

The *psbA* gene family responds differentially to light and UVB stress in *Gloeobacter violaceus* PCC 7421, a deeply divergent cyanobacterium

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Received 19 June 2007; received in revised form 5 September 2007; accepted 7 September 2007

Available online 20 September 2007

Abstract

Gloeobacter violaceus PCC 7421 is a slow-growing cyanobacterium which lacks thylakoid membranes, but whose five-membered *psbA* gene family encodes three isoform variants of the PsbA (D1) reaction center protein of Photosystem II. Under standard culture conditions *Gloeobacter* exhibits photosystem II electron transport, but several clear modifications in the redox potential of key cofactors bound by the PsbA protein are manifested in the flash-fluorescence characteristics. In other cyanobacteria dynamic expression of multiple *psbA* genes and turnover of PsbA isoforms is critical to counter excitation stress. We found that each of *Gloeobacter*'s five *psbA* genes is expressed, with transcript abundances spanning 4.5 orders of magnitude. *psbAI* (*glr2322*) and *psbAII* (*glr0779*), encoding identical PsbA:2 form proteins, are constitutively expressed and dominate the *psbA* transcript pool under control conditions. *psbAIII* (*glr3144*) was strongly induced under photoinhibitory high irradiance stress, thereby contributing to a large increase in the *psbA* transcript pool that allowed cells to maintain their PsbA protein pools and then recover from irradiance stress, within one cellular generation. In contrast, under comparable photoinhibition provoked by UVB the cells were unable to maintain their *psbA* transcript and PsbA protein pools, and showed limited subsequent recovery. *psbAIV* (*glr1706*) and *psbAV* (*glr2656*), encoding two divergent PsbA isoforms, showed consistent trace expression but were never quantitatively significant contributors to the *psbA* transcript pool. © 2007 Elsevier B.V. All rights reserved.

Keywords: *Gloeobacter*; D1 protein; Light; Photosystem II; *psbA* gene family; UV

1. Introduction

Gloeobacter violaceus PCC 7421, along with the related *Gloeobacter violaceus* PCC 8105 [1,2] show deep molecular [3–5] and structural [6] divergence from other known cyanobacteria, and indeed from all known oxygenic photoautotrophs. Their deep violet colour is imparted by unusual phycobiliproteins [7], which are organized into antenna rods or bundles rather than into typical phycobilisomes [8]. They are unique among known cyanobacteria in lacking thylakoid membranes, and so membrane-based energy metabolism, including photosynthetic and respiratory electron transport and ATP

synthesis, is restricted to the cytoplasmic membrane. Furthermore, this lack of thylakoids is reflected in a membrane lipid composition in *Gloeobacter* distinct from other oxygenic photoautotrophs [9] and some evidence for unusual chemiosmotic ion flows [10].

Although *Gloeobacter violaceus* was originally isolated from carbonate-rich terrestrial habitats in Switzerland [1], the cells tolerate only low to moderate irradiance, at least under standard culture conditions. Even under the best culturing conditions achieved to date the cells grow slowly with generation times of at least 72 h or longer [1,9,11].

Photosystem I in *Gloeobacter* shows unusual functional [11–13] and structural [14] features distinct from Photosystem I in other oxygenic photoautotrophs. More recently, *Gloeobacter* Photosystem II has also been shown to be functionally and structurally distinct [15,16] with decreased water splitting

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activity compared to other Photosystem II complexes. These reaction centre differences, coupled with the lack of thylakoids, may hold important information on the evolution of oxygenic photosynthesis, and on the irradiance sensitivity and slow photosynthetic growth of *Gloeobacter*. The *Gloeobacter* genome of 4,659,019 bp includes a family of five *psbA* genes [16–19] encoding three distinct isoforms of the unstable PsbA protein of Photosystem II, including a *psbAV* gene (*glr2656*) which encodes the most divergent known PsbA isoform sequence from any oxygenic photoautotroph.

Cyanobacteria studied to date demonstrate dynamic regulation of multiple *psbA* genes [20–28] at the transcriptional and post-transcriptional levels [29–31]. This regulated *psbA* expression supports the Photosystem II repair cycle [32] with continual synthesis of PsbA protein to counter the photochemical inactivation of Photosystem II, at rates varying depending on environmental conditions [33,34]. Excess irradiance and UVB can each result in net inactivation of Photosystem II with concomitant photoinhibition [27,35–38].

In *Synechococcus* sp. PCC 7942 [33,39–41] and *Anabaena* sp. PCC 7120 [27] changes in *psbA* gene family expression mediate an exchange of two PsbA protein isoforms. PsbA:1 (D1:1) is characterized by a glutamine at position 130, while the photochemically distinct PsbA:2 (D1:2) [27,42–44] which is induced under excitation stress, is characterized by a glutamate at position 130 which interacts with a key phaeophytin co-factor [45,46]. The exchange of these PsbA isoforms is an important element of short-term acclimation to excitation stress [33,35,39,40,47].

In contrast, in *Synechocystis* sp. PCC 6803 two *psbA* genes encoding identical proteins of the PsbA:1 form show differential expression under excitation stress [24,25,48,49,50]. A third *psbA* gene in *Synechocystis* is intact [51] but encodes a divergent PsbA protein that, when artificially expressed, assembles into PSII with aberrant photochemical properties [52]. This divergent *Synechocystis psbAI* gene is naturally expressed only at trace levels, comparable to a divergent *psbA0* gene from *Anabaena* sp. PCC 7120 [27]. Other cyanobacterial *psbA* gene families have been characterized at the genomic level (<http://www.kazusa.or.jp/cyano/>; http://genome.jgi-psf.org/mic_home.html), but not yet at the level of individual gene-specific *psbA* expression.

The five *psbA* genes of *Gloeobacter* form one of the largest known *psbA* families. *Gloeobacter's* deep evolutionary branching, unusual structure, slow growth and sensitivity to excess irradiance make the functions of its *psbA* genes important elements of the functional diversity of *psbA* gene families in cyanobacteria [27]. We have therefore applied two distinct excitation stresses using visible irradiance and UVB to drive comparable degrees of photoinhibition in *Gloeobacter*. In parallel we tracked the PsbA protein pool and the pool of *psbA* transcripts supporting the PSII repair cycle.

2. Methods

2.1. Culture and treatment conditions

Gloeobacter violaceus PCC 7421 cells were obtained from the Pasteur Culture Collection and cultured in BG-11 growth medium [53] supplemented

with Na₂CO₃ (0.38 mM), 10 mM TES buffer pH 8.2, 0.3% Na thiosulfate at 26 °C under incandescent white growth irradiance of 10 μmol photons m⁻² s⁻¹ photosynthetically active radiation measured with a spherical microquantum sensor (Walz) in the culture. These liquid cultures were used directly for flash-fluorescence measurements, which require only small amounts of biomass. For analyses of transcript responses to UVB and irradiance stress, we used liquid culture to inoculate culture plates made with solid BG-11 supplemented with 1% agar. Growth on plates generated more consistent culture quality than did growth in liquid, where cell aggregation and pigmentation states varied, and allowed us to accumulate sufficient quantities of good quality biomass to allow subsequent extraction of RNA. Typically we treated the plates after 5–6 weeks after inoculation, when the cells on plates, based on pigmentation, apparent growth rate and Fv/Fm were at least as healthy as those we grew in liquid. Depending on the nature of the treatment, cells on plates were exposed to 2.5 μmol photons m⁻² s⁻¹ UVB light, provided from Philips TL 20W/12 RS fluorescent lamps with an emission spectrum between 275 and 380 nm, peak, 310–315 nm (Philips Lighting), in addition to the growth irradiance in the case of UVB treatment or increased visible irradiance, 120 μmol photons m⁻² s⁻¹ photosynthetically active in the case of high irradiance treatment. UVB light was measured by a Skye Instruments cosine corrected hemispheric UVB light sensor (no. SKU430) and a Spectrosensometer (no. RS232; Skye Instruments). Visible irradiance was measured with a cosine corrected hemispheric PAR sensor and associated meter (LiCor). We used cellulose-acetate filters to filter out the UVC component of the lamp spectrum below 280 nm.

2.2. PSII functional measurements

A pulse amplitude modulated fluorometer (Xe-PAM, Walz) was used to monitor the chlorophyll a fluorescence parameter ΦPSII [54] as described in [55].

2.3. Flash-fluorescence measurements

To characterize PSII electron transfer properties in control *Gloeobacter* cells we used flash-induced increase and the subsequent decay of chlorophyll fluorescence yield, measured by a double-modulation fluorometer (PSI Instruments, Brno, Czech Republic) [56] in the 150 μs to 100 s time range. Liquid culture samples were dark adapted for 10 min and measurements were performed as described earlier [38]. *Gloeobacter* showed low flash fluorescence signals at the limit of instrumentation resolution, even in control liquid cultures. After UV or high irradiance treatments the residual PSII fluorescence amplitudes were too small for accurate curve deconvolution. We were technically unable to resolve flash fluorescence signals from cultures on plates. Multi component deconvolution of the curves measured from control cultures was done by using a fitting function with three components, two exponentials and one hyperbolic as described in [38]:

$$F(t) - F_0 = A_1 \exp(-t/T_1) + A_2 \exp(-t/T_2) + A_3 / (1 + t/T_3),$$

where $F(t)$ is the variable fluorescence yield, F_0 is the basic fluorescence level before the flash, A_1 – A_3 are the amplitudes, T_1 – T_3 are the time constants. The non-linear correlation between the fluorescence yield and the redox state of Q_A was corrected for by using the Joliot model [57] with a value of 0.5 for the energy-transfer parameter between PSII subunits.

2.4. PsbA protein determination

PsbA, the D1 core subunit of PSII, was quantified from subsamples of culture taken simultaneously with culture samples used for transcript analyses. The slow growth of *Gloeobacter* limited the quantities of culture and forced us to focus our protein and transcript analyses on the control and 60 min excitation stress treatments. Cells were scraped from agar media under control growth and after 60 min of excitation stress treatment, and then flash frozen and stored at –20 °C until analyzed. Total protein was extracted and analyzed by immunoblotting as in [27]. The PsbA protein was quantified as described fully in [27]. Briefly, total cellular proteins were extracted by suspension of the cell pellet in a denaturing, reducing buffer followed by three rounds of sonication and flash freezing. Cell debris was removed by

centrifugation and the solubilized proteins loaded on electrophoretic gels on an equal chlorophyll base [58]. After electrophoretic blotting, immunodetection was performed using an anti-PsbA antibody (AgriSera, www.agrisera.se) which recognizes a highly conserved region of the PsbA protein whose sequence is conserved across all three PsbA isoforms in *Gloeobacter*. In parallel with the sample protein extracts on each gel, three known loads of quantified PsbA protein standard (Environmental Proteomics, www.environmentalproteomics.ca), were run and subject to immunodetection. These standards were then used for comparative quantitation of the PsbA protein from the *Gloeobacter* samples.

2.5. Transcript analyses

Gloeobacter cells proved difficult to break by methods such as hot Trizol or beating with zirconia beads which gave poor and variable yields of RNA. Total RNA was therefore isolated from cells using a Trizol (Invitrogen) phenol–guanidine–isothiocyanate–chloroform extraction protocol modified from [59]. In order to increase RNA yield we sonicated the samples suspended in Trizol for 10 s, avoiding formation of foam, before continuing with the Trizol extraction procedure. Nevertheless, RNA yields from *Gloeobacter* were low compared to other cyanobacteria extracted using comparable methods [27]. The resulting RNA was dissolved in nuclease free water (Invitrogen DEPC treated water). A treatment with 4 U DNA-ase I (Ambion RiboPure Kit, 2130 Woodward, Austin, TX, USA) was applied to remove traces of genomic DNA from RNA solutions. The concentration of our RNA solutions was estimated from the absorbance at 260 nm, assuming one unit absorbance in water to be equivalent to 40 µg RNA/ml.

First strand cDNA synthesis from 1 µg of purified RNA was performed using the BioRad iScript cDNA Synthesis kit (Bio Rad Laboratories Inc). We used a mixture of the reverse primers (Table 1), in order to create a common initial reverse transcriptase reaction covering all the *psbA* and reference gene transcripts. In order to ensure gene specificity for each transcripts detection we designed the forward primers against the divergent transcribed non-translated 5' regions of the *psbA* genes (Table 1). The primer pairs were thus designed to generate amplicons of similar length (600–650 bases) from each *psbA* transcript pool, which nonetheless varied sufficiently to be differentiated by a melting curve. We obtained high and uniform amplification efficiencies (data not shown).

As reference, a specific primer pair was designed to amplify the constitutive, moderate abundance transcripts from the *gll1392* gene encoding adenylate kinase. The expression level of *gll1392* is within the range of *psbA* transcript levels in control and treated cells.

The primer pairs were tested *in silico* using the Amplify software (B. Engels, 2004, University of Wisconsin) and were compared using BLAST against the *Gloeobacter* genome (<http://www.kazusa.or.jp/cyano>) in order to ensure their gene specificity. The *psbA* primer pairs were also tested by PCR using *Gloeobacter* genomic DNA as a template and the expected specific amplicon fragments were verified through agarose gel electrophoresis (data not shown).

Cell samples taken from pre-treatment control plates and the same plates after 60 min of treatment were used to measure the transcripts from the five *psbA* genes and the *gll1392* reference transcript using Real Time Quantitative PCR on

a BioRad iCycler using BioRad iQ SYBR Green Supermix (BioRad Laboratories Inc) to detect accumulation of double-stranded amplicons. For each RNA sample we performed triplicate RT-RT-Q-PCR determinations for each transcript. At the conclusion of the PCR cycling we performed a melt curve analysis to confirm the presence and specificity of the expected amplicon product. The amplification efficiency of the PCR reaction for each triplicate was estimated using the LineReg program [60] and an average of the three was made and used in the subsequent estimation of the expression levels. Amplification efficiency and the detection response was assessed using dilution series and found to be constant over the range of abundance of our transcripts.

We started with the same amount of RNA for each cDNA preparation, as confirmed by absorbance spectroscopy. As an index of transcript abundance we used:

$$\text{Transcript} \propto 1/E^{CT}$$

Where: CT is the cycle value where the amplicon fluorescence emission reached the detection threshold, and E is the amplification efficiency for the reaction. The parameter $1/E^{CT}$ is proportional to the abundance of the template transcript and accounts for variation in amplification efficiency (E) among runs or among genes.

Values of $1/E^{CT}$ for target transcripts were plotted with control values on X axis and treated values on Y axis. We also estimated the total *psbA* transcript pool under control conditions, $\sum 1/E^{CT}$, as the sum of the parameter $1/E^{CT}$ for each transcript. We then expressed the size of each transcript pool, as a proportion of the total control pool of *psbA* transcript, $(1/E^{CT})/\sum 1/E^{CT}_{\text{control}}$, under both control and treatment conditions, to show changes in the pool size and composition during the treatments.

2.6. Data presentation

All the graphs and statistical calculations were done using Origin 7.5 data analysis software (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. *Gloeobacter* exhibits modified flash fluorescence under regular growth conditions

In dark-adapted samples illumination with a single saturating flash forms Q_A^- , which results in a rapid rise of variable fluorescence. In analyses of *Synechocystis* cells for reference comparison, relaxation of the fluorescence yield after the flash is dominated by a fast component (≈ 660 µs, 61%), which is related to the reoxidation of Q_A^- by Q_B (Table 2; Fig. 1a) [38]. The amplitude of this fast phase is greatly decreased in the presence of the inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) which competitively occupies the Q_B binding site. A middle phase (≈ 7 ms, 23%) arises from Q_A^- reoxidation in those centers which had an empty Q_B site at the time of the flash and so have to bind a PQ molecule from the PQ pool; this middle phase is eliminated in the presence of DCMU. Finally, a slow phase (≈ 9 s, 16%) reflects $Q_A^-Q_B^-$ reoxidation through equilibrium with $Q_A^-Q_B$ via a reverse reaction with the S_2 state [38]. The data summarized in Table 2 show the differences in the relaxation kinetics in *Synechocystis* PCC6803 and *Gloeobacter*. Compared with *Synechocystis*, in *Gloeobacter* the fluorescence relaxation is much slower in the ms time scale components and faster in the seconds time scale. The amplitudes of the fast and middle phases are decreased in *Gloeobacter* ($A_1=41\%$, $A_2=12\%$) as compared with *Synechocystis* ($A_1=61\%$, $A_2=23\%$), but the proportion of the slow phase in the *Gloeobacter* is bigger ($A_3=47\%$) than the respective one in *Synechocystis* (16%) (Fig. 1c).

Table 1
Gene specific PCR primers

Gene	Primer	Sequence (5'–3')
glr2322 (<i>psbAI</i>)	Forward	AGCGTAATCTTCGCTCTGAGG
	Reverse	CTTCCATCGACGTCTCACG
glr0779 (<i>psbAII</i>)	Forward	TGCATTATGGATTGTCAGAACA
	Reverse	CTTCCATCGACGTCTCACG
gll3144 (<i>psbAIII</i>)	Forward	TACATAGACAGTTCTTTACCGGATT
	Reverse	CTTCCATCGACGTCTCACG
glr1706 (<i>psbAIV</i>)	Forward	CTTTTCTCACAGGGTTCGATACA
	Reverse	CGAACTTGTAGCCGTAGTTCTG
glr2656 (<i>psbAV</i>)	Forward	GCCTATCCGGTTGCAACGA
	Reverse	CGAACTTGTAGCCGTAGTTCTG
gll1392 (adenylate kinase)	Forward	CACTCGACGGCTGATGTT
	Reverse	CGAACTCGTCAGTTTCTGC

Table 2

Characteristics of chlorophyll fluorescence yield relaxation in *Gloeobacter* and *Synechocystis* PCC6803 cells^a

Strain	Fast phase: τ , ms (amplitude, %)	Middle phase: τ , ms (amplitude, %)	Slow phase: τ , s (amplitude, %)
No addition ^b			
<i>Synechocystis</i>	0.66±0.03 (61±1)	7±0.6 (23±2)	9.7±0.7 (16±0.2)
<i>Gloeobacter</i>	2.75±0.02 (41±1)	194±0.6 (12±2)	3.75±0.4 (47±0.2)
With DCMU ^c			
<i>Synechocystis</i>	1.1±0.001 (1.6±0.01)	– (0)	0.98±0.02 (98.4±0.03)
<i>Gloeobacter</i>	5±0.03 (8±2)	– (0)	2.25±0.2 (92±0.1)

^a Fluorescence was excited by a single turnover flash and relaxation of the fluorescence yield was measured as in Fig. 1.^b The curves measured on untreated cells were analyzed in terms of two exponential components (fast and middle phases) and one hyperbolic component (slow phase).^c The curves measured in the presence of DCMU were analyzed by assuming exponential decay component for the fast phase and hyperbolic decay for the slow phase.

In *Gloeobacter*, in the absence of DCMU, the fast phase is slower (~2.75 ms) than in *Synechocystis* under the same conditions. This shows that the forward electron transfer between Q_A^- and Q_B is slower in *Gloeobacter* than in *Synechocystis*. In addition, the middle phase is also considerably slower (~194 ms)

than in *Synechocystis* (~7 ms), which indicates that binding of PQ to the Q_B site is affected in the *Gloeobacter* reaction centers.

The slow phase of the fluorescence relaxation, measured in the presence and in the absence of DCMU offer information about the free energy gap between Q_A and Q_B . In *Synechocystis*,

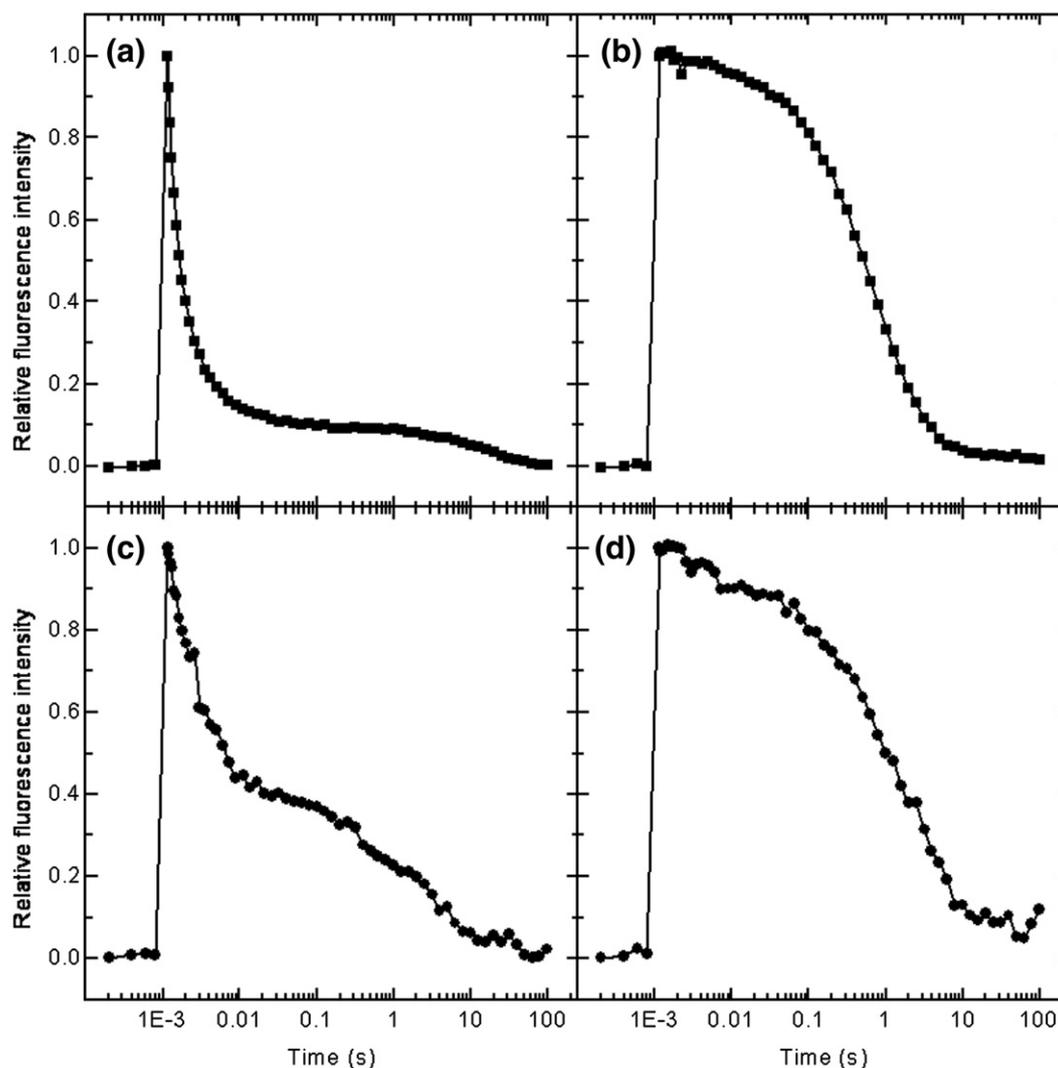


Fig. 1. Flash induced chlorophyll fluorescence in control cultures of *Synechocystis* sp. PCC6803 (squares, panel a) and *Gloeobacter violaceus* PCC 7421 (circles, panel c), and with addition of 10 μ M DCMU (panels b, d). The curves were normalized to the maximum fluorescence amplitude.

the recombination $S_2Q_A^-$ (+DCMU) is 10 times faster than the recombination $S_2Q_B^-$ (no DCMU), which corresponds to $\Delta G=60$ meV between Q_A and Q_B . In *Gloeobacter* the slow phase ratio ($S_2Q_A^-$)/($S_2Q_B^-$) is close to 1.7 which indicates only a 13 meV free energy gap between Q_A and Q_B , which is in agreement with the slower rate of the Q_A to Q_B electron transfer step in *Gloeobacter*.

When fluorescence relaxation is measured in the presence of DCMU (Fig. 1b, d), the reoxidation of Q_A^- occurs via charge recombination with donor side components. In this case, the fluorescence relaxation is dominated by a slow hyperbolic component arising from the recombination of Q_A^- with the S_2 state of the water-oxidizing complex and the time constant is in the order of seconds. The half time of this decay phase is twice as long in *Gloeobacter* (Fig. 1d) than in *Synechocystis* (Fig. 1b and Table 2), which indicates that the $S_2Q_A^-$ charge pair is stabilized by 20 meV in *Gloeobacter* relative to that in *Synechocystis*. In addition, in the presence of DCMU there is a fast phase which arises from $Y_z^+Q_A^-$. This component is also stabilized in *Gloeobacter*.

Taken together, these data indicate that in *Gloeobacter* the acceptor side of PSII is modified which results in slower PQ binding at the Q_B site as well as the decrease of the Q_A to Q_B redox gap due to the shift of the Q_A/Q_A^- and Q_B/Q_B^- redox potentials by about +20 and –30 meV, respectively.

3.2. Progressive Inactivation of PSII function in *Gloeobacter* cells exposed to UVB or high irradiance

To assess the roles of the 5-membered *psbA* gene family of *Gloeobacter* in response to excitation stress we applied two distinct treatments to achieve similar degrees of photoinhibitory

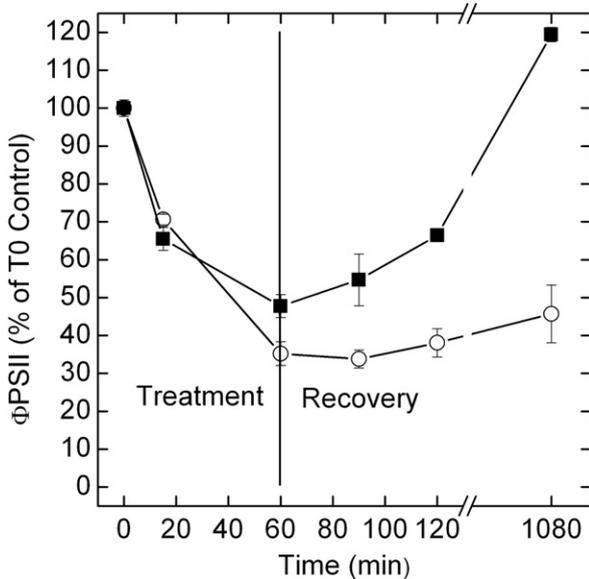


Fig. 2. Loss and recovery of PSII function (Φ PSII) in *Gloeobacter violaceus* PCC 7421 cells exposed to $2.5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ UVB light under continuous visible irradiance at the growth level of $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (open circles) or exposed to $120 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ visible irradiance (solid squares). $N=4$, \pm S.E.M.

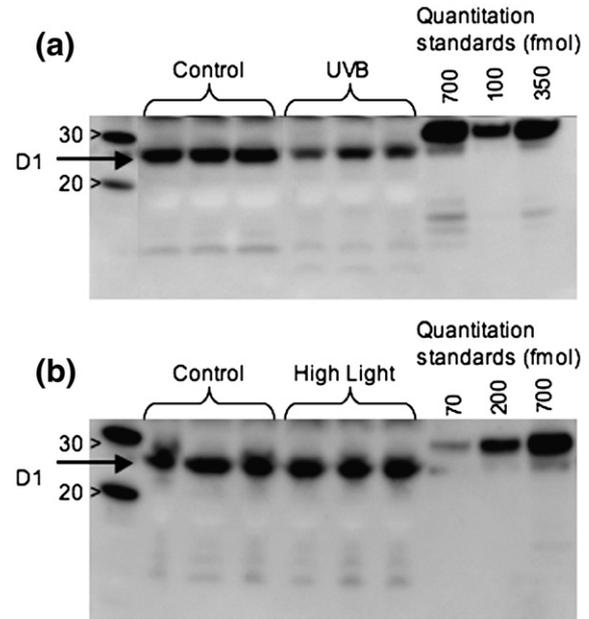


Fig. 3. Change in total PsbA (D1) protein content during UVB and high irradiance exposure of *Gloeobacter violaceus* PCC7421 cells and the subsequent recovery. We detected the total PsbA protein pool using a PsbA Global antibody (www.agrisera.se) on control, 60 min UVB (a) and 60 min high light (b) treated samples. Quantitation of *Gloeobacter* PsbA protein was made using PsbA quantitation standards co-detected on the same immunoblots.

stress (Fig. 2). We grew cells on media plates under low growth irradiance ($10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and then exposed them to a supplemental $2.5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of UVB or to $120 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of visible irradiance for a period of 60 min. The functional effects of these treatments were monitored by measuring the Φ PSII chlorophyll a fluorescence parameter at T0 control, and after 15 and 60 min of treatment (Fig. 2). The subsequent functional recovery was then assessed at 30, 60 and 1020 min after the treatment ended and cells were returned to their previous growth conditions. Over the initial 15 min the two excitation stresses caused similar drops in Φ PSII, but by 60 min the UVB decreased Φ PSII to about 35% of control, while the high irradiance treatment decreased Φ PSII to about 45% of control (Fig. 2). The UVB treated cells then showed only limited capacity to recover and by 1020 min after the end of the UVB stress treatment their Φ PSII reached only 45% of that observed at T0. The high irradiance treated cells showed partial recovery of their Φ PSII function within 120 min of the end of the high irradiance treatment, and then surpassed the T0 control value within 1020 min of the end of the high irradiance treatment (Fig. 2). Note that the generation time for these cells was approximately 72 h (4320 min) and so the observed inhibition and recovery periods were well within the span of cellular generation.

3.3. Quantitation of PsbA content in *Gloeobacter* cells after UVB and high irradiance stress

The total pool of PsbA protein was assessed using a quantitative immunodetection technique on control and samples after 60 min of UVB (Fig. 3a) or high irradiance treatment (Fig.

3b). Following UVB treatment the PsbA protein pool (0.5 pmol PsbA /1000 pmol chl_a) declined to 50% of the level in T0 control cultures (0.95 pmol PsbA /1000 pmol chl_a) (Fig. 3a). In contrast, no significant difference was detected in the size of the PsbA protein pool between the T0 control (1.9 pmol PsbA /1000 pmol chl_a) and the high irradiance treated samples (2.0 pmol PsbA /1000 pmol chl_a) (Fig. 3b), even though these cultures showed nearly as much photoinhibition of ϕ PSII as did the UVB treated cultures (Fig. 2).

3.4. *Gloeobacter psbA* transcripts under control growth, UVB and high irradiance stress

We used gene specific primer pairs and RT-RT-Q-PCR to quantify transcripts from the five *psbA* genes in *Gloeobacter*. Our lower limit of reliable transcript quantitation corresponded to about $1/E^{CT} = 10^{-11}$, because after about 36 or more PCR cycles, amplicon accumulation from residual DNA contamination of the RNA preparations sometimes began to interfere with the quantitation.

We plotted this $1/E^{CT}$ parameter for each *psbA* transcript pool from the control *Gloeobacter* cultures on a log scale (Figs. 4 and 5, X axis) to assess the relative abundance of the transcripts from each *psbA* gene. Transcripts from each *psbA* gene were readily detectable. *glr2322* generated the most *psbA* transcripts under control conditions ($1/E^{CT} = 10^{-5}$), and so we termed it *psbAI*. *glr0779* also generated abundant *psbA* transcripts under

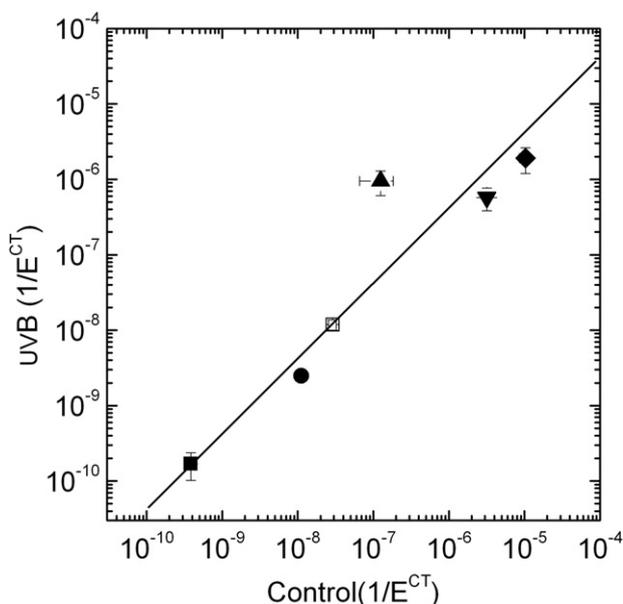


Fig. 4. Control pattern and UVB induced changes in transcripts from the five *psbA* genes of *Gloeobacter violaceus* PCC7421 cells. The transcript level estimations from gene specific RT-RT-Q-PCR ($1/E^{CT}$) were plotted on logarithmic scales with transcript levels from control cultures on the X axis and from 60 min UVB treated cultures on the Y axis. *psbAI* (solid diamond), *psbAII* (solid down triangle), *psbAIII* (solid up triangle), *psbAIV* (solid circle) and *psbAV* (solid square). The reference gene *glr1392* is shown as well (open square). The diagonal drawn through the reference gene represents no specific change in a transcript between the control and UVB treated cultures. Genes that move up from the diagonal are specifically induced by UVB, genes that move down from the diagonal are specifically repressed by high light. $N=4$, \pm S.E.M.

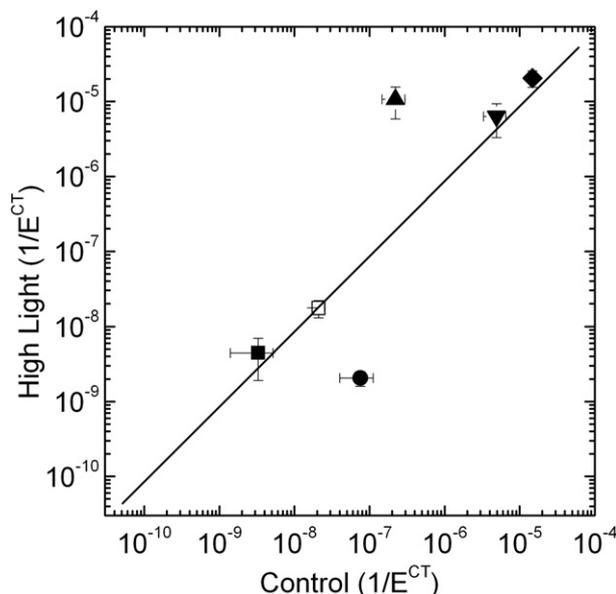


Fig. 5. Control pattern and high-irradiance induced changes in transcripts from the five *psbA* genes of *Gloeobacter violaceus* PCC7421 cells. The transcript level estimations from gene specific RT-RT-Q-PCR ($1/E^{CT}$) were plotted on logarithmic scales with transcript levels from control cultures on the X axis and from 60 min high irradiance treated cultures on the Y axis. *psbAI* (solid diamond), *psbAII* (solid down triangle), *psbAIII* (solid up triangle), *psbAIV* (solid circle) and *psbAV* (solid square). The reference gene *glr1392* is shown as well (open square). The diagonal drawn through the reference gene represents no specific change in a transcript between the control and treated cultures. Genes that move up from the diagonal are specifically induced by high irradiance, genes that move down from the diagonal are specifically repressed by high irradiance. $N=4$, \pm S.E.M.

control conditions ($1/E^{CT} = 5-6 \times 10^{-6}$), and so we termed it *psbAII*. Next were much lower control transcript levels from *glr3144* (termed *psbAIII*; $1/E^{CT} = 1-2 \times 10^{-7}$). *psbAI*, *psbAII* and *psbAIII* encode identical PsbA polypeptides, classed as a PsbA:2 isoform based on their glutamate at position 130. Transcripts from *glr1706* (termed *psbAIV*; $1/E^{CT} = 1-8 \times 10^{-8}$) and *glr2656* (termed *psbAV*; $1/E^{CT} = 5 \times 10^{-10}$ to 5×10^{-9}) were present at trace levels, but still at least 50 fold higher than our lower limit of reliable quantitation ($1/E^{CT} = 10^{-11}$). *psbAIV* and *psbAV* each encode different PsbA proteins, both PsbA:2 isoforms based on their glutamate at position 130 but nonetheless divergent from typical PsbA sequences. In summary, the control transcript abundances from the five *psbA* genes spanned 4.5 orders of magnitude, with *psbAI* and *psbAII* dominating the pool under control conditions.

Fig. 4 (Y axis) plots the changes in transcripts from the five *psbA* genes after 60 min of UVB treatment, while Fig. 5 (Y axis) plots changes in transcripts from the five *psbA* genes after 60 min of high irradiance treatment. In each case, a transcript pool unchanged during the treatment falls along a 1:1 diagonal line. Transcripts specifically induced by the treatment fall above the 1:1 line, while transcripts specifically repressed by the treatment fall below the 1:1 line.

For the UVB experiments the reference gene transcripts showed a small drop in abundance from control to the UVB treated samples, and so we offset the diagonal line to compensate

for this general drop in transcript levels (Fig. 4). UVB treatment caused a moderate specific decrease in both the *psbAI* and *psbAII* transcripts, by about 2.5 to 2.3 fold, respectively. *psbAIII* transcripts, in contrast, accumulated to about 18 fold above their control level. Under UVB the low abundance *psbAIV* transcripts dropped slightly while the trace *psbAV* transcript pool did not change detectably (Fig. 4).

High irradiance stress did not cause significant changes in the levels of the abundant *psbAI* and *psbAII* transcripts, however *psbAIII*, encoding the same PsbA protein sequence, was induced 48 fold within 60 min of high irradiance treatment (Fig. 5), thus going from a minor to a major component of the *psbA* transcript pool. High irradiance also decreased the levels of the low abundance *psbAIV* transcripts (Fig. 5). High irradiance did not significantly alter the levels of the trace *psbAV* transcripts (Fig. 5).

3.5. The *psbA* transcript pool in *Gloeobacter* in control, UVB and high irradiance treated cells

We estimated the total *psbA* transcript pool under control conditions by summing the transcript amounts from the five genes, and then represented the UVB and high irradiance transcript pools as percentages of the control transcript pool (Fig. 6). Under control conditions over 98% of the *psbA* transcript pool was provided by *psbAI* (*glr2322*) and *psbAII* (*glr0779*) (Fig. 6). The other three genes together accounted for only about 2% of the *psbA* transcript pool, with *psbAIII* (*gll3144*) a little over 1%, *psbAIV* (*glr1706*) 0.3% and *psbAV* (*glr2656*) at trace but detectable amounts about 15–20 times lower than *psbAIV* (Fig. 6).

UVB induced a significant decrease in the total transcript pool to only 17% of the control level, mainly because the abundant *psbAI* and *psbAII* transcripts dropped significantly. The *psbAIII* transcript increased under UVB, but not enough to compensate

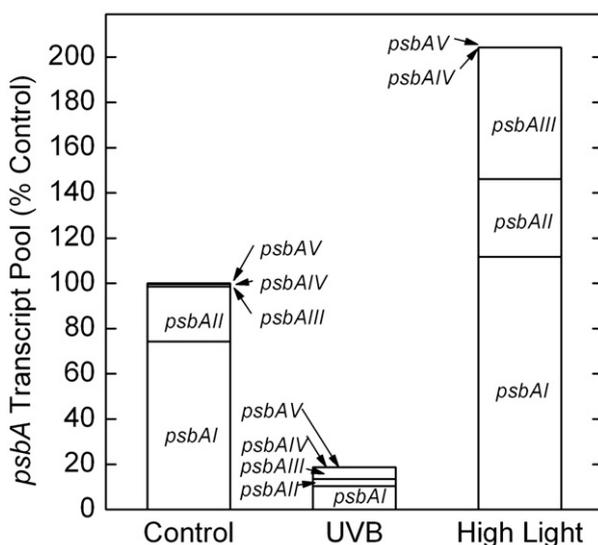


Fig. 6. Changes in the total *psbA* transcript pool and its composition in control, 60 min UVB and 60 min high light treated *Gloeobacter violaceus* PCC 7421 cells. The abundance of each of the five *psbA* genes was estimated relative to the total *psbA* pool in control samples, and summed for the total pool under each condition. $N=4$, \pm S.E.M.

for the drop in *psbAI* and *psbAII* transcripts. There was a small but reproducible drop in the minor *psbAIV* transcript, while the *psbAV* transcript remained at trace levels (Fig. 6).

In marked contrast, high irradiance induced a large increase in the total *psbA* transcript pool (Fig. 5) to 200% of the control transcript pool size, resulting from moderate increases in *psbAI* and *psbAII* transcripts, but more importantly through a large increase in *psbAIII* transcripts, encoding the same PsbA protein, which went from a minor, ca. 1% component of the control transcript pool to a major fraction of the transcript pool under high irradiance stress. As with UVB stress, under high irradiance there was a reproducible drop in the minor *psbAIV* transcript, while *psbAV* remained at trace levels (Fig. 6).

4. Discussion

4.1. PSII electron transport in *Gloeobacter*

The rate of PSII supported oxygen evolution is significantly lower in *Gloeobacter* than in other cyanobacteria [15], which indicates some functional modifications in the PSII complex. Our flash-induced fluorescence yield measurements show that the rates of forward electron transport from Q_A^- to Q_B , as well as of PQ binding to the Q_B site are significantly lower in *Gloeobacter* than in *Synechocystis* 6803. These effects are accompanied by a decrease of the redox gap between Q_A and Q_B relative to that observed in *Synechocystis* 6803, which could lead to an acceptor side limitation of PSII electron transfer at least partly responsible for decreased oxygen evolving activity.

4.2. Expression of the *psbA* genes in *Gloeobacter*: a five member gene family

By using gene specific primers we quantified the individual expression of each of the five *psbA* genes in this cyanobacterium under control and stress conditions. An alignment of the five protein sequences (Fig. 7) shows *psbAI* (*glr2322*), *psbAII* (*glr0779*) and *psbAIII* (*gll3144*) encode the same PsbA protein. All of these proteins have a glutamate at position 130 pointing to a photochemical resemblance to the stress-inducible PsbA:2 proteins found in *Synechococcus* sp. PCC 7942 [39,40,42–46] *Anabaena* sp. PCC 7120 [27] and, based on genomic sequences, in other cyanobacteria.

Interestingly, known eukaryotic chloroplasts contain a glutamate at position 130 and photochemically resemble the stress-inducible PsbA:2 from cyanobacteria [45]. There is no *Gloeobacter* analogue of the PsbA:1 protein isoform characterized by a glutamine at position 130 and found as the excitation-sensitive, constitutively expressed protein in *Synechococcus* sp. PCC 7942 [35,39,40,42–46], *Anabaena* sp. PCC 7120 [27] and *Synechocystis* sp. PCC 6803 [25,49]. Under control conditions the transcripts from *psbAI* and *psbAII* are in vast majority with the other three genes contributing quantitatively insignificant amounts to the total transcript pool. High irradiance induces a significant increase in *psbAIII* transcripts, making it a significant contributor to the increased total transcript pool encoding the constitutive PsbA isoform encoded by these three genes. The

	1		100
psbAI	MTATLERRSSQGLWDRFADWVTS	TNNRFYVGVWFVGLMIPTLLSATICFVVAFAVAAAPPVDMGIREPISGSLLYGNNIITGAVIPSSNAIGLHFYPIWEAA	
psbAII	MTATLERRSSQGLWDRFADWVTS	TNNRFYVGVWFVGLMIPTLLSATICFVVAFAVAAAPPVDMGIREPISGSLLYGNNIITGAVIPSSNAIGLHFYPIWEAA	
psbAIII	MTATLERRSSQGLWDRFADWVTS	TNNRFYVGVWFVGLMIPTLLSATICFVVAFAVAAAPPVDMGIREPISGSLLYGNNIITGAVIPSSNAIGLHFYPIWEAA	
psbAIV	MATILRRRLGNPWEQFANWITS	TDRNRFYIGVWFVGLMVPTLLSATICFVVAFAVAAAPPVDMGIREPISGSLLYGNNIITGAVIPSSNAIGLHFYPIWEAA	
psbAV	MTATLERRQAQGLWDRFADWVTS	THNRFYVGVWFVGLMIPTLLSATICFVIAFAVAAAPPVDMGIREPISGSLLYGNNIITGAVIPSSNAIGLHFYPIWEAA	
	101	130	200
psbAI	SMDEWLYNGGPYQLVVFHFLIGVFCYLGREWELSYRLGLRPWICIAYSAPVAAAAVFLIYPIGQGSFSDGMPLGISGTFNFMFVQAEHNILNHPFHML		
psbAII	SMDEWLYNGGPYQLVVFHFLIGVFCYLGREWELSYRLGLRPWICIAYSAPVAAAAVFLIYPIGQGSFSDGMPLGISGTFNFMFVQAEHNILNHPFHML		
psbAIII	SMDEWLYNGGPYQLVVFHFLIGVFCYLGREWELSYRLGLRPWICIAYSAPVAAAAVFLIYPIGQGSFSDGMPLGISGTFNFMFVQAEHNILNHPFHML		
psbAIV	SMDEWLYNGGPYQLVVFHFLIGVFCYLGREWELSYRLGLRPWICIAYSAPVAAAAVFLIYPIGQGSFSDGMPLGISGTFNFMFVQAEHNILNHPFHML		
psbAV	SMDEWLYNGGPYQLVVFHFLIGVFCYLGREWELSYRLGLRPWICIAYSAPVAAATAVFLVYPIGQGSFSDGMPLGISGTFNFMFVQAEHNILNHPFHML		
	201	Qb binding pocket region	300
psbAI	GVAGVFGGSLFSAMHGSLVTS	SSLIRETSMEESQNYGYKFGQEEETYNIAAHGYFGRLIFQYASF--NNSRSLHFFLAAPVIGIWF TALGISVMAFNLN	
psbAII	GVAGVFGGSLFSAMHGSLVTS	SSLIRETSMEESQNYGYKFGQEEETYNIAAHGYFGRLIFQYASF--NNSRSLHFFLAAPVIGIWF TALGISVMAFNLN	
psbAIII	GVAGVFGGSLFSAMHGSLVTS	SSLIRETSMEESQNYGYKFGQEEETYNIAAHGYFGRLIFQYASF--NNSRSLHFFLAAPVIGIWF TALGISVMAFNLN	
psbAIV	GVAGVFGGSLFAAMHGSLVTS	SLIKATSYEESQNYGYKFGQEEETYNIAAHGYFGRLIFQYASF--TNSRSLHFFLAAPVIGIWF TALGISVMAFNLN	
psbAV	GVAGVFGGSLISAMHGSLVTS	SVSETSYEESQNYGYKFGQEEETYNIAAHGYISRLVQYFAFWGANSRSLHFFLAAPVIGIWF TALGISVMAFNLN	
	301		362
psbAI	GFNFNSSIVDSQGRAIYTWADIVN	NRANLGMVMEHNAHNFPDL LAGTESAPVAVGNADLNG	
psbAII	GFNFNSSIVDSQGRAIYTWADIVN	NRANLGMVMEHNAHNFPDL LAGTESAPVAVGNADLNG	
psbAIII	GFNFNSSIVDSQGRAIYTWADIVN	NRANLGMVMEHNAHNFPDL LAGTESAPVAVGNADLNG	
psbAIV	GFNFNASITDNQGRITWADIVN	NRANLGEVMEHNAHNFPDL LAGTESAPVAVFAALGDG	
psbAV	GFNFNSSIVDSQGRAIYTWADIVN	NRANLGMVMEHNAHNFPDL LAGTESAPVAVSTAKVGG	

Fig. 7. Amino acid sequence alignment of the five *PsbA/D1* polypeptide sequences predicted from the *psbA* gene family from *Gloeobacter violaceus* PCC 7421. *psbAI* (*glr2322*), *psbAII* (*glr0779*) and *psbAIII* (*glr3144*) encode the same *PsbA* protein. *psbAIV* (*glr1706*) encodes a distinct *PsbA* isoform, while *psbAV* (*glr2656*) encodes the most deeply branching of all known *PsbA* proteins, with a two amino acid insertion at position 265. Note that all five polypeptides have a glutamate at position 130, like the *PsbA* proteins of eukaryotes and the excitation-inducible forms found in other cyanobacteria.

Gloeobacter pattern of *psbA* induction under irradiance stress thus parallels that of *Synechocystis* sp. PCC6803 where two *psbA* genes encode a single *PsbA* form whose production is enhanced by stress conditions [25,49]. The constitutively expressed *PsbA* in *Gloeobacter*, however, has a glutamate at position 130, rather than the glutamine at position 130 found in *psbA* genes from *Synechocystis*. Amino acid substitutions between the *Synechocystis psbAII/III* genes and the *Gloeobacter psbAI/II/III* genes at positions 228–230, within the Q_B binding pocket [61], could contribute to the distinct plastoquinone binding properties of the *Gloeobacter* PSII in comparison to the more typical *Synechocystis* pattern.

The *psbAIV* (*glr1706*) and *psbAV* (*glr2656*) genes of *Gloeobacter* are intact and their expression is readily detectable, indeed *psbAIV* shows reproducible down regulation under both UVB and high irradiance stress. Yet they have divergent *PsbA* coding regions; *psbAIV* encodes *PsbAIV*, with amino acid substitutions near or within the region of *PsbA* forming the Q_B binding pocket [61], while *psbAV* encodes *PsbAV*, the most deeply branching of all known *PsbA* sequences, with amino acid substitutions and an unusual 2 amino acid insertion sequence, also within the Q_B binding pocket [61]. These divergent transcripts make quantitatively insignificant contributions to the bulk *psbA* transcript pool under the control, UVB stress and high irradiance stress conditions we tested. This suggests these transcripts have a role other than as core participants in the PSII repair cycle. Interestingly we recently found that both *Synechocystis* sp. PCC 6803 and *Anabaena* sp PCC 7120 [27] also contain one divergent but intact *psbA* gene which is constitutively expressed at trace levels, well above background. A similar gene is also present in the genome of *Nostoc punctiforme* PCC 73102 (<http://genome.jgi-psf.org>). The presence of these trace, divergent *psbA* transcripts across such a wide range of the cyanobacterial diversity suggests that trace expression of divergent *PsbA* proteins has an as yet unknown functional role aside from supporting bulk PSII photochemistry.

4.3. *Gloeobacter* cells induce *psbA* expression to counter high irradiance but not UVB photoinhibition

Gloeobacter cells exposed to increased visible irradiance showed a high initial rate of PSII function loss which became slower in the later phases of irradiance treatment. As soon as the high irradiance stress stopped PSII function recovered as newly synthesized *PsbA* proteins replaced the photoinactivated *PsbA* in the inactive centers at a rate much faster than the slow cellular growth rate. Therefore, *Gloeobacter* has a functional PSII repair cycle to counter PSII photoinactivation by visible irradiance. Other cyanobacteria usually repair PSII damage induced by visible irradiance and moderate UV-B via the same mechanism [35,37,27]. *Gloeobacter*, however, lacks the capacity to efficiently repair PSII centers once they are damaged by UVB, although PSII repair works efficiently after photodamage by visible irradiance. During the initial 15 min of treatment UVB impaired slightly fewer centers compared to high irradiance but by 60 min of treatment the UVB treated sample lost more functional PSII centers. During the recovery period, the UVB-induced loss of PSII function ceased, but PSII function only slowly recovered at a rate comparable to that of slow cell division rather than through repair of impaired PSII centers.

The cells lost about 50% of their *PsbA* protein following 60 min of UVB treatment, but showed no *PsbA* protein loss in case of high irradiance exposure, even though the degree of PSII inactivation was similar under the two excitation stresses. Therefore, under UVB, *Gloeobacter* has the ability to efficiently remove impaired *PsbA* protein from PSII centers, but lacks the capacity to replace the *PsbA* proteins.

High irradiance photoinhibition resulted in a large increase in the *psbA* transcript pool, particularly through a large increase in transcripts from *psbAIII* encoding a *PsbA* protein identical to that encoded by the dominant constitutive transcripts from *psbAI* and *psbAII*. This increased *psbA* transcript pool allowed *Gloeobacter* to limit the loss of *PsbA* protein under high

irradiance photoinhibition and then recover rapidly after the end of the high irradiance stress. In contrast, under UVB the *psbA* transcript pool dropped, since the limited induction of *psbAIII* was insufficient to counter drops in the dominant constitutive transcripts from *psbAI* and *psbAII*. With a depleted *psbA* transcript pool the cells had limited capacity to recover their PSII activity after the UVB treatment ended. We suspect that UVB inhibits the overall transcriptional process in *Gloeobacter* because our reference transcripts dropped somewhat under UVB treatment, and the *psbA* transcript pool dropped even more, in spite of a qualitative pattern of induction of *psbAIII* which was similar to the high irradiance stress response.

5. Conclusions

In spite of slow growth and susceptibility to high irradiance stress *Gloeobacter* has an active PSII repair cycle supported by dynamic regulation of at least three *psbA* genes and capacity for rapid clearance of photoinactivated PsbA protein. The cells however suffer susceptibility to UVB due to inhibition of the transcript generation essential to support the PSII repair process. The resulting Photosystem II, while functional, shows altered cofactor redox potentials for the plastoquinone co-factors, which impose slower electron transfers in comparison to the *Synechocystis* Photosystem II.

Acknowledgments

This study was supported by an NSERC of Canada Discovery grant, CRC and CFI funding (DAC), a NATO Post-Doctoral Fellowship (CIS), an NSERC IPS and New Brunswick Innovation Foundation RAI (CMB). We thank Laurel McIntyre for expert maintenance of the *Gloeobacter* cultures. Global anti-PsbA antibody was a kind gift of Agriser (www.agriser.se); PsbA protein quantitation standard was a kind gift of Environmental Proteomics (www.environmentalproteomics.ca).

References

- [1] R. Rippka, J. Waterbury, G. Cohen-Bazire, A cyanobacterium which lacks thylakoids, *Arch. Microbiol.* 100 (1974) 419–436.
- [2] R. Rippka, H. Herdman, Catalogue of Strains, Pasteur culture collection of cyanobacterial strains in axenic culture, Institut Pasteur Paris, 1992/1993, p. 14.
- [3] B. Nelissen, Y. Vandepeer, A. Wilmotte, R. Dewachter, An early origin of plastids within the cyanobacterial divergence is suggested by evolutionary trees based on complete 16 s ribosomal-RNA sequences, *Mol. Biol. Evol.* 12 (1995) 1166–1173.
- [4] S. Turner, K.M. Pryer, V.P.W. Miao, J.D. Palmer, Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis, *J. Eukaryot. Microbiol.* 46 (1999) 327–338.
- [5] A. Wilmotte, Molecular evolution and taxonomy of the cyanobacteria, in: Kluwer (Ed.), *The Molecular Biology of Cyanobacteria*, D.A. Bryant, Dordrecht, 1994, pp. 1–25.
- [6] S. Schneider, U.J. Jürgens, Cell-wall and sheath constituents of the cyanobacterium *Gloeobacter violaceus*, *Arch. Microbiol.* 156 (1991) 312–318.
- [7] D.A. Bryant, G. Cohen-Bazire, A.N. Glazer, Characterization of the biliproteins of *Gloeobacter violaceus*. Chromophore content of a cyanobacterial phycoerythrin carrying phycourobilin chromophore, *Arch. Microbiol.* 129 (1981) 190–198.
- [8] G. Guglielmi, G. Cohen-Bazire, D.A. Bryant, The structure of *Gloeobacter violaceus* and its phycobilisomes, *Arch. Microbiol.* 129 (1981) 181–189.
- [9] E. Selstam, D. Campbell, Membrane lipid composition of the unusual cyanobacterium *Gloeobacter violaceus* sp. PCC 7421, which lacks sulfoquinovosyl diacylglycerol, *Arch. Microbiol.* 166 (1996) 132–135.
- [10] I. Brown, Is H⁺ the unique ion for photosynthesis energy transduction? *Photosynth. Res. Suppl.* 1 (1995) 10–13.
- [11] F. Koenig, M. Schmidt, *Gloeobacter violaceus* — investigation of an unusual photosynthetic apparatus. Absence of the long wavelength emission photosystem I in 77K fluorescence spectra, *Physiol. Plant.* 94 (1995) 621–628.
- [12] D. Mangels, J. Kruip, S. Berry, M. Rogner, E.J. Boekema, F. Koenig, Photosystem I from the unusual cyanobacterium *Gloeobacter violaceus*, *Photosynth. Res.* 72 (2002) 307–319.
- [13] M. Mimuro, T. Ookubo, D. Takahashi, T. Sakawa, S. Akimoto, I. Yamazaki, H. Miyashita, Unique fluorescence properties of a cyanobacterium *Gloeobacter violaceus* PCC 7421: reasons for absence of the long-wavelength PSI Chl a fluorescence at –196 degrees C, *Plant Cell Physiol.* 43 (2002) 587–594.
- [14] H. Inoue, T. Tsuchiya, S. Satoh, H. Miyashita, T. Kaneko, S. Tabata, A. Tanaka, M. Mimuro, Unique constitution of photosystem I with a novel subunit in the cyanobacterium *Gloeobacter violaceus* PCC 7421, *FEBS Lett.* 578 (2004) 275–279.
- [15] M. Mimuro, T. Sakawa, S. Akimoto, I. Yamazaki, M. Akiyama, M. Kobayashi, H. Miyashita, Photosynthetic properties of a cyanobacterium, *Gloeobacter violaceus* PCC7421, Proceedings of the 12th International Congress on Photosynthesis Brisbane, 2001, pp. S9–S010.
- [16] T. Nguyen, R. van Willigen, G.C. Dismukes, Genome-wide analysis of the PSII reaction center D1 protein: Is *Gloeobacter violaceus* the missing link in evolution of oxygenic photosynthesis? 13th International Congress of Photosynthesis Montreal, 2004, (P7B-4 - Abstract number: 582.).
- [17] T. Kaneko, Y. Nakamura, S. Sato, M. Mimuro, H. Miyashita, T. Tsuchiya, S. Tabata, Complete genome structure of the unicellular cyanobacterium *Gloeobacter violaceus* PCC 7421, *Plant Cell Physiol.* 45 (2004) S129.
- [18] Y. Nakamura, T. Kaneko, S. Sato, M. Mimuro, H. Miyashita, T. Tsuchiya, S. Sasamoto, A. Watanabe, K. Kawashima, Y. Kishida, C. Kiyokawa, M. Kohara, M. Matsumoto, A. Matsuno, N. Nakazaki, S. Shimpo, C. Takeuchi, M. Yamada, S. Tabata, Complete genome structure of *Gloeobacter violaceus* PCC 7421, a cyanobacterium that lacks thylakoids, *DNA Res.* 10 (2003) 137–145.
- [19] Y. Nakamura, T. Kaneko, S. Sato, M. Mimuro, H. Miyashita, T. Tsuchiya, S. Sasamoto, A. Watanabe, K. Kawashima, Y. Kishida, C. Kiyokawa, M. Kohara, M. Matsumoto, A. Matsuno, N. Nakazaki, S. Shimpo, C. Takeuchi, M. Yamada, S. Tabata, Complete genome structure of *Gloeobacter violaceus* PCC 7421, a cyanobacterium that lacks thylakoids, *DNA Res.* 10 (2003) 181–201.
- [20] S.A. Bustos, M.R. Schaefer, S.S. Golden, Different and rapid responses of four cyanobacterial *psbA* transcripts to changes in the light intensity, *J. Bacteriol.* 172 (1990) 1998–2004.
- [21] D. Campbell, A.K. Clarke, P. Gustafsson, G. Öquist, Oxygen-dependent electron flow influences photosystem II function and *psbA* gene expression in the cyanobacterium *Synechococcus* sp. PCC 7942, *Physiol. Plant.* 105 (1999) 746–755.
- [22] A.K. Clarke, D. Campbell, P. Gustafsson, G. Öquist, Dynamic responses of photosystem II and phycobilisomes to changing light in the cyanobacterium *Synechococcus* sp. PCC 7942, *Planta* 197 (1995) 553–562.
- [23] S.S. Golden, J. Brusslan, R. Haselkorn, Expression of a family of *psbA* genes encoding a photosystem II polypeptide in the cyanobacterium *Anacystis nidulans* R-2, *EMBO J.* 5 (1986) 2789–2798.
- [24] A. Mohamed, C. Jansson, Influence of light on accumulation of photosynthesis specific transcripts in the cyanobacterium *Synechocystis* sp. PCC 6803, *Plant Mol. Biol.* 16 (1989) 891–897.
- [25] A. Mohamed, J. Eriksson, H.D. Osiewacz, C. Jansson, Differential expression of the *psbA* genes in the cyanobacterium *Synechocystis* 6803, *Mol. Gen. Genet.* 238 (1993) 161–168.

- [26] M.R. Schaefer, S.S. Golden, Differential expression of members of a cyanobacterial *psbA* gene family in response to light, *J. Bacteriol.* 171 (1989) 3973–3981.
- [27] C.I. Sicora, S.E. Appleton, C.M. Brown, J. Chung, J. Chandler, A.M. Cockshutt, I. Vass, D.A. Campbell, Cyanobacterial *psbA* families in *Anabaena* and *Synechocystis* encode trace, constitutive and UVB-induced D1 isoforms, *Biochim. Biophys. Acta* 1757 (2006) 47–56.
- [28] N.F. Tsinoremas, M.R. Schaefer, S.S. Golden, Blue and red-light reversibly control *psbA* expression in the cyanobacterium *Synechococcus* sp. strain PCC 7942, *J. Biol. Chem.* 269 (1994) 16143–16147.
- [29] J. Komenda, M. Koblizek, J. Masojidek, The regulatory role of photosystem II photoinactivation and *de novo* protein synthesis in the degradation and exchange of two forms of the D1 protein in the cyanobacterium *Synechococcus* PCC 7942, *J. Photochem. Photobiol.* 48 (1999) 114–119.
- [30] R.D. Kulkarni, M.R. Schaefer, S.S. Golden, Transcriptional and posttranscriptional components of *psbA* response to high light-intensity in *Synechococcus* sp. strain PCC 7942, *J. Bacteriol.* 174 (1992) 3775–3781.
- [31] K. Sippola, E.M. Aro, Expression of *psbA* genes is regulated at multiple levels in the cyanobacterium *Synechococcus* sp PCC 7942, *Photochem. Photobiol.* 71 (2000) 706–714.
- [32] E.-M. Aro, I. Virgin, B. Andersson, Photoinhibition of photosystem II. Inactivation, protein damage and turnover, *Biochim. Biophys. Acta* 1143 (1993) 113–134.
- [33] D. Campbell, G.Q. Zhou, P. Gustafsson, G. Öquist, A.K. Clarke, Electron-transport regulates exchange of 2 forms of photosystem-II D1 protein in the cyanobacterium *Synechococcus*, *EMBO J.* 14 (1995) 5457–5466.
- [34] K. Sippola, E. Kanervo, N. Murata, E.M. Aro, A genetically engineered increase in fatty acid unsaturation in *Synechococcus* sp. PCC 7942 allows exchange of D1 protein forms and sustenance of photosystem II activity at low temperature, *Eur. J. Biochem.* 251 (1998) 641–648.
- [35] D. Campbell, M.J. Eriksson, G. Öquist, P. Gustafsson, A.K. Clarke, The cyanobacterium *Synechococcus* resists UV-B by exchanging photosystem II reaction centre D1 proteins, *Proc. Natl. Acad. Sci. U. S. A.* 95 (1998) 364–369.
- [36] T.M. MacDonald, L. Dubois, L.C. Smith, D.A. Campbell, Sensitivity of cyanobacterial antenna, reaction center and CO₂ assimilation transcripts and proteins to moderate UVB: light acclimation potentiates resistance to UVB, *Photochem. Photobiol.* 77 (2003) 405–412.
- [37] C. Sicora, Z. Mate, I. Vass, The interaction of visible and UV-B light during photodamage and repair of photosystem II, *Photosynth. Res.* 75 (2003) 127–137.
- [38] I. Vass, D. Kirilovsky, A.-L. Etienne, UV-B radiation- induced donor- and acceptor-side modifications of photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803, *Biochemistry* 38 (1999) 12786–12794.
- [39] A.K. Clarke, V.M. Hurry, P. Gustafsson, G. Öquist, Two functionally distinct forms of the photosystem II reaction-center protein D1 in the cyanobacterium *Synechococcus* sp. PCC 7942, *Proc. Natl. Acad. Sci. U. S. A.* 90 (1993) 11985–11989.
- [40] A.K. Clarke, A. Soitamo, P. Gustafsson, G. Öquist, Rapid interchange between two distinct forms of cyanobacterial photosystem II reaction-center protein D1 in response to photoinhibition, *Proc. Natl. Acad. Sci. U. S. A.* 90 (1993) 9973–9997.
- [41] R.D. Kulkarni, S.S. Golden, Form II of D1 is important during transition from standard to high light intensity in *Synechococcus* sp strain PCC 7942, *Photosynth. Res.* 46 (1995) 435–443.
- [42] D. Campbell, D. Bruce, C. Carpenter, P. Gustafsson, G. Öquist, Two forms of the photosystem II D1 protein alter energy dissipation and state transitions in the cyanobacterium *Synechococcus* sp. PCC 7942, *Photosynth. Res.* 47 (1996) 131–144.
- [43] P.V. Sane, A.G. Ivanov, D. Sveshnikov, N.P.A. Huner, G. Öquist, A transient exchange of the photosystem II reaction center protein D1:1 with D1:2 during low temperature stress of *Synechococcus* sp. PCC 7942 in the light lowers the redox potential of Q (B), *J. Biol. Chem.* 277 (2002) 32739–32745.
- [44] M. Tichy, L. Lupinkova, C. Sicora, I. Vass, S. Kuvikova, O. Prasil, J. Komenda, *Synechocystis* 6803 mutants expressing distinct forms of the photosystem II D1 protein from *Synechococcus* 7942: relationship between the *psbA* coding region and sensitivity to visible and UV-B radiation, *Biochim. Biophys. Acta* 1605 (2003) 55–66.
- [45] P.J. Nixon, M. Rogner, B.A. Diner, Expression of a higher plant *psbA* gene in *Synechocystis* 6803 yields a functional hybrid photosystem-II reaction center complex, *Plant Cell* 3 (1991) 383–395.
- [46] F. Rappaport, M. Guergova-Kuras, P.J. Nixon, B.A. Diner, J. Lavergne, Kinetics and pathways of charge recombination in photosystem-II, *Biochemistry* 41 (2002) 8518–8527.
- [47] J. Porankiewicz, E. Selstam, D. Campbell, G. Öquist, Membrane lipid unsaturation and recovery of photosynthesis during low temperature acclimation in *Synechococcus* sp. PCC 7942, *Physiol. Plant.* 104 (1998) 405–412.
- [48] L. Huang, M.P. McCluskey, H. Ni, R.A. LaRossa, Global gene expression profiles of the cyanobacterium *Synechocystis* sp strain PCC 6803 in response to irradiation with UV-B and white light, *J. Bacteriol.* 184 (2002) 6845–6858.
- [49] Z. Mate, L. Sass, M. Szekeres, I. Vass, F. Nagy, UV-B induced differential transcription of *psbA* genes encoding the D1 protein of photosystem II in the cyanobacterium *Synechocystis* PCC 6803, *J. Biol. Chem.* 273 (1998) 17439–17444.
- [50] T. Tyystjarvi, I. Tuominen, M. Herranen, E.M. Aro, E. Tyystjarvi, Action spectrum of *psbA* gene transcription is similar to that of photoinhibition in *Synechocystis* sp PCC 6803, *FEBS Lett.* 516 (2002) 167–171.
- [51] G.F. Salih, C. Jansson, Activation of the silent *psbAI* gene in the cyanobacterium *Synechocystis* sp. strain 6803 produces a novel and functional D1 protein, *Plant Cell* 9 (1997) 869–878.
- [52] C. Sicora, R. Wiklund, C. Jansson, I. Vass, Charge stabilization and recombination in photosystem II containing the D1' protein product of the *psbAI* gene in *Synechocystis* 6803, *Phys. Chem. Chem. Phys.* 6 (2004) 4832–4837.
- [53] R. Rippka, J. Deereelles, J.B. Waterbury, M. Herdman, R.Y. Stanier, Generic assignments, strain histories and properties of pure cultures of cyanobacteria, *J. Gen. Microbiol.* 111 (1979) 1–61.
- [54] B. Genty, J.M. Briantais, N.R. Baker, The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence, *Biochim. Biophys. Acta* 990 (1989) 87–92.
- [55] D. Campbell, V.M. Hurry, A.K. Clarke, P. Gustafsson, G. Öquist, Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation, *Microbiol. Mol. Biol. Rev.* 62 (1998) 667–683.
- [56] M. Trtilek, D.M. Kramer, M. Koblizek, L. Nedbal, Dual-modulation LED kinetic fluorometer, *J. Lumin.* 72–74 (1997) 597–599.
- [57] A. Joliot, P. Joliot, Etude cinétique de la réaction photochimique libérant l'oxygène au cours de la photosynthèse, *C. R. Acad. Sci.* 258 (1964) 4622–4625.
- [58] J.D. Barnes, L. Balaguer, E. Manrique, A reappraisal of the use of DMSO for the extraction and determination of chlorophylls-A and chlorophylls-B in lichens and higher plants, *Environ. Exp. Bot.* 32 (1992) 85–100.
- [59] P.J. McGinn, G.D. Price, R. Maleszka, M.R. Badger, Inorganic carbon limitation and light control the expression of transcripts related to the CO₂-concentrating mechanism in the cyanobacterium *Synechocystis* sp. strain PCC 6803, *Plant Physiol.* 132 (2003) 218–229.
- [60] C.J. Ramakers, M. Ruijter, R.H.L. Deprez, A.F.M. Moorman, Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data, *Neurosci. Lett.* 339 (2003) 62–66.
- [61] J. Kern, B. Loll, A. Zouni, W. Saenger, K.D. Irrgang, J. Biesiadka, Cyanobacterial Photosystem II at 3.2 Å resolution — the plastoquinone binding pockets, *Photosynth. Res.* 84 (2005) 153–159.