Photosynth Res (2007) 94:265–274
DOI 10.1007/s11120-007-9237-2

REGULAR PAPER

Global gene expression of a ΔPsbO:ΔPsbU mutant and a spontaneous revertant in the cyanobacterium *Synechocystis* sp. strain PCC 6803

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Received: 4 July 2007 / Accepted: 25 August 2007 / Published online: 8 November 2007

Abstract The photosystem II (PSII) double mutant ΔPsbO:ΔPsbU was unable to grow photoautotrophically at pH 7.5, but growth was restored at pH 10. We have isolated a pseudorevertant of this strain, which exhibited photoautotrophic growth at pH 7.5. PSII-specific oxygen evolution and assembled PSII centers in the pseudorevertant and the original ΔPsbO:ΔPsbU strains were similar at pH 7.5. Comparison of global gene expression of the two strains at pH 7.5 revealed that <4% of genes differed. In the pseudorevertant, up-regulated transcripts included stress-responsive genes, many of which were shown previously to be under the control of Hik34. Elevated transcripts included those encoding heat shock proteins (HspA, DnaK2 and HtpG), two Deg proteases (DegP and DegQ), and the orange carotenoid protein (OCP, Slr1963). Up-regulated genes encoded proteins localized to different cell compartments, including the thylakoid, plasma and outer membranes. We suggest that the cell wide up-regulation of stress response genes in the pseudorevertant may limit the impact of PSII instability that is observed in the ΔPsbO:ΔPsbU strain. Futhermore, the OCP has a photoprotective role mediating phycobilisome-associated nonphotochemical quenching, such that increased OCP levels in the pseudorevertant may reduce photons reaching these impaired centers. These two responses, in combination with uncharacterized stress responses, are sufficient to permit the growth of pseudorevertant at pH 7.5.

Keywords Cyanobacteria · DNA microarray · Oxygen-evolving complex · Histidine kinase · pH · Photosystem II · PsbO · PsbU · Stress response · *Synechocystis*

Abbreviations

OEC	Oxygen-evolving complex
PSII	Photosystem II
Hik	Histidine kinase
ROS	Reactive oxygen species

Introduction

High resolution X-ray crystallographic structures of photosystem II (PSII) from the cyanobacterium *Thermosynechococcus elongatus* have confirmed the association of PsbO, PsbU and PsbV with the lumenal face of the oxygen-evolving complex (OEC) (Ferreira et al. 2004; Loll et al. 2005). In cyanobacteria, PsbO can be deleted, although the PSII centers in ΔPsbO strains are more susceptible to photoinactivation (Bockholt et al. 1991; Burnap and Sherman 1991; Mayes et al. 1991; Philbrick et al. 1991). A similar result was obtained in strains lacking PsbV (Morgan et al. 1998; Shen et al. 1998). The ΔPsbO and ΔPsbV strains also exhibit a strict dependence on Ca^{2+} and Cl^- for photoautotrophic growth and the stability, as well as the activity, of the Mn_4Ca cluster of the OEC is perturbed (Burnap et al. 1996; Z. Li et al. 2004).

Electronic supplementary material The online version of this article (doi:10.1007/s11120-007-9237-2) contains supplementary material, which is available to authorized users.

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Removal of the PsbU subunit also increases the susceptibility of PSII to photoinactivation (Clarke and Eaton-Rye 1999; Inoue-Kashino et al. 2005). However, the requirement for PsbU is less stringent than that observed for the removal of PsbO or PsbV. Nevertheless, the absence of PsbU resulted in slowed growth under conditions where Ca^{2+} or Cl^{-} was limiting (Shen et al. 1997), whereas photoautotrophic growth was abolished in $CaCl_2$-limiting conditions (Inoue-Kashino et al. 2005; Summerfield et al. 2005a). Moreover, in the ΔPsbU mutant, O_2 evolution was reduced by 20% when compared to wild type (Clarke and Eaton-Rye 1999; Kimura et al. 2002). Removal of PsbU has also been shown to affect both energy transfer and electron transport in the phycobilisome/PSII assembly (Veerman et al. 2005), and a *Synechococcus* sp. PCC 7942 mutant lacking PsbU exhibited elevated resistance to oxidative stress (Balint et al. 2006). Additionally, the PsbO, PsbU, and PsbV proteins are required for thermal stability of PSII and for the development of cellular thermotolerance (Nishiyama et al. 1999; Kimura et al. 2002). Proteins with sequence similarity to the PsbP and PsbQ lumenal proteins of plants are also associated with *Synechocystis* sp. strain PCC 6803 (hereafter *Synechocystis*) PSII centers, but are absent from the crystal structures (Kashino et al. 2002). These proteins are not essential for growth and appear to be involved in optimizing PSII activity (Thornton et al. 2004; Ishikawa et al. 2005; Summerfield et al. 2005a, b; Roose et al. 2007).

Mutants lacking any one of the extrinsic proteins were able to grow photoautotrophically. The removal of PsbV resulted in the concomitant loss of PsbU such that the phenotypes of the ΔPsbV and ΔPsbV:ΔPsbU strains were indistinguishable (Shen and Inoue 1993; Eaton-Rye et al. 2003). However, the mutant lacking both PsbO and PsbV is an obligate photoheterotroph (Shen et al. 1995). In *Synechocystis*, PsbU retains a functional association with PSII in the absence of PsbO, since the ΔPsbO:ΔPsbU mutant exhibited impaired photoautotrophic growth and O_2 evolution when compared with the ΔPsbO and ΔPsbU single mutants. In addition, the ΔPsbO:ΔPsbU mutant is an obligate photoheterotroph at pH 7.5. Furthermore, the ΔPsbO:ΔPsbU strain was found to have a similar number of assembled PSII centers as the ΔPsbO mutant and it was subsequently discovered that photoautotropic growth of this strain was possible at pH 10.0 (Eaton-Rye et al. 2003). To investigate this alkaline pH-induced recovery, we isolated a pseudorevertant strain that lacked both PsbO and PsbU, but which was able to grow photoautotrophically at pH 7.5. In this report, we compare the global gene expression at pH 7.5 between the ΔPsbO:ΔPsbU mutant and pseudorevertant using a full-genome DNA microarray of *Synechocystis*.

Materials and methods

Bacterial strains and growth conditions

The glucose tolerant strain of *Synechocystis* was used throughout this study (Williams 1988). Cultures were maintained on BG-11 plates containing 5 mM glucose, 20 μM atrazine and buffered with 10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES)–NaOH (pH 8.2) and 0.3% sodium thiosulfate. Liquid cultures were grown mixotrophically in unbuffered BG-11 with 5 mM glucose and shaking at 125 rpm, at 30 ± 2°C, using cool white fluorescent light at an intensity of ~30 μE m^{-2} s^{-1}, unless otherwise stated. The antibiotic concentrations used for the mutant strains were 25 μg/ml spectinomycin and 15 μg/ml chloramphenicol.

The ΔPsbO:ΔPsbU strain was constructed as described in Eaton-Rye et al. (2003). In order to generate a pseudorevertant strain, the ΔPsbO:ΔPsbU mutant was used to inoculate BG-11 medium at pH 7.5 containing 25 μg/ml spectinomycin and 15 μg/ml chloramphenicol. At this pH, the ΔPsbO:ΔPsbU strain could not grow photoautotrophically; however, the inoculated cells remained in a non-growing phase. After 150 h, the cells were harvested and resuspended in fresh BG-11 at pH 7.5, with appropriate antibiotics. Growth of cells was observed following ~100 h incubation in the fresh medium and these cells were plated on BG-11 and a single colony from one plate was used to inoculate a fresh liquid culture which was grown up and used for subsequent characterization. PCR was used to confirm the ΔPsbO:ΔPsbU genotype of the isolated pseudorevertant. The cell density of the cultures was determined by optical density at 750 nm, as previously described (Colon-Lopez et al. 1997; Meunier et al. 1997).

Physiological characterization

Oxygen evolution was measured at 30°C using a Clark-type electrode (Hansatech) in buffered BG-11 at 10 μg/ml chlorophyll *a*, 1.0 mM $K_3Fe(CN)_6$, 0.2 mM 2,5-dimethyl-*p*-benzoquinone (DMBQ) and actinic light at 2.0 mE m^{-2} s^{-1}, as described in Summerfield et al. (2005a). The relative levels of assembled PSII centers was estimated on a chlorophyll basis employing [^{14}C]-atrazine, as described in Summerfield et al. (2005a).

RNA isolation

Total RNA was extracted and purified using phenol-chloroform extraction and CsCl gradient purification, as

previously described by Reddy et al. (1990) and Singh and Sherman (2002).

Microarray design

The microarray platform and construction was as described in Postier et al. (2003). The cDNA labeling, glass treatment, prehybridization, and hybridization protocols were performed as described in Singh et al. (2003). Biological variation was sampled by pooling RNA extracted from three experiments prior to labeling and hybridization. The ΔPsbO:ΔPsbU strain and the pseudorevertant were grown under photoautotrophic conditions with constant light in BG-11 medium at pH 10, until an $OD_{750\ nm}$ of ~0.25 was obtained. Cells were centrifuged and resuspended in BG-11 medium, pH 7.5, and returned to photoautotrophic conditions for 2 h prior to the harvesting by centrifugation, and RNA extraction.

Data analysis

Spot intensities of the images were quantified by using Quantarray 3.0 (Packard BioChip Technologies). The local background was subtracted from each spot and the spots were normalized using the total signal intensity of the appropriate dye for that technical replicate. Each slide contained three copies of the chromosomal genes, and two slides were used in this dye swap experiment, representing a total of six technical replicates. The data were analyzed using the software Cyber-T (http://visitor.ics.uci.edu/cgi-bin/genex/cybert/CyberT-8.0.form.pl?DATATYPE=CE); this procedure employs a Bayesian probabilistic framework where the variance of each gene is combined with the local background variance of neighboring genes to determine the strength of background variance (Baldi and Long 2001; Long et al. 2001). Genes with a P value <0.01 and that exhibited a change of at least 1.5-fold were considered interesting and retained for further analysis. The complete dataset for this experiment is shown in Supplemental Table 1.

Results and discussion

Physiological characterization of the ΔPsbO:ΔPsbU pseudorevertant

The ΔPsbO:ΔPsbU pseudorevertant was isolated based on its ability to grow photoautotrophically in BG-11 medium at pH 7.5, unlike the original ΔPsbO:ΔPsbU strain. Under these growth conditions, the pseudorevertant strain (ΔPsbO:ΔPsbU (Rev)) had a doubling time of ~14 h, slightly reduced compared to wild type which had a doubling time of 11–12 h at pH 7.5 (Fig. 1). The pseudorevertant doubling time was not altered between pH 7.5 or pH 10 media, and, at pH 10, pseudorevertant growth was similar to that of the ΔPsbO:ΔPsbU strain which was previously reported to grow with a doubling time of ~15 h at pH 10 (data not shown) (Eaton-Rye et al. 2003).

Table 1 Rates of oxygen evolution and PSII assembly of *Synechocystis* sp. PCC 6803 strains at pH 7.5

Strain	Rate of oxygen evolution[a]	Chlorophyll/PSII ratio[b]
Wild type	464 ± 13	833 ± 23
ΔPsbO:ΔPsbU	139 ± 3	1577 ± 26
ΔPsbO:ΔPsbU (Rev)	171 ± 10	1663 ± 90

The data are the average ± the standard error of three independent experiments

[a] Oxygen evolution was supported by 1.0 mM $K_3Fe(CN)_6$ and 0.2 mM 2,5-dimethyl-*p*-benzoquinone and normalized to the wild-type rate of 478 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1}

[b] As determined by [^{14}C]-atrazine binding

In order to gain an insight into the physiological changes involved in growth of the pseudorevertant at pH 7.5, we investigated PSII activity and assembly in this strain compared to wild type and the ΔPsbO:ΔPsbU strain (Table 1). At pH 7.5, the pseudorevertant and the ΔPsbO:ΔPsbU strain had both similar O_2 evolution rates

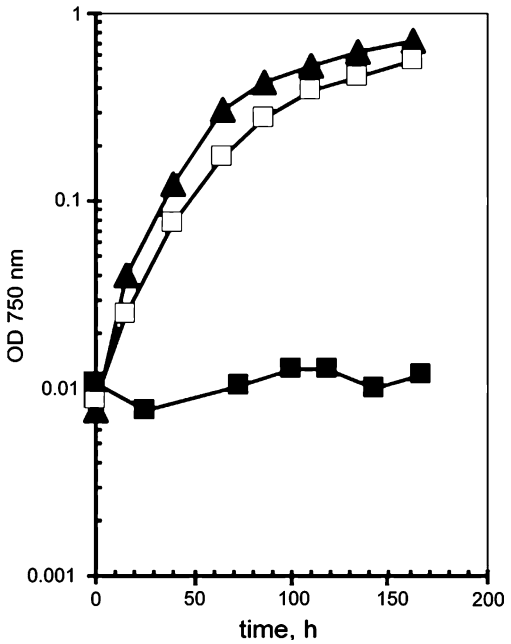

Fig. 1 Photoautotrophic growth curve of *Synechocystis* sp. PCC 6803 strains in BG-11 at pH 7.5. Wild type (closed triangles), ΔPsbO:ΔPsbU (closed squares) and the ΔPsbO:ΔPsbU pseudorevertant (open squares)

and similar relative levels of PSII centers. These data indicate that the ability of the pseudorevertant to grow at pH 7.5 was not likely a result of increased assembly of PSII centers.

The PSII-specific oxygen evolution rates of the pseudorevertant and ΔPsbO:ΔPsbU strain at pH 7.5 (30 and 37% of wild type) were similar to those of the ΔPsbO:ΔPsbU strain at pH 10 (38% of wild type) when measured in the presence of 2.0 mE m^{-2} s^{-1} light (Summerfield et al. 2005a). In contrast, the pH 10 recovery of the ΔPsbO:ΔPsbU strain coincided with an increased number of PSII centers from ~50% of wild type at pH 7.5 to >90% of wild type at pH 10 (Eaton-Rye et al. 2003; Summerfield et al. 2005a). Thus, the physiological response of the ΔPsbO:ΔPsbU strain at elevated pH is not identical to that of the pseudorevertant at pH 7.5. In order to better understand the basis of growth of the pseudorevertant at pH 7.5, we undertook a DNA microarray experiment comparing gene expression of the pseudorevertant and the ΔPsbO:ΔPsbU strain following incubation in BG-11 medium at pH 7.5.

Differentially regulated genes in the ΔPsbO:ΔPsbU pseudorevertant compared to the ΔPsbO:ΔPsbU strain at pH 7.5

As described in Materials and methods, the two strains were grown photoautotrophically in BG-11 medium at pH 10 (to an $OD_{750\ nm}$ of ~0.25), cells were then transferred to BG-11 medium at pH 7.5 and harvested following a 2 h incubation. Using a 1.5-fold and $P < 0.01$ cut off, <4% of chromosomal genes were differentially expressed in the pseudorevertant compared to the ΔPsbO:ΔPsbU strain (124/3165 genes) (Table 2). Approximately half of these genes were up-regulated in the pseudorevertant strain (63/124 genes). Both up- and down-regulated transcripts represented genes from a number of functional categories (Table 2). Forty of the up-regulated genes were increased by 2-fold or greater in the pseudorevertant strain, whereas only 5 of the 61 down-regulated transcripts showed a 2-fold or greater difference between the two strains. This paper will focus on genes up-regulated in the pseudorevertant and data for all genes is available in Supplemental Table 1.

Stress-responsive genes are up-regulated in the pseudorevertant

Genes encoding a number of proteins involved in stress responses were up-regulated in the pseudorevertant compared to the ΔPsbO:ΔPsbU strain. Transcripts encoding the heat shock proteins HspA, DnaK2 and HtpG, the histidine kinase Hik34 and the sigma factors SigB and SigD were up-regulated 1.8–4.5 fold (Table 3). These transcripts were previously shown to be elevated following exposure to osmotic stress (Paithoonrangsarid et al. 2004; Shoumskaya et al. 2005), oxidative stress (H. Li et al. 2004; Z. Li et al. 2004; Kanesaki et al. 2007) and heat shock (Suzuki et al. 2005; Singh et al. 2006) and only the *htpG* transcript was not reported to be increased under salt stress conditions (Shoumskaya et al. 2005) (Table 3). In addition, four genes involved in protease activity were up-regulated in the pseudorevertant. Two of these genes, *clpB1* and *htrA (degP)*, up-regulated 3.2 and 4.0 fold, respectively, have also been reported to be up-regulated following osmotic, salt and oxidative stress, as well as, in response to heat shock (H. Li et al. 2004; Paithoonrangsarid et al. 2004; Shoumskaya et al. 2005; Singh et al. 2006). In addition, 45

Table 2 Functional categories of differentially regulated genes[a] in the ΔPsbO:ΔPsbU pseudorevertant compared to the ΔPsbO:ΔPsbU mutant at pH 7.5

General pathways	Number of genes	Differentially regulated genes (Up)[c] Rev/ΔPsbO:ΔPsbU
Amino acid biosynthesis	97	0
Biosynthesis of cofactors, prosthetic groups, and carriers	124	8 (1)
Cell envelope	67	4 (0)
Cellular processes	76	5 (5)
Central intermediary metabolism	31	0
DNA replication, restriction, recombination, and repair	60	1 (0)
Energy metabolism	132	3 (0)
Hypothetical	1076	51 (30)
Other categories	306	4 (4)
Photosynthesis and respiration	141	8 (0)
Purines, pyrimidines, nucleosides, and nucleotides	41	0
Regulatory functions	146	5 (3)
Transcription	30	2 (2)
Translation	168	7 (6)
Transport and binding proteins	196	2 (1)
Unknown	474	24 (11)
Total number	3165[b]	124 (63)

[a] Genes were considered differentially regulated when $P < 0.01$, fold change >1.5 fold

[b] Total number of genes based on Kazusa annotation prior to May, 2002

[c] (Up) represents the number of differentially regulated genes that were up-regulated in the Revertant compared to the ΔPsbO:ΔPsbU strain

Table 3 Selected upregulated genes[a] in the ΔPsbO:ΔPsbU pseudorevertant compared to the ΔPsbO:ΔPsbU *Synechocystis* sp. PCC 6803 strain at pH 7.5

Gene	Gene function	Fold	*P*	Stress [b]	Hik[c]
Cellular processes: chaperones					
sll1514	16.6 kDa small heat shock protein, HspA	4.5	8.72E-04	O, S, P, H	Hik34
sll0170	DnaK protein 2, heat shock protein 70	1.8	8.29E-05	O, S, P, H	Hik34
sll0430	HtpG, heat shock protein 90	2.5	1.22E-03	O, P, H	Hik34
Regulatory functions					
slr1285	Hik34, two-component sensor histidine kinase	3.3	3.90E–04	O, S, P, H	
Transcription: RNA synthesis, modification, and DNA transcription					
sll0306	SigB, group2 RNA polymerase sigma factor	2.7	4.43E–05	O, S, P, H	
sll2012	SigD, group2 RNA polymerase sigma factor	2.2	8.95E–05	O, S, P, H	Hik33
Translation: degradation of proteins, peptides, and glycopeptides					
sll0020	ATP-dependent Clp protease ATPase subunit	1.7	2.02E–03	P	
slr1641	ClpB1 protein	3.2	5.82E-05	O, S, P, H	Hik34
sll1679	HhoA periplasmic protease (DegQ)	1.6	6.33E-03		
slr1204	HtrA serine protease (DegP)	4.0	6.16E-07	O, S, P, H	Hik10
Hypothetical and unknown proteins					
sll0939	Hypothetical protein	4.2	5.18E-05	O, S, P, H	Hik16
sll1723	Probable glycosyltransferase	1.9	2.06E–03	O	
slr0959	Hypothetical protein	2.3	9.13E–03	O, S	Hik34
slr1603	Hypothetical protein	3.1	8.47E–04	O, S, P, H	Hik34
slr1915	Hypothetical protein	1.7	1.07E–04	O, S, P, H	Hik34
slr1963	OCP, orange carotenoid protein	2.4	1.05E–05	O, S, H	Hik34
ssr2153	Unknown protein	2.5	7.41E–05	S	
slr0924	Periplasmic protein, function unknown	1.7	7.42E–03		
sll0319	Periplasmic protein, function unknown	2.7	3.63E–04		
sll0314	Periplasmic protein, function unknown	2.5	1.55E–03		
slr1608	Putative periplasmic glucose dehydrogenase-B	1.7	7.95E–03		
slr0559	Periplasmic binding protein of ABC transporter	1.6	5.38E–03		
Gene clusters					
sll1693	Hypothetical protein	1.6	4.27E–03		
sll1694	Pilin polypeptide PilA1	1.8	9.15E–03	H*[e]	Hik34[d]
sll1695	Pilin polypeptide PilA2	2.0	7.78E–03	H*[e]	Hik34[d]
sll1696	Hypothetical protein	3.3	3.07E–04	H*[e]	Hik34[d]
sll0788	Hypothetical protein	2.9	8.67E–04	P	Hik34
sll0789	Two-component response regulator	2.7	1.02E–03	P	Hik34
slr0581	Unknown protein	5.4	7.68E–05	O, P	
slr0582	Unknown protein	4.0	9.20E–05	P	
slr1674	Hypothetical protein	3.4	2.20E–04	P, H	
slr1675	Putative hydrogenase expression protein HypA1	2.4	6.94E-04	P, H	
slr1751	Periplasmic carboxyl-terminal protease CtpC	1.9	6.60E–06		
slr1752	Hypothetical protein	2.4	1.89E–04		

[a] Genes were considered differentially regulated when $P < 0.01$, fold change >1.5 fold

[b] Genes previously shown to be induced by osmotic (O), salt (S), hydrogen peroxide (P) or heat (H) (Paithoonrangsarid et al. 2004; Shoumskaya et al. 2005; H. Li et al. 2004; Kanesaki et al. 2007; Singh et al. 2006)

[c] Expression of these genes was previously shown to be altered in the absence of histidine kinase (Paithoonrangsarid et al. 2004; Shoumskaya et al. 2005; Kanesaki et al. 2007)

[d] Expression of these genes was previously shown to be increased when *hik34* was overexpressed (Suzuki et al. 2005)

[e] H* indicates down-regulation following heat shock (Singh et al. 2006)

transcripts encoding hypothetical, other or unknown genes were up-regulated in the pseudorevertant, and 12 of these genes have been reported to be elevated under one or more stress condition (H. Li et al. 2004; Paithoonrangsarid et al. 2004; Shoumskaya et al. 2005; Singh et al. 2006).

In cyanobacteria, two-component systems play an important role in perception and response to environmental signals, including stress responses (Murata and Suzuki 2006). There are 47 histidine kinases and 45 response regulators in *Synechocystis* (Kaneko et al. 1996, 2003), and their functions, and the complexity of their interactions are beginning to be characterized (Paithoonrangsarid et al. 2004; Shoumskaya et al. 2005; Suzuki et al. 2005; Kanesaki et al. 2007). Thirteen of the genes induced in the pseudorevertant have previously been identified as having Hik-mediated stress responses and ten of these transcripts were shown to be under control of Hik34 (Table 3). In the pseudorevertant, the role of Hik34 in the regulation of gene expression is consistent with the elevated *hik34* transcript (Table 3). These up-regulated transcripts in the pseudorevertant represent only a subset of stress responsive genes controlled by Hik34 under osmotic or salt stress (Shoumskaya et al. 2005). However, the transcripts regulated by a particular Hik may differ according to the perceived stress, e.g., *htpG* is up-regulated by Hik34 under osmotic stress, but not salt stress. Therefore, it is possible that alternative genes may be under the control of Hik34 in the pseudorevertant. For example, transcripts encoding the genes sll1694–6 were up-regulated in the pseudorevertant. However, these transcripts were down-regulated following heat shock (Singh et al. 2006) and up-regulated in a strain over expressing *hik34* (Suzuki et al. 2005), but were not altered by the absence of Hik34 under osmotic or salt stress (Shoumskaya et al. 2005).

Cell envelope proteins

The cell envelope is comprised of the plasma membrane, a peptidoglycan layer and an outer membrane, and, not surprisingly, bacterial cells exhibit a cell envelope stress response. This response involves the sensing and degradation of misfolded proteins (reviewed in Raivio and Silhavy 2001; Duguay and Silhavy 2004). In *E. coli*, two stress response systems have been well characterized, and one of these involves the Deg proteases that are also found in cyanobacteria. Two of the three Deg proteases (DegP and DegQ) in *Synechocystis* were up-regulated in the pseudorevertant. Proteomics data had suggested that DegP was associated with the outer membrane (Huang et al. 2004), whereas DegQ had been identified in both plasma membrane (Huang et al. 2006) and periplasmic fractions (Fulda et al. 2000). In *Synechocystis*, inhibition of PSII reduced light stress in a Deg mutant that lacked all three Deg proteases (Barker et al. 2006). This led to the suggestion that these proteases may be involved in processing proteins damaged by reactive oxygen species (ROS), generated during linear photosynthetic electron transfer (Barker et al. 2006). An additional periplasmic protease, CtpC, was up-regulated in the pseudorevertant; the role of this carboxyl-terminal protease is not known, but its transcript has been shown to be up-regulated under salt stress (Jansen et al. 2003). An alternative function of these proteases may be connected to the role of the plasma membrane in PSII biogenesis. The identification of D1, D2, cytochrome b_{559} and PsbO in the plasma membrane led Zak et al. (2001) to propose that this is the site of initial PSII biogenesis. Therefore, it is possible the absence of PsbO may be detrimental to this biogenesis and the elevated levels of periplasmic proteases in the pseudorevertant could be involved in the removal of abnormal core complexes.

Proteomics data suggest that another five proteins that were up-regulated in the pseudorevertant are periplasmic (Fulda et al. 2000). These proteins are: a subunit of an ABC transporter (slr0559), one putative glucose dehydrogenase (slr1608) and three proteins annotated as hypothetical (sll0314, sll0319 and slr0924). Proteomic data indicated that Slr1608, the putative glucose dehydrogenase, was induced by salt stress and this suggested that it might play a role in modification of the cell wall in response to stress. All six putative periplasmic proteins contain predicted signal peptides that are consistent with transport via the general secretory (Sec) pathway. Two peptides of the Sec system were identified in the thylakoid and plasma membranes of *Synechococcus* sp. PCC 7942 using immunoblotting (Nakai et al. 1993, 1994), thus indicating that this system may be utilized in both these membranes.

The gene cluster sll1693–sll1696, containing two putative pilin genes, was up-regulated in the pseudorevertant. These four transcripts were also elevated in the absence of IsiA (Singh and Sherman 2007) and three of the transcripts, sll1694–6, were decreased following heat shock (Singh et al. 2006). The gene sll1694 (*pilA1*) is essential for motility and thick pili formation; however, sll1695 (*pilA2*) has no impact on pili formation or motility (Bhaya et al. 2000). The proteins Sll1694 and Sll1695 were suggested to be involved in chlorophyll transfer to photosystems (He and Vermaas 1999). These data indicate the possibility of an additional role of these genes under specific stress conditions, especially under oxidative stress conditions.

Thylakoid associated proteins

Proteomics data indicated that three proteins with transcripts up-regulated in the pseudorevertant may be associated with thylakoid membranes. These proteins were not predicted to be transmembrane and did not have signal

peptides, thus leading the authors to suggest that they may be associated with the cytoplasmic side of the thylakoid membrane (Srivastava et al. 2005). These transcripts encode Dnak2, an ATP-dependent Clp protease ATPase subunit (sll0020) and the orange carotenoid protein (OCP, slr1923). Under high light conditions, the OCP has a photoprotective role mediating phycobilisome-associated nonphotochemical quenching (Wilson et al. 2006). In the pseudorevertant, the increased *ocp* transcript may lead to decreased energy transfer from the phycobilisomes to PSII, resulting in less damage to these PSII centers that lack PsbO and PsbU and that are sensitive to photoinhibition.

Concluding remarks

The pseudorevertant was isolated as a spontaneous revertant after long-term incubation under non-permissive conditions and the strain studied is likely one of many such revertants that may be obtained under these conditions. The Venn diagram in Fig. 2 depicts some of the genes that were induced in the pseudorevertant and the location of their encoded proteins. These genes fall into three overlapping classes: general stress response, Hik34 regulated and proteases. Additionally, Fig. 2 illustrates that genes upregulated in the pseudorevertant compared to the ΔPsbO:ΔPsbU strain encoded proteins localized to the cell envelope and the thylakoid membrane. In particular, the pseudorevertant induced proteases that localized to the outer, cytoplasmic or thylakoid membranes, in addition to one protease (DegQ) and a number of hypothetical proteins that were targeted to the periplasm. These results indicate that the cell targets both the thylakoid and cytoplasmic membrane systems in order to compensate for the inability to synthesize two lumenal proteins involved in oxygen evolution. Such results strongly suggest that the proteins up-regulated in this strain may be involved with protection against ROS.

Thus, we hypothesize that the deletion of both PsbO and PsbU may increase the level of ROS in this strain. This is in agreement with the proposal of Balint et al. (2006) that increased resistance to exogenously applied oxidative stress exhibited by a ΔPsbU strain in *Synechococcus* sp. PCC 7942 was due to enhanced antioxidative mechanisms in response to increased ROS produced by this strain. However, these authors did not demonstrate elevated ROS

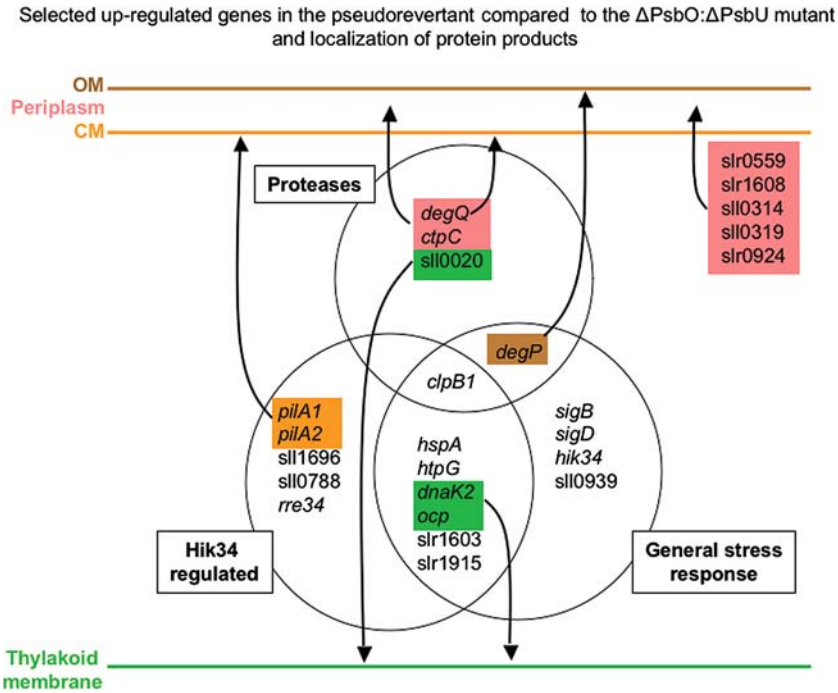

Fig. 2 Selected up-regulated genes (>1.5 fold induction, $P < 0.01$) in the pseudorevertant compared to the ΔPsbO:ΔPsbU mutant grown at pH 7.5. The results are presented in the form of a Venn diagram that highlights the overlap among Hik34 regulated genes, general stress response genes and genes encoding proteases. Genes designated as general stress responsive were reported previously to exhibit elevated transcripts following exposure to at least 3 of the 4 stress treatments: salt, osmotic, oxidative stress or heat shock (Paithoonrangsarid et al. 2004; Shoumskaya et al. 2005; H. Li et al. 2004; Kanesaki et al. 2007; Singh et al. 2006). Hik34 regulated transcripts had altered transcript abundance in either the absence or following the overexpresson of *hik34* (Paithoonrangsarid et al. 2004; Shoumskaya et al. 2005; Suzuki et al. 2005; Kanesaki et al. 2007). Arrows indicate localization of the proteins encoded by these up-regulated genes to the outer membrane (OM), periplasm, cytoplasmic membrane (CM) or thylakoid membrane, based on proteomics data (Fulda et al. 2000; Huang et al. 2004; Huang et al. 2006; Srivastava et al. 2005)

in the ΔPsbU strain or increased levels of proteins involved in antioxidative repair mechanisms. In the pseudorevertant, cells exhibited up-regulation of transcripts involved in stress responses, many of which have been shown to be regulated by Hik34. These transcripts included genes encoding chaperones and proteases, and increased transcript abundance of these genes may limit the impact of damage due to ROS. The gene encoding OCP was also up regulated in the pseudorevertant. This protein dissipates excess energy from the phycobilisomes and therefore may reduce the number of photons reaching the impaired reaction centers. These two responses, probably in combination with other uncharacterized stress responses (such as that involving sll1693-6), may be sufficient to permit growth of the pseudorevertant. As ROS have been proposed to inhibit repair of PSII (Nishiyama et al. 2001, 2006), it would be interesting to compare PSII turnover and repair in the pseudorevertant to the ΔPsbO:ΔPsbU mutant.

Preliminary data indicate that many of the genes up-regulated in the pseudorevertant were also increased in the wild type at pH 10 compared to pH 7.5. The *hik34* transcript is up-regulated in both the wild type and the ΔPsbO:ΔPsbU strain at pH 10. The ten genes elevated in the pseudorevertant that were shown to be regulated by Hik34 were all increased in the wild type, but only four were increased in the ΔPsbO:ΔPsbU strain (data not shown). In the ΔPsbO:ΔPsbU strain a different subset of genes were up-regulated at pH 10, including a dramatic increase in the *hik31* transcript (data not shown). Initial results suggested that alkaline pH may induce a similar stress response in the wild type as seen in the pseudorevertant at pH 7.5, but that the ΔPsbO:ΔPsbU mutant's growth at pH 10 may include an alternative set of genes with *hik31* playing an important role.

Acknowledgements This work was supported by a grant from the Department of Energy (DE-F002-99ER20342) to Louis A Sherman and in part by a New Zealand Marsden grant (UOO309) to Julian Eaton-Rye. This article honors the various contributions of Govindjee to photosynthesis and fluorescence from cyanobacteria beginning in the mid 1960s.

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