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ORIGINAL PAPER

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DNA photolyase homologs are the major UV resistance factors in the cyanobacterium *Synechocystis* sp. PCC 6803

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Abstract In this study, the unicellular photosynthetic cyanobacterium *Synechocystis* sp. PCC 6803 was used as a model phototroph to study the contribution of enzymatic photoreactivation to the overall protection against UV irradiation. We have isolated genes encoding two DNA photolyase homologs, *phrA* and *phrB*, from *Synechocystis* 6803. *phrA* encodes an 8-hydroxy-5-deazariboflavin (HDF) type, Class I DNA photolyase. By complementing a photolyase-deficient mutant strain of *Escherichia coli*, we demonstrated that PhrA is a DNA photolyase. Analysis of a *phrA* knockout mutant strain suggested that this gene is responsible for the majority of the observed UV resistance in *Synechocystis* 6803. Similar studies on *phrB* demonstrated that it also contributes to photoreactivation, but to a much lesser degree. Based on these findings, we conclude that enzymatic photoreactivation is the primary process used for repairing UV-induced damage in *Synechocystis* 6803.

Key words Cyanobacteria · DNA photolyase · DNA repair · Photoreactivation · UV stress

Introduction

Photoreactivation, an enzyme-mediated and light-dependent process that repairs DNA photoproducts induced by UV radiation (Sancar 1994), is the subject of the present study. It is known that this process greatly enhances the survival of cyanobacteria and many other organisms subjected to severe UV stress. The only known class of proteins involved in this process are the DNA photolyases. DNA photolyases and related proteins have been identified in a wide range of organisms from eubacteria and Archaea to eukaryotes, including human and plants (Kanai et al. 1997). In human, DNA photolyase-like proteins do not encode DNA photolyase, but probably play a role in the entrainment of the circadian clock. In plants, genes encoding DNA photolyases specific for cyclobutane pyrimidine dimers (CPDs) (Ahmad et al. 1997) and pyrimidine (6-4) pyrimidone photoproducts [(6-4) photoproducts] (Britt et al. 1993; Nakajima et al. 1998) have recently been identified. In addition, blue light receptors from plants and animals have been found to share a high degree of homology with DNA photolyases (Cashmore et al. 1999).

The unicellular cyanobacterium *Synechocystis* sp. PCC 6803 offers an ideal experimental system for the study of enzymatic photoreactivation in a photosynthetic organism. This bacterium is naturally transformable, and is amenable to various genetic manipulations (Williams 1988). Moreover, *Synechocystis* 6803 is the first phototrophic organism whose complete genome sequence has been determined (Kaneko et al. 1996). Cyanobacteria are highly resistant to UV radiation (Van Baalen 1980; O'Brien and Houghton 1982; Levine and Thiel 1987). The recent elucidation of the crystal structure of DNA photolyase from another cyanobacterium, *Synechococcus* sp. PCC 6301 (also called *Anacystis nidulans*), has provided important insights into the structure and function of the HDF-type of DNA photolyase (Tamada et al. 1997). We report here the identification and isolation of the two genes that encode DNA photolyase homologs from the cyanobacterium *Synechocystis* 6803. Using mutational analysis, we provide evidence for the essential role of these DNA photolyase homologs in UV repair in this photosynthetic organism.

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Materials and methods

Bacterial strains and culture conditions

In these studies, a glucose-tolerant isolate of *Synechocystis* 6803 (Williams 1988) was used as the wild-type strain. *Synechocystis*

6803 wild-type and mutant cultures were grown in BG-11 medium (Allen 1968) at 30 °C. The media for the *phrA* mutant (T1011) and the *phrB* mutant (T1099) were supplemented with 20 μg/ml spectinomycin dihydrochloride and 2 μg/ml gentamycin sulfate, respectively. The *phrA-phrB* double mutant (T1011–1099) was cultured in the presence of both antibiotics. During photoautotrophic growth, cultures were grown under 40 μmol/m^2 per s white light (General Electric cool white fluorescent lamps). Any radiation below 400 nm was filtered out with a plastic UF 5 filter (UV cut off = 383 nm; Cope Plastics, St. Louis, Mo.). Glucose was added (5 mM) to the media to maintain heterotrophic growth in the dark.

The *Escherichia coli* strains INVαF′ [F′ *endA1 recA1 hsdR17* (r_k^-, m_k^+) *supE44 thi-1 gyrA96 relA1* ϕ80*lacZ*Δ*M15* Δ(*lacZYA-argF*)*U169*; Invitrogen, Carlsbad, Calif., USA] and TG1 [*supE hsd*Δ*5 thi* Δ(*lac-proAB*) F′(*traD36 proAB*$^+$ *lacI*^{q} *lacZ*Δ*M15*); Sambrook et al. 1989] were used for the general propagation of plasmids and the construction of the cyanobacterial genomic library, respectively. All *E. coli* strains were grown in Luria-Bertani (LB) medium (Sambrook et al. 1989) at 37 °C. The *E. coli* strain NKJ3002 [*endA1 hsdR17 supE44 thi-1 gyrA96 relA1 thi* Δ(*lac-proAB*) F′(*traD36 proAB*$^+$ *lacI*^{q} *lacZ*Δ*M15*) *phr20*::Kan *uvrA*::Kan Δ*recA*; Nakajima et al. 1998], used in the functional complementation experiment, was grown in medium supplemented with 50 μg/ml kanamycin sulfate.

DNA manipulations

Southern hybridization, PCRs and other routine DNA manipulations were performed as described by Sambrook et al. (1989). To confirm the *phrA* mutation, genomic DNA preparations from *Synechocystis* 6803, the *phrA* mutant and the *phrA phrB* double mutant were digested with *Eco*RI. To confirm the *phrB* mutation, genomic DNA preparations from *Synechocystis* 6803, the *phrB* mutant and the *phrA phrB* double mutant were digested with *Acc*I. Digested DNA preparations were fractionated on 0.8% agarose gels, blotted onto nitrocellulose membranes, and hybridized to ^{32}P-labelled *phrA* or *phrB* probes individually. Radioactivity on the membranes was visualized with a PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.). The PhosphorImager signals were quantified with the ImageQuant software (Molecular Dynamics). DNA sequencing was carried out by the dideoxynucleotide chain termination procedure using the Fidelity DNA sequencing system (Oncor, Gaithersburg, Md.). The final sequence was determined from both strands of DNA. Analysis of nucleotide sequences was performed using the DNASTAR programs (DNASTAR, Madison, Wis.). For database searches, the BLAST (Altschul et al. 1990) network service at the National Center for Biotechnology Information (Bethesda, Md.) was used.

Cloning and over-expression of DNA photolyase genes

For PCRs, Klentaq I DNA polymerase was obtained from Dr. Wayne Barnes (Washington University, St. Louis). Two degenerate primers, PHRP1 (5′-TGGYWYMGVCRBGAYYTVCG-3′) and PHRP2 (5′-GARGCGSYCCACTGCCARCCRCC-3′) were used to amplify part of the *phrA* gene from *Synechocystis* 6803 genomic DNA. The primers were designed based on conserved regions in DNA photolyases (Yasui et al. 1994). The amplified fragment was then used as a probe to screen a pUC119 library made from *Eco*RI- and *Hin*dIII-digested *Synechocystis* 6803 genomic DNA. A 2.5-kb *Eco*RI-*Hin*dIII clone (pSL966) containing the entire ORF of *phrA* was isolated. The second DNA photolyase gene, designated here as *phrB* (CyanoBase accession number: sll1629; Kaneko et al. 1996), was amplified by PCR using the primers PHRP9 (5′-CGAAAACTGGGCCATGG-3′) and PHRP10 (5′-TAATTCTTCCATGGCGC-3′). The resulting PCR product was digested with *Nco*I and then cloned into the *Nco*I and *Sma*I sites in pUC21 (pSL1086). The genomic clone of *phrB* was isolated using the same strategy as described above, but using the *phrB* PCR product as the probe. A 2.9-kb *Sac*I-*Nar*I insert in pUC119 (pSL1192) was found to contain the entire *phrB* ORF.

To over-express the *phrA* and *phrB* genes in *E. coli* cells, PCR-based mutagenesis was used to introduce an *Nde*I site at the initiation codon of each of these genes. The modified coding regions of the *phrA* and *phrB* genes were then cloned in the pTrcHisC vector (Invitrogen) to form the plasmids pSL1055 and pSL1356, respectively.

Complementation of the *E. coli* photolyase-deficient mutant

Each of the plasmids pMS2 (Sancar et al. 1983), pBR322, pSL1055, pSL1356 and pTrcHisC was transformed into *E. coli* NKJ3002. For pSL1055 and pTrcHisC, cultures were induced for 4 h with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Cultures were diluted and 100 μl of each dilution was plated on an agar plate. With lids removed, plates were irradiated under a General Electric germicidal lamp (λ_{max} = 254 nm) with a total dose of 0.2 J/m^2. Plates that received the photoreactivation treatment were immediately placed about 30 cm from two General Electric black lights (λ_{max} = 350 nm) that were wrapped with five layers of Mylar, for 1 h at 37 °C. Plates that were not photoreactivated were kept in light-tight boxes and incubated in the dark at 37 °C. The number of colonies on each plate was determined after 12 h.

Construction of *Synechocystis* 6803 DNA photolyase mutants

A 2-kb *Bam*HI Ω fragment containing the spectinomycin resistance cassette from pHP45Ω (Prentki and Kirsch 1984) was subcloned into pUC119 (pSL994). Then the *Sac*I-*Sma*I fragment from pSL994 containing the Ω fragment was inserted into between the *Sac*I and *Hin*cII sites in the *phrA* gene in pSL966. The resulting construct, pSL1011, was used to transform *Synechocystis* 6803. The resulting *phrA* deletion mutant is designated as T1011.

To construct a *phrB*-deficient mutant strain, a *Sma*I fragment of the gentamycin cassette from pUGM (Schweizer 1993) was inserted into the *Hpa*I site of pSL1086, yielding pSL1099. pSL1099 was transformed into *Synechocystis* 6803 to generate the insertion mutant of *phrB*, T1099. Transformation of T1011 with pSL1099 generated the double mutant, T1011–1099, in which both *phrA* and *phrB* have been inactivated.

Photoreactivation of *Synechocystis* 6803

Aliquots (100 μl) of various serial dilutions of log-phase cultures of the wild-type and mutant cells of *Synechocystis* 6803 were plated on BG-11 medium supplemented with 5 mM glucose. With lids removed, the plates were irradiated with a General Electric germicidal lamp at a fluence rate of 3.7 W/m^2 for different lengths of time. The fluence rate of UV-C (200–280 nm) was determined with an UVX radiometer equipped with a 254 nm sensor (Ultra-violet Products, Upland, Calif.). To photoreactivate irradiated cells, plates were immediately placed under UV-filtered (UF5) cool white fluorescent lamps (40 μmol/m^2 per s). To avoid inducing photoreactivation, control plates were kept in the dark for 24 h prior to returning them to visible light. Cells were allowed to grow for 7–10 days, after which the number of colonies on each plate was determined.

Mixed-culture competition experiments under UV-B stress

Log-phase cultures of *Synechocystis* 6803 and the *phrA* mutant, T1011, were harvested by centrifugation and then washed twice with fresh BG-11. The harvested cells were mixed and then used to inoculate fresh BG-11. The mixed cultures were then allowed to grow without antibiotics under different light regimes. For the UV-B (280–320 nm) treatment, cells were grown in polystyrene Corning culture flasks under 42 μmol/m^2 per s of white light and 0.12 μW/m^2 of UV-B from a UV-B lamp (Villa Lighting, St. Louis, Mo.).

The transmittance of UV-B through polystyrene is about 40% at 310 nm. The UV-B intensity was measured with an UVX radiometer equipped with a 300 nm sensor (Ultra-violet Products). For treatment with visible light, cells were grown under white light (40 μmol/m^2 per s) and all the UV light was screened out by a UF5 filter. All of the cultures were maintained on 12-h light and 12-h dark cycles. To assess the representation of each cell type, aliquots of the cultures were plated on BG-11 medium periodically. Colonies were counted after 10 days (total colony count). These colonies were then transferred to BG-11 medium supplemented with 20 μg/ml spectinomycin dihydrochloride. The spectinomycin-resistant colonies represent *phrA* mutant (T1011) cells in the mixed culture. The representation of wild-type cells was calculated from the difference between the total colony count and the spectinomycin-resistant cell count. To assay for potential reversion of the *phrA* mutant (T1011), the mutant cells were grown for 5 days without spectinomycin dihydrochloride. Cells were checked for spectinomycin resistance as described above. All recovered colonies remained spectinomycin resistant, indicating that this strain did not revert under these experimental conditions.

Results

Cloning of *Synechocystis* 6803 DNA photolyase genes

The cloning of the photolyase genes was completed before the genome sequence of *Synechocystis* 6803 became publicly available. In the absence of any genome sequence information, we decided to clone the photolyase genes from *Synechocystis* 6803 by exploiting amino acid motifs that are conserved among Class I DNA photolyases (Yasui et al. 1994). Using the degenerate primers PHRP1 and PHRP2, a 1.16-kb PCR fragment with a high degree of sequence homology to a *Synechococcus* 6301 DNA photolyase gene was amplified from the genomic DNA of *Synechocystis* 6803. The use of this PCR fragment as a probe to screen a genomic library of *Synechocystis* 6803 resulted in the identification of a single 2.5-kb *Eco*RI-*Hin*dIII clone (pSL966). Upon sequencing, it was found that pSL966 contains a complete ORF encoding a DNA photolyase homolog. This photolyase homolog is designated as *phrA* (GenBank Accession No. U51943) and corresponds to the slr0854 locus in the Cyanobase database (http://www.kazusa.or.jp/cyanobase). The ORF identified is 1467 nucleotides long and encodes a protein of 488 amino acids. The genome sequence of *Synechocystis* 6803 revealed the presence of a gene encoding another DNA photolyase homolog, sll1629. Based on its sequence similarity to known DNA photolyases, we tentatively designate this gene as *phrB*.

Synechocystis 6803 PhrA is highly homologous to HDF-type photolyases

In general, PhrA shares a higher degree of homology to the HDF-type, Class I DNA photolyases (such as *Synechococcus* sp. strain PCC 6301 DNA photolyase, *Halobacterium halobium* DNA photolyase and *Streptomyces griseus* DNA photolyase) than to the methenyltetra-hydrofolate (MTHF)-type DNA photolyases (such as *E. coli* DNA photolyase) (data not shown). The highest sequence homology is found between *Synechocystis* 6803 PhrA and *Synechococcus* 6301 DNA photolyase. The binding sites for the cofactors are highly conserved between these two DNA photolyases. Among the 12 amino acids that are involved in the binding of HDF in *Synechococcus* 6301 DNA photolyase (Tamada et al. 1997), 10 are perfectly conserved in PhrA. For the binding of the catalytic cofactor, $FADH^-$, 13 out of 14 amino acids are conserved between the two DNA photolyases. Notably absent from the primary sequence of PhrA is the C-terminal extension found in blue-light receptors of plants (Ahmad and Cashmore 1993), the green alga *Chlamydomonas reinhardtii* (Small et al. 1995), human (van der Spek et al. 1996), mouse (Kobayashi et al. 1998) and *Drosophila melanogaster* (Emery et al. 1998; Okano et al. 1999). This fact, taken together with the above information, suggests that *phrA* encodes an HDF-type Class I DNA photolyase.

The PhrB protein is shorter than the PhrA protein. Like PhrA, PhrB lacks the C-terminal extension found in blue-light receptors. Of the 14 amino acids implicated in the binding of the $FADH^-$ in *Synechococcus* 6301 (Tamada et al. 1997), 11 are identical or similar in PhrB. It is therefore likely that PhrB binds $FADH^-$. Despite the fact that PhrA and PhrB are from the same organism, PhrA is more closely related to the DNA photolyase from *Synechococcus* 6301 than to PhrB from *Synechocystis* 6803. Only 26% sequence identity is found between PhrA and PhrB. This large degree of sequence divergence between the two proteins may indicate that they have different functions. Moreover, PhrB has only four out of the twelve amino acids known to be involved in the binding of HDF (Tamada et al. 1997) and one out of the seven amino acids for the binding of MTHF (Park et al. 1995). Based on this information, we suspect that PhrB may lack an antenna cofactor or carry a cofactor other than MTHF or HDF.

Functional complementation of an *E. coli* photolyase mutant

To assess the predicted DNA photolyase function of the *phrA* and *phrB* gene products from *Synechocystis* 6803, these genes were used to complement a photolyase-deficient mutant strain of *E. coli*, NKJ3002 (Nakajima et al. 1998). Note that, in spite of the absence of HDF in *E. coli*, Takao et al. (1989) have successfully complemented the photolyase-deficient *E. coli* CSR603 mutant using the *Synechococcus* 6301 DNA photolyase gene.

The results of the complementation experiment are shown in Table 1. Over-expression of the *Synechocystis* 6803 PhrA protein in *E. coli* NKJ3002 cells transformed with pSL1055 was confirmed by SDS-PAGE (data not shown). Both pMS2 (containing the *E. coli* DNA photolyase gene; Sancar et al. 1983) and pSL1055 (containing the *Synechocystis* 6803 *phrA* gene) restored photoreactivation in *E. coli* NKJ3002 cells. As expected,

Table 1 Functional complementation of NKJ3002, a photolyase-deficient mutant strain of *E. coli*

Strain	Survival rate (%)[a]		Photoreactivation factor[c]
	PR[b]	NPR[b]	
NKJ3002	0.0037 ± 0.0001	0.0017 ± 0.0005	2.2
NKJ3002 (pSL1055)[c]	2.5051 ± 1.6884	0.0108 ± 0.0026	232.0
NKJ3002 (pMS2)[d]	25.0697 ± 0.3924	0.0005 ± 0.0001	50139.4

[a] The data shown (mean ± standard deviation) are the average of three independent experiments
[b] Ratio of survival rates with and without photoreactivation (PR/NPR)
[c] pSL1055 is an over-expression plasmid containing the *phrA* gene from *Synechocystis* 6803, inserted in the vector pTrcHisC
[d] pMS2 contains the *phr* gene from *E. coli*, inserted in pBR322

the extent of complementation obtained with the *Synechocystis* 6803 *phrA* gene was less than with the *E. coli* DNA photolyase gene. The results for the controls, NKJ3002 (pBR322) and NKJ3002 (pTrcHisC), did not differ significantly from those for NKJ3002 itself (data not shown). Despite repeated trials, we have not been able to express the PhrB protein using the pTrcHisC plasmid. However, using an alternative over-expression plasmid, pET3xc, we were able to over-express the PhrB protein to a high level. Unfortunately, the pET3xc construct cannot function in NKJ3002 and hence we could not test whether *phrB* can complement NKJ3002.

Photolyase-deficient mutants of *Synechocystis* 6803 are hypersensitive to UV

To assess the respective contributions of *phrA* and *phrB* to the overall UV resistance in *Synechocystis* 6803, knockout mutants were constructed for both genes. The *phrA* and *phrB* genes were inactivated by targeted insertion of a spectinomycin resistance and a gentamycin resistance cassette, respectively. Insertional mutagenesis of each of the two genes was verified by Southern analysis (data not shown). *Synechocystis* 6803 is highly resistant to UV-C (Fig. 1A). When photoreactivating light was provided, *Synechocystis* 6803 cells survived up to 111 J/m^2 UV-C without loss of viability. Wild-type *Synechocystis* 6803 cells exhibited photoreactivation as observed previously with other cyanobacterial species (Van Baalen and O'Donnell 1972; O'Brien and Houghton 1982; Levine and Thiel 1987). When exposed to up to 111 J/m^2 of UV-C, the survival of wild-type *Synechocystis* 6803 cells, whether photoreactivated or not, was very similar to that observed in a related cyanobacterium, *Synechocystis* sp. PCC 6308 (O'Brien and Houghton 1982) and the unicellular cyanobacterium *Agmenellum quadruplicatum* strain PR-6 (Van Baalen and O'Donnell 1972).

The *phrA* mutant (T1011), on the other hand, was extremely sensitive (over a million times) to UV (Fig. 1A) and exhibited very little photoreactivating activity. The UV survival curves for the *phrA* mutant are biphasic. Below 37 J/m^2 of UV-C, cells recovered fully, irrespective of whether or not photoreactivating light was provided (Fig. 1A). This is due to photoreactivation and other light-independent processes that repair UV-induced damage. These repair processes appeared to be saturated at a low UV dose. At an UV-C dose above 37 J/m^2, cell survival decreased exponentially. At moderate doses of UV-C (55.5–111 J/m^2), a residual photoreactivating activity was observed in the *phrA* mutant cells (Fig. 1A). The highest residual photoreactivating activity was observed at 74 J/m^2 UV-C, where the survival rate for *phrA* mutant cells was enhanced by 100-fold higher under photoreactivating conditions.

At 111 J/m^2 of UV-C, the survival rate for the wild type cells is 1000 times higher than that of the *phrA* mutant cells under non-photoreactivating conditions (Fig. 1A), suggesting that PhrA may have a role in dark repair. Similar effects of DNA photolyases on dark repair have been observed in *E. coli* and *Saccharomyces cerevisiae* cells (Yamamoto et al. 1983a, b; Sancar and Smith 1989). It is known that such effects are nucleotide excision repair dependent, and are mediated by interactions between the DNA photolyase enzyme and components of the nucleotide excision repair pathway in the dark (Yamamoto et al. 1983a, b; Sancar and Smith 1989).

Photoreactivation is largely intact in the *phrB* mutant, T1099 (Fig. 1B). With or without photoreactivation, the *phrB* mutant survived essentially to the same level as the wild-type cells. Therefore, inactivation of *phrB* alone does not result in any discernible change in UV sensitivity in *Synechocystis* 6803. The UV response of the *phrA phrB* double mutant (T1011–1099) is very similar to that of the *phrA* mutant (Fig. 1B). However, between 37 and 111 J/m^2 of UV-C, the double mutant is somewhat more sensitive (10 times) than the *phrA* mutant (T1011) under non-photoreactivating conditions. Another notable difference between the two mutants is the significant decrease in the residual photoreactivation in the double mutant. In the double mutant, this residual photoreactivation is still present at 55 J/m^2, but it is absent between 74 and 111 J/m^2 of UV-C.

Mixed culture competition experiment

The mixed culture experiment was conducted to investigate the physiological role of *phrA* in the natural environment. To simulate stress due to solar UV, UV-B

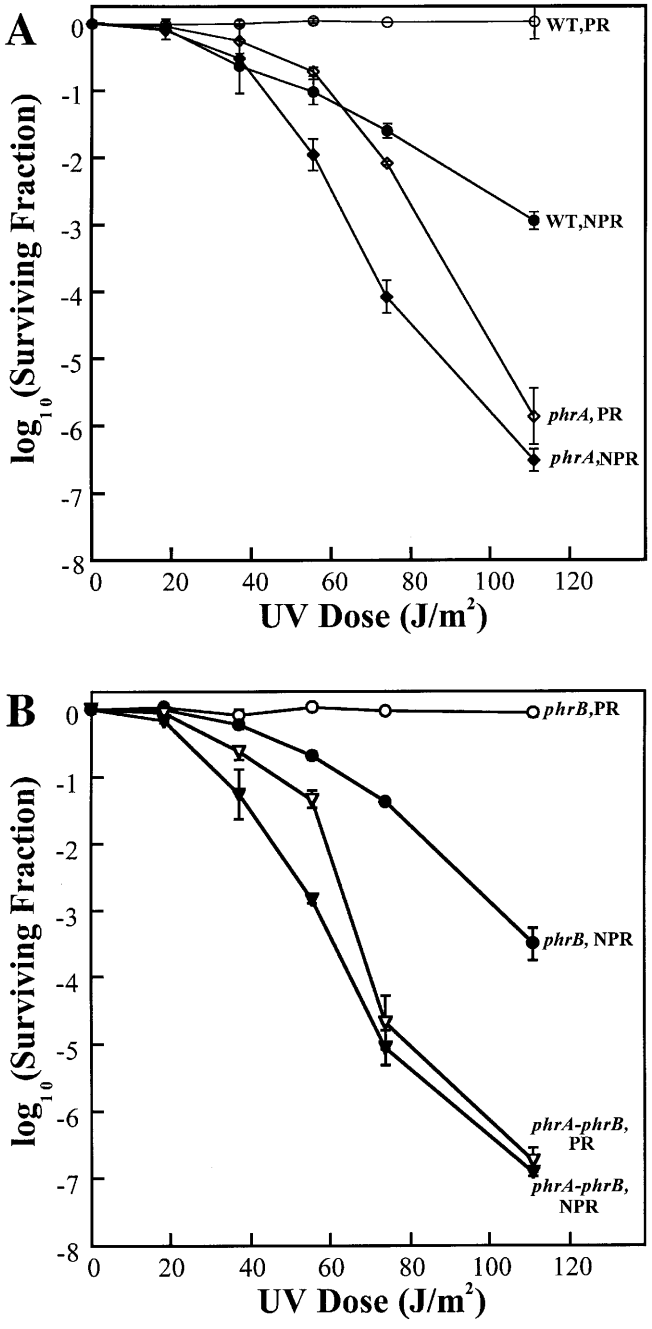

Fig. 1 **A** UV sensitivity of *Synechocystis* 6803 wild type (WT) and the *phrA* mutant. **B** UV sensitivity of the *phrB* mutant and the *phrA phrB* double mutant. Cells were treated as described in Materials and methods. Each data point represents the mean of three independent replicates. Error bars denote one standard deviation from the mean. Abbreviations: PR, photoreactivation; NPR, no photoreactivation

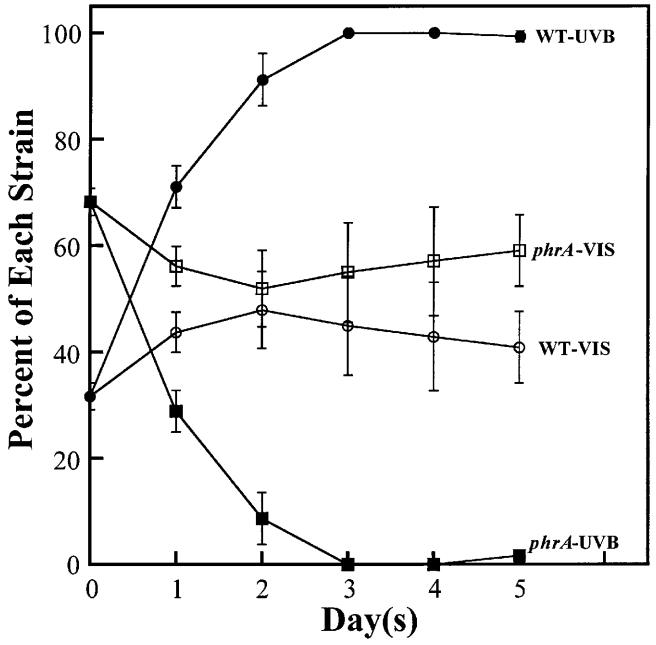

Fig. 2 Mixed culture competition experiment with *Synechocystis* 6803 wild type (WT) and the *phrA* mutant strain. Both strains of cells were cultured together in liquid BG-11. Cells were either exposed to visible light only (VIS) or to visible light supplemented with UV-B (UVB). Total daily UVB fluence was 5184 J/m^2. The relative representation of each strain was determined at the indicated times according to Materials and methods. Each data point represents the average of three replicates and each error bar denotes one standard deviation from the mean

was used as the source of UV. Cells were grown under visible light on a 12-h light/12-h darkness cycle. For the UV-B treatment, cells were exposed to UV-B at a fluence rate of 0.12 J/m^2 per s during the 12-h light period. The resulting daily UV-B dose (5184 J/m^2) is comparable to that to which photosynthetic organisms are exposed in eastern North America during the summer months [data from the National UV Monitoring Center Website, http://oz.physast.uga.edu/docs/Rtp/Rtp98.htm; (last accessed on 16 June 1999)]. The relative representation of the wild type and *phrA* mutant cells changed very little when they were grown under visible light alone (Fig. 2). However, under chronic UV-B stress, the representation of the *phrA* mutant (T1011) quickly declined from an initial 66% to 0% in just 3 days. The doubling time of the *phrA* mutant under these conditions of UV-B stress is about 19 h (data not shown). Therefore, the *phrA* mutant cells were eliminated from the mixed culture within less than four generations.

Discussion

In this study, we have provided genetic evidence that photoreactivation is the major process employed for the repair of UV-induced damage in *Synechocystis* 6803. We have identified a gene for a photolyase homolog, *phrA*, from this photosynthetic cyanobacterium. The deduced amino acid sequence of its product exhibits the highest degree of homology with the HDF-type DNA photolyases and, hence, we predict that *phrA* encodes a HDF-type, Class I DNA photolyase. This prediction is further supported by the fact that the *phrA* gene product can

complement a photolyase-deficient *E. coli* mutant. In addition, we have recently demonstrated that the PhrA protein purified from *Synechocystis* 6803 cells exhibits a cyclobutane-pyrimidine-dimer-specific DNA photolyase activity (Ng et al. 2000). The PhrB protein, on the other hand, shows far less homology with known DNA photolyases. Although it has most of the conserved residues required for $FADH^-$ binding, it lacks the amino acids required for the binding of either HDF or MTHF.

Like many cyanobacteria, *Synechocystis* 6803 exhibits a high degree of resistance to UV (Fig. 1A). In this study, we have demonstrated that this high resistance to UV is due largely to the photoreactivating activity of a single gene product, PhrA. In the absence of *phrA*, cells are as much as a million times more sensitive to UV. Through the inactivation of *phrA*, the predominant source of DNA photolyase activity in *Synechocystis* 6803, we have uncovered a significant residual photoreactivating activity in this cyanobacterium (Fig. 1A). In *Synechocystis* 6803, inactivation of both the *phrA* and *phrB* genes in the double mutant (T1011–1099; Fig. 1B) led to a large reduction in the residual photoreactivation. This suggests that *phrB* contributes most of the residual photoreactivating activity observed in the *phrA* mutant.

Recent studies on photorepair in higher eukaryotes (Todo et al. 1996; Nakajima et al. 1998) have confirmed the presence of a second type of DNA photolyases that specifically repair (6-4) photoproducts, one of the most abundant DNA photoadducts found in UV-irradiated DNA. In addition, cryptochromes in eukaryotes also share a high degree of sequence similarity to DNA photolyases. Based on this information, we speculate that PhrB may function either as a (6-4) DNA photolyase or a cryptochrome. Further experiments will be needed to clarify the function of PhrB.

In most of our experiments we used UV-C, but such radiation is absent in the biosphere. Hence, though we could demonstrate that the DNA photolyase mutants are sensitive to UV-C, the *phrA* gene might not confer any UV resistance to *Synechocystis* 6803 in its natural environment. The mixed culture experiment was designed to address this issue. To mimic the ambient UV stress normally encountered by the organism, the mixed culture was exposed to UV-B at a dose comparable to the solar UV-B dose. Under these conditions, mutant cells lacking *phrA* were quickly eliminated from the culture, thus demonstrating that *phrA* is indeed physiologically important for counteracting UV stress in *Synechocystis* 6803.

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