

psbA gene family coding D1 proteins of photosystem II of cyanobacteria *Synechocystis* PCC 6803 is essential for DNA repair/protection

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***Synechocystis* PCC 6803 wild type (WT) switches from UV-resistant to UV-sensitive state on shifting from photoautotrophic to photoheterotrophic mode of growth. The strain also displays the differential UV sensitivity between the light and dark phase in the photoautotrophic mode of growth. Here we provide genetic evidence for the role of D1 polypeptide in repair/protective activity in the UV sensitivity switching phenomenon. The strain used can switch between photoautotrophy and photoheterotrophy, thereby permitting assessment of the role of photosystem II (PSII) components in functions other than photosynthesis. We show that D1 mutants selected for altered UV sensitivity are novel and different from those known to affect the electron transport function or PSII assembly. To our knowledge this is the first report on the involvement of any PSII constituent in genome housekeeping activity. The observation suggests a new pathway for stress management in cyanobacteria and, by implication, also in higher plants.**

Keywords: Cyanobacteria, D1 protein, DNA repair/protection, photosystem II, ultraviolet sensitivity switch.

AMONG prokaryotes ultraviolet (UV) resistance of cyanobacteria is high, presumably because of adaptation to stress from solar radiation comprising unfiltered high UV component in the absence of ozone shield for a major period of their evolutionary history, that is estimated to be at least 2.3 billion years¹⁻⁵. They occupy niches covering a wide range of abiotic and biotic stresses⁶⁻⁸. At high intensities of solar radiation, UV light adds to the stress by inflicting photo-oxidative damage to the electron transport system and the genome⁹. The mechanisms for maintaining functional states of these two targets are complex and of vital significance, particularly in obligate photoautotrophic cyanobacteria, because they are dependent

solely on light for supply of organic carbon. Their growth in the presence of organic carbon sources exogenously added to the medium is either blocked or poor in the dark. Obligate nature of photoautotrophy, apparently, has evolved in these organisms as the preferred mode of growth, presumably because it maximizes survival through enhanced dark repair of DNA damage accumulated during growth in light¹⁰.

The complexity of UV stress response in cyanobacteria is also evident from its effect in a wide spectrum of physiological conditions of cultures of obligate photoautotrophs, prominent among which are: (i) the level of UV sensitivity switches reversibly in a distinctly different manner on transition between the light and dark phase¹¹; (ii) UV sensitivity is affected by perturbation of redox level on addition of herbicides, DCMU or atrazine, that specifically interact with core polypeptide D1 of reaction centre (RC) of photosystem II (PSII) that blocks photoreduction of plastoquinon Q_b (refs 11 and 12); (iii) high sensitivity develops following arrest of protein synthesis in light by chloramphenicol, but not in the dark phase^{13,14}; (iv) dominant effect of restoration of resistance by DCMU in photoautotrophic culture blocked for protein synthesis by chloramphenicol¹¹; (v) UV sensitivity unrelated to replicative state of DNA¹¹, and (vi) development of high resistance under near anaerobic condition created by prolonged exposure to argon gas in light¹⁴.

Detailed treatment of a model to explain the above-mentioned complex response of cyanobacteria to UVC has been published elsewhere¹². Briefly, the model assumes: (i) DNA and functional PSII complexes are independent lethal targets, and (ii) PSII exists in two UV sensitivity states depending on a functional association of the high turnover RC polypeptide D1 with a UV-resistant subcomplex of PSII in dynamic equilibrium during growth in light, the ratio of the two kinds of PSII complexes within a cell determining its viability when repair of DNA damage is saturated by prolonged dark incubation. The model makes several predictions. These are: (i) exclusive DNA repair system should not interfere with

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UV sensitivity switching process during transition between the light and dark phase in autotrophic mode of growth; (ii) it should be possible to obtain mutants with altered UV sensitivity compared to wild type (WT), which should map the mutations to genes of some constituent(s) of functional PSII complex, that confers altered UV sensitivity measured by lethal effect on colony-forming ability without losing the capability to grow photoautotrophically; (iii) in mutants with functional PSII knocked out, UV sensitivity switch should be eliminated and the survival curves of cells from the light and dark phase should be identical, and the mutants should be at least as resistant as WT, if not more. The last prediction follows from the assumption in the model that damage to UV-sensitive functional PSII leads to loss of viability even when DNA is fully repaired, since DNA and PSII are considered independent lethal targets¹².

In order to test prediction (i), we considered knocking out the best-studied regulatory network of multiple DNA repair systems in prokaryotes, similar to the SOS response in *Escherichia coli* controlled by *recA*–*lexA* gene pair, if present in cyanobacteria. If the light/dark UV sensitivity switching was due to some specific DNA repair system that evolved under the umbrella cover of the putative SOS repair system of cyanobacteria, knocking out *recA* or *lexA* would disable the switch. Such an outcome would falsify the first assumption of the model. The homozygous *recA* mutant was, as expected, highly UV-sensitive, but the light/dark sensitivity switch was not disabled¹⁰. Similarly, an *uvrA* homozygous knockout mutant was sensitive to UV, as expected, but this too retained the light/dark sensitivity switch¹⁵ (Appendix 1). The results are consistent with prediction (i) of the model. However, they do not rule out the possibility of the yet unknown novel DNA repair system sensitive to light/dark shifts. Prediction (ii) was also tested by screening for mutants with altered UV-sensitivity among randomly mutated *psbA2* allele collection in a vector that was transformed into a *psbA* deletion derivative of *Synechocystis* PCC 6803, and the transformants selected for photoautotrophic growth as shown in the present study. Several UV-sensitive and UV-resistant autotrophic mutants with altered *psbA2* allele sequences were obtained. Mutations were in regions different from those known from earlier studies to investigate the mechanism of electron transport function of D1. This is consistent with prediction (ii). In experiments reported here we show that only the first part of prediction (iii) is consistent with the results. The second part that follows from the assumption that DNA and PSII are independent lethal targets of UV, is inconsistent with the results of the experiment designed to test the prediction of the model. Interestingly, as reported here, this discrepancy leads to the unexpected conclusion that the products of *psbA* gene family are required for protection or repair of damage to

the genome from UV stress, even when it is not a part of a functional PSII complex.

Synechocystis PCC 6803 has been used in this study since it is a facultative photoautotroph that can utilize exogenous glucose in light, thereby making carbon fixation through photosynthetic electron transfer function of PSII nonessential for growth^{16–19}. Thus it was possible to check the effect of inactivation of photosynthetic CO₂ fixation without blocking the growth of *Synechocystis* PCC 6803 in light. This permitted determining UV sensitivity of the strain in the absence of PSII core constituents, D1 and CP47, by deletion analysis and construction of mutant *psbA2* alleles with altered UV sensitivity that we report here.

Materials and methods

Bacterial strains and culture conditions

Synechocystis PCC 6803 was used as the WT strain (kindly provided by Takakazu Kaneko)²⁰. The *ΔpsbA*, *ΔpsbB* and D1:ctrl strains were a gift from Julian Eaton-Rye (Table 1). All cultures were grown in BG-11 medium²¹ with light intensity of ~800 lux, at 33°C in the presence of appropriate antibiotics at concentrations of chloramphenicol 15 µg/ml, spectinomycin 25 µg/ml and kanamycin 25 µg/ml. A constant mixed illumination of white light and tungsten lamp was used for growth in liquid as well as on plates. Air from an aquarium pump was filtered and used for bubbling the liquid cultures. The WT and mutant cultures were stored at –80°C in sterile BG-11 containing 15% glycerol. Solid media for plates were prepared by addition of 1.5% (w/v) agar, 10 mM TES/NaOH, pH 8.2, and 0.3% sodium thiosulphate²² to the liquid medium. Glucose (5 mM) was added to both liquid and solid media for photoheterotrophic growth. Atrazine (20 µM) was added to the media of cultures of the PSII mutant strains for photoheterotrophic growth to ensure blocking of photosynthetic electron transfer function.

UV sensitivity and photoreactivation

Aliquots (100 µl) of various serial dilutions of log-phase cultures of the WT and the mutants were spread on the BG-11 plates. With lids removed, the plates were immediately irradiated with UV (254 nm, fluence rate: 9.2 J m⁻² s⁻¹) for different lengths of time. All the plates were then kept in the dark overnight at room temperature (about 22°C) to prevent photoreactivation. After dark incubation, the plates were transferred to the light growth chamber till growth was visible (~10–14 days). For photoreactivation experiments, the plates were immediately transferred to the light growth chamber after irradiation with no post-UV dark incubation period. For determining the change in UVC sensitivity on light to

Table 1. *Synechocystis* sp. PCC 6803 strains

Strain	Relevant genotype	Source
<i>Synechocystis</i> sp. PCC 6803	Wild type (WT)	Takakazu Kaneko
D1:ctrl	$\Delta psbA1$, $\Delta psbA3$, $psbA2^+$, Kan ^r	Julian J. Eaton-Rye
$\Delta psbA$	$\Delta psbA1$, $\Delta psbA2$, $\Delta psbA3$, Cam ^r	Julian J. Eaton-Rye
$\Delta psbB$	$\Delta psbB$, Spec ^r	Julian J. Eaton-Rye
RKM118	$\Delta psbB$, $phrA::cam$, Cam ^r , Spec ^r	The present study

dark transition, the growing culture in light was divided into two flasks, one covered with aluminum foil, both being otherwise identically treated for 24 h. UV irradiation and post-UV dark incubation were carried out as described above. Unless otherwise mentioned, we refer to viability measured by colony counts after allowing a 24 h incubation in the dark to eliminate photoreactivation following UV irradiation as UV-sensitivity or dark UV-sensitivity.

Cloning and inactivation of the *phrA* gene of *Synechocystis* PCC 6803

The slr0854 (*phrA*) gene was PCR amplified using Pwo DNA polymerase (Roche Molecular Biochemicals) from genomic DNA of *Synechocystis* sp. PCC 6803 using the primers P15 (5'-GCCAGTTGCCAGAATCATCAGTGT-3') and P16 (5'-GGAGTTCTACTCTTCGGCCTAGTG-3'). The resultant fragment was directly used for cloning into the *EcoRV* site of pCR-Script™ Amp SK (+) plasmid (Stratagene), yielding the plasmid pphrA.

The *phrA* gene in the plasmid pphrA was inactivated by insertion of chloramphenicol resistance cassette into the unique *HpaI* site (1355 bp downstream of the start codon), yielding the plasmid pphrAcam. The chloramphenicol resistance cassette (hereafter referred to as cam) was PCR amplified from *Tm9*, using the primers: P17 (5'-TGA CGG AAG ATC ACT TCG CA-3') and P18 (5'-CAC ACG GTC ACA TTG CTT CC-3'). The point of insertion of the cam cassette was verified by sequencing using the outward primers: P30 (5'-AGC TGA ACG GTC TGG TTA TAG GTA-3') and P31 (5'-GCT TCC ATG TCG GCA GAA TGC TTA-3').

Cyanobacterial chromosomal DNA was isolated from cultures grown to late log phase by the method described by Porter²³. Transformation in *E. coli* was performed according to Sambrook *et al.*²⁴.

Transformation and selection of *phrA::cam* derivative of $\Delta psbB$ mutant

The plasmid pphrAcam was used to transform $\Delta psbB$ strain of *Synechocystis* PCC 6803, according to Minda *et al.*¹⁰. Transformants were selected on BG-11 agar plates containing chloramphenicol 7.5 µg/ml, glucose 5 mM, spectinomycin 25 µg/ml and atrazine 20 µM. Colonies appeared in about 10–12 days.

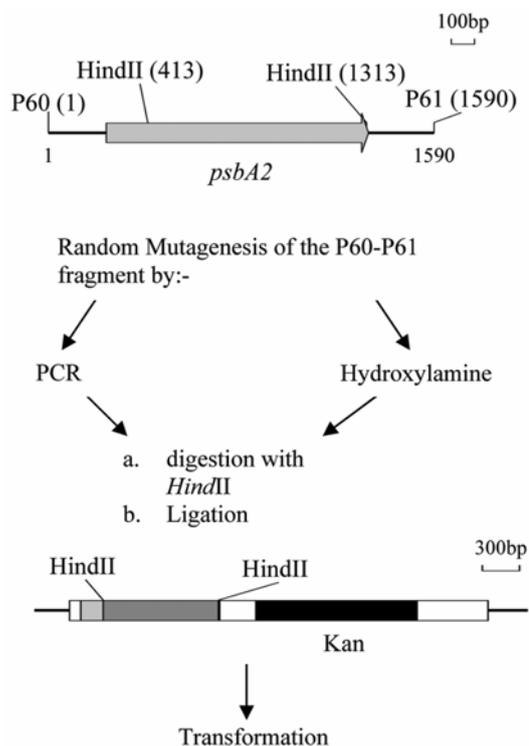


Figure 1. Schematic diagram for introducing random mutagenesis into *psbA2* allele for D1 protein. The P60–P61 fragment of *psbA2* was subjected to random mutagenesis by PCR or hydroxylamine treatment, as described in text. It was then digested with *HindII* and ligated with pTZ2-K digested with *HindII*, and used for the transformation of competent DH5 α cells. Plasmid was extracted from all the transformants and used for the transformation of *Synechocystis* PCC 6803 $\Delta psbA$ strain.

Construction and sequencing of mutant *psbA2* alleles giving altered UV sensitivity

Figure 1 shows a schematic diagram of steps in the generation of random D1 mutants. Random mutagenesis by PCR was performed using GeneMorph Random Mutagenesis kit. The reaction mix was prepared by adding to 37.5 ml of sterile DDW, 5 µl of 10× mutazyme reaction buffer, 4.0 ml of 10 mM dNTP mix, 1.0 µl (15 pmol) of each primer, 1.0 µl of template (10 pg–100 ng) DNA, and 1 µl of mutazyme DNA polymerase.

For hydroxylamine mutagenesis, samples of pure plasmid DNA were added to 5 volumes of 0.1 M sodium phosphate buffer (pH 6.0) containing 1 mM EDTA, and 4 volumes of 1 M hydroxylamine hydrochloride (pH 6.0)

Table 2. Photoautotrophic growth rates of UV-sensitivity altered D1 mutants of *Synechocystis* PCC 6803. Altered amino acids in the sequences with respect to D1:ctrl strain are shown adjacent to the strain name. The location of the mutations on the known D1 structure motifs is mentioned in the second column

Strain	Position of the mutations in the helices of D1 protein	Photoautotrophic growth rate (h)
D1:ctrl	Not applicable	20.6
RKM112 (Leu353Ser)	C-terminal tail	30.6
RKM113 (Leu114Ser), (Phe255Leu)	B helix, DE box	24.8
RKM114 (Ala287Val)	E Helix	28.3
RKM115 (Leu341Pro)	C-terminal tail	44.4
RKM116 (Tyr262Cys), (Phe295Leu)	DE box, E helix	21.7
RKM117 (Gly166Ala)	CD loop	26.3

containing 1 mM EDTA; the volume of the mixture was adjusted to 200 μ l and contained 3 μ g of plasmid DNA. The tube was kept on ice for 45 min, followed by incubation at 75°C for 30 min. After incubation, the mixture was dialysed extensively against 10 mM CaCl₂ at 2°C and DNA was then used for transformation.

In order to isolate the UV sensitivity determining amino acid residues from those of the electron transport function required for photoautotrophic growth, random mutants of D1 were simultaneously screened for photoautotrophic growth and altered dark-survival following UVC irradiation. From about 800 independent transformants (kan-r), 35 UV-resistant and 49 UV-sensitive clones were homogenized and further screened for UV sensitivity by spot test. Among these, 18 were selected for sequencing. The results revealed amino acid substitutions in seven of them, of which two had identical changes. Thus, a total of six unique putative D1 mutants were obtained. All except one, RKM115 (Leu341Pro) (Table 2), turned out to be UV-sensitive compared to D1:ctrl. These were further analysed for photoautotrophic growth rate and UV dark-survival. The remaining 11 mutants had nucleotide substitutions but no amino acid change and, therefore, were not studied further.

For sequencing, the PCR product was first treated with two hydrolytic enzymes, shrimp alkaline phosphate (SAP) and exonuclease I (Exo I) to remove the excess primers and dNTPs in the PCR reaction mixture. This was performed by adding 1.0 μ l of SAP (2 units/ μ l) and 1.0 μ l of Exo I (10 units/ μ l) to 5 μ l of PCR reaction mixture (~1.0 pmol DNA molecules). The contents were mixed well and incubated at 37°C for 15 min. The enzymes were inactivated by heat at 80°C for 15 min. Automated fluorescent sequencing was carried out on the ABI-Prism 377 Automated sequencer (PE Applied Biosystems).

Results

The WT strain used in this study was selected as a glucose-tolerant variant by Williams¹⁸ from a culture that was sensitive to glucose in light. Although this strain can utilize glucose, it still requires light for growth, but is independent of photosynthetic electron transport function

of PSII. However, the original strain PCC 6803 is listed as being capable of utilizing glucose¹⁶. The genetic basis of this variation is not known, but is suspected to be due to metabolic imbalance^{25,26}.

We refer to survival observed after allowing a 24 h dark incubation immediately following UV irradiation before transferring to light for colony development, as dark survival. The level of dark survival is the measure of 'UV sensitivity' or 'dark-UV sensitivity' mentioned here. In the photoheterotrophic mode of growth with glucose as the carbon source, the differential UV sensitivity is abolished in WT as well as in *psbA* and *psbB* deletion mutants (Figure 2). However, WT shows clear differential UV sensitivity in photoautotrophic mode of growth. Therefore, we infer that development of UV resistance on transition occurs when in the preceding light phase the PSII complexes were proficient in light-dependent electron transport function, and not inactive.

We determined the dark survival of two deletion mutants, *ΔpsbA* and *ΔpsbB*, to test if the PSII complex or its constituent component(s) is required for UV resistance development during light to dark transition, without involving photosynthetic electron transport. *psbA* and *psbB* code for PSII RC subunits D1 and CP47 respectively. Indeed differential UV sensitivity in light–dark transition is absent in both the deletion mutants and is more sensitive than WT under photoheterotrophic mode of growth when photosynthetic electron transport is blocked (Figure 2). The mutant strain harbouring *ΔpsbA* can not assemble even partial PSII complex, because the D–E luminal loop of D1 is essential for initiation of the reaction centre core subassembly formation^{27,28}. These results, however, do not rule out other components of photosynthetic electron transport system, rather than only the functional PSII complex per se in affecting the UV sensitivity switching process.

Genetic evidence that DNA alone is the lethal target in PSII defective strains

The model to explain light–dark switching of UV sensitivity assumed that DNA and PSII are both lethal

targets¹². This assumption underlies the manifestation of differential UV sensitivity attributed to kinetics of transient change in the state of PSII. Therefore, in a PSII deleted strain the differential sensitivity should not occur and only DNA should be the lethal target. We introduced a *phrA* knockout gene replacing the WT allele in the $\Delta psbB$ mutant strain in order to check whether it presented only DNA as a lethal target since the second lethal target, PSII, was missing. Figure 3 shows that the double mutant *phrA::cam* $\Delta psbB$ is homozygous for *phrA* insertion mutation. The difference in L/L and L/D of $\Delta psbB$ is because of photoreactivation of damages caused only to the DNA (Figure 4). The dark survival of the single $\Delta psbB$ mutant (L/D curve) following UV irradiation is nearly the same as survival of the double mutant under photoreactivation condition (L/L curve). The slightly higher survival at high UV fluence of the double mutant may be because of minor photoreactivation of DNA damage by the product of the second allele, *phrB* (ref 29). *PhrA*, the photolyase enzyme, is known to specifically photoreactivate damage to DNA^{29,30}. Therefore, we conclude that prolonged dark incubation following UV irradiation eliminates DNA damage by dark-repair system in $\Delta psbB$, implicating that DNA is the only lethal target in this strain.

Effect of multiple alleles of psbA on the dark UV sensitivity

A small but statistically significant difference in dark survival between the two deletion strains suggested that

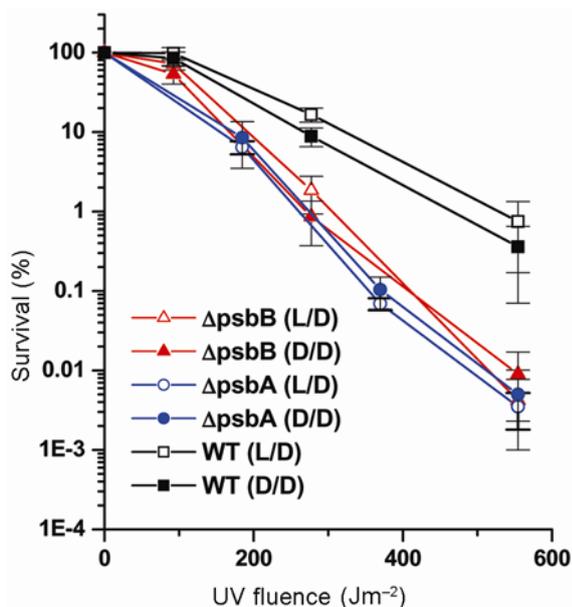


Figure 2. Inactivation of UV sensitivity switch in WT and PSII disrupted mutants under photoheterotrophic growth with glucose in the medium. L/D, Pre-UV light/post-UV in dark for 24 h; D/D, Pre-UV in dark for 24 h/post-UV in dark for 24 h. Bars represent SE values from three or more independent experiments.

$\Delta psbB$ deletion mutant may be helped in protection against UV-induced damage because of the presence of all the alleles of *psbA* that are absent in mutant carrying $\Delta psbA$ (Figure 5). The absence of CP47 presumably lowered the expression of *psbA* that resulted in lower resistance of $\Delta psbB$ strain compared to WT. We therefore

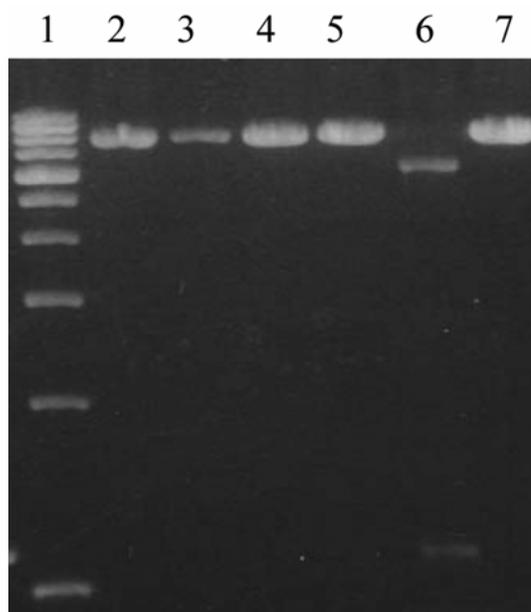


Figure 3. Agarose gel analysis of PCR product from genomic DNA of *Synechocystis* sp. PCC 6803 wild-type and four *phrA::cam* $\Delta psbB$ mutants. Lane 1, 500 bp molecular weight marker; lanes 2–5, Insertionally inactivated *phrA* amplified from four putative *phrA::cam* $\Delta psbB$ mutants (4.2 kb); lane 6, *phrA* amplified from wild-type *Synechocystis* sp. PCC 6803 (3.1 kb) and lane 7, Insertionally inactivated *phrA* amplified from *pphr::Acam*.

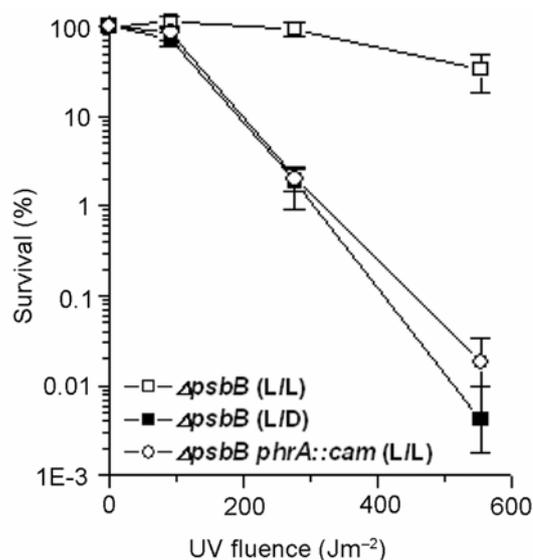


Figure 4. Lack of photoreactivation in PSII disrupted strain harbouring a *phrA* knockout mutation. L/D, Pre-UV light/post-UV in dark for 24 h; L/L, Pre-UV light/post-UV light. All the strains were grown photoheterotrophically. Bars represent SE values from three or more independent experiments.

tested the effect of the presence of only one, the most active WT allele of *psbA*, i.e. *psbA2* (ref. 17), in the presence of *psbB* with electron transport inactivated by addition of atrazine. The introduction of *psbA2* allele is expected to increase the D1 pool strength to over that of $\Delta psbA$. The alleles *psbA1* and *psbA3* were deleted in D1:ctrl strain, but harboured a functional *psbA2* WT allele. The strain is able to grow photoautotrophically and also shows UV sensitivity switching between the light

and dark phase (data not shown). In photoheterotrophic mode of growth, this strain clearly has an intermediate level of UV sensitivity between that of WT and the $\Delta psbA$ mutant lacking all the three alleles (Figure 4). The UV sensitivity is identical in both the light and dark phase. Therefore, the effect on UV sensitivity is unrelated to electron transport function as in other strains in heterotrophic mode of growth. Significantly, the gradation of UV sensitivity correlates with multiplicity of the *psbA* allele, showing a dosage effect of the gene.

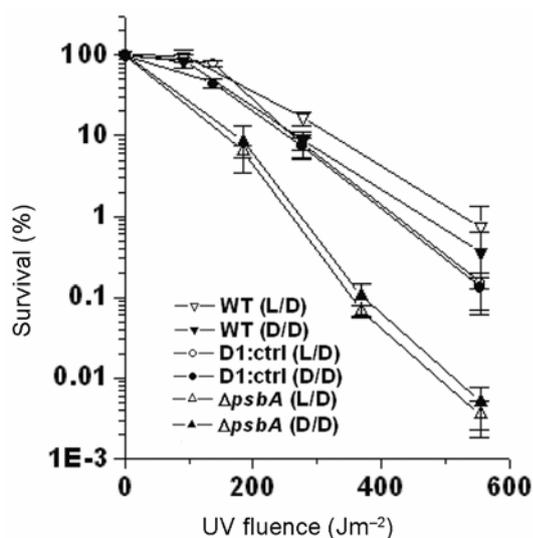


Figure 5. Dosage effect of *psbA* alleles in determining dark UV resistance in cultures growing photoheterotrophically with glucose and atrazine in the medium. L/D and D/D refer to pre- and post-UV light-dark regime as in Figure 2. Relevant genotype: WT (*psbA1*⁺, *psbA2*⁺, *psbA3*⁺); D1:ctrl (*ΔpsbA1*, *psbA2*⁺, *ΔpsbA3*); $\Delta psbA$ (*ΔpsbA1*, *ΔpsbA2*, *ΔpsbA3*). Bars represent SE values from three or more independent experiments.

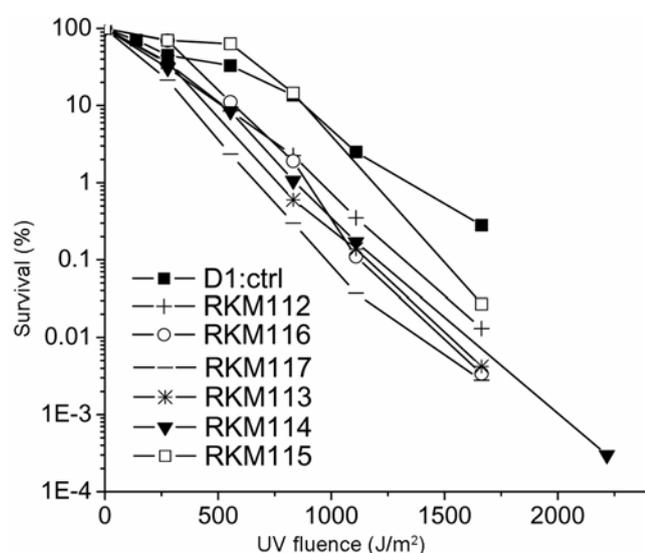


Figure 6. UV survival of six D1 mutants (RKM112–RKM117) and D1:ctrl strain of *Synechocystis* sp. PCC 6803. After irradiation the plates were incubated in dark for 24 h at room temperature (~22°C).

UV sensitivity and growth phenotypes of *psbA2* mutants

The six mutants carried eight changes in amino acid residues. A literature survey showed that none of these substitutions generated by random or site-directed mutations was reported among the residues studied earlier for their role in electron transport function generation or oxygen evolution.

Figure 6 shows the alteration in UV sensitivity due to amino acid changes in the mutants. Table 2 shows the growth rates in the exponential region of the growth phase. It is not possible to attribute the changes in phenotype to one or both mutations in the strains RKM113 and RKM116, which harbour two mutations and on different motifs of the D1 protein. None of these mutations has been reported to be associated with changes in photosynthetic electron transport function characteristics in the literature. It is, however, possible that UV sensitivity changes or decreased growth rate in some mutants may be an indirect effect of altered electron transport function under the growth conditions used. Nevertheless, since the mutants isolated in this study were selected for changes in UV sensitivity, we believe that these are primarily affected in DNA repair/protection function. The other phenotype changes could be secondary effects of DNA repair/protection defects.

Discussion

The kinetics of UV-sensitivity change during light–dark transition in *Anacystis nidulans* could be explained semi-quantitatively by assuming that DNA and PSII are independent lethal targets^{11,12}. The obligate nature of its photoautotrophy rules out designing experiments in which the phenomenon of UV-sensitivity switching could be decoupled from photosynthetic electron transport function of PSII that is essential for growth. In this article we report the effect on the mixotrophic strain of cyanobacteria, *Synechocystis* PCC 6803, of decoupling obligatory growth requirement of light from that of the UV sensitivity switch using glucose as the sole carbon source in mutants deleted for *psbA* and *psbB* genes which code

PSII RC constituents D1 and CP47 respectively. Consistent with the prediction of the model, the light–dark switching of UV sensitivity of WT *Synechocystis* PCC 6803 occurred in photoautotrophic but not in photoheterotrophic growth³¹ (Figure 2). Surprisingly, however, UV resistance is significantly lower in photoheterotrophic than in photoautotrophic growth, indicating that PSII plays a critical role in dark survival. Clear implication is that DNA and PSII are not totally independent lethal targets, since redundancy of PSII function for CO₂ fixation should have eliminated the UV sensitivity contributed by the complex as a lethal target assumed for autotrophic mode of growth. Significantly, the deletion mutants, $\Delta psbA$ and $\Delta psbB$, were more sensitive than WT (Figure 2).

The higher UV sensitivity of PSII deletion mutants compared to WT under photoheterotrophic growth can be explained by one of the following two hypotheses: (1) Photosynthetic electron transport function of PSII is essential for repair. (2) Some constituents of PSII complex or its subassemblies containing D1 and CP47 are required for DNA protection/repair that does not involve photosynthetic electron transport function needed for CO₂ fixation.

That the mechanism of switching to higher resistance in light to dark transition is likely to be independent of electron transfer function is indicated by several observations: (i) In autotrophic growth, adding DCMU/atrazine in light enhances UV resistance¹¹. (ii) D1 degradation is blocked in dark or light in the presence of DCMU/atrazine in cyanobacteria as well as in the chloroplasts that stabilize the D1 pool³². Transition to dark decreases the UV sensitivity of the WT *Synechocystis* PCC 6803. (iii) In double inhibitor experiment sharp fall in UV resistance on blocking protein synthesis during autotrophic growth can be reversed quantitatively by addition of DCMU¹². The above observations indicate that if D1 degradation is blocked, UV resistance develops in light even in the absence of protein synthesis and photosynthetic electron transport function. Therefore, hypothesis (1) mentioned above is ruled out.

Hypothesis (2) is based on the inference that in photoheterotrophic growth, differential UV sensitivity is abolished between light and dark-adapted cells because PSII is not a lethal target, which is consistent with the prediction of the model. In PSII deletion mutants, DNA is the only lethal target. This is supported by the observation that on knocking out *phrA* gene in $\Delta psbB$ strain, the UV sensitivity difference between dark and photoreactivation condition following UV irradiation is abolished (Figure 4). The minor difference at high UV fluence is presumably due to the expression of the second photolyase gene, *phrB*, coding a cryptochrome^{29,30}. Therefore, the difference in dark survival between WT and PSII deletion mutants suggests a housekeeping role in the dark of PSII or its subassembly containing D1 and CP47.

We checked this possibility in the D1:ctrl strain that has only one allele, *psbA2* (ref. 15). Figure 5 shows that the D1:ctrl harbouring only the WT *psbA2* allele has a level of dark UV sensitivity, intermediate between that of WT which has all the three alleles (*A1*, *A2* and *A3*) and $\Delta psbA$ that has none. Clearly dark UV resistance increases with the increasing number of *psbA* alleles under heterotrophic mode in which photosynthetic electron transport and oxygen evolution are dispensable. The high UV sensitivity of $\Delta psbB$ compared to WT in spite of the presence of all the three alleles of *psbA*, suggests a critical role of CP47 independent of the D1 or as a component of the subassembly of PSII complex containing D1 in dark repair/protection against DNA damage. A minor but clear difference in UV survival characteristics of the two deletion strains, $\Delta psbA$ and $\Delta psbB$, is consistent with hypothesis (ii), if we assume that the expression of *psbA* gene is essential for protection against/repair of DNA damage in WT in the photoheterotrophic mode of growth (Figure 3). The above interpretation is also consistent with the extensive literature on expression studies of PSII constituents in cyanobacteria and chloroplasts as discussed in the following.

A number of studies reveal a complex regulation pattern of *psbA* alleles and their dependence on environmental stimuli^{21,25,33–43}. Though core reaction centre polypeptide D1 of PSII complex is a high turnover protein in both cyanobacteria and eukaryotic photosynthetic microbes like *Chlamydomonas* and higher plants, the PSII assembly and stability in cyanobacteria are not known to be controlled by epistasy of synthesis (CES), but largely regulated by degradation kinetics under stress⁴⁴. Transcripts of *psbA* in WT *Synechocystis* PCC 6803 culture grown in dark or light-activated heterotrophy, exist though at a lower level than in photoautotrophic growth, and the PSII complex is also formed in the dark⁴⁰. In the $\Delta psbA$ mutant CP43, CP47, D2 and cytochrome b559 were synthesized, but proteins did not assemble⁴³. These observations suggest that the D1 protein or RC assemblies containing it in the $\Delta psbB$ mutant may be responsible for slightly higher UV resistance than in $\Delta psbA$ under non-photoreactivating condition, indicating its significance in dark repair/protection. The proposed critical role of D1 in light-independent (dark) repair/protection of DNA is consistent with the observation that transcripts of *psbA2* and *psbA3* alleles of *Synechocystis* PCC 6803 are maintained at high and stable level in dark condition compared to that in light under autotrophic growth in which *psbA2* transcript is processed to mature form in the dark³⁴.

The D1 protein has received considerable attention since it is not only a key polypeptide of RC of functional PSII complexes in photosynthesis in all oxygenic photosynthesis-dependent microbes and plants, but also because of its enigmatic high turnover characteristics that have been only recently understood at the molecular level as a cue to a complex set of stress-management mecha-

nisms that protect against and/or repair damage to the PSII complex^{32,43,45–48}. Our earlier and present studies show that it is also responsible for switching of dark UV sensitivity during light–dark transition in both *A. nidulans* and *Synechosystis* PCC 6803 in photoautotrophic mode of growth^{10–12}. Indeed, the high turnover of D1 under normal growth conditions may be a consequence of its being drawn into another pathway that protects or repairs the damage to DNA from photo-oxidative stress with basic chemistry similar to that in functional PSII.

In the present study, we have shown that D1 affects the UV sensitivity by selecting homozygous mutants of D1 which are altered in UV sensitivity as measured by dark survival that reflects the net effect of DNA damage and repair in the absence of light following UV exposure. This implies, irrespective of the mechanism we postulate, that D1 is involved in DNA repair or protection, whether or not the association of D1 and DNA is transient. We have obtained several such mutants and sequenced six which show altered UV sensitivity. As would be expected, if D1 is involved in housekeeping activity to repair damage to DNA even under normal growth conditions, most mutants are more sensitive to UV (Figure 6) and also grow at a slower rate compared to WT (Table 2). The mutant RKM112 having the mutation Leu353Ser, provides the clinching evince for our model. The residue 353 is within the last 16 residues of the C-terminal region that is eliminated in the mature D1 protein 360 amino acids long, before its assembly into a functional PSII complex of *Synechocystis* PCC 6803 (refs 49, 50). Therefore, UV sensitivity of the mutant RKM112, with substitution of serine for leucine at position 353, clearly indicates the role of unprocessed D1 in housekeeping activity and not in electron transport function or oxygen evolution. We believe that all the UV sensitive mutants reported here are most likely structurally defective in the formation of a complex that is needed for DNA repair or protection.

Form 1 of D1 protein, a product of *psbAI* allele in *Cynotheca* sp. ATCC51142, is maintained at a steady level in the light–dark cycle³⁸. It is indicative of the role of D1 presumably in the dark phase as well and may be required for protection against DNA damage throughout the cell cycle. Form 2 of D1, product of alleles *psbAII* and *psbAIII*, in light, has been attributed to photoinhibition-resistant PSII complex in *Synechococcus* strain PCC 7942, whereas the specific role of form 1 is suggested to support efficient photosynthesis³⁷. Similar function in *Synechocystis* PCC 6803 is also attributed to form 1 product^{35,40–44}. The difference between form 1 and form 2 of D1 in *Synechocystis* PCC 6803 is less distinct, though their transcripts are differentially regulated³⁵. Therefore, it is likely that both forms may be involved in photoprotection and photosynthesis. Genetic evidence presented here, however, clearly suggests that at least form 2 or a subassembly containing it is essential also for dark resistance against UVC-induced lethality from DNA damage.

The high UVC resistance of *A. nidulans* under argon atmosphere in light is similar to that of dark phase in aerobic atmosphere¹³. This resistance in microaerobic condition in light is presumably due specifically to form 1 of the D1 protein. It has been shown that *psbA* allele coding for form 1 in some stains of both *Synechococcus* and *Synechocystis* is actively transcribed in microaerobic conditions and is known to be silent in a wide variety of stresses⁵¹. The authors suggest that such a regulation may be useful for survival of cyanobacteria trapped in ice for long periods, where aerobic environment may not exist. However, it is possible that this is the original and only gene with its signature for transcription unaltered and active early in evolution of cyanobacteria when the atmosphere was still reducing, devoid of gaseous oxygen, and so effectively microaerobic. With the gradual build-up of oxygen in the atmosphere, the family of *psbA* alleles evolved by duplication and/or horizontal gene transfers without losing the original phenotype in some of the extant species. The process could accelerate adaptation to changing quality and intensity of solar radiation available with concomitant decrease in UV flux because of emergence of ozone in the atmosphere from oxygen discharged by its own photosynthetic water oxidation activity.

The reason for the existence of several constituents of PSI and PSII in the plasma membrane (PM) is not known^{52–54}. D1 and D2 proteins have been clearly located in the PM. Recent studies have also shown that the assembly of PSII, though not under CES regulation, involves a sequence of multiple steps in cyanobacteria as well as in higher plants^{43,48,55–57}. It is intriguing that in cyanobacteria the initial subcomplex of PSII is formed in the PM, the first subassembly being D1/D2, and that the incorporated D1 is required to be processed before it acquires competence for electron transport and is presumably carried to the thylakoid membrane^{54,56,58}. We speculate that a fraction of the RC subassemblies is dedicated to DNA repair and is functionally associated with the PM harbouring a pool separate from that associated with thylakoid membrane. We know little about chromosomal replication of cyanobacteria^{59,60}. It is reasonable to assume that in cyanobacteria PM plays an analogous role in DNA replication as in other prokaryotes⁶¹. Taken together with our findings, the current evidence suggests a role of D1 in a protective/repair role against damage to DNA and perhaps also in DNA replication. Significantly, *recA* insertion mutant though highly sensitive to UVC, can grow nearly as well as the WT in low light (~800 lux) autotrophically and also display the light–dark switching of UV sensitivity⁹. We, therefore, conclude that RecA and D1 or a subassembly of RC of PSII containing D1 formed in the PM⁵⁴ (discussed above) are involved in DNA housekeeping activity in at least two mutually exclusive, but essential pathways. A recent proteomic analysis showed that expression of *lexA*, a component of

putative and presumably modified *recA*–*lexA* system of SOS regulation homologous to that in *E. coli*, is enhanced on shifting from photoautotrophic to heterotrophic growth that also downregulates photosynthesis-associated genes of the WT⁶². It seems the down-regulation of PSII constituents for DNA repair is compensated by induction of SOS-like system in cyanobacteria that occurs in heterotrophic growth. Domain *et al.*⁶³ have reported down-regulation of *recA* and *lexA* genes on UV irradiation under photoautotrophic mode of growth and concluded that the SOS system does not operate for DNA repair⁶² in *Synechocystis* PCC 6803. Presumably two different DNA repair regulatory systems operate that are switched on/off depending upon the mode of growth. The PSII constituent plays a critical role in autotrophic mode, as this study indicates, and presumably SOS-like system dominates in heterotrophic growth in relatively low light intensity, conditions likely to prevail during growth in mats abundantly found in stromatolites¹.

There is at least one more important phenomenon of ecological and evolutionary significance that may be interpreted in the light of the findings reported here as a protective function of D1. Most of the cyanobacterial phage and chloroplast genomes harbour one *psbA* allele. The phages may have acquired *psbA* gene from their respective hosts for enhancing photosynthesis in the early stages of infection as suggested by Lindell *et al.*⁶⁴. However, in view of our findings reported here, we suggest that the advantage of protecting their own while destroying the genome of the host, at a later stage of development, including the phage genome packing step may be a more stringent selective pressure to maintain their own genome free of oxidative and UV-induced damage, when repair systems of the host are compromised by lytic growth of the phage. Consistent with this hypothesis,

Appendix 1. Evidence for light–dark UV sensitivity switching in UV sensitive *uvrA::cam* knockout mutant of *Synechocystis* PCC 6803 (Figure 7)¹⁵.

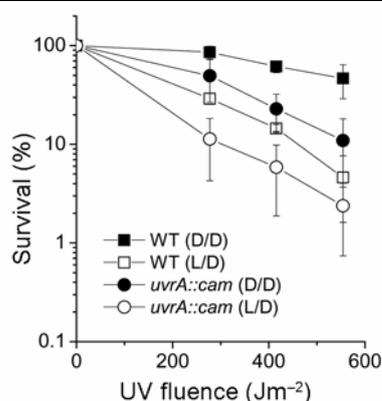


Figure 7. Dark survival characteristics of WT and *uvrA* knockout mutant in light and dark phase. L/D; Pre-UV light/post-UV dark; D/D, Pre-UV dark/post-UV dark. The strain was constructed and verified to be homozygous as described in the text for constructing *phrA::cam*.

there are no alleles of *psbA* gene found in cyanobacterial plasmids, though some are larger than the phages that carry a copy of one from their hosts⁶⁵. Chlororespiration in cyanobacteria can reduce plastoquinones in the absence of PSII complex to carry out photosynthetic electron transport using the PSI complex⁶⁶. Therefore, it is reasonable to assume that under stress, PSII constituents may be harnessed for housekeeping activity unrelated to its electron transport function for water oxidation, as suggested in this study. Obligate photoautotrophy, presumably, ensured a high-level expression of the *psbA* genes necessary for protection of the genomes of both the hosts and their phages under high intensity of solar radiation in the course of evolution of cyanobacteria.

- Schopf, W. J., Fossil evidence of Archaean life. *Philos. Trans. R. Soc. London, Ser. B*, 2006, **361**, 869–885.
- Rasmussen, B., Fletcher, I. R., Brocks, J. J. and Kilburn, M. R., Reassessing the first appearance of eukaryotes and cyanobacteria. *Nature*, 2008, **455**, 1101–1104.
- Schidlowski, M., The beginning of life on earth: evidence from the geological records. In *The Chemistry of Life's Origin* (eds Greenberg *et al.*), (NATO Science, Series C), Kluwer Publisher, 1993, pp. 389–414.
- Shi, T. and Falkowski, P. G., Genome evolution in cyanobacteria: the stable core and the variable shell. *Proc. Natl. Acad. Sci. USA*, 2008, **108**, 2511–2515.
- Bjorn, L. O. and Govindjee, The evolution of photosynthesis and chloroplast. *Curr. Sci.*, 2009, **96**, 1466–1474.
- Rothschild, L. J. and Mancinelli, R., Life in extreme environments. *Nature*, 2001, **409**, 1092–1101.
- Nisbet, E. G. and Sleep, N. H., The habitat of early life. *Nature*, 2001, **409**, 1083–1091.
- Whitton, B. A. and Potts, M. (eds), *The Ecology of Cyanobacteria – their Diversity in Time and Space*, Kluwer, 2000.
- He, Y.-Y. and Haeder, D.-P., Reactive oxygen species and UV-B: effect on cyanobacteria. *Photochem. Photobiol. Sci.*, 2002, **1**, 729–734.
- Minda, R., Ramchandani, J., Joshi, V. P. and Bhattacharjee, S. K., A homozygous *recA* mutant of *Synechocystis* PCC 6803: construction strategy and characteristics eliciting a novel RecA independent UVC resistance in dark. *Mol. Gen. Genomics*, 2005, **274**, 616–624.
- David, K. A. V., Rane, S. S., Mathur, M. and Bhattacharjee, S. K., Herbicide mediated UV resistance in cyanobacteria: On the role of photosynthetic electron transport system rather than replicative DNA as lethal target determining dark-survival of *Anacystis nidulans*. *Photochem. Photobiol.*, 1988, **47**, 107–113.
- Bhattacharjee, S. K., Sensitivity to far-UV light as a parameter for the study of *in vitro* regulation of development of PSII particles in cyanobacteria: an analytical model. In *Photosynthesis: Molecular Biology and Bioenergetics* (eds Singhal, G. S. *et al.*), Narosa Publishing House, New Delhi, 1989, pp. 267–281.
- Bhattacharjee, S. K., Unstable protein mediated ultraviolet light resistance in cyanobacteria *Anacystis nidulans*. *Nature*, 1977, **269**, 82–83.
- Bhattacharjee, S. K. and David, K. A. V., Unusual resistance to ultraviolet light in dark phase of blue-green bacterium *Anacystis nidulans*. *Nature*, 1977, **265**, 183–184.
- Minda, R., Molecular biology of photo-regulation of UV sensitivity in cyanobacteria, Ph D thesis, Mumbai University, 2005.
- Ripka, R., Deruelles, J., Waterbury, J. B., Herdman, M. and Stanier, R. Y., Generic assignments, strain histories and properties

- of pure cultures of cyanobacteria. *J. Gen. Microbiol.*, 1979, **111**, 1–61.
17. Jansson, C., Debus, R., Osiewacz, H. D., Gurevitz, M. and McIntosh, L., Construction of an obligate photoheterotrophic mutant of the cyanobacterium *Synechocystis* 6803. *Plant Physiol.*, 1987, **85**, 1021–1025.
 18. Williams, J. G. K., Construction of specific mutations in photosystem II photosynthetic reaction center by genetic engineering methods in *Synechocystis* 6803. *Methods Enzymol.*, 1988, **167**, 766–778.
 19. Nilsson, F., Simpson, D. J., Jansson, C. and Anderson, B., Ultrastructural and biochemical characterization of *Synechocystis* 6803 mutant with inactivated *psbA* genes. *Arch. Biochem. Biophys.*, 1992, **295**, 340–347.
 20. Kaneko, T. *et al.*, Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803. Sequence determination of the entire genome and assignment of potential protein-coding regions. *DNA Res.*, 1996, **3**, 109–136.
 21. Allen, M. M., Simple conditions for growth of unicellular blue-green algae on plates. *J. Phycol.*, 1968, **4**, 1–4.
 22. Pakrasi, H. B., Williams, J. G. K. and Arntzen, C. J., Targeted mutagenesis of the *psbE* and *psbF* blocks photosynthetic electron transport: Evidence for a functional role of cytochrome b-559 in photosystem II. *EMBO J.*, 1988, **7**, 325–332.
 23. Porter, R. D., DNA transformation. *Methods Enzymol.*, 1988, **167**, 703–712.
 24. Sambrook, J., Fritsch, E. F. and Maniatis, T., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, 1989, 2nd edn.
 25. Golden, S. S., Light-responsive gene expression and the biochemistry of photosystem II reaction center. In *The Molecular Biology of Cyanobacteria* (ed. Donald, A. B.), Kluwer, 1994, pp. 693–714.
 26. Kobayashi, M., Okada, K. and Ikeuchi, M., A suppressor mutation in the α -phytylcyanin gene in the light/glucose-sensitive phenotype of the *psbK*-disruptant of the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Cell Physiol.*, 2005, **46**, 1561–1567.
 27. Eaton-Rye, J. J. and Vermass, W. F. J., Oligonucleotide-directed mutagenesis of *psbB*, the gene encoding CP47, employing a deletion strain of the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Mol. Biol.*, 1991, **17**, 1165–1177.
 28. Komenda, J. and Masojedex, J., Functional and structural changes of the photosystem II complex induced by high irradiance in cyanobacterial cells. *Eur. J. Biochem.*, 1995, **233**, 677–682.
 29. Hitomi, K. *et al.*, Cyanobacterial cryptochrome and photolyase: characterization of two photolyase-like genes of *Synechocystis* sp. PCC 6803. *Nucleic Acids Res.*, 2000, **28**, 2353–2362.
 30. Ng, W.-O. and Pakrasi, H. R., DNA photolyase homologs are the major UV resistance factors in the cyanobacterium *Synechocystis* sp. PCC 6803. *Mol. Gen. Genet.*, 2001, **264**, 924–970.
 31. Minda, R., Joshi, V. P. and Bhattacharjee, S. K., The evolutionary significance of 'obligate' photoautotrophy of cyanobacteria. *Curr. Sci.*, 2008, **94**, 850–853.
 32. Mattoo, A. K., Hoffman-Falk, H., Marder, J. B. and Edelman, M., Regulation of protein metabolism: Coupling of photosynthetic transport to *in vivo* degradation of rapidly metabolized 32-kilodalton protein of the chloroplast membrane. *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 1380–1384.
 33. Vermaas, W. F. J., Ikeuchi, M. and Inoue, Y., Protein composition of the photosystem II core complex in genetically engineered mutants of cyanobacterium *Synechocystis* sp. PCC 6803. *Photosynth. Res.*, 1988, **17**, 97–113.
 34. Mohamed, A. and Jansson, C., Influence of light on accumulation of photosynthesis specific transcripts in the cyanobacterium *Synechocystis* 6803. *Plant Mol. Biol.*, 1989, **13**, 693–700.
 35. Mohamed, A., Eriksson, J., Osiewacz, H. D. and Jansson, C., Differential expression of the *psbA* genes in the cyanobacterium *Synechocystis* 6803. *Mol. Gen. Genet.*, 1993, **238**, 161–168.
 36. Garcia-Fernandez, J. M., Hess, W. R., Howmard, J. and Partensky, F., Expression of the *psbA* gene in marine oxyphotobacteria *Prochlorococcus* spp. *Arch. Biochem. Biophys.*, 1998, **359**, 17–23.
 37. Agrawal, G. K., Kato, H., Asayama, M. and Sirai, M., An AU-box motif upstream of SD sequence of light-dependent *psbA* transcripts confer mRNA instability in darkness in cyanobacteria. *Nucleic Acids Res.*, 2001, **29**, 1835–1843.
 38. Colon-Lopez, M. S. and Sherman, L. A., Transcriptional regulation of photosystem I and II genes in light-dark and continuous-light-grown cultures of unicellular cyanobacterium *Cyanothece* sp. strain ATCC 51142. *J. Bacteriol.*, 1998, **180**, 519–526.
 39. Kulkarni, R. D. and Golden, S. S., Adaptation to high light intensity in *Synechococcus* sp. strain PCC 7942: regulation of three *psbA* genes and two forms of D1 protein. *J. Bacteriol.*, 1994, **176**, 959–965.
 40. Smart, L. B. and McIntosh, L., Expression of photosynthesis genes in the cyanobacterium *Synechocystis* sp. PCC 6803: *psaA*–*psaB* and *psbA* transcripts accumulate in dark-grown cells. *Plant Mol. Biol.*, 1991, **17**, 959–971.
 41. Alfonso, M., Perewoska, I., Constant, S. and Kirilovsky, D., Redox control of *psbA* expression in cyanobacteria *Synechocystis* strains. *J. Photochem. Photobiol. B*, 1999, **48**, 104–113.
 42. Eaton-Rye, J. J., Shand, J. A. and Nicoll, W. S., pH-dependent photoautotrophic growth of specific photosystem II mutants lacking luminal extrinsic polypeptides in *Synechocystis* PCC 6803. *FEBS Lett.*, 2003, **543**, 148–153.
 43. Komenda, J., Reisinger, V., Muller, B. C., Dobakova, B. G. and Eichacker, L. A., Accumulation of the D₂ protein is a key regulatory step for assembly of the photosystem II reaction center complex in *Synechocystis* PCC 6803. *J. Biol. Chem.*, 2004, **279**, 48620–48629.
 44. Choquet, Y. and Wollman, F.-A., The CES process. In *The Chlamydomonas Resource Book* (ed. Stern, D. B.), Academic Press, 2009, vol. 2, pp. 1027–1063.
 45. Kyle, D. J., Ohad, I. and Arntzen, C. J., Membrane protein damage and repair: selective loss of a quinone-protein function in chloroplast membranes. *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 4070–4074.
 46. Maenpaa, P., Kallio, T., Mulo, P., Salih, G., Aro, E.-M., Tyystjarvi, E. and Janssoon, C., Site-specific mutations in the D1 polypeptide affect the susceptibility of *Synechocystis* 6803 cells to photoinhibition. *Plant Mol. Biol.*, 1993, **22**, 1–12.
 47. Allakhverdiev, S. I., Nishiyama, Y., Takahashi, S., Miyairi, S., Suzuki, I. and Murata, N., Systematic analysis of the relation of electron transport and ATP synthesis to photodamage and repair of photosystem II in *Synechocystis*. *Plant Physiol.*, 2005, **137**, 263–273.
 48. Aro, E.-M., Sousa, M., Rokka, A., Paakkarinen, V., Saleem, A., Bhattachikova, N. and Rintamaki, E., Dynamics of photosystem II: a proteomic approach to thylakoid protein complexes. *J. Exp. Bot.*, 2005, **56**, 347–356.
 49. Satoh, K. and Yamamoto, Y., The carboxyl-terminal processing of precursor D1 protein of the photosystem II reaction center. *Photosynth. Res.*, 2007, **94**, 203–215.
 50. Nixon, P. J., Trost, J. T. and Diner, B. A., Role of the carboxy terminus of polypeptide D1 in the assembly of a functional water-oxidizing manganese cluster in photosystem II of the cyanobacterium *Synechocystis* sp. PCC 6803: assembly requires a free carboxyl group at C-terminal position 344. *Biochemistry*, 1992, **31**, 10859–10871.
 51. Sicora, C. I., Ho, F. M., Salminen, T., Styring, S. and Aro, E.-M., Transcription of a 'silent' cyanobacterial *psbA* gene is induced by microaerobic conditions. *Biochim. Biophys. Acta*, 2009, **1787**, 105–112.
 52. Smith, D. and Howe, C. J., The distribution of photosystem I and photosystem II polypeptides between the cytoplasmic and thylakoid membranes of cyanobacteria. *FEMS Lett.*, 1993, **110**, 341–348.

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53. Zak, E., Norling, B., Maitra, R., Huang, F., Anderson, B. and Pakrasi, H. B., The initial steps of biogenesis of cyanobacterial photosystems occur in plasma membranes. *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 13443–13448.
54. Huang, F., Parmryd, I., Nilsson, F., Persson, A. L., Pakrasi, H. B., Adersson, B. and Norling, B., Proteomics of *Synechocystis* sp. Strain PCC 6803: identification of plasma membrane proteins. *Mol. Cell. Proteomics*, 2002, **1**, 956–966.
55. Liberton, M., Berg, R. H., Heuser, J. and Pakrasi, H. B., Ultrastructure of the membrane systems in the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803. *Protoplasma*, 2006, **227**, 129–138.
56. Keren, N., Liberton, M. and Pakrasi, H. B., Photochemical competence of assembled photosystem II core complex in cyanobacterial plasma membrane. *J. Biol. Chem.*, 2005, **280**, 6548–6553.
57. Suorsa, M., Regal, R. E., Paakkarinen, V., Battchikova, N., Herrman, R. J. and Aro, E.-M., Protein assembly of photosystem II and accumulation of subcomplexes in the absence of low molecular mass subunits PsbL and PsbJ. *Eur. J. Biochem.*, 2004, **271**, 96–107.
58. Roose, J. L. and Pakrasi, H. B., Evidence that D1 processing is required for manganese binding and extrinsic protein assembly into photosystem II. *J. Biol. Chem.*, 2004, **279**, 45417–45422.
59. Asato, Y., Towards an understanding of cell growth and the cell division cycle of unicellular photoautotrophic cyanobacteria. *CMLS Cell. Mol. Life Sci.*, 2003, **60**, 663–687.
60. Yoshida, T. Y., Maki, M., Okamoto, H. and Hiroshi, S., Coordination of DNA replication and cell division in cyanobacteria *Microcystis aeruginosa*. *FEMS Microbiol. Lett.*, 2005, DOI 10.1016/j.femste.2005.07.041
61. Hase, A. and Nakamura, H., Importance of the plasma membrane in the cellular origin and evolution. *Viva Origino* 2003, **31**, 233–242.
62. Kurian, D., Jansen, T. and Manpa, P., Proteomic analysis of heterotrophy in *Synechocystis* PCC 6803. *Proteomics*, 2006, **5**, 1483–1494.
63. Domain, F., Houot, L., Chauvat, F. and Cassier-Chauvat, C., Function and regulation of cyanobacterial genes *lexA*, *recA*, and *ruvB*: LexA is critical to the survival of cells facing inorganic starvation. *Mol. Microbiol.*, 2004, **53**, 65–80.
64. Lindell, D., Sullivan, M. B., Johnson, Z. I., Tolonen, A. C., Rohwer, F. and Chisholm, S. W., Transfer of photosynthesis genes to and from *Prochlorococcus* viruses. *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 11013–11018.
65. Paul, J. H., Sullivan, M. B., Segal, A. M. and Rohwer, F., Marine phage genomics. *Comp. Biochem. Physiol. B*, 2002, **133**, 463–476.
66. Rumeau, D., Peltier, G. and Cournac, L., Chlororespiration and cyclic electron flow around PSI during photosynthesis and plant stress response. *Plant Cell Environ.*, 2007, **30**, 1041–1051.

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