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Responses of *psbA*, *hli* and *ptox* genes to changes in irradiance in marine *Synechococcus* and *Prochlorococcus*

Gry Mine Berg[1,*], Jeff Shrager[2], Gert van Dijken[1], Matthew M. Mills[1], Kevin R. Arrigo[1], Arthur R. Grossman[2]

[1]Department of Environmental Earth System Science, Stanford University, Stanford, California 94305, USA
[2]Department of Plant Biology, The Carnegie Institution for Science, 260 Panama Street, Stanford, California 94305, USA

ABSTRACT: Expression of 3 gene families involved with photoacclimation — *psbA* (encoding the photosystem II reaction center protein D1), *hli* (encoding the high-light inducible proteins), and *ptox* (encoding the plastid terminal oxidase) — was compared in the marine cyanobacteria *Synechococcus* WH8102 and *Prochlorococcus* MED4 acclimated to either low or high light. These 2 strains, adapted for growth in oligotrophic marine environments, have distinct light-harvesting systems and respond differently to changes in irradiance. In response to growth at higher irradiance, *Synechococcus* WH8102 increased expression of the *psbA* multigene family (*psbA1–4*) 5-fold. Within this gene family, the expression of *psbA2* increased 60-fold. Expression of 4 *hli* genes increased 2- to 5-fold, whereas expression of the *ptox* gene decreased 3-fold. In comparison, expression of the *psbA* gene increased 2-fold in *Prochlorococcus* MED4 cultures grown at higher irradiances. Expression of the *Prochlorococcus* MED4 *hli6–9* and *hli16–19* operons increased 11- to 14-fold, while *ptox* expression increased 3-fold. Using *psbA* induction as a standard for acclimation to changes in irradiance, we observed that the induction ratio of *ptox*:*psbA1* and *hli*:*psbA1* was 144 and 70 times greater, respectively, in *Prochlorococcus* MED4 compared with *Synechococcus* WH8102. These observations suggest that induction of *ptox* and *hli* may play a key role in the phototolerance of *Prochlorococcus* MED4. Conversely, the induction of *psbA*, and the synthesis of the PSII reaction center protein D1, may be critical for the acclimation of *Synechococcus* WH8102 to high irradiances.

KEY WORDS: *Synechococcus* WH8102 · *Prochlorococcus* MED4 · Photoacclimation · Photoinhibition · Gene expression · *psbA* · *hli* · *ptox* · Fluorescence characterization · Carbon fixation

INTRODUCTION

Marine cyanobacteria dominate the brightly lit surface waters of ocean gyres where nutrient availability is highly restricted (Bouman et al. 2006, Fuller et al. 2006). Two clades that are adapted to growth under these conditions, and that overlap in their distributions, are represented by the strains *Synechococcus* WH8102 (Palenik et al. 2003) and *Prochlorococcus marinus* MED4 (Rocap et al. 2003, Scanlan et al. 2009). Despite their coexistence under similar environmental conditions (Zwirglmaier et al. 2007, 2008), the organisms in these genera are defined by distinctly different light-harvesting systems and nutrient requirements (Ting et al. 2002). *Synechococcus* WH8102 harvests light energy mostly through absorption by phycobilisomes (PBS) — macromolecular protein complexes that are peripheral to the thylakoid membrane and are associated mainly with photosystem II (PSII) (Grossman et al. 1993, 2001). PBS are composed of a central core of the pigmented biliprotein allophycocyanin (AP, encoded by the *apc* genes), from which rods of the pigmented biliproteins phycocyanin (PC, encoded by the *rpc* genes) and phycoerythrin (PE, encoded by the *cpe* genes) radiate outwards (Fig. 1). AP binds the chromophore phycocyanobilin (PCB), PC binds the chro-

*Email: mine.berg@gmail.com

mophores PCB and phycoerythrobilin (PEB), and PE binds the chromophores PEB and phycourobilin (PUB) to harvest light. In contrast, *Prochlorococcus* relies on integral thylakoid membrane proteins (Pcbs) that bind divinyl chlorophyll *a* and *b* to harvest light energy. The Pcbs of *Prochlorococcus* MED4 form a ring around PSII (Bibby et al. 2003), their Fig. 1.

High irradiance in the uppermost layer of the ocean can lead to photoinhibition and loss of function of the photosynthetic apparatus. During photosynthesis, an electron from an excited chlorophyll molecule is transferred to the primary electron acceptor pheophytin and then on to the quinone molecules (Q_A and Q_B) of the plastoquinone (PQ) pool. From the PQ pool, electrons are transferred to cytochrome b_{6f}, plastocyanin, and finally, through photosystem I (PSI), to reduce $NADP^+$ in the classical z scheme (Fig. 1). Photoinhibition results when the PQ pool becomes over-reduced (Vass et al. 1992) or during charge recombination between PSII acceptor and donor sides (Keren et al. 1997). Over-reduction of the PQ pool can generate singlet oxygen species that ultimately cleave and damage the PSII

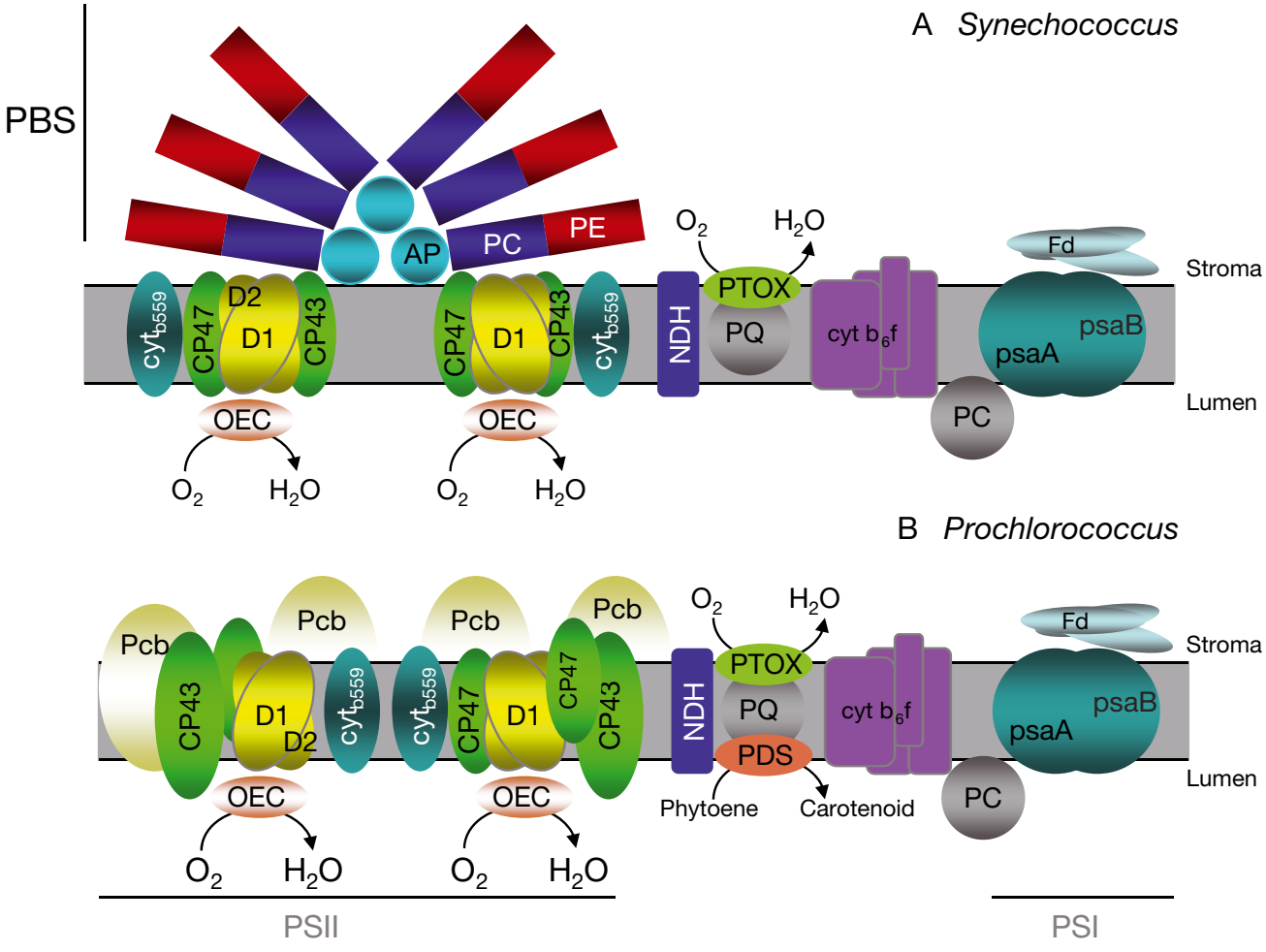

Fig. 1. Schematic illustration of light-harvesting and electron-transport proteins in *Synechococcus* and *Prochlorococcus* (adapted from Ting et al. 2002). (A) *Synechococcus*. The phycobilisome (PBS) light-harvesting system is composed of a core protein, allophycocyanin (AP, shown in turquoise), an inner rod protein, phycocyanin (PC, shown in blue), and the outer rod (shown in red) composed of either 1 or 2 structurally distinct forms of phycoerythrin (PE). (B) *Prochlorococcus*. The prochlorophyte chlorophyll-binding proteins form a ring around PSII. The NDH (in blue) complex represents a number of possible reductases that reduce the plastoquinone (PQ) pool using stromal reductant (i.e. derived from glycolysis), including a putative NAD(P)H-plastoquinone oxidoreductase which occurs adjacent to plastid terminal oxidase (PTOX) in the *Prochlorococcus* MED4 genome (McDonald & Vanlerberghe 2005), NAD(P)H dehydrogenase, ferredoxin-quinone oxidoreductase and glycolate-quinone oxidoreductase, as described in Carol & Kuntz (2001). D1 and D2 = core proteins of the PSII reaction center; CP43 and CP47 = chl *a*-binding core antenna proteins associated with the PSII reaction center; OEC = oxygen evolving complex; cyt_{b559} = cytochrome b_{559}; cyt b_6f = cytochrome b_{6f} complex; Pcb = prochlorophyte chlorophyll-binding protein; PC (blue) = phycocyanin; PC (grey) = plastocyanin; PE = phycoerythrin; PQ = plastoquinone pool; NDH = NAD(P)H plastoquinone oxidoreductase; PTOX = plastid terminal oxidase; PDS = phytoene desaturase; psaA = PSI reaction center apoprotein; Fd = ferredoxin

core protein D1 (Vass et al. 1992, Clarke et al. 1993, Nishiyama et al. 2006). Damage to D1 results in photoinhibition, which is manifested as a decrease in PSII photochemical efficiency (evidenced by a decrease in the PSII fluorescence yield (F_v/F_m)) and in the rate of photosynthetic carbon fixation (Clarke et al. 1995, Behrenfeld et al. 1998, Six et al. 2007a, Garczarek et al. 2008).

The redox state of the PQ pool plays a key role in the regulation of photosynthetic electron transport and in the susceptibility of the photosynthetic apparatus to photoinhibition. Therefore, photosynthetic organisms have developed a variety of strategies to prevent the PQ pool from becoming over-reduced. One strategy is to dissipate excess absorbed light energy as heat, possibly involving the high-light inducible proteins (He et al. 2001, Havaux et al. 2003). A second strategy involves transferring electrons from the PQ pool to molecular oxygen via the action of terminal oxidases (Bennoun 1982, 2002, Berry et al. 2002, Hart et al. 2005, Bailey et al. 2008). When these strategies are not sufficient to prevent photoinhibition, D1 protein synthesis is upregulated to replace damaged D1 proteins and restore function to the PSII reaction center (Schaefer & Golden 1989a,b, Clarke et al. 1993,1995, Long et al. 1994, Campbell et al. 1998a). With respect to cyanobacteria, genes encoding 3 families of proteins have been shown to be important for the management of absorbed excitation energy: (1) the *hli* genes that encode the high-light inducible proteins (HLIPs); (2) the *ptox* gene that encodes the plastid terminal oxidase (PTOX); and (3) the *psbA* genes that encode the PSII reaction center core protein D1.

Investigations into the function of HLIPs in freshwater cyanobacteria suggest that these proteins absorb and dissipate excess energy (He et al. 2001, Havaux et al. 2003). It has also been suggested that they transiently bind chlorophyll (Funk & Vermaas 1999) and play a role in the regulation of tetrapyrrole biosynthesis (Xu et al. 2002). Although most probably integral to the thylakoid membranes, it is not clear with which photosystem these proteins associate. While earlier investigations linked them with PSII (Promnares et al. 2006, Yao et al. 2007), a more recent investigation has shown that 3 of the 4 extant HLIPs of *Synechocystis* PCC 6803 are associated with PSI (Wang et al. 2008). Marine cyanobacteria have a greater number of HLIPs compared with their freshwater counterparts, and some of these may respond to stressors other than light, including viral infection (Lindell et al. 2007) and nitrogen limitation (Su et al. 2006, Tolonen et al. 2006). While *Synechococcus* WH8102 has 8 *hli* genes, *Prochlorococcus* MED4 has 22, the greatest number of *hli* genes observed in any of the cyanobacteria sequenced thus far (Bhaya et al. 2002).

In addition to the HLIPs, there are a number of oxidases that serve as electron valves, extracting electrons between PSII and PSI. These enzymes include PTOX (McDonald et al. 2003), cytochrome *c* oxidase (Berry et al. 2002), and the alternative quinol oxidases (Hart et al. 2005). Cytochrome *c* oxidase is essential for respiration and provides energy needed for maintenance of cellular functions in the dark. The alternative quinol oxidase is hypothesized to have a similar function to cytochrome *c* oxidase but is much less well characterized and is absent in marine *Prochlorococcus* (Hart et al. 2005). In contrast, PTOX plays potentially important roles in photosynthetic electron transport, as well as in respiratory electron transport, and also in reactions critical for the synthesis of carotenoids. In the light, PTOX may mediate the re-oxidation of the PQ pool reduced by PSII (Cournac et al. 2000, Bailey et al. 2008). In the dark, PTOX may oxidize the PQ pool reduced internally via NADP(H) dehydrogenase (NDH), PQ oxidoreductase (Bennoun 1982, 2002, Rumeau et al. 2007), or phytoene desaturase (Carol & Kuntz 2001) (Fig. 1). Putative *ptox* genes encoded by both *Synechococcus* WH8102 and *Prochlorococcus* MED4 have been characterized in natural cyanobacterial assemblages in ocean gyres (McDonald & Vanlerberghe 2005), and recent investigations in *Prochlorococcus* sp. have demonstrated that the *ptox* gene is regulated by high-light exposure (Steglich et al. 2006).

Active repair of the PSII reaction center protein D1, involving rapid changes in *psbA* gene expression and D1 protein synthesis, is an effective short-term strategy to cope with rapidly changing light intensities (Six et al. 2007b). While *Prochlorococcus* MED4 has a single D1 isoform (D1:1) encoded by 1 *psbA* gene, *Synechococcus* WH8102 has 2 D1 isoforms (D1:1 and D1:2) encoded by 4 *psbA* genes (Palenik et al. 2003). In the latter clade, the ratio of D1:1 to D1:2 isoforms may change with light intensity and with increased photodamage (Garczarek et al. 2008). Despite the difference in *psbA* gene copy number, increases in total *psbA* transcript accumulation are observed in both marine *Prochlorococcus* and *Synechococcus* upon exposure to higher irradiance (Steglich et al. 2006, Six et al. 2007a, Garczarek et al. 2008), and the level of the *Prochlorococcus* MED4 *psbA* transcript is directly correlated with light intensity over the diel cycle (Zinser et al. 2009).

To gain insight into whether *Prochlorococcus* MED4 and *Synechococcus* WH8102 acclimate to changes in irradiance in a similar manner given their very different light harvesting systems, we shifted cultures from very low light to a higher irradiance. We also hoped to establish whether or not changes in cell physiology — such as carbon fixation and the efficiency of excitation energy capture by closed PSII (F_v/F_m) — correlated

with changes in gene expression. To this end, we monitored changes in the levels of transcripts of genes unique to each species, including genes encoding components of their respective light-harvesting systems, as well as genes common to both species, including *psbA*, *hli* and *ptox*. We specifically tested the hypothesis that homologous genes, i.e. genes with a certain sequence similarity, encoding proteins of similar function, would have similar patterns of expression in both species.

MATERIALS AND METHODS

Culture growth conditions. Axenic, semi-continuous cultures of *Synechococcus* WH8102 (CCMP2370) were grown at 22°C in 0.2 µm filtered and autoclaved SN medium (Waterbury et al. 1986) under continuous white light at intensities of 15 and 50 µmol quanta m^{-2} s^{-1}. Axenic, semi-continuous cultures of *Prochlorococcus* MED4 (CCMP2389) were grown in Pro99 medium at 19°C exposed to continuous irradiance of either 20 or 160 µmol quanta m^{-2} s^{-1}. Both species were grown in triplicate, aerated, glass flasks (volume: 2 l) and were acclimated to the lower or higher growth irradiance for 3 wk before samples were collected and analyzed. The higher light intensities represented the maximum intensities that the cells could tolerate when shifted in 1 step from the lower growth irradiances. *Synechococcus* WH8102 has been successfully grown in up to 650 µmol quanta m^{-2} s^{-1} of continuous white light when progressively acclimated by no more than 2-fold increases in irradiation (Six et al. 2004). Increases in irradiance of 5-fold or more in 1 step will typically lead to photoinhibition of cell growth and death (Six et al. 2004). This has also been observed for *Synechococcus* WH7803, which can be shifted by only 2- or 3-fold in irradiance at a time (Kana & Glibert 1987). Once the cultures were acclimated they were sampled for carbon fixation, fluorescence yield, and the levels of specific transcripts using quantitative polymerase chain reaction (qPCR) analyses.

Fluorescence yield. The PSII fluorescence yield (F_v/F_m) was measured using a pulse-amplitude-modulated (PAM) fluorometer (Water-PAM, Walz). Prior to measurement, the PAM was zeroed with 4 ml sterile-filtered (pore size 0.2 µm) culture medium. Approximately 4 ml culture was collected and dark-adapted for 15 min before the modulated (non-actinic) measuring light (peaking at 460 nm) was switched on to determine the minimum fluorescence (F_0) of the dark-adapted sample. Subsequently, the maximum fluorescence (F_m) of that sample was determined following a saturating light pulse of 4000 µmol quanta m^{-2} s^{-1}, peaking at 660 nm, for 0.8 ms. F_v/F_m was calculated as $(F_m - F_0)/F_m$. Because the light source used to determine F_m was saturating, closing all the reaction centers, the spectral quality of this light source should have a minimal effect on this measurement. PBS-containing cyanobacteria have lower fluorescence yields than do vascular plants, prochlorophytes or eukaryotic phytoplankton because F_0 includes emissions from phycobiliproteins in addition to PSII (Campbell et al. 1996, 1998b). As such, we cannot directly compare the F_v/F_m values between PBS- and non-PBS-containing cyanobacteria. Nevertheless, we can compare relative changes within each taxon as an indicator of PSII function.

Photosynthesis–irradiance (*P–E*). *P–E* relationships were determined from ^{14}C-bicarbonate incorporation as described in Arrigo et al. (2010) with the exception that the samples were incubated for 1 h at the culture growth temperature (23 or 19°C). Chlorophyll *a* (chl *a*)-normalized carbon uptake was calculated from radioisotope incorporation and the concentration of chl *a* (Arrigo et al. 2010). Data were fitted by least-squares non-linear regression to the *P–E* equation of Platt et al. (1980) to derive photosynthetic parameters. The photosynthetic parameters discussed here include $P^{\star}_{max}$ (µg C µg^{-1} chl *a* h^{-1}), the maximum chl *a*-normalized rate of carbon fixation; $\alpha^{\star}$ (µg C µg^{-1} chl *a* h^{-1}) (µmol photons m^{-2} $s^{-1})^{-1}$, the chl *a*-normalized initial slope of the *P–E* curve, an index of photosynthetic efficiency; and E_k ($E_k = P^{\star}_{max}/\alpha^{\star}$, µmol photons m^{-2} s^{-1}), the irradiance at which photosynthesis approaches saturation, an index of photoacclimation.

Chl *a* concentration. The concentration of chl *a* in the *Prochlorococcus* MED4 cultures was determined by filtering triplicate 5 ml culture aliquots onto 25 mm Whatman GF/F filters and extracting the filters in the dark at 4°C for 24 h in 5 ml of 90% acetone. Chl *a* was measured fluorometrically using a Turner Fluorometer 10-AU (Turner Designs) according to Holm-Hansen et al. (1965). The concentration of chl *a* in the *Synechococcus* WH8102 cultures was determined by filtering triplicate 5 ml culture aliquots onto 25 mm Whatman GF/F filters that were placed into bead-beater tubes. After addition of 1 ml 90% acetone, the filters were extracted overnight. The following day, a volume corresponding to 50 µl glass beads was added to the tubes and the samples were processed at maximum speed for 30 s in a Mini Beadbeater (Biospec Products) followed by centrifugation at 13 400 rpm (12 100 × *g*) in a microfuge for 1 min at 4°C. The supernatant was diluted 1:5 in 90% acetone and measured fluorometrically.

RNA isolation. Total RNA from cultures acclimated under low light (LL) and high light (HL) was extracted using phenol–chloroform (Sambrook et al. 1989).

Briefly, pelleted cells were lysed in 500 µl SDS-EB buffer (2% SDS, 400 mM NaCl, 40 mM EDTA, 100 mM Tris HCl, pH 8.0), 50 µl 3 M sodium acetate (pH 5.0), and 600 µl phenol–chloroform–isoamyl alcohol (25:24:1, v/v) by pipetting the cell suspension up and down. Following centrifugation (13 400 rpm [12 100 × *g*], 4°C, 5 min), the aqueous supernatant (~600 µl) was transferred to a new tube and extracted a second time with 1 vol (600 µl) of phenol–chloroform–isoamyl alcohol. After a second centrifugation (13 400 rpm [12 100 × *g*], 4°C, 5 min), 1 vol chloroform was added to the supernatant and the sample was centrifuged once more. Following transfer of the aqueous supernatant, 2 vols of 100% ice-cold ethanol and 50 µl 10 M $LiCl_2$ were added to the sample which was incubated at −20°C for 1 h to precipitate the RNA. Nucleic acids were pelleted by centrifugation (12 000 rpm [9700 × *g*], 4°C, 25 min). The supernatant was discarded and the pellet was rinsed with 80% ethanol, air-dried, and resuspended in sterile distilled water. The total RNA concentration was determined by absorption at 260 nm, and 20 µg aliquots were treated twice with 5 U of RNase-free DNase I (Qiagen) in a total volume of 50 µl according to the manufacturer's instructions. RNA quality was assessed from the A_{260}/A_{280} nm ratio and from denaturing agarose gel electrophoresis.

qPCR. Three µg of total RNA and 200 U of Superscript III reverse transcriptase (Invitrogen) were used in reverse transcription reactions to generate cDNA. With each reaction, we performed a negative control in which Superscript III was omitted from the reaction. The products of the reactions, with and without Superscript III, were diluted 50-fold and used as templates for qPCR amplifications using the DyNAmo HS SYBR green kit (Finnzymes) and specific primer pairs designed to amplify 200 bp regions of selected genes (Table 1). Each qPCR reaction contained 10 µl of DyNAmo HS SYBR green mixture, 1.5 µM each of the forward and reverse primers, 4 µl of water and 2 µl of 1:50 diluted reverse transcription product in a total volume of 20 µl.

The levels of specific transcripts were quantified using an MJ Research DNA engine with a Chromo-4 continuous fluorescence detector (Bio-Rad) using the following amplification protocol: 1 cycle at 95°C for 10 min, 40 cycles at 94°C for 10 s, 55°C for 15 s, 72°C for 15 s, and a final incubation at 72°C for 10 min. The melting curve thermal profile, from 65 to 95°C, was used to distinguish specific from non-specific amplification (Ririe et al. 1997). The PCR threshold cycle (C_t value) was determined following baseline subtraction at a constant fluorescence level. Duplicate qPCRs were performed for each sample, and all reactions were repeated independently to evaluate the reproducibility of the results. Data were normalized to the expression of the housekeeping gene *rnpB*, encoding RNaseP. The relative difference between the target gene and the housekeeping gene (control) was calculated using the ΔC_t method according to $\Delta C_t = 2^{-[C_t(\text{target}) - C_t(\text{control})]}$ and the relative fold change between the HL and LL condition (HL/LL) was calculated using the $\Delta\Delta C_t$ method, where $\Delta\Delta C_t = 2^{-\{[C_t(\text{HL}) - C_t(\text{control})] - [C_t(\text{LL}) - C_t(\text{control})]\}}$.

Gene-specific primers (Table 1) were designed using the Primer3 program (Rozen & Skaletsky 2000) with default parameters (http://frodo.wi.mit.edu/primer3/). We designed gene-specific primers for 3 of the 4 *Synechococcus* WH8102 *psbA* gene sequences (*psbA3* and *psbA4* were indistinguishable at the nucleotide level and were treated as a single gene) by creating 50 primer pairs for each of the SYN0983 (*psbA1*), SYN1470 (*psbA2*) and SYN2151/SYN 1919 (*psbA3*/*psbA4*) sequences using Primer3. The resulting table of 50 primer pairs per gene, along with the sequence for each gene, was uploaded into the BioBike biocomputing platform (www.biobike.org), and special purpose Lisp code was used to pull all the sub-sequences that would be extracted by these primers from the genes. These sub-sequences (virtual PCR products) were cross-blasted with one another, and a set of primers was chosen that created a set of resulting sequences with the least combined overlap. To ensure similar amplification efficiencies (difference < 8%) for target and housekeeping genes, standard curves were generated for each set of primers according to Berg et al. (2008).

RESULTS

PSII photochemical efficiency

Maximal F_v/F_m was lower in both taxa in the HL compared with the LL treatment (Fig. 2A). Whereas the decrease in F_v/F_m under HL was a modest 23% for *Prochlorococcus* MED4, the decrease in F_v/F_m was >90% for *Synechococcus* WH8102 (Fig. 2). In *Synechococcus* WH8102 Replicates B and C, the decrease in F_v/F_m was driven by a 70% increase in F_0, from 577 ± 66 to 984 ± 46 (Fig. 2B). In these replicates, F_m under HL was indistinguishable from F_0. In Replicate A, F_0 increased 31% from 550 to 723, but F_m also decreased slightly from 810 to 749 (Fig. 2B). While F_0 increased in *Prochlorococcus* MED4 under HL compared to LL (approximately 50%), it remained much lower than in *Synechococcus* WH8102 (Fig. 2B). Despite the very slight variable fluorescence in HL-exposed *Synechococcus* WH8102, carbon fixation and growth remained relatively high (Fig. 3).

Table 1. Primers used for quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis of transcript levels in *Synechococcus* WH8102 (designated SYNW) and *Prochlorococcus* MED4 (designated PMM)

Putative function	Gene identifier	Gene name	Forward primer	Reverse primer
Ammonium transport	SYNW0253	*amt1*	CCT GGT TCC CAC TGT TGA TT	CTG CCT GCA ACA GGG TAA AT
	PMM0263	*amt1*	CAC GCA GCT GAT TTA CCT GA	ATC CGT CAG CAA CAC TTC CT
Urea transport	SYNW2441	*urtB*	GAC GTG GTT CCT CAA CAA A	CCG AGT AGG GAG ACT GCA AC
	PMM0970	*urtA*	TAC TGC CTC TCT TGC CGT TT	GCT TTT TCC ACC AAC GGT TA
Nitrate transport	SYNW2463	*nrtP*	CGA TCC TCG AGC TCA CCT AC	CAT GCT CAT CAC CAG GTA GCC
Rubisco large subunit	SYNW1718	*rbcL*	GTC TGG AAG ACA TCC GCT TC	GTC CTT GGT GAA GTC CAG ACC
	PMM0550	*rbcL*	CGA TTT GAG TTC GTT GCT G	CCA GTT TGC AAG GCC AGT AT
PSII apoprotein D1:1	SYNW1470	*psbA-1*	GGG AAG CTT TTT GTC AGT GG	CAC CGG AGA TGA TGT TGT TG
PSII apoprotein D1:2	SYNW0983	*psbA-2*	CTC CAC CAA CAA CCG TCT TT	CAT TGC TGG AAG GAA CAA CA
	SYNW2151	*psbA-3*		
	SYNW1919	*psbA-4*	CAA GTT TGG CCA AGA GGA AG	GGT TGA AGT TGA AGC CGT TC
PSII apoprotein D1	PMM0223	*psbA*	ACC AGT TGC TGG TTC ATT CC	CAC TGA CGT CCC ATG TAT GC
PSII chl *a*-binding protein CP47	SYNW1982	*psbB*	GAG TCC ATC CCT GAA AAG C	CGA CAG GGA AGT TCT CGA AG
	PMM0315	*psbB*	CTG GAG CTT CGA AGG TGT TG	GAA AAG CTC CAA ATC CAA AGC
PSII chl *a*-binding protein CP43	SYNW0676	*psbC*	CGC TCT CTA ATC CCG GTC	CGT ACA TGG GTT TGT CGA AG
	PMM1158	*psbC*	GGC CCA AAG CTT TAC TTT CC	CAA GGC TCA ATC CGT TAG G
PSI apoprotein	SYNW2124	*psaA*	ACC TGC TGG CAC AGC TCT ATC	GTA CAT GTG ACC AGC GAC GAT G
PSI apoprotein	PMM1524	*psaA*	TAT GGG CCC AAT CTT CTC AG	CTT GGT TGA ATT GTT GGA GC
Light-harvesting complex protein	PMM0627	*pcb*	CCG GTG CTT TCA CTC TTT TC	ATC CAC CAG CTG CAA GTA CC
Allophycocyanin beta chain	SYNW0484	*apcB*	GCC AAG GCT CTG CTG TAC TC	CAC ACC CAG GGA GTT GTA GG
Phycocyanin II beta chain	SYNW2022	*rpcB*	CGC GAC ATG GAG ATC ATT C	GCG GAA ATA CCA GCT TTG TC
Phycoerythrin I beta chain	SYNW2017	*cpeB*	CGC TAG CCT CCG TTC TTA TG	GCG AAG AAC GAT CTC ACC ATC
Plastid terminal oxidase	SYNW0887	*ptox*	CGA GGC AGT CAA GAC GTA CA	GCT ATT GGC TTT GAC GTG GT
	PMM0336	*ptox*	GCA AGG TCT CCT TAT TTT GC	GGT GCC TTG CTA AAA ACC TG
High-light inducible protein	SYNW1449	*hli1*	GAC TCC GCC TCC AAG ACT C	GCA ATC CGA AGC CGA TCT G
	SYNW0330	*hli2*	GCT TTT GGC TGG AGT GGT TAC	CCA ATG CAG GAA GGT GTC TC
	SYNW0616	*hli3*	GCT TGA GCC CAC TGA CAT C	CAC CAG ATC AGC AGA CCA TG
	SYNW0817	*hli4*	GAC TCC TTC CAC CGA TGC	CAG TTT TTC AGC CCG TTC
	SYNW2403	*hli5*	CAA CTC AAA AGG GGC AAT CAG	CTT CGG TGA TGA TCC CAA TC
	SYNW2180	*hli6*	GTC TGA CAA CGC ACG CTT C	CCG ATC TGG GAC AGG ATG
	SYNW1331	*hli7*	GGC TTT CCA GTA CGA GCA AC	CCC AGT TCG AGA TAC CAA CG
	SYNW1833	*hli8*	GGC TGA GCA ACT CGA AAA G	CAG TTC CCG GCA AAC AC
	PMM1118	*hli4*	GTT TTA ATG GTT GGG CAG CA	GAA CCA ACC TGG AAT GAT TTG
	PMM1404	*hli5*	CGA AAC AAA AAC AGT TGA GAA GG	CGA ATC CAG GAA TAA TTT GAC C
	PMM1398	*hli7*	GAG TCT GGC GGA AGA CAA AA	CCT GGA ATG ATT TGA CCT GTT
	PMM1396	*hli9*	CTG GCA AAA TGC AGA AAG AA	CCC AGG TAT GAT CCA GCC
	PMM1385	*hli11*	ATG AAG AAC AAC GAA CCA AAA	CCA GGA ATA ATT TGA CCT GTG G
	PMM1384	*hli12*	GTT TTA ATG GTT GGG CAG CA	CCA ACC AGG GAT GAT TTG AC
	PMM0816	*hli18*	GCT TTA CTC GTT GCT TCC CTAA	CGA AAC CTG GAA TGA TTT GA
	PMM0690	*hli21*	GGC AAA AAT CAA ATC TGT TGA A	CGA ATC CAG GAA TAA TTT GAC C
Protoporphyrin chelatase subunit	SYNW0716	*chlI*	GAT CCT GAA CTA CGG GTC C	GTC CAT CCA CAT CCA GCT C
	PMM1055	*chlI*	GGC CTT TGA ACC AGG CTT AT	CTC TTC TGG ATT GCC AGA GC
Chlorophyll synthase subunit	PMM0428	*chlG*	GAG CGG ACC ACT TTT AGC AG	CCC ACC TAA AGC CAG AAG AA
Low-light inducible protein	SYNW0667	*irtA*	GAG GGA CTA CAC CCA GAC C	GAT GCT GGC GTA CAG GTT C

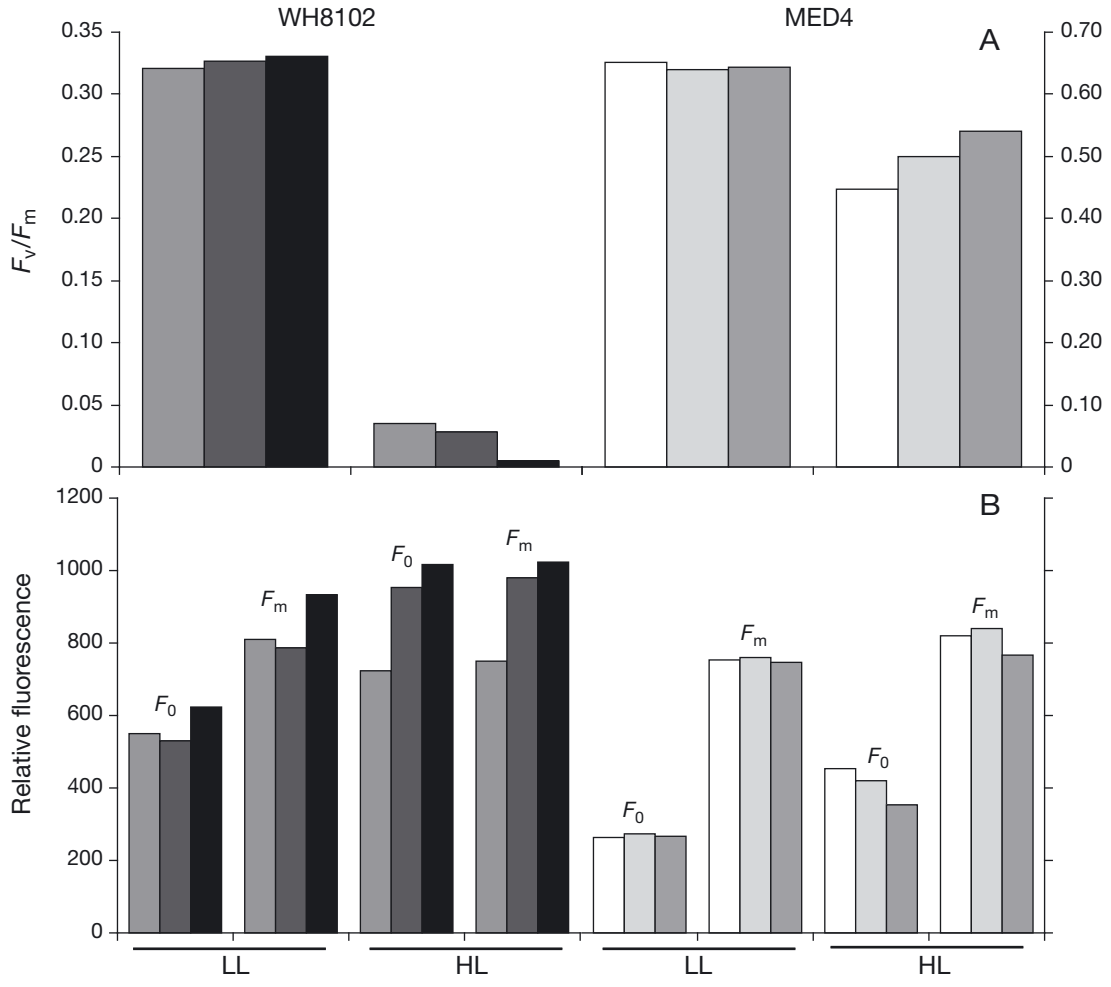

Fig. 2. Fluorescence characteristics of *Synechococcus* WH8102 and *Prochlorococcus* MED4. (A) Photosynthetic efficiency (F_v/F_m) of triplicate *Synechococcus* WH8102 (grey, dark grey and black bars) and *Prochlorococcus* MED4 (white, light grey and grey bars) grown under low-light (LL) and high-light (HL) conditions. (B) F_0 and F_m: fluorescence of triplicate *Synechococcus* WH8102 (grey, dark grey and black bars) and *Prochlorococcus* MED4 (white, light grey and grey bars) grown under low-light (LL) and high-light (HL) conditions

Carbon fixation

Although the P^*_{max} in *Prochlorococcus* MED4 was 3-fold greater under HL than under LL (Fig. 3A), there was no difference in α^* between the 2 light treatments (Table 2). In contrast, P^*_{max} and α^* for *Synechococcus* WH8102 were >2-fold and 5-fold higher, respectively, in the LL treatment than in the HL treatment (Fig. 3B, Table 2). Under LL, P^*_{max} was 5-fold greater in *Synechococcus* WH8102 than in *Prochlorococcus* MED4. Under HL, P^*_{max} was nearly the same for *Synechococcus* WH8102 and *Prochlorococcus* MED4 (Fig. 3, Table 2). Interestingly, both taxa displayed similar E_k values for growth under HL (124 to 174 µmol photons m^{-2} s^{-1}) and LL (52 to 54 µmol photons m^{-2} s^{-1}) conditions (Table 2).

Table 2. Photosynthesis–irradiance variables. P^*_{max} = the maximum chl *a*-normalized rate of carbon fixation (µg C μg^{-1} chl *a* h^{-1}); α^* = the chl *a*-normalized initial slope of the photosynthesis–irradiance (P–E) curve, an index of photosynthetic efficiency (µg C μg^{-1} chl *a* h^{-1}) (µmol photons m^{-2} $s^{-1})^{-1}$; E_k = the irradiance at which photosynthesis approaches saturation, an index of photoacclimation (P^*_{max}/α^*, µmol photons m^{-2} s^{-1}). WH8102 = *Synechococcus* WH8102; MED4 = *Prochlorococcus* MED4; HL: high light; LL: low light

Parameter	Organism	HL	LL
P^*_{max}	WH8102	2.11	4.3
	MED4	2.44	0.84
α^*	WH8102	0.017	0.08
	MED4	0.014	0.016
E_k	WH8102	124	54
	MED4	174	52

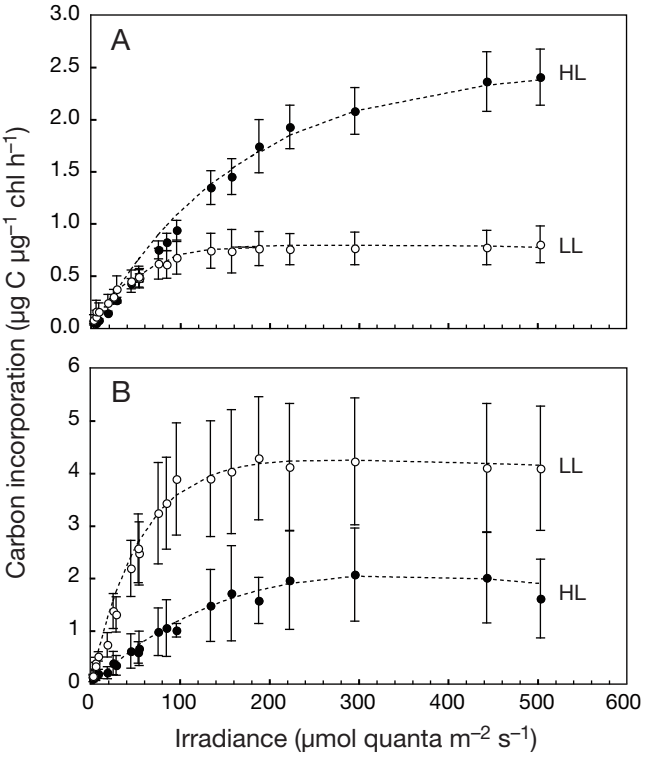

Fig. 3. Carbon fixation of (A) *Prochlorococcus* MED4 and (B) *Synechococcus* WH8102 under low-light (LL, ○) and high-light (HL, ●) treatments. Means ±SD of triplicate cultures are shown

Relative transcript abundance (ΔC_t)

Based on preliminary results we identified a number of genes involved in photosynthesis and the HLIPs (data not shown) that were differentially regulated under HL. For example, we identified 8 *hli* genes out of the 22 present in *Prochlorococcus* MED4 that were upregulated under HL compared to LL. In addition to these genes, we assayed the expression of genes encoding PSI and PSII reaction center proteins, light-harvesting proteins and biliproteins, chl *a* synthesis proteins, and nitrogen transporters. We chose primers with similar priming efficiencies (see 'Materials and methods') to facilitate comparison of expression across genes. However, we also compared absolute transcript abundance, calculated from the slope of the standard curve (noted in parentheses), with ΔC_t in Fig. 4. The most abundant transcripts in LL-acclimated *Prochlorococcus* MED4 were *amt1* (0.0105 ± 0.001 ng mRNA µg^{-1} TRNA) encoding the ammonium transporter, *pcb* (0.0110 ± 0.008 ng mRNA µg^{-1} TRNA) encoding the light-harvesting antenna protein, and *psbA* (0.0098 ± 0.0009 ng mRNA µg^{-1} TRNA) encoding the PSII reaction center protein D1 (Fig. 4A). Other transcripts that accumulated to high levels were *ptox* (0.00062 ± 0.0002 ng mRNA µg^{-1} TRNA) encoding the plastid terminal oxidase, and *rbcL* (0.0002 ± 0.00003 ng mRNA µg^{-1} TRNA) encoding Rubisco (Fig. 4A). In relative terms, the *hli* genes comprised the least expressed of the genes we assayed (Fig. 4A). Similar to *Prochlorococcus* MED4, the most abundant transcript in *Synechococcus* WH8102 under LL was *amt1* (0.0117 ± 0.005). However, the second most abundant transcript was *rbcL* (0.0042 ± 0.0004), followed by *psbA3/4* (0.0032 ± 0.001) and *psaA* (0.0016 ± 0.0003), the latter encoding the PSI reaction center protein (Fig. 4B). The abundance of the *ptox* transcript in *Synechococcus* WH8102 was relatively low (0.00005 ± 0.000008), similar to the levels measured for the *hli* transcripts (Fig. 4B).

Transcript induction ($\Delta\Delta C_t$) HL/LL

Here we define the term induction as the fold difference in relative transcript abundance in HL-acclimated over LL-acclimated cells. Transcripts from genes encoding proteins involved in N uptake, C fixation, and chl *a* synthesis were either not induced or were depressed in both species (Fig. 5A). A notable exception was *nrtP*, encoding the nitrate transporter in *Synechococcus* WH8102, which was induced 4-fold (Fig. 5A). Transcripts from the chl *a* synthesis genes *chlI* and *chlG* were depressed, as was the transcript from the LL inducible gene *irtA*, which was 2-fold depressed in *Prochlorococcus* MED4 and 5-fold depressed in *Synechococcus* WH8102 (Fig. 5A). Although no physiological function has been assigned to the IrtA protein, the gene has been shown to be induced under LL (relative to HL) in *Prochlorococcus* sp. and, as such, was used here as a negative control. In both species, the *rbcL* transcript was depressed under HL. However, it was significantly more depressed in *Synechococcus* WH8102 (17-fold) compared with *Prochlorococcus* MED4 (3.5-fold), consistent with changes in $P^{\star}_{\max}$ (Fig. 3, Table 2).

In *Prochlorococcus* MED4, the *hli* gene transcripts assayed here were induced 4- to 14-fold, and the 4 most highly induced were *hli7* (14 ± 2), *hli18* (13 ± 4), *hli9* (11 ± 10) and *hli5* (7 ± 6). In *Synechococcus* WH8102, the most highly induced *hli* gene transcripts were *hli6* (5 ± 2), *hli4* (4 ± 1), *hli1* (3 ± 0.3) and *hli5* (2 ± 0.3) (Fig. 5B). Average induction for the 4 most highly induced *hli* gene transcripts was 11-fold in *Prochlorococcus* MED4 and 3.5-fold in *Synechococcus* WH8102. Not all the *hli* gene transcripts were induced under HL in *Synechococcus* WH8102; *hli8* and *hli2* were depressed 1.5-fold whereas the levels of *hli3 and hli7* transcripts did not vary between LL and HL (Fig. 5B). The *ptox* gene transcript was induced 2.5 ± 1.3 in *Prochlorococcus* MED4 and depressed 2.5-fold in *Synechococcus* WH8102 (Fig. 5B).

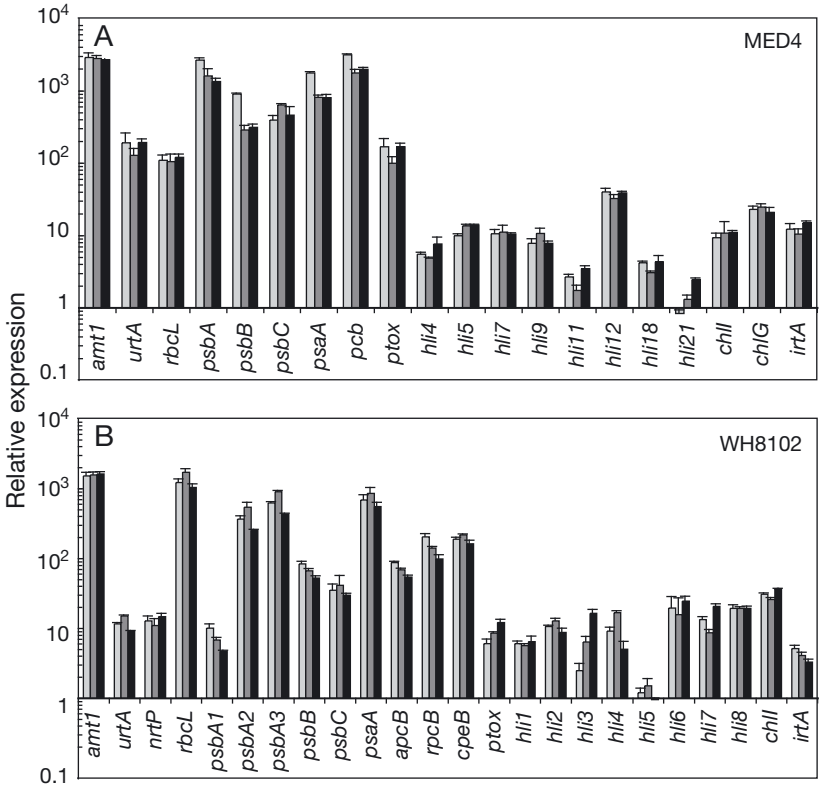

Fig. 4. Relative log expression (ΔC_t) of transcripts associated with nitrogen uptake (*amt1*, *urtA*, *nrtP*), carbon fixation (*rbcL*), photosynthetic reaction centers (*psbA*, *psbB*, *psbC*, *psaA*), light harvesting (*pcb*, *apcB*, *rpcB*, *cpeB*), excess energy dissipation (*ptox* , *hli*), chlorophyll synthesis (*chlI, chlG*) and low light (*irtA*) in (A) *Prochlorococcus* MED4 and (B) *Synechococcus* WH8102 grown under low-light (LL) conditions. Duplicate measurements (+SD) for each of 3 cultures (shaded light grey, grey, black) are shown. Transcript *psbA3/4* has been abbreviated to *psbA3*

Synechococcus WH8102 *psbA* composition

The differential induction of the *psbA* gene transcripts in *Synechococcus* WH8102 resulted in a change in the composition of the total *psbA* transcript pool between the LL and HL treatments. Under LL, *psbA3/4* contributed 80%, *psbA2* contributed 17%, and *psbA1* contributed 3% of total *psbA* transcript abundance (Fig. 6). Whereas the contribution of *psbA3/4* remained at 80% under HL, the *psbA2* contribution decreased to 1% and the contribution of *psbA1* increased to 18% of total *psbA* transcript abundance under HL (Fig. 6).

DISCUSSION

Changes in the relative transcript abundances of the *psbA*, *hli* and *ptox* gene families in response to increased irradiance differed significantly between *Synechococcus* WH8102 and *Prochlorococcus* MED4. Our results suggest that the regulation of these gene families, and in some cases their functions, also varies between these 2 cyanobacterial species.

Compared with the induction of the *hli* gene transcripts, the induction of the gene transcripts encoding the reaction center proteins was low in *Prochlorococcus* MED4. Only *psbA*, which encodes the D1:1 isoform, was induced (2.5 ± 0.5). In contrast, *psbA2* (encoding D1:2) was significantly induced (60 ± 5) in *Synechococcus* WH8102 followed by *psbA3/4*, also encoding D1:2 (7 ± 1). In contrast, *psbA1*, which encodes D1:1, was depressed 3-fold (Fig. 5C). Both *psbB* (2 ± 0.1) and *psbC* (8 ± 1) genes, encoding the CP47 and CP43 chlorophyll-binding proteins, respectively, were induced in *Synechococcus* WH8102. In contrast, *psbB* was depressed 2.6-fold and *psbC* (1.5 ± 0.3) was not significantly regulated in *Prochlorococcus* MED4 (Fig. 5C). In both species, *psaA* (encoding the PSI reaction center apoprotein) was depressed (Fig. 5C) while in *Prochlorococcus* MED4, *pcb* (encoding the light-harvesting antenna proteins) was slightly depressed. There was no change in the *apcB*, *rpcB* and *cpeB* genes encoding the biliproteins allophycocyanin, phycocyanin and phycoerythrin, respectively, in *Synechococcus* WH8102 (Fig. 5C).

psbA transcript induction and photoinhibition

HL-acclimated *Prochlorococcus* MED4 cultures recovered their F_v/F_m to near maximum following an 8-fold shift in light intensity after a short period of acclimation. In contrast, a >90% reduction in PSII photochemical efficiency in *Synechococcus* WH8102 cultures following a 3-fold shift in light intensity suggested that they were severely photoinhibited. In the latter cultures, the individual *psbA* transcripts exhibited marked changes in abundance between LL and HL. Following exposure of cells to HL, the D1:1 isoform encoded by *psbA1* declined, while the D1:2 isoform encoded by *psbA2–4* increased 7- to 60-fold. In freshwater *Synechococcus* PCC 7942, an increase in D1:2 transcription and D1:2 polypeptide composition of the PSII reaction center is associated with greater resistance to photoinhibition through improved dissipation of excess absorbed light energy (Schaefer & Golden 1989a, Krupa et al. 1991, Clarke et al. 1993, Campbell et al. 1996). Unlike the situation in freshwater *Synechococcus*

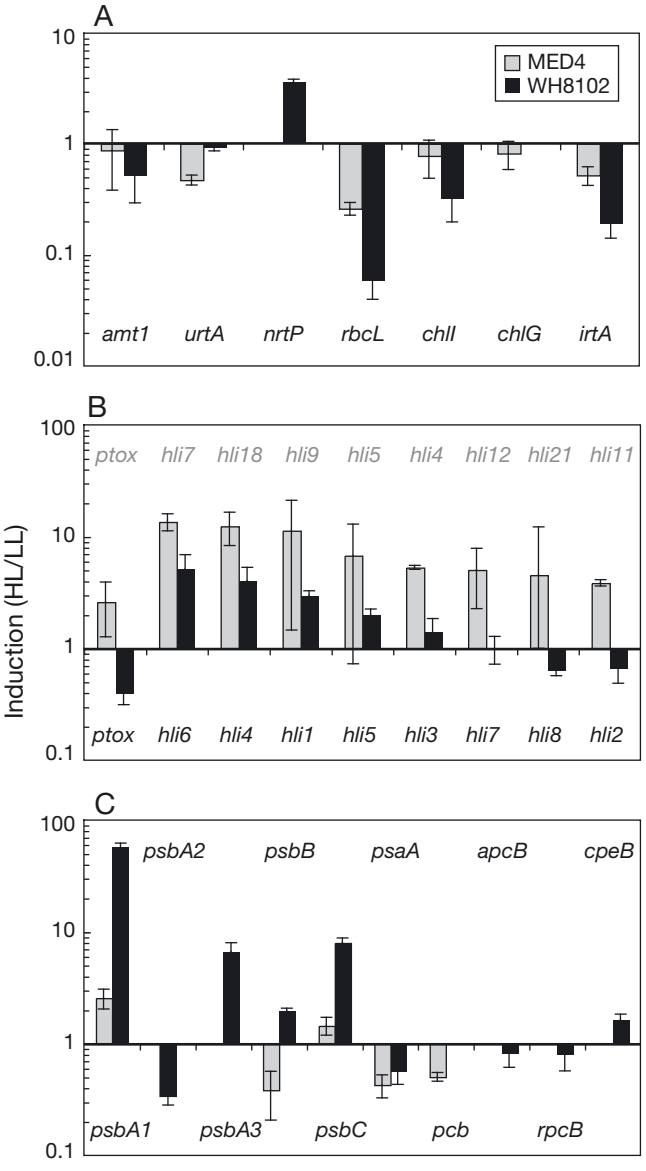

Fig. 5. Induction (comparative relative expression, $\Delta\Delta C_t$, high light (HL)/low light (LL)) of transcripts associated with (A) nitrogen uptake (*amt1*, *urtA*, *nrtP*), carbon fixation (*rbcL*), chlorophyll synthesis (*chlI, chlG*) and low light (*irtA*); (B) excess energy dissipation (*ptox*, *hli*); and (C) photosynthetic reaction centers (*psbA*, *psbB*, *psbC*, *psaA*) and light harvesting (*pcb*, *apcB*, *rpcB*, *cpeB*) in *Prochlorococcus* MED4 and *Synechococcus* WH8102. Means ±SD of triplicate cultures are shown

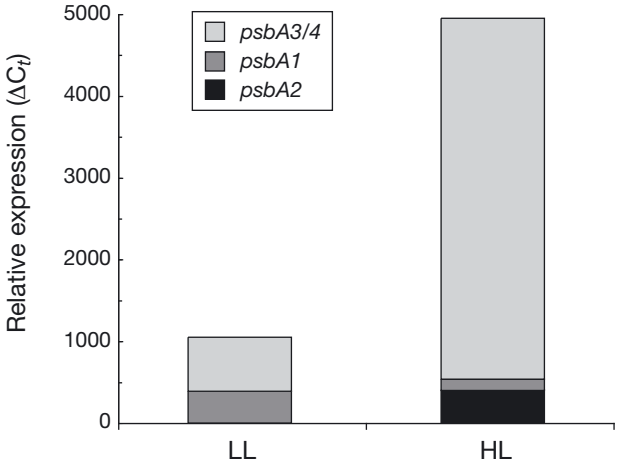

Fig. 6. Absolute changes in the *psbA* gene pool in *Synechococcus* WH8102 grown under low-light (LL) and high-light (HL) conditions

PCC 7942, PSII photochemical efficiency does not appear to be restored by increased transcription of D1:2 polypeptide in marine *Synechococcus* WH8102 or in *Synechococcus* WH7803 (Six et al. 2007a, Garczarek et al. 2008). In both of these marine species, the F_v/F_m remains depressed despite substantial increases in *psbA* mRNA following HL and UV treatments (Six et al. 2007a, Garczarek et al. 2008). Potential explanations for this include the fact that either damaged D1 proteins are not being replaced rapidly enough to overcome photodamage (Six et al. 2007a), or a high level of intracellular oxidative stress elicits damage of newly synthesized D1:2 proteins before they have had a chance to substitute the damaged D1:1 proteins in the reaction center (Garczarek et al. 2008).

If most D1 proteins in PSII were non-functional, resulting in an F_v/F_m approaching zero, we would expect that most cells would be unable to perform photosynthesis and rapidly lose viability. Contrary to this expectation, HL-acclimated *Synechococcus* WH8102 cultures continued to grow fairly well despite their severely reduced F_v/F_m. This finding may be explained by factors that serve to depress F_v/F_m independently of PSII damage. As mentioned previously, F_v/F_m is not directly comparable between species that have a PBS light-harvesting system and those that have a chlorophyll-based antenna. One reason for this is that the PBS contributes to F_0 fluorescence, thereby decreasing the amount of variable fluorescence and the value of F_v/F_m. Therefore, it is important to determine whether a decrease in F_v/F_m in *Synechococcus* is caused by a decrease in F_m, an increase in F_0, or both. As noted in Fig. 2B, the decrease in F_v/F_m in HL-acclimated *Synechococcus* WH8102 was driven mostly by an increase in F_0, in striking contrast to the situation for *Prochlorococcus* MED4, suggesting that the decrease in F_v/F_m resulted from an increase in PBS fluorescence. Such an increase could result from a decoupling of PBS subcomplexes from the thylakoid membranes, potentially reducing the transfer of energy from the PBS to the PSII reaction center, thereby elevating fluorescence emissions. Six et al. (2007a) suggested — on the basis of degradation of the L_{CM} linker polypeptide (large chromophorylated protein that an-

chors the PBS to the thylakoid membrane) — that a large number of PBS units become disconnected from the membranes following exposure of *Synechococcus* WH8102 to UV irradiation. These authors also suggested that the release of highly fluorescent phycobiliproteins in the cytosol constitutes a photoprotective mechanism that may prevent excess photons from reaching PSII. As such, PBS uncoupling and the ensuing increase in F_0 fluorescence may mask the functionality of the PSII reaction center by depressing F_v/F_m in a manner that is not proportional to D1 damage.

While changes in F_v/F_m do not appear proportional to PSII damage in *Synechococcus* WH8102, elevated *psbA* transcript abundance coupled with a 2-fold decrease in P^*_{max} and a 5-fold reduction in α^* indicated that the HL-acclimated *Synechococcus* WH8102 cultures were suffering from D1 photodamage (cf. Behrenfeld et al. 1998). Our results also indicated that *Synechococcus* WH8102 can acclimatize to, and grow under, conditions that lead to persistent photoinhibition. However, it is still unclear why *Synechococcus* WH8102 was not able to recover fully from photoinhibition. Perhaps the answer lies in the efficiency with which the light-harvesting antennae funnel excitation energy to the PSII reaction center. Photoinhibition can result from even moderate light exposure if excess light-harvesting antennae are present relative to chl *a*. For example, in *Synechococcus* PCC 7942 the extent of photoinhibition following a light shift depends, in a linear fashion, on the ratio antennae pigment:chl *a* (Clarke et al. 1995). Because the amount of pigment is, to a large extent, dictated by concentrations of external nitrogen (N) (Yamanaka & Glazer 1980, Wyman et al. 1985, Glibert et al. 1986, Collier & Grossman 1992), shifting marine *Synechococcus* grown under N-sufficient conditions from low to high light may result in a degree of photodamage that the cells cannot recover from due to the large ratio of antennae pigment to chl *a* existing in the cells prior to the light shift. In nature, conditions of high nutrients and high irradiance very seldom co-occur, and cyanobacteria in the upper layers of the oligotrophic ocean potentially never develop large antennae pigment:chl *a* ratios.

hli transcript induction and photoprotection

In addition to the efficient transfer of excitation energy by the PBS to the PSII reaction center, the ease with which *Synechococcus* WH8102 cultures became photoinhibited relative to *Prochlorococcus* MED4 cultures may be related to differences in their respective strategies of photoprotection, potentially driven by differences in *hli* transcript induction. On average, *hli* transcript induction was 5-fold greater in HL-acclimated *Prochlorococcus* MED4 than in HL-acclimated *Synechococcus* WH8102. The genes associated with the 3 most upregulated transcripts (*hli7*, *hli18* and *hli9*) belong in the *hli6–9* and *hli16–19* operons in the *Prochlorococcus* MED4 genome (Bhaya et al. 2002). Of all the *Prochlorococcus* MED4 *hli* genes, the genes in these 2 operons have been reported to be the most responsive to shifts in light intensity (Steglich et al. 2006). Expression of *hli5* and *hli4* also increased significantly, consistent with earlier observations by Steglich et al. (2006). We did not survey the levels of *hli1*, *hli2*, *hli10*, *hli13* and *hli20* transcripts because preliminary tests suggested that light intensity did not significantly alter their expression. In an investigation by Steglich et al. (2006), the levels of transcripts from these genes were similar under all light treatments, suggesting that factors other than light may regulate expression of these genes. In contrast to *Prochlorococcus* MED4, only 2 *hli* genes (*hli6* and *hli4*) were induced to a significant extent in HL-acclimated *Synechococcus* WH8102. If the *hli* genes do encode proteins that dissipate excess light energy (He et al. 2001, Havaux et al. 2003), then this result is surprising because all genes encoding proteins involved in excess energy dissipation would be expected to increase under conditions of persistent photoinhibition. The difference in the number of *hli* genes that were induced in *Synechococcus* WH8102 and *Prochlorococcus* MED4, and the extent to which they were induced in response to growth at higher irradiance, suggests that a greater number of the HLIPs are involved in excess energy dissipation and photoprotection in *Prochlorococcus* MED4 than in *Synechococcus* WH8102. However, translational regulation of HLIP activity may or may not be related to transcription such that greater transcriptional HLIP regulation in *Synechococcus* WH8102 may make up for the decreased transcription compared with *Prochlorococcus* MED4.

ptox transcript abundance and excess energy dissipation

PTOX represents another putative pathway for dissipating excess absorbed excitation energy (Bailey et al. 2008). Based on the differences in transcript levels measured in this study, the *ptox* gene appears to be more active in *Prochlorococcus* MED4 than in *Synechococcus* WH8102; in the former, the basal *ptox* transcript level under LL was on par with that of *rbcL*, and it increased in the HL-acclimated cultures, suggesting that irradiance plays a role in its expression. This is consistent with the results of Steglich et al (2006), who demonstrated a 3- to 4-fold increase in *ptox* transcript levels in *Prochlorococcus* MED4 following exposure to light after a dark period. In contrast, *ptox* expression in

Synechococcus WH8102 was barely detected in cells maintained under LL (150-fold lower than *rbcL* expression) and it decreased in the HL-acclimated cells; these results do raise questions about the function of PTOX in *Synechococcus* WH8102.

The interpretation of the role of PTOX is complicated by the fact that this oxidase is at the intersection of many redox pathways associated with the PQ pool. These pathways include oxidation of the PQ pool reduced by PSII (Shahbazi et al. 2007, Bailey et al. 2008), by NDH or other reductases (Bennoun 1982, Peltier et al. 1987, Bennoun 2002, Rumeau et al. 2007), and by phytoene desaturase, which is associated with the desaturation reactions of carotenoid biosynthesis (Wetzel et al. 1994, Carol et al. 1999, Josse et al. 2000, Kuntz 2004). It appears that the importance of PTOX in these various pathways differs among photosynthetic organisms. For example, in *Arabidopsis*, changes in PTOX expression and protein accumulation do not alter the redox state of the PQ pool and do not prevent photoinhibition (Rosso et al. 2006), as has been suggested for tomatoes and cyanobacteria (Shahbazi et al. 2007, Bailey et al. 2008). Rather than preventing PSII photoinhibition, it has been suggested that PTOX, in concert with NDH, regulates cyclic electron flow around PSI by maintaining the redox state of the PQ pool in a manner that prevents PSI photoinhibition (Rumeau et al. 2007).

Most organisms that exhibit PTOX activity also synthesize carotenoids (Carol & Kuntz 2001). If carotenoid synthesis is upregulated in HL-acclimated *Prochlorococcus* MED4 cultures, it may be difficult to distinguish the role of PTOX in re-oxidizing the PQ pool that has been reduced by PSII versus its role in the desaturation of phytoene, or other carotenoid synthesis reactions. It may well be that, in cyanobacteria, PTOX has a dual role (Shahbazi et al. 2007). To date, most investigations show that *ptox* is transcribed in the light, and that its transcription increases under increased irradiance (Simkin et al. 2003, Steglich et al. 2006). Because α-carotene is an important pigment in *Prochlorococcus* MED4, and its cellular concentration is modulated by growth light intensity (Moore et al. 1995), the potential role of PTOX in the carotenoid synthesis pathway deserves closer examination.

Using *psbA* induction as a proxy for acclimation to changes in irradiance (cf. Clarke et al. 1993, Campbell et al. 1998b, Six et al. 2007a,b), we observed that the induction ratio of *ptox*:*psbA1* and *hli*:*psbA1* was 144 and 70 times greater, respectively, in *Prochlorococcus* MED4 than in *Synechococcus* WH8102 (Fig. 7). These observations suggest that induction of *hli* and *ptox* genes may help to alleviate excitation pressure in *Prochlorococcus* MED4 and reduce the need for *psbA* induction. In contrast, induction of *psbA* and synthesis of the PSII reaction center protein D1 may play a principal role in the acclimation of *Synechococcus* WH8102 to changes in irradiance, which may explain why cells experience photoinhibition and PBS uncoupling following a shift to higher growth light intensities (Six et al. 2004, 2007a).

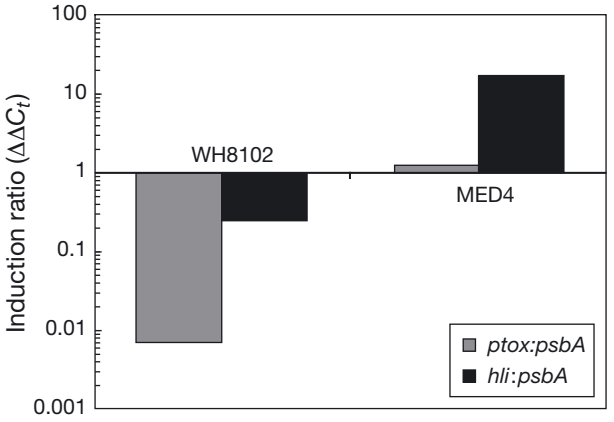

Fig. 7. Ratio of ΔΔC_t*ptox*:ΔΔC_t*psbA* and ΔΔC_t*hli*:ΔΔC_t*psbA* in *Synechococcus* WH8102 and *Prochlorococcus* MED4

In summary, it appears that the 2 cyanobacterial species investigated here have adapted different photoacclimation strategies to life in the oligotrophic ocean, raising the possibility that factors other than irradiance control the distribution of these phytoplankton in the water column. *Synechococcus* WH8102 appears optimized for high rates of carbon fixation and relies on synthesis and replacement of the PSII reaction center protein D1 as its primary mechanism of photoprotection. In contrast, *Prochlorococcus* MED4 appears to have evolved photoprotective strategies potentially involving energy dissipation via HLIP synthesis and via alternative electron flow to PTOX, although alternative electron flow to PTOX in *Synechococcus* WH8102 has also been noted (Bailey et al. 2008). Because both the *hli* and *ptox* gene families are present in these 2 species, but are regulated differently by irradiance, it is likely that the genes in these families have different functions in *Synechococcus* WH8102 and *Prochlorococcus* MED4.

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