

## ORIGINAL ARTICLE

# *In situ* dynamics of O<sub>2</sub>, pH and cyanobacterial transcripts associated with CCM, photosynthesis and detoxification of ROS

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**The relative abundance of transcripts encoding proteins involved in inorganic carbon concentrating mechanisms (CCM), detoxification of reactive oxygen species (ROS) and photosynthesis in the thermophilic cyanobacterium *Synechococcus OS-B'* was measured in hot spring microbial mats over two diel cycles, and was coupled with *in situ* determinations of incoming irradiance and microenvironmental dynamics of O<sub>2</sub> and pH. Fluctuations in pH and O<sub>2</sub> in the mats were largely driven by the diel cycle of solar irradiance, with a pH variation from ~7.0 to ~9.5, and O<sub>2</sub> levels ranging from anoxia to supersaturation during night and day, respectively. Levels of various transcripts from mat cyanobacteria revealed several patterns that correlated with incident irradiance, O<sub>2</sub> and pH within the mat matrix. Transcript abundances for most genes increased during the morning dark–light transition. Some transcripts remained at a near constant level throughout the light period, whereas others showed an additional increase in abundance as the mat underwent transition from low-to-high light (potentially reflecting changes in O<sub>2</sub> concentration and pH), followed by either a decreased abundance in the early afternoon, or a gradual decline during the early afternoon and into the evening. One specific transcript, *psbA1*, was the lowest during mid-day under high irradiance and increased when the light levels declined. We discuss these complex *in situ* transcriptional patterns with respect to environmental and endogenous cues that might impact and regulate transcription over the diel cycle.**

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## Introduction

In light-exposed extreme environments, such as hypersaline ponds or geothermal springs, perennial surface-associated microbial communities form densely populated mm–cm thick mats composed of an exopolymer matrix harboring a strictly microbial assemblage of photosynthetic primary producers and heterotrophic microorganisms (Teske and Stahl, 2002). Owing to the absence of bioturbation and grazing by metazoans, such mats are ideal

model systems for ecophysiological and ecological studies of microbes, their metabolic interactions, adaptation and evolution (Ward *et al.*, 2008).

Mass transfer in cyanobacterial mats is strongly diffusion limited, causing development of steep chemical gradients (de Beer and Kühl, 2001; Wieland *et al.*, 2001), and strong light attenuation typically confines the euphotic zone from sub mm to a few mm thick layer in most mats (Kühl *et al.*, 1997). As a consequence of a high density of oxygenic phototrophs and the impedance of mass transfer within the exopolymer matrix, there is O<sub>2</sub> supersaturation, high pH and marked CO<sub>2</sub> depletion in the upper layers of photosynthetic microbial mats under high irradiance (de Beer *et al.*, 1997; Wieland and Kühl, 2006). These conditions may result in photorespiration, severe CO<sub>2</sub> limitation, as well as significant photoinhibition and reactive

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oxygen species (ROS) formation. However, microbial mats can sustain high rates of photosynthesis under high irradiance, suggesting that the cyanobacteria in the mats have efficient inorganic carbon concentrating mechanisms (CCM) and can either control the production of ROS or ameliorate its potential toxic effects. Yet, both the presence and regulation of CCM activity and ROS detoxification are virtually unstudied in mat-dwelling cyanobacteria (cf. recent reviews by Price *et al.*, 2008; Latifi *et al.*, 2009; but see Kilian *et al.* (2007)).

Studies of CCM in cyanobacteria, its regulation by CO<sub>2</sub> levels, the ways in which it has been tailored to growth in specific environments and how it impacts other cellular reactions, including those that generate ROS, have mostly been performed with the freshwater planktonic model organisms *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942 (Price *et al.*, 2008; Raven *et al.*, 2008). These organisms have been maintained in culture for decades under laboratory conditions in which there is little fluctuation in pH, O<sub>2</sub> and CO<sub>2</sub> levels.

Studies of the ways in which cyanobacteria adjust to low CO<sub>2</sub> conditions have primarily focused on the CCM, which allows cells to concentrate inorganic carbon (Ci; in this study defined as CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup>) against a concentration gradient and to generate a high concentration of CO<sub>2</sub> around ribulose-1,5-bisphosphate carboxylase (Tchernov *et al.*, 2003; Kaplan *et al.*, 2008). Cyanobacteria possess a number of different genes that are induced during Ci deprivation, which allow cells to acquire and fix Ci more efficiently (Volokita *et al.*, 1984; Badger and Price, 1990; Omata *et al.*, 1999; Shibata *et al.*, 2001; Maeda *et al.*, 2002; Price *et al.*, 2004; Zhang *et al.*, 2004; Koropatkin *et al.*, 2007; Folea *et al.*, 2008; Xu *et al.*, 2008); these low Ci-induced genes encode polypeptides that are considered as components of the CCM (Kaplan *et al.*, 2008). The majority of ribulose-1,5-bisphosphate carboxylase in cyanobacteria is localized to a specialized microcompartment, the carboxysome, and the structural and catalytic components of the carboxysome are also considered as part of the CCM (Price *et al.*, 2008).

The high O<sub>2</sub> tension found within cyanobacterial mats during mid-day may result in elevated ROS generation (Jamieson *et al.*, 1986). Levels of ROS may also increase as a consequence of both ultraviolet irradiation and excess excitation of the photosynthetic apparatus by high levels of photosynthetically active radiation (400–700 nm). ROS can severely damage cellular components, including the photosynthetic apparatus itself (Aro *et al.*, 2005; Bouchard *et al.*, 2006). Photoinhibition, a consequence of photodamage to photosystem II, can lead to a further rise of ROS levels. Oxidative stress in phototrophs can be alleviated by the synthesis of various enzymes (for example, glutathione peroxidase, glutathione S-transferases, thioredoxins and superoxide dismutase) that help minimize ROS-elicited damage (Goyer *et al.*, 2002; Sarkar *et al.*,

2005; Dayer *et al.*, 2008). Harmful effects of light on phototrophs will also depend on Ci availability, as well as on environmental levels of other nutrients, for example, nitrogen, phosphorus, sulfur and iron (Takahashi and Murata, 2008).

The microbial mats in the effluent channels of the alkaline siliceous hot springs, Mushroom and Octopus Springs (Yellowstone National Park, WY, USA), have been the subject of detailed biogeochemical and microbiological investigations for >40 years (Brock, 1967; Ward *et al.*, 1998, 2006). At temperatures >55°C, these mats are exclusively colonized by prokaryotes, including oxygenic photoautotrophic cyanobacteria in the genus *Synechococcus* spp. and anoxygenic filamentous phototrophic bacteria, *Roseiflexus* spp. and *Chloroflexus* spp.; many other functional groups inhabit these microbial ecosystems, but the photosynthetic microbes are dominant inhabitants (Ward *et al.*, 1998, 2006; Bhaya *et al.*, 2007). Two ecologically relevant cyanobacteria, *Synechococcus* OS-B' and *Synechococcus* OS-A (designated Syn OS-B' and Syn OS-A), have been isolated from the mats of Mushroom and Octopus Springs (Allewalt *et al.*, 2006), and their genomes were sequenced along with metagenomic sequencing of DNA isolated from microbes in the top layer of the microbial mat (Bhaya *et al.*, 2007). This genomic information provides a strong foundation for studies of cyanobacterial *in situ* gene expression over the diel cycle (Steunou *et al.*, 2006, 2008).

We investigated the *in situ* dynamics of the abundance of transcripts from Syn OS-B' (and cyanobacteria with similar genes) encoding proteins involved in CCM, photosynthesis and ROS detoxification, to resolve diel expression patterns and gain insights into mechanisms controlling expression of these genes in mat-dwelling, thermophilic cyanobacteria. These studies were combined with detailed mapping of temperature, incident irradiance and microenvironmental analysis of pH and O<sub>2</sub> conditions in the mat over the diel cycle.

## Materials and methods

### Sample site and collection

Sampling and *in situ* measurements were performed over two diel cycles on 30 June–1 July 2005 and 13–14 September 2008 in an effluent channel of Mushroom Spring, Yellowstone National Park. Temperature at the sampling site was 56–61°C during each diel cycle. The downwelling quantum irradiance was measured continuously with a datalogger equipped with a quantum irradiance sensor (LI-1400D, LI190, LI-COR Biosciences, Lincoln, NE, USA). Mat samples (~1.0 cm<sup>2</sup>) were collected, the top 2 mm were excised, cut in half and frozen in liquid N<sub>2</sub>, as described previously (Steunou *et al.*, 2008). Although extensive sampling in 2005 was allocated toward the dark-to-light and

light-to-dark transition periods, more samples were collected during daytime in 2008.

#### Microsensor measurements

Microprofiles of the O<sub>2</sub> concentration in the microbial mat were measured *in situ* in vertical steps of 50–200 µm with a Clark-type O<sub>2</sub> microelectrode (Revsbech, 1989) connected to a battery-operated pA meter (PA2000, Unisense A/S, Århus, Denmark), as described in detail elsewhere (Steunou *et al.*, 2006, 2008). Based on data from numerous O<sub>2</sub> microprofiles, isopleth diagrams of O<sub>2</sub> levels in the mat over the diel cycle were generated with the software package Origin Pro 7.5 (OriginLab Corp., Northampton, MA, USA).

Microprofiles of pH were measured *in situ* using a pH glass microelectrode (Thomas, 1978; Kühl and Revsbech, 2001) and a standard calomel reference electrode connected to a high impedance mV-meter (VF-4, WPI Inc., Sarasota, FL, USA). Linear calibrations of the mV readings were performed in standard buffers at pH 4.0, 7.0 and 10.0 at *in situ* temperatures, yielding near-Nernstian sensor responses of 54–57 mV per pH unit change.

#### Gene annotation

The genome of Syn OS-B' was examined to identify genes encoding proteins involved in CCM and

detoxification of ROS; resources used to identify these genes were Genbank annotation, KEGG genomic data (Kanehisa and Goto, 2000) and homologies to well studied genes in model organisms. Primers used to analyze transcript abundances (Table 1) were designed to specifically target transcripts from Syn OS-B', based on full genome sequence information (NC\_007776 for Syn OS-B' and NC\_007775 for Syn OS-A; Bhaya *et al.*, 2007), and homologous sequences likely associated with variant mat cyanobacteria observed in the metagenomic database (<http://blast.jcvi.org/yellowstone-blast/index.cgi?project=gym>; Bhaya *et al.*, 2007). Primers were tested on genomic DNA from an axenic culture of Syn OS-B' and a unicyanobacterial culture of Syn OS-A, as positive and negative controls, respectively. All primers were specific except for those used to quantify transcripts from CYB\_1611 (putative *bicA*), which amplified the same fragment from the genomes of Syn OS-A and Syn OS-B'.

#### RNA extraction and DNase treatment

RNA from microbial mat samples collected in 2005 was extracted as described (Steunou *et al.*, 2008). A slightly modified protocol was used to extract the RNA from samples collected in 2008. In this protocol the initial vortexing and cell harvesting steps were removed. Instead, mat pieces were

**Table 1** Primers used for qPCR analysis of *in situ* transcript levels in *Synechococcus* OS-B' (genome accession number NC\_007776)

Gene	Locus tag	Forward primer	Reverse primer
<i>Carboxysome assembly proteins</i>			
<i>ccmM</i>	CYB_1794	5'-CCACAGCCTACGTCATTCT-3'	5'-AGTAGGGTTGCCGTCTTCT-3'
<i>ccmN</i>	CYB_1793	5'-TCGCTATTTTGTTCAGTGGTGA-3'	5'-CCCATTCCCAAGGTTGCT-3'
<i>Ci-fixation</i>			
<i>rbcL</i>	CYB_2579	5'-GTGGAGCGGGATAAGTTGAA-3'	5'-CAGCCTCCATCACGAACAAGT-3'
<i>CO<sub>2</sub> hydration</i>			
<i>chpX</i>	CYB_1810	5'-GGGCTTGGGGATCTTGTTT-3'	5'-GCAGGGAGCGTAGTGATAG-3'
<i>chpY</i>	CYB_2877	5'-CACCTTTTGGCGATGTAGT-3'	5'-TTGGCCAGCTTCAAATATTCTT-3'
<i>Putative bicarbonate transporters</i>			
<i>cmpA</i>	CYB_0604	5'-CGCAAGTCAAAGCTGCCTAT-3'	5'-GGGCCAAATCTGTTGAGTAAGA
Put. <i>bicA</i> <sup>a</sup>	CYB_1611	5'-CAGAACCTGCCGACCTACCT-3'	5'-AGCTCTGGTCCGAGTCGT-3'
<i>Superoxide dismutase</i>			
<i>sodB</i>	CYB_2514	5'-TCTGGCCAACAAGTCTCTGG-3'	5'-GTAACCTTCAGCGTGCCATC-3'
<i>Peroxidase</i>			
	CYB_2254	5'-CATGGAATTCAAGACGGTGAA-3'	5'-TATTCGCTGTCCACCGAGAC-3'
<i>Carotenoid synthesis</i>			
<i>crtP</i>	CYB_1694	5'-GACAAGCCGGGCACCTAT-3'	5'-CAGCCATTCCAAGAGGCTA-3'
<i>crtO</i>	CYB_0017	5'-CTGCGACCGGAGATCATT-3'	5'-ATCGCGGCTGCATTAAG-3'
<i>Gluthathione synthesis</i>			
	Cyb_0556	5'-ACCCTTCTGCCAGGGAGT-3'	5'-CGCTCAGAGCTAGGTAGAGAGA-3'
<i>Photosystem genes</i>			
<i>psbA1</i>	CYB_0216	5'-TGATGATCCCGACGCTGT-3'	5'-GAGCCATTTCATCCAAGAAGC-3'
<i>psbA2</i>	CYB_0371	5'-AACATCATTTCTGGGGCTGTA-3'	5'-GTAGCTCAGCTCCCATTTCG-3'
<i>psbA3</i>	CYB_0433	Same as for <i>psbA2</i> /CYB_0371	
<i>Potential regulatory protein</i>			
<i>ccmR</i>	CYB_1948	5'-AAGCAATTGAGCAAAGCTGTAG-3'	5'-TTGAGCACCTTAAGAGCTACGTC-3'

Abbreviations: Ci, inorganic carbon; qPCR, quantitative PCR.

<sup>a</sup>The primers will anneal to the *bicA* gene in both *Synechococcus* OS-B' and *Synechococcus* OS-A.

disrupted at full power in the bead-beater for 15–30 s after the addition of 250  $\mu$ l NaOAc and lysis buffer (see steps 1, 3 in Supplementary Methods 1). We also extracted the sample once with 1 vol chloroform after the final phenol:chloroform extraction, as described in Supplementary Methods 1.

#### Reverse transcription–quantitative PCR

Random primers (Invitrogen Corp., Carlsbad, CA, USA) were annealed to 1  $\mu$ g of total RNA and extended for 10 min (25  $^{\circ}$ C) and 30 min (55  $^{\circ}$ C) using Transcriptor Reverse Transcriptase (Roche Diagnostics Corp., Indianapolis, IN, USA), according to the manufacturer's instructions. The synthesized complementary DNA was diluted 10-fold and 2  $\mu$ l of the diluted complementary DNA served as template for quantitative PCR using the FastStart SYBR Green Master system (Roche Diagnostics Corp.) and the Engine Opticon System (Bio-Rad Laboratories Inc., Hercules, CA, USA) according to the manufacturer's instructions. Specific amplification of target genes was 1 cycle at 95  $^{\circ}$ C for 10 min, and 40 cycles at 94  $^{\circ}$ C for 10 s, 60  $^{\circ}$ C or 64  $^{\circ}$ C for 15 s, 72  $^{\circ}$ C for 30 s, 80  $^{\circ}$ C for 10 s, and a final incubation for 10 min at 72  $^{\circ}$ C during the last cycle. The relative abundance of individual transcripts was determined by using the same amount of RNA in the reverse transcription-reactions and by normalizing each time point over the diel cycle to the lowest amplification signal for each specific transcript (Whelan *et al.*, 2003; Steunou *et al.*, 2008).

## Results and discussion

#### Gene annotation

Investigated genes of Syn OS-B' and the gene clusters in which they occur are shown in Figure 1.

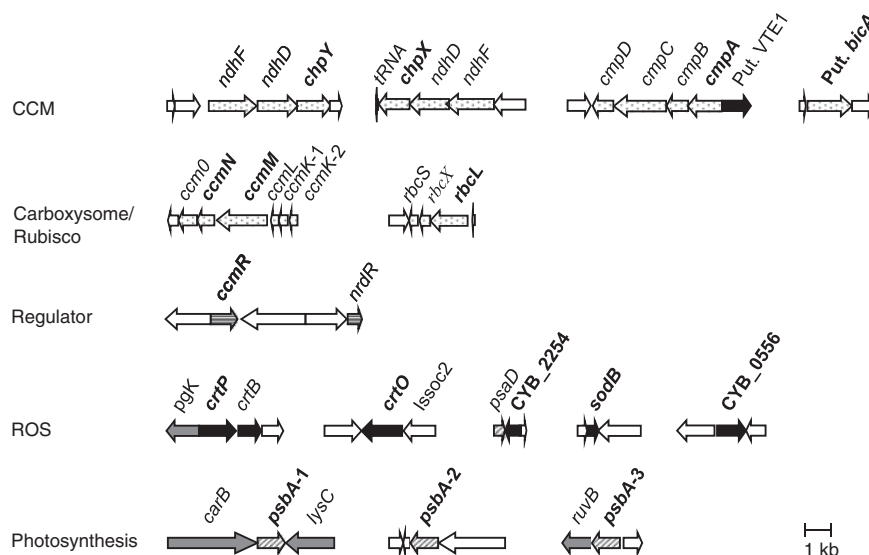
Amino acid identities to putative orthologs encoded by the various reference cyanobacterial genomes (*Synechococcus* sp. PCC 7942; *Synechococcus* sp. PCC 7002 and *Synechocystis* sp. PCC 6803) are shown in Supplementary Table 1. In all cases, the deduced protein sequences were most identical to homologs in Syn OS-A, except for CrtO, which is not present on the Syn OS-A genome. Additional information regarding genes selected for expression analysis can be found in Supplementary Information 1 and Supplementary Figures 1 and 2.

#### Environmental analysis

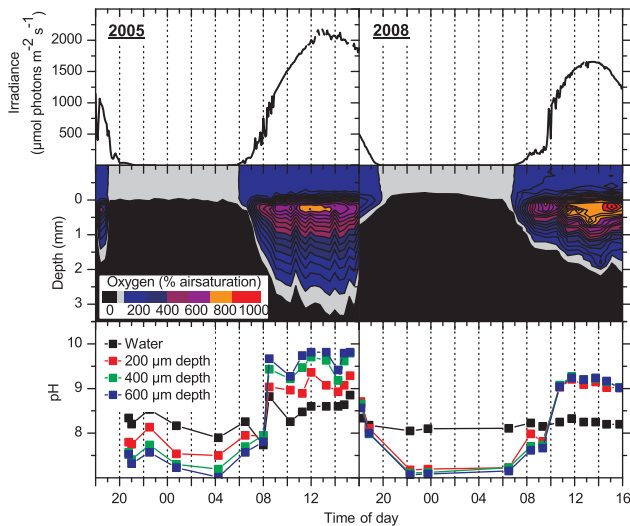
The O<sub>2</sub> and pH levels in the uppermost mat layers (~50–150  $\mu$ m depth) closely tracked the incident irradiance, where O<sub>2</sub> fluctuated between anoxia during the night, suboxic conditions during a prolonged period of dim light in the early morning, O<sub>2</sub> supersaturation during most of the day, and a period of fluctuating oxic and suboxic conditions during late afternoon (Figure 2). The pH in the mat spanned >2 units, from pH ~7.0 during the night (2005 and 2008) to pH ~9.2 (2008) and ~9.3–9.8 (2005) during periods of high light in the day; the pH of the overlying water changed much less (Figure 2, bottom panel). The pronounced daytime increase in pH indicates severe CO<sub>2</sub> depletion in the photic zone during periods of high photosynthetic CO<sub>2</sub> fixation as reported in studies of other microbial mats (de Beer *et al.*, 1997).

#### In situ gene expression

Our *in situ* investigations of diel gene expression patterns involved monitoring levels of specific



**Figure 1** Clusters of genes analyzed for *in situ* gene expression over two diel cycles in Mushroom Spring (bold type). Genes involved in CCM and Ci fixation (dotted or green arrows), detoxification of reactive oxygen species (ROS; black or red arrows), putative regulators (horizontal lines or yellow arrows) and photosynthetic electron transport (oblique or blue arrows) in *Synechococcus* OS-B' (genome accession number NC\_007776). Gray arrows indicate genes not known to be directly or indirectly involved in these functions, and white arrows encode hypothetical proteins. The color reproduction of this figure is available on the html full text version of the manuscript.



**Figure 2** *In situ* measurements of incident downwelling irradiance ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ; top panels),  $\text{O}_2$  levels (% air saturation; central panels) and pH (bottom panels) at different mat depths, as measured over two diel cycles at a  $60^\circ\text{C}$  sampling site in Mushroom Spring in July 2005 (left panels) and September 2008 (right panels).

cyanobacterial transcripts associated with CCM, ROS detoxification and photosynthesis in the microbial mats. The abundance of these transcripts changed significantly in concert with the extremely dynamic physico-chemical conditions of the microbial mat. Analyses of these patterns allow insights into regulatory mechanisms that modulate physiological activities and growth of mat-dwelling thermophilic cyanobacteria.

**CCM.** Transcripts encoding proteins involved in bicarbonate and  $\text{CO}_2$  uptake (*chpY*, *cmpA*, putative *bicA* and *chpX*) increased in the mat during the transition from total darkness to low light (Figure 3), and stayed at this level throughout the low-light period (irradiance of  $< 200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) in the early morning (Figures 3b-e). A further increase in the levels of the *chpY*, *cmpA*, putative *bicA* and *chpX* transcripts was observed as irradiance levels rose (08:00 and 10:00 hours in 2005 and 2008, respectively). In the early afternoon, the levels of *chpY*, *cmpA* and *bicA* transcripts declined (14:00 hours in 2005 and 15:00 hours in 2008), even though irradiance,  $\text{O}_2$  concentration and pH were still elevated (Figures 2 and 3a). The *chpX* transcript also declined somewhat in the early afternoon, although it tended to be maintained at a higher level relative to the other transcripts (Figure 3e).

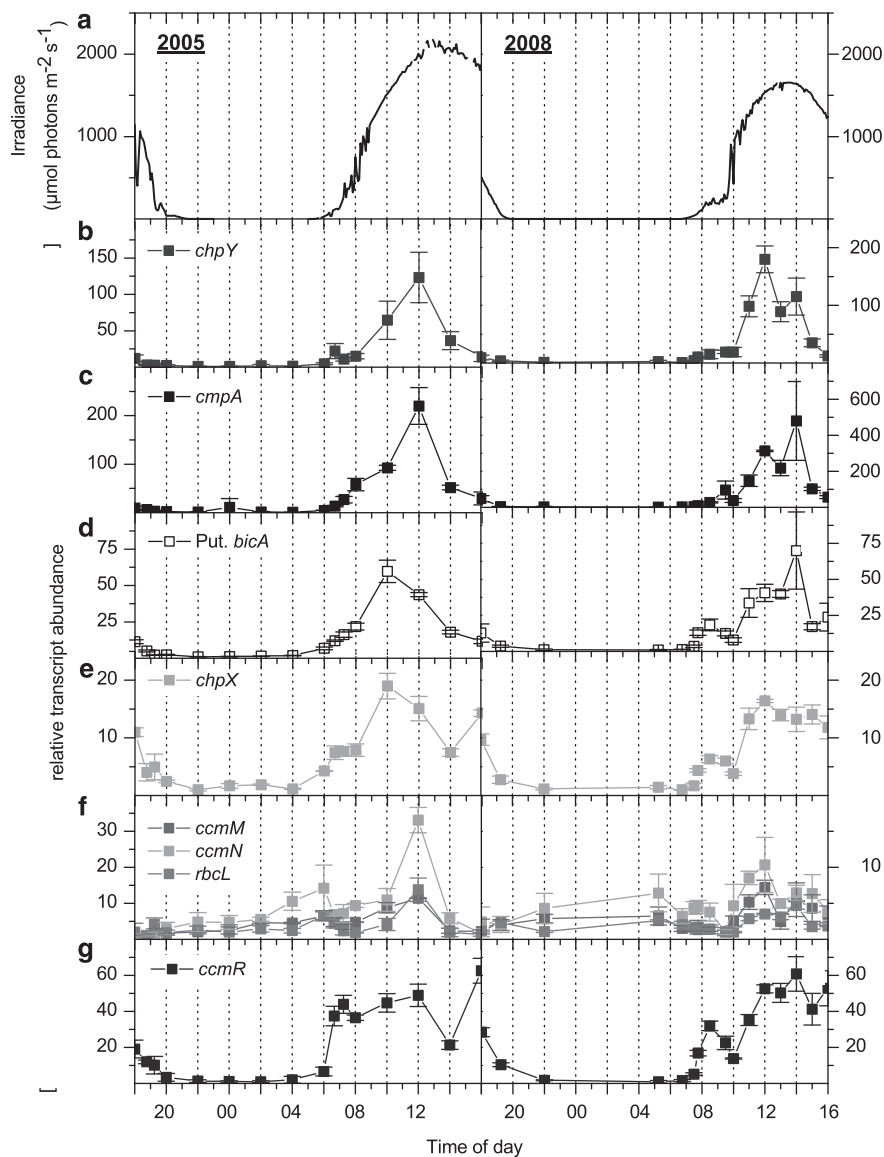
These first observations of diel patterns of transcript abundances from genes encoding cyanobacterial Ci transporters in hot spring mats are largely consistent with what has been observed for cultured cyanobacteria. The darkness to light transition elicited an increase in abundance of CCM

transcripts; such results could be explained by photoreceptor control or changes in the redox state of the cells (for example, the plastoquinone pool) upon initial light exposure. Both possibilities are in accord with the finding that active Ci-transport is light/energy dependent (Ogawa *et al.*, 1985; Li and Calvin, 1998; Tchernov *et al.*, 2001), and studies of laboratory cultures of *Synechocystis* sp. PCC 6803 have demonstrated that light is a prerequisite for elevation of CCM transcript levels (McGinn *et al.*, 2003). The additional increase in CCM transcript levels under the low-to-high irradiance transition has a number of potential explanations:

(1) Several investigations on changes in CCM transcript levels suggest that a limiting internal Ci pool can serve as a signal that activates gene expression during illumination (Omata *et al.*, 1999, 2001; Shibata *et al.*, 2001; McGinn *et al.*, 2003; Woodger *et al.*, 2003, 2005). This possibility is plausible for mat cyanobacteria as, during the shift from low-to-high light, the pH in the upper layers of the microbial mat increased from  $\sim 7.5$  to  $> 9.0$  (Figure 2), indicating a severe depletion of the Ci pool. Depletion of external Ci as a consequence of high photosynthetic demand for Ci by the cyanobacteria would lead to a marked depletion of internal Ci, at least until the CCM proteins facilitate Ci uptake against the concentration gradient. However, Ci limitation, as well as limitation for other nutrients, can also cause a decline in the utilization of reductants produced by photosynthetic electron transport, which in turn might lead to elevated redox levels, ROS production and cellular damage (Takahashi and Murata, 2008); all of these factors would likely impact gene expression.

(2) Part of the elevated CCM transcript levels may also reflect a further light-induced activation of genes in Syn OS-B' variants that reside at the bottom of the cyanobacterial layer of the mat; these organisms may not have experienced light saturation of gene activity during the low-light period in the morning owing to strong light attenuation in the microbial mat (Ward *et al.*, 2006).

(3) Increased transcript levels may also reflect elevated energy levels required to fuel gene activity in the cyanobacteria and an increase in the cellular redox state with increasing photosynthetic activity as morning progresses (McGinn *et al.*, 2004); these features are also related to (4) elevation of the  $\text{O}_2$  concentration in the mat (Figure 2), which would impact cyanobacterial respiration, photosynthesis and energy status (Woodger *et al.*, 2005). It is also possible that specific metabolites like 2-phosphoglycolate, accumulating under increasing mat  $\text{O}_2$  concentrations, could act as co-inducers of gene expression (Marcus *et al.*, 1983; Nishimura *et al.*, 2008). (5) High pH within the microbial mat during midday (Figure 2) may lead to elevated intracellular pH (Ritchie, 1991, 2008) and a potential shift in the intracellular  $\text{CO}_2/\text{HCO}_3^-$  ratio, which might impact gene activity; this ratio may also be influenced by



**Figure 3** Incident downwelling irradiance (400–700 nm) (a;  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and relative abundance of transcripts from CCM genes (b; *chpY*, c; *cmpA*, d; putative *bicA*, e; *chpX*, f; *ccmM*, *ccmN*, *rbcL* and g; putative *ccmR*), as measured over two diel cycles at a 60 °C sampling site in Mushroom Spring in July 2005 (left panels) and September 2008 (right panels). Transcript abundance data show the mean  $\pm$  s.e. ( $n = 3$ ).

the intracellular ionic composition and carbonic anhydrase activity.

The pronounced decline in CCM transcript levels in the early afternoon as observed for *chpY*, *cmpA* and *bicA* (Figure 3), despite high irradiance and an elevated  $\text{O}_2$  concentration and pH (Figure 2), suggests that the system may be able to auto-regulate its gene expression as a consequence of induced CCM activity and efficient acquisition of  $\text{C}_i$ . Changes in levels of other cellular metabolites and circadian signals could also have regulatory consequences. The decreasing abundance of transcripts for the  $\text{C}_i$  transporters during the night may reflect both a marked decrease in light levels and an increase in both internal and external  $\text{C}_i$  levels as photosynthetic  $\text{CO}_2$  fixation stops.

Interestingly, the *chpX* transcript, reported to be constitutively expressed in cyanobacterial cultures (McGinn *et al.*, 2003; Woodger *et al.*, 2003), accumulated in the mat during early morning as well as under the low-to-high light shift, and remained elevated in the afternoon unlike other CCM genes, which declined in the early afternoon (Figure 3). Both irradiance and altered  $\text{C}_i$  availability may impact *chpX* transcript differently relative to the other CCM genes, but it is also possible that *chpX* mRNA degradation is slower than that of other transporter mRNAs. Culture studies of Syn OS-B' gene expression might help distinguish such differences, although differences in specific transcript abundances observed *in situ* in the mat and in cell cultures are not unexpected, owing to our

limited ability to establish laboratory conditions that mimic the biotic and abiotic conditions in microbial mats.

Genes encoding carboxysome assembly proteins (*ccmM*, *ccmN*) and ribulose-1,5-bisphosphate carboxylase (*rbcL*) increased somewhat pre-dawn (reaching the highest night value at ~04:00 and ~05:30 hours in 2005 and 2008, respectively), as well as after the low-to-high light transition (Figure 3f, after 10:00) for both samplings. Carboxysomes are evenly segregated between dividing cells (Savage *et al.*, 2010), and a pre-dawn increase in transcript abundance may prepare the cells for division, which would require division of the carboxysome or for the onset of photosynthetic activity at sunrise. Thus, a circadian element may influence the expression of genes critical for the fixation of CO<sub>2</sub> during the day. Such regulation was previously suggested for the *rbcL* gene based on *in situ* data (Wyman, 1999), and the levels of *ccm* and *rbcL* transcripts (and other transcripts) exhibit circadian oscillations in *S. elongatus* PCC 7942 (Vijayan *et al.*, 2009).

The rise in *ccmM*, *ccmN* and *rbcL* transcript levels during the low-to-high light transition (Figure 3f) could be explained by the same factors as those discussed above for transcripts encoding the Ci transporters, that is, depletion of internal Ci and an altered redox state, but expression may also be linked to cell division, the timing of which is still unresolved for the hot spring mat cyanobacteria (Ward *et al.*, 1998, 2006). The carboxysome content increases in cultured cyanobacteria after a shift from high-to-low external Ci concentrations (Turpin *et al.*, 1984; McKay *et al.*, 1993), and *ccm* transcripts have also been shown to increase somewhat in response to low Ci (Woodger *et al.*, 2003). Other studies report no change in the levels of *ccm* transcripts in response to low Ci-conditions (McGinn *et al.*, 2003), but if the activities of the *ccm* and *rbcL* genes are also under circadian control or linked to cell division, the lack of responsiveness could be a consequence of asynchronous cell growth under continuous light.

#### *CcmR*, a putative regulator of CCM

Transcripts encoding the putative regulator CcmR markedly increased with the onset of light, and the transcript levels remained relatively high over the entire light period (Figure 3g). The rapid accumulation of transcripts after the onset of light and the near constancy of the transcript level throughout the light period (most apparent in the 2005 samples) suggests that expression of this gene may be modulated by small changes in redox-conditions, photoperception by receptors such as phytochrome, or other cellular processes that are saturated throughout the cyanobacterial layer even at low irradiance. Our results are, to some extent, at odds with results obtained in studies of *cmpR/ndhR/*

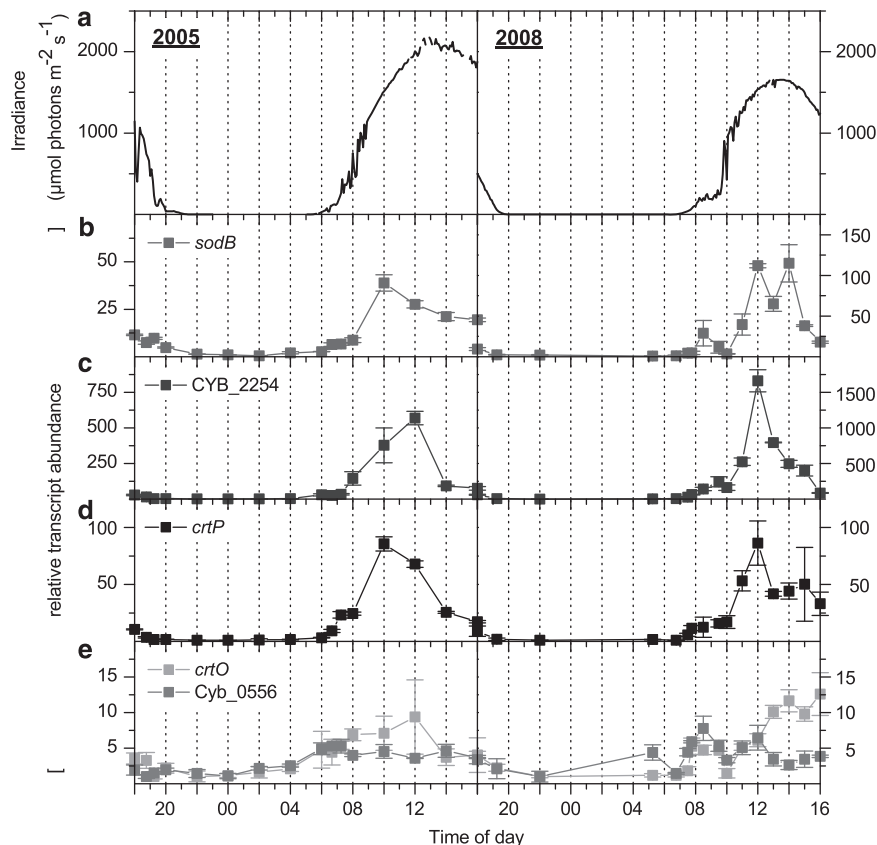
*ccmR* in cultured cyanobacteria (McGinn *et al.*, 2003; Woodger *et al.*, 2003, 2007), where transcript abundances were influenced by Ci levels.

#### ROS

All transcripts for genes encoding enzymes involved in ROS detoxification increased during the transition from darkness to low light. Some transcripts (*sodB*, *crtP* and CYB\_2254, which encodes a putative Prx) stayed at a fairly constant level during the low-light period of the early morning (Figure 4) and then increased with increasing irradiance (08:00 and 10:00 hours in 2005 and 2008, respectively); this pattern is similar to that observed for genes involved in Ci uptake. Later in the afternoon, that is, at 14:00 hours, under high irradiance and O<sub>2</sub> levels, transcript levels started to decline (*sodB*, CYB\_2254 and *crtP*). Levels of other ROS-related transcripts (*crtO* and CYB\_0556, encoding a putative glutamate-cysteine ligase) exhibited a relatively small increase over the light period (Figure 4e). The influence of high irradiance on *sodB*, *crtP* and *crtO* transcript levels have been investigated in axenic liquid cultures of Syn OS-B' (Kilian *et al.*, 2007), and our *in situ* data are in accord with the results from this laboratory study, in which there was an influence of high light on *sodB* and *crtP* transcript levels, whereas the abundance of the *crtO* transcript appeared independent of irradiance (darkness was not investigated).

However, the changing irradiance might not directly influence levels of specific transcripts as several variables in the mat environment (for example, O<sub>2</sub>, ROS levels and pH) and within the cytoplasm of cyanobacteria co-vary with irradiance. The increasing abundance of transcripts encoding ROS-associated proteins during the darkness to low light transition (Figure 4) is likely regulated in a similar way as that of CCM genes; such regulatory features were reported for the *sodB* gene in *Synechocystis* sp. PCC 6803 (Kim and Suh, 2005). On the other hand, changes in transcript levels observed during the transition from low-to-high light could be influenced by various factors, many of which have already been discussed for the Ci transporters. Some specific regulatory mechanisms that might be associated with this transition in cyanobacteria are a change in cellular redox state (for example, of the plastoquinone pool), an increased flux of electrons through the cytochrome b6f complex, photosystem I-mediated thioredoxin signaling (Zeller and Klug, 2006; Oelze *et al.*, 2008), and the accumulation of various ROS (Li *et al.*, 2004).

It should be noted that light-induced H<sub>2</sub>O<sub>2</sub> accumulation is not only biologically mediated, but can also be abiotically generated by the action of ultraviolet irradiation on dissolved organic carbon (Wilson *et al.*, 2000b). H<sub>2</sub>O<sub>2</sub> formation and the dynamics that control its accumulation are yet to



**Figure 4** Incident downwelling irradiance (400–700 nm) (a;  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and relative abundance of transcripts associated with ROS detoxification (b, *sodB*; c, CYB\_2254 which encodes a type 2-Cys Prx; d, *crtP*; e, *crtO* and CYB\_0556, which encodes a putative glutamate–cysteine ligase), as measured over two diel cycles at a 60 °C sampling site in Mushroom Spring in July 2005 (left panels) and September 2008 (right panels). Transcript abundance data show the mean  $\pm$  s.e. ( $n = 3$ ).

be investigated in photosynthetic microbial mats. However, there is increased formation of  $\text{H}_2\text{O}_2$  in waters flowing over the surface of hot spring microbial mats during the day relative to the night (Wilson *et al.*, 2000a). Such elevated  $\text{H}_2\text{O}_2$  from photochemical reactions in the upper layers of microbial mats could significantly impact photosynthetic processes.  $\text{H}_2\text{O}_2$  has a relatively long lifetime and can diffuse into cells from the surrounding medium; this contrasts with singlet oxygen which has a short lifetime, although it is often considered to be the most damaging ROS formed by cyanobacteria (Triantaphylides *et al.*, 2008; Triantaphylides and Havaux, 2009).

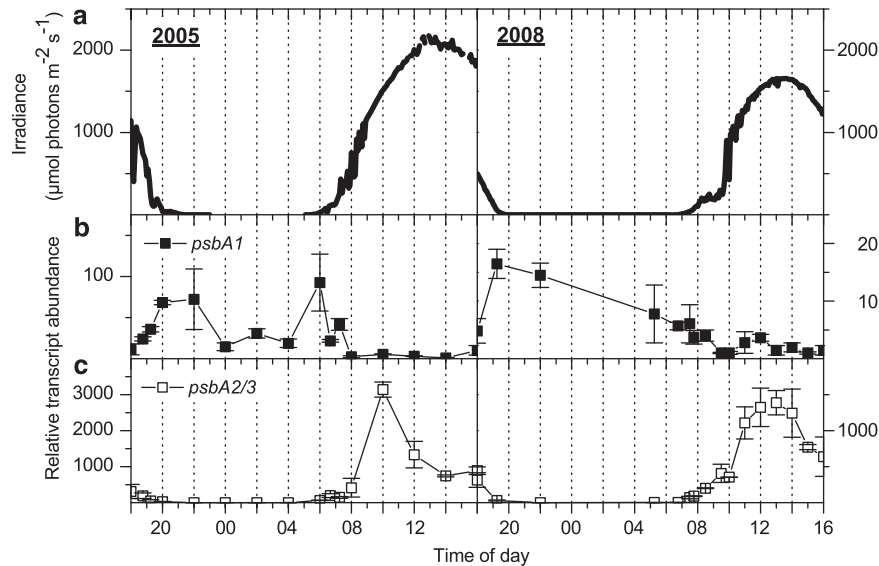
Based on our *in situ* data, it is difficult to identify specific regulatory mechanisms that modulate transcript accumulation for ROS-associated genes, as several physiological features of the system are changing simultaneously. However, our data show that at least some genes associated with ROS detoxification (for example, *sodB*, *crtO*, CYB\_0556) are probably not controlled in the same manner like most CCM genes, as the levels of transcripts from these genes, unlike those encoding most CCM polypeptides, do not decline in the mid-afternoon. Expression patterns of ROS-associated genes appear to largely

respond to irradiance and subsequent changes in the mat chemistry (for example, supersaturating  $\text{O}_2$  levels, high pH and  $\text{C}_i$  depletion), although they may also be impacted by circadian control.

#### Photosynthesis

Transcripts from the *psbA2/3* genes (reverse transcription quantitative PCR analyses did not distinguish transcripts from these genes because of their near identical nucleotide sequences; two nucleotide differences in coding region) were elevated at the onset of light with the highest levels directly correlating with high incident irradiance ( $\sim 1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ; Figures 5a and c). In contrast, the *psbA1* transcript levels increased in the early evening and generally declined slowly during the night (Figure 5b). In 2005, the lowest *psbA1* transcript levels were observed during the high-light mid-day periods, whereas highest levels were observed under low irradiance in the late afternoon to early evening and in the early morning (Figure 5b). Our *in situ* results are consistent with culture studies of Syn OS-B', where *psbA2/3* transcript levels increased during the transition from low-to-high light, whereas *psbA1* transcript





**Figure 5** Incident downwelling irradiance (400–700 nm) (a;  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and relative abundance of transcripts associated with photosynthetic function (b; *psbA1* and c; *psbA2/3*), as measured over two diel cycles at a 60 °C sampling site in Mushroom Spring in July 2005 (left panels) and September 2008 (right panels). Transcript abundance data show the mean  $\pm$  s.e. ( $n = 3$ ).

levels increased when the cells were shifted from high-to-low light (Kilian *et al.*, 2007); similar results were reported for other cultivated cyanobacteria (Schaefer and Golden, 1989).

The high *psbA2/3* transcript levels in the microbial mat during mid-day do not necessarily indicate a higher cyanobacterial D1 protein level than in cyanobacteria experiencing low-light conditions (for example, early morning). The D1 protein is susceptible to ROS/high-light triggered damage and is rapidly degraded when cells are absorbing excess excitation energy (Krieger-Liszkay *et al.*, 2008). A rapid turnover coupled with the fact that ROS may inhibit D1 repair processes (Nishiyama *et al.*, 2001) raises the possibility that the actual level of functional D1 may be lower during high-light exposure in spite of very high transcript accumulation. Our  $\text{O}_2$  micro-profiles show a slight decline in  $\text{O}_2$  levels in the upper mat layers at around mid-day (Figure 2). However,  $\text{O}_2$  levels in the mat remained at supersaturating levels throughout the day, indicative of sustained photosynthetic activity, which suggests that the level of *psbA2/3* transcripts and the protein encoded by these transcripts are sufficient to ensure the regeneration of active photosystem II reaction centers following damage resulting from high light/ROS.

### Conclusion

This study presents the first investigation of *in situ* transcript abundance of genes involved in CCM and ROS detoxification in conjunction with precise measurements of environmental parameters over a diel cycle in a photosynthetic microbial mat. Gene expression patterns were linked to dynamic shifts of the physico-chemical conditions (pH and  $\text{O}_2$ ) in the mat,

largely driven by the diel cycle of solar irradiance. The results provide insights into complex *in situ* variation in gene expression over the course of the day, and point toward potential regulatory mechanisms that control the dynamics of transcript accumulation.

Variations in transcript abundances in Syn OS-B' under *in situ* conditions were largely consistent with patterns observed for cyanobacteria grown as axenic liquid cultures under controlled laboratory conditions. This provides validation for the idea that important, ecologically-relevant information can be derived from laboratory studies, although the many factors that co-occur *in situ* should be considered when performing experiments with axenic cultures in the laboratory. In some cases this complexity, for example, potential integration of circadian control with control through environmental signals, may be lost under culture conditions because of the way in which the cells are grown, that is, continuous light versus light–dark cycling and modulation of light levels over the diel cycle, and differences in features of the surrounding environment including interactions with other microorganisms and the presence of an organic matrix, which can highly impact diffusion rates and lead to the formation of steep chemical gradients. Some of these complexities can be dissected with specific manipulations of axenic cultures, although key aspects of *in situ* environmental features should always be considered in the interpretation of the data.

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## References

- Allewalt JP, Bateson MM, Revsbech NP, Slack K, Ward DM. (2006). Effect of temperature and light on growth of and photosynthesis by *Synechococcus* isolates typical of those predominating in the Octopus spring microbial mat community of Yellowstone National Park. *Appl Environ Microbiol* **72**: 544–550.
- Aro E-M, Suorsa M, Rokka A, Allahverdiyeva Y, Paakkarinen V, Saleem A et al. (2005). Dynamics of photosystem II: a proteomic approach to thylakoid protein complexes. *J Exp Bot* **56**: 347–356.
- Badger MR, Price GD. (1990). Carbon oxysulfide is an inhibitor of both CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> uptake in the cyanobacterium *Synechococcus* PCC7942. *Plant Physiol* **94**: 35–39.
- Bhaya D, Grossman AR, Steunou A-S, Khuri N, Cohan FM, Hamamura N et al. (2007). Population level functional diversity in a microbial community revealed by comparative genomic and metagenomic analyses. *ISME J* **1**: 703–713.
- Bouchard JN, Roy S, Campbell DA. (2006). UVB effects on the photosystem II-D1 protein of phytoplankton and natural phytoplankton communities. *Photochem Photobiol* **82**: 936–951.
- Brock TD. (1967). Relationship between standing crop and primary productivity along a hot spring thermal gradient. *Ecology* **48**: 566–571.
- Dayer R, Fischer BB, Eggen RIL, Lemaire SD. (2008). The peroxiredoxin and glutathione peroxidase families in *Chlamydomonas reinhardtii*. *Genetics* **179**: 41–57.
- de Beer D, Glud A, Epping E, Kühl M. (1997). A fast-responding CO<sub>2</sub> microelectrode for profiling sediments, microbial mats, and biofilms. *Limnol Oceanogr* **42**: 1590–1600.
- de Beer D, Kühl M. (2001). Interfacial microbial mats and biofilms. In: Boudreau BP, Jørgensen BB (eds). *The Benthic Boundary Layer. Transport Processes and Biogeochemistry*. Oxford University Press: New York, pp 374–394.
- Folea IM, Zhang P, Nowaczyk MM, Ogawa T, Aro E-M, Boekema EJ. (2008). Single particle analysis of thylakoid proteins from *Thermosynechococcus elongatus* and *Synechocystis* 6803: localization of the CupA subunit of NDH-1. *FEBS Lett* **582**: 249–254.
- Goyer A, Haslekås C, Miginiac-Maslow M, Klein U, Le Marechal P, Jacquot J-P et al. (2002). Isolation and characterization of a thioredoxin-dependent peroxidase from *Chlamydomonas reinhardtii*. *Eur J Biochem* **269**: 272–282.
- Jamieson D, Chance B, Cadenas E, Boveris A. (1986). The relation of free radical production to hyperoxia. *Annu Rev Physiol* **48**: 703–719.
- Kanehisa M, Goto S. (2000). KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res* **28**: 27–30.
- Kaplan A, Hagemann M, Bauwe H, Kahlon S, Ogawa T. (2008). Carbon acquisition by cyanobacteria: mechanisms, comparative genomics, and evolution. In: Herrero A, Flores E (eds). *The Cyanobacteria. Molecular biology, Genomics and Evolution*. Caister Academic Press: Norfolk, UK, pp 305–334.
- Kilian O, Steunou A-S, Fazeli F, Bailey S, Bhaya D, Grossman AR. (2007). Responses of a thermophilic *Synechococcus* isolate from the microbial mat of octopus spring to light. *Appl Environ Microbiol* **73**: 4268–4278.
- Kim J-H, Suh KH. (2005). Light-dependent expression of superoxide dismutase from cyanobacterium *Synechocystis* sp. strain PCC 6803. *Arch Microbiol* **183**: 218–223.
- Koropatkin NM, Koppelaar DW, Pakrasi HB, Smith TJ. (2007). The structure of a cyanobacterial bicarbonate transport protein, CmpA. *J Biol Chem* **282**: 2606–2614.
- Krieger-Liszkay A, Fufezan C, Trebst A. (2008). Singlet oxygen production in photosystem II and related protection mechanism. *Photosynth Res* **98**: 551–564.
- Kühl M, Lassen C, Revsbech NP. (1997). A simple light meter for measurements of PAR (400 to 700 nm) with fiber-optic microprobes: application for P vs E<sub>0</sub> (PAR) measurements in a microbial mat. *Aquat Microb Ecol* **13**: 197–207.
- Kühl M, Revsbech NP. (2001). Biogeochemical micro-sensors for boundary layer studies. In: Boudreau BP, Jørgensen BB (eds). *The Benthic Boundary Layer. Transport Processes and Biogeochemistry*. Oxford University Press: New York, pp 180–210.
- Latifi A, Ruiz M, Zhang C-C. (2009). Oxidative stress in cyanobacteria. *FEMS Microbiol Rev* **33**: 258–278.
- Li H, Singh AK, McIntyre LM, Sherman LA. (2004). Differential gene expression in response to hydrogen peroxide and the putative PerR regulon of *Synechocystis* sp. strain PCC 6803. *J Bacteriol* **186**: 3331–3345.
- Li Q, Calvin DT. (1998). Energy sources for HCO<sub>3</sub><sup>-</sup> and CO<sub>2</sub> transport in air-grown cells of *Synechococcus* UTEX 625. *Plant Physiol* **116**: 1125–1132.
- Maeda S-i, Badger MR, Price GD. (2002). Novel gene products associated with NdhD3/D4-containing NDH-1 complexes are involved in photosynthetic CO<sub>2</sub> hydration in the cyanobacterium, *Synechococcus* sp. PCC7942. *Mol Microbiol* **43**: 425–435.
- Marcus Y, Harel E, Kaplan A. (1983). Adaptation of the cyanobacterium *Anabaena variabilis* to low CO<sub>2</sub> concentration in their environment. *Plant Physiol* **71**: 208–210.
- McGinn PJ, Price GD, Badger MR. (2004). High light enhances the expression of low-CO<sub>2</sub>-inducible transcripts involved in the CO<sub>2</sub>-concentrating mechanism in *Synechocystis* sp. PCC6803. *Plant Cell Environ* **27**: 615–626.
- McGinn PJ, Price GD, Maleszka R, Badger MR. (2003). Inorganic carbon limitation and light control the expression of transcripts related to the CO<sub>2</sub>-concentrating mechanism in the cyanobacterium *Synechocystis* sp. strain PCC6803. *Plant Physiol* **132**: 218–229.
- McKay RML, Gibbs SP, Espie GS. (1993). Effect of dissolved inorganic carbon on the expression of carboxysomes, localization of Rubisco and the mode of inorganic carbon transport in cells of the cyanobacterium *Synechococcus* UTEX 625. *Arch Microbiol* **159**: 21–29.
- Nishimura T, Takahashi Y, Yamaguchi O, Suzuki H, Maeda S, Omata T. (2008). Mechanism of low CO<sub>2</sub>-induced activation of the cmp bicarbonate transporter

- operon by a LysR family protein in the cyanobacterium *Synechococcus elongatus* strain PCC 7942. *Mol Microbiol* **68**: 98–109.
- Nishiyama Y, Yamamoto H, Allakhverdiev SI, Inaba M, Yokota A, Murata N. (2001). Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery. *EMBO J* **20**: 5587–5594.
- Oelze M-L, Kandlbinder A, Dietz K-J. (2008). Redox regulation and overreduction control in the photosynthesizing cell: complexity in redox regulatory networks. *Biochim Biophys Acta* **1780**: 1261–1272.
- Ogawa T, Miyano A, Inoue Y. (1985). Photosystem-I-driven inorganic carbon transport in the cyanobacterium, *Anacystis nidulans*. *Biochim Biophys Acta* **808**: 77–84.
- Omata T, Gohta S, Takahashi Y, Harano Y, Maeda S-I. (2001). Involvement of a CbbR homolog in low CO<sub>2</sub>-induced activation of the bicarbonate transporter operon in cyanobacteria. *J Bacteriol* **183**: 1891–1898.
- Omata T, Price GD, Badger MR, Okamura M, Gohta S, Ogawa T. (1999). Identification of an ATP-binding cassette transporter involved in bicarbonate uptake in the cyanobacterium *Synechococcus* sp. strain PCC 7942. *Proc Natl Acad Sci USA* **96**: 13571–13576.
- Price GD, Badger MR, Woodger FJ, Long BM. (2008). Advances in understanding the cyanobacterial CO<sub>2</sub>-concentrating-mechanism (CCM): functional components, Ci transporters, diversity, genetic regulation and prospects for engineering into plants. *J Exp Bot* **59**: 1441–1461.
- Price GD, Woodger FJ, Badger MR, Howitt SM, Tucker L. (2004). Identification of a SulP-type bicarbonate transporter in marine cyanobacteria. *Proc Natl Acad Sci USA* **101**: 18228–18233.
- Raven JA, Giordano M, Beardall J. (2008). Insights into the evolution of CCMs from comparisons with other resource acquisition and assimilation processes. *Physiol Plant* **133**: 4–14.
- Revsbech NP. (1989). An oxygen microsensor with a guard cathode. *Limnol Oceanogr* **34**: 474–478.
- Ritchie R. (1991). Membrane-potential and pH control in the cyanobacterium *Synechococcus* R-2 (*Anacystis nidulans*) PCC 7942. *J Plant Physiol* **137**: 409–418.
- Sarkar N, Lemaire S, Wu-Scharf D, Issakidis-Bourguet E, Cerutti H. (2005). Functional specialization of *Chlamydomonas reinhardtii* cytosolic thioredoxin h1 in the response to alkylation-induced DNA damage. *Eukaryot Cell* **4**: 262–273.
- Savage DF, Afonso B, Chen AH, Silver PA. (2010). Spatially ordered dynamics of the bacterial carbon fixation machinery. *Science* **327**: 1258–1261.
- Schaefer MR, Golden SS. (1989). Differential expression of members of a cyanobacterial *psbA* gene family in response to light. *J Bacteriol* **171**: 3973–3981.
- Shibata M, Ohkawa H, Kaneko T, Fukuzawa H, Tabata S, Kaplan A *et al.* (2001). Distinct constitutive and low-CO<sub>2</sub>-induced CO<sub>2</sub> uptake systems in cyanobacteria: genes involved and their phylogenetic relationship with homologous genes in other organisms. *Proc Natl Acad Sci USA* **98**: 11789–11794.
- Steunou A-S, Bhaya D, Bateson MM, Melendrez MC, Ward DM, Brecht E *et al.* (2006). *In situ* analysis of nitrogen fixation and metabolic switching in unicellular thermophilic cyanobacteria inhabiting hot spring microbial mats. *Proc Natl Acad Sci USA* **103**: 2398–2403.
- Steunou A-S, Jensen SI, Brecht E, Becraft ED, Bateson MM, Kilian O *et al.* (2008). Regulation of *nif* gene expression and the energetics of N<sub>2</sub> fixation over the diel cycle in a hot spring microbial mat. *ISME J* **2**: 364–378.
- Takahashi S, Murata N. (2008). How do environmental stresses accelerate photoinhibition? *Trends Plant Sci* **13**: 178–182.
- Tchernov D, Helman Y, Keren N, Luz B, Ohad I, Reinhold L *et al.* (2001). Passive entry of CO<sub>2</sub> and its energy-dependent intracellular conversion to HCO<sub>3</sub><sup>-</sup> in cyanobacteria are driven by a photosystem I-generated ΔμH<sup>+</sup>. *J Biol Chem* **276**: 23450–23455.
- Tchernov D, Silverman J, Luz B, Reinhold L, Kaplan A. (2003). Massive light-dependent cycling of inorganic carbon between oxygenic photosynthetic microorganisms and their surroundings. *Photosynth Res* **77**: 95–103.
- Teske AP, Stahl D. (2002). Microbial mats and biofilms: evolution, structure, and function of fixed microbial communities. In: Staley JT, Reysenbach AL (eds). *Biodiversity of Microbial Life: Foundations of Earth's Biosphere*. Wiley-Liss: New York, pp 49–100.
- Thomas RC. (1978). *Ion-sensitive intracellular microelectrodes: how to make and use them*. Academic Press: London, New York.
- Triantaphylides C, Havaux M. (2009). Singlet oxygen in plants: production, detoxification and signaling. *Trends Plant Sci* **14**: 219–228.
- Triantaphylides C, Krischke M, Hoerberichts FA, Ksas B, Gresser G, Havaux M *et al.* (2008). Singlet oxygen is the major reactive oxygen species involved in photooxidative damage to plants. *Plant Physiol* **148**: 960–968.
- Turpin DH, Miller AG, Calvin DT. (1984). Carboxysome content of *Synechococcus leopoliensis* (cyanophyta) in response to inorganic carbon. *J Phycol* **20**: 249–253.
- Vijayan V, Zuzow R, O'Shea EK. (2009). Oscillations in supercoiling drive circadian gene expression in cyanobacteria. *Proc Natl Acad Sci USA* **106**: 22564–22568.
- Volokita M, Zenvirth D, Kaplan A, Reinhold L. (1984). Nature of the inorganic carbon species actively taken up by the cyanobacterium *Anabaena variabilis*. *Plant Physiol* **76**: 599–602.
- Ward DM, Bateson MM, Ferris MJ, Köhl M, Wieland A, Koepfel A *et al.* (2006). Cyanobacterial ecotypes in the microbial mat community of mushroom spring (Yellowstone National Park, Wyoming) as species-like units linking microbial community composition, structure and function. *Phil Trans R Soc B* **361**: 1997–2008.
- Ward DM, Cohan FM, Bhaya D, Heidelberg JF, Köhl M, Grossman A. (2008). Genomics, environmental genomics and the issue of microbial species. *Heredity* **100**: 207–219.
- Ward DM, Ferris MJ, Nold SC, Bateson MM. (1998). A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. *Microbiol Mol Biol Rev* **62**: 1353–1370.
- Whelan JA, Russell NB, Whelan MA. (2003). A method for the absolute quantification of cDNA using real-time PCR. *J Immunol Methods* **278**: 261–269.
- Wieland A, de Beer D, Damgaard LR, Köhl M, van Dusschoten D, Van As H. (2001). Fine-scale measurement of diffusivity in a microbial mat with nuclear magnetic resonance imaging. *Limnol Oceanogr* **46**: 248–259.
- Wieland A, Köhl M. (2006). Regulation of photosynthesis and oxygen consumption in a hypersaline cyanobacterial

- mat (Camargue, France) by irradiance, temperature and salinity. *FEMS Microbiol Ecol* **55**: 195–210.
- Wilson CL, Hinman NW, Cooper WJ, Brown CF. (2000a). Hydrogen peroxide cycling in surface geothermal waters of Yellowstone National Park. *Environ Sci Tech* **34**: 2655–2662.
- Wilson CL, Hinman NW, Sheridan RP. (2000b). Hydrogen peroxide formation and decay in iron-rich geothermal waters: the relative roles of abiotic and biotic mechanisms. *Photochem Photobiol* **71**: 691–699.
- Woodger FJ, Badger MR, Price GD. (2003). Inorganic carbon limitation induces transcripts encoding components of the CO<sub>2</sub>-concentrating mechanism in *Synechococcus* sp. PCC7942 through a redox-independent pathway. *Plant Physiol* **133**: 2069–2080.
- Woodger FJ, Badger MR, Price GD. (2005). Sensing of inorganic carbon limitation in *Synechococcus* PCC7942 is correlated with the size of the internal inorganic carbon pool and involves oxygen. *Plant Physiol* **139**: 1959–1969.
- Woodger FJ, Bryant DA, Price GD. (2007). Transcriptional regulation of the CO<sub>2</sub>-concentrating mechanism in a euryhaline, coastal marine cyanobacterium, *Synechococcus* sp. strain PCC 7002: role of NdhR/CcmR. *J Bacteriol* **189**: 3335–3347.
- Wyman M. (1999). Diel rhythms in ribulose-1,5-bisphosphate carboxylase/oxygenase and glutamine synthetase gene expression in a natural population of marine picoplanktonic cyanobacteria (*Synechococcus* spp.). *Appl Environ Microbiol* **65**: 3651–3659.
- Xu M, Ogawa T, Pakrasi HB, Mi H. (2008). Identification and localization of the CupB protein involved in constitutive CO<sub>2</sub> uptake in the cyanobacterium, *Synechocystis* sp. strain PCC 6803. *Plant Cell Physiol* **49**: 994–997.
- Zeller T, Klug G. (2006). Thioredoxins in bacteria: functions in oxidative stress response and regulation of thioredoxin genes. *Naturwissenschaften* **93**: 259–266.
- Zhang P, Battchikova N, Jansen T, Appel J, Ogawa T, Aro E-M. (2004). Expression and functional roles of the two distinct NDH-1 complexes and the carbon acquisition complex NdhD3/NdhF3/CupA/Sll1735 in *Synechocystis* sp PCC 6803. *Plant Cell* **16**: 3326–3340.

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