REP27, a Tetratricopeptide Repeat Nuclear-Encoded and Chloroplast-Localized Protein, Functions in D1/32-kD Reaction Center Protein Turnover and Photosystem II Repair from Photodamage[[OA]]

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The goal of this research is elucidation of the molecular mechanism for the unique photosystem II (PSII) damage and repair cycle in chloroplasts. A frequently occurring, irreversible photooxidative damage inhibits the PSII charge separation reaction and stops photosynthesis. The chloroplast PSII repair process rectifies this adverse effect by selectively removing and replacing the photoinactivated D1/32-kD reaction center protein (the chloroplast-encoded psbA gene product) from the massive (>1,000 kD) water-oxidizing and O₂-evolving PSII holocomplex. DNA insertional mutagenesis in the model organism *Chlamydomonas reinhardtii* was applied for the isolation and characterization of *rep27*, a repair-aberrant mutant. Gene cloning and biochemical analyses in this mutant resulted in the identification of REP27, a nuclear gene encoding a putative chloroplast-targeted protein, which is specifically required for the completion of the D1 turnover process but is not essential for the de novo biogenesis and assembly of the PSII holocomplex in this model green alga. The REP27 protein contains two highly conserved tetratricopeptide repeats, postulated to facilitate the *psbA* mRNA cotranslational insertion of the nascent D1 protein in the existing PSII core template. Elucidation of the PSII repair mechanism may reveal the occurrence of hitherto unknown regulatory and catalytic reactions for the selective in situ replacement of specific proteins from within multiprotein complexes.

Chloroplast development and differentiation in the unicellular green alga *Chlamydomonas reinhardtii* can take place either under autotrophic or (photo) heterotrophic conditions. Biogenesis and assembly of functional PSII, and of the other thylakoid membrane complexes, including PSI, the cytochrome b₆-f complex, and the ATP synthase (Wollman et al., 1999; Minai et al., 2006), can take place in the dark in C. reinhardtii, provided that organic carbon, e.g. acetate, is supplied to the cells (Guenther et al., 1990). Whether in the light or in the dark, assembled thylakoid membrane complexes are fairly stable and do not turnover with physiologically relevant rates. An exception to this rule is the D1/32-kD PSII reaction center protein (the *psbA* gene product), which is known for its frequent turnover in the light (Mattoo and Edelman, 1987; Vasilikiotis and Melis, 1994).

The PSII holocomplex performs the functions of light absorption and excitation energy transfer to P680, leading to water oxidation, the release of O₂ and protons, and the transport of electrons from water to reduce plastoquinone molecules in the thylakoid membrane. The transient formation of strong oxidants, the abundance of O₂, and the presence of excitation energy are conditions that may lead to photooxidative damage (Ohad et al., 1984; Barber, 1994; Melis, 1999), causing irreversible inactivation in the electron-transport function of the D1 protein and inhibiting the function of PSII (Kok, 1956; Powles, 1984; Melis, 1991; Aro et al., 1993). An elaborate repair mechanism operates in organisms of oxygenic photosynthesis and restores the functional status of PSII. The PSII damage and repair cycle, as the phenomenon has come to be known (Guenther and Melis, 1990), operates in tandem with photosynthesis and appears to be conserved in cyanobacteria, algae, and crop plants.

The rate constant of photodamage is proportional to the incident light intensity (Baroli and Melis, 1996; Tyystjarvi and Aro, 1996; Hakala et al., 2005). Thus, photodamage can occur with a half time as slow as 24 h under very low light intensity conditions, or as fast as 30 min under bright sunlight (Kim et al., 1993; Yokthongwattana and Melis, 2006). The rate-limiting step in the enzymatic repair of PSII occurs with a half time of about 2 h (Sundby et al., 1993; Vasilikiotis and Melis, 1994; Neidhardt et al., 1998).
This defines the overall rate constant for the repair process and is largely independent of the light intensity to which the photosynthetic organism is exposed.

In PSII repair-aberrant mutants of *C. reinhardtii*, water oxidation and electron transport capacity depend on the balance between de novo biogenesis/assembly of PSII complexes (presence of acetate) and the rate of photodamage. Therefore, in repair-aberrant mutants, the degree of photoinhibition of photosynthesis can be manipulated by the relative dominance of these two players, i.e. de novo biogenesis/assembly and photodamage of PSII. In the presence of acetate and under weak light intensities, when the rate of de novo biogenesis/assembly of PSII is comparable to that of photodamage, intermediate levels of photoinhibition will manifest in relation to a wild-type control. Stronger light intensities, however, would make the rate of photodamage far exceed the rate of de novo biogenesis/assembly, causing a quantitative accumulation of photodamaged PSII and the end of detectable photosynthetic water oxidation activity.

In this study, DNA insertional mutagenesis of *C. reinhardtii* was employed to generate and isolate PSII repair mutants. The appropriate screening steps (Zhang et al., 1997) resulted in the isolation of *rep27*, a *C. reinhardtii* strain that is defective in photoautotrophic growth, which, however, greens normally in the presence of a strain that is defective in photoautotrophic growth, mutants. The appropriate screening steps (Zhang et al., 1997) suggested that in the presence of acetate, growth, Chl accumulation, as well as the assembly and acclimation properties of the photosynthetic apparatus were not affected by the *rep27* mutation. When cultivated in TAP media under either 10- or 50-µE conditions, the wild type displayed a slightly faster rate of growth than the *rep27* mutant (Fig. 1B).

Pivotal in the successful screening of repair mutants in *C. reinhardtii* is the differential biogenesis/assembly of functional PSII units in the presence of acetate and the subsequent inability of the chloroplast to repair them once photodamage has occurred (Zhang et al., 1997). It is thus expected that, under low-light (10 µE) growth in the presence of acetate, the rate of de novo biogenesis/assembly of the PSII holocomplex would be faster than the rate of PSII photodamage. Under these conditions, repair mutants will possess functional PSII reaction centers, the abundance of which will decline with growth irradiance faster than that in the wild type. This differential irradiance response can be monitored from the in vivo variable to maximal (Fv/Fm) fluorescence yield ratio and by the light-saturated rate of oxygen evolution in the two strains. The Fv/Fm ratio provides a convenient and direct method for the measurement of the photochemical charge separation efficiency of PSII reaction centers (Kitajima and Butler, 1975), while the light-saturated rate of oxygen evolution provides a measure of PSII electron transport capacity and, therefore, of the number of fully assembled functional PSII units.

The Fv/Fm fluorescence yield ratio was measured in *C. reinhardtii* cells grown under different irradiance regimes (10–150 µE; Fig. 1C). The ratio was lower in the *rep27* mutant than in the wild type under all growth irradiance conditions, suggesting fewer functional PSII reaction centers in the former than in the latter. This was confirmed by sensitive absorbance difference spectrophotometry (ΔΔA320; Melis, 1989), which revealed a substantially lower concentration of the functional primary quinone acceptor (QA) in the mutant than in the wild type. For cells grown at 150 µE, analysis showed QA/Chl (wild type) = 1:560 mol:mol; QA/Chl (*rep27*) = 1:180 mol:mol, whereas P700/Chl ratios were similar at about 1:650 mol:mol in each of these the two strains. Accordingly, the operational PSII/PSI ratio was lowered in the *rep27* mutant in an irradiance-dependent fashion (Table I). These results are consistent with the interpretation that the *rep27* strain is a putative PSII repair mutant.

To independently assess the functional capacity of PSII, the light-saturated rate of photosynthesis (Pmax) was measured (Fig. 1D). Wild type and *rep27* mutant were grown at three different irradiances (10, 50, and 150 µE) and Pmax was measured from the rate of oxygen evolution in a Clark-type oxygen electrode.

### RESULTS

**Isolation and Characterization of *rep27*, a Putative PSII Repair Mutant**

A putative PSII repair mutant, termed *rep27*, was isolated following DNA insertional mutagenesis and on the basis of a stringent two-step screening protocol (Zhang et al., 1997). Acetate requirement was the first screening step in this protocol. Whereas both wild-type and *rep27* mutant strains grew well on acetate-containing Tris-acetate phosphate (TAP) media, only the wild type grew on minimal Tris-bicarbonate-phosphate (TBP) media (Table I; Fig. 1A). Cellular chlorophyll (Chl) content was similar in wild type and *rep27* mutant, about $3.4 \times 10^{-15}$ mol/cell when grown under 10 µmol photons m$^{-2}$ s$^{-1}$ (10 µE) and about $1.7 \times 10^{-15}$ mol/cell when grown under 150 µmol photons m$^{-2}$ s$^{-1}$ (150 µE). Moreover, wild type and *rep27* displayed similar Chl a/b ratios (data not shown). These results suggested that in the presence of acetate, growth, Chl accumulation, as well as the assembly and acclimation properties of the photosynthetic apparatus were not affected by the *rep27* mutation. When cultivated in TAP media under either 10- or 50-µE conditions, the wild type displayed a slightly faster rate of growth than the *rep27* mutant (Fig. 1B).
under saturating illumination conditions. Figure 1D shows that Pmax for the wild type increased with growth irradiance, as is expected upon acclimation of the photosynthetic apparatus to the level of irradiance (Anderson, 1986; Baroli and Melis, 1996). On the contrary, Pmax for the rep27 declined with growth irradiance and dropped below the isosbestic point when grown at 150 μE, consistent with the repair-aberrant phenotype of this mutant. Under higher than 150 μE growth irradiance conditions, photosynthetic O2 evolution in the rep27 mutant became negligible, and the only gas exchange reaction recorded was that of cellular respiration that consumed oxygen from the medium. These results are consistent with the notion that, at irradiance levels greater than 150 μE, the rate of photodamage was substantially greater than that of de novo PSII biogenesis/assembly, resulting in a quantitative PSII photoinhibition. Survival of the organism under these conditions is ensured by the presence of acetate, which supports oxidative phosphorylation by the cells.

The Fv/Fm ratio of the rep27 mutant did not exceed 0.4, irrespective of whether cells were grown under low light or in the dark. At present, it is not clear why the rep27 cells have a lower than wild type Fv/Fm ratio. The possibility cannot be excluded that, although PSII biogenesis/de novo assembly and the PSII repair processes occur in different regions of the chloroplast thylakoid membrane and likely involve substantially different mechanisms, they may nevertheless utilize at least some common enzymatic reactions, possibly including the one catalyzed by the REP27 protein. Further work is needed to delineate these points.

Biochemical Analysis of Wild-Type and rep27 Strains

Figure 2A (D1) shows that the rep27 mutant has lower than wild-type steady-state levels of the D1/32-kD reaction center protein in its thylakoid membranes, consistent with earlier finding on this mutant (Zhang et al., 1997). Figure 2A also shows that subunits of the PSII holocomplex, other than the D1 reaction center

Table 1. Differential growth, irradiance screening, and functional characterization of the C. reinhardtii wild-type and rep27 mutant strains

| Parameter Measured                        | Wild Type, 10 μE | rep27, 10 μE | Wild Type, 150 μE | rep27, 150 μE |
|-------------------------------------------|-----------------|-------------|------------------|--------------|
| Growth in TBP (bicarbonate) media         | Yes             | No          | Yes              | No           |
| Growth in TAP (acetate) media             | Yes             | Yes         | Yes              | Yes          |
| Chl/cell (mol x 10^-15)                   | 3.2             | 3.6         | 1.6              | 1.8          |
| PSII/PSI ratio                            | 1.3/1           | 0.6/1       | 1.16/1           | 0.36/1       |
Transcription and D1 Translation

Figure 2. A, Western-blot analysis of 50-μE grown C. reinhardtii wild type and rep27 total protein cell extract. The steady-state level of PSII subunits was determined from the intensity of the antibody cross-reaction in the western blots with specific polyclonal antibodies generated against D1, D2, CP47, and PsbO, respectively. B, Western-blot analysis of 50-μE grown wild type and rep27 total protein cell extract. The steady-state level of these thylakoid membrane proteins was determined from the intensity of the antibody cross-reaction in the western blots with specific polyclonal antibodies generated against PsaK, Cyt f, PetC, AtpA, and Hsp70B.

Uncoupled psbA Transcription and D1 Translation in the rep27 Mutant

One possible scenario for the presence of lower steady-state levels of the D1 protein in the rep27 mutant might be a defect in the incorporation of nascent D1 in the photodamaged PSII core complex. This hypothesis requires that psbA gene transcription and mRNA levels are about the same in wild type and rep27 mutant. Steady-state levels of psbA and psbD mRNA were measured by northern-blot analysis (Fig. 3A), showing that psbA mRNA levels in mutant and wild type were indistinguishable from each other. Interestingly, rep27 showed somewhat enhanced steady-state levels of psbD mRNA relative to the wild type, underlining the greater relative amounts of D2 protein in the mutant (Fig. 3A) relative to that in the wild type.

The preceding suggested that a lower level of the D1 protein in the rep27 mutant, relative to that in the wild type, may be a consequence of the unimpeded degradation of photodamaged D1, coupled with the chloroplast inability to replace the lost D1 in the PSII-core complex. It should thus be possible to delineate between the de novo biogenesis/assembly of PSII from the selective D1 turnover in the chloroplast of the rep27 mutant. This was tested by comparative [35S]-sulfate pulse-labeling experiments with the cw15 wild type and rep27 mutant (Fig. 3B). In such experiments, cycloheximide was added just prior to the application of the radioactivity to inhibit synthesis of cytosolic proteins.

Figure 3B shows about equivalent amounts of large subunit of Rubisco, ATP synthase α-subunit (CF1-α), and D2 protein that were labeled in wild type and mutant. These provide a measure of the de novo biogenesis/assembly and accumulation of chloroplast components as a function of cell growth in the presence of acetate. Interestingly, in the rep27, but not in the wild type, radio labeled D1 and D2 accumulated in about equal amounts, evidence that de novo biogenesis/assembly of D1 is not affected in the mutant. However, the wild type showed a much greater accumulation of radiolabeled D1 than the mutant, consistent with the active turnover of this PSII reaction center protein. These results are evidence of selective inhibition of the D1 turnover/PSII repair and lack of impairment in
the de novo biogenesis/assembly of the PSII complex during cell growth in the mutant. In sum, except for the remarkable disparity of D1 radiolabeling in wild type and rep27, the labeling of other PSII and thylakoid membrane proteins was found to occur in equivalent amounts in the two strains.

Deletion of Genomic DNA upon pJD67 Plasmid DNA Insertion in rep27

Backcrosses of rep27 (mt+) and strain CC1068 (mt−) revealed that the repair-aberrant rep27 phenotype cosegregated with the ARG7 tag that was employed to generate the insertional mutagenesis library (data not shown). To determine the copy number of pJD67 plasmid insertions in rep27, Southern-blot analysis with the wild type and rep27 genomic DNA was carried out, as follows. ARG7-Ndel primer set (Table II) was used to PCR amplify a 0.75-kb Ndel/Ndel DNA fragment, derived from the 3′ end of the ARG7 plasmid, as indicated in Figure 4. This DNA fragment of the Arg-7 gene was employed as a probe in Southern-blot analysis of wild-type and rep27 genomic DNA, digested with NcoI or HpaI restriction enzymes. Figure 4A shows a single hybridization band between the probe and the wild-type genomic DNA corresponding to the endogenous inactive ARG7 gene. The rep27 showed polymorphism with two distinct hybridization bands between probe and mutant genomic DNA. In addition to the endogenous ARG7 gene, a single extra DNA band was observed, confirming a single pJD67 plasmid insertion in the rep27 mutant genome (Fig. 4A; see also Yokthongwattana and Melis, 2006).

Southern hybridization and PCR analyses were conducted to test for the presence of the pBluescript origin of replication and ampicillin resistance, to be used in a plasmid rescue effort for the cloning of the genomic DNA flanking the pJD67 insertion site (Gumpel and Purton, 1994). Such analysis revealed that a significant portion of the 5′ end of the plasmid was deleted, including the important ori and amp sites (Fig. 4B). Deletion of the 5′ end of the transforming linearized plasmid is a common occurrence in DNA insertional mutagenesis (Tetali et al., 2007). As an alternative approach by which to clone the genomic DNA flanking the pJD67 insertion site, a thermal asymmetric interlaced (TAIL)-PCR was employed with rep27 genomic DNA (Liu et al., 1995; Dent et al., 2005). TAIL-amplified PCR products were blasted against the Ver. 3.0 annotated C. reinhardtii genomic database (http://genome.jgi-psf.org/Chlre3/Chlre3.home.html). We found that the 3′ end of the pJD67 plasmid interrupted the coding sequence of an exostosin-like gene (open reading frame [ORF]0 in Fig. 4B) at contig 25 on scaffold 8.

![Southern-blot analysis of C. reinhardtii genomic DNA using the Ndel/Ndel 3′ end of the Arg-7 gene on NcoI or HpaI digested wild-type and rep27 genomic DNA. Black arrows indicate the position of the intrinsic inactivated ARG7 genes in the wild-type and rep27 DNA and white arrows show the position of a single additional band resulting from the insert DNA in the rep27 mutant. B, C. reinhardtii genomic DNA map, derived from the JGI Chlamydomonas Genome Project, showing the relative position of five ORFs (scaffold 8, contigs 25 and 26) and the insertion locus of pJD67. Restriction enzyme sites (NcoI and HpaI) used in the digestion of genomic DNA and the position of probes for Southern-blot analysis (Ndel/Ndel) are indicated on the pJD67 plasmid. The dotted rectangle in the 5′ end of the pJD67 shows the missing pBluescript portion, apparently deleted during the pJD67 insertion. Also indicated by opposite-facing arrows is the location of six primer sets (RT0–RT5). Four subclones (1A, 2A, 4A, and 8) isolated from BAC clone 10A5 were used for the rep27 complementation experiments. TAIL-PCR amplified fragment (TAIL) and the position of the TPB primer for the TAIL-PCR also shown.](http://www.plantphysiol.org/doi/abs/10.1104/pp.106.104693)
To define the opposite (5’ end) site of the pJD67 insertion locus, we designed six marker primer sets for PCR-based diagnostic analysis (RT0–RT5; Fig. 4B; Table II). Reverse transcription (RT)-PCR results with the RT0 to RT5 set of primers revealed that a 25-kb fragment of the *C. reinhardtii* genomic DNA, encompassing the RT1-RT4 loci, was missing and apparently deleted during the pJD67 insertional event (Fig. 4B). Four ORFs were annotated in the missing fragment of the *Chlamydomonas* genomic DNA. Of those, ORF1 (Fig. 4B, shaded) turned out to be the gene necessary and sufficient to alleviate the acetate-requiring phenotype of the rep27 strain (please see below).

The TAIL-PCR-amplified genomic DNA flanking the insertion site was subsequently used as a probe for the screening of a *C. reinhardtii* bacterial artificial chromosome (BAC) genomic library, obtained from the Clemson University Genomics Institute (https://www.genome.clemson.edu/). Four BAC clones (27N13, 10C5, 10A5, and 10K12) hybridized to the TAIL-PCR-amplified genomic DNA fragment (data not shown). Restriction patterns of these BAC clones showed that they were identical to each other and contained the missing 25-kb genomic DNA fragment of rep27 (data not shown).

**Complementation of rep27 with BAC Clone and Subcloned Genomic DNA**

BAC clone 10A5 was used to complement the rep27 acetate-requiring phenotype. Cotransformation of the rep27 mutant with the BAC clone 10A5 DNA was implemented with the pSL18 plasmid, conferring paramomycin resistance (Depege et al., 2003). Transformants were selected on TAP-agar plates containing 5 μg/mL paramomycin. To test whether BAC clone 10A5 complements the acetate-requiring phenotype of rep27, each BAC transformant (approximately 200 colonies in total) was replica plated onto paramomycin containing TAP-agar and TBP-agar media. One such transformant, which showed robust and unequivocally photoautotrophic growth in TBP media (Fig. 5A, BAC-T), was recovered and further analyzed by PCR (Fig. 5B). A randomly selected BAC clone 10A5 transformant (BAC-C), which did not grow on TBP media, was chosen as a negative control for the molecular and PCR analysis. Genomic DNA from BAC-T and BAC-C were used as templates in these PCR amplifications. Figure 5B shows that RT0 and RT5 primer sets, probing a DNA region outside the deleted 25 kb in the rep27 strain, generated PCR products, consistent with the presence of this genomic DNA in the mutant. The RT1 primer set failed to generate PCR products in either BAC-T or BAC-C transformants, suggesting that ORF0 may not be responsible for the autotrophic growth properties of the BAC-T strain. RT2 primer set successfully generated PCR products from the BAC-T genomic DNA only, but not from the BAC-C DNA, suggesting that ORF1 is present in the BAC-T and might encode a protein that confers autotrophic growth properties of the BAC-T strain (Fig. 5B). RT3 primer set successfully generated PCR products in both BAC-T and BAC-C transformants. Conversely, RT4 primer set generated PCR products in the BAC-C but not in the BAC-T transformant, suggesting that ORF3 may not be responsible for the autotrophic growth properties of the BAC-T strain. These results point to ORF1 as the likely gene candidate that confers autotrophic growth properties to the rep27 mutant. The results also provide testimony to the complex nature and unpredictable outcome of *C. reinhardtii* complementation by BAC clone DNA.

**Table II. Primers used in the PCR and cloning analysis of the C. reinhardtii rep27 strain**

| Purpose of Primers | Primer Names | PCR Primer Sequence | PCR Primer Sequence | Estimated Amplification Product Size in Nucleotides |
|--------------------|--------------|---------------------|---------------------|---------------------------------------------------|
| TPB1               | GGATGACGATGACCGCCATTGTTAGATTC | TAIL TPB2 | CACGGTGGCTCTGATCTGACG  | 198 |
| PCR                | CACGGTGGCTCTGATCTGACG | PCR TPB3 | TTACAGCTTCAAGGACCTGTG | 605 |
| TPDeg1             | NTCGGWCTSCNAGC | TPDeg2 | WGNCTCGACGGCGCG  | 362 |
| RT0                | GACGCCGAGTATGAAGGAG | Deleted RT1 | AAGTTTGTACATGCACAGAAGGAC | 254 |
| RT1                | TTACAGCTTCAAGGACCTGTG | RT2 | TTCAAGGGTTTATGGGAATCTGG | 280 |
| RT4                | GACGCCGAGTATGAAGGAG | RT5 | GACGAACAAGGCGACCTGTG | 856 |
| RT5                | GACCCGACCGCAGCAGCAG | Full size 27P-F3 | CTAGTGCAAGCCAGACATG | 672 |
| REP27              | 27E4-F | cDNA 27E5-R | GAACGAATCTCGTACGACACC | 1,059 |
| cDNA               | GAACGAATCTCGTACGACACC | Cloning 27E11-R1X | GCCTCTGACTGCTCAGGCGCGCCG | 1,057 |
| Probe              | ARG7-Ndel | Prep PSBA | ATGCAGCAGCTTCAAGGACCTGTG | 672 |
| Prep               | ATGCAGCAGCTTCAAGGACCTGTG | PSBD | ATGCAGCAGCTTCAAGGACCTGTG | 1,059 |
| RACE               | RCCAGTTCACCGGCAACACG | RACE-A | GCATTTCCAAGCAGCCACG | 1,057 |
| PCR                | RACE-C | RACE-B | CGTTGCGGCTGCTTCCAGAC | 1,059 |

To define the opposite (5’ end) site of the pJD67 insertion locus, we designed six marker primer sets for PCR-based diagnostic analysis (RT0–RT5; Fig. 4B; Table II). Reverse transcription (RT)-PCR results with the RT0 to RT5 set of primers revealed that a 25-kb fragment of the *C. reinhardtii* genomic DNA, encompassing the RT1-RT4 loci, was missing and apparently deleted during the pJD67 insertional event (Fig. 4B). Four ORFs were annotated in the missing fragment of the Chlamydomonas genomic DNA. Of those, ORF1 (Fig. 4B, shaded) turned out to be the gene necessary and sufficient to alleviate the acetate-requiring phenotype of the rep27 strain (please see below).

The TAIL-PCR-amplified genomic DNA flanking the insertion site was subsequently used as a probe for the screening of a *C. reinhardtii* bacterial artificial chromosome (BAC) genomic library, obtained from the Clemson University Genomics Institute (https://www.genome.clemson.edu/). Four BAC clones (27N13, 10C5, 10A5, and 10K12) hybridized to the TAIL-PCR-amplified genomic DNA fragment (data not shown). Restriction patterns of these BAC clones showed that they were identical to each other and contained the missing 25-kb genomic DNA fragment of rep27 (data not shown).
Because the mutant phenotype of rep27 was successfully complemented by BAC clone 10A5, PstI partially digested 10A5 DNA fragments were ligated onto the pBR322/PstI vector to isolate subclones, which contained the various deleted ORFs (ORF0–ORF3). Four different subclones, each containing ORFs from the deleted 25-kb region, were identified by DNA hybridization and direct sequencing analysis (Fig. 4B, subclones 1A, 8, 4A, and 2A). Transformants of the rep27 mutant with each of these subclones were tested for autotrophic growth on TBP minimal media. Only subclone 4A, containing a 6.2-kb PstI partial-genomic DNA fragment that includes ORF1, successfully rescued the acetate-requiring phenotype of the rep27 mutant. Transformation of the rep27 mutant with any other subclone (1A, 8, and 2A) failed to rescue the acetate-requiring phenotype of rep27 (data not shown). Accordingly, ORF1 was designated as the putative REP27 gene.

The REP27 Gene Product Is Required for the PSII Repair

For further molecular and biochemical analysis, three independent transformant lines with subclone 4A were selected (rep27-complemented [rep27-comp] strains). In the subsequent physiological and biochemical analyses, to alleviate crowding and enhance clarity of the presentation, results from only one line are reported. However, all three lines displayed similar properties to those of the rep27-comp strain shown.

RT-PCR with the RT2 primer set confirmed the presence of the REP27 transcript in the rep27-comp strains (data not shown). Because the rep27-comp strains were selected on the basis of growth on TBP-agar minimal media, which lack acetate, it was initially assumed that transcription of REP27 is necessary and sufficient to rescue the acetate-requiring phenotype of the rep27 mutant. To further test this hypothesis, the $F_o/F_m$ fluorescence yield ratio and photosynthetic activity in wild-type, rep27 mutant, and rep27-comp strains were measured under a variety of growth conditions.

A phenotype of the rep27 mutation is the lower-than-wild-type $F_o/F_m$ ratio under all growth irradiance conditions (Fig. 1C), consistent with the interpretation that the rep27 strain is a PSII repair-aberrant mutant (Zhang et al., 1997). The effect of light-shift experiments on the $F_o/F_m$ value of the rep27-comp strains was compared to that of the wild-type and rep27 mutant (Fig. 6A). Cells grown under 10 $\mu$E were transferred to approximately 1,000 $\mu$E. Chl fluorescence yield parameters were measured as a function of exposure time to 1,000 $\mu$E (Fig. 6A). Prior to the light shift, $F_o/F_m$ for the wild type and rep27-comp strain was high (about 0.78), whereas it was considerably lower for the rep27 mutant (0.37). $F_o/F_m$ for rep27 decreased to nearly zero within 1 h under a 1,000-$\mu$E irradiance, whereas the $F_o/F_m$ for the wild type and rep27-comp strains declined to about 0.45 in the same period of time. These results are consistent with the notion that rep27 is a PSII repair-aberrant mutant and that complementation of this strain with a wild-type copy of the REP27 gene rescues the mutation.

The activity of photosynthesis and the response of wild-type, rep27, and rep27-comp strains to irradiance were further evaluated from the measurement of the light-saturation curve of photosynthesis (Fig. 6B). In the wild-type and rep27-comp strains, photosynthetic activity increased as a function of light intensity with identical slopes (quantum yield of photosynthesis) and reached a Pmax of approximately 40 mmol $O_2$ (mol Chl$^{-1}$ s$^{-1}$) at about 1,000-$\mu$E light intensity. This pattern was nearly indistinguishable among wild-type and rep27-comp strains and independent of growth irradiance in the 10 to 50 $\mu$E region. Contrary to the wild-type and rep27-comp strains, the rep27 mutant showed a quantum yield of photosynthesis (initial slope in the photosynthesis versus irradiance curve) that was only about 50% of that in the wild-type and
the rep27-comp strains (Fig. 6B), consistent with the approximately 50% lower \( F_v/F_m \) and with the notion of a state of chronic photoinhibition in the mutant. It is also of interest to observe that a lower \( P_{\text{max}} \) of approximately 15 mmol O2 (mol Chl)\(^{-1}\) s\(^{-1}\) in the rep27 mutant was reached at about 1,000-\( \mu \)E light intensity. Upon exposure to progressively higher light intensities, \( P_{\text{max}} \) declined in the rep27, a consequence of the progressively faster rates of PSII photodamage coupled with the mutant’s inability to complete the repair reactions. It is also of interest to note that this photoinhibitory effect was more pronounced with the mutant strain grown at 50 rather than 10 \( \mu \)E (Fig. 6B, white versus black triangles).

The preceding analysis suggested that rep27 undergoes a normal biogenesis/assembly of functional PSII units when grown in the presence of acetate, but cannot repair them when PSII photodamage occurs. There is thus a competition between the de novo biogenesis/assembly of functional PSII units in the presence of acetate and the irreversible inhibition of PSII due to photodamage in the rep27 mutant. This interpretation requires that either photodamaged and inactive D1 accumulates in the thylakoid membrane or, if the lesion is in a step subsequent to inactive D1 degradation, a depletion of the D1 protein in the mutant relative to that in the wild type. To delineate between these two different mechanistic alternatives, western-blot analysis was applied to assay steady-state levels of D1 protein in wild-type, rep27, and rep27-comp strains (Fig. 6C). It is seen that rep27 has substantially lower steady-state amounts of the D1 protein relative to the wild type, whereas the rep27-comp strain displayed levels of D1 that were comparable to those of the wild type. These results strengthened the notion that the rep27 lesion does not prevent degradation of the photodamaged and inactive D1, but it does interfere with the next step, i.e. the biosynthesis and/or cotranslational insertion of the nascent D1, thereby impeding the completion of the D1 protein turnover and the PSII repair process.

**REP27 Gene Identification and Structure**

To identify the transcription start site of the REP27 gene, 5’ RNA ligase mediated-RACE was employed and resulted in the identification of 455 coding region nucleotides, in addition to those reported by the U.S. Department of Energy Joint Genome Institute (JGI) for ORF1 (Fig. 4). These occurred in the upstream region of the JGI-reported ORF1 translation initiation site. A full-length REP27 cDNA was subsequently isolated by RT-PCR amplification using 27P-F3 and 27E11-R1X primer set (Table II), which confirmed the extended REP27 coding sequence. The REP27 cDNA nucleotide sequence was deposited in the GenBank with accession number EF127650, predicted to encode a 449-amino acid putative precursor protein (Fig. 7A), with a molecular mass of 49.5 kD for the precursor polypeptide and 45.6 kD for the mature protein. The REP27 genomic
DNA was also predicted to contain a minimum of 11 exons and 10 introns. 

*REP27* appears in the JGI Chlamydomonas Genome Project database as an unknown gene whose product contains a single tetratricopeptide repeat (TPR) motif with no assigned function. However, our complete cDNA sequence showed that this gene also contains a plastid-targeted transit peptide and includes an additional TPR motif, both of which are missing from the JGI annotation. In agreement, database searches revealed the occurrence of *REP27* orthologs in the genomic DNA of other plant species, including the transit peptide and the two pairs of the TPR domains, which appear to be highly conserved (Fig. 7A).

Amino acid sequence analysis by ChloroP1.1 software (Emanuelsson et al., 1999) predicted that *REP27* contains a chloroplast transit peptide. Similarly, application of the HMMTOP v2.0 software (Tusnady and Simon, 2001) pointed to the existence of two putative transmembrane helices in *REP27* (amino acid regions

Figure 7. Deduced amino acid sequence alignments (ClustalW analysis; Higgins et al., 1994) of *REP27* and its homologs. A, Database searches revealed that the deduced amino acid sequence of rice (Os01g0358300), Arabidopsis (At1g02910), and Ostreococcus (CAL55849) gene products are orthologs to that of *REP27*. Asterisks mark absolutely conserved residues among the four protein sequences. High and low similarity residues are marked by double or single dots, respectively. Vertical arrow indicates the predicted cleavage site of the chloroplast transit peptide targeting sequence. Solid lines and dotted lines over the amino acid sequences indicate the TPR motifs and predicted transmembrane helices, respectively. B, Phylogenetic tree showing the relative evolutionary proximity of the *REP27* ortholog (Os01g0358300, At1g02910, and CAL55849) and paralog proteins (Os04g0507100 [rice], At2g28740 [Arabidopsis], CAL58275 [Ostreococcus], and C_142189 [Chlamydomonas]) based on the amino acid sequence comparisons.
TMH1 and TMH2 including amino acids 180–202 and 217–239, respectively; Fig. 7A), which may suggest that REP27 is a thylakoid membrane integral protein. Application of Inter-ProScan 13.1 software analysis (Quevillon et al., 2005) identified two distinct TPR motifs in REP27 (TPR1A/TPR1B and TPR2A/TPR2B; Fig. 7A). The helix-rich TPR motifs have been described in the literature to form a compact unit of two helices interacting with each other in the antiparallel direction. It is thought that such conserved TPR motifs are involved in a variety of critical protein–protein interactions in the living cell (for review, see Lamb et al., 1995) and are consistent with the putative role of the REP27 in the D1 turnover and PSI repair.

A search of the National Center for Biotechnology Information database revealed the occurrence of six REP27 homologs, including two copies each in rice (Oryza sativa), Arabidopsis (Arabidopsis thaliana), and Ostreococcus, a small unicellular green alga whose genomic DNA sequence and annotation recently became available (Derelle et al., 2006). Moreover, a search of the Ostreococcus, a small unicellular green alga whose genomic DNA sequencing in the JGI. A search of the GenBank database revealed the occurrence of a REP27 homolog in this green alga. Accordingly, it appears that two copies of REP27 homologs are present in each of these photosynthetic organisms. These can be divided into two distinct groups: REP27 orthologs (Os01g0358300, rice; At1g02910, Arabidopsis; and CAL55849, Ostreococcus) contain the chloroplast transit peptide and two TPR motifs (Fig. 7A), while REP27 paralogs (Os04g0507100 for rice; At2g28740 for Arabidopsis; CAL58275 for Ostreococcus; and C_142189 for Chlamydomonas) are devoid of both a transit peptide and the TPR domains (data not shown). REP27 had about a 35% amino acid sequence identity with its orthologs (CAL55849, Os01g0358300, and At1g02910). However, it had limited similarity and that was only in the C-terminal half of the protein with the paralogs, which were devoid of transit peptide and TPR motifs.

The REP27 protein appears to exist only in grana-containing organisms of oxygenc photosynthesis. The phylogenetic comparison diagram in Figure 7B shows that REP27, CAL55849, Os01g0358300, and At1g02910 are grouped together and, therefore, closely related. Likewise, REP27 paralog proteins Os04g0507100, At2g28740, CAL58275, and C_142189 are closely related among them and distantly related to the former. This may suggest a distant phylogenetic relationship between the two groups of genes and proteins and points to the possibility that REP27-like genes in photosynthetic eukaryotes exist in pairs, one copy encoding a putative chloroplast-targeted protein, the other encoding a nonplastid counterpart.

Induction of REP27 Gene Expression by Irradiance and Requirement for D1 Protein Turnover But Not for de Novo PSII Biogenesis/Assembly

To test whether expression of the REP27 gene is constitutive or induced by irradiance, steady-state REP27 mRNA levels were measured before and after a shift in light intensity. Northern-blot analysis with a probe specific for the REP27 gene showed that 1,000 μE-treated wild-type cells had about 5 times more transcript accumulation than that of 50 μE-acclimated control cells (Fig. 8A). This observation is consistent with the well-documented, irradiance-induced increase in repair gene expression, including ELIP/Cbr (Fig. 8B) and HSP70B (Yokthongwattana et al., 2001). Such increase in gene expression is though to be a meaningful response of the cells to the increased rates of photodamage and the need for enhanced rates of repair with increasing irradiance (Melis, 1999; Yokthongwattana and Melis, 2006).

DISCUSSION

It is known that specific D1-less (ΔpsbA) or D2-less (ΔpsbD) mutants fail to assemble the entire PSII core complex (Bennoun et al., 1986; Erickson, 1986; Cohen et al., 2001; Baena-Gonzalez et al., 2003). The rep27 is distinct from such mutants, as it undergoes biogenesis/assembly of a functional PSII holocomplex but fails to perform the D1 reaction center protein turnover. Therefore, under weak growth irradiance, rep27 displays a limited water oxidation and O₂ evolution capacity. Under moderate to high rates of photodamage, PSI activity ceases to exist.

Due to incomplete annotation of the C. reinhardtii genomic DNA sequencing in the REP27 locus, the true transcription initiation site for this gene was not available. 5’ RACE PCR was performed in this work, resulting in the proper identification of the transcription initiation site and translation start locus of REP27, which is 261 bp upstream from the tentative translation start site reported by the JGL. A search of the GenBank database identified a pair of REP27 homologs in photoautotrophic eukaryotes, including Chlamydomonas, Arabidopsis, rice, and Ostreococcus (Fig. 7). REP27 orthologs contained both the transit peptide for chloroplast targeting of the protein, the two apparently conserved TPR motifs, and the two transmembrane domains. REP27 paralogs lacked both the transit peptide for chloroplast targeting of the protein and the two apparently conserved TPR motifs and are apparently cytosolic proteins.

An Arabidopsis T-DNA mutant was isolated in which the REP27 ortholog gene was interrupted by the T-DNA insertion. This mutant, termed lpa1 for low PSI accumulation (Peng et al., 2006), had many characteristics similar to those reported in this work for the rep27 mutant. However, contrary to the conclusions reached in this work, Peng et al. (2006) assigned a PSII biogenesis function to the LPA1 protein in Arabidopsis, as opposed to the specific D1 turnover function of the REP27 gene in this work.

TPR motif-containing proteins are widely encountered among a variety of organisms, including nonphotosynthetic bacteria, cyanobacteria, yeast (Saccharomyces cerevisiae), fungi, plants, animals, and humans (Blatch
REP27, a TPR Protein, Functions in D1 Turnover and PSII Repair

The work presented in this article suggests that the REP27 protein is essential for the D1 reaction center protein turnover, probably facilitating translation and/or insertion of the nascent D1 in the vacated PSII reaction center template. It is of interest to speculate about the mechanistic role of TPR motifs in this process. TPR motifs are thought to be important factors in both transcription and translation processes. For example, Chlamydomonas Mbb1 and its Arabidopsis ortholog, HCF107, contains 10 and 11 TPR motifs, respectively, thought to be responsible for the mRNA stability and translation initiation of the plastidic psbH (Vaistij et al., 2000; Sane et al., 2005). NAC2, which has nine TPR motifs, is required for stable accumulation of the psbD mRNA in Chlamydomonas (Boudreau et al., 2000). A regulator of Chl biosynthesis, FLU, harbors two TPR motifs in its C terminus, which are required for interaction with glutamyl-tRNA reductase (Meskauskiene et al., 2001; Meskauskiene and Apel, 2002). A novel chloroplast protein, TCP34, with three TPR motifs, was localized in the fraction of transcriptionally active chloroplast chromosome, indicating that TPR motifs possessed the ability to bind plastid DNA (Weber et al., 2006). The Ycf3 mutant analysis also showed that TPR motifs are involved in the PSI stability via a direct interaction with PsaA and PsAD (Naver et al., 2001).

It is well known that molecular chaperones, such as the cytoplasmic HSP70 and HSP90, interact with a number of cochaperones that contain TPR motifs (for review, see Blatch and Lassle, 1999). In the chloroplast, Tic40 may bind to chloroplast ClpC and HSP70B (Chou et al., 2003). In the green algae Dunaliella salina, the presence of chloroplast-localized HSP70B was detected as a component of the transiently forming 320-kD PSII repair intermediate (Kim et al., 1993; Melis and Nemson, 1995; Yokthongwattana et al., 2001). It is possible (currently under investigation) that REP27 is also a component of this PSII repair intermediate, in a structure that may serve to preserve the conformation of the disassembled PSI core complex, from which the D1 protein has been temporarily vacated. Therefore, a working hypothesis is that the TPR motifs contained by the REP27 protein act as a receptor of the nascent D1 and are required for the proper guidance and insertion of the de novo-synthesized D1 protein in the existing PSII template. The REP27 and HSP70 proteins may act coordinately in the scaffold of the PSII repair intermediate in

Figure 8. A, Northern-blot analysis probed with C. reinhardtii REP27-specific DNA. Wild-type cells, grown under 50-μE conditions (L), were incubated under high light (1,000 μE, H) for 1 h. Agarose gel lanes were loaded with 4 or 8 μg of total RNA from the 50 μE-grown (L) and 1,000 μE-exposed (H) cells, transferred to nylon membrane, and probed with a radiolabeled full-size REP27 cDNA probe. A (bottom), rRNA loading control. B, Northern-blot analysis probed with C. reinhardtii ELIP-specific DNA. Wild-type and rep27 mutant grown under 50-μE conditions were incubated under high light (1,000 μE) for 1 h. Agarose gel lanes were loaded with 4 μg of total RNA from the 50 μE-grown (L) and 1,000 μE-exposed (H) cells, transferred to nylon membrane, and probed with a radiolabeled full-size ELIP cDNA probe. Ribosomal RNA loading controls are shown at the bottom.

Figure 9. Schematic of the putative function of the REP27 protein in the D1 protein turnover and PSII repair process. REP27 is shown to play a role in the cotranslational insertion of a nascent D1 (pD1) in the D1-less PSII template during the PSII repair cycle. This step follows the PSII disassembly and specific degradation of photodamaged D1 in the thylakoid membrane.
a process that facilitates the stability of the D1-less PSI complex and guides psbA cotranslational insertion of the de novo-synthesized D1 protein in the PSI template. Figure 9 shows a preliminary schematic, aiming to illustrate the step(s) in the PSI repair process and the possible mechanism of action for the REP27 protein. Consistent with the evidence presented in this work, de novo biogenesis/assembly of PSI in the presence of acetate is possible in C. reinhardtii, leading to the formation of functional PSI. Photodamage by excess irradiance leads to irreversible inhibition in the function of the D1 protein, occurring in the appressed thylakoid membranes of the chloroplast. PSI disassembly, followed by degradation of the inactive D1, takes place in stroma-exposed thylakoids (Mels, 1991, 1999; Ossenbuhl et al., 2002), leading to a D1 vacancy from the PSI reaction center complex. The latter serves as a template for the insertion of a de novo-synthesized nascent D1 protein, a process catalyzed by the REP27 protein. According to this working hypothesis, the REP27 protein is essential for the incorporation of the nascent D1 but not for the de novo biogenesis/assembly of the PSI holocomplex. This model is consistent with the psbA mRNA association with thylakoid membranes, which is accentuated under conditions of enhanced photodamage and repair (Kettunen et al., 1997), underlining a cotranslational assembly of the D1 protein during the PSI repair (Zhang et al., 1999, 2000). In the rep27 mutant, incorporation of the nascent D1 in the PSI template during the D1 turnover process is inhibited, leading to the phenomenology described in this work.

MATERIALS AND METHODS

Strains and Media

Chlamydomonas reinhardtii mutants were generated by transformation (Kindle, 1999; Tam and Leebry, 1993) of an Arg auxotroph, strain CC-425 (arg7-8 cu5 mt + su-a-2-66; Chlamydomonas Center, Duke University), with pJD67 plasmid DNA (linearized with HindIII) containing the complementing arginosuccinate lyase gene (argS7), as described by Davies and Grossman (1994) and Davies et al. (1996). Procedures for the screening, isolation, and maintenance of putative repair mutants, including the rep27 strain, have also been described (Zhang et al., 1997). Wild type and rep27 of the green alga C. reinhardtii were grown mixotrophically in a TAP medium (Gorman and Levine, 1965), either in liquid cultures or on 1% agar plates. To test photoautotrophic growth to the early exponential growth phase (about 1–2×10^6 cells/mL) prior to harvesting and measurements of photosynthesis. Chlamydomonas BAC genomic DNA library filters and clones were acquired from the Clemson University Genomics Institute (https://www.chlre3/chlre3.home.html). The cell suspension was incubated at 65°C for 2 h, extracted twice with phenol-chloroform-isomylalcohol (25:24:1), and precipitated with isopropanol (Chen and Mels, 2004). Total RNA was isolated from the isopropanol pellet of the cell extract using Invitrogen’s Trizol Reagent and by following the manufacturer’s recommended procedure. 5' RACE by PCR was performed using FirstChoice RNA ligase mediated-RACE kit (Ambion) by following the manufacturer’s recommended procedure.

Southern- and northern-blot analyses were carried out according to standard protocol (Sambrook et al., 1989). The high light induction of REP27 gene expression was tested upon shifting cultures from 50 to 1,000 μmol photons m^(-2) s^(-1) for 1 h prior to RNA isolation and by using the full size of the cDNA fragment as a probe in the measurement of transcript abundance. Northern-blot analysis with early light-inducible protein (ELIP)-specific DNA was performed with a radiolabeled, full-size ELIP cDNA probe, which was amplified from C. reinhardtii with primer sets P02F (GGGGGGATCCCTCGGCTGATCGCTTAG) and P03R (GGGGGATCCGGGACATTTGTCGTCTT). A possible mechanism of action for the REP27 protein. According to this working hypothesis, the REP27 protein is essential for the incorporation of the nascent D1 but not for the de novo biogenesis/assembly of the PSI holocomplex. This model is consistent with the psbA mRNA association with thylakoid membranes, which is accentuated under conditions of enhanced photodamage and repair (Kettunen et al., 1997), underlining a cotranslational assembly of the D1 protein during the PSI repair (Zhang et al., 1999, 2000). In the rep27 mutant, incorporation of the nascent D1 in the PSI template during the D1 turnover process is inhibited, leading to the phenomenology described in this work.

Cloning of the Flanking Regions of the pJD67 Insertion Site

C. reinhardtii genomic DNA flanking the plasmid insertion site was amplified using a TAIL-PCR procedure, optimized for Chlamydomonas genomic DNA, by a modification of the method described (Dent et al., 2005). The primers used for the TAIL-PCR are listed in Table II. Briefly, flanking genomic DNA was amplified by PCR from the region adjacent to the inserted pJD67 plasmid that was used for DNA insertional mutagenesis (Zhang et al., 1997). For HindIII-digested pJD67 transformants, specific primers for primary, secondary, and tertiary reactions were designed and named TPB1, TPB2, and TPB3, respectively (Table II). Two arbitrary degenerate primers were tested for amplification, TPdE1 and TPdE2, as previously described (Dent et al., 2005). The general TAIL-PCR protocol of Liu et al. (1995) was used with some minor modifications for the various PCR amplification reactions. Nucleotide sequences of the resulting PCR products were obtained via an ABI3100 sequencer. Chlamydomonas genomic sequence information was obtained from the Chlamydomonas Genome Project Web site (http://genome.jgi-psf.org/Chlr3/Chlr3.home.html).

Insertional Mutagenesis and Clamydomonas Transformation

Generation of a C. reinhardtii insertional mutagenesis library and screening for the identification of specific PSI repair mutants was implemented as previously described (Zhang et al., 1997). Complementation of the rep27 putative repair mutant was achieved by cotransformation of the mutant with BAC clone 10A5 and with subclones derived from this BAC with plasmid pSL18 (Depege et al., 2003), using the conventional glass bead transformation method (Kindle, 1990). pSL18 contains the paromomycin resistance gene (selectable marker) operated under the control of the C. reinhardtii Hsp70A and RbcS2 promoters (Sizova et al., 2001) and linked to the PsA promoter and terminator that can be used to express ORFs in C. reinhardtii (Depege et al., 2003). C. reinhardtii transformants carrying the above-mentioned BAC clone(s) were selected on TAP (acetate-containing) or TBP (minimal media) plates containing 5 μg mL^(-1) paromomycin (Sigma Chemical).

Photosynthesis Measurements

Procedures for the measurement of the Fv/Fm variable-to-maximal fluence yield ratio and for the Pmax have been described (Zhang et al., 1997). To test for the effect of high light exposure on photosynthesis and PSI parameters, cw15, rep27, and rep27-comp strains were incubated under 1,000 μE for variable periods of time. The photosynthetic activity of strains cw15, rep27, and rep27-comp lines was estimated from the light-saturation curve of photosynthesis, measured as the oxygen evolution activity of the cells at different actinic intensities. Measurements of the light-saturation curve of photosynthesis commenced with the measurement of the rate of oxygen evolution at 50, 1,000, 1,500, 2,000, and 2,500 μE. Registration of the rate (slope) of oxygen evolution at each light intensity step was recorded for about 5 min. Therefore, in the 30-min duration of these measurements, samples were exposed to progressively higher light intensities (0–2,500 μE).
Protein Analysis

For the isolation of total cellular protein, C. reinhardtii strains were grown in liquid TAP media under continuous illumination (50 μE). Cell biomass equivalent to 100 μg Chl were collected by centrifugation (5,000 g) and resuspended in 400 μL of 0.1 M dithiothreitol and 0.1 M NaN3. Following incubation for 5 min, 400 μL of 2× sample solubilization buffer containing 10% SDS, 10% glycerol, and 10% β-mercaptoethanol was added and incubated for 30 min at room temperature. To enhance the solubilization efficiency of SDS, the mix was incubated in boiling water for 2 min. Unsolubilized material was removed by centrifugation at 15,000 g for 5 min prior to loading samples onto the SDS-PAGE.

The Chl concentration of the various suspensions was measured as previously described (Lichtenthaler, 1987). Aliquots corresponding to an equal amount of Chl were loaded in the wells of the stacking gel and electrophoresed through 12.5% SDS-polyacrylamide running gels as described by Tetalji et al. (2007). The electrophoretically separated proteins were transferred onto nitrocellulose (Immobilon-NC, Millipore) and probed sequentially with primary specific polyclonal antibodies and horseradish peroxidase conjugated secondary antibodies (Bio-Rad).

Antibodies to D1, PeC, and AtpA subunit were described in Park and Rodermeier (2004), and HisPib was described in Yokkhangwattana et al. (2001). Antibodies to Psak, CytF, and PsbO were obtained as a kind gift from Dr. Paraq Chinis. Specific polyclonal antibodies against D2 and CP47 were also described (Kim et al., 1993; Yokkhangwattana et al., 2001). Cross-reactions between protein bands and antibodies were visualized by use of the SuperSignal ECL (Pierce) detection kit following the manufacturer’s specifications. Quantification of northern- and western-blot bands was made through application of the ImageJ 1.37v software (http://rsb.info.nih.gov/ij/).

In Vivo (Pulse) Labeling with [35S]-Sulfate

Radioactivity-labeling procedures were performed as previously described (Vasilikiotis and Melis, 1994; Preiss et al., 2001). Exponential growth phase cells of C. reinhardtii, equivalent to 60 μg Chl, were harvested by centrifugation at 5,000 g for 5 min at room temperature. Pelleted cells were resuspended in 300 μL sulfur-deprived TAP to yield 0.2 μg Chl/μL and incubated for 2 h at room temperature under 50 μM cycloheximide (10 μg/mL) was added to block cytoplasmic protein synthesis and cells were incubated for 15 min prior to addition of 100 μCi/μL [35S]-sulfate. At the end of a 10-min labeling period, 100 μg ml−1 lincomycin and cold sulfate (1 μM final concentration) were added to terminate the labeling reaction. Radioactivity-labeled samples were collected for 30 min, vacuum dried, exposed to a phosphor screen, and analyzed by a Storm PhosphorImager (Molecular Dynamics). Specific polyclonal antibodies against D2 and CP47 were also described in Park and Melis (2003) Deletion of the tobacco plastid psbA gene triggers an upregulation of the thylakoid-associated NADPH dehydrogenase complex and the plastid terminal oxidase (PTOX). Plant J 35: 704–716.

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CORRECTIONS

Due to a software error, six articles in the April 2007 issue were printed with an incorrect DOI (digital object identifier) number at the bottom of the first page. These numbers allow the articles to be uniquely identified online. The online versions of the articles have been updated, and the correct numbers are listed below.

Chen et al., Vol. 143: 1954–1967, www.plantphysiol.org/cgi/doi/10.1104/pp.107.095588
Deng et al., Vol. 143: 1660–1668, www.plantphysiol.org/cgi/doi/10.1104/pp.107.095521
Kalituho et al., Vol. 143: 1861–1870, www.plantphysiol.org/cgi/doi/10.1104/pp.107.095562
Marino et al., Vol. 143: 1968–1974, www.plantphysiol.org/cgi/doi/10.1104/pp.107.097139
Park et al., Vol. 143: 1547–1560, www.plantphysiol.org/cgi/doi/10.1104/pp.107.096396
Schaarschmidt et al., Vol. 143: 1827–1840, www.plantphysiol.org/cgi/doi/10.1104/pp.107.096446