UV-B-induced Differential Transcription of \( \text{psbA} \) Genes Encoding the D1 Protein of Photosystem II in the Cyanobacterium \( \text{Synechocystis} \) 6803*

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Zoltán Máté, László Sass, Miklós Szekeres, Imre Vass‡, and Ferenc Nagy

From the Institute of Plant Biology, Biological Research Center of the Hungarian Academy of Sciences, P. O. Box 521, H-6701 Szeged, Hungary

UV-B irradiation of intact \( \text{Synechocystis} \) sp. PCC 6803 cells results in the loss of photosystem II activity, which can be repaired via de novo synthesis of the D1 (and D2) reaction center subunits. In this study, we investigated the effect of UV-B irradiation on the transcription of the \( \text{psbA2} \) and \( \text{psbA3} \) genes encoding identical D1 proteins. We show that UV-B irradiation increases the level of \( \text{psbA2} \) mRNA by 2-3-fold and, more dramatically, it induces a 20-30-fold increase in the accumulation of the \( \text{psbA3} \) mRNA even at levels of irradiation too low to produce losses of either photosystem II activity or D1 protein. The induction of \( \text{psbA3} \) transcript accumulation is specific for UV-B light (290–330 nm). Low intensity UV-A irradiation (290–330 nm) and white light induce only a small, at most, 2-3-fold enhancement, whereas no effect of blue light was observed. Expression patterns of chimeric genes containing the promoter regions of the \( \text{psbA2} \), \( \text{psbA3} \) genes fused to the firefly luciferase (luc) reporter gene indicate that (i) transcription of \( \text{psbA2} / \text{luc} \) and \( \text{psbA3} / \text{luc} \) transgenes was elevated, similarly to that of the endogenous \( \text{psbA} \) genes, by UV-B irradiation, and that (ii) a short, 80-base pair \( \text{psbA3} \) promoter fragment is sufficient to maintain UV-B-induced transcription of the luc reporter gene. Furthermore, our findings indicate that UV-B-induced expression of the \( \text{psbA2} \) and \( \text{psbA3} \) genes is a defense response against UV-B stress, which is regulated, at least, partially at the level of transcription and does not require active electron transport.

Recent reductions in the stratospheric ozone layer allowing more ultraviolet-B (UV-B, 280–320 nm) radiation to reach the Earth’s surface and ecologically significant depths of the ocean (1) have initiated extensive research efforts to elucidate molecular mechanisms regulating responses of various organisms to UV irradiation (2). In photosynthetic organisms, a crucial part of the overall UV-B effect is related to damage in the photosynthetic apparatus leading to decreased oxygen evolution and \( \text{CO}_2 \) fixation (3–6), reduction in dry weight, secondary sugars, starch, and total chlorophyll (7, 8). Within the photosynthetic apparatus, the most sensitive UV-B target is the light energy converting complex of photosystem II (PSII) (9) (for reviews, see Refs. 9 and 10), although photosystem I (4, 11), ribulose-bisphosphate carboxylase/oxygenase (12, 13), and ATPase (14) can also be damaged. PSII is a large pigment-protein assembly embedded in the thylakoid membrane. Inhibition of PSII activity by UV-B radiation is accompanied by the degradation of the D1 and D2 protein subunits (15–18), which form the heart of the PSII reaction center by contributing ligands to cofactors that mediate primary photochemistry (for review, see Ref. 19).

Photoheterotrophic cyanobacterium species are very useful model organisms for studying the adverse effects of UV-B irradiation on the activity of PSII because (i) cyanobacteria were among the earliest oxygenic photosynthetic organisms that were exposed early in their evolution to UV-B fluxes much higher than the relatively low present day UV levels (20), and (ii) they have evolved efficient mechanisms to cope with the stress of UV exposure (21). Indeed, in a previous study, we have provided evidence that intact \( \text{Synechocystis} \) 6803 cells are able to repair UV-B-induced damage of PSII via de novo synthesis of the D1 and D2 proteins (22). Just recently, Campbell et al. (23) have reported that the cyanobacterium \( \text{Synechococcus} \) appears to resist UV-B-induced PSII damage by selectively exchanging the PSII reaction center D1:1 protein (encoded by \( \text{psbA1} \)) for the different D1:2 form (encoded by \( \text{psbAII} \) and \( \text{psbAIII} \) genes).

In \( \text{Synechocystis} \) 6803, the D1 protein is also encoded by three genes, \( \text{psbA1}, \text{psbA2}, \) and \( \text{psbA3} \) (24). However, in contrast to \( \text{Synechococcus} \), \( \text{Synechocystis} \) has only one form of the D1 polypeptide encoded by the \( \text{psbA2} \) and \( \text{psbA3} \) genes. The expression of both \( \text{psbA2} \) and \( \text{psbA3} \) is under light control (25, 26), with \( \text{psbA2} \) accounting for the majority (>90%) of the \( \text{psbA} \) transcripts under normal growths conditions (26). The third \( \text{psbA} \) gene, \( \text{psbA1} \), encodes a slightly different D1 protein (see Ref. 27), but there are no indications that it is expressed in wild-type cells (25, 26, 28).

In this study, we show that expression of the two active \( \text{psbA} \) genes of \( \text{Synechocystis} \) 6803 is induced, although differentially, in response to UV-B light. UV-B-induced elevation of the expression of the endogenous \( \text{psbA} \) genes or the chimeric \( \text{psbA1/luc} \) genes is regulated, at least partially, at the level of transcription, and elevation of \( \text{psbA} \) transcript levels does not require de novo protein synthesis or active electron transport. These data suggest that transcriptionally regulated expression of the \( \text{psbA2} \) and especially that of the \( \text{psbA3} \) helps to maintain functional PSII under conditions of UV-B exposure and provide evidence for the role of duplicate \( \text{psbA} \) genes in cyanobacteria.

**EXPERIMENTAL PROCEDURES**

**Growth Conditions**—\( \text{Synechocystis} \) 6803 cells were routinely grown at 40 \pm \mu m \cdot s^{-1} \text{ light intensity in BG-11 medium at 30 °C, gently bubbled with air containing 5% CO}_2. Cells in the exponential growth phase (A_{730} nm of 0.6–0.8) were harvested by centrifugation for 10 min

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‡ To whom correspondence and reprint requests should be addressed.

The abbreviations used are: PSII, photosystem II; \( \mu \text{E}, \text{ microeinsteins(s)}; \text{PCR}, \) polymerase chain reaction; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; nt, nucleotide(s).
at 4000 × g at room temperature. The chlorophyll a content was determined by methanolic extraction, and the cells were diluted to 30 µg of chlorophyll a ml⁻¹ by fresh BG-11 medium.

**Light Source**—UV-B light was produced by a Vilbert-Lourmat VL-215M lamp in combination with a 0.1-mm cellulose acetate filter (Claroform, Courtaulds Chemicals, UK) in order to screen out any UV-C contribution, below 290 nm. Under these conditions, our UV-B source provided maximal emission at 312 nm with 60 µE m⁻² s⁻¹ intensity at the surface of the cell suspension. For some experiments, this intensity was decreased to 6 µE m⁻² s⁻¹ by applying eight layers of cellulose acetate filters. Due to the high optical density of the irradiated samples, the average UV-B irradiation within the cell suspension was attenuated by about a factor of 30, as calculated by taking into account the absorption by the optically dense sample according to Ref. 29, corresponding to 2 and 0.2 µE m⁻² s⁻¹ average intensity in the above irradiation protocols, respectively. These intensities are in the physiologically relevant range, because in full sunshine of about 2000 µE m⁻² s⁻¹ the UV-B contribution is approximately 7 µE m⁻² s⁻¹.

**Irradiation Conditions**—UV-B irradiation was performed in open, flat glass containers in which 330 ml of cell suspension (30 µg of chlorophyll/ml) of 15 mm depth was continuously stirred at room temperature. Before UV-B treatment, the cells were incubated under visible light of 40 µE m⁻² s⁻¹ intensity for 1 h. For monitoring the recovery process after UV-B exposure, the irradiated cells were transferred to visible light of 40 µE m⁻² s⁻¹ intensity and were kept at room temperature under continuous stirring. Steady-state rates of oxygen evolution were measured using a Hansatech DW2 O₂ electrode at a light intensity of 1000 µE m⁻² s⁻¹ in the presence of 0.5 mM 2,5-dimethyl-p-benzoquinone as electron acceptor. D1 protein analysis was performed as described previously by Sass et al. (22).

**mRNA Extraction, S1 Nuclease Protection Assay**—Total RNA was extracted as described by Nagy et al. (30). S1 nuclease protection assay was performed according to Fejes et al. (31). Sequences of the synthetic single-stranded oligonucleotides employed in these assays were: (i) luc, 5’-ATAGAATGGCCGCGGCTTTCTCTATTTGGCTGTCTTCCATTACAGCTTACAT-3’; (ii) psbA2, 5’-CGCTGTTGGAGAGTCGTTGTCATTTGGTTATAATTCCTTATGATTTTGTCGATG-3’; (iii) psbA2, 5’-CGCTGTTGGAGAGTCGTTGTCATTTGGTTATAATTCCTTATGATTTTGTCGATG-3’. The length of the gene specific protected fragments, 78 nt for psbA2, 72 nt for psbA3, and 85 nt for psbA3 when using the probe homologous to psbA2 (psbA3), 72 nt for psbA2 when using the probe homologous to psbA3 (psbA2), 72 nt for psbA2 when using the probe homologous to psbA2 (psbA2), and 58 nt for psbA3 when using the probe homologous to psbA2 (psbA2), respectively, and plotted as a function of time. The time point of transferring the cells from UV-B to visible light is shown by the dashed vertical line. The transcript levels are normalized to 1 in the nonirradiated control cells. For comparison, changes in the rate of oxygen evolution (●) and in the amount of the D1 protein (●) are also plotted. These values are expressed as percentage of those in the nonirradiated control cells.

**RESULTS**

**Differential Induction of the psbA Genes by UV-B Light**—Repair of PSII via de novo synthesis of the D1 reaction center protein following its damage by UV-B radiation is a multistep process (22). Here, we studied in detail the first step of this process and monitored changes in psbA transcript levels during UV-B exposure and throughout the following recovery period. To ensure gene-specific detection of the psbA2, psbA3 transcripts, synthetic oligonucleotides were employed in S1 nuclease protection assays. First, we showed that UV-B radiation elevated the expression level of the psbA genes. Fig. 1A (lanes 1–3) shows that after 90 min of UV-B irradiation the level of the accumulated psbA mRNA increased about 5–7-fold. More importantly, Fig. 1B (lanes 1–3) indicates that the level of psbA3 mRNA was elevated about 20–25-fold. In contrast, Fig. 1C (lanes 1–3) shows only a 2–3-fold induction of the psbA2. When the UV-irradiated cells were transferred back to white light of 40 µE m⁻² s⁻¹, the amount of psbA3 transcripts decreased again dramatically, whereas the amount of psbA2 transcripts showed a more moderate, about 2–3-fold decrease (Fig. 1, B and C, lanes 4–6).

In order to compare the relative abundance of the two psbA transcripts with PSI1 activity, the autoradiograms shown in Fig. 1 (B and C) were quantified by a phosphoimage analyzer and plotted together with changes in oxygen evolving activity and D1 protein amount. Fig. 1D illustrates that during UV-B irradiation the rate of oxygen evolution, the measure of electron transport activity through PSI1, and the net amount of the D1 protein declined in a closely parallel manner. After 180 min of UV-B irradiation, both the rate of oxygen evolution and the amount of the D1 protein decreased to about 15% of the respective control values. Under the same experimental conditions,
Fig. 2. Effect of spectral composition of irradiation on the activity of psbA genes. A, cells were kept under growth conditions at 40 \( \mu \text{E m}^{-2} \text{s}^{-1} \) visible light for 1 h (lane 1) and were subsequently exposed to different light regimes for 30 min: 100 \( \mu \text{E m}^{-2} \text{s}^{-1} \) visible light (lane 2), UV-B plus 100 \( \mu \text{E m}^{-2} \text{s}^{-1} \) visible light (lane 3), UV-B (lane 4), UV-B without any blue or visible component (lane 5), UV-A (lane 6), and UV-A (lane 7). The transcript levels shown were determined as described in Fig. 1. B, the spectral characteristics of the light regimes. a, irradiance from the UV-B lamp through a cellulose acetate filter, which blocks the UV-C region, below 290 nm; b, the same as a in combination with a UG-11 filter, which blocks the blue and visible region in the 390–690 nm range (dark shading); c, irradiance from the UV-B lamp through a plastic filter, which blocks UV-B light below 330 nm (light shading); d, transmission of the narrow-band blue filter.

the relative amount of psbA3 transcripts was increased 20-fold, as compared with that in nonirradiated control cells. After transferring the UV-B-irradiated cells to white light, the damaged PSII protein structure and the inhibited oxygen evolving activity were rapidly restored. During a 3-h recovery period, the rate of oxygen evolution and the amount of the D1 protein reached about 80% of the control values. In the recovery phase of the experiment, the relative amount of psbA3 transcripts gradually declined to levels close to those in the nonirradiated control cells. In contrast, the level of psbA2 transcripts changed only to a small extent during recovery and stayed at the slightly elevated level up to 3 h.

UV-B Specificity—The signal transduction pathway that leads to UV response of photosynthetic genes in cyanobacteria has not been studied in detail, and no specific UV-B receptor has been reported yet. However, data in the literature indicate that blue light of low fluence triggers a differential response in the psbA gene family in Synechococcus sp. PCC 7942 (36). In order to exclude any accidental effect of blue light, which might be emitted from our lamp, as well as to probe the effect of other spectral ranges on the psbA3 gene response, six different irradiation protocols were compared. Exposure of cells, incubated at 10 \( \mu \text{E m}^{-2} \text{s}^{-1} \) white light as control condition, to white light of 100 \( \mu \text{E m}^{-2} \text{s}^{-1} \) resulted in only a small increase in psbA3 transcript level (Fig. 2A, lane 2). Illumination of the cells with the same white light through a narrow band blue filter with maximal transmission at 450 nm (Fig. 2B, dotted line) induced a response (lane 5) similar to that evoked by white light. Exposure of cells to UV-B light, whose irradiance profile is shown in Fig. 2B (solid line a) alone (lane 4) or in combination with visible light (lane 3) led to the already described (Fig. 1) immense induction of the psbA3 gene. Combination of the UV-B light source with an UG-11 filter in order to cut all blue and visible light in the 390–690-nm range, producing the irradiance profile shown in Fig. 2B, line b (dark shading), resulted in the same extent of psbA3 induction (lane 6) as nonfiltered UV-B light (lane 3). This excludes the possibility of an accidental blue component of our lamp being responsible for the induction of the psbA3 gene. The possible effect of the small but not negligible UV-A emission from the lamp in the 330–400-nm range was also checked. To this end, the UV-B component of the lamp was blocked by a 330-nm cut-off filter, which produced the irradiance profile shown by line c (light shading) in Fig. 2B. Illumination with this light protocol resulted in some enhancement of the psbA3 transcript level (lane 7), which was, however, much smaller than that induced by UV-B (plus UV-A) light (lane 4). These results demonstrate that the remarkable induction of psbA3 gene expression observed in response to illumination with our UV source was specific for the UV-B component (290–330 nm) of the lamp, and unrelated to the small UV-A emission or any accidental blue (visible) illumination.

Effect of Electron Transport Activity—In order to check the possible involvement of redox regulation in the UV-B-induced expression of psbA3, two different experimental approaches were used. In the first experiment, the intensity of UV-B light was decreased to 10% of that used in Fig. 1 and de novo protein synthesis was blocked by the translation inhibitor lincomycin. Under these conditions, the oxygen evolving activity was not decreased by up to 60 min of irradiation (data not shown). This indicates that the applied UV-B light did not damage the function and protein structure of PSII complexes, because in the absence of protein repair any damage should have led to a decreased oxygen evolution rate. Surprisingly, even this very low fluence of UV-B light was sufficient for a strong induction of the psbA3 gene after a 10-min exposure, as shown in Fig. 3A. Because the very weak UV-B light applied (average intensity within the cell suspension 0.2 \( \mu \text{E m}^{-2} \text{s}^{-1} \)) was certainly below the limit of driving PSII electron transport, this finding indicates that direct redox control via reduction of the plastoquinone pool is unlikely to play a role in UV-B induction of psbA3.
This hypothesis was further corroborated by another experiment, showing that inhibition of electron transport through PSII by DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) did not affect the induction of \( \text{psbA3} \) in response to UV-B light either. According to the data in Fig. 3B, 60-min exposure of cells to UV-B light in the absence (lane 2) and in the presence of DCMU (lane 4) induced \( \text{psbA3} \) expression to similar extents.

**Reporter Gene Constructs**—To determine whether the UV-B-induced expression of the \( \text{psbA3} \) and \( \text{psbA2} \) genes is regulated at the level of transcription as well as to identify important regulatory regions, a series of chimeric genes were constructed and their expression in transgenic cyanobacteria cells analyzed. These chimeric genes contained various fragments of the 5′-upstream regions of the \( \text{psbA2} \) and \( \text{psbA3} \) genes fused to the \( \text{luc} \) gene encoding firefly luciferase. For these experiments, a low intensity UV-B light was used in order to ensure that gene expression is monitored under conditions when damage to PSII is minimal. The most important results obtained are summarized in Fig. 4. It was first established that the induction of the \( \text{psbA2} \) and \( \text{psbA3} \) genes followed the same pattern as observed under strong UV-B light (shown in Fig. 1), i.e. \( \text{psbA3} \) mRNA accumulation was enhanced to 20–30-fold by UV-B irradiation and declined gradually to control levels during a 4.5-h recovery period. In contrast, \( \text{psbA2} \) mRNA accumulation pattern showed only 2–3-fold changes either during induction or the recovery phase (Fig. 4, top left).

The two chimeric reporter strains containing the longest \( \text{psbA2} \) and \( \text{psbA3} \) upstream regions (including the 5′-nontranslated leader sequences) fused to the \( \text{luc} \) gene (\( \text{psbA2/luc} \) and \( \text{psbA3/luc} \), respectively) exhibited the same expression patterns as observed for the endogenous \( \text{psbA} \) genes, i.e. \( \text{luc} \) mRNA levels increased strongly upon UV-B exposure and declined under white light for the \( \text{psbA3/luc} \) construct and showed only minor fluctuations for the \( \text{psbA2/luc} \) construct. Truncation of the \( \text{psbA3} \) promoter to positions −160 and −80 (+1 being the transcription start site) had no effect on the UV-B-induced accumulation of the \( \text{luc} \) mRNA; however, the decline during recovery seemed to occur more rapidly. Furthermore, replacement of the \( \text{psbA3} \) nontranslated leader sequence with that of \( \text{psbA2} \) (in the \( \text{psbA3/luc} \) construct where the \( \text{psbA3} \) promoter is truncated to the −160 position) did not change significantly either the UV-B-induced accumulation or the following rapid decline of \( \text{luc} \) mRNA levels during the recovery period. Neither did the replacement of the leader sequence of \( \text{psbA2} \) with that of \( \text{psbA3} \) in the \( \text{psbA2/luc} \) construct alter the expression pattern of the \( \text{psbA2/luc} \) mutant. These data indicate that (i) UV-B-induced expression of \( \text{psbA3} \) is regulated mainly at the transcription level, (ii) the majority of important cis-regulatory elements or site(s) mediating this defense response against UV-light stress should be located within a short 80-base pair region, and (iii) the leader sequences of the \( \text{psbA} \) transcripts do not have important regulatory functions.

**DISCUSSION**

In the present work, the effect of UV-B light on the expression of the *Synechocystis* \( \text{psbA} \) genes encoding the D1 protein was studied as an approach to gain information about the early steps of the protein repair process that was shown by Saas et al. (22) to be required for restoring the activity of the UV-B damaged PSII. It was found that *Synechocystis* 6803 cells responded to UV-B by the differential expression of the two active \( \text{psbA} \) genes, i.e. transcript levels of \( \text{psbA3} \) were increased 20–30-fold in contrast to those of \( \text{psbA2} \), which were increased only 2–3-fold. The immense induction of the \( \text{psbA3} \) gene was highly specific for the UV-B spectral range (290–330 nm in our conditions), but this response was not influenced by the presence of background visible light (Fig. 2). Any major contribution to

\[ \text{psbA3} \] induction by the low intensity UV-A component of our lamp or by an accidental blue illumination can be excluded. These data strongly suggest that up-regulation of the \( \text{psbA3} \) gene can occur under natural conditions where UV-B light is always accompanied by visible illumination and provide the first demonstration for the existence of a specific UV-B-responsive gene in *Synechocystis* cells.

Besides the different extent of induction under UV-B exposure, the \( \text{psbA2} \) and \( \text{psbA3} \) transcript levels also showed different kinetic behavior during the course of UV-B and subsequent visible illumination. The 20–30-fold induction of \( \text{psbA3} \) was

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**Fig. 4.** UV-B-induced expression of the \( \text{psbA2} \), \( \text{psbA3} \), and the chimeric \( \text{psbA2/luc} \) genes. The cells were exposed to UV-B light at 10 times lower intensity than that applied in Fig. 1 and were subsequently transferred to growth conditions under visible light of 40 \( \mu \text{E m}^{-2} \text{s}^{-1} \). Transcript levels were determined after 0, 10, and 20 min of UV-B exposure (lanes 1, 2, and 3) as well as after 30, 60, and 270 min of recovery in visible light (lanes 4, 5, and 6). The \( \text{psbA3} \), \( \text{psbA2} \), and \( \text{luc} \) mRNA levels were detected by S1 nuclease protection assays; the lengths of the specific protected fragments (78 nt, 72 nt, and 45 nt, respectively) are shown. Top right, structure of the \( \text{psbB2A} \) vector (32); below, the structure of the chimeric \( \text{psbA2/luc} \) transgenes used in these studies. To create the \( \text{psbA2/luc} \) transgenes, an NcoI site was introduced by PCR mutagenesis at the ATG of the \( \text{psbA2} \) and \( \text{psbA3} \) genes as well as at the ATG of the \( \text{luc} \) reporter gene. Therefore, all of these chimeric genes contain only the coding region of the \( \text{luc} \) reporter gene and the full-length, unchanged leader sequences, except the last two nucleotides before the ATG of the \( \text{psbA2} \) and \( \text{psbA3} \) genes. The lengths of the promoter fragments are indicated by numbers, checkered bars indicate \( \text{psbA2} \), filled bars \( \text{psbA3} \) specific sequences, and arrows the transcription start sites.
more rapid, and after having reached a transient maximum exhibited a slight decline even under UV-B light. This decline was accelerated after transferring the cells to visible light, and the enhanced psbA3 transcripts decreased close to control levels after a 2–3-h recovery period (Fig. 1). In contrast, the 2-fold induction of psbA2 appeared to be slower, and the slightly higher levels induced by UV-B exposure were maintained for a longer period of time under the conditions of recovery in visible light. These observations support the idea that the elevation of psbA3 transcript levels is a rapid response to UV-B light, which may be very important for cyanobacterial cells in coping with the stress situation caused by enhanced UV-B fluxes from solar radiation.

Our data on the chimeric reporter strains suggest that the UV-B-induced differential accumulation of the psbA2 and psbA3 transcripts is likely regulated at the level of transcription for the following reasons: (i) the expression pattern of the psbA2/luc and psbA3/luc transgenes is identical with those of the respective endogenes and (ii) exchange of the psbA2 and psbA3 leader sequences in the reporter gene constructs does not affect the expression pattern (Fig. 4), which places the site of the UV-B-responsive element(s) in the psbA3 promoter. Furthermore, we show that truncation of the psbA3 promoter region to -160 and -80 positions does not eliminate the basic UV-B response. These latest observations indicate that cis-regulatory element(s) responsible for UV-B-induced transcription should reside within the first 80 bases of the psbA3 promoter.

The psbA2 and psbA3 genes encode identical D1 polypeptides (the psbA1 gene is not expressed under conditions checked so far), and the physiological role of producing the same protein from two different genes is an intriguing open question. Based on our data, we postulate that the Synechocystis 6803 cyanobacterium regulates the repair of its-UV-damaged PSII reaction center by increasing the pool of psbA mRNA available for producing new D1 proteins by switching on the psbA3 gene, which we consider to be a UV-stress gene. This hypothesis is supported by the following observations: (i) under normal growth conditions, in visible light, the majority (at least 90%) of psbA transcripts arises from the psbA2 gene (25, 26) and most likely the majority of the D1 protein is also translated from these messages; (ii) mainly because of the highly induced transcription of the psbA3 genes by UV-B irradiation, the overall level of psbA mRNA is about 5–7-fold higher in UV-B-irradiated as compared with nonirradiated cells (Fig. 1A); and therefore it is likely (iii) that under these conditions the amount of D1 protein translated from the psbA3 message is also significantly increased. Considering, however, that even under the conditions of maximal UV-B induction the psbA3 and psbA2 transcript levels are more or less identical, it follows that a considerable amount of D1 protein mobilized for repair should also be translated from the psbA2 mRNA. Therefore, we suggest that the psbA3 gene serves as a fast-reacting backup copy that can be relied on under conditions of UV-B stress in order to comply with the demand for higher D1 protein production.

The utilization of duplicate psbA genes seems to be a common trait of various cyanobacteria. However, there are species, like Synechococcus 7942, that contain three active psbA genes, which produce two distinct forms of the D1 protein. The psbAII genes encode the D1:1 protein, and the psbDII and psbIII genes encode identical D1:2 proteins (37). Upon exposure to high intensity visible light, the constitutive D1:1 form is transiently replaced by the D1:2 form in the PSII reaction center (38). Moreover, parallel with our work, Campbell et al. (23) reported that UV-B irradiation of Synechococcus 7942 cells also induces expression of the otherwise lowly expressed psbAIII and psbAIII genes and leads to a transient exchange of D1:1 for D1:2 protein in the PSII reaction center. Furthermore, these authors found that constitutive overexpression of the psbAII and psbAIII genes renders the cells to be UV-B resistant, whereas deletion of these genes results in an increased UV-B sensitivity. Based on these observations, Campbell et al. (23) concluded that resistance to UV-B irradiation is mediated by the exchange of the D1:1 and D1:2 forms in Synechococcus cells. This explanation is clearly not applicable to Synechocystis cells, where the various psbA genes encode identical D1 proteins. To resolve this apparent contradiction, we propose the following hypothesis. In both Synechococcus and Synechocystis cells, resistance to UV-B irradiation is brought about mainly by the selectively induced transcription of one or two of the psbA genes. Induced transcription leads to an increased psbA mRNA pool that is used by the translation machinery to produce large amounts of new D1 proteins to replace the damaged D1 molecules in the PSII. Accordingly, we suggest that UV-B resistance in different cyanobacterial species is controlled mainly at the level of transcription, and the exchange of the various forms of D1 proteins merely represents the ratio of various psbA mRNAs available for translation.

Cyanobacteria were among the earliest oxygentic photosynthetic organisms, which most likely experienced much higher UV-B fluxes early in their evolution (2.5–0.6 × 10^6 years ago) than the relatively low present day UV levels. The development of a readily inducible spare gene copy for the D1 protein, located in the heart of the highly UV sensitive PSII complex, is most likely one of the strategies applied by cyanobacteria for coping with the stress of UV exposure. The fact that this gene arrangement has been maintained over 1–2 billion years indicates that UV stress has been a sustained condition during evolution. At present, the components of the signal transduction pathway or the UV-B receptor(s) mediating the UV-B-induced transcription of the psbA3, psbAII, psbAIII, or other genes in cyanobacteria are not known. Redox control of photosynthetic gene expression, including psbA, has recently been supported by various observations (41), pointing to the central role of electron flow through PSI that modifies the redox level of the plastoquinone pool, which is then transmitted by a not yet clarified mechanism. Our results demonstrate the induction of psbA3 in the absence of PSI electron transport, observed at very low levels of UV-B light, or in the presence of the electron transport inhibitor DCMU (Fig. 3). This argues against a direct involvement of redox signaling in the transcriptional activation of the psbA3 gene under UV-B light. From these data, it is more likely that a specific UV-B-related signal transduction pathway is involved in the induction of psbA3.

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17444  UV-B-induced Transcription of psbA in Cyanobacterium

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