Transcription profiling of the model cyanobacterium *Synechococcus* sp. strain PCC 7002 by Next-Gen (SOLiD™) sequencing of cDNA

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The genome of the unicellular, euryhaline cyanobacterium *Synechococcus* sp. PCC 7002 encodes about 3200 proteins. Transcripts were detected for nearly all annotated open reading frames by a global transcriptomic analysis by Next-Generation (SOLiD™) sequencing of cDNA. In the cDNA samples sequenced, ∼90% of the mapped sequences were derived from the 16S and 23S ribosomal RNAs and ∼10% of the sequences were derived from mRNAs. In cells grown photoautotrophically under standard conditions [38°C, 1% (v/v) CO₂ in air, 250 μmol photons m⁻² s⁻¹], the highest transcript levels [up to 2% of the total mRNA for the most abundantly transcribed genes; e.g., *cpcAB*, *psbA*, *psaA*) were generally derived from genes encoding structural components of the photosynthetic apparatus. High-light exposure for 1 h caused changes in transcript levels for genes encoding proteins of the photosynthetic apparatus, Type-1 NADH dehydrogenase complex and ATP synthase, whereas dark incubation for 1 h resulted in a global decrease in transcript levels for photosynthesis-related genes and an increase in transcript levels for genes involved in carbohydrate degradation. Transcript levels for pyruvate kinase and the pyruvate dehydrogenase complex decreased sharply in cells incubated in the dark. Under dark anoxic (fermentative) conditions, transcript changes indicated a global decrease in transcripts for respiratory proteins and suggested that cells employ an alternative phosphoenolpyruvate degradation pathway via phosphoenolpyruvate synthase (*ppsA*) and the pyruvate:ferredoxin oxidoreductase (*nifJ*). Finally, the data suggested that an apparent operon involved in tetrapyrrole biosynthesis and fatty acid desaturation, *acsF2–ho2–hemN2–desF*, may be regulated by oxygen concentration.

**Keywords:** cyanobacteria, transcription profiling, *Synechococcus* 7002, cDNA sequencing, fermentation

INTRODUCTION

*Synechococcus* sp. strain PCC 7002 (hereafter *Synechococcus* 7002) is a euryhaline, unicellular cyanobacterium, which tolerates extremely high-light intensities and grows over a wide range of NaCl concentrations (Batterton Jr. and van Baalen, 1971; Sakamoto and Bryant, 2002; Nomura et al., 2006b). It has the fastest reported doubling time for any cyanobacterium: ∼2.6 h when grown on urea or ammonia and ∼4.0 h when grown on nitrate under optimal conditions [38°C, 1% (v/v) CO₂ in air at a saturating light intensity of ∼250 μmol photons m⁻² s⁻¹]. The organism is easily transformable genetically (Stevens and Porter, 1980), the complete genome sequence is available (see http://www.ncbi.nlm.nih.gov/), and a versatile system for genetic complementation and gene over-expression is available (Xu et al., 2011). Collectively, these traits make *Synechococcus* 7002 a robust and promising platform for biotechnological applications, including the production of biofuels.

Using energy provided by sunlight, cyanobacteria produce the reducing equivalents required for CO₂ reduction and cellular metabolism from the oxidation of water molecules via two photosystems, denoted photosystem II (PS II) and photosystem I (PS I; Bryant, 1994). The resulting reducing equivalents are mostly used for CO₂ fixation and subsequent generation of carbohydrates and other metabolite building blocks, from which other cellular constituents are made. In addition to reducing equivalents, the photosynthetic apparatus generates a proton gradient across the thylakoid membrane, which is used for ATP synthesis. Because light is not continuously available, all phototrophic organisms must switch to another metabolic mode at night to produce the maintenance energy to support basic cell functions. Cyanobacteria have a complete respiratory electron transport chain that allows respiration with oxygen as terminal electron acceptor (Pescheck et al., 2004; Bernroitner et al., 2008). However, in many natural environments, oxygen is rapidly consumed in the dark by cyanobacteria or other organisms (Stal, 1995; Steunou et al., 2008), and thus the local environmental conditions may quickly become anoxic. Under these conditions, most cyanobacteria can perform fermentation of stored carbohydrates to produce maintenance energy (Stal and Moezelaar, 1997; McNeely et al., 2010a,b). Thus, cyanobacteria must rapidly adjust to diurnal light availability, changing light intensities, and the availability of inorganic nutrients (N, P, S, Fe, etc.). A consequence of these fluctuating conditions is that cells experience rapid changes in cellular redox states due to changing photosynthetic and respiratory electron transfer processes, as well as oxygen levels, over the course of a day.

In order to maintain cellular levels of reducing equivalents generated by the photosystems within acceptable boundaries, cyanobacteria adjust their cellular contents of PS I, PS II, and light-harvesting...
phycoobilisomes to maximize both ATP and NADPH production (Fujita et al., 1994). However, the availability of CO₂, which is the major sink for the electrons produced, is an equally important factor. Thus, it is critical that cells balance reductant generation and CO₂ fixation rates to avoid over-reduction of electron carriers, which can lead to the production of reactive oxygen and nitrogen species that can cause potentially lethal phototoxic damage to cells. Such adjustments can be performed at many different stages of gene expression, including mRNA synthesis (transcription); protein biosynthesis (translation); protein maturation, assembly, and stability; post-translational modification and allostery.

Because the complete genome of *Synechococcus 7002* is available and the complete set of its predicted genes is known, gene expression in this cyanobacterium can be studied at different levels. This study focused on the transcriptional level and was performed to characterize the expression of all predicted mRNA-encoding open reading frames (ORFs) of *Synechococcus 7002* cells grown under selected physiological states. Several methods are available to obtain information about transcript levels. Microarrays have been extensively used to study global gene expression patterns in several cyanobacteria, including *Synechocystis* sp. PCC 6803, *Nostoc* sp. PCC 7120, *Nostoc punctiforme*, *Synechococcus* sp. PCC 7942, and *Synechococcus* sp. WH8102 (Hihara et al., 2001, 2003; Gill et al., 2002; Ehira et al., 2003; Postier et al., 2003; Singh et al., 2003; Sato et al., 2004; Kuchroo et al., 2005; Campbell et al., 2007, 2008; Foster et al., 2007; Nodop et al., 2008; Summerfield et al., 2008; Stuart et al., 2009; Ito et al., 2009; Ostrowski et al., 2010; Rowland et al., 2010). However, microarrays suffer from a number of problems, including their relatively low sensitivity, accuracy, specificity, and reproducibility (see Draghici et al., 2006 for a review). Quantitative-RT-PCR allows a high degree of specificity and accuracy, but this method is not suitable for global analyses of transcription. Because Next-Generation (Next-Gen) sequencing can provide very large numbers of randomly distributed cDNA sequences over an entire cDNA sample (Cloonan et al., 2008), cDNA sequencing was employed to obtain global transcription information for *Synechococcus 7002*. In the studies described here, the global transcriptome of *Synechococcus 7002* cells that had been grown under a well-defined, standard photolithoautotrophic conditions was determined. Additionally, the transcriptomes of cells that had been subjected to selected perturbations, including exposure to high-light intensity or darkness under oxic respiratory or anoxic fermentative conditions were compared to that of cells grown under standard conditions. Finally, the transcriptome of *Synechococcus 7002* cells grown photolithoautotrophically under micro-oxic conditions was determined. The resulting datasets were compared to provide some initial insights into the patterns of gene expression that might be responsible for some of the physiological properties exhibited by *Synechococcus 7002*.

**MATERIALS AND METHODS**

**SAMPLE PREPARATION**

*Synechococcus 7002* was grown in 20-mm culture tubes containing medium A (25 mL) supplemented with 1 mg of NaNO₃ mL⁻¹ as nitrogen source (designated as medium A⁺; Stevens and Porter, 1980). Medium A is a Tris-buffered (pH 8.2) medium containing 0.3 M NaCl and 20 mM magnesium-sulfate; the exact composition of medium A is 18 g L⁻¹ NaCl, 0.6 g L⁻¹ KCl, 1.0 g L⁻¹ NaNO₃, 5.0 g L⁻¹ MgSO₄·7H₂O, 50 mg L⁻¹ KH₂PO₄, 266 mg L⁻¹ CaCl₂, 30 mg L⁻¹ Na₂EDTA-2H₂O, 3.89 mg L⁻¹ FeCl₃·6H₂O, 1 g L⁻¹ Tris/HCl (pH 8.2), 1 mL L⁻¹ P1 trace metal solution, 4 μg L⁻¹ vitamin B₁₂, 0.03 g L⁻¹ MoO₃ (85%), 0.003 g L⁻¹ CaSO₄·5H₂O, 0.01215 g L⁻¹ CoCl₂·6H₂O. Unless otherwise specified, cultures were grown at 38°C with continuous illumination at 250 μmol photons m⁻² s⁻¹ and were sparged with 1% (v/v) CO₂ in air (these optimal growth conditions are here defined as “standard conditions”). Pre-cultures were grown under these “standard conditions” under continuous illumination. Cultures for RNA analyses were inoculated at an OD₇₃₀ of between 0.05 and 0.1; and cells were subsequently grown under these conditions to OD₇₃₀ = 0.7 (see Figure 1). For high-light-intensity treatment or for incubation in darkness, cells were grown to OD₇₃₀ = 0.7 under the specified standard conditions and immediately prior to harvest were illuminated at ~900 μmol photons m⁻² s⁻¹ or incubated in the dark for 1 h. For the latter treatment, cells were incubated in the dark while sparging with 1% (v/v) CO₂ in N₂ for 1 h. Photolithoautotrophic growth under micro-oxic conditions was performed by growing cells in the light while sparging with 1% (v/v) CO₂ in N₂ under otherwise standard conditions. To identify transcription changes during the growth of a batch culture, cells were harvested at OD₇₃₀ = 0.4, 0.7, 1.0, 3.0, and 5.0 (see Figure 1). Cells were rapidly chilled and centrifuged (5 min, 5000 × g, 4°C) and the cell pellets were quickly frozen in liquid nitrogen and stored at −80°C until required for further processing.

**RNA EXTRACTION**

Frozen cells from 20 to 30 mL culture were resuspended in 10 mM Tris–HCl, pH 8.0 (400 μL) and lysozyme (40 μL of a 50-mg/mL stock) was added. The sample (220 μL, each) was dispensed into two 1.5-ml reaction tubes and incubated for 2 min at room temperature. For protein denaturation and cell lysis, acidic phenol–chloroform solution [400 μL; I:1 (v/v), pH 4.3] and BugBuster Protein Extraction Reagent (400 μL; Novagen) were added to each reaction tube, and the samples were vortexed two times for 1 min and stored on ice between treatments. The samples were centrifuged (2 min, 10,000 × g, 22°C), the aqueous phase was extracted once more with phenol–chloroform solution, and the combined extracts were finally extracted once with chloroform–isoamyl alcohol (24:1, v/v). The RNA was precipitated from the aqueous phase by adding one tenth volume of 3.0 M sodium acetate, pH 5.2 and 2.5 volumes of ethanol, and the precipitated RNA was washed twice with 70% (v/v) ethanol. After the resulting pellet was air-dried, the RNA was further processed using the High Pure RNA Isolation Kit (Roche) according to the recommendations of the manufacturer. Briefly, the RNA pellet was resuspended in DNase incubation buffer (460 μL), and RNAsin RNase inhibitor (4 μL, Promega) and DNase I (60 μL) were added to the samples, which were incubated for 1 h at 22°C. The RNA was purified by size exclusion on the provided spin-columns, which also removed small RNAs (approximately <300 bp). When rRNA depletion was performed, the RNA was dissolved in TE buffer (10 mM Tris–HCl, pH 8.0, 1 mM EDTA) or twice-distilled H₂O when it was...
used directly for cDNA library synthesis. Depletion of rRNA was performed using MICROBExpress Bacterial mRNA Purification Kit (Ambion) with cyanobacteria-specific capture oligonucleotides. RNA concentrations were determined using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific); a Qubit System (Invitrogen) was also used to determine RNA and DNA concentrations separately.

**eDNA LIBRARY CONSTRUCTION AND SOLID™ SEQUENCING**

Construction of cDNA libraries and SOLID™ sequencing was performed in the Genomics Core Facility at The Pennsylvania State University (University Park, PA, USA). The cDNA libraries were constructed from 0.5 μg RNA sample using SOLID™ Whole Transcriptome Analysis Kit (Applied Biosystems) and were barcoded by using the SOLID™ Transcriptome Multiplexing Kit (Applied Biosystems). SOLID™ ePCR Kit and SOLID™ Bead Enrichment Kit (both Applied Biosystems) were used for processing the samples for sequencing, and either the SOLID™ 3 or 3Plus protocol (Applied Biosystems) was used for sequencing.

The cDNA sequence data have been submitted to the NCBI Sequence Read Archive (SRA) under accession number SRP004049.

**DATA ANALYSES**

The sequencer datasets were first converted into fastq format using the PERL script supplied with the BWA software package (Li and Durbin, 2009). The resulting fastq sequences were mapped in colorspace against the Synechococcus 7002 genome using the Burrows–Wheeler algorithm, allowing four mismatches (>90% sequence identity). Prior to further analyses, the sequences mapping to rRNA-coding regions (the major portion of all datasets) and non-uniquely mapping reads were removed from the output files generated by BWA. All protein-coding regions were analyzed for cDNA sequences mapping entirely within or partially covering the respective ORF (by at least one nucleotide), and the resulting hits for each ORF were counted. The relative transcript abundances for all ORFs were calculated as the number of sequences mapping in a given ORF divided by the total number of sequences mapping within any protein-coding region. To monitor differences in transcript level between two conditions, the relative transcript abundances under the two conditions were compared for all ORFs. The ratio is given as the relative transcript abundance under condition 2 divided by the relative transcript abundance under condition 1. For the “standard” growth conditions defined above, cDNA sequence data for three independent biological replicates were obtained. Based on these three “standard” datasets the mean and standard deviation for the relative transcript abundance was calculated for each ORF. When “standard” conditions served as the basis for a comparison, the probability for equal transcription was calculated for each ORF using the z-test (Ott and Longnecker, 2000). When conditions were compared, for which only single datasets were available, the chi-square test was applied to determine the probability level for equal transcription for each ORF (Monaghan et al., 2009).

To compare the mRNA levels for different genes within one sample, the number of aligned sequences for a given ORF was normalized by the length of the ORF, and the results are reported as aligned sequences (“hits”) per kilobase. For comparisons of the same gene but in different samples, the number of aligned sequences was normalized relative to the total number of mRNA counts, because the gene length is constant but the total number of mRNA counts was variable and was also dependent upon the sequencing depth.

**RESULTS AND DISCUSSION**

**ANALYZING THE TRANSCRIPTOME OF SYNECHOCOCCUS 7002 BY SOLID™ SEQUENCING OF cDNA**

Under the experimental design and conditions defined as “standard” (see Materials and Methods), cultures of Synechococcus 7002 grow exponentially up to an optical density at 730 nm (OD$_{730\,nm}$) of ~0.7 (see Figure 1; and Sakamoto and Bryant, 1998). Growth slows at higher OD$_{730\,nm}$ values because of light limitation imposed by self-shading. Thus, cultures were harvested at OD$_{730\,nm}$ = 0.7 to produce the maximal yield of cells in exponential phase. RNA was extracted as described and used for cDNA sequencing. Depending on how many samples were pooled and barcoded for one sequencing run, and the version of the sequencing chemistry employed (SOLID™ 3 or 3Plus), between ~7 and 30 million mapped sequence reads were obtained with SOLID™ sequencing (Table 1). In the absence of any depletion of rRNA, 89.9–94% of the sequences mapped to the 16S and 23S rRNAs. Because of the presence of some small, multigene families (e.g., psbA, psbD) that have similar sequences, the percentage of uniquely mapped sequences ranged from 94.9 to 98.3%. However, cDNA sequencing allows unique mapping even if only one base is different, and transcription differences can therefore be detected even for very similar genes. This is not possible when using microarrays, at least not with such a high accuracy, because that method is based on hybridization (Hihara et al., 2001).

In some early experiments, RNA samples were processed in attempts to remove some of the rRNA sequences (see Materials and Methods for details). In one sample from cells grown under standard conditions, rRNA was depleted to a level about 20% lower than the average value for untreated samples (Table 1), but on average, the depletion resulted in a much smaller reduction in rRNA, generally only ~5%. Because the depletion protocol employed was relatively ineffective, rRNA depletion was not employed prior to cDNA synthesis for most samples.

![FIGURE 1 | Growth curve for Synechococcus 7002. Growth curve under “standard” conditions in medium A; indicating the OD$_{730\,nm}$ values at which samples for transcription analyses were taken (arrow).](image-url)
Table 1 | Number of sequences obtained by SOLID™ sequencing for the samples analyzed in this study.

| Sample      | Mapped reads | Mapped in rDNA regions | Percent rDNA | Remaining mapped reads | Uniquely mapped reads | Percent unique reads |
|-------------|--------------|------------------------|--------------|-----------------------|----------------------|---------------------|
| Standard 1  | 18,238,746   | 13,193,499             | 72.3            | 5,045,247             | 4,886,185            | 96.8               |
| Standard 2  | 29,450,401   | 27,109,346             | 92.1           | 2,341,055             | 2,270,856            | 970                |
| Standard 3  | 25,062,458   | 22,558,727             | 89.9           | 2,523,731             | 2,455,018            | 973                |
| Darkoxic    | 7,375,663    | 6,406,311              | 86.9           | 969,352               | 949,679             | 98.0               |
| Dark anoxic | 16,252,102   | 13,828,205             | 85.1           | 2,423,897             | 2,382,528            | 98.3               |
| High light  | 8,112,975    | 7,249,329              | 89.4           | 863,646               | 819,321             | 94.9               |
| OD 0.4      | 6,834,732    | 6,251,835              | 91.5           | 582,897               | 569,435             | 97.7               |
| OD 1.0      | 16,964,973   | 15,545,651             | 91.6           | 1,419,322             | 1,381,923            | 97.4               |
| OD 3.0      | 11,375,065   | 10,530,750             | 92.6           | 844,315               | 821,743             | 973                |
| OD 5.0      | 9,375,497    | 8,813,140              | 94.0           | 562,357               | 548,269             | 975                |

The number of reads obtained for the different samples, number of mapped reads, number of reads mapping within the rDNA regions and outside rDNA regions and the number of reads mapping uniquely (outside rDNA regions) are given for the individual samples.

*These samples were treated to deplete rRNAs.

Table 2 | The 15 most abundant mRNA species in the “standard 1” sample.

| Locus tag      | Counts | Counts/total counts | Gene name | Gene product                                      |
|----------------|--------|---------------------|-----------|---------------------------------------------------|
| SYNPC7002_A0957 | 19392  | 0.00610             | psbT      | Photosystem II reaction center, PsbT protein      |
| SYNPC7002_A2210 | 70907  | 0.02231             | cpcA      | Phycocyanin, alpha subunit                         |
| SYNPC7002_A2209 | 52347  | 0.01647             | cpcB      | Phycocyanin, beta subunit                         |
| SYNPC7002_A1929 | 43223  | 0.01360             | apcB      | Allophycocyanin, beta subunit                      |
| SYNPC7002_A2579 | 28125  | 0.00886             | –         | Hypothetical protein                              |
| SYNPC7002_C0011 | 13311  | 0.00419             | –         | Hypothetical protein                              |
| SYNPC7002_A1930 | 32380  | 0.01019             | apcA      | Allophycocyanin alpha subunit                      |
| SYNPC7002_A1589 | 15162  | 0.00477             | psaC      | Photosystem I iron-sulfur center subunit VII       |
| SYNPC7002_A1418 | 63774  | 0.02006             | psbA      | Photosystem qb protein                            |
| SYNPC7002_A2804 | 33226  | 0.01045             | –         | General secretion pathway protein                  |
| SYNPC7002_A1928 | 9781   | 0.00308             | apcC      | Allophycocyanin-associated phycobilisome           |
| SYNPC7002_A0272 | 12032  | 0.00379             | –         | 7.8-kDa core-linker polypeptide                    |
| SYNPC7002_A2326 | 12655  | 0.00398             | petF      | RNA-binding protein                               |
| SYNPC7002_A1008 | 21979  | 0.00691             | psaF      | Ferredoxin I (2Fe-2S)                              |
| SYNPC7002_A0167 | 14787  | 0.00465             | petJ      | Photosystem I reaction center subunit III, PsAF    |

In the “standard 1” sample, which had the highest number of non-rRNA sequences, transcripts were detected for nearly all of the 3235 predicted ORFs in the *Synechococcus 7002* genome. Table 2 presents the 15 genes that had the highest number of mapped cDNA sequences in cells grown under standard conditions. As expected, most of these genes encode structural components of the phycobilisomes and the two photosystems. For example, *cpcA* (SYNPC7002_A2210), encoding the alpha subunit of phycocyanin (de Lorimier et al., 1984), accounted for 2.2% of the mapped cDNA sequences and *psbA* (SYNPC7002_A1418) accounted for 2.0% of the mapped cDNA sequences. Due to the very high number of total sequences provided by SOLiD™ sequencing, transcripts could be detected even for genes transcribed at very low levels; there were only a 12 ORFs (SYNPC7002_A2187, A2712, A2746, SYNPC7002_D0029, D0030, SYNPC7002_F0015, F0016, SYNPC7002_G0039, G0040, G0050, G0051, and G0052) with no mapped cDNA sequences under standard growth conditions; for two of those (SYNPC7002_A2187 and A2746) transcripts were found under at least one of the other conditions tested here, but at very low level (one or two mapped sequences only). These ORFs are either annotated as hypothetical proteins or integrases and resolvases, which suggests that they might be wrongly annotated or do not represent functional genes. The transcript levels for all genes and all conditions described in this study are provided in the Table S1 in Supplementary Material.

Some cDNA samples were also analyzed by pyrosequencing (data not shown). Although similar results were obtained for the most abundant transcripts, because of the much smaller...
number of sequences obtained and the much greater cost per sequence, pyrosequencing was not pursued further because it was not a cost-effective method for global transcription profiling.

To assess the reproducibility of the cDNA sequencing approach, RNA samples isolated from three independent cultures grown under "standard conditions" were independently converted to cDNA and subjected to SOLiD™ sequencing. Two of these samples were processed without rRNA depletion, while as described above, one sample (standard 1) was treated to deplete the rRNA sequences in the sample. Figure 2A shows a scatter plot comparing the transcript abundances calculated for the two non-depleted samples (standard 2 and standard 3). The majority of the data points occurred on a line with a slope of ∼1.0, which indicated that the mapped sequence values for each gene obtained from the independent samples were very similar. Very few data points fell outside the lines indicating a two-fold difference (Figure 2A), and most of those points are associated with genes that are transcribed at low levels, for which stochastic processes would produce the largest variation. These data suggested that, much like microarrays, differences reflecting greater than two-fold changes can be considered to be significant. Moreover, much smaller differences (∼25%) might be statistically significant for highly transcribed genes (note that p-values are reported for all comparisons in Table S2 in Supplementary Material).

Figures 2B,C show scatter plot comparisons of the results obtained for the sample subjected to rRNA depletion plotted against the results for the two samples which were not depleted of rRNA. These two scatter plots are very similar to one another,
but differ from that shown in Figure 2A by showing lightly greater variance. A larger number of genes showed transcript levels that differed by a factor of 2. This result suggested that the rRNA depletion process slightly altered the levels of some mRNAs. For this reason, and because the depletion method was variable and incomplete, rRNA depletion was abandoned after some initial trials. Because the values obtained for all three datasets were highly comparable, the transcript levels from the three “standard conditions” samples were averaged to produce a list of average transcript abundances for each gene for cells grown under standard conditions. These averages were subsequently used as the basis for comparisons of transcript abundances for other conditions (see Table 3; and Table S2 in Supplementary Material). Further, the high similarity of the datasets obtained for three “standard conditions” samples shows that reliable comparisons can be made even with a single dataset for a specific condition.

**TRANSCRIPTION CHANGES IN CELLS SUBJECTED TO HIGH-LIGHT INTENSITY**

*Synechococcus* 7002 is known to be extremely tolerant to high-light intensity (Sakamoto and Bryant, 2002; Nomura et al., 2006b), and sunlight intensity is one of the most important environmental factors in natural habitats of cyanobacteria. A light intensity of 250 μmol photons m⁻² s⁻¹, which is saturating for standard growth of dilute *Synechococcus* 7002 cultures, is often regarded as a high-light condition for *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis* 6803; e.g., Hihara et al., 2001; Mizusawa et al., 2009). To induce high-light stress in *Synechococcus* 7002, a culture which was grown under standard conditions was exposed for 1 h to a light intensity of 900 μmol photons m⁻² s⁻¹. Table S2 in Supplementary Material provides comparisons for all genes under all conditions reported here, and it includes the p-values for the respective comparisons derived from the statistical analyses. Figure 3A shows a scatter plot of the relative transcript abundances for all ORFs in cells exposed to high-light intensity compared to the average value for standard conditions. Many genes show differences greater than two-fold, and the values for a few specific genes are indicated on the panel. The transcript levels of many genes involved in the Calvin–Benson–Bassham cycle were two- to four-fold higher in cells exposed to high light than in standard conditions (Table 3). However, the transcript levels of the other fatty acid desaturases (desB, desE, desF) were unchanged. These results suggested that cells might increase the proportion of polysaturated fatty acids after high-light treatment. It has previously been shown that a desA mutant strain for *Synechococcus* 7002 exhibited a temperature-sensitive phenotype when cultures were grown at high-light intensity (Sakamoto and Bryant, 2002). Transcripts for *psbS*, encoding the substrate-binding protein of the phosphate transport system, were six-fold higher after high-light treatment (Table 3). However, the transcript levels for other genes for this ABC transport system (*psaA, psbB, pstC*) only increased approximately two-fold. Together with the higher transcript levels for genes involved in bicarbonate/CO₂ uptake, this could point to an increased cell growth. However, the genes coding for components of nitrate and sulfate assimilation mechanisms do not show an increase in the mRNA levels (see Table S2 in Supplementary Material), and it has been reported that the growth rate of *Synechococcus* 7002 remains constant under moderately high-light conditions (Nomura et al., 2006a). Therefore,
Table 3 | Changes in the transcript level for genes of selected metabolic pathways.

| Locus tag       | Ratio high light/standard | Ratio dark oxic/standard | Ratio dark anoxic/dark oxic | Ratio microoxic/dark oxic | Gene name | Gene product                                      |
|-----------------|---------------------------|--------------------------|-----------------------------|--------------------------|-----------|---------------------------------------------------|
| **GLYCOLYSIS**  |                           |                          |                             |                          |           |                                                   |
| SYNPC7002_A2438 | 1.31                      | 1.51                     | 0.29                        | 0.19                     | gik       | Glucokinase                                       |
| SYNPC7002_A0964*| 0.53                      | 3.27                     | 1.62                        | 0.50                     | pgi       | Glucose-6-phosphate isomerase                      |
| SYNPC7002_A0162*| 0.39                      | 2.96                     | 0.80                        | 0.27                     | pfkA      | 6-Phosphofructokinase PfkA                         |
| SYNPC7002_A0886 | 0.06                      | 1.48                     | 0.08                        | 0.05                     | –         | PfkB family of fructokinase                       |
| SYNPC7002_A0329*| 0.43                      | 0.70                     | 0.57                        | 0.82                     | fbp       | Fructose-1,6-bisphosphatase                       |
| SYNPC7002_A0010 | 0.85                      | 4.41                     | 1.05                        | 0.24                     | fbaB      | Fructose-bisphosphate aldolase class I            |
| SYNPC7002_A2697 | 0.07                      | 5.23                     | 0.63                        | 0.12                     | gap       | Glycerolaldehyde-3-phosphate DH, type I            |
| SYNPC7002_A1585*| 2.58                      | 0.46                     | 0.39                        | 0.84                     | pgk       | Phosphoglycerate kinase                           |
| SYNPC7002_A2233 | 1.03                      | 1.23                     | 0.27                        | 0.22                     | gpm       | 2,3-Bisphosphoglycerate-independent phosphoglycerate mutase |
| SYNPC7002_A2560 | 1.66                      | 0.34                     | 0.26                        | 0.76                     | gpmB      | Phosphoglycerate mutase                           |
| SYNPC7002_A0073 | 2.30                      | 0.50                     | 0.11                        | 0.21                     | eno       | 2-Phosphoglycerate hydratase (enolase)             |
| **PYRUVATE METABOLISM** |                       |                           |                             |                          |           |                                                   |
| SYNPC7002_A1658 | 1.56                      | 0.36                     | 0.11                        | 0.31                     | pyk       | Pyruvate kinase                                   |
| SYNPC7002_A0250 | 0.18                      | 19.27                    | 177.82                      | 9.23                     | ppsA      | Phosphoenolpyruvate synthase                      |
| SYNPC7002_A0353 | 1.47                      | 0.38                     | 0.10                        | 0.25                     | pdhA      | Pyruvate dehydrogenase E1 alpha chain             |
| SYNPC7002_A0655 | 1.78                      | 0.53                     | 0.23                        | 0.43                     | pdhB      | Pyruvate dehydrogenase E1 beta chain              |
| SYNPC7002_A1126 | 1.23                      | 0.59                     | 0.47                        | 0.60                     | ipdA      | Dihydrolipoamide dehydrogenase                    |
| SYNPC7002_A0101 | 1.52                      | 0.40                     | 0.11                        | 0.27                     | –         | Dihydrolipoamide S-acetyltransferase              |
| SYNPC7002_A1443 | 0.33                      | 24.68                    | 10755.5                     | 4.36                     | nifU      | Pyruvate:ferredoxin (flavodoxin) oxidoreductase    |
| **OXIDATIVE PENTOSE PHOSPHATE CYCLE** |                       |                           |                             |                          |           |                                                   |
| SYNPC7002_A0964*| 0.53                      | 3.27                     | 1.62                        | 0.50                     | pgi       | Glucose-6-phosphate isomerase                      |
| SYNPC7002_A1459 | 0.25                      | 3.08                     | 2.64                        | 0.85                     | zvf       | Glucose-6-phosphate 1-dehydrogenase               |
| SYNPC7002_A0928 | 0.33                      | 2.54                     | 0.32                        | 0.13                     | plg       | 6-Phosphogluconolactonase                         |
| SYNPC7002_A0221 | 0.39                      | 3.55                     | 3.46                        | 0.97                     | gnd       | 6-Phosphogluconate DH, decarboxylating            |
| SYNPC7002_A1269*| 2.45                      | 0.09                     | 0.06                        | 0.66                     | rpiA      | Ribose 5-phosphate isomerase A                    |
| SYNPC7002_A0324*| 2.99                      | 0.47                     | 0.22                        | 0.47                     | rpe       | Ribulose-phosphate 3-epimerase                    |
| SYNPC7002_A1022*| 1.31                      | 0.40                     | 0.55                        | 1.36                     | tkt       | Transketolase                                     |
| SYNPC7002_A0010*| 0.85                      | 4.41                     | 1.05                        | 0.24                     | fbaB      | Fructose-bisphosphate aldolase class I            |
| SYNPC7002_A0329*| 0.43                      | 0.70                     | 0.57                        | 0.82                     | fbp       | Fructose-1,6-bisphosphatase                       |
| SYNPC7002_A0162*| 0.39                      | 2.95                     | 0.80                        | 0.27                     | pfkA      | 6-Phosphofructokinase PfkA                         |
| SYNPC7002_A1460 | 0.31                      | 3.38                     | 5.53                        | 1.64                     | tal       | Transaldolase                                     |
| SYNPC7002_A2558 | 1.38                      | 0.59                     | 0.44                        | 0.74                     | talC      | Transaldolase                                     |
| **CBB CYCLE**   |                           |                          |                             |                          |           |                                                   |
| SYNPC7002_A1796 | 3.93                      | 0.35                     | 0.48                        | 1.38                     | rbcS      | RuBisCO, small subunit                            |
| SYNPC7002_A1798 | 3.07                      | 0.28                     | 0.35                        | 1.24                     | rbcL      | RuBisCO, large subunit                            |
| SYNPC7002_A1797 | 3.01                      | 0.44                     | 0.50                        | 1.13                     | rbcX      | RuBisCO protein, RuBisCO chaperone                |
| SYNPC7002_A1585*| 2.58                      | 0.46                     | 0.39                        | 0.84                     | pgk       | Phosphoglycerate kinase                           |
| SYNPC7002_A0106 | 1.94                      | 0.25                     | 0.04                        | 0.17                     | gap       | Glycerolaldehyde-3-phosphate DH, type I            |
| SYNPC7002_A1352 | 2.64                      | 0.53                     | 0.20                        | 0.37                     | fba       | Fructose-bisphosphate aldolase, class II          |
| SYNPC7002_A0329 | 0.43                      | 0.70                     | 0.57                        | 0.82                     | fbp       | Fructose-1,6-bisphosphatase                       |
| SYNPC7002_A1301 | 2.34                      | 0.34                     | 0.10                        | 0.31                     | glpX      | Bacterial fructose-1,6-bisphosphatase             |
| SYNPC7002_A1022*| 1.31                      | 0.40                     | 0.55                        | 1.36                     | tkt       | Transketolase                                     |
| SYNPC7002_A0595 | 2.09                      | 0.46                     | 0.09                        | 0.19                     | tpiA      | Triosephosphate isomerase                         |
| SYNPC7002_A1269*| 2.45                      | 0.09                     | 0.06                        | 0.66                     | rpiA      | Ribose 5-phosphate isomerase A                    |
| SYNPC7002_A0324*| 2.99                      | 0.47                     | 0.22                        | 0.47                     | rpe       | Ribulose-phosphate 3-epimerase                    |
| SYNPC7002_A2657 | 2.34                      | 0.39                     | 0.03                        | 0.09                     | prk       | Phosphoribokinase                                 |
| SYNPC7002_A2665 | 1.36                      | 1.10                     | 0.40                        | 0.36                     | prk       | Phosphoribokinase                                 |

(Continued)
Table 3 | Continued

| Locus tag   | Ratio high light/ std | Ratio dark oxic/ std | Ratio dark anoxic/ std | Ratio micro-oxic/ std | Gene name | Gene product |
|-------------|-----------------------|----------------------|------------------------|------------------------|-----------|--------------|
| **CO2 CONCENTRATION** |                       |                      |                        |                        |           |              |
| SYNPC7002_A1805  | 0.63                  | 0.89                 | 0.30                   | 0.33                   | ndhF4     | NADH Dh subunit F4 |
| SYNPC7002_A1806  | 0.53                  | 0.60                 | 0.14                   | 0.23                   | ndhD4     | NADH Dh subunit D4 |
| SYNPC7002_A1807  | 0.54                  | 0.57                 | 0.13                   | 0.22                   | cupB      | CO2 hydration protein |
| SYNPC7002_A0172  | 1.08                  | 0.39                 | 0.31                   | 0.80                   | ndhF3     | NADH Dh (plastoquinone) chain 5 |
| SYNPC7002_A0173  | 1.07                  | 0.27                 | 0.17                   | 1.30                   | ndhD3     | NADH-quinone oxidoreductase (subunit 4) |
| SYNPC7002_A0174  | 1.49                  | 0.76                 | 0.19                   | 0.25                   | cupA      | CO2 hydration protein |
| SYNPC7002_A0175  | 2.39                  | 0.79                 | 0.26                   | 0.33                   | cupS      | Conserved hypothetical protein |
| SYNPC7002_A0171  | 1.39                  | 0.66                 | 0.70                   | 1.05                   | rbcR      | Transcription regulator RbcR |
| SYNPC7002_A2371  | 0.96                  | 1.15                 | 1.25                   | 1.09                   | bicA      | Bicarbonate transporter, BicA |
| SYNPC7002_A0470  | 3.43                  | 1.02                 | 0.29                   | 0.28                   | sbbA      | Sodium-dependent bicarbonate transporter |
| SYNPC7002_A2612  | 1.55                  | 0.62                 | 0.07                   | 0.11                   | ccmK      | CO2 concentrating mechanism protein |
| SYNPC7002_A2613  | 1.71                  | 0.61                 | 0.51                   | 0.84                   | ccmK      | CO2 concentrating mechanism protein |
| SYNPC7002_A1802  | 2.22                  | 0.37                 | 0.02                   | 0.06                   | ccmK      | CO2 concentrating mechanism protein |
| SYNPC7002_A1803  | 3.56                  | 0.46                 | 0.03                   | 0.07                   | ccmK      | CO2 concentrating mechanism protein |
| SYNPC7002_A2389  | 0.95                  | 1.59                 | 1.59                   | 1.00                   | ccmK      | CO2 concentrating mechanism protein |
| SYNPC7002_A1801  | 1.92                  | 0.38                 | 0.03                   | 0.07                   | ccmK      | CO2 concentrating mechanism protein |
| SYNPC7002_A1800  | 2.69                  | 0.31                 | 0.04                   | 0.14                   | ccmK      | CO2 concentrating mechanism protein |
| SYNPC7002_A1799  | 1.57                  | 0.15                 | 0.05                   | 0.35                   | ccmK      | CO2 concentrating mechanism protein |
| SYNPC7002_A1997  | 2.27                  | 0.60                 | 0.15                   | 0.26                   | icfA      | Carbonic anhydrase |
| **PHOTOSYSTEM II** |                       |                      |                        |                        |           |              |
| SYNPC7002_A1418  | 2.03                  | 0.73                 | 0.20                   | 0.27                   | psbA      | Photosystem q(b) protein |
| SYNPC7002_A2164  | 0.71                  | 0.57                 | 0.62                   | 1.08                   | psbA      | Photosystem q(b) protein |
| SYNPC7002_A0157  | 5.20                  | 0.70                 | 1.17                   | 1.67                   | psbA-II   | PS II D1 subunit PsbA-II (Qb protein) |
| SYNPC7002_A1759  | 2.03                  | 0.79                 | 0.61                   | 0.78                   | psbB      | PS II protein |
| SYNPC7002_A1559  | 1.66                  | 0.51                 | 0.40                   | 0.79                   | psbC      | PS II 44 kDa subunit reaction center protein |
| SYNPC7002_A1560  | 1.81                  | 0.50                 | 0.22                   | 0.45                   | psbD      | PS II D2 protein (photosystem q(a) protein) |
| SYNPC7002_A2199  | 1.62                  | 0.60                 | 0.12                   | 0.19                   | psbD      | PS II D2 protein |
| SYNPC7002_A0230  | 1.25                  | 0.57                 | 0.13                   | 0.24                   | psbE      | Cytochrome b559, alpha subunit (PsbE) |
| SYNPC7002_A0231  | 0.82                  | 0.33                 | 0.26                   | 0.78                   | psbF      | Cytochrome b559, beta subunit (PsbF) |
| SYNPC7002_A0808  | 0.93                  | 0.37                 | 0.42                   | 1.14                   | psbH      | Phosphoprotein of PS II |
| SYNPC7002_A0233  | 1.02                  | 0.56                 | 0.40                   | 0.72                   | psbJ      | PS II subunit PsbJ |
| SYNPC7002_A2279  | 0.95                  | 0.27                 | 0.08                   | 0.31                   | psbK      | PS II 4 kDa reaction center component |
| SYNPC7002_A0232  | 0.85                  | 0.39                 | 0.29                   | 0.74                   | psbL      | PS II subunit PsbL |
| SYNPC7002_A2151  | 0.89                  | 0.15                 | 0.07                   | 0.46                   | psbM      | PS II reaction center M protein |
| SYNPC7002_A0809  | 4.54                  | 0.36                 | 0.22                   | 0.61                   | psbN      | PS II reaction center N protein-related protein |
| SYNPC7002_A0269  | 1.72                  | 0.17                 | 0.17                   | 1.03                   | psbO      | PS II manganese stabilizing protein PsbO |
| SYNPC7002_A1303  | 0.73                  | 0.68                 | 0.22                   | 0.33                   | psbP      | PsbP |
| SYNPC7002_A0957  | 0.38                  | 0.40                 | 0.23                   | 0.57                   | psbT      | PS II reaction center, PsbT protein |
| SYNPC7002_A0322  | 1.11                  | 0.39                 | 0.07                   | 0.18                   | psbU      | PS II 12 kDa extrinsic protein (PsbU) |
| SYNPC7002_A0112  | 0.77                  | 0.30                 | 0.11                   | 0.36                   | psbV      | Cytochrome c-550 precursor (cytochrome c550) |
| SYNPC7002_A1258  | 0.90                  | 0.65                 | 0.30                   | 0.46                   | psbW      | PS II reaction center W protein |
| SYNPC7002_A1312  | 1.01                  | 1.14                 | 4.87                   | 4.27                   | psbW2     | PS II complex subunit |
| SYNPC7002_A1347  | 1.95                  | 0.44                 | 0.19                   | 0.43                   | psbY      | PS II PsbY protein |
| SYNPC7002_A0135  | 1.17                  | 0.28                 | 0.27                   | 1.51                   | psbZ      | PS II subunit PsbZ |
| SYNPC7002_A2533  | 0.82                  | 0.19                 | 0.13                   | 0.45                   | psbZ      | PS II 11 kDa protein |
| **PHOTOSYSTEM I** |                       |                      |                        |                        |           |              |
| SYNPC7002_A1961  | 0.42                  | 0.52                 | 0.56                   | 1.08                   | psaA      | PS I P700 chlorophyll A apoprotein A1 |
| SYNPC7002_A1962  | 0.56                  | 0.39                 | 1.21                   | 3.08                   | psaB      | PS I protein A2 |
| SYNPC7002_A1589  | 0.32                  | 0.17                 | 0.06                   | 0.33                   | psaC      | PS I iron-sulfur center subunit VII |
| SYNPC7002_A0682  | 0.73                  | 0.70                 | 0.11                   | 0.16                   | psaD      | PS I subunit II |
| SYNPC7002_A1393  | 0.69                  | 0.32                 | 0.04                   | 0.12                   | psaE      | PS I reaction center subunit IV |

(Continued)
| Locus tag | Ratio high light/ std | Ratio dark oxid/ std | Ratio dark anoxic/ std | Ratio dark anoxic/ dark oxid | Ratio micro-oxic/ std | Gene name | Gene product |
|-----------|-----------------------|----------------------|------------------------|-------------------------------|----------------------|-----------|--------------|
| **PHOTOSYSTEM I** | | | | | | | |
| SYNPC7002_A1008 | 1.31 | 0.49 | 0.12 | 0.25 | 2.14 | psaF | PSI reaction center subunit III, PsaF |
| SYNPC7002_A2621 | 0.35 | 0.32 | 0.08 | 0.24 | 1.31 | psaI | PS I reaction center subunit VIII |
| SYNPC7002_A1009 | 0.36 | 0.22 | 0.12 | 0.53 | 0.64 | psaJ | PS I reaction center, subunit IX/PsaJ |
| SYNPC7002_A2401 | 0.28 | 0.30 | 0.04 | 0.13 | 1.29 | psaK | PS I reaction center subunit X |
| SYNPC7002_A2620 | 0.35 | 0.44 | 0.02 | 0.04 | 1.12 | psaL | PS I reaction center subunit XI |
| SYNPC7002_A1834 | 0.27 | 0.32 | 0.41 | 1.28 | 0.28 | psaM | PS I protein M |
| SYNPC7002_A0975 | 1.92 | 0.38 | 0.19 | 0.49 | 0.92 | btpA | PS I biogenesis protein btpA |
| **PHOTOSYNTHETIC ELECTRON TRANSPORT** | | | | | | | |
| SYNPC7002_A1910 | 2.05 | 0.17 | 0.06 | 0.37 | 1.07 | petA | Apocytochrome f precursor |
| SYNPC7002_A0842 | 2.27 | 0.68 | 0.13 | 0.19 | 1.31 | petB | Cytochrome b6 |
| SYNPC7002_A1909 | 2.25 | 0.19 | 0.14 | 0.74 | 1.25 | petC | Rieske FeS protein |
| SYNPC7002_G0076 | 0.28 | 0.00 | 0.50 | n.d. | 0.00 | petC-II | Rieske iron-sulfur protein paralog |
| SYNPC7002_A0841 | 1.30 | 0.60 | 0.12 | 0.21 | 1.13 | petD | cytb6/f complex subunit IV |
| SYNPC7002_A1097 | 0.84 | 0.69 | 0.23 | 0.33 | 0.76 | – | Cytochrome b6/f complex, alt. iron-sulfur subunit |
| SYNPC7002_A0374 | 1.41 | 0.22 | 0.07 | 0.30 | 0.74 | petG | Cytochrome b6-f complex subunit 5 |
| SYNPC7002_A1311 | 1.31 | 0.66 | 0.11 | 0.17 | 0.58 | petM | Cytochrome b6-f complex subunit VII |
| SYNPC7002_A2391 | 1.55 | 0.86 | 0.50 | 0.58 | 1.10 | petJ | Cytochrome c6 |
| SYNPC7002_A0167 | 0.82 | 0.53 | 0.25 | 0.47 | 0.71 | petJ | Cytochrome c6 precursor (cytochrome c553) |
| SYNPC7002_A0624 | 0.77 | 0.55 | 1.31 | 2.36 | 0.75 | petF | Ferredoxin (2Fe-2S) II |
| SYNPC7002_A2192 | 1.44 | 0.60 | 0.70 | 1.17 | 1.37 | petF | Ferredoxin (2Fe-2S) |
| SYNPC7002_A2325 | 4.08 | 0.69 | 0.14 | 0.20 | 1.15 | petF | Ferredoxin |
| SYNPC7002_A2326 | 2.81 | 0.40 | 0.11 | 0.27 | 1.53 | petF | Ferredoxin I (2Fe-2S) |
| SYNPC7002_A2548 | 0.66 | 1.38 | 1.01 | 0.73 | 0.36 | petF2 | Ferredoxin PetF2 |
| SYNPC7002_A0853 | 2.17 | 0.29 | 0.21 | 0.72 | 1.35 | petH | Ferredoxin-NADP reductase |
| **PHYCOCYANISMES, LYASES AND DEGRADATION** | | | | | | | |
| SYNPC7002_A1930 | 0.67 | 0.20 | 0.05 | 0.23 | 1.55 | apcA | Allophycocyanin alpha subunit |
| SYNPC7002_A1929 | 1.01 | 0.20 | 0.05 | 0.23 | 1.99 | apcB | Allophycocyanin, beta subunit |
| SYNPC7002_A1928 | 0.49 | 0.26 | 0.07 | 0.26 | 0.78 | apcC | Allophycocyanin-associated core-linker |
| SYNPC7002_A2140 | 0.86 | 0.89 | 0.33 | 0.37 | 1.27 | apcD | Allophycocyanin B alpha subunit |
| SYNPC7002_A2009 | 0.51 | 0.51 | 0.27 | 0.52 | 1.41 | apcE | Phycobilisome core-membrane linker ApcE |
| SYNPC7002_A1831 | 0.92 | 0.75 | 0.21 | 0.28 | 0.96 | apcF | Allophycocyanin beta-18 subunit |
| SYNPC7002_A2210 | 0.53 | 0.25 | 0.10 | 0.40 | 2.46 | cpcA | Phycocyanin, alpha subunit |
| SYNPC7002_A2209 | 0.39 | 0.13 | 0.03 | 0.21 | 1.82 | cpcB | Phycocyanin, beta subunit |
| SYNPC7002_A2211 | 0.10 | 0.23 | 0.25 | 1.09 | 1.62 | cpcC | Phycocyanin-associated rod linker protein |
| SYNPC7002_A2212 | 0.23 | 0.20 | 0.02 | 0.09 | 1.35 | cpcD | Phycocyanin-associated, rod-terminating linker |
| SYNPC7002_A0811 | 0.45 | 0.42 | 0.12 | 0.29 | 1.22 | cpcG1 | Phycobilisome rod-core linker CpcG |
| SYNPC7002_A0639 | 0.23 | 1.04 | 0.15 | 0.14 | 0.93 | cpcG2 | Phycocyanin-associated rod-core linker |
| SYNPC7002_A2010 | 0.48 | 0.26 | 0.03 | 0.13 | 1.13 | cpcM | Phycobiliprotein beta subunit methylase CpcM |
| SYNPC7002_A2213 | 0.51 | 0.20 | 0.03 | 0.14 | 0.94 | cpcE | Phycocyanin alpha subunit lyase, CpcE |
| SYNPC7002_A2214 | 0.55 | 0.40 | 0.10 | 0.26 | 0.80 | cpcF | Phycocyanin alpha subunit lyase, CpcF |
| SYNPC7002_A1822 | 1.07 | 0.74 | 0.13 | 0.18 | 1.03 | cpcS | Phycobiliprotein lyase |
| SYNPC7002_A2053 | 0.68 | 1.19 | 0.75 | 6.66 | 0.76 | cpcU | Phycobiliprotein lyase |
| SYNPC7002_A2095 | 1.67 | 0.55 | 0.16 | 0.28 | 1.80 | cpcT | Bilin lyase |
| SYNPC7002_A2772 | 1.67 | 0.36 | 0.39 | 1.06 | 0.96 | cpcV | Similar to phycobiliprotein lyase CpcS and CpcU |
| SYNPC7002_A1821 | 2.17 | 4.77 | 0.75 | 0.16 | 1.21 | nbIA | Phycobilisome degradation protein |
| SYNPC7002_A0348 | 0.79 | 2.68 | 6.62 | 2.47 | 1.02 | nbIB | Phycobiliprotein lyase related protein |
| **PORPHYRIN/CHLOROPHYLL BIOSYNTHESIS** | | | | | | | |
| SYNPC7002_A1302 | 0.66 | 0.47 | 0.15 | 0.32 | 1.45 | hemA | Glutamyl-tRNA reductase |
| SYNPC7002_A2206 | 1.69 | 0.47 | 0.09 | 0.19 | 0.69 | hemL | Glutamate-1-semialdehyde-2,1-aminomutase |
| SYNPC7002_A1753 | 0.44 | 0.36 | 0.24 | 0.67 | 0.81 | hemB | Delta-aminolevulinic acid dehydratase |
| SYNPC7002_G0127 | 0.92 | 0.00 | 0.17 | n.d. | 0.14 | hemB-II | Delta-aminolevulinic acid dehydratase |

(Continued)
Table 3 | Continued

| Locus tag       | Ratio high light/std | Ratio dark oxic/std | Ratio dark anoxic/microoxic/std | Gene name | Gene product                                                                 |
|-----------------|----------------------|---------------------|---------------------------------|-----------|-----------------------------------------------------------------------------|
| **PORPHYRIN/CHLOROPHYLL BIOSYNTHESIS** |                      |                     |                                 |           |                                                                             |
| SYNPCC7002_A1610 | 1.07                 | 0.91                | 0.10                            | hemC      | Porphobilinogen deaminase                                                   |
| SYNPCC7002_A1192 | 0.36                 | 1.56                | 2.34                            | cygG/     | Uroporphyrin-III synthase/methyltransferase                                |
|                 |                      |                     |                                 | hemD      |                                                                             |
| SYNPCC7002_A0823 | 0.91                 | 0.40                | 0.30                            | hemE      | Uroporphyrinogen decarboxylase                                              |
| SYNPCC7002_A1828 | 0.40                 | 0.66                | 0.09                            | hemF      | Coproporphyrinogen III oxidase, aerobic                                      |
| SYNPCC7002_A1990 | 1.02                 | 1.08                | 1.20                            | hemN2     | O2-independent coproporphyrinogen III oxidase                              |
| SYNPCC7002_A2831 | 1.44                 | 1.47                | 1.85                            | hemN      | O2-independent coproporphyrinogen III oxidase, putative                     |
| SYNPCC7002_A0644 | 0.56                 | 1.67                | 0.37                            | hemJ      | Protoporphyrinogen oxide                                                   |
| SYNPCC7002_A2476 | 0.43                 | 1.05                | 0.23                            | chiP      | Geranylgeranyl reductase                                                    |
| SYNPCC7002_A0596 | 1.90                 | 0.41                | 0.09                            | chiD      | Mg-protoporphyrin IX chelatase subunit D                                   |
| SYNPCC7002_A2256 | 1.71                 | 0.32                | 0.11                            | chiI      | Mg-chelatase ATPase subunit I                                              |
| SYNPCC7002_A1000 | 1.04                 | 1.45                | 3.65                            | chiH      | Mg-chelatase, subunit H                                                     |
| SYNPCC7002_A1018 | 0.68                 | 0.78                | 0.52                            | chiH      | Mg-protoporphyrin IX chelatase subunit H                                   |
| SYNPCC7002_A0908 | 1.92                 | 0.49                | 0.46                            | chiM      | Magnesium-protoporphyrin O-methyltransferase                               |
| SYNPCC7002_A0707 | 0.82                 | 0.58                | 0.08                            | acsF      | Magnesium-protoporphyrin IX monomethyl ester aerobic oxidative cyclase      |
|                 |                      |                     |                                 |           | (oxygen-dependent)                                                         |
| SYNPCC7002_A1982 | 1.49                 | 0.31                | 11.09                           | acsF2     | Mg-protoporphyrin IX monomethyl ester cyclase                               |
| SYNPCC7002_A0210 | 0.41                 | 0.34                | 0.10                            | por       | Light-dependent protochlorophyllide reductase                              |
| SYNPCC7002_A1652 | 0.08                 | 3.88                | 5.79                            | chiB      | Light-independent protochlorophyllide reductase, B subunit                 |
| SYNPCC7002_A2345 | 0.16                 | 4.76                | 0.78                            | chiN      | Light-independent protochlorophyllide reductase, N subunit                 |
| SYNPCC7002_A2347 | 0.15                 | 8.20                | 0.41                            | chiL      | Light-independent protochlorophyllide reductase, iron-sulfur ATP-binding   |
|                 |                      |                     |                                 |           | protein                                                                      |
| SYNPCC7002_A0548 | 2.59                 | 0.59                | 0.19                            | chiG      | Chlorophyll synthase, ChlG                                                  |
| SYNPCC7002_A2589 | 1.17                 | 0.68                | 0.40                            | hemH      | Ferrochelatase                                                             |
| SYNPCC7002_A2508 | 0.53                 | 0.31                | 0.05                            | hox1      | Heme oxygenase (decyclizing)                                                |
| SYNPCC7002_A1991 | 0.59                 | 0.14                | 17.46                           | ho2       | Heme oxygenase 2                                                           |
| SYNPCC7002_A2228 | 0.55                 | 0.78                | 0.26                            | acsF      | Phycocyanobilin-ferredoxin oxidoreductase                                  |
|                 |                      |                     |                                 |           |                                                                             |
| **ATP SYNTHASE** |                      |                     |                                 |           |                                                                             |
| SYNPCC7002_A0734 | 3.01                 | 0.29                | 0.29                            | atpA      | ATP synthase F1, alpha subunit                                              |
| SYNPCC7002_A0739 | 1.60                 | 0.07                | 0.02                            | atpB      | ATP synthase F0, A subunit                                                  |
| SYNPCC7002_A0750 | 2.80                 | 0.28                | 0.06                            | atpC      | ATP synthase F1, epsilon subunit                                            |
| SYNPCC7002_A0749 | 2.52                 | 0.33                | 0.51                            | atpD      | ATP synthase beta chain                                                    |
| SYNPCC7002_A0738 | 4.47                 | 0.23                | 0.21                            | atpE      | ATP synthase C chain (Lipid-binding protein)                                |
| SYNPCC7002_A0736 | 2.01                 | 0.13                | 0.08                            | atpF      | ATP synthase B chain (Subunit II)                                           |
| SYNPCC7002_A0733 | 2.18                 | 0.23                | 0.23                            | atpG      | ATP synthase F1, gamma subunit                                              |
| SYNPCC7002_A0737 | 1.45                 | 0.13                | 0.09                            | atpH      | ATP synthase F1, delta subunit                                              |
| SYNPCC7002_A0735 | 2.03                 | 0.10                | 0.16                            | atpI      | ATP synthase subunit I                                                     |
| SYNPCC7002_A0740 | 1.65                 | 0.14                | 0.04                            | atpA-II   | ATP synthase F1, alpha subunit                                              |
| SYNPCC7002_G0151 | 0.21                 | 1.58                | 0.33                            | atpB-II   | ATP synthase F0, A subunit                                                  |
| SYNPCC7002_G0148 | 0.15                 | 1.26                | 0.55                            | atpC-II   | ATP synthase epsilon subunit                                                |
| SYNPCC7002_G0145 | 0.43                 | 3.39                | 0.35                            | atpD-II   | ATP synthase epsilon subunit                                                |
| SYNPCC7002_G0144 | 0.31                 | 1.88                | 0.88                            | atpE      | ATP synthase F1, beta subunit                                              |
| SYNPCC7002_G0146 | 0.25                 | 2.88                | 0.35                            | atpF      | ATP synthase b subunit                                                     |
| SYNPCC7002_G0147 | 0.08                 | 1.1                 | 0.17                            | atpG      | ATP synthase F1, gamma subunit                                              |
| SYNPCC7002_G0150 | 0.30                 | 2.68                | 0.80                            | atpH      | ATP synthase F1, gamma subunit                                              |
| SYNPCC7002_G0152 | 0.11                 | 0.98                | 0.16                            | atpI      | ATP synthase subunit I                                                     |
| SYNPCC7002_G0149 | 0.34                 | 2.64                | 0.93                            | atpA      | ATP synthase F1, alpha subunit                                              |

(Continued)
| Locus tag | Ratio high light/std | Ratio dark oxic/std | Ratio dark anoxic/std | Ratio dark anoxic/dark oxic | Ratio micro-oxic/std | Gene name | Gene product |
|-----------|---------------------|--------------------|----------------------|---------------------------|---------------------|-----------|-------------|
| **NADH DEHYDROGENASE** | | | | | | | |
| SYNPCC7002_A0926 | 1.19 | 0.64 | 0.26 | 0.42 | 1.34 | ndhA | NADH dehydrogenase subunit A |
| SYNPCC7002_A2547 | 1.63 | 1.16 | 0.46 | 0.39 | 0.83 | ndhB | NADH dehydrogenase subunit B |
| SYNPCC7002_A2748 | 2.23 | 0.90 | 1.85 | 2.06 | 1.29 | ndhC | NADH dehydrogenase subunit C |
| SYNPCC7002_A2327 | 1.68 | 0.30 | 0.08 | 0.27 | 0.94 | ndhD | NADH dehydrogenase subunit 4 |
| SYNPCC7002_A2000 | 0.22 | 1.16 | 0.63 | 0.54 | 1.04 | ndhD1 | NADH dehydrogenase subunit D1 |
| SYNPCC7002_A1973 | 15.62 | 0.84 | 0.20 | 0.24 | 1.48 | ndhD2 | NADH dehydrogenase subunit D2 |
| SYNPCC7002_A0923 | 1.16 | 0.61 | 0.07 | 0.11 | 1.04 | ndhE | NADH dehydrogenase subunit E |
| SYNPCC7002_A0854 | 1.61 | 0.85 | 0.93 | 0.97 | 1.05 | ndhF | NADH dehydrogenase subunit 5 |
| SYNPCC7002_A0924 | 1.21 | 0.85 | 0.16 | 0.18 | 1.31 | ndhG | NADH dehydrogenase subunit G |
| SYNPCC7002_A2541 | 1.39 | 1.00 | 0.33 | 0.34 | 0.80 | ndhH | NADH dehydrogenase subunit H |
| SYNPCC7002_A0925 | 1.11 | 0.77 | 0.10 | 0.13 | 1.10 | ndhl | NADH-plastoquinone oxidoreductase, I subunit |
| SYNPCC7002_A2750 | 1.98 | 1.08 | 1.03 | 0.95 | 0.99 | ndhJ | NADH dehydrogenase subunit J |
| SYNPCC7002_A2198 | 0.65 | 0.85 | 1.86 | 2.18 | 1.07 | ndhK | NADH dehydrogenase subunit K |
| SYNPCC7002_A0560 | 2.22 | 0.78 | 0.63 | 0.81 | 1.30 | ndl | NADH DH subunit L (inorganic carbon transporter) |
| SYNPCC7002_A0569 | 1.71 | 1.10 | 0.44 | 0.40 | 1.77 | ndhM | Conserved hypothetical protein |
| SYNPCC7002_A1143 | 1.17 | 1.49 | 1.02 | 0.69 | 0.80 | ndhN | Conserved hypothetical protein |
| SYNPCC7002_A2094 | 1.19 | 0.84 | 1.23 | 1.47 | 0.83 | ndhO | Conserved hypothetical protein |
| **CYTOCHROME OXIDASE** | | | | | | | |
| SYNPCC7002_A1162 | 0.39 | 1.09 | 0.09 | 0.09 | 0.77 | ctaCl | Cytochrome oxidase subunit II |
| SYNPCC7002_A1163 | 0.50 | 1.57 | 0.34 | 0.22 | 1.06 | ctaDl | Cytochrome oxidase large subunit (subunit I) |
| SYNPCC7002_A1164 | 0.33 | 1.15 | 0.45 | 0.39 | 1.00 | ctaEl | Cytochrome oxidase small subunit (subunit III) |
| SYNPCC7002_A0727 | 0.62 | 0.45 | 0.11 | 0.24 | 1.12 | ctaC1l | Cytochrome C oxidase subunit II |
| SYNPCC7002_A0726 | 1.13 | 0.86 | 0.20 | 0.23 | 0.96 | ctaD1l | Cytochrome oxidase II large subunit |
| SYNPCC7002_A0725 | 1.09 | 0.81 | 0.29 | 0.35 | 1.26 | ctaEl | Cytochrome oxidase II small subunit |
| **TRANSDHYDROGENASE** | | | | | | | |
| SYNPCC7002_A0986 | 0.08 | 3.80 | 1.31 | 0.34 | 0.85 | pntA | Transhydrogenase, chain alpha, part 1 |
| SYNPCC7002_A0984 | 0.13 | 6.80 | 0.60 | 0.09 | 0.93 | pntB | Transhydrogenase, subunit beta |
| SYNPCC7002_A0985 | 0.15 | 7.36 | 0.84 | 0.11 | 1.26 | pntC | Transhydrogenase, chain alpha, part 2 |
| **LACTATE DEHYDROGENASE, ALCOHOL DEHYDROGENASE AND HYDROGENASE** | | | | | | | |
| SYNPCC7002_G0164 | 0.48 | 0.24 | 0.14 | 0.61 | 0.63 | ldhA | Fermentative lactate dehydrogenase |
| SYNPCC7002_A1838 | 0.64 | 1.29 | 0.67 | 0.83 | 0.65 | acsA | Acetate-CoA ligase |
| SYNPCC7002_A2015 | 0.77 | 2.37 | 0.46 | 0.19 | 0.94 | acs | Acetyl-CoA synthetase |
| SYNPCC7002_A0888 | 0.59 | 1.10 | 0.41 | 0.38 | 0.90 | – | Alcohol DH, Zn-binding family |
| SYNPCC7002_A2590 | 0.88 | 0.60 | 0.11 | 0.19 | 0.72 | – | Short-chain alcohol dehydrogenase family |
| SYNPCC7002_A0195 | 0.30 | 6.62 | 16.39 | 2.47 | 1.24 | hoxE | Hydrogenase subunit E |
| SYNPCC7002_A0196 | 0.26 | 7.23 | 4.78 | 0.66 | 1.06 | hoxF | Hydrogenase large diaphorase subunit F |
| SYNPCC7002_A0197 | 0.33 | 4.88 | 2.15 | 0.44 | 0.76 | hoxU | Hydrogenase small diaphorase subunit U |
| SYNPCC7002_A0198 | 0.37 | 4.32 | 0.99 | 0.23 | 1.05 | hoxY | Hydrogenase small subunit Y |
| SYNPCC7002_A0200 | 0.37 | 4.32 | 0.99 | 0.23 | 1.05 | hoxH | Hydrogenase large subunit H |
| **FATTY ACID DESATURASES** | | | | | | | |
| SYNPCC7002_A2756 | 2.92 | 0.14 | 0.05 | 0.39 | 1.82 | desA | Phosphatidylcholine desaturase |
| SYNPCC7002_A0159 | 9.49 | 0.00 | 0.05 | n.d. | 1.20 | desB | Omega-3 acyl-lipid desaturase |
| SYNPCC7002_A2198 | 0.81 | 0.25 | 0.07 | 0.28 | 1.02 | desC | Delta-9 acyl-lipid desaturase |
| SYNPCC7002_A2833 | 1.56 | 1.40 | 0.39 | 0.28 | 1.55 | desE | Fatty acid desaturase |
| SYNPCC7002_A1989 | 0.59 | 0.27 | 1.40 | 5.19 | 20.88 | desF | syn-2, delta 9 acyl-lipid fatty acid desaturase |
| **IRON UPTAKE** | | | | | | | |
| SYNPCC7002_A2507 | 1.01 | 0.61 | 0.16 | 0.26 | 5.39 | suF | Iron transport protein |
| SYNPCC7002_G0079 | 1.05 | 0.97 | 2.51 | 2.59 | 6.23 | – | ABC transporter, permease, FecCD family |
| SYNPCC7002_G0080 | 0.31 | 1.44 | 3.66 | 2.55 | 5.05 | – | Iron ABC transporter, ATP-binding protein |
| SYNPCC7002_G0081 | 0.36 | 0.33 | 0.93 | 2.83 | 5.01 | – | Outer membrane TonB-dependent receptor |

(Continued)
the up-regulation of the phosphate ABC transport system probably reflects an increased requirement for phosphate in cells exposed to high light, but this requirement probably is not directly linked to faster cell growth. Genes showing much lower transcript levels after high-light treatment included glyceraldehyde-3-phosphate dehydrogenase (gap, SYNCC7002_A2697, ~15-fold) and a bacterial/plant-type fructokinase (pfkB, SYNCC7002_A0886, ~15-fold; Table 3). Transcript levels for an alternative glyceraldehyde-3-phosphate dehydrogenase (gap, SYNCC7002_A0106) were slightly higher (approximately two-fold) and for a second phosphofructokinase gene (pfkA, SYNPCC7002_A0162) were slightly lower (approximately two-fold). Interestingly, transcript levels for pntA, pntB, and pntC, encoding subunits of pyridine nucleotide transhydrogenase, were 7- to 13-fold lower after high-light treatment (Table 3). These results suggested that electron exchange between the NADH and NADPH pools might be less important in cells under high-light conditions.

The reducing equivalents required for cell growth are provided by the photosystems in the light, and in the absence of photoinhibition effects, high-light intensity should provide more reducing equivalents. On the other hand, when the light intensity is too high, the photosynthetic apparatus could produce excess reducing equivalents, which should cause cells to increase the transcript levels for genes involved in electron-consuming processes, e.g., the enzymes of the Calvin–Benson–Bassham cycle (see above). Phycobilisomes serve as the main antennae for photosynthesis in cyanobacteria, and they transfer excitation energy to both photosystems (Ashby and Mullineaux, 1999; Dong et al., 2009). Acclimation of this system directly affects the efficiency of excitation usage. Further, acclimative changes in the PS II to PS I ratio determines the ratio of linear electron transport (producing reductants (e.g., NADPH) from water oxidation) to cyclic electron transport (involving PS I and the cytochrome b6f complex), which generates proton motive force across the thylakoid membrane for ATP synthesis (Fujita et al., 1994).

| Locus tag       | Ratio high light/std | Ratio dark anoxic/std | Ratio dark anoxic/std | Ratio micro-oxic/std | Gene name | Gene product |
|-----------------|----------------------|-----------------------|-----------------------|---------------------|-----------|--------------|
| SYNCC7002_G0083| 0.50                 | 1.84                  | 1.52                  | 0.82                | 7.54      | –            | ABC transporter, iron-binding lipoprotein |
| SYNCC7002_G0086| 1.13                 | 0.50                  | 0.41                  | 0.82                | 3.32      | –            | ATP-binding protein of ABC transporter for iron |
| SYNCC7002_G0087| 0.52                 | 0.48                  | 0.36                  | 0.75                | 3.02      | fecD         | Iron compound ABC transporter (FecCD) |
| SYNCC7002_G0088| 0.67                 | 0.65                  | 0.11                  | 0.16                | 3.96      | fecC         | FecCD transport (permease) family |
| SYNCC7002_G0090| 0.85                 | 0.93                  | 0.17                  | 0.18                | 4.57      | –            | TonB family C-terminal domain protein |
| SYNCC7002_G0091| 0.29                 | 0.54                  | 1.02                  | 1.89                | 1.27      | fecB         | Iron(III) dicitrate-binding periplasmic protein |
| SYNCC7002_G0092| 0.63                 | 0.63                  | 1.08                  | 1.70                | 0.90      | –            | Iron(III) dicitrate-binding periplasmic protein |
| SYNCC7002_G0093| 0.00                 | 0.99                  | 1.64                  | 1.65                | 2.91      | –            | TonB-dependent receptor |
| SYNCC7002_G0096| 0.97                 | 0.45                  | 0.42                  | 0.94                | 2.36      | –            | Ferric aerobactin receptor |
| SYNCC7002_G0102| 0.16                 | 0.61                  | 1.52                  | 2.47                | 0.90      | –            | Periplasmic binding protein; for iron siderophore |
| SYNCC7002_G0138| 0.13                 | 0.55                  | 0.15                  | 0.27                | 11.93     | –            | TonB-dependent siderophore receptor |

The ratio of relative transcript abundance under high light, dark anoxic incubation, dark fermentative incubation, and low O2 conditions compared to “standard” conditions is given. The ratio for the dark anoxic sample is additionally compared to the dark oxic sample. Genes listed in more than one biochemical pathway are indicated by *+, #, and $, respectively. n.d., not defined (due to division by 0); std, standard conditions.
The transcript levels of the genes encoding PS I subunits, PS II subunits and other photosystem-related proteins changed upon high-light treatment. Transcript levels for PS II genes generally increased, whereas the transcript levels of PS I genes generally decreased (Figure 4). Similar observations were made for Synechocystis 6803 exposing cells to high light (300 μmol photons m$^{-2}$ s$^{-1}$ for 15 min or 1 h; Hihara et al., 2001). In this study for Synechococcus 7002, transcripts for \textit{psbN} and \textit{psbA-II} (SYNPCC7002_A0157) increased approximately five-fold, and those for the other two \textit{psbA} genes (SYNPCC7002_A1418 and SYNPCC7002_A2164) either increased slightly (approximately two-fold) or remained constant, respectively. Due to the high sequence similarity of SYNPCC7002_A0157 and SYNPCC7002_A1418, many \textit{psbA} sequences did not map uniquely which made inferences difficult. However, transcripts for \textit{psbA} gene SYNPCC7002_A2164 were present at a very low level compared to those for the other two \textit{psbA} genes. Compared to the other two \textit{psbA} paralogs (SYNPCC7002_A0157 and SYNPCC7002_A1418), which encode proteins of nearly identical amino acid sequence, the SYNPCC7002_A2164 PsbA paralog has many differences in the amino acid sequence. Multiple copies of \textit{psbA} also occur in other cyanobacteria (e.g., Synechocystis 6803, Nostoc sp. PCC 7120, and Thermosynechococcus elongatus), and it has been reported that the transcription of these genes is modulated in response to

**FIGURE 3** Changes in the relative transcript abundance after different treatments or growth. The scatter plots show the relative transcript abundances (A) after a 1-h high-light treatment, (B) after 1 h incubation in the dark (oxic conditions), and (C) after 1 h incubation in the dark under anoxic conditions compared to that for “standard conditions” (mean of three biological replicates). Scatter plot (D) shows the relative transcript abundances for a culture grown under micro-oxic conditions compared to a culture grown under “standard conditions.” The gray lines give two-fold changes in either direction. Selected genes are identified by name/locus tag number.
changes in light intensity and $O_2$ level (Schaefer and Golden, 1989; Summerfield et al., 2008; Sander et al., 2010). Our data suggest that transcripts of SYNCC7002_A0157 increased five-fold at high-light intensities (Figure 3A), whereas the other tested conditions did not affect its transcription very much. Transcript levels for the other psbA genes were more or less constant under the conditions tested in this study or were slightly lower under dark fermentative conditions (in the case of SYNCC7002_A1418). Considering the genes for all subunits of both photosystems, the data clearly showed that significant changes in the ratio of PS I and PS II begin with changes at the mRNA level (Figure 4). These changes are well correlated with the observation that the PS II to PS I ratio increases when cells are grown at higher light intensity (Fujita et al., 1994). When energy transfer from phycobilisomes to PS II is impaired in Synechococcus 7002, the PS II content of the cells increases (Zhao et al., 2001), whereas dark acclimation of wild type cells resulted in a state 2 transition (i.e., direct energy transfer from phycobilisomes to PS I; Huang et al., 2003; Dong et al., 2009). A recent microarray study in Synechocystis 6803 showed that direct excitation of either PS I or PS II caused significant changes in transcription. The genes with increased transcript levels were not only restricted to those genes of photosystem that was not being excited, but included genes for diverse metabolic processes as well (Singh et al., 2009).

Among the components of the photosynthetic electron transport chain, the petH gene, encoding ferredoxin:NADP+ oxidoreductase, showed approximately two-fold higher transcript levels in cells exposed to high light (Table 3). Transcript levels for genes for other components of electron transfer, such as the cytochrome $b_6f$ complex and cytochrome $c$, also increased up to two-fold. The situation for petF, encoding the ferredoxin electron acceptor for PS I, was more complex, because several genes are annotated as ferredoxins. Some of these genes showed elevated transcript levels at high light (four-fold at maximum), whereas transcript levels for others remained constant. There was an overall decrease in transcript levels for phycocyanin and phycocyanin-associated linker proteins (2- to 10-fold; Figures 3A and 5), whereas the transcript level of allophycocyanin-associated genes decreased to a lesser extent (maximally approximately two-fold reduction; Figure 5). Transcripts for genes encoding the enzymes of heme and chlorophyll biosynthesis generally did not change very much after a 1-h exposure to high light with one major exception. Transcripts for chlb, chlL, and chlN genes, encoding the light-independent (dark-active) protochlorophyllide reductase, decreased ~6- to 12-fold upon high-light treatment.

The genes encoding the $F_{\text{p}}F_{\text{i}}$-type ATP synthase showed higher transcript levels (up to four-fold) after high-light treatment than cells grown under standard conditions. This observation suggested that the higher level of electron transport and proton motive force produced under these conditions is probably used to enhance ATP synthesis. Finally, there are two gene clusters, $ctal$ and $ctalla$, that encode cytochrome oxidases in Synechococcus 7002 (Nomura et al., 2006a). Transcript levels for the $ctal$ genes were considerably higher compared to those for the $ctalII$ genes (4- to 20-fold higher under standard conditions); this finding agrees with previous results showing that cytochrome oxidase I is the major terminal oxidase and is responsible for most oxygen uptake in the dark (Nomura et al., 2006b). Furthermore, the cytochrome oxidases are required to maintain cellular redox balance in the light. Previous studies had suggested that cytochrome oxidase II might play a role as a signal transducer to measure redox balance and trigger an oxidative stress response (Nomura et al., 2006b). Transcript levels for the $ctal$ genes decreased slightly (approximately two-fold) after high-light treatment, whereas the transcript levels for the $ctalII$ genes were essentially unchanged (Table 3).
Flavoproteins related to SYNPC7002_A1321 and SYNPC7002_A1743 have previously been reported to act as oxygen photoreductases in *Synechocystis* sp. PCC 6803 (Helman et al., 2003; Hackenberg et al., 2009). Interestingly, high-light treatment did not increase the transcript levels for these two genes, and in fact, transcript levels for SYNPC7002_A1743 might have decreased slightly (~1.5-fold). This observation suggested that these flavoproteins might have functions in addition to their roles in the dissipation of excess electrons via the Mehler reaction.

**TRANSCRIPTION CHANGES IN CELLS UNDER DARK OXIC (RESPIRATORY) CONDITIONS**

Light is obviously the key factor for photolithoautotrophic growth because it provides the reducing equivalents for CO₂ reduction. Light powers the electron transport reactions that generate protonmotive force for ATP synthesis, and thus directly fuels the biosynthesis of all cell components. For cyanobacteria in natural environments, cells are exposed to alternating periods of light and dark during a diel cycle, and light intensity may also increase or decrease depending on cloud cover and other factors. Changes in light availability will produce large changes in the supplies of ATP and reducing power in cyanobacterial cells. Therefore, gene expression patterns must be constantly readjusted to enable cells to cope with these changing circumstances.

To simulate the light-to-dark transition that would occur at dusk, a culture that had been grown under standard conditions was transferred into darkness for 1 h while sparging with 1% (v/v) CO₂ in air (i.e., under oxic conditions). Figure 3B shows a scatter plot comparing transcript levels in dark oxic conditions to those for standard conditions. The transcriptional changes that occurred upon dark oxic treatment were generally more severe compared to those associated with high-light treatment (Figure 3A). Both the number of genes showing a greater than two-fold change in transcript abundance, as well as the magnitude of the observed differential transcription changes, were higher. Transcripts for genes encoding components of the photosynthetic apparatus were still relatively abundant after a 1-h dark oxic incubation. Although transcript levels for a few genes remained unchanged, the relative transcript levels for genes encoding components of the photosynthetic apparatus were significantly lower (up to approximately six-fold) compared to their levels in cells under standard conditions (Table 3). These observations for transcript levels of photosystem-related genes are consistent with a comparable study in *Synechocystis* 6803, in which a culture was incubated in the dark and transcription changes were subsequently monitored as a function of time (Gill et al., 2002).

The transcript levels of genes encoding the enzymes of heme and chlorophyll biosynthesis were generally slightly lower upon dark incubation (approximately two- to three-fold for many of the genes); however, there was one exception: the three genes encoding the light-independent protochlorophyllide reductase (Kada et al., 2003; Bröcker et al., 2010; Muraki et al., 2010). The mRNA levels for the subunits of this enzyme (*chlL*, *chlB*, *chlN*) were four- to eight-fold higher in cells under dark oxic conditions. In the dark the transcript levels of genes encoding the structural subunits of the phycobilisomes and the bilin lyases were either approximately constant [e.g., *apcD*, *cpcG2* (SYNPC7002_A0639), *apcF*] or were lower by up to seven-fold (Figure 5). Although transcripts for both allophycocyanin and phycocyanin-related genes were lower in the dark, transcripts for phycocyanin and the peripheral rods were generally affected to a greater extent than genes encoding components of the phycobilisome core. Transcripts for *nblA* increased about five-fold upon dark incubation. Similar observations were made in a microarray study for the transcript levels for *apcAB*, *cpcBA*, and *nblA* genes in *Synechocystis* 6803 when a culture was incubated in the dark (Gill et al., 2002). Because NblA causes phycobiliproteins to become sensitive to degradation by Clp proteases (Baier et al., 2004; Karradt et al., 2008), this observation suggests that phycobilisomes and phycobiliproteins are actively degraded in the dark in order to reduce the cellular content of phycobiliproteins and to recycle the reduced carbon and nitrogen contained within the proteins of these antenna structures. Interestingly, the mRNA level for *nblA* also increased approximately two-fold after high-light treatment, which might be indicative of a similar reduction of light-harvesting components under high light.

After 1 h in the dark the mRNA levels for the Type-1 NADH dehydrogenase genes and for the cytochrome oxidase genes (both *ctfA* and *ctfB*) were similar to those in cells grown under standard conditions. However, transcript levels for most genes encoding the F0F1-type ATP synthase were significantly lower, 3- to 14-fold, after 1 h in the dark (Table 3). This correlates well with respiratory electron transport rates that are about 10-fold lower than the rate of oxygen evolution in the light (Nomura et al., 2006a). The *Synechococcus* 7002 genome encodes a second set of genes for an F0F1-type ATP synthase (annotated as ATPase II; SYNPC7002-G0144 – SYNPC7002-G0152). This plasmid-located (on plasmid pQ7) gene cluster has been suggested to encode a Na⁺-translocating N-ATPase (Dibrova et al., 2010). The transcript levels for the genes in this particular gene cluster were very low under standard conditions (about 5% of the corresponding ATPase I levels) and were even lower in cells exposed to high light (Table 3). Transcript levels for the ATPase II genes, however, increased upon dark incubation (approximately three-fold). The transcription data thus showed that the genes encoding this putative N-ATPase are transcribed and are regulated; however, the biological function of this putative ATPase is currently unknown.

Genes involved in CO₂ uptake, concentration and fixation showed lower transcript levels after 1 h of dark incubation (Table 3); transcripts for the *rbcL* and *rbcS* genes, encoding RuBisCO, were about three-fold lower and transcripts for genes encoding components of the carboxysome were up to six-fold lower. Transcript levels for two genes (*ndhD3*, *ndhF3*) encoding subunits of the inducible CO₂-concentrating complex were lower in the dark (approximately three-fold), and even the gene coding for a transcriptional regulator (*rbcR*/*cmrR*; Woodger et al., 2007) showed a slightly lower transcript level. However, the mRNA levels encoding subunits of the constitutive CO₂-concentrating complex remained more or less constant. These data illustrate that genes involved in CO₂ fixation, the major electron sink, are regulated at the transcriptional level and that this regulation includes not only the essential central components but also those for the more peripheral, inducible CO₂ uptake system.

The transcript levels for many genes involved in carbohydrate degradation (e.g., glycolysis) increased in cells after a 1-h dark treatment (Table 3). Transcripts increased three-fold for *pgi* (glucose-6-phosphate isomerase) and *pfkA* (6-phosphofructokinase), four-fold
for \( fbaB \) (fructose-bisphosphate aldolase class I), and five-fold for \( gap \) (SYNPCC7002_A2697; glyceraldehyde-3-phosphate dehydrogenase, type I). Some of the reactions of glycolysis or the oxidative pentose phosphate cycle and the Calvin–Benson–Bassham cycle are common to more than one of these pathways. Cyanobacteria usually have one enzyme that is used for the oxidative pentose phosphate cycle and another enzyme that is used for the Calvin–Benson–Bassham cycle (Knowles and Plaxton, 2003). The transcription data for genes encoding glyceraldehyde-3-phosphate dehydrogenase (\( gap \)), fructose-bisphosphate aldolase (\( fba \)), and fructose-1,6-bisphosphatase (\( fbp \)) reflect this phenomenon. Transcription of one set of \( gap \) and \( fba/fbp \) genes (\( gap \)/SYNPCC7002_A0106, \( fba/\)SYNPCC7002_A1352, \( glpX/\)SYNPCC7002_A1301) was regulated in the same way as the RuBisCO genes (i.e., increased transcript levels in high-light-treated cells and lower transcript levels in cells under dark oxic conditions). Transcript levels for the other set of genes was regulated oppositely: i.e., transcript levels for these genes (\( gap/\)SYNPCC7002_A2697, \( fbaB/\)SYNPCC7002_A0010, \( fbp/\)SYNPCC7002_A0329) were higher in cells after dark oxic treatment (or at least at about the same level in the case of \( SYNPCC7002_A0329 \)) and lower in cells exposed to high light. Thus, although these biochemical pathways share certain reactions, the transcription data suggest that there are probably distinctive enzymes, which are differentially expressed, that change in response to light and/or the availability of reducing equivalents provided through the action of light.

The so-called light-repressed transcript (\( lrtA \)), which encodes the “light-repressed protein (Singer and Doolittle, 1974; Tan et al., 1994),” became extremely abundant after dark incubation. The \( lrtA \) transcripts increased nine-fold after dark, oxic treatment and increased to an even greater level under dark, fermentative conditions (Table 3). Conversely, the \( lrtA \) transcripts were about five-fold lower in cells exposed to high light for 1 h. It has previously been demonstrated that \( lrtA \) transcripts are actively degraded after cells are exposed to light (Samartzidou and Widger, 1998).

Two genes in pyruvate metabolism were among those genes for which transcript levels increased the most upon dark incubation (see Figures 3B and 6). Transcripts for \( ppsA \), encoding phosphoenolpyruvate synthase increased 20-fold, and those for \( nifH \), encoding pyruvate:ferredoxin oxidoreductase increased 25-fold (Figure 6). Conversely, high-light treatment for 1 h caused transcripts for these two genes to decrease about three- and five-fold respectively. After cells were exposed to dark oxic conditions for 1 h, transcript levels for pyruvate kinase (\( pyk \)) and pyruvate dehydrogenase (\( pdhA, pdhB, ipdA \), and \( SYNPCC7002_A0110 \)) decreased approximately two-fold. However, the transcript levels for the genes encoding pyruvate kinase and pyruvate dehydrogenase did not change much (1.5-fold higher) after cells were exposed to high light for 1 h. Micro-oxic growth conditions (see below) likewise did not alter the transcript levels for genes involved in the pyruvate metabolism. The possible implications of these differences for pyruvate metabolism will be discussed below when describing transcription changes that occur during fermentative (dark anoxic) conditions.

As noted above, high-light treatment caused transcript levels for pyridine nucleotide transhydrogenase subunits (\( pntA, pntB, pntC \)) to decrease sharply. Dark oxic incubation had the opposite effect on the transcription of the transhydrogenase genes: mRNA

![Diagram of pyruvate metabolism](image-url)

**FIGURE 6 | Changes in the transcript levels for genes coding for proteins involved in pyruvate metabolism.** Relative transcript levels for genes encoding proteins/enzymes involved in pyruvate metabolism (pyruvate (Pyr) kinase (\( pyk \)), phosphoenolpyruvate (PEP) synthase (\( ppsA \)), pyruvate dehydrogenase (Pyr-DH complex (E1 alpha, E1 beta, E2 and E3 proteins; encoded by \( pdaA, pdhB, SYNPCC7002_A0110, ipdA \), and pyruvate:ferredoxin oxidoreductase (Pyr:Fd OR; \( nifJ \ ))) are shown. The proposed reaction pathways from PEP to acetyl-CoA with the respective enzymes are shown in (A,C); and the change in the mRNA level for the respective genes upon dark incubation is indicated by arrows. (B) The ratio of the transcript levels for cells after a 1-h dark oxic treatment (dark gray), a 1-h dark anoxic treatment (fermentative conditions; black), a 1-h high-light treatment (light gray), and photolithoautotrophic growth under micro-oxic conditions (medium gray) are compared to transcript levels in cells under standard conditions.
levels increased approximately seven-fold for pntB and pntC and increased approximately four-fold for pntA. These transcriptional changes suggested that cells have a much higher requirement for NADH/NADPH electron exchange under dark respiratory conditions than in the light.

The mRNA level for SYNPCC7002_A1442, which encodes dihydroorotate dehydrogenase, an enzyme involved in pyrimidine metabolism, was 30-fold higher after a 1-h dark incubation; under dark fermentative conditions (see below) its transcript level was 50-fold higher than in standard conditions. The gene encoding this particular dihydroorotate dehydrogenase and nifJ are immediate neighbors, have the same orientation, and are separated by only 70 bp; these observations suggest that they maybe transcribed as an operon under a common regulatory mechanism. The transcript level for a second pyrD gene (SYNPC7002_A2195) did not change much upon dark oxic treatment, but was about five-fold lower after 1 h under dark anoxic incubation when compared to cells grown under standard conditions. The transcript levels of both pyrD genes were similar to those of standard conditions when cells were grown under micro-oxic conditions (see below), suggesting that the regulation is not directly related to oxygen. The biochemical rationale for this exchange of PyrD proteins is not clear, but darkness is usually associated with lower oxygen levels for cyanobacteria. This could result in an indirect acclimation of pyrD transcription in response to changing oxygen levels or cellular redox potential.

Transcription of the genes coding for the two flavoproteins (SYNPC7002_A1321 and SYNPC7002_A1743) increased (three- and two-fold, respectively) after dark oxic incubation. A similar change was also observed upon dark anoxic incubation for SYNPC7002_A1321 (three-fold higher), although no increase of the mRNA level for SYNPC7002_A1743 was observed (see below). Growth under micro-oxic conditions (see below) resulted in a slight decrease (−1.5-fold) in transcript levels for both genes, whereas almost no change was observed after high-light incubation. As noted above, these observations suggest that these flavoproteins might not exclusively function as catalysts to eliminate excess electrons in the light.

**TRANSCRIPTIONAL CHANGES IN CELLS UNDER DARK ANOXIC (FERMENTATIVE) CONDITIONS**

Because PS II cannot oxidize water and evolve O₂ in the dark, O₂ levels typically decrease in natural habitats of oxygenic photosynthetic organisms at night. The remaining O₂ in these environments is often rapidly consumed by the respiratory activities of cyanobacteria and/ or other microorganisms, which imposes dark anoxic (i.e., fermentative) conditions on the cyanobacteria. To simulate these conditions, cultures were placed in the dark and were sparged with 1% CO₂ (v/v) in N₂. Figure 3C shows a scatter plot in which the transcript level for each gene in cells exposed to dark anoxic conditions was plotted against the transcript level in cells grown under standard conditions. It is apparent that a larger number of genes exhibited greater than two-fold changes in transcription level than for dark oxic conditions (compare Figures 3B,C) and that many genes additionally showed transcript levels that were markedly higher or lower than this threshold. This is perhaps not surprising because two important environmental parameters, light and oxygen, were changed in this experiment. The transcript levels for genes encoding the two photosystems, electron transport proteins, phycobilisome components, and heme and chlorophyll biosynthesis were even lower in cells exposed to these fermentative conditions than in cells from dark oxic conditions (Table 3). A few exceptions, e.g., psbW2 and chlH (SYNPC7002_A1000), had increased transcript levels under fermentative conditions compared to standard conditions. For each of these genes, there is a paralog (psbW/SYNPC7002_A1258, chlH/SYNPC7002_A1018) for which transcript levels decreased in cells exposed to fermentative conditions. Furthermore, transcripts for ho2 and aefP2 were much higher under dark anoxic conditions than under standard or dark oxic conditions (see further discussion concerning micro-oxic conditions below).

Transcripts for ppsA (phosphoenolpyruvate synthetase) and nifJ (pyruvate:ferredoxin oxidoreductase) increased dramatically, 180- and 110-fold, respectively, under fermentative conditions. The latter value was in excellent agreement with results from quantitative-RT-PCR, which showed that nifJ transcripts increased 130 ± 23-fold when cells were incubated for 30-min under dark anoxic conditions (McNeely et al., 2010a; Xu, 2010). Conversely, transcripts for pyk (pyruvate kinase) and the pyruvate dehydrogenase complex (pdhA, pdhb, ipdA, and SYNPC_A0110) were as much as 10-fold lower under dark anoxic conditions compared to standard conditions. Assuming that these very large transcriptional changes also reflect changes in enzyme activity levels in the cells, these results strongly suggested that the normal glycolytic pathway for phosphoenolpyruvate conversion to acetyl-coenzyme A (CoA) via pyruvate kinase and pyruvate dehydrogenase is replaced by an alternative pathway comprising phosphoenolpyruvate synthase and pyruvate:ferredoxin oxidoreductase. Transcription of nifJ has been reported under oxic conditions in several cyanobacteria (Schmitz et al., 2001); however, inactivation of pdhA in *Synechococcus* 7002 resulted in an acetate-requiring auxotrophic mutant strain (Xu, 2010). This result implies that pyruvate:ferredoxin oxidoreductase (NifJ) activity in cells grown under oxic conditions is insufficient to provide adequate acetyl-CoA to support autotrophic growth.

Phosphoenolpyruvate synthase (PpsA) is the main enzyme involved in the conversion of phosphoenolpyruvate to pyruvate in a modified Embden–Meyerhof pathway that occurs in the archaeon *Thermococcus kodakarensis*, although both pyruvate kinase and phosphoenolpyruvate synthase are also present in this organism (Imanaka et al., 2006). The conversion of phosphoenolpyruvate into pyruvate by pyruvate kinase generates one ATP from ADP, whereas phosphoenolpyruvate synthase produces one ATP and pyruvate from phosphoenolpyruvate, AMP and phosphate (Imanaka et al., 2006). This means that phosphoenolpyruvate synthase conserves more energy than pyruvate kinase, which is very important under energy-limited conditions such as fermentation. Pyruvate decarboxylation via the pyruvate dehydrogenase complex yields one equivalent of each of NADH, acetyl-CoA and CO₂. Pyruvate decarboxylation by the pyruvate:ferredoxin oxidoreductase also generates acetyl-CoA but instead of NADH produces two molecules of reduced ferredoxin, which have a much lower redox potential than the NADH/NAD⁺ couple (Tittmann, 2009). Reduced ferredoxin can be used directly to reduce nitrate, sulfate, and other substrates or can be used to produce NADPH via ferredoxin:NADP⁺ oxidoreductase. The availability of reducing equivalents at lower redox potentials should also benefit cells.
energetically when respiration is not possible. In summary, it appears quite likely that *Synechococcus* 7002 uses alternate pathways for the conversion of phosphoenolpyruvate into acetyl-CoA in the light and in the dark under fermentative conditions (Figure 6). The fermentative pathway might conserve more energy for cellular metabolism than the pathway that operates in the light or under dark oxic conditions.

Under dark anoxic conditions, no oxidative phosphorylation can be performed because the terminal electron acceptor for the respiratory chain (O₂) is missing. These conditions caused dramatic changes in mRNA level for genes coding for the subunits of enzyme complexes involved in electron transport and ATP synthesis. The relative mRNA levels for the genes encoding the Type-1 NADH dehydrogenase complex, cytochrome oxidase and ATP synthase decreased more than 10-fold for some genes when compared to levels in cells under standard conditions (Table 3). Under fermentative conditions ATP synthesis for growth and cellular maintenance is produced by substrate-level phosphorylation. These reactions also typically produce reduced coenzymes or redox proteins, which must be regenerated by reduction of some substrate molecule. As for dark oxic conditions, transcripts for genes encoding components of the CO₂ concentration and fixation pathways are lower than in cells grown under standard conditions. Reduction of nitrate and nitrite and excretion of ammonia could potentially occur under these conditions (McNeely et al., 2010a,b). Nevertheless, the reduction of protons to produce hydrogen does not happen during glycolysis can also be used to reduce protons to hydrogen. Like many other cyanobacteria, *Synechococcus* 7002 produces a so-called bidirectional, NAD(P)H-oxidizing [NiFe]-hydrogenase (McNeely et al., 2010a,b; Xu, 2010). When cells were incubated under dark oxic conditions, transcripts for the genes encoding the hydrogenase increased three- to seven-fold. Interestingly, under dark fermentative conditions, transcripts for the three genes that encode the diaphorase moiety of the enzyme (ho2, hemN2, desF) showed strongly increased mRNA levels. Specifically, transcripts for *acsF2*, *acsF2*, and *desF* were up-regulated, whereas *acsA*, *desF*, and *hemN2* showed a strong up-regulation, whereas *hemN2* and *desF* transcript levels were similar to those of the standard samples, which were grown at ambient O₂ (1% (v/v) CO₂ in air; Figure 3D). However, a few genes showed strongly increased mRNA levels. Specifically, transcripts for *acsF2*, *ho2*, *hemN2*, and *desF* were 15- to 20-fold higher than in cells grown under standard conditions (Table 3). These four genes are clustered (Figure 7) and could potentially be transcribed as an operon. However, under dark fermentative conditions only *acsF* and *ho2* showed a strong up-regulation, whereas *hemN* and *desF* transcript levels were similar to those for cells in standard conditions. This could either mean that multiple promoters exist that are differentially used, that transcription terminates at different places dependent upon growth conditions, or that transcript segments have different stabilities under some growth conditions. Three of these genes (hemN2, acsF2, ho2) code for enzymes involved in heme, chlorophyll and phycocyanobilin biosynthesis, respectively. The *Synechococcus* 7002 genome harbors multiple genes for each of these enzymes: *acsF* (SYNPCC7002_A0707) and *acsF* (SYNPCC7002_A0707), encoding magnesium-protoporphyrin IX monomethyl ester oxidative cyclases; *ho2* (SYNPCC7002_A2508) and *ho2* (SYNPCC7002_A1991), encoding heme oxygenases that synthesize biliverdin; and *hemF* (SYNPCC7002_A1382), *hemN* (SYNPCC7002_A2831), and *hemN* (SYNPCC7002_A1990) encoding coproporphyrinogen III oxidases. In contrast to *acsF*, *ho2*, and *hemN*, which exhibited much higher mRNA levels under

Interestingly, transcript levels for *ldhA* (SYNPCC7002_G0164, d-lactate dehydrogenase), *acsA* (SYNPCC7002_A1838, acetyl-CoA ligase), *acs* (SYNPCC7002_A2015, acetyl-CoA synthetase), and two putative *adh* genes (alcohol dehydrogenases, SYNPCC7002_A0868 and SYNPCC7002_A2590) were similar under all conditions tested in this study. Transcript levels for *ldhA* and the *adh* genes actually decreased somewhat under dark fermentative conditions. Because it is known that d-lactate is the major fermentation product produced by *Synechococcus* 7002 (McNeely et al., 2010b), these observations further establish that post-transcriptional regulation processes are important in establishing the fermentative capabilities of *Synechococcus* 7002.

**TRANSCRIPTION CHANGES IN CELLS UNDER MICRO-OXIC CONDITIONS**

In order to distinguish if transcription changes were resulting from the effects of light or directly from the O₂ levels, transcription profiling was performed with cells grown photoautotrophically under micro-oxic conditions. Micro-oxic conditions were achieved by sparging the culture with 1% (v/v) CO₂ in N₂ to continuously remove the O₂ produced by PS II. Interestingly, transcript levels for this sample were similar to those of the standard samples, which were grown at ambient O₂ (1% (v/v) CO₂ in air; Figure 3D). However, a few genes showed strongly increased mRNA levels. Specifically, transcripts for *acsF2*, *ho2*, *hemN2*, and *desF* were 15- to 20-fold higher than in cells grown under standard conditions (Table 3). These four genes are clustered (Figure 7) and could potentially be transcribed as an operon. However, under dark fermentative conditions only *acsF* and *ho2* showed a strong up-regulation, whereas *hemN* and *desF* transcript levels were similar to those for cells in standard conditions. This could either mean that multiple promoters exist that are differentially used, that transcription terminates at different places dependent upon growth conditions, or that transcript segments have different stabilities under some growth conditions. Three of these genes (hemN2, acsF2, ho2) code for enzymes involved in heme, chlorophyll and phycocyanobilin biosynthesis, respectively. The *Synechococcus* 7002 genome harbors multiple genes for each of these enzymes: *acsF* (SYNPCC7002_A0707) and *acsF* (SYNPCC7002_A0707), encoding magnesium-protoporphyrin IX monomethyl ester oxidative cyclases; *ho2* (SYNPCC7002_A2508) and *ho2* (SYNPCC7002_A1991), encoding heme oxygenases that synthesize biliverdin; and *hemF* (SYNPCC7002_A1382), *hemN* (SYNPCC7002_A2831), and *hemN* (SYNPCC7002_A1990) encoding coproporphyrinogen III oxidases. In contrast to *acsF*, *ho2*, and *hemN*, which exhibited much higher mRNA levels under

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**FIGURE 7 | Organization of the *acsF2* gene cluster.** The *acsF2* gene cluster comprises four genes (*acsF2*, *ho2*, *hemN2*, and *desF*). There is an ORF encoding a putative transcriptional regulator (SYNPCC7002_A1993) in the region upstream from *acsF2* but encoded on the opposite strand.
micro-oxic conditions, the transcript levels of acsF, hox1, and hemF were essentially the same as for cells in standard conditions. Dark incubation, on the other hand, resulted in lower mRNA levels for acsF, hox1, and hemF (two- to three-fold lower under dark oxic conditions and 10- to 20-fold lower under dark anoxic conditions). The transcript levels of hemN, however, were somewhat higher under both micro-oxic (less than two-fold) and dark incubation (both oxic and anoxic; 1.5- to 2-fold) compared to levels in cells grown under standard conditions.

*Synechocystis* 6803 also has an acsF2–ho2–hemN2 gene cluster encoded by ORFs sll1874, sll1875, sll1876. The expression and/or the activity of the respective enzymes has been reported to depend on oxygen levels (Minamizaki et al., 2008; Yilmaz et al., 2009; Goto et al., 2010). It has further been shown that the oxygen-dependent coproporphyrinogen III oxidase (HemF) is the major enzyme under ambient O2 levels, whereas one of the oxygen-independent HemN (sll1876) proteins is most active under micro-oxic conditions (Goto et al., 2010). The role of the second hemN (sll1917) gene product in *Synechocystis* 6803, with highest similarity (50% identity) to SYNPCC7002_A2831 (HemN) in *Synechococcus* 7002, is still unclear. In *Synechocystis* 6803 transcript levels of the clustered ORFs sll1874 (acsF2), sll1875 (ho2), and sll1876 (hemN2) coordinate increase under micro-oxic conditions (Minamizaki et al., 2008). Our data indicate that in *Synechococcus* 7002 orthologs of these three genes are possibly transcribed together under micro-oxic conditions with a fourth gene, desF, which encodes a acyl-lipid/fatty acid desaturase, a di-iron containing enzyme that uses O2 as a substrate.

Interestingly, transcripts for isiA, which encodes the iron-stress-induced, PS I-associated, chlorophyll a-binding IsiA protein, and isiB, which encodes flavodoxin, increased 15- and 30-fold, respectively, under micro-oxic conditions. Transcript levels for isiA and isiB, which are cotranscribed as an operon, greatly increase under Fe-limiting growth conditions (Leonhardt and Straus, 1992). This observation can be explained by lower Fe2+ availability under more reducing conditions than at ambient O2 levels. This suggestion is additionally supported by the fact that transcript levels for many genes coding for iron uptake systems also increased (up to 12-fold) under micro-oxic conditions compared to standard conditions (Table 3). Under both dark oxic and anoxic conditions, the isiA and isiB mRNA levels were lower compared to standard conditions, but genes encoding various iron uptake systems were also transcribed at levels similar to standard conditions, which suggested that a sufficient iron supply is available under these conditions. The requirement for iron is much lower in the dark, because synthesis of the photosynthetic apparatus, which consumes the majority of the iron taken up by cyanobacteria, is down-regulated in the dark (see above).

**TRANSCRIPTIONAL CHANGES IN A CULTURE UNDERGOING BATCH GROWTH UNDER STANDARD CONDITIONS**

To monitor changes in transcription during the transition from exponential growth to almost stationary phase, cells were harvested at different cell densities (OD730 nm = 0.4, 1.0, 3.0, and 5.0) during batch growth (see Figure 1; and Sakamoto and Bryant, 1998). When the transcription profiles for these samples were compared to those for standard conditions (OD730 nm = 0.7), it was found that transcript levels were relatively constant throughout the exponential growth phase (Figure 8). At OD730 nm values of 0.4 and 1.0, transcription changes differed by at most ~10-fold compared to transcript levels at OD730 nm = 0.7. However, the reliability for many of these differences was low, because they were associated with ORFs whose expression levels were low. As the cell density increased beyond OD730 nm = 1.0, more differences in transcript levels were observed. At OD730 nm = 3.0 the maximal transcript-level differences increased to ~17-fold, and at OD730 nm = 5.0, the maximal differences were ~20-fold. Differences of these magnitudes are likely to be highly significant, but these differences are generally smaller than many of the changes in response to light and oxygen.

Transcripts for the pstS gene, encoding the substrate-binding protein of the phosphate transport system, increased about 20-fold at OD730 nm = 5.0 (Table 4). Transcripts for other genes associated with this phosphate uptake system (pstA, pstB, pstC) also increased two- to four-fold. Furthermore, transcript levels for a predicted phosophatase (SYNPCC7002_A0893) and alkaline phosphatase (SYNPCC7002_A2352) increased about 10-fold. Transcript levels for all of these genes were nearly constant at lower cell densities. Collectively, the results at higher cell densities resembled microarray data that were obtained from *Synechococcus* sp. WH8102 after phosphate limitation (Tetu et al., 2009; Ostrowski et al., 2010).

As the cell density increased in the batch culture, transcript levels for nblA also increased. For example, at OD730 nm = 1.0, and 3.0, nblA transcripts were about two-fold higher than in cells under standard conditions, and at OD730 nm = 5.0, nblA transcripts were five-fold higher. NblA expression is associated with “chlorosis,” which occurs when phycobiliproteins are degraded by cyanobacterial cells in response to nutrient deprivation, high-light intensity and oxidative stress (Collier and Grossman, 1992, 1994; Bienert et al., 2006; Karradt et al., 2008). Nitrogen, sulfur, and carbon limitation generally produce the greatest increases in nblA expression (Collier and Grossman, 1994), but phosphate limitation can also cause chlorosis. For example, previous studies have shown that phosphate starvation was accompanied by extensive degradation of phycobiliproteins in *Synechococcus* sp. PCC 6301 and PCC 7002 (Batterton and van Baalen, 1968; Stevens et al., 1981). Because transcript levels for nblA and phosphate acquisition increased in parallel at higher cell densities during batch growth, it is likely that cells growing in medium A+ in batch culture become phosphate-limited at OD730 nm values above ~3.0. Interestingly, the transcript levels of genes involved in photosynthesis are not subject to major changes with increasing culture density (Table S2 in Supplementary Material), which is different from observations that have been made in *Synechocystis* 6803 (Foster et al., 2007).

**CONCLUSION**

The data presented in this study, which were obtained by deep sequencing of cDNA via SOLiD™ Next-Gen sequencing, identified mRNAs for nearly all annotated genes in the genome of *Synechococcus* 7002. The reproducibility of the method appears to be as good or better than microarrays, and the sequencing depth can be adapted to achieve virtually any desired dynamic range. The data clearly showed that cells significantly altered their transcription patterns for major metabolic processes (photosynthesis, CO2 fixation, sugar degradation, respiration) in response to changes in light and oxygen, and the observed changes were consistent with previous studies in this...
Figure 8 | Changes in the relative transcript abundance throughout standard batch growth. The scatter plots show the relative transcript abundances (A) at OD\textsubscript{730 nm} = 0.4, (B) at OD\textsubscript{730 nm} = 1.0, (C) at OD\textsubscript{730 nm} = 3.0, and (D) at OD\textsubscript{730 nm} = 5.0 compared to those for standard conditions (at OD\textsubscript{730 nm} = 0.7). The gray lines indicate two-fold changes in either direction; selected genes are identified by name/locus tag number.

Table 4 | Prominent changes in transcript levels at different culture densities of a batch culture.

| Locus tag          | Ratio OD 0.4/std | Ratio OD 1.0/std | Ratio OD 3.0/std | Ratio OD 5.0/std | Gene name | Gene product                                      |
|--------------------|------------------|------------------|------------------|------------------|-----------|---------------------------------------------------|
| SYNPCC7002_A2284   | 1.85             | 1.40             | 1.42             | 18.26            | pstS      | Phosphate transport system substrate-binding protein |
| SYNPCC7002_A2286   | 0.87             | 1.23             | 1.13             | 2.55             | pstA      | Phosphate ABC transporter, permease protein        |
| SYNPCC7002_A1895   | 0.90             | 0.87             | 0.73             | 2.09             | pstB      | Phosphate import ATP-binding protein               |
| SYNPCC7002_A2285   | 1.49             | 1.18             | 1.07             | 4.21             | pstC      | Phosphate ABC transporter, permease protein        |
| SYNPCC7002_A0893   | 0.60             | 0.61             | 1.23             | 11.20            | –         | Predicted phosphatase                              |
| SYNPCC7002_A2352   | 1.14             | 0.55             | 0.78             | 10.75            | –         | Alkaline phosphatase                               |
| SYNPCC7002_A1821   | 0.93             | 2.03             | 1.90             | 5.20             | nblA      | Putative phycobilisome degradation protein         |

The ratio of relative transcript abundance at OD\textsubscript{730 nm} values of 0.4, 1.0, 3.0, and 5.0 compared to the “standard conditions” sample at OD\textsubscript{730 nm} = 0.7 is given. std, standard conditions.
cyanobacterium and others. Greatly increased transcript levels for the genes encoding an alternative pathway for the conversion of phosphoenolpyruvate into acetyl-CoA suggests that this process occurs by completely different routes in Synechococcus 7002 during light/oxic and dark/anoxic conditions. Transcription changes for a putative actF2–hho2–hemN2–desF operon suggest that the transcription of these genes may be directly regulated by oxygen concentration. If this proves to be the case, the transcription of these genes can be monitored as a reporter for attempts to modulate the intracellular oxygen concentrations in cyanobacteria is a very important consideration in attempts to engineer cells for light-driven hydrogen production.

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REFERENCES

Appel, L., and Schulz, R. (1996). Sequence analysis of an operon of a NAD(P)-reducing nickel hydrogenase from the cyanobacterium Synechocystis sp. PCC 6803 gives additional evidence for direct coupling of the enzyme to NAD(P)H-dehydrogenase (complex I). Biochim. Biophys. Acta 1298, 141–147.

Ashby, M. K., and Mullineaux, C. W. (1999). The role of ApoD and ApoF in energy transfer from phycobilisomes to PS I and PS II in a cyanobacterium. Photosynth. Res. 61, 169–179.

Badger, M. R., and Price, G. D. (2003). CO2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. J. Exp. Bot. 54, 609–622.

Baier, K., Lehmann, H., Stephan, D. P., and Lockau, W. (2004). NbNa is essential for phycobilisome degradation in Anabena sp. strain PCC 7120 but not for development of functional heterocysts. Microbiology 150, 2739–2749.

Battichkova, N., and Aro, E. M. (2007). Cyanobacterial NDH-1 complexes: multiplicity in function and subunit composition. Physiol. Plant. 131, 22–32.

Bationt, J. C., and van Baalen, C. (1968). Phosphorus deficiency and phosphate uptake in the blue-green alga Anacystis nidulans. Can. J. Microbiol. 14, 341–348.

Battorent, J. C. Jr., and van Baalen, C. (1971). Growth responses of blue-green algae to sodium chloride concentration. Arch. Microbiol. 76, 151–165.

Bernrotnier, M., Zamocky, M., Payer, M., Furtmüller, P. G., Peschek, G. A., and Obinger, C. (2008). Heme-copper oxidasases and their electron donors in cyanobacterial respiratory electron transport. Chem. Biodivers. 5, 1927–1961.

Bienert, R., Baier, K., Volkmer, R., Lockau, W., and Heinemann, U. (2006). Crystal structure of NbNa from Anabaena sp. PCC 7120, a small protein playing a key role in phycobilisome degradation. J. Biol. Chem. 281, 5216–5223.

Bröcker, M., J. Schomburg, S., Heinz, D. W., Jahn, D., Schubert, W. D., and Mosen, J. (2010). Crystal structure of the nitrogenase-like dark operative protocellulphyllide oxidoreductase catalytic complex (Chln/Chlb)2. J. Biol. Chem. 285, 27336–27345.

Bryant, D. A. (1994). The Molecular Biology of Cyanobacteria. Dordrecht: Kluwer Academic Publishers.

Campbell, E. L., Christman, H., and Meeks, J. C. (2008). DNA microarray comparisons of plant factor- and nitrogen deprivation-induced homogonina reveal decision-making transcriptional regulation patterns in Nostoc punctiforme. J. Bacteriol. 190, 7382–7391.

Campbell, E. L., Summers, M. L., Christman, H., Martin, E. M., and Meeks, J. C. (2007). Global gene expression patterns of Nostoc punctiforme in steady-state dinitrogen-grown heterocyst-containing cultures and at single time points during the differentiation of akinetes and homogonina. J. Bacteriol. 189, 5247–5256.

Cannon, G. C., Heinhorst, S., and Kerfeld, C. A. (2009). Carboxysomal carbonic anhydrases: structure and role in microbial CO2 fixation. Biochem. Biophys. Acta 1804, 382–392.

Clooman, N., Forrest, A. R., Kolle, G., Gardiner, B. B., Faulkner, G. J., Brown, M. K., Taylor, D. E., Stepco, A. L., Wani, S., Bethel, G., Robertson, A. J., Perkins, A. C., Bruce, S. J., Lee, C. C., Ranade, S. S., Peckham, H. E., Manning, J. M., McKernan, K. J., and Grimmond, S. M. (2008). Stem cell transcriptome profiling via massive-scale mRNA sequencing. Nat. Methods 5, 613–619.

Collier, J. L., and Grossman, A. R. (1992). Chlorosis induced by nitrogen deprivation in Synechococcus sp. strain PCC 7942: not all bleaching is the same. J. Bacteriol. 174, 4718–4726.

Collier, J. L., and Grossman, A. R. (1994). A small polyepptide triggers complete degradation of light-harvesting phycobiliproteins in nutrient-deprived cyanobacteria. EMBO J. 13, 1039–1047.

de Lorimier, R., Bryant, D.A., Porter, R.D., Liu, W. Y., Jay, E., and Stevens, S. E. Jr. (1984). Genes for the alpha and beta subunits of phycocyanin. Proc. Natl. Acad. Sci. U.S.A. 81, 7946–7950.

Dihrova, D. V., Galperin, M. Y., and Multikidjanian, A. Y. (2010). Characterization of the N-ATPase, a distinct, laterally transferred βα′-translocating form of the bacterial F-type membrane ATPase. Bioinformatics 26, 1473–1476.

Dong, C., Tang, A., Zhao, J., Mullineaux, C. W., Shen, G., and Bryant, D. A. (2009). ApoD is necessary for efficient energy transfer from phycobilisomes to photosystem I and helps to prevent photoinhibition in the cyanobacterium Synechocystis sp. PCC 6803. J. Bacteriol. 181, 3671–3681.

Goto, T., Aoki, R., Minamizaki, K., and Fujita, Y. (2010). Functional differentiation of two analogous coproporphyrinogen III oxides for heme and chlorophyll biosynthesis pathways in the cyanobacterium Synechocystis sp. PCC 6803. Plant Cell Physiol. 51, 650–663.

Gutthann, F., Egert, M., Marques, A., and Appel, J. (2007). Inhibition of respiration and nitrate assimilation enhances photosynthetic light evolution under low oxygen concentrations in Synechocystis sp. PCC 6803. Biochim. Biophys. Acta 1767, 161–169.

Hachenberg, C., Engelhardt, A., Matthijs, H. C., Wittink, F., Bause, H., Kaplan, A., and Hagemann, M. (2009). Photoregulatory 2-phosphoglycolate metabolism and photoreduction of O2 cooperate in high-light acclimation of Synechocystis sp. strain PCC 6803. Planta 230, 625–637.

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Helman, Y., Tchernov, D., Reinhold, L., Shibata, M., Ogawa, T., Schwarz, R., Ohad, I., and Kaplan, A. (2003). Genes encoding A-type flavoproteins are essential for photooxidation of O2 in cyanobacteria. *C. R. Biologies* 13, 230–235.

Hihara, Y., Kamei, A., Kanehisa, M., Kaplan, A., and Ikeuchi, M. (2001). DNA microarray analysis of cyanobacterial gene expression during acclimation to high light. *Plant Cell* 13, 793–806.

Hihara, Y., Sanoike, K., Kanehisa, M., and Ikeuchi, M. (2003). DNA microarray analysis of redox-responsive genes in the genome of the cyanobacterium *Synechocystis* sp. strain PCC 6803. *J. Bacteriol.* 185, 1719–1725.

Huang, C., Yuan, X., Zhao, J., and Bryant, K. (2008). An essential role for glycolysis in the photosynthesis and differential decrease of photosystems I and II in a cyanobacterium. *Appl. Environ. Microbiol.* doi: 10.1128/AEM.02792-10. [Epub ahead of print].

McNeely, K., Xu, Y., Anamay, G., Bennett, N., Bryant, D. A., and Dismukes, G. C. (2010a). Characterization of a nif mutant of *Synechococcus* sp. strain PCC 7002 lacking pyruvate:ferredoxin oxidoreductase. *Appl. Environ. Microbiol.*

Minamizaki, K., Mizoguchi, T., Goto, T., Tamaki, H., and Fujita, Y. (2008). Identification of two homologous genes, chlA1 and chlAI1, that are differentially involved in isocyclic ring formation of chlorophyll a in the cyanobacterium *Synechocystis* PCC 6803. *J. Biol. Chem.* 233, 2684–2692.

Mizoguchi, T., Nakagiri, I., Sato, N., and Wada, H. (2009). Lack of di-galactosyl-diaclylglycerol increases the sensitivity of *Synechocystis* sp. PCC 6803 to high light stress. *FEBS Lett.* 583, 718–722.

Monaghan, J. R., Epp, L. G., Putta, S., Page, R. B., Walker, J. A., Beachy, C. K., Zhu, W., Pao, G. M., Verma, I. M., Hunter, T., Bryant, S. V., Gardiner, D. M., Harkins, T. T., and Voss, S. R. (2009). Microarray and cDNA sequence analysis of transcription during nerve-deprived limb regeneration. *BMC Biol.* 7, 1. doi: 10.1186/1741-7007-7-1

Muraki, N., Nomata, J., Ebata, K., Mizoguchi, T., Shiba, T., Tamaki, H., Kurihisa, G., and Fujita, Y. (2010). X-ray crystal structure of the light-independent protoclorophyllide reductase. *Nature* 465, 110–114.

Nodop, A., Pietz, D., Höcker, R., Becker, A., Pistorius, E. K., Forchhammer, K., and Michel, K. F. (2008). Transcript profiling reveals new insights into the acclimation of the mesophilic freshwater cyanobacterium *Synechococcus elongatus* PCC 7942 to iron starvation. *Plant Physiol.* 147, 747–763.

Nomura, C. T., Persson, S., Shen, G., Inoue-Sakamoto, K., and Bryant, D. A. (2006a). Characterization of two cytochrome oxidase operons in the marine cyanobacterium *Synechococcus* sp. PCC 7002: inactivation of ctaDI affects the PS II L/P ratio. *Photosyn. Res.* 87, 215–228.

Nomura, C. T., Sakamoto, T., and Bryant, D. A. (2006b). Roles for heme-copper oxidases in extreme high-light and oxidative stress response in the cyanobacterium *Synechocystis* sp. strain PCC 7002. *Arch. Microbiol.* 185, 471–479.

Ogawa, T., and MiH. (2007). Cyanobacterial NADPH dehydrogenase complexes. *Photores.* 93, 69–77.

Ostrowski, M., Mraz, S., Tetu, S. G., Phillippy, K., Johnson, A., Palenik, B., Paulsen, I. T., and Scanlan, D. J. (2010). *Pra* is required for coordinate regulation of gene expression during phosphate stress in a marine cyanobacterium. *ISME J.* 4, 908–921.

Ott, R. L., and Longnecker, M. (2000). *An Introduction to Statistical Methods and Data Analysis*. Pacific Grove, CA: Duxbury Press.

Peschek, G. A., Obinger, C., and Paumann, M. (2004). The respiratory chain of blue-green algae (cyanobacteria). *Physiol. Plant.* 120, 358–369.

Postier, B. L., Wang, H. L., Singh, A., Impson, L., Andrews, H. L., Khlan, J., Li, H., Rissing, G., Pesta, D., Deyholos, M., Galbraith, D. W., Sherman, L. A., and Burnap, R. L. (2003). The construction and use of bacterial DNA microarrays based on an optimized two-stage PCR strategy. *Nat. Genet.* 33, 23. doi: 10.1101/1471-2164-2-4-2

Rowland, J. G., Pang, X., Suzuki, L., Murata, N., Simon, W. J., and Slabas, A. R. (2010). Identification of components associated with thermal acclimation of photosystem II in *Synechocystis* sp. PCC6803. *PloS One* 5, e10151. doi: 10.1371/journal.pone.001051

Sakamoto, T., and Bryant, D. A. (1998). Growth at low temperature causes nitrogen limitation in the cyanobacterium *Synechocystis* sp. PCC 7002. *Arch. Microbiol.* 169, 10–19.

Sakamoto, T., and Bryant, D. A. (2002). Synergistic effect of high-light and low temperature on cell growth of the *Δ12 fatty acid desaturase* mutant of *Synechocystis* PCC7002. *Physiol. Plant.* 132, 1825–1839.

Stal, L. J. (1995). Physiological ecology of cyanobacteria in microbial mats and other communities. *New Phytol.* 131, 1–32.

Stal, L. J., and Moezelhaar, R. (1997). Fermentation in cyanobacteria. *FEBS Lett.* 402, 19–21.

Steunou, A. S., Jensen, S. I., Brecht, E., Becraft, E. D., Bateson, M. M., Kilian, O., Bhaya, D., Ward, D. M., Peters, J. W., Grossman, A. R., and Kuhl, M. (2008). Regulation of nif gene expression and the energetics of N2 fixation over the diel cycle in a hot spring microbial mat. *ISME J.* 2, 364–378.

Stevens, S. E., Paone, D. A. M., and Barkwill, D. L. (1981). Accumulation of cyanophycin granules as a result of phosphate limitation in *Aphanememod quadruplicatum*. *Plant Physiol.* 67, 716–719.

Stevens, S. E., and Porter, R. D. (1980). *Transformation in Aphanememod quadruplicatum*. *Proc. Natl. Acad. Sci. U.S.A.* 77, 6052–6056.

Stuart, R. K., Dupont, C. L., Johnson, D. A., Paulsen, I. T., and Paleink, B. (2009). Coastal strains of marine cyanobacterium *Synechococcus* species exhibit increased tolerance to copper shock and a distinctive transcriptional
response relative to those of open-ocean strains. Appl. Environ. Microbiol. 75, 5047–5057.

Summerfield, T. C., Toepel, L., and Sherman, L. A. (2008). Low-oxygen induction of normally cryptic psbA genes in cyanobacteria. Biochemistry 47, 12939–12941. 

Tan, X., Varughese, M., and Widger, W. R. (1994). A light-repressed transcript found in Synechococcus PCC 7002 is similar to a chloroplast-specific small subunit ribosomal protein and to a transcription modulator protein associated with sigma 54. J. Brol. Chem. 269, 20905–20912. 

Tetu, S. G., Brahamsha, B., Johnson, D. A., Tai, V., Phillippy, K., Palenik, B., and Paulsen, I. T. (2009). Microarray analysis of phosphate regulation in the marine cyanobacterium Synechococcus sp. WH8102. ISME J. 3, 835–849. 

Tittmann, K. (2009). Reaction mechanisms of thiamin diphosphate enzymes: redox reactions. FEBS J. 276, 2454–2468. 

Woodger, F. J., Bryant, D. A., and Price, G. D. (2007). Transcriptional regulation of the CO2-concentrating mechanism in a euryhaline, coastal marine cyanobacterium, Synechococcus sp. strain PCC 7002: role of NdHR/CcmR. J. Bacteriol. 189, 3335–3347. 

Xu, Y. (2010). Synechococcus sp. PCC7002: A Robust and Versatile Cyanobacterial Platform for Biofuels Development. Ph.D. thesis, The Pennsylvania State University, University Park, PA. 

Xu, Y., Alvey, R. M., Byrne, P. O., Graham, J. E, Shen, G., and Bryant, D. A. (2011). Expression of genes in cyanobacteria: adaptation of endogenous plasmids as platforms for high-level gene expression in Synechococcus sp. PCC 7002. Methods Mol. Biol. 684, 273–293. 

Yeates, T. O., Kerfeld, C. A., Heinhorst, S., Cannon, G. C., and Shively, J. M. (2008). Protein-based organelles in bacteria: carboxysomes and related microcompartments. Nat. Rev. Microbiol. 6, 681–691. 

Yilmaz, M., Kang, I., and Beale, S. I. (2009). Heme oxygenase 2 of the cyanobacterium Synechocystis sp. PCC 6803 is induced under a microaerobic atmosphere and is required for microaerobic growth at high light intensity. Photosyn. Res. 103, 47–59. 

Zhao, J., Shen, G., and Bryant, D. A. (2001). Photosystem stoichiometry and state transitions in a mutant of the cyanobacterium Synechococcus sp. PCC 7002 lacking phycocyanin. Biochim. Biophys. Acta 1505, 248–257. 

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