LOW PHOTOSYNTHETIC EFFICIENCY 1 is required for light-regulated photosystem II biogenesis in Arabidopsis

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Photosystem II (PSII), a multisubunit protein complex of the photosynthetic electron transport chain, functions as a water-plastoquinone oxidoreductase, which is vital to the initiation of photosynthesis and electron transport. Although the structure, composition, and function of PSII are well understood, the mechanism of PSII biogenesis remains largely elusive. Here, we identified a nuclear-encoded pentatricopeptide repeat (PPR) protein LOW PHOTOSYNTHETIC EFFICIENCY 1 (LPE1; encoded by At3g46610) in Arabidopsis, which plays a crucial role in PSII biogenesis. LPE1 is exclusively targeted to chloroplasts and directly binds to the 5′ UTR of psbA mRNA which encodes the PSII reaction center protein D1. The loss of LPE1 results in less efficient loading of ribosome on the psbA mRNA and great synthesis defects in D1 protein. We further found that LPE1 interacts with a known regulator of psbA mRNA translation HIGH CHLOROPHYLL FLUORESCENCE 173 (HCF173) and facilitates the association of HCF173 with psbA mRNA. More interestingly, our results indicate that LPE1 associates with psbA mRNA in a light-dependent manner through a redox-based mechanism. This study enhances our understanding of the mechanism of light-regulated D1 synthesis, providing important insight into PSII biogenesis and the functional maintenance of efficient photosynthesis in higher plants.

Significance

Photosystem II (PSII) reaction center protein D1 is encoded by chloroplast gene psbA and is crucial to the biogenesis and functional maintenance of PSII. D1 proteins are highly dynamic under varying light conditions and thus require efficient synthesis, but the mechanism remains poorly understood. We reported that Arabidopsis LPE1 directly binds to the 5′ UTR of psbA mRNA in a light-dependent manner through a redox-based mechanism and facilitates the association of HCF173 with psbA mRNA to regulate D1 translation. These findings fill a major gap in our understanding of the mechanism of light-regulated D1 synthesis in higher plants and imply that higher plants and primitive photosynthetic organisms share conserved mechanisms but use distinct regulators to regulate biogenesis of PSII subunits.

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The 32-kDa D1 protein is encoded by psbA, a gene in the chloroplast genome, whose expression is controlled by a complex regulatory process requiring nuclear-encoded proteins (9). Light is the major signal regulating psbA gene expression. In cyanobacteria, expression of psbA is mainly regulated by light control of the transcription and stability of psbA mRNA (10); however, in plant chloroplasts, light-regulated initiation of translation plays a primary role in regulating psbA gene expression (11). In the unicellular alga Chlamydomonas reinhardtii, initiation of psbA mRNA translation may be regulated by the binding of a complex made up of four proteins (RB47, RB38, RB60, and RB55) to the 5′ UTR of the mRNA (12–15). In addition, a serine/threonine protein phosphotransferase associated with the psbA 5′ UTR binding complex (RB47/RB38/RB60/RB55) is able to inactivate the complex’s RNA-binding properties through ADP-dependent phosphorylation of RB60. This inactivation requires high ADP levels, and thus attenuation of translation in the dark may be achieved by the concomitant increase in the AMP/ATP ratio (16). In the higher plant Arabidopsis thaliana, only two regulators of psbA mRNA translation, HCF173 and
HCF244, have been identified (17, 18), and the mechanism of regulation is largely unclear.

Pentatricopeptide repeat (PPR) proteins form a large family of helical repeat proteins that are ubiquitously expressed and are deeply involved in the coevolution of the nucleus with the mitochondria and plastids. Each PPR motif consists of a 35-aa degenerate consensus related to the tetratricopeptide motif (19). PPR proteins harbor between 2 and 30 PPR motifs, and their tandem alignment allows the modular recognition of specific RNA sequences (20, 21). A number of recent studies show that PPR motifs have direct RNA-binding activity (22–24). Genetic data implicate PPR proteins in every step of organellar gene expression: transcription, RNA stabilization, RNA cleavage, RNA splicing, RNA editing, and translation (19, 25–27). The role of PPR proteins in PSI biogenesis remains unknown.

In vivo chlorophyll fluorescence is a powerful, noninvasive technique used to identify mutations affecting photosynthesis (28–31). Alterations in chlorophyll fluorescence indicate defects in the photosynthetic electron transport chain resulting from changes in the structure and function of the thylakoid membrane (31). Screening for altered chlorophyll fluorescence therefore provides a means of obtaining and characterizing photosynthetic mutants. In this study, we characterized the low photosynthetic efficiency1 (lpe1–At3g46610) mutant, which has reduced PSI activity, using chlorophyll fluorescence analysis and found that LPE1 was involved in PSI biogenesis. LPE1 encoded a chloroplast PPR protein that, by directly associating with the 5′ UTR of psbA mRNA in a light-dependent manner through redox regulation, was required for the translation of D1 protein. LPE1 also interacted with HIGH CHLOROPHYLL FLUORESCENCE 173 (HCF173), which is involved in regulating psbA mRNA translation, and facilitated the association of HCF173 with psbA mRNA to promote the activation of psbA mRNA translation. These results suggest that the chloroplast PPR protein LPE1 interacts with HCF173 and participates in light-regulated translation of psbA mRNA in a redox-dependent manner in higher plants.

Results

PSII Activity Is Reduced in lpe1 Mutants. To gain insight into the regulation of PSI function and identify auxiliary factors required for PSII, we surveyed many pools of Arabidopsis mutants using a chlorophyll fluorescence video imaging system (32). Arabidopsis plants showing aberrant maximum photochemical efficiency [variable fluorescence/maximum fluorescence (Fv/Fm)] of PSI were identified to obtain a series of low photosynthetic efficiency (lpe) mutants. One of these, lpe-1 (SALK_059367, At3g46610), had a lower Fv/Fm than wild-type (Col-0) plants (Fig. 1 A and B). To confirm that disruption of At3g46610 was responsible for the observed phenotype, we analyzed two other independent homozygous transfer DNA (T-DNA) insertion lines (SALK_030882 and SALK_110539) from the sequence-indexed Arabidopsis T-DNA insertion mutant stocks. We refer to SALK_059367, SALK_030882, and SALK_110539 as lpe-1, lpe-1-2, and lpe-1-3, respectively, throughout. The T-DNA insertion was located in the 5′ UTR in lpe-1 and lpe-2 mutant alleles and in the coding sequence (CDS) in the lpe-3 mutant allele of LPE1 (SI Appendix, Fig. S1 A and B). Quantitative real-time RT-PCR analysis showed that transcription of At3g46610 was down-regulated in lpe-1 and lpe-1-2, and the transcript could not be detected in lpe-1-3 plants (Fig. L4). This analysis of three independent lpe1 alleles expressing different levels of LPE1 suggested that the changes in PSI function and plant growth depended on LPE1; in particular, lpe-1-3, the LPE1-knockout mutant, showed a drastic retardation of photoautotrophic growth (Fig. 1B and SI Appendix, Fig. S1 C and D). The lpe1 phenotype thus appears to result from the inactivation of At3g46610.

Initial Fv/Fm measurements suggested that PSI activity was disturbed in the lpe1 mutants. To further investigate the primary effect of lpe1 mutations, the minimum (F0) and maximum (Fm) fluorescence of dark-adapted leaves, Fv/Fm, and other chlorophyll parameters were quantitatively determined under a fixed light intensity (SI Appendix, Table S1). Our results indicated that the reduction of Fv/Fm in lpe1 mutants was primarily caused by the increase in F0. In addition, the lpe1 mutants showed much higher levels of nonphotochemical quenching (NPO) than wild-type plants, indicating that they dissipated more excess excitation energy via nonphotochemical pathways. NPO primarily includes energy-dependent quenching (qE) and photoinhibitory quenching (qI), according to their relaxation kinetics, because state-transition quenching (qT) is significant only under very low light in most plants (29, 31). We found that the higher levels of NPO in lpe1 mutants were primarily due to an increase in qI (SI Appendix, Table S1), indicating higher photoinhibition. By contrast, qE decreased in lpe1 mutants, suggesting a reduced ability to use heat dissipation for photoprotection. To further characterize the photosynthetic apparatus, we analyzed the light intensity dependence of three chlorophyll fluorescence parameters, the light–response curves of PSI quantum yield (ΦPSII), the electron transport rate (ETR), and the redox state of the O2 electron acceptor of PSI (1-qP). The ΦPSII and ETR were much lower in lpe1 mutants than in wild-type plants (Fig. 1C), as also observed for Fv/Fm (Fig. 1B). Interestingly, 1-qP, which reflects the redox state of the O2 electron acceptor of PSI, was higher in the lpe1 mutants (Fig. 1C), suggesting a more highly oxidized plastoquinone pool in the plants. This is more likely to result from a PSI deficiency than from downstream defects (33). These results suggest that disruption of LPE1 specifically affected photosynthetic activity of PSI.

LPE1 Deficiency Specifically Affects PSI Biogenesis and the Formation of Grana Thylakoid in Chloroplast. Our results showed that PSI photosynthetic activity was reduced in lpe1 mutants, suggesting that LPE1 was involved in the functional regulation of the PSI photosynthetic apparatus. To investigate structural alterations in
thylakoid proteins, chlorophyll–protein complexes were solubilized from thylakoid membranes using dodecyl-β-D-maltopyranoside (DM) and were separated using blue native PAGE (BN-PAGE). After separation in the first dimension, five major PSII complexes were resolved (Fig. 2A), apparently representing PSII–LHCCI supercomplexes, dimeric PSII, monomeric PSII, CP43 minus PSII, and trimeric LHCCI (34). The BN-PAGE analysis indicated that the amount of PSII per unit of chlorophyll was lower in thylakoid preparations from lpe1 mutants, especially lpe1-3, than in corresponding preparations from wild-type plants (Fig. 2A and B). To confirm that PSII complexes were specifically decreased in lpe1 mutants, thylakoid membranes were analyzed following BN-PAGE separation by immunoblotting with antibodies specific for subunits of each protein complex. Immunoblotting with anti-CP43 and anti-D1 antisera indicated that thylakoid membranes from lpe1 mutants contained lower levels of PSII complex than those from wild-type plants (Fig. 2 C and D).

The levels of Cytb6/f and ATP synthase complex, which are not associated with light-harvesting pigments, did not differ between lpe1 mutants and wild-type plants (SI Appendix, Fig. S2), suggesting that the absence of LPE1 affected the formation and stability of the PSII complex.

To examine the accumulation of thylakoid proteins, thylakoid membranes were isolated from wild-type plants and lpe1 mutants, and immunoblot analysis was performed using antibodies raised against subunits of the photosynthetic thylakoid membrane protein complexes. The relative protein levels of the different subunits were calculated on an equal chlorophyll basis. Marked reductions in the PSII subunits D1, D2, CP43, PsbE, PsbF, and PsbO were detected in lpe1 mutants (Fig. 2E and SI Appendix, Fig. S3). Protein levels of the PSI core subunits PsAa and PsBb were also reduced slightly. By contrast, levels of the PSI protein PsDa, the LHCCI chlorophyll a/b-binding proteins LHClA1 and LHClB1, ATP synthase subunit B, and cytochrome f were relatively consistent between wild-type and mutant plants (Fig. 2E and SI Appendix, Fig. S3). These results suggest that reductions in LPE1 levels perturb the accumulation of PSII complexes.

PSII and LHCCI are restricted to grana thylakoids and therefore are segregated from PSI, LHCl, and ATP synthase, which, for steric reasons, are located only in stroma lamellae (35). The ultrastructure of chloroplasts in leaves of 4-wk-old wild-type and lpe1-3 plants was compared using transmission electron microscopy (SI Appendix, Fig. S4). Wild-type chloroplasts displayed well-developed membrane systems comprised of grana connected by the stroma lamellae; however, in lpe1-3 chloroplasts, the thylakoid membrane systems were partially disturbed, and the membrane spacing was less clear (SI Appendix, Fig. S4). In addition, some grana appeared to be enlarged in lpe1 mutants compared with the wild-type plants (SI Appendix, Fig. S4); this phenotype of lpe1 mutants is similar to that of other PSI-deficient mutants, such as hcf136 (36). This suggests that the absence of LPE1 affects the formation of grana thylakoids in chloroplasts.

**LPE1 Encodes a Chloroplast PPR Protein and Associates with the 5’ UTR of psbA mRNA.** LPE1 is predicted to encode a 665-aa protein of unknown function (The Arabidopsis Information Resource, www.arabidopsis.org/) which possesses an N-terminal chloroplast transit peptide (amino acids 1–68) (37) as well as 13 PPR motifs (SI Appendix, Fig. S5) (38). To understand the role of LPE1 in PSII accumulation, we first determined the subcellular localization of the protein. Analysis of LPE1-GFP fusion proteins using confocal laser-scanning microscopy found that LPE1 was specifically localized in the chloroplast (Fig. 3A). Due to a failure to generate an antibody against LPE1, we generated transgenic Arabidopsis plants containing a LPE1-FLAG construct in wild-type plants to determine the location of LPE1. Chloroplasts were extracted from transgenic plants, and the thylakoid membrane and stroma fractions were separated and used to identify the precise sublocation of LPE1. Immunoblot analyses of the soluble and membrane fractions from Percoll-purified chloroplasts showed that LPE1 was associated with the thylakoid membranes of isolated chloroplasts and also was found in the stroma (Fig. 3B).

To confirm that the observed association of LPE1 with membranes was a genuine feature and explore whether LPE1 was an intrinsic membrane protein, thylakoid membrane fractions were isolated from chloroplasts extracted from LPE1-FLAG transgenic plants and subjected to immunoblot analysis. After the membrane preparations were sonicated in the presence of various salts, LPE1 remained associated with NaCl-treated membranes, confirming its association with thylakoid membrane. However, a considerable amount of LPE1 was released from thylakoid membranes following treatment with CaCl2, and it was effectively removed by 0.2 M Na2CO3 or urea (SI Appendix, Fig. S6). PsbO (the 33-kD luminal protein of PSI) and D1 (the PSI core protein) were used as controls (SI Appendix, Fig. S6). As LPE1 behaved like the peripheral protein PsbO but not like the integral protein D1, LPE1 appeared to be peripherally associated with the thylakoid membrane rather than an intrinsic membrane protein.
and E and SI Appendix, Fig. S7). We therefore concluded that LPE1 specifically associates with psbA mRNA. The 5′ UTR of psbA mRNA contains the crucial cis-acting RNA elements and can associate with transacting protein factors involved in post-transcriptional regulation (11). To confirm the direct association between LPE1 and psbA mRNA, we performed an EMSA. The mature form of wild-type LPE1 without the putative plastid transit peptide was expressed as a His-tagged fusion protein in Escherichia coli (SI Appendix, Fig. S8). The purified recombinant LPE1 was incubated with a psbA 5′ UTR RNA probe. The LPE1–RNA complex was detected as a band that migrated more slowly than the free probe in the gel; increasing retardation of the band was detected as the amount of recombinant LPE1 was increased (Fig. 3F). The association of LPE1 with the psbA 5′ UTR was also confirmed by competition experiments with an unlabeled psbA 5′ UTR-specific RNA probe (Fig. 3F). Thus, LPE1 directly associates with the 5′ UTR of psbA mRNA in vitro, implying that psbA mRNA is a direct target of LPE1.

Ribosomal Loading of psbA mRNA Is Impaired in lpe1 Mutants. To understand the role of LPE1 binding to psbA mRNA, we first analyzed transcript levels of psbA mRNA using quantitative RT-PCR and Northern blot analyses. There were no obvious differences in the expression of psbA mRNA in lpe1 mutants and wild-type plants (Fig. 4 A and SI Appendix, Fig. S9), suggesting that LPE1 mutations did not affect the accumulation of psbA transcript.

To check whether LPE1 mutations affected the association of psbA mRNA with ribosomes during translation, we examined ribosomal loading of this transcript. Leaf extracts were fractionated in sucrose gradients under conditions that maintained intact polysomes (39). Efficiently translated ribonucleic acids will migrate deep into the gradient, as they are strongly associated with ribosomes. RNA gel blot hybridizations were performed using RNA purified from gradient fractions to localize the position of specific mRNAs within the gradients. When the distribution of plastidic and cytosolic rRNAs from wild-type and lpe1 plants across a sucrose gradient was determined, an equal pattern of rRNA distribution was observed, indicating that there was no general difference in ribosome distribution between mutant and wild-type plants (Fig. 4B). By contrast, different sedimentation patterns were observed between psbA mRNA extracted from wild-type plants and lpe1 mutants, as extracts from lpe1 mutants showed significantly decreased amounts of psbA mRNA in the polysome fractions (Fig. 4 C and D). We also examined the polysomal association of psbE mRNA, encoded by the psbE-psbF-psbI-psbL polycistronic transcription unit and found comparable levels of association between lpe1 and wild-type plants (Fig. 4D). To confirm the distribution of small RNA particles (monosomes and free RNA) and polymbosomes in sucrose gradients, crude polysomal RNAs isolated from lpe1 and wild-type plants were treated with EDTA, which disrupts ribosomal association of mRNAs. This treatment resulted in a shift of psbA and psbE mRNA into the monosome fractions in both wild-type and lpe1 mutant plants (Fig. 4E), thus confirming the pattern of polysomes. These results suggest that LPE1 promotes ribosomal loading of psbA mRNA, probably via direct association with psbA mRNA in vivo.

To confirm the effect of defects of ribosomal loading of psbA mRNA in lpe1 mutants on the synthesis of D1 protein encoded by psbA, we performed in vivo protein-labeling experiments. We followed a previous methodology for studying translation of psbA mRNA (18), using a 20-min pulse to label the synthesis of thylakoid proteins. The rates of synthesis of the PSII subunits D2 and CP43 and of the α- and β-subunits of the chloroplast ATP synthase (CF1-αβ) were almost unchanged in mutant plants. However, incorporation of [35S]Met into D1 was greatly

PPR proteins regulate RNA by direct binding via PPR motifs (22–24). As LPE1 affected PSII biogenesis, we used RNA immunoprecipitation (RIP) to screen all PSII-associated mRNAs encoded by the plastid genome to identify the RNA target(s) of LPE1 in wild-type and LPE1-FLAG plants (Fig. 3 C–E and SI Appendix, Fig. S7). The psbA mRNA was enriched in the LPE1-FLAG plants, as the ratio of mRNA from LPE1-FLAG to wild-type plants was higher than all other transcript ratios (Fig. 3D)
reduced in the lpe1-3 mutant (Fig. 4F), indicating a drastic reduction in the synthesis of this protein. Furthermore, we found that the amounts of D1 aggregate, dimer, and monomer were all drastically decreased in lpe1 mutants relative to wild-type plants (SI Appendix, Fig. S10). Together, these results suggest that LPE1 is involved in the translation of psbA mRNA.

**LPE1 Interacts with HCF173 and Facilitates the Association of HCF173 with psbA mRNA.** Previous studies indicated that HCF173 and HCF244 are key regulators of psbA mRNA translation (17, 18). As LPE1 also regulates psbA mRNA translation in a similar manner, we explored the interaction of LPE1 with HCF173 and HCF244. To determine whether LPE1 interacts with HCF173 and HCF244, we first performed bimolecular fluorescence complementation (BiFC) analysis using an *Arabidopsis* protoplast transient expression system. We used two chloroplast proteins, HHL1 (HYPERSENSITIVE TO HIGH LIGHT1) fused with the N terminus of YFP (YN) and LQY1 (LOW QUANTUM YIELD OF PHOTOSYSTEM II) fused with the C terminus of YFP (YC), as a positive control, as reported in our previous work (32). Coexpression of LPE1-YN and HCF173-YC resulted in significant fluorescence in protoplast chloroplasts, but coexpression of LPE1-YC with HCF244-YN did not (Fig. 5A), suggesting that LPE1 interacted with HCF173 but not with HCF244. No fluorescence was detected in protoplasts cotransformed with LPE1-YC and HHL1-YN or with HCF173-YN and LQY1-YC (Fig. 5A), suggesting that LPE1 interacted specifically with HCF173. To confirm this, we performed a coimmunoprecipitation (CoIP) assay using HCF173 antibody (SI Appendix, Fig. S11). This also indicated that LPE1 interacted with HCF173 (Fig. 5B), and the interaction was further corroborated by yeast two-hybrid (Y2H) analysis (Fig. 5C).

HCF173 associates with other proteins as part of a higher molecular weight complex (18). To investigate whether LPE1 was part of this complex, solubilized chloroplast membrane proteins from LPE1-FLAG and wild-type plants were analyzed using nondenaturing BN-PAGE in the first dimension followed by separation on denaturing SDS gels in the second dimension. The gels were subjected to immuno blot analysis to detect fusion proteins. LPE1 colocalized with HCF173 (Fig. 5D), suggesting that they were part of the same supercomplex and supporting the idea that LPE1 and HCF173 form a complex to regulate the translation of psbA mRNA. Considering the association of LPE1 with psbA mRNA, we further analyzed whether the interaction between LPE1 and HCF173 was affected by psbA mRNA. The degradation of psbA mRNA via RNase treatment or the addition of in vitro-transcribed psbA mRNA in RIP assays (SI Appendix, Fig. S12A) revealed that the LPE1–HCF173 interaction was unaffected by psbA mRNA abundance (SI Appendix, Fig. S12B).

HCF173 associates with psbA mRNA and regulates its translation (18), although the mechanism remains unclear. RIP and EMSA analyses indicated a direct interaction between LPE1 and psbA mRNA, suggesting that the association between HCF173 and psbA mRNA may be mediated by LPE1. To test this, we used RIP analysis to determine the effect of LPE1 deficiency on the association between HCF173 and psbA mRNA. This association was significantly lower in lpe1-3 mutants than in wild-type plants (Fig. 5E). The LPE1 deficiency did not affect the abundance of HCF173 (SI Appendix, Fig. S13), excluding the possibility that the decreased association between HCF173 and psbA mRNA resulted from lower levels of HCF173. To further check
whether HCF173 deficiency affected the association of LPE1 with psbA mRNA, we used virus-induced gene silencing (VIGS) to suppress the expression of HCF173 in LPE1-FLAG transgenic plants. Compared with LPE1-FLAG transgenic plants transformed with VIGS-HCF173 vector, the LPE1-FLAG transgenic plants transformed with VIGS-HCF173 vector exhibited >90% reduction in HCF173 expression (SI Appendix, Fig. S14 A and B) and a significant reduction in the level of D1 protein (SI Appendix, Fig. S14 C), implying that D1 translation mediated by LPE1 contributes to the recovery of PSI activity.

Next, we determined whether, 8, 24, or 48 h of light-induced greening induced the transcription of PSI-related genes including psbA and LPE1. We found significant levels of transcription of the plastid genes, including psaA, psaB, psbA, psbB, and psbD, in etiolated seedlings in constant-dark conditions. Their transcription increased slightly following light exposure (Fig. 5 A and SI Appendix, Fig. S15 A and B), implying that D1 translation mediated by LPE1 contributes to the recovery of PSI activity.

Light Promotes the Association of LPE1 with psbA mRNA in a Redox-Dependent Manner. Previous studies showed that D1 synthesis is light regulated in higher plants (6), but the mechanism is unclear. To determine whether LPE1 is involved, we first established a system of light induction to control photosystem biogenesis in Arabidopsis. After 5 d of etiolated growth in the dark, seedlings were exposed to growth light (100 μmol photons m−2 s−1) for 0, 8, 24, or 48 h. The leaves of wild-type seedlings gradually turned green when exposed to increasing periods of illumination, and Fv/Fm, ΦPSII, and ETR increased simultaneously (SI Appendix, Fig. S15 A and B), confirming the reliability of the system of light induction. However, LPE1-deficient lpe1-3 seedlings showed no response in Fv/Fm, ΦPSII, and ETR during light-induced greening (SI Appendix, Fig. S15 A and B), implying that D1 translation mediated by LPE1 contributes to the recovery of PSI activity.

The resolved proteins were immunodetected using anti-D1, anti-D2, anti-CP43, anti-CP47, and anti-HCF173 antibodies. I: PSII-LHCII; II: PSII core monomer; III: PSII core monomer minus CP43; IV: LHCII trimer; V: unassembled protein. (E) The effect of LPE1 deficiency on the association of HCF173 with psbA mRNA. The y axis represents the amount of RNA that coimmunoprecipitates with HCF173 in the lpe1-3 mutants compared with the amount in the wild type (Col-0) in a quantitative RT-PCR assay (**P < 0.01; Student’s t test). All experiments were repeated at least three times with similar results.
Vitis vinifera mRNA was detected in the dark Populus trichocarpa mRNA. A BLASTP search using the full-length LPE1 se-
mRNA in mature LPE1-FLAG plants. Leaves were harvested from 4-wk-old LPE1-FLAG transgenic green leaves in both light and dark conditions (Fig. 6).

We further confirmed the effect of light and redox state on the association of LPE1 with psbA mRNA in mature LPE1-FLAG transgenic green leaves in both light and dark conditions (Fig. 6F). First, similar to light-induced greening, LPE1 protein associated with a greater amount of psbA mRNA following light exposure for 6 or 12 h, although a significant level of basal association of LPE1 with psbA mRNA was detected in the dark (Fig. 6G). Next, the redox state of LPE1 was determined from its mobility using nonreducing SDS/PAGE upon the binding of 4-acetamido-4-maleimidylstibene-2,2-disulfonic acid (AMS). This approach allowed us to distinguish between the oxidized and reduced forms of LPE1 protein (42). The reduced form of LPE1 protein was increased following DTT treatment, confirming that LPE1 is regulated by the stromal redox state. In comparison with dark conditions, the amount of the reduced form of LPE1 protein gradually increased following light exposure for 6 or 12 h (Fig. 6H). Additionally, the reduced form of LPE1 protein was also detected in dark conditions in mature plants (Fig. 6H), which is consistent with a significant level of basal association of LPE1 with psbA mRNA in the dark, implying that mature chloroplasts possess a basal level of reducing power in the dark. These results suggest that light regulates the association of LPE1 with psbA mRNA through the modulation of the redox state of LPE1 protein.

**LPE1 Homologs Are Found Exclusively in Land Plants.** HCF173 homologs are present in land plants, green algae, and cyanobacteria (18). Based on the functional similarity of LPE1 and HCF173 in terms of D1 synthesis, we determined whether LPE1, like HCF173, has been evolutionarily conserved, as might be expected for a protein involved in regulating the translation of psbA mRNA. A BLASTP search using the full-length LPE1 sequence was performed to search the genomes of other photosynthetic species for LPE1 homologs. Homologs were identified in many land plants, including the bryophyte moss Physcomitrella patens, the dicots Papulus trichocarpa, Vitis vinifera, Cucumis...
sativus, Ricinus communis, Glycine max, Fragaria vesca, and Solanum lycopersicum, and the monocots Oryza sativa, Zea mays, Hordeum vulgare, and Aegilops tauschi. However, LPE1 homologs were not found in more primitive photosynthetic organisms such as cyanobacteria and algae (SI Appendix, Figs. S21 and S22), and thus the evolutionary distribution of LPE1 differs from that of HCF173.

Discussion

PSII biogenesis requires the efficient synthesis of PSII subunits, especially of the reaction center protein D1 that exhibits highly dynamic turnover under variable light conditions. Although the regulation of D1 degradation is well known (4), the mechanism of D1 synthesis remains poorly understood. We identified a nuclear-encoded chloroplast PPR protein, LPE1, required for light-regulated D1 translation during PSII biogenesis.

Several lines of evidence support a vital role for LPE1 in D1 translation during PSII biogenesis. First, the specific decrease in PSII complexes and subunits (observed in BN-PAGE and Western blot analyses; Fig. 2 and SI Appendix, Figs. S2 and S3) together with significantly reduced PSI RNA activity (Fig. 1 and SI Appendix, Table S1) in lpe1 mutants suggested that LPE1 is involved in the biogenesis of PSII complexes but not in other photosynthetic complexes. Second, a systematic RIP analysis of all the plastid-encoded PSII-related RNAs suggested that psbA mRNA, which encodes D1 protein, specifically associates with LPE1, a chloroplast PPR protein (Fig. 3 and SI Appendix, Fig. S5). Their direct association was confirmed using EMSA analysis (Fig. 3F).

These results were consistent with psbA mRNA being the major target of LPE1. Third, in vivo protein labeling revealed a drastic reduction in D1 levels in lpe1 mutants (Fig. 4F) but unaltered expression levels or transcript patterns of psbA (Fig. 4A and SI Appendix, Fig. S9), suggesting that LPE1 is required for D1 translation. Finally, polymosome association experiments found impaired ribosomal loading of psbA mRNA in lpe1 mutants (Fig. 4 B–E), supporting the conclusion that LPE1 plays a key role in D1 translation during PSII biogenesis. The synthesis and membrane insertion of D1 occur in a concerted manner at the thylakoid membrane (5). This was confirmed by the distribution of LPE1 in thylakoids and stroma (Fig. 3B and SI Appendix, Fig. S6), which resembles that of HCF173, a known activator of D1 translation (18), and by comigration of LPE1/ HCF173 with PSI monomer and PSII supercomplexes of higher molecular weight (Fig. 5D). These data all imply that psbA mRNA translation involves migration from stroma to thylakoids.

We could not exclude the possibility that LPE1 has other targets or roles, as RNA targets were screened based on the assumption that LPE functioned mainly as a PPR protein in RNA regulation (19). Transcript levels of many NDH-related genes and of a few PSII genes such as psbM decreased to different extents in lpe1 mutants (SI Appendix, Fig. S9). In addition, synthesis of Psal/B and CP47 was reduced slightly, although these reductions were much smaller than the reduction in D1 synthesis (Fig. 4F). RIP analysis did not find an obvious association between LPE1 and psal/B or psbB mRNA, which encodes CP47 (Fig. 3 C–E and SI Appendix, Fig. S7), suggesting that the reduction in Psal/B and CP47 might be an indirect effect of LPE1 deficiency. It is also possible that defects in one PSI subunit can delay the assembly of PSII complexes and hence disturb the synthesis of other subunits, according to a previous study (34).

Furthermore, delayed assembly of PSII complexes also can accelerate the degradation of PSI subunits (34), which is consistent with increased degraded D1 fragment in lpe1 mutants in this study (SI Appendix, Fig. S10). PSI repair involves the disassembly and reassembly of the PSII complex. As D1 exhibits a high turnover (2), its synthesis is essential for PSI reassembly. We found that exposure to high light aggravated the defect in D1 accumulation in lpe1 mutants due to faster degradation but inefficient synthesis of D1 (SI Appendix, Fig. S10) and also caused more serious photoinhibition of PSII (qY) (SI Appendix, Table S1), implying that LPE1 deficiency also may affect PSI repair. Thus, the reduced growth and photosynthetic activity of lpe1 mutants (Fig. 1) probably resulted from comprehensive effects including D1 defects and other indirect targets.

Regulation of translation in the chloroplast involves cis-acting RNA elements located in the 5′ UTR of mRNA and a set of corresponding transacting protein factors (43). A previous study showed that chloroplast PPR proteins (such as PGR3) are involved in RNA translation in chloroplasts through binding to the 5′ UTR of target mRNAs, including petL and ndhA (23). Although the 5′ UTR is important for activating the translation of psbA mRNA (44), little is known about the proteins that bind the 5′ UTR in higher plants. As our EMSA analysis indicated that LPE1 bound the 5′ UTR of psbA mRNA directly (Fig. 3F), it may be an activator of psbA translation. However, LPE1 differs from typical PPR proteins, which have conserved amino acid sequences. The specific sequences in the 5′ UTR of psbA mRNA recognized by LPE1 therefore remain uncertain, due to the atypical codons encoding the protein’s PPR motifs, and we could not exclude the possibility that it was the overall protein structure of LPE1, rather than particular amino acids, that enabled its association with the 5′ UTR of psbA mRNA.

HCF173, together with HCF244, plays a role in initiating D1 translation in higher plants such as Arabidopsis thaliana (17, 18). BiC experiments showed a direct and specific interaction of LPE1 with HCF173 but not with HCF244 (Fig. S4). The direct interaction between LPE1 and HCF173 was confirmed by Y2H and CoIP analyses (Fig. 5 B and C) and by their comigration (Fig. 5D), suggesting that LPE1 and HCF173 form a complex functioning in D1 translation. Although previous studies show that HCF173 is a key regulator of D1 translation, HCF173 lacks a distinguishable RNA-binding motif (18), and thus the details of its association with psbA mRNA remain elusive. Our results showed that LPE1 deficiency significantly reduces the association of HCF173 with psbA mRNA (Fig. 5E) but does not affect HCF173 abundance (SI Appendix, Fig. S13). However, HCF173 deficiency does not affect the association of LPE1 with psbA mRNA.
mRNA (SI Appendix, Fig. S14). These data suggest that LPE1 acts as a bridging factor to facilitate the association of HCF173 with psbA mRNA. As a proportion of psbA mRNA associates with HCF173 in LPE1-knockout mutant plants (Fig. 5E), HCF173 may bind mRNA directly by uncharacterized RNA-binding motifs or other unknown regulators; functional redundancy of HCF173 with LPE1 may also facilitate its association with psbA mRNA. However, our results indicate that the interaction between LPE1 and HCF173 does not depend on psbA mRNA (SI Appendix, Fig. S12), thus reducing the likelihood of direct binding of HCF173 with psbA mRNA. To further clarify the mechanism of D1 protein synthesis, additional regulators of psbA mRNA translation need to be identified.

Light is a vital environmental signal that regulates the expression of plastid genes and photosystem biogenesis (11). Our results indicate that D1 synthesis is controlled mainly by light at the translational level in Arabidopsis (Fig. 6 A and B), consistent with previous reports in barley (6, 40, 45). Genetic and biochemical studies in Chlamydomonas indicate that light regulates translation by modulating the binding of activator proteins to the 5′ UTR of psbA mRNAs (12). Translation of psbA mRNA is also regulated by light via the 5′ UTR in higher plants such as tobacco (44, 46), although the regulators have not been identified. We found that the association between LPE1 and the 5′ UTR of psbA mRNA was light dependent (Fig. 6 C and G); this observation, together with the slow and slight response of PSII activity in the absence of LPE1 during PSII biogenesis (SI Appendix, Fig. S15 A and B), support the idea that LPE1 mediates light-regulated D1 translation by regulating its association with psbA mRNA (Fig. 7). We further found that redox state affects the association between LPE1 and psbA mRNA (Fig. 6 D and E). A bioinformatics analysis found several conserved cysteines in LPE1 (SI Appendix, Fig. S22), and AMS labeling assays indicate that light regulates the redox state of LPE1 (Fig. 6H), implying that light regulates RNA-binding activity through redox modulation of disulfides of LPE1. This resembles the mechanism by which translation activators associate with psbA mRNA in Chlamydomonas (41), although the trans regulatory factors differ greatly (12–15) due to the large differences in psbA mRNA 5′ UTR sequences between the two species (47). In addition, redox-dependent binding of unidentified trans factors to the Arabidopsis psbA 5′ UTR confirms the importance of redox regulation in chloroplast translation in higher plants (47). The limited recovery of association between LPE1 and psbA mRNA following DTT treatment (Fig. 6 D and E) implies that their association is also regulated by changes other than redox state, including pH homeostasis, ADP/ATP ratio, and proton gradient.

Our results also show that LPE1 interacts with HCF173, and its association with the 5′ UTR of psbA mRNA is stimulated by light in a redox-independent manner (Fig. 6 D and E). The greater association of HCF173 with LPE1 may have resulted in part from the higher expression of HCF173 induced by light (Fig. 6 A–C), although LPE1 transcription was not affected (Fig. 6A and SI Appendix, Figs. S17 and S18). D1 translation may thus be regulated by light at several levels, including control of association of psbA mRNA with activators mediated by LPE1 and modulation of transcription in the nucleus through HCF173. However, LPE1 and of HCF173 have diverse roles in D1 synthesis during PSII biogenesis. First, HCF173 affects the abundance of psbA mRNA as well as initiating translation of D1, but LPE1 is involved specifically in D1 translation (18). Second, although LPE1 homologs are found exclusively in land plants (SI Appendix, Figs. S21 and S22), HCF173 homologs are also present in other photosynthetic organisms such as algae, suggesting different evolutionary distributions of LPE1 and HCF173. [However, it should be noted that HCF173 homologs in primitive photosynthetic organisms had low sequence similarity and may also have different functions (18).] These findings indicate that higher plants and primitive photosynthetic organisms share conserved mechanisms to regulate D1 synthesis during PSII biogenesis but employ distinct regulatory factors.

Concluding Remarks

This study provides insights into PSII biogenesis in higher plants by identifying a crucial regulator of psbA mRNA translation and thus fills a major gap in the understanding the mechanism of light-regulated D1 synthesis (Fig. 7). These findings indicate that controlling plastid gene translation by light may be a vital strategy regulating the biogenesis and functional maintenance of the PSII complex in higher plants.

Materials and Methods

Plant Materials and Growth Conditions. All the T-DNA and transgenic Arabidopsis thaliana lines used in this study were in the Col-0 background. The lpe1-1, lpe1-2, and lpe1-3 mutants were obtained from the Arabidopsis Biological Resource Center (stock numbers SALK_095367, SALK_030882, and SALK_110539). Further details can be found in SI Appendix, SI Materials and Methods.

Analysis of Chlorophyll and Chlorophyll Fluorescence. Chlorophyll was extracted from 3-wk-old plants using 80% acetone in 2.5 mM Hepes, pH 7.5; the chlorophyll content was determined as previously described (48). Further details can be found in SI Appendix, SI Materials and Methods.

Details of additional experimental procedures, such as transmission electron microscopy, isolation of thylakoid membranes, RT-PCR and quantitative real-time RT-PCR, BR-BSAPAGE and immunoblot analyses, in vivo labeling of chloroplast proteins, RNA gel blot and polysome association analyses, subcellular localization of GFP fusions and BiFC, chloroplast fractionation and immunolocalization studies, analysis of D1 protein accumulation under high light, immunoprecipitation, RIP, EMSA assays, generation of antibodies, VIGS assay, determination of the redox state of LPE1 protein in vivo, and accession numbers can be found in SI Appendix, SI Materials and Methods.

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