

Characterization of the cyanobacterial *ycf37*: mutation decreases the photosystem I content

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We have constructed and analysed a cyanobacterial mutant that lacks the putative homologue of *ycf37*, the chloroplast open reading frame 37, which is conserved in different algae, but missing in the plastome of higher plants. In this report we show that Ycf37 of *Synechocystis* sp. PCC 6803 contains three tetratricopeptide repeat (TPR) units resembling the structural organization of Ycf3, a protein that has been suggested to function as a chaperone during photosystem (PS) I complex formation. We demonstrate a light-activated transcript accumu-

lation of this gene. Inactivation of *ycf37* leads to a lower PSI/PSII ratio and a higher phycocyanin/chlorophyll ratio in *Synechocystis* cells. The observed alterations in the *ycf37* mutants and the structural organization of the gene product suggest a functional role in PSI stability or assembly.

Key words: conserved chloroplast ORF, photosystem I biogenesis, *Synechocystis* sp. PCC 6803, TPR domain.

INTRODUCTION

Nucleotide sequencing of plastid genomes from higher plants as well as from green and non-green algae has revealed the presence of several conserved chloroplast open reading frames (ORFs), or *ycf* genes, whose functions remained unknown. Among these, some putative genes are absent from higher-plant chloroplast genomes, but still present in the plastid genomes of algae. Targeted inactivation of these ORFs in *Chlamydomonas reinhardtii* as well as in tobacco by biolistic transformation was shown to be a powerful tool for analysing the function of chloroplast-encoded proteins (reviewed in [1]). The chromosome of *Synechocystis* sp. PCC 6803 [2] contains putative genes with homology to most of the conserved plastid *ycfs*. Thus, targeted mutation of these ORFs in *Synechocystis* sp. PCC 6803 may be another approach to the elucidation of *ycf* functions [3,4].

Most of the generated chloroplast mutants are impaired in photosynthetic functions (for a review see [1]). Interestingly, some of these *ycfs* may encode chaperones involved in the assembly of the photosynthetic apparatus. The inactivation of *ycf4* in *C. reinhardtii* results in a complete loss of photosystem I (PSI) [5]. Also *ycf3* mutants in tobacco and *C. reinhardtii* lack detectable amounts of photosystem (PS) I, although PSI genes are normally expressed, suggesting a role of this gene during PSI assembly [5,6]. Interestingly, the Ycf3 amino acid sequence contains a so-called tetratricopeptide repeat (TPR) domain. The TPR is defined as a degenerate motif of 34 amino acids present in a variety of structurally and functionally diverse proteins [7]. TPRs contain two short amphipathic α -helices. They are typically present in tandem arrays of 2–16 motifs packed together in a parallel arrangement, thereby providing scaffolds for protein–protein interactions and often for the assembly of multiprotein complexes [8]. It has been suggested that Ycf3 and Ycf4 function as chaperones during PSI complex formation [5]. In the cyanobacterium *Synechocystis* sp. PCC 6803 disruption of *ycf4* only leads to reduced levels of PSI, which is still active and supports photoautotrophic growth on the wild-type level [3]. This indicates that despite the fact that most cyanobacterial and chloroplast

genes show a remarkable degree of sequence similarity, their inactivation can have different phenotypic consequences in respective organisms.

ycf37 is one of the ORFs that is conserved in the non-green algae *Cyanophora paradoxa* (accession no. NC_001675), *Cyanidium caldarium* (accession no. NC_001840), *Porphyra purpurea* [9] and *Guillardia theta* [10], but missing in the plastomes of higher plants. A highly similar ORF (*str0171*, called *ycf37* in the following) was found on the chromosome of *Synechocystis* sp. PCC 6803 [2]. In this report we show that Ycf37 contains three TPR units resembling the structural organization of Ycf3. We demonstrate a light-activated transcript accumulation for this gene. Furthermore, we have analysed the consequences of the inactivation of *ycf37* in *Synechocystis* sp. PCC 6803. Resultant mutants exhibit a lower PSI/PSII ratio and a higher phycocyanin/chlorophyll (PC/Chl) ratio compared with the wild type. Based on similar effects on PSI of the previously characterized cyanobacterial *ycf4* mutant [3] and the newly generated *ycf37* mutant, we propose that also Ycf37 is involved in PSI assembly or stability.

MATERIALS AND METHODS

Growth conditions

Synechocystis sp. PCC 6803 wild-type and mutant strains were grown in batch cultures at 30 °C in BG-11 medium [11] at a continuous photon irradiance of 30 μmol of photons $\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (standard conditions). Transformants of *Synechocystis* sp. PCC 6803 were selected on media with increasing amounts of chloramphenicol (2–7 $\mu\text{g}\cdot\text{ml}^{-1}$) or kanamycin (5–40 $\mu\text{g}\cdot\text{ml}^{-1}$). Growth of the wild-type and the mutant strains was monitored by attenuation measurements at 750 nm (Uvikon, Munich, Germany) and by cell counts using a haemocytometer (Thoma, Bad Blankenburg, Germany). For RNA preparations, 50 ml of exponentially growing *Synechocystis* sp. PCC 6803 cultures (D_{750} , 0.6–0.7) were either placed in a dark room or irradiated at 30 μmol of photons $\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with white light for 30 min.

Abbreviations used: Chl, chlorophyll; PC, phycocyanin; PS, photosystem; TPR, tetratricopeptide repeat; ORF, open reading frame.

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RNA isolation and RNA gel-blot analysis

Total *Synechocystis* sp. PCC 6803 RNA was isolated from cells using the TRIzol kit (Gibco-BRL, Rockville, MD, U.S.A.) according to the manufacturer's instructions, followed by purification via column chromatography (Qiagen, Hilden, Germany). Separation and analysis of RNA molecules by RNA gel-blot hybridization was performed as described previously [12]. Antisense RNA probes were transcribed from a *yef37*-specific DNA fragment using the MAXIscript kit (Ambion, Austin, TX, U.S.A.) and [α - 32 P]UTP (Amersham, Braunschweig, Germany). The DNA fragment was amplified from genomic *Synechocystis* sp. PCC 6803 DNA using primers P1 5'-TCTCTACCCATTGTTTATCTTTCCG-3' and P2 5'-TTTCAAGGAATTGGCCCGC-3'. T7 promoter sequences were generated using the Lig'n Scribe Kit (Ambion).

Mutagenesis

The *yef37* gene (locus *slr0171* in the CyanoBase [2]) was amplified using primers P1 and P2 (see above). The PCR product was cloned in vector pUC57 (MBI Fermentas, Vilnius, Lithuania). The chloramphenicol-resistance cassette from pACYC184 (New England Biolabs, Frankfurt am Main, Germany) was ligated into a single *BalI* site. The resulting construct was used to transform *Synechocystis* sp. PCC 6803 as described in [13]. For reversion of the mutants to the wild-type phenotype, a 1.4 kb fragment bearing *slr0169* and *slr0171* sequences was amplified using primers P3 5'-TGGAGGCTCGTACAATACAT-3' and P4 5'-GAAAGAATTAACGGGCGACA-3'. The PCR product was cloned into the pGEM-T vector (Promega, Madison, WI, U.S.A.) and a kanamycin-resistance cartridge from pUC4K [14] was inserted into a *BsaAI* site 110 bp upstream of the *yef37* start codon. The *yef37* mutant was transformed with this construct.

Absorption, 77 K fluorescence emission and P700 measurements

In vivo absorption spectra of whole cells of wild-type *Synechocystis* sp. PCC 6803 and its *yef37* mutant were measured at room temperature using an M500 Specord spectrophotometer (Zeiss, Jena, Germany). PC/Chl ratios were determined from absorption spectra as described previously. Low-temperature fluorescence emission spectra (77 K) were recorded using an F-4500 Hitachi spectrophotometer (Hitachi, Tokyo, Japan) as described in [3]. Chl was measured by extraction of whole cells in 100% methanol and determined according to [15]. PSI content was estimated spectrophotometrically from thylakoid membranes of wild-type *Synechocystis* sp. PCC 6803 and the *yef37* mutant. Thylakoid membranes were isolated as described in [16]. An absorption difference spectrum was recorded for thylakoid membranes (10 μ g of Chl) with 10% (v/v) Triton X-100 in the presence of sodium ascorbate (0.3 mM) or ferricyanide (0.3 mM). The concentration of P700, the primary electron donor of PSI, was estimated from the maximum absorption difference (at \approx 702 nm) using an extinction coefficient of 64 mM $^{-1}$ ·cm $^{-1}$ [17].

Oxygen-evolution measurements

Oxygen evolution from whole cells under continuous white light was determined polarographically with a Clark-type electrode at 30 °C with 10 mM NaHCO $_3$ as an acceptor. Oxygen evolution was measured with saturating illumination.

Protein extraction and Western-blot analyses

Cultures (50 ml) of the wild-type and two different *yef37* mutant clones (*yef37*-#1, *yef37*-#3) were harvested by centrifugation,

resuspended in 500 μ l of lysis buffer (50 mM Tris/HCl, pH 7.0, and 2%, w/v, SDS) and sonicated in the presence of 0.3 g of glass beads. After centrifugation, Western-blot analyses of proteins from the supernatant (equivalents of 7 μ g of Chl) were carried out as described in [18]. Signal intensities were quantified densitometrically by using an integration control unit (ICU-1) and the Image Doc/EASY Win2 software from Herolab (Wiesloch, Germany). Relative amounts of PsaA and PsbO proteins were calculated after standardization on the internal RbcL-derived signal.

RESULTS

Structure and expression of the *yef37* gene from *Synechocystis* sp. PCC 6803

Computer-assisted similarity searches with the TPR-consensus motif have revealed a total of 22 TPR domains encoding ORFs within the completely sequenced genome of *Synechocystis* sp. PCC 6803 (F. Ossenbühl and J. Nickelsen, unpublished work). These include the *yef3* gene (*slr0823*), which previously has been reported to be a TPR protein [5], and the as-yet-uncharacterized *yef37* gene (*slr0171*). Figure 1 shows the three TPR domains present in both Ycf3 and Ycf37. In contrast to the previous report, our alignment predicts an unsplit third TPR motif for Ycf3 that is separated by 14 amino acids from the second TPR. In Ycf37, the first and second TPRs are separated by five amino acids, whereas the second and third ones are in tandem. All TPRs contain two short amphipathic α -helices and, thus, the two polypeptides share a similar overall structure, suggesting that they might fulfil related functions. The same holds for the plastid *yef37* genes from non-green algae. All of them contain three TPR domains with the second and third TPRs in tandem and a spacer between the first and second, ranging from four amino acids in *C. paradoxa* to nine in *P. purpurea*.

Transcript analysis was performed to provide information regarding the expression of *yef37*. RNA was isolated from wild-type *Synechocystis* sp. PCC 6803 cells grown under white light and from cells that were transferred to darkness for 30 min. RNAs were hybridized with a radiolabelled antisense probe complementary to *yef37*. Only one band of 0.9 kb could be detected, implying monocistronic transcription of this gene. Co-transcription of *yef37* with *slr0169* (the ORF located upstream) would need a minimum of 1400 kb, and that with the downstream ORF (*slr0172*) at least 1100 bp. Nevertheless, polar effects could also occur, when the 5' end of the downstream transcript (*slr0172*,

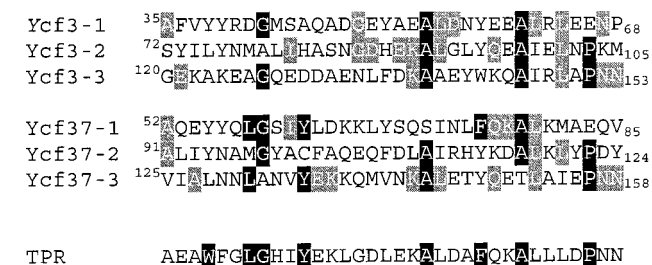


Figure 1 Alignment of the three TPR units in Ycf3 and Ycf37 from *Synechocystis* sp. PCC 6803 with the general TPR consensus sequence (TPR [23])

Highly conserved amino acid positions characteristic of TPR proteins are shown against a black background, and less-conserved ones are shown on grey. Amino acid positions are given at the beginning and end of each repeat.

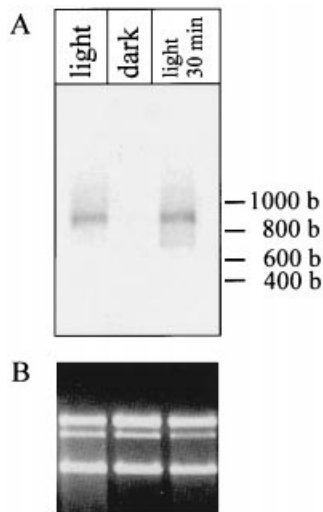


Figure 2 RNA gel-blot analysis

Ycf37 mRNA level was examined in wild-type *Synechocystis* sp. PCC 6803. Cells were collected from light-grown mid-logarithmic-phase cultures (lane 1), or after 30 min of dark incubation (lane 2) followed by illumination for 30 min (lane 3). Total RNA (2.5 μ g) was loaded per lane (B) and hybridized with a *ycf37*-specific RNA probe to detect sense transcripts (A). Sizes of marker bands (low-range RNA ladder, MBI Fermentas) are indicated.

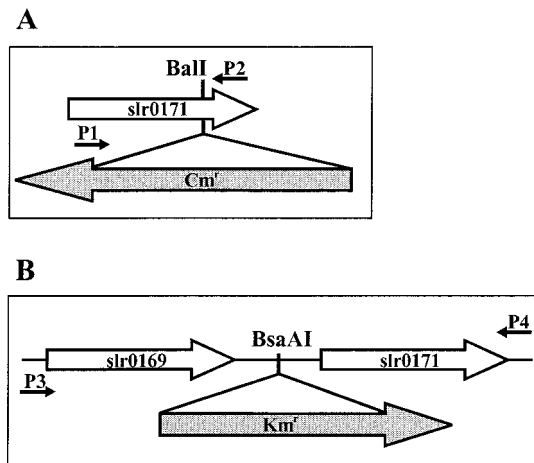


Figure 3 Inactivation of *ycf37* in *Synechocystis* sp. PCC 6803

The strategy for constructing donor plasmids with inactivated *ycf37* copy (A) and with wild-type copy (B) is shown. Arrows within the boxes indicate the direction of transcription. P1, P2, P3 and P4 indicate the primers used to amplify the DNA fragment and check for complete segregation of the mutant strain (see text). Cm^r , chloramphenicol-resistance cartridge; Km^r , kanamycin-resistance cartridge (shaded arrows).

guaB, a putative IMP dehydrogenase) extended into the *ycf37* region that was destroyed. Using an antisense RNA probe starting at the stop codon of the *ycf37* gene, no other bands could be detected (results not shown). It is, therefore, unlikely that the promoter region of *guaB* extends into *ycf37* gene region. Moreover, the phenotype of the mutants can hardly be explained by a mutation in a gene that potentially functions in purine ribonucleotide biosynthesis.

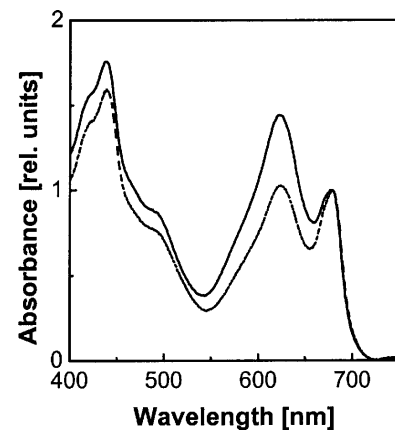


Figure 4 Absorption spectra of wild-type and *ycf37* mutant cells

In vivo absorption spectra were measured using whole cells of wild-type *Synechocystis* sp. PCC 6803 (dashed line) and its *ycf37* mutant (solid line). The peaks at 440 nm and 679 nm mark the maxima of Chl *a* absorption, and the peak at 625 nm reflects the absorption maximum of PC. The spectra were corrected for light scattering at 750 nm. The PC/Chl ratios were 0.9 for the wild type and 1.3 for the mutant when calculated from the spectra according to [24].

Table 1 Pigment content, electron-transfer rates and measurement of P700 in wild-type and *ycf37* mutant cells

Estimation of Chl content, PSI content and oxygen-evolution rates was performed using liquid cultures at a D_{750} value of 0.5–0.7 (mid-logarithmic phase). Means \pm S.D. from three independent experiments are shown. Values in parentheses were calculated on a per-cell basis (μ mol of $O_2 \cdot cell^{-1} \cdot h^{-1}$).

Parameter	Wild type	<i>ycf37</i> mutant
Chl content (mmol/cell)	$4.3 \times 10^{-14} \pm 2.5 \times 10^{-15}$	$2.9 \times 10^{-14} \pm 1.7 \times 10^{-15}$
PSI content (molecules/cell)	258000 ± 17400	187000 ± 2800
Electron-transfer rate ($H_2O \rightarrow NaHCO_3$; μ mol of $O_2 \cdot mg$ of Chl $^{-1} \cdot h^{-1}$)	150 ± 8 (5.7×10^{-9})	226 ± 12 (5.8×10^{-9})

Northern-blot analyses indicated a light-induced transcript accumulation of *ycf37*, whereas dark incubation led to fast reduction of the transcript levels (Figure 2).

Inactivation of the *ycf37* gene

We disrupted the *Synechocystis* sp. PCC 6803 *ycf37* homologous gene (*slr0171*) by inserting a chloramphenicol-resistance cassette from pACYC184 into the single *BaII* site 326 bp downstream from the translation start site (Figure 3). After transformation of *Synechocystis* sp. PCC 6803 wild-type cells with this construct, complete segregation of the mutation was confirmed by PCR as well as by Southern-blot hybridization (results not shown).

The photoautotrophic growth rate of the *ycf37* mutant was indistinguishable from that of the wild type under standard growth conditions (results not shown). However, mutant cultures differed clearly from the wild type by their distinct blue colour. As is apparent from the absorption spectra shown in Figure 4, the mutation of *ycf37* leads to a significant increase in the PC absorbance peak at 625 nm. In addition, Chl content per cell was remarkably reduced in the *ycf37* mutants (Table 1). This is

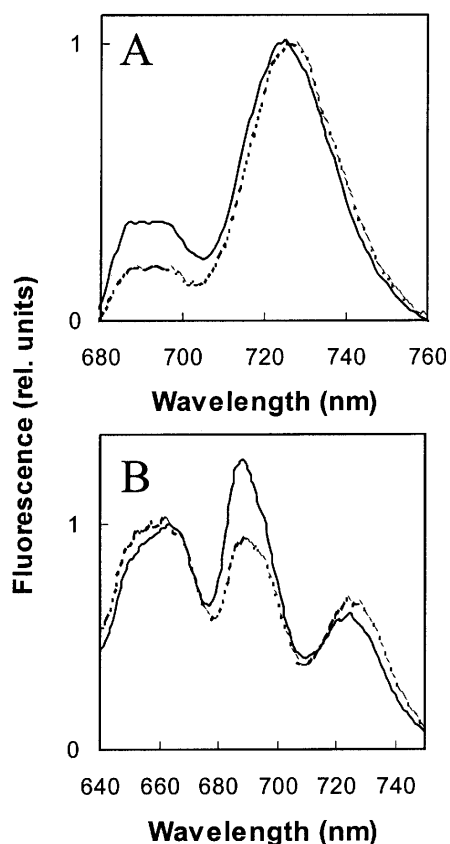


Figure 5 77 K fluorescence emission spectra

The 77 K fluorescence emission spectra of whole cells of wild-type *Synechocystis* sp. PCC 6803 (dashed line) and *ycf37* mutants (solid line) are shown after excitation of Chl *a* at 440 nm (A) and after excitation of phycobilisomes at 590 nm (B). The spectra were normalized to the emission maxima at 725 nm (A) or 665 nm (B).

reflected by the higher PC/Chl ratio of the *ycf37* mutant in comparison with the wild type (Figure 4).

ycf37 mutants show an altered PSI/PSII ratio

Cyanobacteria are able to change their PSI/PSII ratio in response to altered light conditions. Since an increase of the PC/Chl ratio could reflect such changes, we performed fluorescence measurements. Low-temperature (77 K) fluorescence emission spectra of intact cells after Chl excitation are shown in Figure 5(A). The large emission peak at 725 nm was predominantly derived from PSI, whereas the two peaks at 685 and 695 nm originated mainly from Chls of the PSII core complexes. When spectra were normalized to the PSI emission peak, a decrease in the PSI/PSII fluorescence emission ratio became obvious in the *ycf37* mutant. The excitation wavelength of 590 nm was absorbed mainly by PC in the phycobilisomes (Figure 5B). Three characteristic peaks were evident, corresponding to fluorescence emission from PC and allophycocyanin (650–660 nm), the PSII core Chls and the long-wavelength terminal emitters of the phycobilisome core (680 nm) and the PSI core Chls (725 nm). As compared with the wild type, the *ycf37* mutant showed increased fluorescence from the phycobilisome terminal emitters and decreased fluorescence from PSI. These fluorescence spectra suggest an alteration in the PSI/PSII ratio in the mutant strain. This finding is supported by the results of Western-blot analysis using an antibody directed

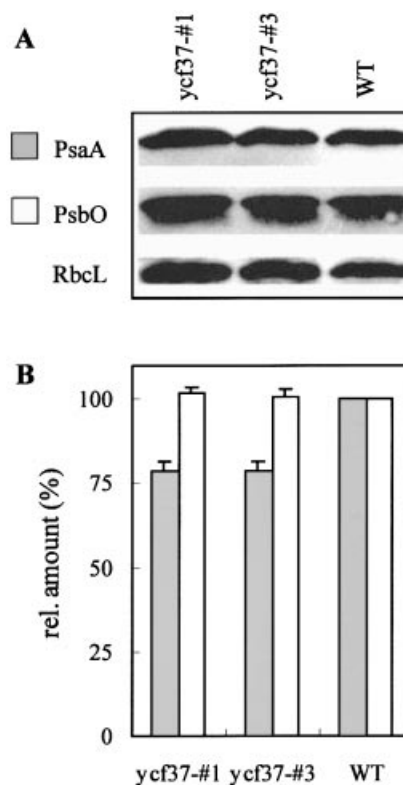


Figure 6 Western-blot analysis of photosynthetic complexes in *ycf37* mutants

(A) Total proteins from two independent *ycf37* mutant clones (*ycf37*-#1 and *ycf37*-#3) and the wild type (WT) were separated by SDS/PAGE, blotted on to filters and probed with antibodies against PsaA, PsbO or RbcL. (B) Signal intensities were quantified and standardized to the RbcL signal. Relative amounts of PsaA (grey bars) and PsbO (white bars) compared to the wild type (100%) are shown. Membranes were exposed to film for 1 min for all antibodies used. Means \pm S.D. from three independent Western blots are shown.

against the PSI subunit PsaA. The immunoblot in Figure 6 shows that the level of PsaA (normalized to the RbcL signal) was reduced to about 80% of the wild-type level in two independently constructed *ycf37* mutants. The amount of PSII was not affected in *ycf37* mutants, as was shown by Western-blot analysis using an antibody against PsbO (Figure 6).

As judged from spectrophotometric measurements of P700 concentration in the *ycf37* mutant there was no significant decrease in the number of PSI reaction centres when calculated on the basis of Chl (Table 1). Taking into account the significant decrease of the Chl content in the *ycf37* mutant (Table 1), a prominent decrease in the amount of PSI reaction centres per cell was evident. The measurement of O₂ evolution revealed that the rate of light-saturated whole-chain electron transfer was about 33% higher in the mutant than in the wild type when normalized to Chl content (Table 1). Due to the lower Chl content in the mutant, the electron-transfer rates in the wild type and mutant were nearly identical when related to the cell number.

To confirm that the observed phenotype was indeed due to the inactivation of the *ycf37* gene we attempted a reversion of the *ycf37* mutant strain to the wild-type phenotype by transformation with the wild-type gene. A kanamycin-resistance gene cartridge was inserted upstream of the *ycf37* gene to facilitate selection of transformants (Figure 3). In homozygous revertants the mutant version of *ycf37* with the inserted cassette was replaced by the

wild-type gene. Therefore, revertants were resistant only to kanamycin and lost their chloramphenicol resistance. Transformants showed the wild-type phenotype, as revealed by absorption and fluorescence spectra (results not shown). These results demonstrate that the phenotype of *ycf37* mutants is due to the inactivation of this gene rather than to a compensatory mutation or a polar effect on transcription of the 5' ORF. Polar effects of insertion of the resistance cassettes on the transcription of downstream genes cannot be totally excluded. However, they are very unlikely, as transcription analysis showed a monocistronic *ycf37* messenger RNA. Moreover, no additional band resulting from a potential extension of the 3' gene (*guaB*) into the *ycf37* gene region could be detected in Northern-blot experiments.

DISCUSSION

The chloroplast ORF *ycf37* is so far known to be conserved in the plastid genomes of four algal species *C. paradoxa* (accession no. NC_001675), *C. caldarium* (accession no. NC_001840), *P. purpurea* [9] and *G. theta* [10]. The hypothetical protein encoded by the *Synechocystis* sp. PCC 6803 ORF *slr0171* shares high similarity with the conserved hypothetical chloroplast protein Ycf37. A predicted translation product of an *Arabidopsis thaliana* EST shows similarity to the *Synechocystis* sp. PCC 6803 *ycf37* gene product (35% identity, 62% similarity), suggesting a transfer of this gene to the nuclear genome later in evolution of plants. The conservation of *ycf37* across relatively large phylogenetic distances implies an important role of the gene product in chloroplast and cyanobacterial cell function.

To study the function of *ycf37*, we created a mutant strain in the cyanobacterium *Synechocystis* sp. PCC 6803 by targeted insertional mutagenesis of *slr0171* (*ycf37*). We could not observe differences in growth rates between wild-type and mutant strains under photoautotrophic conditions. Nevertheless, the observed light-induced transcript accumulation of this gene may suggest a role for *ycf37* in photosynthetic processes. As was shown, inactivation of *ycf37* in *Synechocystis* sp. PCC 6803 gave rise to a fully segregated mutant strain that showed an alteration of the PSI/PSII ratio. The decrease of this ratio was the result of a lower PSI content. Because in cyanobacteria most of the Chl is associated with PSI, whereas PSII has a phycobilisome antenna, the observed changes in the stoichiometry of the PSs coincide well with the increase in the PC/Chl ratio in the mutants. In mutant cells, there was a reduction of the Chl content that can be related to the lower PSI content. Interestingly, the reduction of the PSI content had no effect on O₂-evolution rate in the mutant cells when calculated on a per-cell basis. This implies that the number of PSI centres is not a limiting factor for electron transfer in *ycf37* mutant cells.

The *ycf4* mutant strain of *Synechocystis* sp. PCC 6803 [3] has a very similar phenotype. Nevertheless, there are differences between the *ycf4* and *ycf37* mutants. In the *ycf4* mutant the decrease in the PSI/PSII ratio appeared to be a result of both a reduction of the PSI content and an increase in the PSII content [3]. In contrast, the reduction of the PSI content in the *ycf37* mutant was not paralleled by an increase in PSII content. To further rule out the possibility that a *ycf4* mutant was selected together with the knocked-out *ycf37*, we performed a sequence analysis of *ycf4*. We found no changes in the *ycf4* nucleotide sequence in *ycf37* mutants. Moreover, the wild-type phenotype could be restored by back transformation of the *ycf37* mutant with the wild-type gene. Thus the observed phenotype is the result of the inactivation of *ycf37*. Similar phenotypical effects, such as a blue colour and a reduction of the PSI content, were also shown for mutations of the *btpA* [19] and *ftsH* genes [20].

Proteins that are encoded by these genes were suggested to function in PSI biogenesis. The inactivation of *ycf3* and *ycf4* in *C. reinhardtii* [5] had more drastic effects, resulting in the complete loss of functional PSI. Obviously, inactivation of different genes leads to similar effects: a reduction or complete loss of PSI reaction centres. Three of them are conserved in plastid and cyanobacterial genomes.

It remains unclear why part of the PSI complex seems to assemble and function correctly in the *ycf37* mutant. However, there are several examples where oligomeric proteins lacking one subunit assemble in cyanobacteria, while they are degraded rapidly in *C. reinhardtii* chloroplasts. This holds true for PSII complexes, lacking for instance PsbK, or for PSI subunits when expressed in the absence of PsaC (reviewed in [21]). The same phenotypic differences between *Synechocystis* sp. PCC 6803 and *C. reinhardtii* mutants could be detected in *ycf4* mutants of both organisms [3,5]. Thus photosynthetic eukaryotes may have developed a higher proteolytic activity in order to regulate biogenesis of multi-subunit complexes [21].

In conclusion, the data presented here show that inactivation of a *Synechocystis* sp. PCC 6803 gene with similarity to the conserved chloroplast ORF *ycf37* results in a reduction of the PSI content. The relatively small decrease (about 30%, see Table 1) of the PSI content in *ycf37* mutant cells is not surprising when the phenotype of this mutant is compared with other *Synechocystis* sp. PCC 6803 knockouts of genes that were shown to be involved in the biogenesis of PSI [3,19,20]. In the *Synechocystis* sp. PCC 6803 genome there are several ORFs with similarity to putative chloroplast genes. Two of the gene products (Ycf3, Ycf4) seem to be involved in assembly of PSI [5]. They can be cross-linked to subunits of the PSI complex in *Synechocystis* sp. PCC 6803 (J. Miao, unpublished work), suggesting a direct interaction between these putative chaperones and PSI complexes. Another ORF (*slr0399*, with similarity to *ycf39*) was suggested to have a chaperone-like function, possibly in the insertion of the quinone Q_A into PSII reaction centres [22]. Based on our observation that inactivation of the light-induced *ycf37* gene in *Synechocystis* sp. PCC 6803 leads to a reduction of the PSI content we propose a function of *ycf37* in assembly or, more likely, stability of PSI.

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