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.

chloroplast | photosynthesis | photosystem II biogenesis | D1 synthesis | light regulation

The photosynthetic apparatus of cyanobacteria, eukaryotic algae, and vascular plants is located in the specialized thylakoid membrane system and includes the integral membrane protein complexes photosystem II (PSII), cytochrome b_6/f, photosystem I (PSI), and ATP synthase, which harvest light and transduce solar energy into chemical energy. PSII is one of three multisubunit protein complexes of the photosynthetic electron transport chain (1). It is localized in the thylakoid membranes of photosynthetically active organisms and functions as a water-plastoquinone oxidoreductase, which is vital for the initiation of photosynthesis and promotion of electron transport (2).

The PSII reaction center complex is composed of D1 and D2 proteins, the α and β subunits of cytochrome b_{559}, and the PsbI protein, which is capable of primary charge separation and subsequent electron transfer (3). The D1 protein has a higher turnover rate than any other thylakoid protein in the light. D1 protein turnover operates to repair PSII centers damaged by light (2), and replacement of damaged D1 protein is a tightly synchronized process (4). In plant chloroplasts, the insertion of D1 protein into the thylakoid membrane and assembly with other PSII proteins occurs cotranslationally (5). In addition, the accumulation of D1 protein depends on environmental signals, including light and developmental stage (6–8). Thus, regulation of D1 protein synthesis is important both for the correct biogenesis of PSII during chloroplast development and for maintenance of a functional photosystem.

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 ADP/ATP ratio (16). In the higher plant *Arabidopsis thaliana*, only two regulators of *psbA* mRNA translation, HCF173 and

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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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 ubiquitous in eukaryotes 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 PSII 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 PSII activity, using chlorophyll fluorescence analysis and found that LPE1 was involved in PSII 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 PSII function and identify auxiliary factors required for this process, we screened many pools of *Arabidopsis* mutants using a chlorophyll fluorescence video imaging system (32). *Arabidopsis* plants showing aberrant maximum photochemical efficiency [variable fluorescence/maximum fluorescence (F_v/F_m)] of PSII were identified to obtain a series of *low photosynthetic efficiency* (*lpe*) mutants. One of these, *lpe1-1* (SALK_059367, At3g46610), had a lower F_v/F_m 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 *lpe1-1*, *lpe1-2*, and *lpe1-3*, respectively, throughout. The T-DNA insertion was located in the 5′ UTR in *lpe1-1* and *lpe1-2* mutant alleles and in the coding sequence (CDS) in the *lpe1-3* 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 *lpe1-1* and *lpe1-2*, and the transcript could not be detected in *lpe1-3* plants (Fig. 1*A*). This analysis of three independent *lpe1* alleles expressing different levels of *LPE1* suggested that the changes in PSII function and plant growth depended on LPE1; in particular, *lpe1-3*, the *LPE1*-knockout mutant, showed a drastic retardation of photoautotrophic growth (Fig. 1*B* and *SI Appendix*, Fig. S1 *C* and *D*). The *lpe1* phenotype thus appears to result from the inactivation of At3g46610.

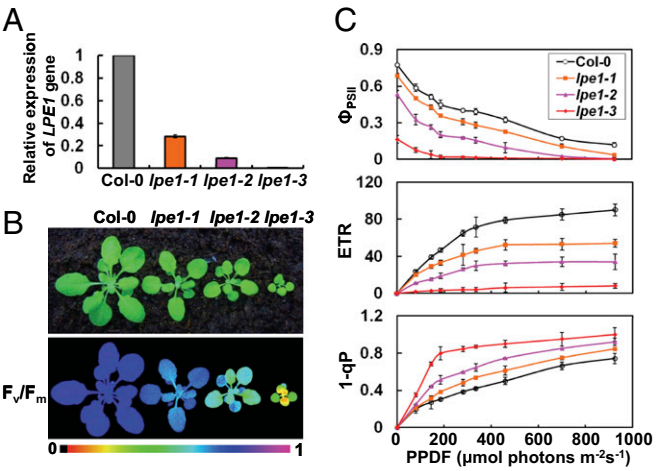

Fig. 1. Mutations in *LPE1* reduce PSII activity. (*A*) Quantitative real-time RT-PCR analysis of *LPE1* transcription in 4-wk-old wild-type (Col-0), *lpe1-1*, *lpe1-2*, and *lpe1-3* plants. (*B*) Images and false-color images representing F_v/F_m of 4-wk-old wild-type (Col-0), *lpe1-1*, *lpe1-2*, and *lpe1-3* plants. The false-color scale ranges from black (0) via red, orange, yellow, green, blue, and violet to purple (1) as indicated below the false-color images. (*C*) Light–response curves Φ_{PSII}, ETR, and 1-qP in 4-wk-old wild-type (Col-0), *lpe1-1*, *lpe1-2*, and *lpe1-3* plants. The measurements were performed at light intensities of 0, 81, 145, 186, 281, 335, 461, 701, and 926 μmol photons·m^{-2}·s^{-1}. Six biological replicates were performed in all experiments, and similar results were obtained.

Initial F_v/F_m measurements suggested that PSII activity was disturbed in the *lpe1* mutants. To further investigate the primary effect of *lpe1* mutations, the minimum (F_0) and maximum (F_m) fluorescence of dark-adapted leaves, F_v/F_m, and other chlorophyll parameters were quantitatively determined under a fixed light intensity (*SI Appendix*, Table S1). Our results indicated that the reduction of F_v/F_m in *lpe1* mutants was primarily caused by the increase in F_0. In addition, the *lpe1* mutants showed much higher levels of nonphotochemical quenching (NPQ) than wild-type plants, indicating that they dissipated more excess excitation energy via nonphotochemical pathways. NPQ 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 NPQ 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 PSII quantum yield (Φ_{PSII}), the electron transport rate (ETR), and the redox state of the Q_A electron acceptor of PSII (1-qP). The Φ_{PSII} and ETR were much lower in *lpe1* mutants than in wild-type plants (Fig. 1*C*), as also observed for F_v/F_m (Fig. 1*B*). Interestingly, 1-qP, which reflects the redox state of the Q_A electron acceptor of PSII (30), was higher in the *lpe1* mutants (Fig. 1*C*), suggesting a more highly oxidized plastoquinone pool in the plants. This is more likely to result from a PSII deficiency than from downstream defects (33). These results suggest that disruption of *LPE1* specifically affected photosynthetic activity of PSII.

LPE1 Deficiency Specifically Affects PSII Biogenesis and the Formation of Grana Thylakoid in Chloroplast. Our results showed that PSII photosynthetic activity was reduced in *lpe1* mutants, suggesting that LPE1 was involved in the functional regulation of the PSII 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. 2*A*), apparently representing PSII–LHCII supercomplexes, dimeric PSII, monomeric PSII, CP43 minus PSII, and trimeric LHCII (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. 2 *A* 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.

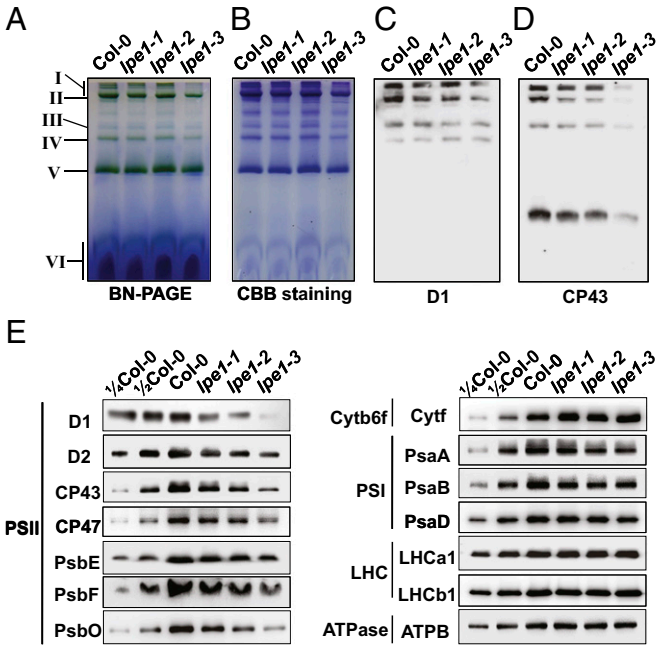

Fig. 2. Analysis of PSII biogenesis in wild-type plants and *lpe1* mutants. (*A*) Representative unstained BN-PAGE gel. Thylakoid membranes from wild-type (Col-0) and *lpe1* mutant plants were solubilized with 2% DM and separated using native PAGE. Samples containing equal amounts of chlorophyll (10 μg) were loaded in each lane. I: PSII-LHCII; II: PSII dimer; III: PSII core monomer; IV: PSII core monomer minus CP43; V: LHCII trimer; VI: unassembled protein. (*B*) Representative BN-PAGE gel stained with Coomassie brilliant blue (CBB). (*C*) Representative example of immunoblotting using anti-D1 antiserum to probe a BN-PAGE gel; 1.5 μg of chlorophyll was loaded in each lane. (*D*) Representative example of immunoblotting using anti-CP43 antiserum to probe a BN-PAGE gel; 1.5 μg of chlorophyll was loaded in each lane. All experiments involved three independent biological replicates, which produced similar results. (*E*) Thylakoid membrane proteins from wild-type (Col-0) and *lpe1* mutant plants were separated using 15% SDS-urea-PAGE. Gels were electroblotted to PVDF membranes and probed with antisera against specific thylakoid membrane proteins. Samples were loaded on an equal chlorophyll basis. ATPase, ATP synthase complex; ATPB, β subunit of ATP synthase complex; Cytb6/*f*, cytochrome b6/f complex; LHC, light-harvesting complex. Similar results were obtained from four independent biological replicates.

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, CP47, PsbE, PsbF, and PsbO were detected in *lpe1* mutants (Fig. 2*E* and *SI Appendix*, Fig. S3). Protein levels of the PSI core subunits PsaA and PsaB were also reduced slightly. By contrast, levels of the PSI protein PsaD, the LHCII chlorophyll a/b-binding proteins LHCa1 and LHCb1, ATP synthase subunit B, and cytochrome *f* were relatively consistent between wild-type and mutant plants (Fig. 2*E* and *SI Appendix*, Fig. S3). These results suggest that reductions in LPE1 levels perturb the accumulation of PSII complexes.

PSII and LHCII are restricted to grana thylakoids and therefore are segregated from PSI, LHCI, 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 PSII-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. 3*A*). 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. 3*B*).

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 $CaCl_2$, and it was effectively removed by 0.2 M Na_2CO_3 or urea (*SI Appendix*, Fig. S6). PsbO (the 33-kD luminal protein of PSII) and D1 (the PSII 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.

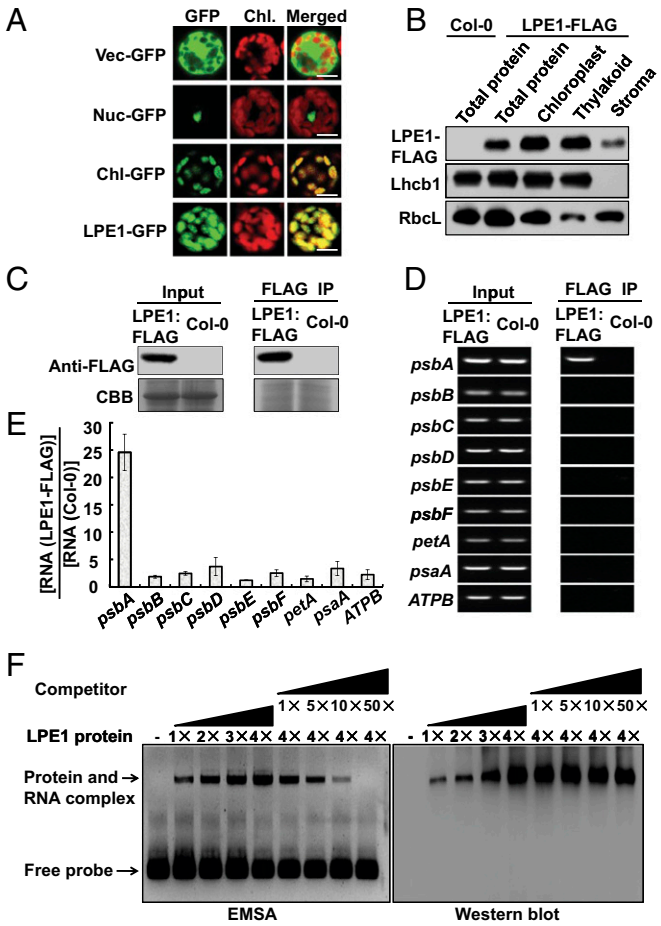

Fig. 3. Subcellular localization of LPE1 and association of LPE1 with *psbA* mRNA in vivo and in vitro. (*A*) Localization of LPE1 within the chloroplast by GFP assay. Chl, chlorophyll; Chl-GFP, chloroplast control; LPE1-GFP, LPE1-GFP fusion; Nuc-GFP, nuclear control; Vec-GFP, empty vector control. (Scale bars: 10 μm.) (*B*) LPE1 localizes to the thylakoid membrane and stromal fraction. Intact chloroplasts were isolated from leaves of LPE1-FLAG transgenic plants and then were separated into thylakoid membrane and stromal fractions. Polyclonal antibodies were used against the integral membrane protein Lhcb1, the abundant stroma protein, ribulose bisphosphate carboxylase large subunit (RbcL), and LPE1-FLAG. Total proteins extracted from wild-type and LPE1-FLAG transgenic plants were used to confirm anti-FLAG antibody specificity. (*C*) Western blots of proteins present in crude leaf extracts from Col-0 and LPE1-FLAG transgenic plants and RNA immunoprecipitated using the anti-FLAG antibody. (*D*) Association of *psbA* mRNA with LPE1 detected using RT-PCR. (*E*) Quantification of the association between *psbA* mRNA and LPE1 detected using quantitative RT-PCR. Five additional independent biological replicates were performed, and each produced similar results. (*F*) EMSAs containing the full-length *psbA* 5′ UTR probe. (*Left*) The positions of RNA–protein complexes and unbound RNA are indicated at the left of the panel. (*Right*) The same fractions were analyzed using immunoblotting with an anti-His antibody. Increasing amounts of LPE1 were used for dose-dependent association. Increasing amounts of unlabeled competitors were used for competitive association. Three independent biological replicates were performed; a representative example is shown.

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. 3 *D* 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. 3*F*). 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. 3*F*). 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. 4*B*). 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-psbJ-psbL* polycistronic transcription unit and found comparable levels of association between *psbEFJL* transcript and polysomes in *lpe1* mutants and wild-type plants (Fig. 4 *C* and *D*). To confirm the distribution of small RNA particles (monosomes and free RNA) and polyribosomes 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* mRNAs into the monosome fractions in both wild-type and *lpe1* mutant plants (Fig. 4*E*), 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-α/β) were almost unchanged in mutant plants. However, incorporation of $^{[35S]}$Met into D1 was greatly

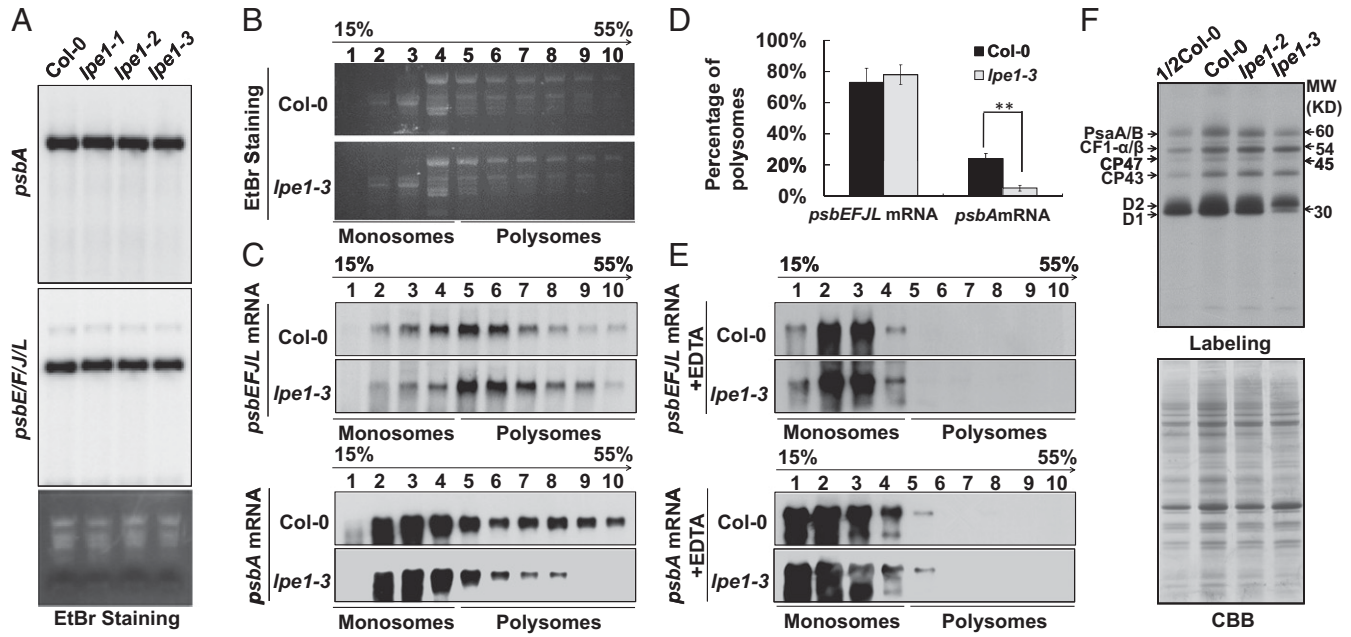

Fig. 4. Analyses of D1 expression in wild-type plants and *lpe1* mutants. (*A*) RNA gel blot analysis of transcript levels in wild-type and *lpe1* mutant plants. Transcripts of *psbA* and *psbEFJL* were detected by probing the filter with the appropriate gene-specific probes. Samples with equal amounts of total RNA from wild-type and *lpe1* plants were size-fractionated using agarose gel electrophoresis, transferred to a nylon membrane, and probed with digoxigenin-labeled probes. (*B–D*) Analysis of the association of *psbA* mRNA with polysomes. Whole-cell extracts were fractionated in a linear sucrose gradient (15–55%) by ultracentrifugation, and 10 fractions of equal volume were analyzed via gel electrophoresis of methylene blue-stained samples (*B*), RNA gel blot analysis using *psbA*- and *psbEFJL*-specific probes (*C*), and quantification of mRNAs associated with polysomes using Phoretix 1D software (*D*). The values (mean ± SE; $n = 3$ independent biological replicates) are given as the ratios of mRNAs associated with polysomes to total mRNAs; $^{**}P < 0.01$; Student's *t* test. (*E*) Cell extracts were treated with EDTA to disrupt polysome association. (*F*) In vivo analysis of protein synthesis in wild-type and *lpe1* plants. $^{[35S]}$Met was incorporated into thylakoid membrane proteins in young wild-type and *lpe1* mutant seedlings. The thylakoid membranes were isolated from *Arabidopsis* seedlings following a 20-min pulse in the presence of cycloheximide. Proteins were separated using SDS-urea-PAGE and visualized autoradiographically. Coomassie brilliant blue (CBB) was used to stain the gel to quantify the amounts of total protein.

reduced in the *lpe1-3* mutant (Fig. 4*F*), 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 II1) fused with the C terminus of YFP (YC), as a positive control, as reported in our previous work (32). Coexpression of LPE1-YC and HCF173-YN resulted in significant fluorescence in protoplast chloroplasts, but coexpression of LPE1-YC with HCF244-YN did not (Fig. 5*A*), 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. 5*A*), 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. 5*B*), and the interaction was further corroborated by yeast two-hybrid (Y2H) analysis (Fig. 5*C*).

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 immunoblot analysis to detect fusion proteins. LPE1 colocalized with HCF173 (Fig. 5*D*), 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. S12*A*) revealed that the LPE1–HCF173 interaction was unaffected by *psbA* mRNA abundance (*SI Appendix*, Fig. S12*B*).

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. 5*E*). 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

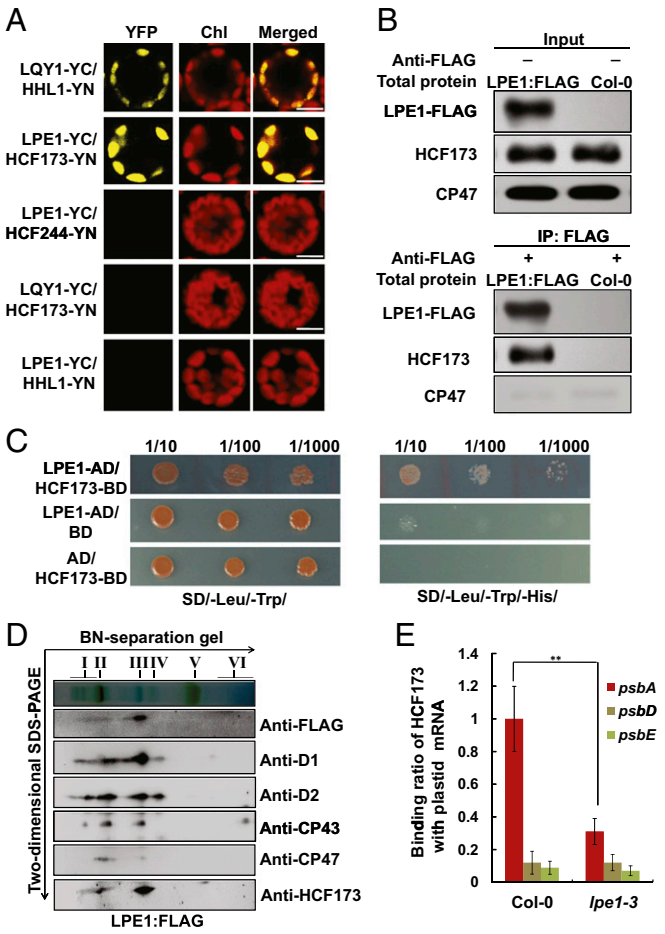

Fig. 5. Analysis of the interaction between LPE1 and HCF173. (*A*) BiFC analysis of *Arabidopsis* protoplasts showing the interaction between LPE1 and HCF173. LPE1-YC and HCF173-YN or HCF244-YN were cotransfected into protoplasts and visualized using confocal microscopy. As a positive control, HHL1-YN and LQY1-YC were cotransfected into protoplasts, as reported by Jin et al. (32). As negative controls, LPE1-YC and HHL1-YN and LQY1-YC and HCF173-YN were cotransfected into protoplasts. (Scale bars: 10 μm.) (*B*) CoIP assays confirming the interaction of LPE1 with HCF173. Chloroplast proteins from wild-type (Col-0) and LPE1-FLAG transgenic plants were incubated with Protein A/G-coupled anti-FLAG antiserum. The immunoprecipitates were probed with specific antibodies, as indicated. Bound proteins were eluted, separated using SDS/PAGE, and subjected to immunoblot analysis with His-tag and LPE1 antibodies. (*C*) Y2H assays confirming that LPE1 interacts with HCF173. LPE, fused with AD vector and HCF173 fused with BD vector were cotransfected into yeast. As negative controls, LPE1 fused with AD and empty vector BD and HCF173 fused with BD and AD were cotransfected into yeast. (*D*) Colocalization analysis of LPE1 and HCF173. Immunoblot analysis of thylakoid proteins from LPE1-FLAG plants separated in the first and second dimensions. The high molecular weight complex was separated in the first dimension using BN-PAGE and in the second dimension by SDS/PAGE. The resolved proteins were immunodetected using anti-D1, anti-D2, anti-CP47, anti-CP43, anti-FLAG, and anti-HCF173 antibodies. I: PSII-LHCII; II: PSII dimer; III: PSII core monomer; IV: PSII core monomer minus CP43; V: LHCII trimer; VI: 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* mutant 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.

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-GFP vector (control plants), the LPE1-FLAG transgenic plants transformed with VIGS-HCF173 vector exhibited >90% reduction in HCF173 expression (*SI Appendix*, Fig. S14*A*) and a significant reduction in the level of D1 protein (*SI Appendix*, Fig. S14*B*) but no change in the association of LPE1 with *psbA* mRNA (*SI Appendix*, Fig. S14*C*). These results suggest that LPE1 regulates *psbA* mRNA translation by facilitating the association between HCF173 and *psbA* mRNA.

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 F_v/F_m, Φ_{PSII}, 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 F_v/F_m, Φ_{PSII}, 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 PSII activity.

Next, we determined whether 8, 24, or 48 h of light-induced greening induced the transcription of PSII-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. 6*A* and *SI Appendix*, Fig. S16). These results are consistent with those of previous reports (40), suggesting that light is not strictly required for the transcription of genes encoding PSII core subunits. However, the expression of the nuclear genes *Lhcb1* and *HCF173* increased greatly following light exposure relative to expression in etiolated control plants. Surprisingly, expression of the nuclear gene *LPE1* was not significantly affected by light (Fig. 6*A* and *SI Appendix*, Figs. S16–S18). Protein levels of D1 and other PSII subunits were monitored during light-induced greening. Levels of D1 and other photosystem proteins, including the PSII core protein D2, CP47, PsbO, and the LHC subunits Lhca1 and Lhcb1, drastically increased to strikingly higher levels following exposure to light (Fig. 6*B*). Levels of HCF173 were also increased by light, although it could be detected in etiolated seedlings under dark conditions (Fig. 6*B*). This suggested that expression of the PSII core proteins, including D1, encoded in the plastid is primarily controlled by light at the translational level during PSII biogenesis.

The suggestion that binding of LPE1 to the 5′ UTR of *psbA* mRNA promoted D1 translation led us to determine whether the association of LPE1 with *psbA* mRNA was affected by light. A given quantity of LPE1 protein associated with a greater amount of *psbA* mRNA following light exposure for 8, 24, or 48 h; the negative controls *psbB*, *psbC*, and *psbD* did not associate with LPE1 regardless of light exposure (Fig. 6*C*), suggesting that light specifically promotes the association between LPE1 and *psbA* mRNA. Furthermore, the quantity of HCF173 associated with LPE1 increased during light exposure (Fig. 6*C* and *SI Appendix*, Fig. S19). As redox potential can transduce a light stimulus to regulate translation of chloroplast mRNA in *Chlamydomonas* (41), we measured the effect of redox reagents on the association of LPE1 with *psbA* mRNA. Analysis using an in vitro EMSA indicated that the association decreased following treatment with the oxidizing agent 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB), but application of the reducing agent DTT enabled recovery of the association (Fig. 6*D*). In vivo RIP analysis also indicated that, under 48-h light exposure, DTT treatment increased the association of LPE1 with *psbA* mRNA, and

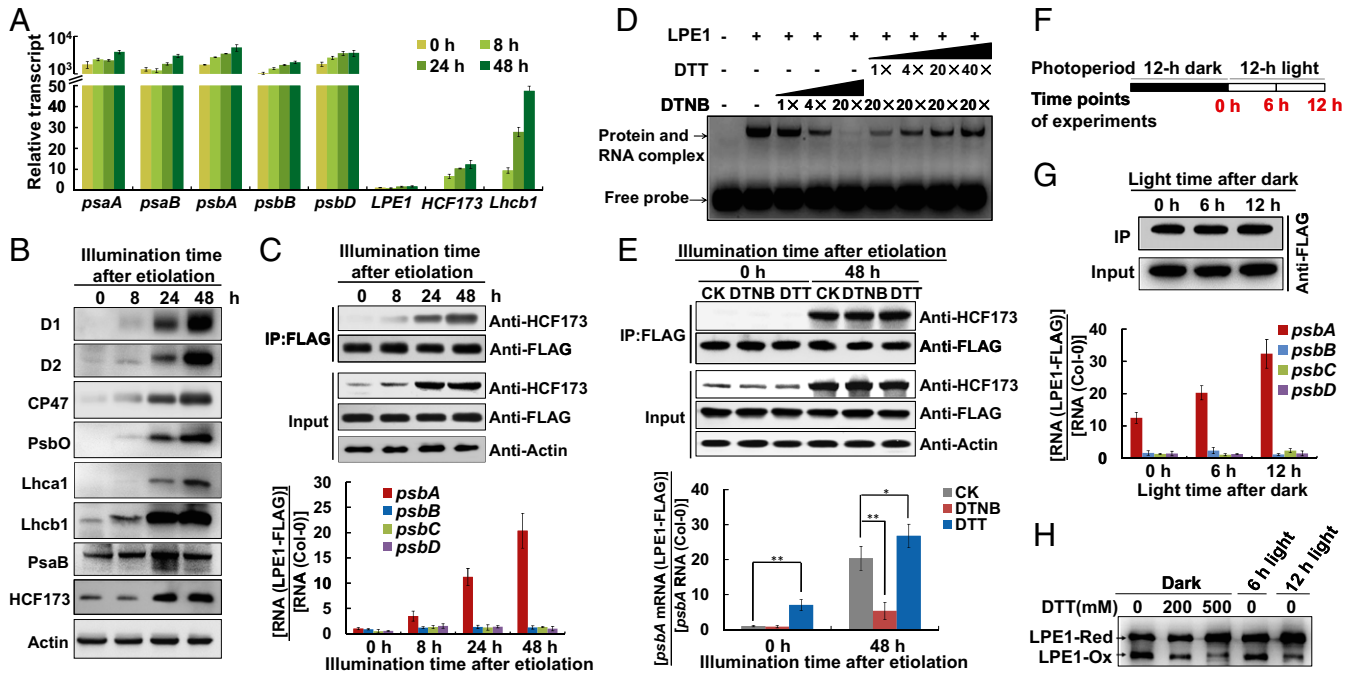

Fig. 6. LPE1 is involved in light-regulated D1 synthesis by associating with *psbA* mRNA in a redox-dependent manner. (*A*) Quantitative PCR analysis of *psbA*, *LPE1*, and other photosynthesis-related genes during light-induced greening of etiolated wild-type seedlings as described in *SI Appendix*, Fig. S15. (*B*) Western blot analysis of D1 and other photosystem proteins isolated from etiolated wild-type seedlings during light-induced greening as described in *SI Appendix*, Fig. S15. (*C*) Analysis of the association of LPE1 with HCF173 and *psbA* mRNA during light-induced greening of etiolated seedlings. Wild-type and *35S::LPE1:FLAG* transgenic seedlings were grown in the dark for 5 d, and etiolated seedlings were illuminated for 0, 8, 24, or 48 h. Protein complexes were immunoprecipitated using anti-FLAG antibody. (*Upper*) Western blot analysis of proteins present in crude leaf extracts and immunoprecipitated (IP) with anti-FLAG antibody. The blot was developed with anti-FLAG and anti-HCF173 antibodies and shows the enrichment of LPE1-FLAG protein in samples derived from the transgenic line. (*Lower*) RIP-qPCR analysis of the association between *psbA* transcripts and LPE1-enriched complexes in comparison with the corresponding input sample. (*D*) EMSA showing the effect of redox reagents on the association of LPE1 with *psbA* mRNA in vitro. A full-length *psbA* 5′ UTR probe was incubated with LPE1 proteins, and the oxidizing agent DTNB or the reducing agent DTT was added to the reaction. (*E*) The effect of redox reagents on light-induced association of LPE1 with HCF173 and *psbA* mRNA in vivo in the presence of DTT or DTNB. (*Upper*) Western blot analysis of the effect of redox reagents on the light-induced association of LPE1 with HCF173. (*Lower*) RIP-qPCR analysis of the effect of redox reagents on the light-induced association of LPE1 with *psbA* mRNA (*$P < 0.05$; **$P < 0.01$; Student's *t* test). (*F*) Schematic representation of the experimental set-up used in *G* and *H*. Four-week-old LPE1-FLAG transgenic *Arabidopsis* plants were maintained under a 12-h light/12-h dark cycle, and leaves were harvested at 0, 6, or 12 h into the subjective day. (*G*) RIP-qPCR analysis of the association of LPE1 with *psbA* mRNA in mature LPE1-FLAG transgenic *Arabidopsis* plants. Leaves were harvested from 4-wk-old LPE1-FLAG transgenic *Arabidopsis* plants maintained in the dark for 12 h and exposed to light for 0, 6, or 12 h, as described in *F*. (*H*) Visualization of the redox status of LPE1 proteins by AMS assay in mature LPE1-FLAG transgenic *Arabidopsis* plants. Protein extracts from different light treatments (as described in *F*) were extracted under nonreducing conditions and labeled with AMS. Protein extracts from plants exposed to 0 h light after 12 h of dark treatment (dark) were treated with DTT. Samples were separated by nonreducing SDS/PAGE and immunoblotted with anti-FLAG antibody. The positions of reduced (LPE1-Red) and oxidized (LPE1-Ox) forms of LPE1 are indicated by arrows. All experiments were repeated at least three times, with similar results.

treatment with DTNB decreased it (Fig. 6*E* and *SI Appendix*, Fig. S20), suggesting a probable dependence on the redox state. Surprisingly, the association of LPE1 with *psbA* mRNA could be restored by DTT treatment in etiolated seedlings to a limited extent (Fig. 6*E* and *SI Appendix*, Fig. S20). By comparison, the interaction between LPE1 and HCF173 was not obviously affected by treatment with DTT and DTNB (Fig. 6*E*), suggesting that their interaction is unlikely to depend on the redox state.

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. 6*F*). 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. 6*G*). Next, the redox state of LPE1 was determined from its mobility using nonreducing SDS/PAGE upon the binding of 4-acetoamido-4-maleimidylstilbene-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. 6*H*). Additionally, the reduced form of LPE1 protein was also detected in dark conditions in mature plants (Fig. 6*H*), 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, had 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 *Populus 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 tauschii*. 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 PSII 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. 3*F*). 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. 4*F*) but unaltered expression levels or transcript patterns of *psbA* (Fig. 4*A* and *SI Appendix*, Fig. S9), suggesting that LPE1 is required for D1 translation. Finally, polysome 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. 3*B* and *SI Appendix*, Fig. S6), which resembles that of HCF173, a known activator of D1 translation (18), and by comigration of LPE1/HCF173 with PSII monomer and PSII supercomplexes of higher molecular weight (Fig. 5*D*). 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 PsaA/B and CP47 was reduced slightly, although these reductions were much smaller than the reduction in D1 synthesis (Fig. 4*F*). RIP analysis did not find an obvious association between LPE1 and *psaA*/*B* or *psbB* mRNA, which encodes CP47 (Fig. 3 *C*–*E* and *SI Appendix*, Fig. S7), suggesting that the reduction in PsaA/B and CP47 might be an indirect effect of LPE1 deficiency. It is also possible that defects in one PSII 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 PSII subunits (34), which is consistent with increased degraded D1 fragment in *lpe1* mutants in this study (*SI Appendix*, Fig. S10). PSII repair involves the disassembly and reassembly of the PSII complex. As D1 exhibits a high turnover (2), its synthesis is essential for PSII 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 (qI) (*SI Appendix*, Table S1), implying that LPE1 deficiency also may affect PSII 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. 3*F*), 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). BiFC experiments showed a direct and specific interaction of LPE1 with HCF173 but not with HCF244 (Fig. 5*A*). The direct interaction between LPE1 and HCF173 was confirmed by Y2H and CoIP analyses (Fig. 5 *B* and C) and by their comigration (Fig. 5*D*), 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. 5*E*) but does not affect HCF173 abundance (*SI Appendix*, Fig. S13). However, HCF173 deficiency does not affect the association of LPE1 with *psbA*

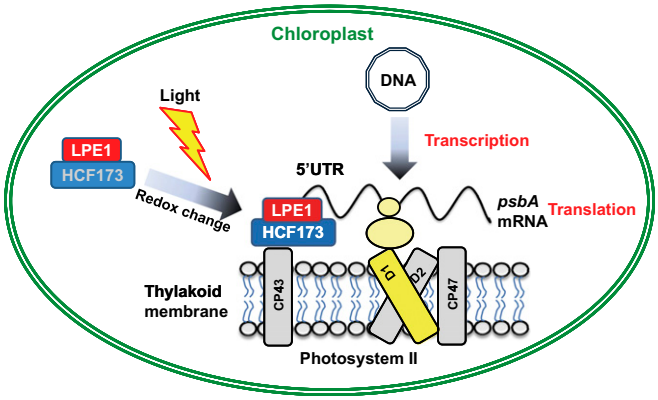

Fig. 7. Proposed working model of the roles played by LPE1 in light-regulated D1 synthesis during PSII biogenesis in higher plants. Light regulates the synthesis of D1 protein mainly by acting at the translational level during PSII biogenesis in chloroplasts of higher plants. *psbA* mRNA is initially transcribed by the plastid genome in a constitutive manner. Light induces the expression of the nuclear-encoded translation factor HCF173, which then interacts with LPE1 in the chloroplast. Light initiates photosynthesis, increasing the reducing power and promoting the reduction of LPE1 protein. The reduced form of LPE1 in complex with the HCF173 protein associates with the 5′ UTR of *psbA* mRNA to recruit the ribosome and activate the initiation of *psbA* mRNA translation (the translated D1 protein is highlighted in yellow). Finally, D1 proteins are inserted into the thylakoid membrane to form the PSII complex in a cotranslational manner to perform photosynthesis.

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. 5*E*), 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. 6*H*), 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. 6*A* 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 control of 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_059367, 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·KOH, 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, BN-SDS/PAGE 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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