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Mechanism of REP27 Protein Action in the D1 Protein Turnover and Photosystem-II Repair from Photodamage

David Dewez1§, Sungsoon Park1§, Jose Gines García-Cerdán2, Pia Lindberg1 and Anastasios Melis1*

1Plant and Microbial Biology, University of California, Berkeley, California, 94720–3102
§These authors contributed equally to this work
*Corresponding author

KEYWORDS
Chlamydomonas reinhardtii; green microalgae; photoinhibition; photosynthesis; protein turnover; photochemical reaction center; repair

ABBREVIATIONS
BN: blue-native; D1: the psbA-encoded 32 kD PSII reaction center protein; HSM: high-salt medium; LHCII: Light-harvesting complex of PSII; RC: reaction center; TAP: tris-acetate-phosphate medium; TBP: tris-bicarbonate-phosphate medium; TMH: transmembrane helix; TPR: tetratricopeptide repeat

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Anastasios Melis (melis@nature.berkeley.edu).

* Corresponding author; e-mail melis@nature.berkeley.edu
2Present address: Institute of Chemistry, Umeå University, Umeå, Sweden, SE-901 87
ABSTRACT

The function of the REP27 protein in the photosystem-II repair process was elucidated. REP27 is a nuclear-encoded and chloroplast-targeted protein containing two tetratricopeptide repeated (TPR) motifs, two putative transmembrane domains, and an extended C-terminal region. Cell fractionation and Western blot analysis localized the REP27 protein in the *C. reinhardtii* chloroplast thylakoids. A folding model for REP27 suggested chloroplast stroma localization for N- and C-termini regions as well as the two tetratricopeptide repeats. A REP27 gene knockout strain of *C. reinhardtii*, termed *rep27* mutant, was employed for complementation studies. The *rep27* mutant was aberrant in the PSII-repair process and had substantially lower than wild type levels of D1 protein. Truncated REP27 cDNA constructs were made for complementation of the *rep27*, whereby TPR1, TPR2, TPR1+TPR2, or the C-terminal domains were deleted. *rep27*-complemented strains minus the TPR motifs showed elevated levels of D1 in thylakoids, comparable to those in the wild-type, but the PSII photochemical efficiency of these strains was not restored, suggesting that the functionality of the PSII reaction center could not be recovered in the absence of the TPR motifs. It is suggested that TPR motifs play a role in the functional activation of the newly integrated D1 protein in the PSII reaction center. *rep27*-complemented strains missing the C-terminal domain showed low levels of D1 protein in thylakoids, as well as low PSII photochemical efficiency, comparable to those in the *rep27* mutant. Therefore, the C-terminal domain is needed for a *de novo* biosynthesis and/or assembly of D1 in the photodamaged PSII template. We conclude that REP27 plays a dual role in the regulation of D1 protein turnover by facilitating co-translational biosynthesis-insertion (C-terminal domain) and activation (TPR motifs) of the nascent D1 during the PSII repair process.

INTRODUCTION

The unicellular green alga *Chlamydomonas reinhardtii* is a good model system to study the regulation of photosynthesis at the molecular level since the chloroplast development and differentiation can take place either under autotrophic, photo-heterotrophic, or dark-heterotrophic conditions. The chloroplast biogenesis and most of the photosynthetic apparatus assembly can occur in the dark, when cells are supplied with organic carbon such as acetate (Guenther et al.,
Vegetative cells are haploid, permitting ready phenotypic manifestation of mutations, or genetic lesions. Photosynthesis deficient mutants can thus be isolated and investigated, conferring to *Chlamydomonas* a significant advantage over other model plant systems. Measurement of the chlorophyll fluorescence with intact cells offers a non-invasive approach to assessing the functionality of photosystem-II (PSII) and of the electron transport process in the thylakoid membrane of photosynthesis. Thus, a number of photosynthesis mutants with defects in the biogenesis and assembly of thylakoid membrane complexes were generated and isolated (Zhang et al., 1997; Wollman et al., 1999; Minai et al., 2006), providing valuable information about the corresponding processes and leading to the isolation and characterization of genes and proteins.

The PSII repair cycle (Guenther and Melis, 1990) is a process essential to photosynthesis and plant growth, occurring in all organisms of oxygenic photosynthesis, and serving to restore the functional status of PSII from a frequently occurring photodamage. Repair entails the unique selective degradation and replacement of the D1/32 kD PSII reaction center protein from the massive (>1,000 kD) PSII holocomplex (Mattoo and Edelman, 1987). The PSII damage and repair mechanism is highly conserved in all organisms of oxygenic photosynthesis, as it maintains the activity of photosynthesis by selectively degrading and replacing the PSII D1/32 kD reaction center protein (Melis, 1991; Aro et al., 1993). The rate constant of photodamage is a linear function of light intensity (Baroli and Melis, 1996; Tyystjärvi and Aro 1996), ranging between 0 in the dark to about 1.2 h\(^{-1}\) under bright sunlight and physiological growth conditions. In contrast, the enzymatic repair process occurs with a light intensity-independent rate constant, equal to about 0.7 h\(^{-1}\) (Neidhardt et al., 1998; Ohnishi et al., 2005; Yokthongwattana and Melis, 2006). Under bright sunlight conditions, the rate of photodamage can be faster than the rate of repair, resulting in accumulation of inactive D1 proteins, loss of photosynthetic yield and chloroplast productivity (Adir et al., 1990; Bailey et al., 2002). The repair entails D1 activation (Guenther et al., 1990; Neale and Melis, 1991) and post-translational modifications to restore the PSII water-splitting activity (Diner et al. 1988; Bowyer et al., 1992).

Biogenesis of the photosynthetic apparatus is a process involving the coordinated expression of genes leading to the biosynthesis and assembly of both chloroplast- and nuclear-encoded proteins. The chloroplast genome of the unicellular green alga *Chlamydomonas reinhardtii* encodes approximately 100 genes, required for protein synthesis of the
photosynthetic apparatus and carbon fixing machinery (Maul et al., 2002). Genetic and biochemical studies of *Chlamydomonas* revealed the involvement of numerous nucleus-encoded factors acting in the transcription/translation or in several post-transcriptional events of chloroplast gene expression, such as mRNA processing, stability and translation into proteins (Barkan and Goldschmidt-Clermont, 2000; Somanchi and Mayfield, 2001). Compared to the present information available on the rapid light-dependent turnover of the D1 protein in PSII (Aro et al., 1993; Yokthongwattana and Melis, 2006), our understanding of the regulation of the PSII repair mechanism is very limited, either at the level of protein translation or post-translational steps leading to a functional PSII. The de novo synthesis, membrane insertion and assembly of D1 processes are most likely to require the participation of nuclear-encoded auxiliary proteins.

In earlier studies from this lab (Zhang et al., 1997; Park et al., 2007), DNA insertional mutagenesis in the model organism *Chlamydomonas reinhardtii* was applied for the isolation of mutants defective in photoautotrophic growth. Isolated from this screening protocol, the *rep27* strain was found to grow normally in the presence of acetate, but displayed low photosynthetic activity even under low light conditions. Under weak growth irradiance, the *rep27* showed a limited water oxidation and O$_2$ evolution capacity, whereas under moderate to high irradiance PSII activity ceased to exist, indicating inability of the chloroplast to perform the D1 protein turnover. Gene cloning and biochemical analysis of the *rep27* strain resulted in the identification of the *REP27* nuclear gene, which was deleted from the *rep27* mutant. The *REP27* cDNA sequence was isolated and used for complementation of the *rep27* mutant, permitting the recovery of the wild-type phenotype (Park et al., 2007). From these preliminary results, it was suggested that *REP27* is a nuclear gene involved in the rapid light-dependent turnover of the D1 protein during the PSII repair process.

The present work investigated and elucidated the mechanism of the REP27 protein action in the D1 protein turnover and PSII repair from photodamage. It was concluded that REP27 plays a dual role in the regulation of the D1 protein turnover by facilitating co-translational biosynthesis-insertion (C-terminal domain) and activation (TPR motifs) of the nascent D1 during the PSII repair process.
RESULTS

BN- and SDS-PAGE analysis of wild type and rep27 mutant

DNA insertional mutagenesis with the model organism Chlamydomonas reinhardtii was applied for the isolation and characterization of putative PSII repair mutants (Zhang et al., 1997; Park et al., 2007). The rep27 strain, defective in photoautotrophic growth, was isolated from this screening library. It was found that rep27 grew in the presence of acetate, but it displayed a lower level of photosynthetic activity under low light conditions. Under moderate to high light intensity, PSII photochemical activity was absent in this mutant. Further physiological and biochemical analyses showed lower steady-state levels of the D1/32-kD reaction center protein in the rep27 than in the wild type, while others subunits of the PSII holocomplex, such as D2, CP47, were only somewhat reduced. Peripheral subunits of PSII, such as the PsbO proteins, were in equivalent amounts in wild type and rep27. Subunits of non-PSII complexes (Cyt b6-f, PSI, and ATP synthase) also occurred in equivalent amounts in wild type and rep27 mutant (Park et al., 2007). Results from the earlier work supported a working hypothesis, whereby de novo biogenesis/assembly of the PSII holocomplex occurred more-or-less normally in the rep27 mutant; however, replacement (turnover) of the photodamaged D1/32 kD protein was severely impaired.

A comparative thylakoid membrane protein profile analysis of wild type and rep27 mutant was undertaken, aiming to identify biochemical and functional differences between the two strains. For better resolution of the integral and hydrophobic components of the thylakoid membrane, holocomplexes were separated in a first dimension via non-denaturing polyacrylamide gel electrophoresis. Fig. 1A (upper lane) shows a Blue-Native gel of wild type thylakoid membrane proteins, compared with the green native gel of the same sample (Fig. 1A, lower lane). The native form of the resolved holocomplexes was then separated into their constituent subunits upon running in a second dimension by denaturing SDS-PAGE and visualized by silver staining (Fig. 1B). Combination of BN-PAGE and SDS-PAGE was previously used to identify specific thylakoid membrane proteins of C. reinhardtii by peptide mass fingerprinting and MALDITOF-MS (Rexroth et al., 2003). This analysis permitted separation and identification of the main PSII core subunits (D1, D2, CP43 and CP47 proteins),
PSI proteins (PsaA, PsaB and PsaF and light-harvesting complex I protein), the light-harvesting complexes of PSII in trimeric and monomeric forms, cytochrome b$_{6}$f complex proteins, and chloroplast ATP synthase complex (CF$_{0}$F$_{1}$) proteins (Rexroth et al., 2003). A selective comparison of the 2-DE proteome of photo-mixotrophically grown Chlamydomonas wild type and rep27 mutant is given in Fig. 2. Demarcated by dashed line are subunits of the PSII supercomplex (Fig. 2, CC425). In the rep27 mutant, these proteins appeared to occur at substantially lower levels (Fig. 2, rep27) relative to the wild type. The 2-DE gel also showed a higher relative amount of LHCII proteins in the rep27 mutant relative to the wild type, demarcated by a dashed line in Fig. 2 (rep27). Since the overall Chl/cell ratio in the rep27 mutant was similar to that in the wild type (Zhang et al., 1997; Park et al., 2007) and the 2-d gels were loaded on the basis of equal Chl, the results suggest a higher LHCII/RCII ratio in the rep27 compared to that in the wild type. This is a consequence of the rep27 mutation, adversely affecting the RCII and PSII-core complexes but not the LHCII.

Quantitative Western blot analysis was employed to more directly compare levels of the PSII core and RC proteins in wild type and rep27 mutant. Fig. 3 shows that D1 and CP43 were depleted from the thylakoid membrane of the rep27 mutant, whereas D2 and CP47 occurred in comparable quantities in wild type and rep27 mutant. The results suggest that PSII-core and RC complexes are not stable in the rep27 mutant but become dissociated easily, even under the mild detergent conditions employed in the Blue Native gel experiments of Figures 1 and 2. This observation would explain the greatly reduced amounts of PSII core and RC proteins in the results of Fig. 2 (rep27).

The above proteome-based results are consistent with previous spectrophotometric and Western-blot analyses probing the steady-state level of PSII and D1 reaction center protein in Chlamydomonas wild-type and rep27 mutant (Zhang et al., 1997; Park et al., 2007).

**Occurrence of REP27 in wild type and photochemical apparatus mutants**

The cDNA sequence of REP27 (GenBank Accession Number EF127650) was used for complementation of the rep27 mutant, which permitted recovery of the wild-type phenotype. Based on these findings, specific polyclonal antibodies were generated in rabbit against a portion of the REP27 protein, defined by amino acids F273-to-L367. SDS-PAGE and Western-Blot
analysis were then applied to probe the occurrence and steady-state level of REP27 proteins in *Chlamydomonas reinhardtii* wild type and selected photochemical apparatus mutants. Results from such comparative analysis are shown in Fig. 4, where a specific cross reaction was detected between the REP27 antibodies and a protein band migrating to 47 kD (Fig. 4, CC425). The REP27 molecular weight, obtained by this Western-blot analysis was similar to that deduced from the amino acid composition of the protein (45.6 kD, Park *et al.*, 2007). The REP27 specific polyclonal antibodies failed to recognize any protein band in extracts from the *rep27* mutant (Fig. 4, *rep27*), consistent with the notion that the latter is a *REP27* knockout. Conversely, *rep27*-complemented strains showed a distinct protein band in the ~48 kD region (Fig. 4, *rep27*-comp) [the recombinant REP27 is larger by about 2 kD due to the presence of a 2xMYC tag]. It is of interest that both the D1-less (ΔpsbA) and D2-less (ΔpsbD) mutant strains of *C. reinhardtii* showed an equal to wild type abundance for the REP27 protein (Fig. 4), in spite of the fact that these strains have no PSII reaction center complex, display no variable Chl fluorescence or oxygen evolution (Bennoun *et al.*, 1986; Erickson, 1986; Minai *et al.*, 2006) and, therefore, do not undergo a photodamage and repair cycle. It is suggested that the *REP27* gene is expressed constitutively in *Chlamydomonas reinhardtii* under all growth conditions. Moreover, it appears that the *REP27* gene transcription and translation are not down regulated by the absence of the D1/D2 reaction center proteins of PSII. It may be concluded that a lack of *de novo* biogenesis/assembly of the PSII reaction center does not adversely affect the synthesis of the REP27 protein.

**Thylakoid membrane fractionation and localization of REP27**

It was previously suggested that REP27 is a putative chloroplast-targeted protein (Park *et al.*, 2007), as the REP27 amino acid sequence analysis (Emanuelsson *et al.*, 1999) predicted a chloroplast transit peptide in the precursor protein. Moreover, two putative transmembrane helices (amino acid regions TMH1 and TMH2 including amino acids 180–202 and 217–239, respectively) were also predicted by HMMTOP v2.0 software analysis (Tusnady and Simon, 2001). It was hypothesized that REP27 is integral to the thylakoid membrane. To experimentally test this hypothesis, a cellular fractionation and analysis of soluble and membrane-bound proteins was undertaken. **Fig. 5** shows a Western-blot analysis of wild type (CC425), *rep27*
mutant, and rep27-complemented strains, probed with specific polyclonal antibodies generated against the REP27, RbcL, or D1 proteins. Total cell extract (TE), soluble (S) and total membrane (TM) fractions were assayed. RbcL and D1 specific polyclonal antibodies served in testing for the enrichment of the soluble and total membrane fractions. Results showed absence of the REP27 protein from the total protein extract of the rep27 mutant (Fig. 5, TE), but presence in the CC425 and rep27-complemented strains (Fig. 5, REP27). Importantly, the REP27 protein was found in exclusive association with the total membrane extract of the CC425 and rep27-complemented strains (Fig. 5, TM) and not with the soluble protein fraction (Fig. 5, S). Resolved thylakoid membranes and isolation of a light membrane fraction, including the chloroplast envelopes, was achieved by differential ultracentrifugation, as recently done in this lab (Lindberg and Melis, 2008). Western blot analysis of these membrane fractions with REP27-specific antibodies showed presence of the REP27 protein in the thylakoid membrane fraction and absence from the lighter envelope-containing fraction (results not shown). These results clearly suggest integral thylakoid membrane localization for the REP27 protein, probably spanning the thylakoid lipid bilayer through the TMH1 and TMH2 regions.

In an effort to more precisely localize the REP27 in the thylakoid membrane of photosynthesis, appressed and non-appressed thylakoid membrane domains were isolated upon mechanical fractionation of C. reinhardtii wild type (Neale and Melis, 1991). It was shown previously that the 10k fraction is enriched in thylakoid grana membranes and the 140k fraction in stroma exposed membranes, as evidenced from direct measurements of PSI and PSII content using P700, QA and pheophytin quantitations (Neale and Melis, 1991). The differential distribution of the photosystems among such thylakoid membrane domains was also reflected in the Chl a/b ratio of the fractions, thus providing an easy to measure assay and convenient marker. Fig. 6 shows that the Chl a/b ratio was 2.60 in the total thylakoid extract, it was lower to 2.39 in the heavy grana-enriched 10k fraction, and higher at 3.24 in the light stroma-exposed thylakoid containing PSI-enriched 140 k fraction. Fig. 6 also shows Western-blot analysis of the various thylakoid membrane fractions with D1-specific (Fig. 6A) and REP27-specific polyclonal antibodies (Fig. 6B). Measurement of the REP27/D1 ratio showed that the REP27 protein is relatively more prevalent in the 40k than in the 10k or 140 k fractions, suggesting that the membrane domain of the REP27 protein is intermediate to the fully appressed and stroma-exposed thylakoids. These could be the “fret” domains of the thylakoid membrane (Morrissey et
al., 1986), where the PSII repair takes place, localized adjacent to the appressed grana membranes, yet exposed to the stroma medium so as to permit the D1 protein turnover.

**Functional role of the REP27 tetratricopeptide motifs and C-terminal domain**

By using Inter-ProScan 13.1 software analysis (Quevillon et al., 2005), two distinct tetratricopeptide (TRP) motifs were also identified in REP27 (Park et al. 2007). These were termed TPR1A/TPR1B and TPR2A/TPR2B, and were shown to occur near the N-terminal end of the protein. TPR motifs form a compact unit of two helices interacting with each other in the antiparallel direction (Blatch and Lässle, 1999). Accordingly, the structure of the REP27 protein entails the N-terminus, followed by two tetratricopeptide repeat motifs (TPR1 and TPR2), the two transmembrane helices and the long C-terminal hydrophilic portion of the protein. On the basis of topology analysis, using the TMAP transmembrane program and the positive inside rule (Persson and Argos, 1994), we propose that the N- and C-termini, including the two TPR motifs, are exposed to the chloroplast stroma phase. A folding model, depicting the structural association of REP27 with the thylakoid membrane was thus constructed (Fig. 7).

To gain a better understanding on the function of REP27 during the D1/32-kD PSII reaction center protein turnover, the role of the TPR motifs and of the C-terminal region were investigated. It has been suggested that TPR motifs are involved in a variety of critical protein-protein interactions in the living cell (Blatch and Lässle, 1999), and this is consistent with a putative functional role of the REP27 in the unique D1 replacement process. Therefore, **REP27 cDNA constructs missing the TPR1 (pSLREP27-ΔT1), TPR2 (pSLREP27-ΔT2), both TPR1 and TPR2 (pSLREP27-ΔT1+2), or the C-terminal region (pSLREP27-ΔCt) were made (Fig. 8, see also Materials and Methods). These were used for transformation of the rep27 mutant strain. Complementation of the rep27 mutant (rep27-comp) with a full-length **REP27 cDNA construct (pSLREP27-comp) was used as a control. The paramomycin resistance cassette was used as the first selectable marker for the isolation of transformants. Putative rep27-ΔT1, rep27-ΔT2, rep27-ΔT1+2, and rep27-ΔCt transformant strains were screened further via Western-blot analysis with specific polyclonal antibodies generated against the REP27 protein. Only strains with a positive expression of the modified REP27 protein were selected for further analysis.**
Positive transformant strains \textit{rep27-\Delta T1}, \textit{rep27-\Delta T2}, \textit{rep27-\Delta T1+2}, and \textit{rep27-\Delta Ct} were tested for photoautotrophic growth on both HSM and TBP minimal media (Fig. 9). It is known that a functional REP27 protein is needed for autotrophic growth of \textit{C. reinhardtii} in minimal media (Park et al. 2007). Accordingly, wild type (Fig. 9A) and \textit{rep27-comp} strains (Fig. 9C) grew viable colonies on both HSM and TBP plates. Conversely, the \textit{rep27} mutant (Fig. 9B) and transformant strains \textit{rep27-\Delta T1} (Fig. 9D), \textit{rep27-\Delta T2} (Fig. 9E), \textit{rep27-\Delta T1+2} (Fig. 9F), and \textit{rep27-\Delta Ct} (Fig. 9G) all failed to rescue the acetate-requiring phenotype of the \textit{rep27} mutant. It is concluded that both TPR motifs and the C-terminal portion of the REP27 protein are needed for the completion of the PSII repair cycle and the survival of \textit{C. reinhardtii} under photoautotrophic growth conditions.

To probe the status of the truncated REP27 protein in transformant strains \textit{rep27-\Delta T1}, \textit{rep27-\Delta T2}, \textit{rep27-\Delta T1+2}, and \textit{rep27-\Delta Ct}, these were grown on TAP media, followed by biophysical and biochemical analysis of the function of PSII. Growth in the presence of acetate (TAP) under weak illumination enables biosynthesis and assembly of functional PSII (Zhang et al. 1997; Park et al. 2007), i.e., under conditions when the rate of photodamage is rather slow (Melis 1999), thereby permitting accumulation of a smaller-than-wild-type fraction of functional PSII reaction centers. Fig. 10 (left panel) shows that wild type, \textit{rep27} mutant, and transformant strains employed in this work, all grew viable colonies and greened normally on TAP plates. To probe the functional properties of PSII in these strains, the fluorescence yield F\textsubscript{V}/F\textsubscript{M} ratio was measured. The F\textsubscript{V}/F\textsubscript{M} ratio provides a measure of the photochemical charge separation efficiency of the PSII reaction centers (Kitajima and Butler, 1975); therefore it offers an indication of the proportion of functional PSII reaction centers under \textit{in vivo} conditions. The F\textsubscript{V}/F\textsubscript{M} ratio of the wild type was equal to 0.72, whereas F\textsubscript{V}/F\textsubscript{M} for the \textit{rep27} mutant (0.30) was only about 40% of that in the wild type, suggesting that only a fraction of the PSII reaction centers were functional in the \textit{rep27} mutant (growth on TAP). As a positive control for the recovery of the REP27 function, the \textit{rep27-comp} strain showed elevated F\textsubscript{V}/F\textsubscript{M} ratio (=0.68) and similar to that of the \textit{C. reinhardtii} wild type. The \textit{rep27-\Delta T1}, \textit{rep27-\Delta T2}, \textit{rep27-\Delta T1+2}, and \textit{rep27-\Delta Ct} transformants all showed low F\textsubscript{V}/F\textsubscript{M} ratios and similar to that of the \textit{rep27} mutant (see Fig. 10, right panel). It is concluded that the TPR motifs and C-terminal region of the REP27 protein are all necessary to restore the proportion of functional PSII reaction centers and electron transport capacity in the chloroplast of this green microalga.
Western-blot analysis was employed with the wild type, rep27, rep27-comp, rep27-ΔT1, rep27-ΔT2, rep27-ΔT1+2 and rep27-ΔCt transformant strains to test for the presence and relative steady-state amounts of the REP27 and D1 proteins (Fig. 11). Consistent with earlier results (Park et al. 2007), the rep27 mutant lacked the REP27 protein and showed low levels of the D1 protein in its thylakoids (Fig. 11, rep27). The rep27-comp strain offered positive evidence of the REP27 protein and substantially enhanced levels of the D1 protein, which were similar to those in the wild type (Fig. 11, rep27-comp). This is consistent with the elevated Fv/Fm ratio (=0.68) in the latter. Interestingly, all truncated REP27 transformant strains (Fig. 11, rep27-ΔT1, rep27-ΔT2, rep27-ΔT1+2, and rep27-ΔCt) clearly showed presence of the modified REP27 protein in the cell. Moreover, they also showed elevated amounts of the D1 protein in the chloroplast thylakoid membranes. Specifically, strains rep27-ΔT1 and rep27-ΔT2 displayed levels of the D1 protein that were nearly equivalent to those in the wild type and rep27-comp. Strains rep27-ΔT1+2 and rep27-ΔCt contained substantial amounts of the truncated REP27 protein, however, they did not accumulate D1 protein to levels equivalent to that in the other transformants.

The results clearly showed that removal of TPR1, TPR2, TPR1+TPR2, or 61 amino acids from the C-terminal domain did not interfere with the synthesis and assembly of the REP27 protein in the thylakoid membrane. Moreover, these transformants had elevated steady-state amounts of the D1 protein in the respective thylakoid membranes. However, evidenced from the low Fv/Fm ratio and inability to grow on minimal media, it may be concluded that synthesis and assembly of the truncated REP27 to the thylakoid membrane is not sufficient by itself to restore function in these transformants. Presence of both TPR motifs, and of the C-terminal portion of the protein, is required for the completion of the D1 protein turnover during the PSII repair process. It is hypothesized (a) that the C-terminal portion of the REP27 protein is needed for the insertion of nascent D1 proteins in the D1-less PSII template, and (b) that TPR motifs are required for the “activation” of newly inserted and bound D1 proteins, which, in the case of the truncated REP27 transformants remained inactive. Such “activation” step was inferred earlier from biophysical studies (Guenther et al., 1990; Neale and Melis, 1990) and could involve a posttranslational modification and/or proper membrane deployment / folding of the nascent D1 protein during its assembly within the PSII template and subsequent maturation. Since the TPR-less strains had a low PSII photochemical charge separation efficiency and lack of photoautotrophic growth capacity, it appears that TPR domains are needed to assure a full
integration of nascent D1 proteins into the PSII reaction center template, leading to a functional PSII reaction center.

The rep27-ΔCt mutant showed the lowest steady-state level of D1 present, compared to the others rep27 transformant strains, but the level was still higher than that of the original rep27 mutant. It is most likely that the REP27 C-terminal domain plays an essential role in the de novo D1 biosynthesis at the level of ribosomal psbA mRNA translation and it may also be required for insertion of nascent D1 proteins in the D1-less PSII template. Taken together, these results illuminate, for the first time and in great detail, the role that is played by the REP27 protein in the D1 protein turnover and PSII repair from photodamage.

DISCUSSION

The D1 protein is subject to a frequent turnover, which far surpasses that of all other thylakoid membrane and PSII subunits. Turnover can take place at all light intensities, but is accelerated under increasing irradiance (Melis, 1999). The D1 protein turnover was demonstrated in pulse-chase experiments, in which the D1 was preferentially labeled over that of other thylakoid membrane proteins (Mattoo and Edelman, 1987; Schuster et al., 1988; Adir et al., 1990). In a previous study, presenting the isolation and characterization of the rep27 mutant strain (Park et al., 2007), comparative [35S]-sulfate labeling experiments showed that the rep27 mutant accumulated radio-labeled D1 in tandem with the accumulation of the other PSII subunits. However, and opposite to that in the wild type, it did not show the prolific and preferential accumulation of D1 over the other PSII subunits. Since the latter is evidence for active D1 turnover (Park et al., 2007), it was suggested that rep27 has a specific defect in the active turnover of this PSII reaction center protein. Furthermore, steady-state levels of psbA and psbD mRNA, measured by northern-blot analysis, were similar in the wild-type and mutant. From these results, it was proposed that the rep27 mutant is defective in the D1 protein turnover and, therefore, unable to complete the PSII repair process. The comparative thylakoid membrane functional and protein analysis of wild type and rep27 mutant in this work confirms and strengthens this hypothesis.
Since the \textit{REP27} knockout mutant contains functional PSII units, when grown in the presence of acetate (Fig. 10) and under weak irradiance, it is likely that the REP27 protein does not play a direct role in the \textit{de novo} biogenesis and assembly of the PSII holocomplex. This conclusion is strengthened by the finding that REP27 is localized on fully assembled thylakoid membrane domains, in which there is a fully assembled PSII and electron-transport apparatus (Figs. 4-6), and where turnover of the D1 protein is the only function requiring “assembly”. On the contrary, \textit{de novo} biosynthesis and assembly of PSII, and of thylakoid membranes, is expected to take place at the chloroplast “polar regions”, where thylakoid membranes begin to emanate, and probably far away from the fully assembled grana complexes. The \textit{de novo} biogenesis/assembly of PSII was in fact proposed to occur in areas of the chloroplast where there is a low density of membranes in a process of assembly biochemically related to the chloroplast inner envelope (Zerges and Rochaix, 1998). The spatially separated \textit{de novo} biogenesis/assembly of PSII from the D1 repair machinery explains why substantial residual O$_2$-evolving photosynthetic activity is encountered even after the knockout inactivation of \textit{REP27}. Consistent with this reasoning is also the finding that regulation of \textit{psbA} translation by auto-feedback repression for PSII biogenesis/assembly is clearly distinct from the D1 biosynthesis for PSII repair (Minai \textit{et al.}, 2006). Therefore, we conclude that the \textit{rep27} mutant performs biogenesis/assembly of a functional PSII holocomplex but fails to undertake the D1 protein turnover, as would be required by the PSII repair process.

Regulation of D1 biosynthesis takes place primarily during the \textit{psbA} mRNA translation, with translation initiation, elongation and co-translational assembly of the D1 protein into PSII all being regulated (Kettunen \textit{et al.} 1997; Zhang \textit{et al.}, 1999; Zhang \textit{et al.}, 2000; Zhang and Aro, 2002). Association of cofactors to the D1 protein during its \textit{de novo} synthesis has been suggested to stabilize and properly fold the nascent D1 chain (Kim \textit{et al.} 1994). Adding to this earlier information, results from the present work showed how the REP27 protein plays a role in facilitating, and probably regulating, different stages of the \textit{de novo} D1 biosynthesis, assembly, and activation during the PSII repair process. For example, the REP27 C-terminal is essential for the \textit{psbA} mRNA translation initiation and assembly of the nascent D1. The TPR domains of REP27 are required for the “activation” of the bound D1 in the PSII reaction center during the PSII repair process.
The REP27 is not the only TPR motif-containing chloroplast protein in *Chlamydomonas reinhardtii*, which is implicated in posttranscriptional steps of the chloroplast gene expression. The nuclear-encoded TPR protein Mbb1 is involved in *psbB* mRNA processing, stability and translation (Vaistij *et al.*, 2000). Moreover, the Nac2 factor is required for the stabilization, processing and translation of the *psbD* mRNA, permitting the proper folding of the D2 protein during the *de novo* biosynthesis-assembly of PSII (Boudreau *et al.*, 2000). It is interesting to observe that the REP27 has a similar functionality with the Mbb1 and Nac2 proteins (i.e., mediating protein-protein interactions) during chloroplast mRNA metabolism, even though the three proteins probably act independently in different signaling pathways.

Other possibly significant TPR motif-containing proteins that play a role in photosynthesis include the periplasmic Prat1 in *Synechocystis* sp. PCC 6803 (Klinkert *et al.*, 2004; Schottkowski *et al.*, 2009) and the thylakoid membrane-bound LPA1 in *Arabidopsis thaliana* (Peng *et al.*, 2006). Klinkert *et al.* (2004) showed that targeted inactivation of PratA resulted in drastically reduced PSII content in the cyanobacterium cell. Additional experimentation further supported the notion of a specific role for the PratA protein in the D1 biosynthesis, as this occurred during the *de novo* assembly of newly synthesized PSII holocomplexes. Consistent with this view was the finding that PratA is a cell periplasmic protein, i.e., occurs in a domain where *de novo* biosynthesis of photosynthetic complexes is taking place, and not associated with the mature thylakoid membrane, where the repair of PSII occurs. Comparison of the deduced amino acid sequence (CLUSTAL 2.0.8 analysis, supplemental Fig. 1S, A) of REP27 with PratA suggested a low similarity, further strengthening the notion of two different proteins.

There is greater similarity between the deduced amino acid sequence alignment of REP27 and LPA1 (CLUSTAL 2.0.8 analysis, supplemental Fig. 1S, B). LPA1 has been localized to the thylakoid membrane of *Arabidopsis* chloroplasts (Peng *et al.* 2006). However, and somewhat at variance with the conclusions drawn from this work, the latter authors assigned a primary function to LPA1 in the *de novo* biosynthesis and assembly of the PSII holocomplex, rather than to a specific function in the turnover of the D1 protein, occurring during the PSII damage and repair cycle. More work is needed to delineate between these alternative possibilities of the three proteins in the different photosynthetic systems.
In conclusion, REP27, a nuclear encoded protein, is essential for the D1 reaction center protein turnover, permitting a completion of the translation process, maturation and activation of D1 into a functional PSII reaction center holocomplex. Figure 12 is a schematic illustration of the role of the REP27 protein during the PSII repair from photodamage. It portrays the critical role played by the REP27 protein in the translation of the psbA mRNA, insertion of the nascent D1 protein in the D1-less PSII template, and in the activation of the newly assembled reaction center complex. According to this proposed mechanism, REP27 C-terminal is permitting the initiation of ribosomal psbA mRNA translation and protein insertion, whereas the TPR motifs enable functional activation of the newly assembled D1 within the existing PSII template.

MATERIALS AND METHODS

Growth media and culture conditions

Wild-type, rep27 mutant and related transformants of the green alga C. reinhardtii were grown mixotrophically in acetate-containing TAP media (Gorman and Levine, 1965) at 25°C under illumination of 50 µmol photons m⁻² s⁻¹ provided by cool white fluorescent lamps. Algal cultures in early exponential growth phase were used for experiments, with cells either in liquid culture or on 1.5% agar plates. To test photoautotrophic growth of strains, cells were grown on TBP minimal media, in which sodium bicarbonate (25 mM, pH 7.4) replaced the acetate as the growth carbon source (Polle et al., 2003). Cells were collected by centrifugation at 5,000 x g for 5 min at 20°C. Cells pellet was stored at -80°C, or used immediately for extraction of total proteins. Chlorophyll concentration was determined in 80% acetone extracts according to Arnon (1949), with equations corrected as in Melis et al. (1987). Each experiment was repeated three times with independently grown cell cultures.

Mutant strain generation

Generation of truncated REP27 proteins and the corresponding cDNA constructs were implemented using the QuikChange II XL Site-directed Mutagenesis Kit (Stratagene), according to the manufacturer’s instructions. Oligonucleotides (Bioneer, CA) carrying the desired mutations are listed in Table 1. Plasmids carrying the targeted mutations were identified by sequencing, isolated and reintroduced into pSL18 for complementation of C. reinhardtii rep27 mutant.
Deletion of the specific amino acids from the mature REP27 protein in the various constructs is as follows: ΔTPR1, A58-E87; ΔTPR2, A97-Y126; ΔTPR1+2, A58-Y126; ΔCt, L389-E449.

**Primers used in this work**

| Use of primer | Name of primers | Primer sequence 1 | Primer sequence 2 |
|---------------|-----------------|-------------------|-------------------|
| Antibody generation | F273F & L367R | cccatagtccgcggccaggtccgccccg | gtcgggtctgggcgcctgtggatcccc |
| Construction of truncated REP27 | TPR1 | CAGCAATATCAACCGACCAGCATGCAGCCCAACGAGGATGAG | CTCATCGTCGTTGGGCTGATGCTGTCGTTGATATTGCT |
| | TPR2 | CCAACGAGGATGAGGCTCGTGCCCTGAAGCTGATTGTGGCT | AGCCAAATACGCTTTCAAGCCACGGAGCCTCATCTGTCGTTGGG |
| | TPR1+2 | CAGCAATATCAACCGACCAGCGGCCTGAAGCTGATTGTGGCT | AGCCAAATACGCTTTCAAGCCGCTGGTGTCGTTGATATTGCT |
| Chlamy codon optimized 2X MYC tag | Myc-F&R | GGGGTCTAGACATATGGAAGGATGCAGGATCAGCGAGGAGGACCTGAA | TTACAGGTCTCTTTGCCT |

**Complementation studies**

*Chlamydomonas reinhardtii* rep27 mutant was generated via DNA insertional mutagenesis, as described by Park *et al.* (2007). C. *reinhardtii* rep27 complemented transformants were generated by complementation of the *rep27* mutant with the pSL18 plasmid vector containing wild type of truncated *REP27* cDNA, via the conventional glass bead transformation protocol (Kindle, 1990). The pSL18 plasmid contains the paramomycin resistance gene (selectable marker) operated under the control of the *C. reinhardtii* Hsp70A and *RbcS2* promoters (Sizova *et al.*, 2001) and linked to the *PsaD* promoter and terminator that can be used to express ORFs in *C. reinhardtii* (Depege *et al.*, 2003). *C. reinhardtii* transformants were selected on TAP (acetate-containing) plates containing 5 mg mL⁻¹ paramomycin (Sigma Chemical).
Blue-Native gel electrophoresis (BN-PAGE)

Thylakoid membranes were diluted to 0.5 mg Chl per ml in BN-PAGE solubilization buffer (50 mM Bis-Tris–HCl, pH 7.0, 750 mM ε-amino-n-caproic acid and 20% glycerol according to Schägger et al. (1994). Dodecyl-β-d-maltoside was added to a final concentration of 1% (w/v), solubilization was carried out on ice for 40 min, followed by centrifugation at 1,000xg for 10 min. The supernatant was supplemented with Serva blue G-250 from a 5% (w/v) stock in 500 mM ε-amino-n-caproic acid to a detergent/Serva blue G-250 ratio of 4:1 (w:w) and directly loaded onto the gel. BN-PAGE was carried out in a 5-12.5% gradient by using the Hoefer SE 600 and Hoefer SE 250 electrophoresis apparatuses according to Schägger et al. (1994) with modifications according to Thidholm et al. (2002).

Denaturing SDS-poly-acrylamide gel electrophoresis (SDS-PAGE)

For the isolation of total protein, cell biomass equivalent to 100 μg Chl were resuspended in 400 μL of 0.1 M dithiothreitol and 0.1 M Na₂CO₃. Following incubation for 5 min, 400 μL of 2× sample solubilization buffer containing 250 mM Tris-HCl (pH adjusted to 6.8), 7% SDS, 20% glycerol, 2 M urea and 10% β-mercaptoethanol was added and incubated for 1 h at room temperature. Unsolubilized material was removed by centrifugation at 15,000xg for 5 min prior to loading samples onto the SDS-PAGE. Aliquots corresponding to an equal amount of Chl were loaded in the wells of the stacking gel and electrophoresed through the 12.5% SDS-polyacrylamide running gels containing 1 M urea, as described by Tetali et al. (2007).

Generation of REP27 specific polyclonal antibodies and Western-blot analysis

Specific polyclonal antibodies were generated in rabbit against the REP27 protein using a C-terminal portion of the REP27 protein as recombinant antigen. Nucleotide fragments corresponding to F273-L367 of the C. reinhardtii REP27 were amplified using the primers listed in Table 1 and sub-cloned into the pET15b vector (Novagen), and the resulting constructs were expressed in Escherichia coli BL21 (DE3; Novagen). Expressed protein fragment with 6X Histidine tag were purified through Ni-NTA column and injected into rabbits according to the standard protocol of ProSci Inc (San Diego, CA). After three injections, cleared sera were used as antibodies against each antigen. For immunoblot analyses, SDS-PAGE-resolved proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore). The latter were blocked and subsequently incubated with rabbit immune serum containing specific polyclonal antibodies against the REP27 protein. Cross-reactions were visualized with
horseradish peroxidase conjugated secondary antibodies (Bio-Rad). Specific polyclonal antibodies against the D1/32 kD PSII reaction center protein were also employed, as described by Park and Rodermel (2004). Cross-reactions between protein bands and antibodies were visualized by the Supersignal ECL (Pierce) detection kit following the manufacturer’s specifications.

**Measurement of photosynthetic parameters**

The maximum quantum yield of primary PSII photochemistry was determined from measurements of the fluorescence yield ratio $F_V/F_M = (F_M - F_0)/F_M$, performed with a Plant Efficiency Analyzer (PEA) fluorometer (Hansatech Ltd., King’s Lynn, Norfolk, UK). The PEA saturating flash was provided by an array of six light-emitting diodes giving a maximum emission at 650 nm with an intensity of 3,000 µmol photons m$^{-2}$ s$^{-1}$. The fluorescence yield at 50 µs after the flash was considered as the non-variable $F_0$ value and the maximum fluorescence yield attained at later times after the flash as the $F_M$ (Strasser et al., 1995).

**Cell and thylakoid membrane fractionations**

Algal cells were harvested upon centrifugation (Beckman JA-10 Rotor) at 5,000 × g for 5 min. Pelleted cells were resuspended in sonication-buffer containing protease inhibitors (50 mM Tricine/NaOH, pH 7.8, 10 mM NaCl, 5 mM MgCl$_2$, 1 mM aminocaproic acid, 1 mM aminobenzamidine, 0.1 mM phenylmethylsulfonylfluoride and 2 mM Na-ascorbate) and sonicated on ice 3 times for 60 s in a 50 % duty cycle pulse mode, with 60 s cooling intervals in-between (Branson sonifier). The crude homogenate was then centrifuged at 3,000 x g for 5 min in order to remove unbroken cells and large cell fragments. To separate membrane-bound from soluble proteins, the crude homogenate was subjected to centrifugation at 20,000 g for 60 min. The pellet was used as the total thylakoid membrane fraction. The supernatant was used as the soluble fraction. Appressed and non-appressed thylakoid membrane regions were isolated upon mechanical fractionation and differential centrifugation, as described previously (Neale and Melis, 1991). Thylakoid membrane vesicles were precipitated by differential centrifugation at 10,000 × g (10K fraction), 40,000 × g (40K fraction) and 140,000 × g (140K fraction). The Chl alb ratio was determined in all fractions to provide a measure of the differential enrichment of grana and stroma-exposed thylakoid membranes, and an indication of the PSII/PSI ratio. The supernatant of the 140K fraction was used as the soluble fraction. Proteins of all subfractions were separated in SDS-PAGE gel and probed with specific polyclonal antibodies.
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### Table I

**Primers used in this work**

| Use of primer | Name of primers   | Primer sequence       | Primer sequence       |
|---------------|-------------------|-----------------------|-----------------------|
| Antibody generation | F273F & L367R | cccatatgtccgccgcccaggtccgccc | gtcgggtctgggccgccccttgagatcccc |
| Construction of truncated REP27 | TPR1         | CAGCAATATCAACCGACC   | CTCATCGCTGTTGAGGCTG   |
|               |                 | AGCATGCAGCCCCAACGAC  | CATGCTGGTCGGTGTGATAG   |
|               |                 | GATGAG                | TTGCTG                |
|               | TPR2           | CCCAACGCAGATGAGGCT    | AGCCACAATCTAGCTTCA     |
|               |                 | CGTGCCCTGAAGCTGATT   | GCCACGAGCTCCTCATCG    |
|               |                 | GTGGCT                | TCGTGGGG               |
|               | TPR1+2         | CAGCAATATCAACCGACC   | AGCCACAATCTAGCTTCA     |
|               |                 | AGCGGCCTGAAGCTGATT   | GCCGCTGGTGGTGTGATAT   |
|               |                 | GTGGCT                | ATTGCTG                |
| Chlamy codon optimized 2X MYC tag | Myc-F&R       | GGAGGTCTAGACATATGGA   | TTACAGGTCTCTCTCGCT     |
|               |                 | GCAGAAGCTGATCACGGGA  | GATCAGCTTCTGCTCCCC    |
|               |                 | GGAGAGCTGggGAGCA     | CAGGTCCTTCTCGCTGAT    |
|               |                 | GAAGCTGATCGAGCGAGG   | CAGCTTCTGCTCCATATG    |
|               |                 | AGGACCTGTAA          | TCTAGACCCC             |
LEGENDS TO THE FIGURES

FIGURE 1. Solubilized thylakoid membranes of *Chlamydomonas reinhardtii* wild type (CC425) were subjected to PAGE analyses. A (upper lane): Blue Native PAGE (BN-PAGE) of thylakoid membrane proteins stained with Coomassie Brilliant blue. A (lower lane): green native gel of the same sample. B, Two-dimensional gel electrophoresis (2-DE) of the wild type where BN-PAGE was used in the first dimension electrophoresis and a 12% acrylamide SDS-PAGE in the second dimension. The 2-DE gel was visualized by silver staining. PII1~PII3, PSII reaction center subunits (D1, D2, CP43 and CP47 proteins); PI, PSI complex (PsaA, PsaB and PsaF) and light-harvesting complex I protein; Cb, Cytochrome b_{6}-f; cA, Chloroplast ATP synthase (CF0F1); LT, LHCII trimer; LM, LHCII monomer; mA, mitochondrial H^{+}-ATP synthase.

FIGURE 2. Comparison of silver-stained 2-DE gels between wild type (CC425) and *rep27* mutant. The dashed lines demarcate the expected location of the PSII-core complex (CC425 panel) and of the LHCII (*rep27* panel). For more details, see legend of Fig. 1.

FIGURE 3. Western blot analysis of *Chlamydomonas reinhardtii* wild type and *rep27* thylakoid membrane proteins. Cells were grown under continuous irradiance of 100 μmol m^{-2} s^{-1} incident intensity. Steady-state levels of PSII subunits were determined from the intensity of the antibody cross-reaction in Western blots with specific polyclonal antibodies generated against D1, D2, CP47, CP43, REP27, and the ATP synthase. Note the lower D1 and CP43 levels in the *rep27*, and the absence of the REP27 in the mutant.

Figure 4. A, Western-blot analysis of total proteins from *Chlamydomonas reinhardtii* wild type (CC425), *rep27*, *rep27*-comp, D1-less (∆*psbA*) and D2-less (∆*psbD*) mutant strains, probed with specific polyclonal antibodies raised against the REP27 protein. B, SDS-PAGE of proteins (2 μg Chl loaded) separated onto 12% acrylamide and visualized with Coomassie Brilliant blue. The slightly slower electrophoretic mobility of the REP27 protein in the complemented strain (*rep27*-comp) is due to the presence of the MYC tag, introduced into the plasmid-construct used for complementation.
FIGURE 5. Western-blot analysis of total proteins from *Chlamydomonas reinhardtii* wild type (CC425), *rep27* and *rep27*-comp strains. Cells were fractionated into soluble (S) and total membrane (TM) portions. Total extract (TE) was obtained upon breaking the cells with acid-washed glass beads. The total membrane fraction (TM) was obtained by centrifugation at 20,000 g for 60 min. Lanes were loaded with 2 μg Chl. The supernatant was used as the soluble fraction (S). Relative protein content was estimated from the intensity of the antibody cross-reaction in the Western-blots. Specific polyclonal antibodies were used, generated against REP27, RbcL or D1, respectively.

FIGURE 6. Western-blot analysis of proteins from thylakoid membrane fractions isolated upon sonication and differential centrifugation at 10k (10,000×g), 40k (40,000×g) and 140k (140,000×g). Lanes were loaded with 2 μg Chl. Proteins were probed with specific polyclonal antibodies generated against D1 (A) and REP27 (B). (C) Coomassie-stained SDS-PAGE protein profile of the different thylakoid membrane fractions.

FIGURE 7. Folding model of the REP27 protein in the chloroplast thylakoid membrane. This protein contains 449 amino acids from the N- to the C-terminus. The number of positively charged amino acids is indicated for each domain of the protein. Two transmembrane helices (TMH1 and TMH2) are shown spanning the thylakoid membrane such that N- and C-termini are exposed in the chloroplast stroma phase. The model further shows two tetratricopeptide repeat motifs (TPR1 and TPR2) occurring near the N-terminus, and the transit peptide that is cleaved upon chloroplast import. REP27 displays a rather extensive C-terminal portion, which is exposed in the chloroplast stroma.

FIGURE 8. *REP27* cDNA constructs used for complementation of the *rep27* mutant. On the left, the name of each construct is given. In the middle, a map of the structural organization of the respective construct is shown. On the right, transformant strains obtained from the transformation of *rep27* mutant by these cDNA constructs.
FIGURE 9. Photoautotrophic growth of *C. reinhardtii* wild type, *rep27* mutant, and *rep27* transformant strains grown on HSM and TBP medium, respectively. A, CC125 wild type; B, *rep27* mutant; C, *rep27*-comp; D, *rep27*-ΔT1; E, *rep27*-ΔT2; F, *rep27*-ΔT1+2; G, *rep27*-ΔCt.

FIGURE 10. Growth (left) and efficiency of PSII primary photochemistry (Fv/FM, right) of CC125 wild type, *rep27* mutant, and *rep27* transformant strains grown on TAP media. Fv/FM values shown are the average from three independent experiments.

FIGURE 11. Western-blot analysis of total proteins from *Chlamydomonas reinhardtii* wild type (CC425), *rep27* mutant, and *rep27*-comp, *rep27*-ΔCt, *rep27*-ΔT1, *rep27*-ΔT2 and *rep27*-ΔT1+2 transformant strains, probed with specific polyclonal antibodies against REP27 (A) or D1 proteins (B). Also shown is the Coomassie-stained SDS-PAGE profile of the proteins from the various samples. 4 μg Chl were loaded onto 12% acrylamide for the SDS-PAGE and Western blot analysis.

FIGURE 12. Schematic of the REP27 protein function in the D1 protein turnover. The *REP27* C-terminal is essential for the de novo D1 biosynthesis at the level of ribosomal *psbA* mRNA translation and initial assembly in the PSII template. The TPR motifs participate in posttranslational modification (D1 activation). Both TPR motifs are needed to activate the nascent D1 and to confer functional status to the PSII holocomplex.
Fig. 2

CC425

rep27
Fig. 4
Fig. 5

[Image of a gel showing protein bands labeled as REP27, RbcL, and D1 with molecular weights (kD) indicated: 55, 43, 55, 34, and 26.]
Fig. 6
Fig. 7

Diagram showing the structure of a protein with the following features:
- CpTP
- $^1\text{NH}_2$
- TPR1
- TPR2
- TMH1
- TMH2
- Stroma
- Lumen
- +26
- +35
- $^{449}\text{COOH}$
**Fig. 8**

| Construct name | REP27 cDNA constructs | Mutant strains |
|----------------|------------------------|----------------|
| pSLREP27-comp  | P_{PsAD} TPR1 TPR2 REP27 2XMYC T_{PsAD} | rep27-comp |
| pSLREP27-ΔT1   | P_{PsAD} TPR1 TPR2 REP27 2XMYC T_{PsAD} | rep27-ΔT1 |
| pSLREP27-ΔT2   | P_{PsAD} TPR1 TPR2 REP27 2XMYC T_{PsAD} | rep27-ΔT2 |
| pSLREP27-ΔT1+2 | P_{PsAD} TPR1 TPR2 REP27 2XMYC T_{PsAD} | rep27-ΔT1+2 |
| pSLREP27-ΔCt   | P_{PsAD} TPR1 TPR2 REP27 2XMYC T_{PsAD} | rep27-ΔCt |
Fig. 9

HSM

TBP
Fig. 10

| Strains          | F<sub>V</sub>/<F<sub>M</sub> |
|------------------|-----------------------------|
| A: CC125         | 0.72                        |
| B: rep27         | 0.30                        |
| C: rep27-comp    | 0.68                        |
| D: rep27-ΔT1     | 0.29                        |
| E: rep27-ΔT2     | 0.28                        |
| F: rep27-ΔT1+2   | 0.28                        |
| G: rep27-ΔCt     | 0.27                        |
Fig. 11
Fig. 12

The image depicts a diagram illustrating the process of photosystem II (PSII) formation. Key components and processes include:

- **psbA** gene
- **translation** of proteins D2 and pD1
- **insertion** of D1 and D2 into the REP27 complex
- **activation** of the functional PSII complex

The diagram includes representations of proteins D2, pD1, and CP47, which are involved in the assembly of PSII. The final product is the **Functional PSII** complex, composed of D2, D1, CP47, and CP43.