

The Ability of Cyanobacterial Cells to Restore UV-B Radiation Induced Damage to Photosystem II is Influenced by Photolyase Dependent DNA Repair

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ABSTRACT

Damage of DNA and Photosystem-II are among the most significant effects of UV-B irradiation in photosynthetic organisms. Both damaged DNA and Photosystem-II can be repaired, which represent important defense mechanisms against detrimental UV-B effects. Correlation of Photosystem-II damage and repair with the concurrent DNA damage and repair was investigated in the cyanobacterium *Synechocystis* PCC6803 using its wild type and a photolyase deficient mutant, which is unable to repair UV-B induced DNA damages. A significant amount of damaged DNA accumulated during UV-B exposure in the photolyase mutant concomitant with decreased Photosystem-II activity and D1 protein amount. The transcript level of *psbA3*, which is a UV-responsive copy of the *psbA* gene family encoding the D1 subunit of the Photosystem-II reaction center, is also decreased in the photolyase mutant. The wild-type cells, however, did not accumulate damaged DNA during UV-B exposure, suffered smaller losses of Photosystem-II activity and D1 protein, and maintained higher level of *psbA3* transcripts than the photolyase mutant. It is concluded that the repair capacity of Photosystem-II depends on the ability of cells to repair UV-B-damaged DNA through maintaining the transcription of genes, which are essential for protein synthesis-dependent repair of the Photosystem-II reaction center.

Abbreviations: Chl, chlorophyll; CPD, cyclobutane pyrimidine dimer; D1 and D2, reaction center protein subunits of Photosystem-II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PSII, Photosystem-II; qPCR, quantitative PCR; UV-B, ultraviolet-B (280–315 nm) spectral range; 6-4 PPs, thymine–thymine pyrimidine–pyrimidone (6-4) photoproducts.

INTRODUCTION

Photosynthetic organisms are unavoidably exposed to incident solar light, which not only provides energy to the biosphere via photosynthetic energy conversion, but at the same time can cause damage to biological systems. During evolution of photosynthetic organisms several pathways arose to prevent, or repair

the negative effects of solar radiation. The most detrimental component of sunlight that reaches the Earth's surface is the UV-B (280–315 nm) spectral range as the more damaging shorter wavelength UV-C radiation is screened out by the ozone layer and other components of the atmosphere.

DNA is particularly sensitive to UV radiation due to its absorption that extends into the physiologically relevant UV-B (1,2) region. Absorption of UV-B photons causes phototransformations in the DNA molecules leading to formation of cyclobutane pyrimidine dimers (CPDs) and thymine–thymine pyrimidine–pyrimidone (6-4) photoproducts (6-4 PPs) among others (3). CPDs are the most predominant (75–80% of all UV-B induced damages) and biologically the most relevant UV-B induced lesions in the DNA molecule. As DNA and RNA polymerases cannot read through these lesions their accumulation must be prevented by efficient repair and/or UV screening compounds to maintain functional DNA (4). Photoreactivation with the help of the photolyase enzyme is one of the most important and frequently occurring mechanisms to repair UV-induced DNA photodamages in prokaryotes (3). The structure and function of photolyases is fairly well known: They specifically bind to pyrimidine dimers, flip them out of the DNA duplex and with the aid of photons from the 360–530 nm range their FADH⁻ prosthetic group reduces the cyclobutane ring, splitting it up, thus restoring the functional DNA (5). The product of the *phrA* gene, which codes for a CPD-specific DNA photolyase, is the major photoreactivating factor in the cyanobacterium *Synechocystis* sp. PCC 6803 (6,7). Cells lacking this gene are rendered highly sensitive to UV-B radiation (8).

In photosynthetic organisms, pigmented complexes absorb most of the incoming UV-B light (9), therefore besides DNA components the photosynthetic apparatus, especially the Photosystem-II (PSII) complex, is also an important target of UV-B radiation (see Ref.[10]). Within PSII, the primary action site of UV-B radiation is the catalytic manganese cluster of the water oxidizing complex, which leads to inhibition of light-induced electron transport (11–14). This effect is followed by damaging the D1 and D2 protein subunits, which form the backbone of the reaction center complex (15–19). UV-B-induced damage of PSII can be restored via repairing the PSII core complex, which proceeds via *de novo* synthesis of the damaged D1 and D2 subunits (18). The PSII repair cycle is a complicated process, which has been studied in a great detail in case of photodamage induced by visible light, for reviews see (20,21). Repair of UV-B-induced damages occurs basically in the same way as in

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the case of photodamage of PSII by visible light, with the following main steps: (1) Proteolytic removal of the damaged D1 and D2 protein subunits, (2) Transcription of mRNA from the *psbA* and *psbD* genes encoding the D1 and D2 subunits, respectively, (3) Production of new protein subunits from the respective mRNA pools, (4) Incorporation of the newly synthesized protein copies into the PSII complex, and finally (5) Religation of redox cofactors and reactivation of PSII. In the repair process, two UV-B-specific effects are noteworthy. One is the FtsH protease dependent degradation of both D1 and D2 proteins (19). The other such feature is the presence and induction of UV-B-specific *psbA* gene copies in some cyanobacteria, such as *Synechocystis* 6803, *Anabaena* 7120, *Gloeobacter violaceus* (22–24). Although UV-B-induced damage of DNA molecules and photosynthetic complexes obviously occurs in a parallel fashion, as shown by growth retardation under conditions when DNA lesions accumulate (25), there has not been a serious attempt in the literature to study the influence of DNA damage on the PSII repair cycle. The only indication for such an effect so far is the observation that the presence of a UV-C contamination from the UV-B source, which significantly enhances DNA damage, decreases the extent of PSII recovery associated with the D1 turnover (18). However, the molecular background of the mechanism that retards the protein synthesis has not been elucidated.

In the repair cycle of photodamaged PSII complexes *de novo* synthesis of damaged D1 and D2 protein subunits is a highly important step. As this includes the transcription and translation of several genes encoding D1, D2, chaperons, accessory proteins, etc. see (20,21) we hypothesize that following UV-B stress there is a strong connection between the ability of cells to maintain damage free DNA molecules and to regain their photosynthetic activity.

In this study, we investigated the correlation of PSII damage and its protein synthesis-dependent repair with the concomitant DNA damage and its repair. Our results show that DNA repair is a prerequisite of efficient protein synthesis and maintenance of functional PSII.

MATERIALS AND METHODS

Cell cultures and UV-B treatment conditions. *Synechocystis* PCC6803 (denoted as *Synechocystis* hereafter) wild type and the $\Delta phrA$ ($\Delta slr0854$) photolyase deficient mutant were grown in BG-11 medium, in an incubator with an orbital shaker (120 rpm), in 3% CO₂-enriched atmosphere under constant 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR at 30°C.

The photolyase lacking mutant ($\Delta slr0854$) was constructed by interrupting the *slr0854* gene with a spectinomycin cassette.

For the experiments, 200 mL cultures were harvested by centrifugation (6500 g, 5 min, 24°C) in their exponential growth phase, re-suspended in fresh BG-11 medium at 7.5 $\mu\text{g mL}^{-1}$ Chl concentration, and further incubated for an hour. The UV-B treatments were carried out at 30°C, with continuous stirring in square glass containers in which the suspension formed a 2.5 cm high layer. UV-B radiation was provided by a Vilber Lourmat VL-215M lamp in combination with cellulose acetate filter (Clarifoil 0.1 mm, cut off at 290 nm) to screen out any possible UV-C radiation. The UV-B intensity was 3.0 W m⁻² (\approx 8.0 $\mu\text{mol m}^{-2} \text{s}^{-1}$) at the surface of the cell suspension as measured with a Cole-Parmer radiometer (model 97503-00) equipped with a 312 nm sensor. Due to the high optical density of the cell suspension, the average UV-B irradiation intensity was only approximately 0.3 W m⁻² (\approx 0.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$) within the cell suspension, as calculated by taking into account the absorption by the optically dense sample according to Rontó *et al.* (26). Before UV-B treatments, cells were kept for 30 min under 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ white light, which was also used as background

illumination during the UV-B treatments, as well as during the recovery period following the UV-B treatments.

The radiation intensities for the white light and the UV-B illumination were measured using a LiCor Photometer (DMP Ltd.) equipped with a PAR sensor and a Cole-Parmer Radiometer equipped with a UV-B (312 nm) sensor, respectively.

Assessment of photosynthetic activity. Wild type and $\Delta phrA$ mutant cells were exposed to UV-B light (8 $\mu\text{mol m}^{-2} \text{s}^{-1}$) either in the absence, or in the presence of the protein synthesis inhibitor lincomycin (300 $\mu\text{g mL}^{-1}$). The UV-B light was supplemented with white light (WL, 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$) in the initial phase of the experiments (UV-B + WL, for 270 min). This was followed by a recovery period under white light only (WL) for 120 min. In the final phase of the experiment, the UV-B exposure was repeated in the absence of white background illumination (UV-B) for 60 min. In experiments, in which the protein synthesis inhibitor lincomycin was added to the cell cultures only UV-B + WL illumination was applied for 120 min.

PSII activity during and after UV-B treatments and recovery periods was assessed by measuring the changes of variable Chl fluorescence values, as the initial amplitudes of the flash induced fluorescence signals ($F_v = F_m - F_o$) relative to the control ($t = 0$ min) point reflect the relative amount of functional PSII complexes. The flash-induced Chl fluorescence measurements were performed with an FL 3000 Fluorometer (Photon Systems Instruments Ltd.), using 1 mL samples in the absence and presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). Measurements were repeated on three biologically different samples at preset time points (see Fig. 1). The data were visualized and evaluated using the Fluorwin software, version 3.6.3.11 and Origin.

D1 protein analysis. Thylakoid membranes were isolated as described in Ref. (27) from samples taken right before the onset of UV-B + WL irradiation (0 min time point), at the end of the 270 min UV-B + WL treatment, at the end of the subsequent 120 min WL recovery period, as well as at the end of the final 60 min UV-B

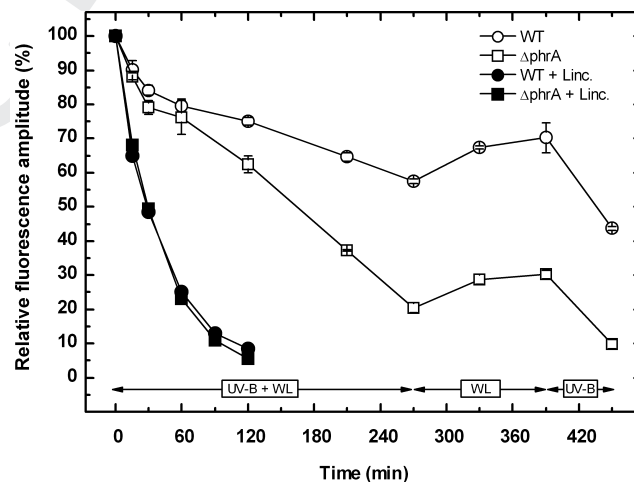


Figure 1. PSII activity changes, assessed by flash-induced Chl fluorescence, of *Synechocystis* cells in response to UV-B exposure. WT (circles) and the photolyase lacking $\Delta phrA$ mutant (squares) were exposed to UV-B light (8 $\mu\text{mol m}^{-2} \text{s}^{-1}$) either in the absence (open symbols) or in the presence (closed symbols) of the protein inhibitor lincomycin. The UV-B light was supplemented with white light (WL, 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$) in the initial phase of the experiment (UV-B + WL), which was followed by a recovery period under white light only (WL). In the final phase of the experiment, the UV-B exposure was repeated in the absence of white background illumination (UV-B). PSII activity was assessed by measuring the initial amplitude of flash-induced Chl fluorescence signals that reflects the amount functional PSII centers. The data are shown in percentage of the control value measured in untreated cells. The error bars indicate standards deviations obtained from three independent experiments. When error bars are not visible, they are either smaller than the size of the closed symbols, or overlap with the outline of the open symbols.

1 treatment. Thylakoid samples, each equalized to contain 1 μg of
 2 Chl, were separated by SDS-PAGE, on a 12% gel, blotted on nitro-
 3 cellulose membrane and visualized with specific antibodies according
 4 to (19). Protein bands were quantified with the help of ImageJ ([http://](http://rsbweb.nih.gov/ij/)
 5 rsbweb.nih.gov/ij/). The measurements were repeated on three biologi-
 6 cally different samples and the mean values were used for assessing
 7 the changes in the D1 protein amounts.

8 *Expression level of the psbA3 gene was assessed by qPCR as*
 9 *described earlier (28).*

10 *Isolation of genomic DNA.* Genomic DNA was isolated using meth-
 11 ods that minimize the risk of inducing single or double strand breaks.
 12 Cells of 15 mL samples were harvested and resuspended in 200 μL of
 13 saturated NaI solution. After incubation for 10 min at 37°C, 1 mL water
 14 was added, and samples were centrifuged. The pellets were resuspended
 15 in 400 μL of Tris buffer I (50 mM Tris, pH 8, 5 mM EDTA, 50 mM
 16 NaCl) and 100 μL of lysozyme (50 mg mL⁻¹) was added. The suspen-
 17 sion was incubated for 10 min at 37°C and then centrifuged (5500 g, 4°
 18 C, 5 min). The pellets were washed with 400 μL Tris buffer I and cen-
 19 trifuged (5500 g, 4°C, 5 min). The pellets were resuspended in 500 μL
 20 Tris buffer I and 100 μL of *n*-lauryl-sarcosine (10% w/v), 4 μL of
 21 RNase A (10 mg mL⁻¹) was added and kept for 20 min at 37°C. From
 22 this point on samples were handled with extra care and wide bore pipette
 23 tips were used. 25 μL of Proteinase K (20 mg mL⁻¹) was added and the
 24 samples were then incubated for 60 min at 60°C, and centrifuged
 25 (17 500 g, 4°C, 20 min). The clear green supernatants were carefully
 26 transferred into new tubes and gently mixed on a tube rotator for
 27 15–20 min with equal volume of Phenol-TE pH 8.0, and centrifuged
 28 (15 000 g, 4°C, 5 min). The upper phases were carefully transferred to
 29 new tubes and the phenol extraction was repeated. The DNA was pre-
 30 cipitated with 1/9 vol of 3M NaOAc and 0.7 vol of *i*-Propanol at –20°C
 31 for at least 30 min, centrifuged (20 000 g, 4°C, 1 h), and washed with
 32 400 μL of 70% ET-OH. The supernatant was discarded and the samples
 33 were left to dry on room temperature, and rehydrated in 20–30 μL of
 34 water at 4°C for overnight.

35 *Detection of UV-B induced DNA damage by qPCR.* This method is
 36 based on the assumption that biologically significant lesions block the
 37 action of Taq polymerase. Therefore, only the intact DNA molecules can
 38 be quantified using qPCR, and the extent of damage can be calculat-
 39 ed from the virtually decreased amount of the DNA. Accordingly,
 40 UV-B-dependent decrease in amplified 16S rDNA was shown in Ref.
 41 (25). To enable exact determination of the extent of DNA damage, we
 42 carried out similar PCR reactions, but using qPCR. Keeping in mind that
 43 pyrimidine dimers are the most abundant type of lesions caused by
 44 UV-B (4), a 1 kbp long region of the *Synechocystis* genome containing
 45 the most pyrimidine neighbors was selected using a custom made PERL
 46 script for amplification in a ABI SDS7000 qPCR equipment. A 50 bp
 47 long genomic sequence with the least number of these neighbors was
 48 used as an internal control and the relative apparent quantities were used
 49 for calculation of the probabilities of DNA lesions. The cells were har-
 50 vested, washed, subjected to three freeze-thaw cycles and used in PCR
 51 reactions as templates like in colony-PCR.

52 *Detection of UV-B-induced DNA damage by alkaline gel method.*
 53 Pyrimidine dimers were detected by incubating gDNA with T4 Endonu-
 54 clease V, which specifically cleaves the DNA molecule at pyrimidine
 55 dimer sites, causing single strand breaks (29). The resulting fragments
 56 were separated on a 0.6% alkaline agarose gel, previously soaked in alka-
 57 line electrophoresis buffer (50 M NaOH, 1 mM EDTA). The same solu-
 58 tion was used as running buffer.

59 The presence of unrepaired pyrimidine dimers yields strand breaks
 60 leading to reduced size of DNA fragments, which show up as a smear
 61 on the alkaline gel. The relative decrease in the apparent average
 length is representative of the encountered direct UV-B damages and
 also allows for quantitative determination of those. Nucleic acids were
 visualized with the help of ethidium-bromide. Gel images were evalu-
 ated using custom made software written in MATLAB for establish-
 ing the average/mean fragment lengths according to the calculations
 in (30).

The two DNA damage quantification methods complement each
 other well. The qPCR-based assay is faster and simpler than the alka-
 line gel method, and it shows the sum of all the lesions; while the alka-
 line gel is more sensitive and allows selective detection of pyrimidine
 dimers.

Significance in dataset variability was tested with one-way ANOVA,
 at $P = 0.05$.

RESULTS AND DISCUSSION

Effect of the lack of photolyase on UV-B induced activity loss of PSII

We aimed to investigate the role of UV-B-induced DNA lesions
 in PSII activity loss during UV-B stress. To achieve this, we
 compared the changes in the amount of functional PSII, quanti-
 fied by flash-induced Chl fluorescence measurements in UV-B
 exposed WT *Synechocystis* and its photolyase deficient mutant
 (ΔphrA), which is defective in its DNA repair capability.

In the initial phase of the UV-B treatment, which was per-
 formed in the presence of 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ visible light, the
 ΔphrA mutant lost its PSII activity somewhat faster than the
 WT. After 120 min of UV-B + WL treatment, the loss of PSII
 activity became significantly more pronounced in the ΔphrA
 mutant than in the WT cells ($F = 0.009$, at $P = 0.05$), in which
 protein synthesis-dependent PSII repair is functional (Fig. 1 open
 symbols). This large difference in PSII activity was retained dur-
 ing the subsequent exposure to UV-B + WL, and after 270 min
 the amount of active PSII decreased to 60 and 20% of their ini-
 tial values in the WT and ΔphrA cells, respectively (Fig. 1 open
 circles and open squares).

When the cells were kept under visible light following the
 UV-B + WL treatment, the PSII activity partially recovered and
 after 120 min it reached 70 and 30% of the initial values in the
 WT and the ΔphrA mutant, respectively. When the cells were
 subsequently exposed to UV-B light in the absence of back-
 ground visible light, PSII activity dropped rapidly to as low as
 10% of its original level in the ΔphrA mutant in 60 min,
 whereas in the WT it dropped only to 45% of its initial value
 (Fig. 1 open squares and open circles, respectively).

When PSII repair was blocked by the protein synthesis inhibi-
 tor lincomycin, the loss of PSII was accelerated in both strains
 and decreased in a parallel fashion to a value lower than 10% of
 their initial values in 120 min (Fig. 1 closed symbols). In the
 presence of lincomycin, the loss of activity reflects the efficiency
 of PSII photodamage in itself without the influence of the protein
 synthesis dependent repair. Therefore, the identical inhibition
 course of PSII in the WT and ΔphrA mutant cells in the pres-
 ence of lincomycin shows that the lack of DNA repair capacity
 does not modify the UV-B sensitivity of the PSII complex. The
 different extent of activity loss in the absence and presence of
 lincomycin in the same strain reflects the efficiency of protein
 synthesis dependent PSII repair during the light treatment. The
 difference between the inhibition curves measured in the absence
 and presence of lincomycin at 120 min is significantly
 ($F = 0.012$, at $P = 0.05$) smaller in the ΔphrA mutant than in
 the WT (Fig. 1). Further into the UV-B + WL treatment (210
 and 270 min), when the PSII activity in the presence of linc-
 omycin is close to zero, the difference between the activities of
 the WT and the ΔphrA mutant increases even more. These data
 show that PSII damage is repaired less efficiently in the absence
 of photolyase in the ΔphrA mutant cells than in the WT cells,
 which have active photolyase.

Effect of the lack of photolyase on UV-B-induced D1 protein loss

UV-B-induced inactivation of PSII function is accompanied with
 the damage and degradation of the D1 subunit of the PSII

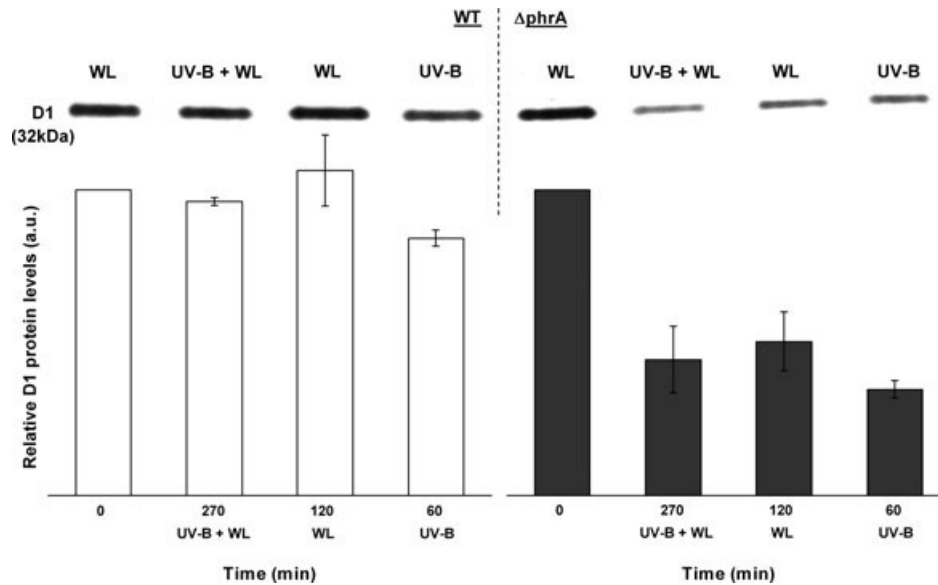


Figure 2. Response of D1 protein to UV-B treatment in WT and $\Delta phrA$ cells. Cells were exposed to 270 min of $8 \mu\text{mol m}^{-2} \text{s}^{-1}$ UV-B, in the presence $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ white light (UV-B + WL), followed by a 120-min WL only recovery period, and an additional UV-B only (UV-B) exposure. Isolated thylakoid membranes from samples taken during the treatment were separated by SDS-PAGE. D1 proteins were detected and visualized *via* immunoblotting. Protein bands were quantified with ImageJ and D1 amounts are shown relative to the untreated control samples (0 min). $n = 3$.

reaction center (18,31). When low intensity white light is applied simultaneously with UV-B illumination, it helps to ameliorate the UV-B-induced activity loss of PSII through enhanced protein turnover (32). Changes in D1 protein levels during the UV-B treatment (Fig. 2) corroborate the fluorescence data. The mutant cells lost a large portion of their D1 pool during UV-B stress, and were unable to recover it even in the absence of UV-B (Fig. 2 right panel), whereas D1 levels in WT cells were much less affected under the same treatment (Fig. 2. left panel), being significantly ($F = 0.0419$, at $P = 0.05$) higher than in the $\Delta phrA$ cells.

Effect of the lack of photolyase on UV-B-induced *psbA* mRNA response

The prerequisite of efficient PSII protein repair is the availability of abundant *psbA* mRNA pool for translation of D1, as well as transcription of other genes whose protein products, such as D2, the FtsH protease, etc. (18,19,33,34) are required for PSII repair. In *Synechocystis*, the *psbA3* copy of the *psbA* family serves as a UV stress gene, and its UV-B-induced transcription contributes significantly to the increase of the total *psbA* mRNA pool (22). Therefore, we used the *psbA3* transcript abundance as a marker to evaluate the effects of photolyase deactivation on UV-B-induced responses at the transcript level of genes essential for PSII repair. UV-B light highly induces the *psbA3* gene both in the WT and the $\Delta phrA$ cells during the first phase of UV-B exposure reaching a steady-state level after 60–120 min (Fig. 3). When UV-B illumination was stopped after 270 min of treatment and cells were exposed to visible light alone the *psbA3* transcript level rapidly decreased in the WT cells, and was inducible again by subsequent UV-B exposure in the absence of visible background light.

In contrast, in the $\Delta phrA$ cells the *psbA3* mRNA pool did not respond to the lack of the UV-B stimulus in the presence of background visible light, or to a repeated UV-B exposure. No

significant ($F = 0.744$, at $P = 0.05$) change could be observed from the level that was reached at the end of the UV-B plus visible light treatment (Fig. 3). This behavior led us to assume that in the absence of the main DNA repair facilitator photolyase, the $\Delta phrA$ cells accumulate so many UV-B-induced DNA damages that prevent the transcription of not only *psbA3* but also of those genes whose protein products are needed to degrade the already existing mRNAs.

Quantification of UV-induced DNA damage

In another cyanobacterium, *Anabaena variabilis*, Rastogi *et al.* (25) have shown that DNA damages accumulated in a UV-B

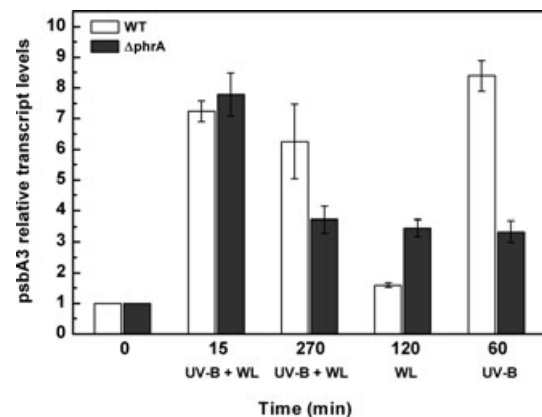


Figure 3. Changes of relative *psbA3* transcript levels in WT and $\Delta phrA$ cells. Cells were exposed to $8 \mu\text{mol m}^{-2} \text{s}^{-1}$ UV-B in the presence of $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ white light (UV-B + WL) for 270 min, followed by a 120-min WL ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$) only recovery period and then a 60-min UV-B ($8 \mu\text{mol m}^{-2} \text{s}^{-1}$) only treatment. The columns represent the induction levels of *psbA3*, after normalization to the transcript amount of the *mpbB* (*slr1311*) housekeeping gene of *Synechocystis*, coding for the RNase P subunit B. $n = 3$.

dose-dependent manner. We used two different methods to assess the extent of UV-induced DNA damage. Our qPCR-based method, described in the Materials and Methods, makes possible to determine the average number of DNA lesions without specifying the type of the lesions. In the WT cells, there was no significant ($F = 0.724$, at $P = 0.05$) accumulation of DNA damages during the UV-B treatment with concomitant visible light present, and the detected average DNA damage remained below 0.1–0.2 lesions per 1 kb long DNA segment (Fig. 4). In contrast, in the $\Delta phrA$ cells DNA lesions gradually accumulated reaching *ca* 1 lesion in average per 1 kb long DNA segment after 270 min UV-B plus visible light treatment (Fig. 4). The level of DNA damage was maintained during the recovery period, which shows that DNA repair is irreversibly inhibited in the absence of photolyase in the $\Delta phrA$ mutant. Although cyanobacteria possess an excision repair mechanism that eliminates UV-induced pyrimidine dimers in the absence of photoreactivating blue/UV-A light (see Ref. [35]), the efficiency of excision repair seems to be negligible under our conditions. This could be due to the slow

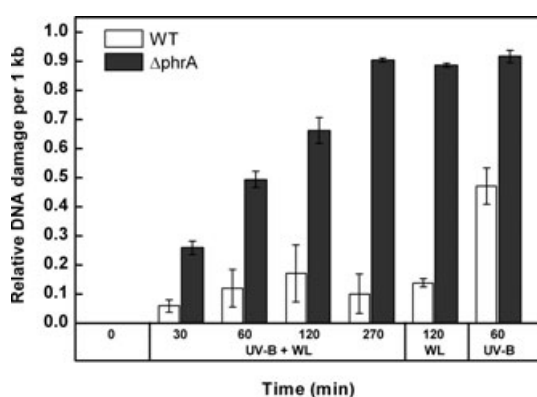


Figure 4. qPCR assessment of DNA damage. WT and $\Delta phrA$ cells were exposed to $8 \mu\text{mol m}^{-2} \text{s}^{-1}$ UV-B in the presence of $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ white light (UV-B + WL) for 270 min, followed by a 120 min WL ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$) only recovery period and then a 60-min UV-B ($8 \mu\text{mol m}^{-2} \text{s}^{-1}$) only treatment. The columns represent the number of DNA polymerase hindering lesions on a 1 kb long DNA sequence. $n = 2$.

response of the excision repair system as indicated by the observation that 15 h was required for removal of pyrimidine dimers by excision repair in another cyanobacterium *Synechocystis* PCC 6308 (35). In addition, it has been shown that the absence of the photolyase enzyme inhibits excision repair in *Saccharomyces cerevisiae* and *Escherichia coli* (36). The interaction of photolyase dependent and excision repair appears to be a general phenomenon (36) and can explain the irreversible inhibition of DNA repair under our conditions (Fig. 4).

During the second phase of UV-B illumination, which was performed in the absence of background visible light the level of average DNA damages was also induced in the WT cells. This is most likely due to the absorption of the second cofactor of the *Synechocystis* CPD photolyase, 7,8-didemethyl-8-hydroxy-5-deazariboflavin (8-HDF) in the 430–450 nm wavelength range (37), which is absent in the UV-B alone illumination protocol. Therefore, the activity of this particular photolyase is lower if no white light is present, leaving room for the accumulation of UV-B-induced DNA damages.

The average gene length in the *Synechocystis* genome is about 1 kb. Thus, the observed 1 DNA lesion/kb implies that by the end of the UV-B treatment virtually all genes in the genome of the $\Delta phrA$ mutant could have a lesion that can stop transcription under the applied irradiation conditions.

The qPCR method cannot discriminate among the different types of UV-induced DNA modifications, and it approximates the quantity of all sorts of DNA damages that could potentially impair gene expression. Therefore, we also applied a method that can specifically detect pyrimidine dimers that represent the most abundant type of UV-B-induced DNA lesion. We treated gDNA isolated from UV-B-treated cells with T4 endonuclease V, which produces single strand breaks at pyrimidine dimer sites, and separated the single stranded DNA fragments using alkaline gel. Quantification of the separated fragments carried out *via* analysis of the gel images with the help of a Matlab software, designed in our laboratory, resulted in DNA-damage amounts (Fig. 5), very similar to those detected by the qPCR method. The high degree overlap of the qPCR and alkaline gel data sets indicates that the overwhelming majority of the DNA lesions which accumulate during UV-B stress are in fact pyrimidine dimers.

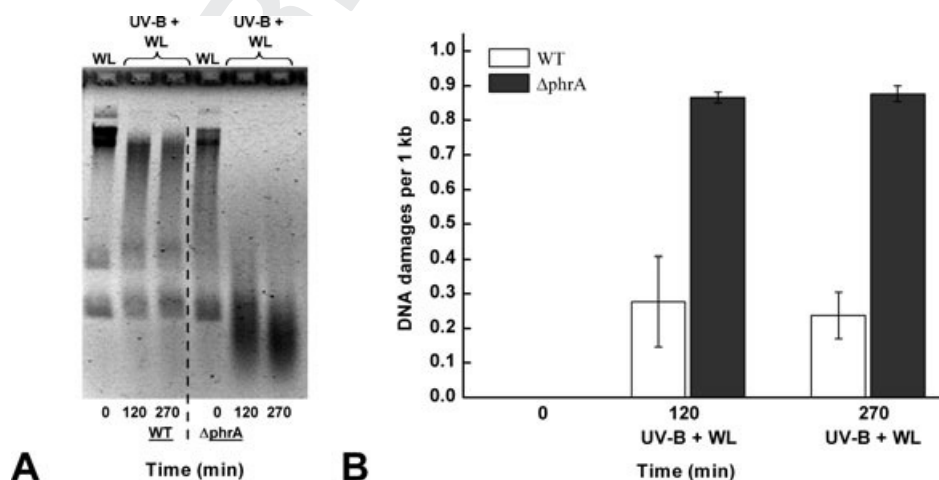
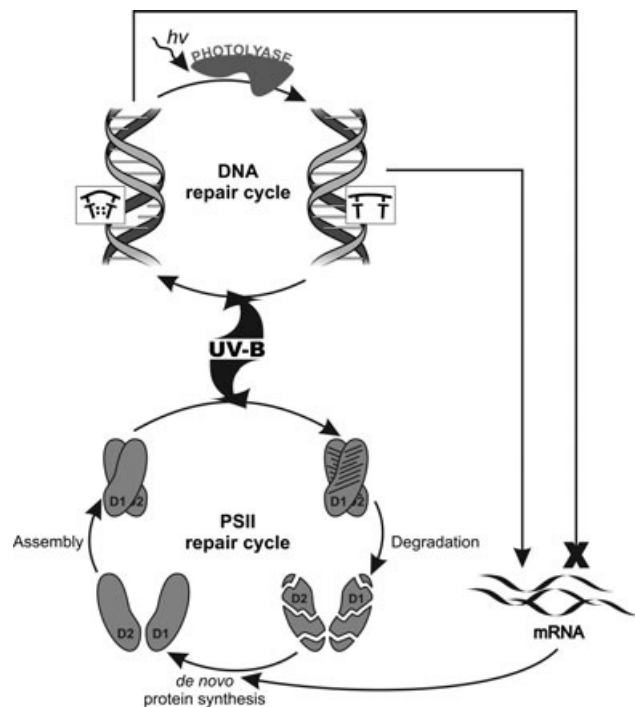


Figure 5. Alkaline gel assessment of DNA damage. Isolated gDNA from UV-B-treated WT and $\Delta phrA$ cells was digested with T4 Endonuclease V and separated on alkaline gels. The gels were neutralized and soaked in ethidium-bromide for visualization. Panel A: gDNA from WT and $\Delta phrA$ cells. Panel B: Calculated amount of DNA lesions. $n = 3$.

Concluding remarks

The results described here represent the first demonstration that UV-B-induced DNA damage affects photosynthetic functions, and show that the absence of the photolyase enzyme encoded by the *phrA* gene enhances photodamage of PSII in *Synechocystis* 6803 cells. This effect is caused by the accumulation of unrepaired DNA lesions, which give rise to inefficient transcription of *psbA* and other key genes of PSII repair, leading to enhanced loss of the D1 reaction center protein and photosynthetic activity during UV-B irradiation. Therefore, the DNA and PSII repair cycles, which represent two highly important defense mechanisms against UV-B-induced damage in photosynthetic organisms, are directly connected (Scheme 1).

The D1 subunit of the PSII complex is the protein with the highest turnover rate in oxygenic photosynthetic organisms, therefore accumulation of transcription limiting DNA lesions represent a key target for the PSII repair cycle. Our data also show that when UV-B exposure occurs in the presence of visible light and at temperatures close to the optimal growth conditions the DNA repair capacity of the photolyase enzyme is sufficient to keep DNA damage at a sufficiently low level that does not interfere with PSII repair. The effect of DNA repair limitation on



Scheme 1. Interaction of DNA damage repair and Photosystem-II damage-repair cycles in photosynthetic organisms. UV-B radiation damages DNA by inducing cyclobutane pyrimidine dimers, which block polymerases and prevent the translation of mRNA. These DNA lesions can be restored by the action of the light-activated enzyme, photolyase, which breaks up the pyrimidine dimers and ensures the translation of DNA into functional mRNA. UV-B damage of Photosystem-II reaction center complex leads to the degradation of the D1 (and D2) reaction center subunits, which have to be resynthesized to restore Photosystem-II structure and function. Protein synthesis can proceed only if sufficient amount of mRNA is available for the translation of new D1 (and D2) subunits, which incorporate into the PSII complex and restore its activity. This critical step of the PSII repair cycle is blocked if unrepaired DNA lesions accumulate and prevent mRNA formation.

PSII repair becomes significant when the photolyase enzyme is inactivated or inhibited. Low temperature is known to decrease the efficiency of photolyase (38,39) and generally the photoreactivation of thymidine dimers (25), which leads to decreased DNA repair activity. Therefore, limitation of DNA repair on PSII function may occur under physiological relevant conditions, such as in polar and alpine environments where elevated UV exposure occurs in combination with low temperature.

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