_793_F11) and *sel1-3* (SALK_054374) were obtained from the Arabidopsis Biological Resource Center.

Complementation Analysis

For the complementation test of the *sel1* mutant, the 2,629-bp wild-type genomic DNA fragment of the At4g18520 gene, containing 645 bp of a putative promoter sequence and 130 bp of 3' untranslated region, was amplified using Pfu-X polymerase (SolGent) and gene-specific primers. After verification by sequencing, the amplified fragment was cloned into a pBIN19 binary vector and subsequently introduced into heterozygous *sel1-3* mutant plants using the *Agrobacterium tumefaciens*-mediated floral dip method (Clough and Bent, 1998). For the generation of FLAG-tagged SEL1 complementation line, DNA encoding three copies of FLAG was amplified and cloned into pCB308 binary vector (Xiang et al., 1999), producing pCB308-FLAG vector. The SEL1 genomic DNA without stop codon, including 645-bp upstream region from ATG, was amplified and cloned into pCB308-FLAG vector and subsequently introduced into heterozygous *sel1-2* mutant plants.

Northern-Blot Analysis

Total RNA was extracted from 7-d-old seedlings using the easy-BLUE Total RNA Extraction Kit (iNtRON Biotechnology), separated on MOPS-formaldehyde agarose gel, and transferred to a nitrocellulose membrane (Whatman). The ³²P-labeled probes were prepared using a DecaLabel DNA Labeling Kit (Fermentas). Prehybridization, hybridization, washes, and signal detection were performed as described previously (Kwon and Cho, 2008).

Subcellular Localization of GFP Fusion Proteins in Protoplasts

Analysis of the subcellular localization of GFP fusion proteins in protoplasts was performed as described previously (Kwon and Cho, 2008).

Chloroplast Isolation and Fractionation

Chloroplasts were isolated and separated into soluble and membrane fractions as described previously (Kwon and Cho, 2008).

Histochemical GUS Staining

Tissues were incubated in cold 90% (v/v) acetone and placed in a staining buffer (50 mM sodium phosphate buffer, pH 7.2, 0.2% Triton-X, 2 mM potassium ferrocyanide, 2 mM potassium ferricyanide, and 2 mM 5-bromo-4-chloro-3-indolyl-β-glucuronic acid). After a vacuum infiltration, samples were incubated overnight at 37°C. GUS-stained tissues were cleared with an ethanol series and fixed in formaldehyde-acetic acid solution (50% [v/v] ethanol, 10% [v/v] acetic acid, and 5% [v/v] formaldehyde). Following fixation, samples were stored in 70% (v/v) ethanol and examined under the dissecting microscope.

TEM Analysis

TEM analysis was performed as described previously (Kwon and Cho, 2008), with minor modification. For analysis of the etioplast ultrastructure, wild-type and *sel1* mutant seedlings were grown on MS agar plates in the dark for 7 d. Under dim green light, cotyledons of the wild type were directly detached from seedlings and then immediately soaked in fixation solution. For the *sel1* mutant, cotyledons were detached and soaked in numbered 1.5-mL tubes containing fixation solution. The genotype of the sample was determined by PCR using the remaining part of the seedling. The detached cotyledons in the corresponding tubes confirmed that homozygous *sel1* mutant was used for TEM analysis.

Western-Blot Analysis

Total proteins were isolated from 7-d-old seedlings as described previously (Barkan, 1998), separated by SDS-PAGE, transferred to polyvinylidene difluoride membrane, immunoblotted with various antibodies, and detected using the enhanced chemiluminescence kit (Santa Cruz Biotechnology). Antibodies were obtained from Agrisera (RbcL, PsaA, D1 protein of PSII, AtpB, and cytochrome subunit f) and from Uniplastomic (AccD and RpoB).

Analysis of Plastid Transcript Abundance

Total RNA was extracted from 7-d-old wild-type and *sel1* mutant seedlings by using the NucleoSpin RNA Plant Kit (Macherey-Nagel). DNA-free RNA (2 μg) was then reverse transcribed with random hexamers by using a RevertAid First-Strand cDNA Synthesis Kit (Fermentas). Quantitative real-time PCR was carried out in an IQ5 Light Cycler (Bio-Rad) using SYBR Premix Ex Taq II (Takara). The relative quantification of gene expression data were analyzed using the IQ5 Optical System Software (Bio-Rad). The data set was normalized using cytosolic 18S rRNA as a reference and setting the median value to 0. The primer sequences used in quantitative RT-PCR analysis are described in Okuda et al. (2009).

Analysis of RNA Editing

HRM analysis was performed as previously described (Chateigner-Boutin and Small, 2007; Okuda et al., 2009). Genomic DNA for HRM analysis was isolated with plant I genomic DNA (iNtRON Biotechnology). RNA isolation and RT were performed as above. The sequence including the *accD* RNA editing sites (*accD-1* and *accD-2*) from the wild type and *sel1* mutant was amplified by PCR with gene-specific primer pairs, and then the RT-PCR products were sequenced directly or cloned into a T-blunt vector using a T-blunt PCR Cloning Kit (SolGent). Plasmids prepared from 25 independent colonies of each sample were sequenced to determine the RNA editing efficiency of *accD-1* and *accD-2*.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession number At4g18520.

Supplemental Data

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

Supplemental Figure S1.

Supplemental Figure S2. The content of chlorophyll and carotenoids in *sel1* mutant.

Supplemental Figure S3. Sucrose gradient sedimentation analysis of SEL1-containing particles in chloroplast.

Supplemental Figure S4. The transcript accumulation and pattern of *atpB* gene in *sel1* mutant.

Supplemental Figure S5. High-resolution melting analysis of plastid RNA editing in *sel1* mutant.

Supplemental Figure S6. RNA editing of plastid transcripts in *sel1* mutant.

Supplemental Figure S7. Polysome association of photosynthesis-related mRNAs in *sel1* mutant.

Supplemental Figure S8. Analysis of the plastid-encoded genes in *sel1* mutant.

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