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Consequences of a Deletion in *dspA* on Transcript Accumulation in *Synechocystis* sp. Strain PCC6803†

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A sensor histidine kinase of *Synechococcus* sp. strain PCC7942, designated *nblS*, was previously identified and shown to be critical for the acclimation of cells to high-light and nutrient limitation conditions and to influence the expression of a number of light-responsive genes. The *nblS* orthologue in *Synechocystis* sp. strain PCC6803 is designated *dspA* (also called *hik33*). We have generated a *dspA* null mutant and analyzed global gene expression in both the mutant and wild-type strains under high- and low-light conditions. The mutant is aberrant for the expression of many genes encoding proteins critical for photosynthesis, phosphate and carbon acquisition, and the amelioration of stress conditions. Furthermore, transcripts from a number of genes normally detected only during exposure of wild-type cells to high-light conditions become partially constitutive in the low-light-grown *dspA* mutant. Other genes for which transcripts decline upon exposure of wild-type cells to high light are already lower in the mutant during growth in low light. These results suggest that DspA may influence gene expression in both a positive and a negative manner and that the *dspA* mutant behaves as if it were experiencing stress conditions (e.g., high-light exposure) even when maintained at near-optimal growth conditions for wild-type cells. This is discussed with respect to the importance of DspA for regulating the responses of the cell to environmental cues.

Photosynthetic organisms have evolved intricate mechanisms for sensing and acclimating to environmental change. Parameters such as light quality, light intensity, and nutrient availability can modulate both the structure and the function of the photosynthetic machinery. Physiological and biochemical changes elicited by external cues include modification of light-harvesting complex (LHC) synthesis and degradation (6, 12, 27, 29, 38, 45, 65, 75), changes in absorption and excitation energy transfer properties of LHC (11, 18, 28, 59), and a modification of reaction center function (59, 80). Intracellular cues critical for controlling cellular processes during acclimation may reflect the cell's growth potential, cellular redox conditions, and/or accumulation of reactive oxygen species (52, 77).

Precise control over the fate of absorbed excitation energy is critical for cell viability during exposure of photosynthetic cells to excess excitation, since energized pigment molecules may trigger the production of damaging, reactive oxygen species (2). Over the short term, cells can dissipate excess absorbed excitation energy as heat by quenching excited pigment molecules in the LHC or by eliciting a state transition in which the LHC of photosystem II (PS II) directs its excitation energy to PS I, where quenching can occur. Over the long term, excess excitation may cause a dramatic reduction in the level of LHC. In cyanobacteria, a reduction in LHC size is reflected in reduced levels of transcripts encoding light-absorbing polypeptides (or phycobiliproteins) of the major LHC (or phycobilisomes). Several studies have shown that light and nutrient conditions modulate levels of phycobiliprotein transcripts (e.g., from the *cpcBA* and *apcAB* genes) (9, 30, 40, 41, 53, 60), enzymes required for pigment biosynthesis (e.g., from *hem* and *chl* genes), and subunits of the photosynthetic reaction centers (e.g., from *psaA*, *psaB*, *psbA*, and *psbD* genes) (36).

PS II is a primary site of damage to the photosynthetic machinery in high light (HL), causing photoinhibition. The PS II reaction center polypeptides are composed of D1 and D2, and damage to the reaction center reflects a rapid loss of function of D1. Cellular processes have evolved to facilitate rapid turnover and exchange of photodamaged D1 subunits (10). In cyanobacteria, the D1 and D2 polypeptides are encoded by two gene families, *psbA* and *psbD*, respectively. *Synechococcus* sp. strain PCC7942 has three distinct *psbA* genes and two distinct *psbD* genes. The three *psbA* genes encode two forms of D1 (7, 25); *psbAI* encodes D1, form I, which is abundant under low-light (LL) conditions, while *psbAII* and *psbAIII* each encode D1, form II, which becomes abundant when cells are shifted from LL to HL. The latter form of D1 is important for maintaining PS II function and the fitness of cells under HL conditions (1, 8, 31, 50, 51). *Synechocystis* sp. strain PCC6803 also has three *psbA* genes, but the expression of only *psbAII* and *psbAIII* has been observed; transcripts from both *psbAII* and *psbAIII* increase during exposure of the cells to HL (13). *Synechocystis* sp. strain PCC6803 also has two *psbD* genes (*psbDI* and *psbDII*) encoding D2 (35, 56).

Another family of cyanobacterial genes that are highly expressed during HL exposure and nutrient deprivation has been designated *hli* (HL induced) (15) or *scp* (small chlorophyll

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binding protein). The genes in this family have homology to light-harvesting polypeptides of vascular plants but have one membrane-spanning helix, rather than three (15). In *Synechocystis* sp. strain PCC6803, there are four *hli* genes, *hliA*, *hliB*, *hliC*, and *hliD* (also called *scpB*, *scpC*, *scpD*, and *scpE*) (21), as well as an *hli* sequence fused to the ferrochelatase coding region (21, 34). All of the Hli polypeptides accumulate following the exposure of cells to HL conditions, although the kinetics of accumulation may be different. These polypeptides also accumulate under conditions of nitrogen or sulfur deprivation and low temperature, suggesting that they are stress-related polypeptides that are synthesized when cells absorb excess excitation energy (34). A null mutant for all four of the *hli* genes (*hli* quadruple mutant) grows well in LL but, unlike wild-type cells, dies upon exposure to HL (34). The Hli polypeptides may be involved in protecting cells from HL damage and may mechanistically facilitate the dissipation of excess absorbed light energy (33) and/or limit the accumulation of free phototoxic intermediates in chlorophyll biosynthesis (81).

Recently, we isolated a photosynthetic mutant of *Synechococcus* sp. strain PCC7942 that exhibited abnormal expression of the light-regulated *hli*, *psbA*, and *cpcBA* genes and that died rapidly following exposure to either HL or nutrient deprivation. The gene altered (by two point mutations) in the mutant encodes a sensor histidine kinase (62, 71, 72), designated NblS (77), that has a PAS domain and may interact with the response regulator NblR (69). The PAS domain may bind a chromophore and function in monitoring light, oxygen, and/or redox conditions (76, 77). We were unable to obtain an *nblS* null mutant of *Synechococcus* sp. strain PCC7942 (77). Recently, a mutant of *Synechocystis* sp. strain PCC6803 that was null for *dspA* (also called *hik33*) (73) was isolated, and the mutant phenotype was rescued by the *Synechococcus* sp. strain PCC7942 *nblS* gene (42). Like the *nblS* mutant, the *dspA* strain showed aberrations in expression of the *psbA* and *hli* genes, although the phenotype of the *dspA* null mutant was somewhat different than that of the *nblS* mutant.

Microarray-based methods for high-throughput monitoring of gene expression have been developed over the last decade (14, 66, 82). Such methods are powerfully used for analyses of gene expression in organisms for which there is a complete genome sequence, such as *Synechocystis* sp. strain PCC6803 (46). Recent microarray studies have exploited *Synechocystis* sp. strain PCC6803 for identifying genes whose activities are modulated by light levels and UV irradiation (22, 36, 43). To further define the role of NblS/DspA/Hik33 (3, 54, 73) in the responses of cells to environmental change, we used microarrays to compare global transcript levels in wild-type *Synechocystis* sp. strain PCC6803 and the *dspA* mutant both in LL and following exposure of cells to HL. The results suggest that DspA has a far-reaching effect on the levels of numerous transcripts, including those encoding polypeptides directly or indirectly associated with photosynthesis and other metabolic processes, and that these effects are apparent under both HL and LL conditions.

MATERIALS AND METHODS

Strains and growth conditions. The nonmotile strain of *Synechocystis* sp. strain PCC6803 and the *dspA* mutant (42) were cultured in BG-11 medium with or without supplementation with 10 mM glucose. Cells were maintained in LL (30 μmol of photon m^{-2} s^{-1}) and bubbled with 3% CO_2 in air. For HL treatment, cells were grown according to a method described previously (34). Briefly, when cultures attained mid-logarithmic growth (optical density at 750 nanometers of ~0.6), they were diluted to an optical density at 750 nanometers of ~0.3 and either placed back in LL or illuminated with 500 μmol of photon m^{-2} s^{-1} of white light for various periods of time. The cells were then cooled in liquid nitrogen and collected by centrifugation (5,000 × *g* for 5 min), and the cell pellets were stored at −80°C until they were used for RNA isolation.

Amplification of gene fragments and array construction. The whole set of PCR-amplified gene-specific fragments used to construct the microarray was generated as previously described (63); all primers used contained an additional universal sequence (forward primer, 5′-CCAGGGGAGCTCTTCCATG-3′; reverse primer, 5′-GCACGGGTGCTCTTCCACC-3′) that allowed for the reamplification of the fragments with a single primer pair. The PCRs were performed in 384-well microtiter plates with 0.4 U of Pfx polymerase (Invitrogen, Carlsbad, Calif.), 10 to 30 ng of the original amplified DNA fragment, 5 μmol of oligonucleotides, 50 μmol of deoxynucleoside triphosphates, and 1.5 mM $MgCl_2$ in a 20-μl reaction volume. For PCR amplification of specific gene fragments from purified *Synechocystis* sp. strain PCC6803 genomic DNA (74), reactions were performed in 96-well microtiter plates using 2 U of *Taq* polymerase (MBI Fermentas, Amherst, N.Y.), 100 ng of genomic DNA, 5 μmol of oligonucleotides, 50 μmol of deoxynucleoside triphosphates, and 1.5 mM $MgCl_2$ in a 100-μl reaction volume. Reactions were cycled 25 times at 94°C for 2 min, 92°C for 30 s, 55°C for 30 s, 72°C for 2 min, and, after the final cycle, 72°C for 5 min. Approximately 5% of the PCR products contained multiple DNA fragments; these fragments were reamplified either at different annealing temperatures or from electrophoretically resolved fragments that were purified from agarose gels. There were still 67 genes for which products did not amplify well; 70-mer oligonucleotides specific for these genes were included on the array. The PCR products, resolved on 1.2% agarose gels and sized with respect to molecular mass markers prepared by Life Technology (Rockville, Md.), were purified by MultiScreen PCR 384-well filter plates (Millipore, Bedford, Mass.), and the nucleic acid concentration was measured with the PicoGreen double-stranded DNA quantification reagents (Molecular Probes, Eugene, Ore.). Purified PCR products were resuspended in 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) to a final concentration of 250 to 300 ng/μl. Eight microliters of each PCR product was transferred from 384-well microtiter plates to the printing plates, also in the 384-well microtiter format (Genetix Limited, Hampshire, United Kingdom). The PCR products were arrayed onto CMT-GAPS slides coated with γ amino propyl silane (Corning Incorporated, New York, N.Y.) by using an Omnigrid Microarrayer (GeneMachines, San Carlos, Calif.) with ChipMaker 2 pins (TeleChem International, Sunnyvale, Calif.). Four complete cDNA sets, each consisting of 3,707 array elements, were printed onto each slide. Printed slides were allowed to dry for 12 h at room temperature (25°C) at <50% relative humidity and then cross-linked by UV irradiation in the Stratalinker 1800 (Stratagene, La Jolla, Calif.) at a total power of 300 mJ. Just prior to prehybridization, arrays were baked at 65°C for 10 min. Following prehybridization for 1 h at 50°C in 3× SSC–0.1% sodium dodecyl sulfate (SDS)–0.1 mg of bovine serum albumin/ml, the arrays were rinsed by immersion in double-distilled H_2O, transferred to isopropanol for 1 min, dried by centrifugation for 5 min in a SpeedVac Plus model SC210A (Savant, Holbrook, N.Y.), and then stored in a desiccator at room temperature until they were used.

Nucleic acid isolation. RNA was isolated from 50-ml cultures of wild-type cells and the *dspA* mutant; cells were collected by centrifugation (5,000 × *g* for 5 min), and cell pellets were resuspended in 500 μl of NAES extraction buffer (50 mM sodium acetate [pH 5.1], 10 mM EDTA, 1% SDS). An equal volume of acidic phenol (equilibrated with NAES) was added to the suspension (approximately 1.5 ml) along with 100 mg of glass beads (0.1-μm average diameter; Sigma, St. Louis, Mo.) prior to agitation (two times for 30 s each) at the maximum speed (~5,000 rpm) in a Mini-Bead Beater (Biospec Products, Bartlesville, Okla.). The beads and unbroken cells were pelleted by a brief centrifugation (5 to 10 s at full speed in an Eppendorf centrifuge 5415C), and the supernatant was subjected to two phenol-chloroform (1:1) and one chloroform extractions. Two volumes of ethanol were added to the aqueous phase of the final chloroform extraction, and the solution was incubated for 2 h at −20°C. Precipitated RNA was collected by centrifugation (10 min at full speed in an Eppendorf Microfuge), washed with 70% ethanol, resuspended in Tris-EDTA, and treated with 25 U of RNase-free DNase I (Amersham, Piscataway, N.J.) at room temperature for 30 min. The DNase I was removed by two phenol-chloroform extractions, and the RNA was precipitated with 2 volumes of ethanol.

Microarray hybridization and data analysis. Hybridization to the microarray was performed according to the method described by Schena et al. (66), with minor modifications. First-strand cDNA was synthesized by incubating 25 μg of

TABLE 1. Primer pairs used for quantitative real-time RT-PCR[a]

Gene	Forward primer	Reverse primer
cpcB (*sll*1577)	CCGGTGGAAACGCCTACA	GCAGCAGCGGCGCGGTCGA
psaA (*slr*1834)	CGTCATCAACTCCTACGGTTC	GTAACAATACCTCCGAGGAGAT
psbAII (*slr*1311)	ACAGCCGTTCCTTGCACTTCTT	TTAACCGTTGACAGCAGGAGCG
pstS (*sll*0680)	ACTGTTGGTCTGGCAGCCTG	CCACGGACTGATAGTTAACTTC
groEL-1 (*slr*2076)	GAGTTAACCGGTGCCCTGATTG	AAGTCACCACCGGGGGCACCA
groEL-2 (*sll*0416)	GCCGACATCATCGCCAAAGCC	CCATGCCACCCATATCGGGCA
ccmK (*sll*1029)	TGACCGCTGGCATTGAAA	GGCGGATGATGGAAGGATTA
rps1B (*slr*1984)	TCCAACAGTGCAGCCTTTTC	GCAATCCTTCCACATCGC

[a] Forward and reverse primers specific for transcripts encoding CpcB, PsaA, PsbAII, PstS, GroEL-1, GroEL-2, CcmK, and the control polypeptide Rps1B that were used for quantitative real-time RT-PCR are shown.

total RNA with mixed gene-specific reverse primers and 30 U of Superscript II RNase H-minus reverse transcriptase (Invitrogen). The reaction was performed at 42°C for 1 h in a volume of 40 μl. After digesting total RNA with RNase H, second-strand synthesis was accomplished by using the Klenow fragment of DNA polymerase (USB, Cleveland, Ohio) and random primers; Cy3- or Cy5-dUTP fluorescent nucleotides were incorporated into the second strand (Amersham) over a labeling period of 3 h at 37°C. Labeled DNA fragments were purified by using the QIAquick spin column (QIAGEN, Valencia, Calif.), and the cDNA derived from 6.25 μg of total RNA was resuspended in hybridization buffer (3× SSC, 0.3% SDS, 0.17 μg of tRNA/μl) to a final volume of 30 μl. The fluorescent cDNA was denatured at 95°C for 90 s, and its application to the array was done by capillary delivery under a glass lifter slip (Eric Scientific, Portsmouth, N.H.). Three spots each of 20 μl of 3× SSC were positioned at the edges of the slides to maintain humidity during hybridization. Slides were sealed in custom-built hybridization chambers, and hybridizations were done at 65°C for 16 h. Following hybridization, the arrays were washed for 2 min at room temperature in low-stringency wash buffer (2× SSC, 0.03% SDS), 2 min in moderate-stringency wash buffer (1× SSC), and 2 min in high-stringency wash solution (0.05× SSC); dried in a Speedvac Plus (Savant); and scanned with a GenePix Laser Scanner 4000 (Axon, Foster city, Calif.). The TIFF images generated by the GenePix Laser Scanner 4000 were imported into the GenePix Pro 3.0 program (Axon), and spot intensities from scanned slides were quantified. Grids were predefined and manually adjusted to ensure optimal spot recognition. Spot signals that were distorted by dust or local high backgrounds were flagged and not included in the analysis. After determining the relative ratios of the fluorescent signals from the two channels (532 and 635 nm), the data files (.gpr files) were imported into the Stanford Microarray Database (http://genome-www.stanford.edu/microarray/) (26, 70) and normalized by using an internal control, *rps1b* (for details, see the Stanford Microarray Database website). The primary data sets may be viewed and downloaded (see above); the experiment identification numbers are 38980, 38981, 38985 to 38988, 39071, 39080, 39085, 39086, 39563, 39578, and 39583 to 39590.

Only those spots with a minimum normalized net intensity for the median (channel 1 net median intensity or normalized channel 2 net median intensities) of >350 for both wild-type cells and the *dspA* mutant, in at least one of the two channels, were included in the analyses. Log (base 2) channel 1/channel 2 ratios after normalization were calculated. The significance of the differences in gene expression observed (at $P = 0.05$) was statistically evaluated by *t* tests using a df of 7 with an α of 2.8, representing $P = 0.05$. The degree of freedom (df) value was calculated from the 8 samples used for the data analysis; for each data point, two slides were analyzed, with four duplicates for each array element on each slide. Two independently isolated RNA samples were used for generating the cDNA that was hybridized to each of the slides.

Microarray experimental design. The effect of glucose on gene expression in wild-type cells and the HL effects on both the wild-type and the *dspA* mutant of the *Synechocystis* sp. strain PCC6803 were examined. For measuring long-term acclimation of wild-type cells to glucose, total RNA was extracted from cells cultured in BG-11 medium or BG-11 medium supplemented with 10 mM glucose. The cells were grown for 2 weeks in LL but were diluted every 2 days to maintain their logarithmic growth. Total RNA isolated from wild-type cells grown in BG-11 medium in the absence of glucose was reverse transcribed, and the second-strand cDNA was labeled with Cy3-dUTP. Total RNA isolated from the cells grown in BG-11 medium supplemented with glucose was used for labeling cDNA with Cy5-dUTP. The Cy3- and Cy5-labeled cDNA samples were mixed and applied to a microarray slide. A "dye-swap" Cy-dye-dUTP labeling was performed with RNA isolated from a duplicate, independent experiment. A similar procedure was used for analyzing transcripts in wild-type cells and the *dspA* mutant grown in glucose-supplemented medium following transfer from LL (zero time point reference) to HL (for 30 min, 1 h, 3 h, and 6 h). We also examined differential transcript accumulation in the *dspA* mutant in LL compared to that of wild-type cells grown under the same conditions. Total RNA from the LL-grown *dspA* mutant was reverse transcribed, and the second-strand cDNA was labeled with Cy5-dUTP and mixed with Cy3-dUTP-labeled cDNA generated from LL-grown wild-type cells and applied to a microarray slide. The experiment was duplicated, but the dyes used to label the cDNAs were swapped; the cDNA generated from LL-grown *dspA* mutant was labeled with Cy3-dUTP, while the cDNA from LL-grown wild-type cells was labeled with Cy5-dUTP. The *dspA* mutant grew extremely slowly and was difficult to maintain in the absence of glucose.

Real-time quantitative RT-PCR. To confirm the validity of the results generated from microarray analyses, we performed real-time reverse transcription (RT)-PCR for 8 specific transcripts and compared the results to the mean of the quantified array measurements for those same transcripts. To remove trace amounts of genomic DNA contamination from RNA samples used for the real-time RT-PCR, RNA (100 ng/μl) was treated with 2 U of RNase-free DNase at 37°C for 30 min (Ambion, Austin, Tex.), and the DNase was removed by DNase removal reagent (Ambion). Levels of specific transcripts in total mRNA isolated from HL-treated cells were quantified by real-time RT-PCR with the LightCycler (Roche Applied Science, Indianapolis, Ind.). Amplifications were performed by incubating 200 ng of DNA-free RNA with specific primer pairs, as given in Table 1, and LightCycler RNA Master SYBR Green I according to the protocols of the manufacturer (Roche Applied Science). Amplifications performed according to the protocols are as follows: 1 cycle of RT reaction at 61°C for 20 min, 1 cycle at 95°C for 2 min, and 45 cycles at 95°C for 5 s, 55°C for 5 s, and 72°C for 13 s. A "no-RT" PCR control reaction was performed by incubating an equal amount of DNA-free RNA samples with a mixture of specific primer pairs and LightCycler DNA Master SYBR Green I at a final volume of 20 μl (Roche Applied Science). Amplification was done for 1 cycle at 95°C for 30 s followed by 45 cycles of 95°C for 5 s, 55°C for 5 s, and 72°C for 10 s.

A number of standards were developed as controls for these experiments. The *rps1b* (*slr*1984) and *rnpB* (*slr*0249) transcripts were used as external standards since the levels of these transcripts did not change in either wild-type cells or the *dspA* mutant following HL exposure (under all conditions that we used). PCR efficiency differences between target and reference transcripts were corrected by generating fit coefficients. A dilution series was used for constructing standard curves that enabled us to determine the fit coefficients for PCR amplification efficiency of target relative to the reference (*rps1b* and *rnpB*) transcripts. Quantification of the relative change (*n*-fold) in mRNA levels was calculated as described by Technical Note no. LC13/2001 (Roche Applied Science, 2001). Each quantitative RT-PCR experiment was performed with at least two replicates.

RESULTS

(i) Effect of glucose on expression in wild-type cells and the *dspA* mutant. In LL, the *dspA* mutant exhibited a markedly reduced growth rate (doubling time of ~40 h) under photoautotrophic conditions relative to wild-type *Synechocystis* sp. strain PCC6803 and was difficult to maintain. This slow growth rate reflected very low light-dependent O_2 evolution (42), demonstrating that a strain lacking DspA is strongly affected in both growth and photosynthetic performance. In contrast, in the presence of glucose, the mutant grew, with a doubling time

of 8 h, compared to a 6-h doubling time for wild-type cells (42). We examined how glucose influenced global transcript accumulation in wild-type *Synechocystis* sp. strain PCC6803. Cells were grown long term (2 weeks with the dilution of cultures to maintain the cells in logarithmic growth phase) in LL in either BG-11 or BG-11 supplemented with 10 mM glucose. Total RNA was purified (5) and reverse transcribed, second-strand cDNAs were labeled with Cy3- or Cy5-dUTP, and equal amounts of the two labeled cDNA samples were hybridized to the arrays (see Materials and Methods). The use of second-strand synthesis for probe preparation, compared to using just first-strand synthesis, increased the array signals generated without significantly changing the relative intensities of the signals.

Of the 3,164 distinct *Synechocystis* sp. strain PCC6803 genes represented on the array, ~95% showed no statistically significant difference in transcript levels between wild-type cells grown photoautotrophically and those grown mixotrophically (supplemented with 10 mM glucose). Statistically significant changes in the levels of transcripts for 5% of the genes were relatively small; all changes were less than twofold (data not shown). These results demonstrate that during long-term growth, the pattern of transcript accumulation in wild-type cells is not dramatically influenced by the inclusion of glucose in the growth medium. Therefore, we compared the effects of HL on gene expression in wild-type cells and the *dspA* mutant during growth in glucose-supplemented medium.

(ii) Gene expression in wild-type cells and the *dspA* mutant. Expression from 3,164 *Synechocystis* sp. strain PCC6803 genes was measured for wild-type cells and the *dspA* mutant grown in LL and then transferred to HL for 0.5, 1, 3, and 6 h. A comparison of transcript abundance in wild-type cells and the *dspA* mutant grown in LL demonstrated that the levels of 84 transcripts were altered by more than twofold in the mutant relative to wild-type cells (data not shown). Nearly all of these transcripts showed increased abundance in the *dspA* mutant relative to wild-type cells during LL growth, with the exceptions of *chlL*, *chlN*, *hofG*, *cpcC*, *psaA*, *psaB*, *cpcG*, *sll*0177, and *slr*0147, for which the transcripts were lower in the mutant strain.

We identified a set of 143 genes for which the transcript abundance changed by at least threefold in either wild-type cells or the *dspA* mutant following the transfer of cells to HL (for at least one of the time points following transfer) or that were already elevated in the *dspA* mutant compared to wild-type cells during LL growth. A hierarchical clustering analysis was used to group the genes on the basis of similarities in their expression patterns and to allow for easy viewing of the changes that we observed; six different groups were established, as shown in Fig. 1. For each gene in the different expression categories, the change in transcript levels over the 6-h time course following the transfer of cells to HL (for both wild-type and *dspA* mutant cells), the difference in the initial expression levels for wild-type cells and the *dspA* mutant under LL conditions (0 h), and the statistical significance of the data are given in Table 2. Table 3 shows trends in expression within specific categories of genes critical for photosynthesis and acclimation of cells to phosphorus deprivation. In this table, all changes of twofold or greater are shown.

Group 1: transcripts in wild-type cells increased, while *dspA* transcripts declined. Seven genes were placed in group 1.

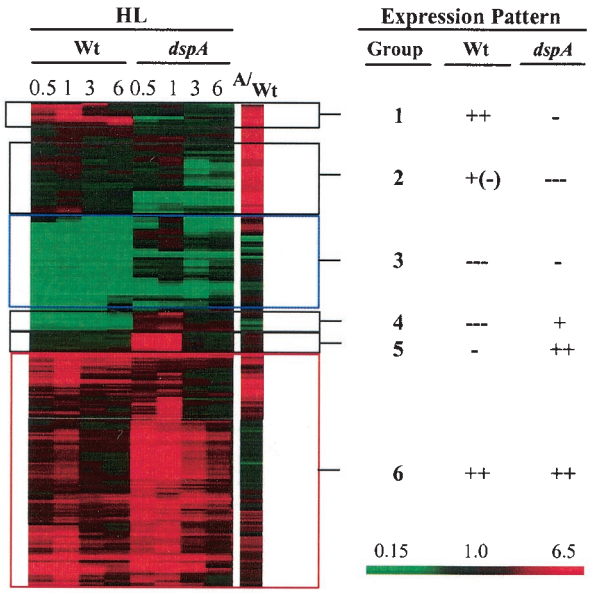

FIG. 1. Gene expression profiles of wild-type *Synechocystis* sp. strain PCC6803 and the *dspA* mutant following HL exposure presented as hierarchical clusters. Global expression patterns of 143 genes whose transcript levels changed by at least threefold in either wild-type (Wt) cells or the *dspA* mutant for at least one time point following the transfer of cells to HL. The time following the transfer of wild-type cells (left) and the *dspA* mutant (right) are 0.5, 1, 3, and 6 h. Also presented is the ratio of transcripts in *dspA* relative to those of wild-type cells (A/Wt) in LL-grown cells. Expression patterns with respect to wild-type cells and the *dspA* mutant were divided into groups 1 to 6, and the trends within each of the groups are presented in the right columns for both wild-type cells and the *dspA* mutant. Increases in transcript abundance are indicated in red (+), and decreases are indicated in green (−). The scale, extending from 0.15- to 6-fold, is shown on the right.

When wild-type cells were exposed to HL, the level for all of the seven genes increased to a significant extent, while they declined by 30 to 70% in the *dspA* mutant. The genes in this group include two genes related to cell division, *ftsH* (or *slr*0228) and *slr*1604. The *ftsH* gene encodes a metalloprotease involved in the replacement of damaged D1 polypeptides in the reaction center of PS II 46a, 64a, 70a). The other genes in this group include *psbD2* (*slr*0927), which encodes a second reaction center polypeptide; *pstS* (*sll*0680), which encodes a phosphate binding protein; *pstC* (*sll*0681), which encodes a subunit of the phosphate permease; and two genes encoding proteins of unknown function (Table 2). Interestingly, transcript levels of genes in this category were 2.6- to 17-fold higher in the mutant than in wild-type cells during LL growth; the *pstS* and the *pstC* transcripts were 17- and 10-fold more abundant, respectively, in the mutant than in wild-type cells during LL growth.

Group 2: transcripts that changed slightly in wild-type cells and declined in the *dspA* mutant. Surprisingly, most of the phosphate starvation-induced genes (*pho*), extensively studied in *E. coli* (78), were in group 2; this group includes *pstA* (*slr*1249), *pstB* (*sll*0684, *sll*0683), and *pstS* (*slr*1247). Some transcripts for *pho* regulon polypeptides exhibited a transient increase in HL, as is clearly observed for *pstC* and *pstS* (Tables 2 and 3). A similar increase in *pstS* mRNA in HL was observed

TABLE 2. Influence of HL on levels of transcripts in wild-type cells and the *dspA* mutant[a]

Gene[b]	Function	Statistical significance of transcript levels after HL treatment (h)								
		Wt				*dspA*				*dspA*/Wt
		0.5	1	3	6	0.5	1	3	6	0
Group 1										
*slr*0228 (*ftsH*)	Protease	**3.69**	**3.81**	**1.36**	0.81	−1.40	0.88	−1.53	−2.48	**2.61**
*slr*1604 (*ftsH*)	Cell division	**2.25**	**2.50**	1.09	0.69	−1.91	−1.49	−1.63	−2.15	**3.24**
*slr*0927 (*psbD2*)	PS II reaction center D2 protein	**1.76**	**2.79**	**1.52**	1.02	−2.47	−1.79	−1.87	−2.39	**3.43**
*sll*0680 (*pstS*)	Phosphate-binding periplasmic protein precursor	**2.66**	**4.17**	**2.32**	**2.58**	−3.37	−3.19	1.21	0.77	**17.48**
*sll*0681 (*pstC*)	Phosphate transport system permease protein	**1.77**	**2.21**	1.24	1.14	−5.48	−4.60	−1.56	−2.45	**10.35**
*sll*1515	Unknown	**1.94**	**2.11**	**2.32**	**3.46**	−2.24	−1.50	−1.66	−2.07	**5.23**
*ssr*0692	Unknown	**1.23**	**1.34**	**1.41**	**2.47**	−1.72	−1.32	−1.41	−1.66	**3.43**
Group 2										
*slr*1247 (*pstS*)	Phosphate-binding periplasmic protein	0.92	1.00	0.95	0.95	−7.30	−6.98	−4.70	−5.76	**14.79**
*sll*0683 (*pstB*)	Phosphate transport ATP-binding protein	**1.46**	**1.63**	1.02	0.87	−3.39	−2.79	−1.68	−2.16	**5.91**
*sll*0684 (*pstB*)	Phosphate transport ATP-binding protein	**1.23**	**1.40**	0.96	−1.78	−3.15	−2.70	−1.72	−2.13	**4.71**
*slr*1248 (*pstC*)	Phosphate transport system permease protein	0.88	0.90	0.89	−1.42	−4.44	−4.38	−4.41	−4.78	**4.61**
*slr*1249 (*pstA*)	Phosphate transport system permease protein	0.85	0.85	0.87	−1.36	−2.43	−2.27	−2.49	−3.14	**2.48**
*sll*0654	Alkaline phosphatase	0.83	0.89	0.88	0.77	−6.82	−5.82	−5.60	−4.90	**9.19**
*slr*0897	Probable endoglucanase	−1.25	0.77	0.87	−1.56	−1.76	−1.67	−2.04	−2.76	**11.98**
*sll*1349 (*cbbZp*)	Phosphoglycolate phosphatase	0.98	1.15	1.04	0.96	−1.86	−1.55	−1.51	−1.89	**4.99**
*sll*1535 (*rfbP*)	Putative sugar transferase	1.18	**1.29**	0.90	−1.32	−1.74	0.82	−1.83	−2.82	**3.23**
*slr*1204 (*htrA*)	Protease	−1.33	0.98	−1.44	0.87	−2.13	−2.00	−2.83	−2.57	**4.70**
*sll*0656	Unknown	−1.30	0.83	0.80	−1.41	−3.71	−3.14	−4.05	−4.38	**6.33**
*slr*2123	Similar to D-3-phosphoglycerate dehydrogenase	−1.32	0.81	0.80	−1.52	−1.69	0.84	−1.75	−2.22	**3.24**
*slr*2122	Unknown	−1.28	0.90	0.91	−1.34	−1.42	0.86	−1.71	−2.11	**3.21**
*ssr*2912	Unknown	−1.22	0.89	0.87	0.81	−1.50	−1.32	−1.66	−2.50	**3.38**
*slr*1116	Unknown	−1.29	−1.21	0.83	−1.19	−1.56	−1.36	−1.27	−1.58	**3.32**
*slr*0238	Unknown	0.91	0.93	−1.23	0.81	0.79	0.82	−1.32	−1.91	**3.74**
*ssl*3769	Unknown	0.99	**1.30**	1.08	1.01	−2.06	−1.49	−3.06	−3.36	**6.87**
Group 3										
*sll*1471 (*cpcG*)	Phycobilisome rod-core linker polypeptide	−10.05	−8.42	−7.85	−11.58	−3.23	−3.14	−2.61	−3.27	−2.97
*ssl*3093 (*cpcD*)	Phycobilisome small rod linker polypeptide	−8.26	−6.77	−5.41	−5.13	−2.48	−1.69	−2.06	−2.26	−1.58
*sll*1580 (*cpcC*)	Phycobilisome rod linker polypeptide	−14.16	−12.11	−16.11	−10.14	−5.19	−3.03	−3.99	−3.34	−2.00
*sll*1579 (*cpcC*)	Phycobilisome rod linker polypeptide	−17.20	−14.42	−12.19	−10.64	−5.87	−3.45	−4.31	−3.79	−1.93
*sll*1578 (*cpcA*)	Phycocyanin alpha subunit	−14.20	−15.06	−22.71	−9.60	−8.55	−4.06	−4.37	−3.14	−1.42
*sll*1577 (*cpcB*)	Phycocyanin beta subunit	−13.59	−16.82	−24.57	−7.86	−9.60	−4.27	−4.07	−2.85	−1.33
*ssr*3383	Phycobilisome small core linker polypeptide	−4.48	−3.41	−2.32	−1.61	−2.76	−2.43	−1.81	−1.78	0.99
*slr*1986	Allophycocyanin beta subunit	−7.50	−5.09	−2.83	−1.57	−5.03	−2.94	−1.93	−1.56	0.89
*slr*2067	Allophycocyanin alpha subunit	−4.36	−3.21	−2.60	−1.40	−2.85	−2.05	−1.36	0.81	0.98
*sll*0819 (*psaF*)	PS I reaction center subunit III precursor	−5.20	−4.77	−4.81	−3.91	−1.97	0.68	−1.68	−1.55	−1.35
*sml*0008 (*psaJ*)	PS I subunit IX	−3.45	−3.52	−3.55	−2.95	−1.50	0.80	−1.50	−1.51	0.83
*slr*1835 (*psaB*)	P700 apoprotein subunit Ib	−3.83	−5.46	−5.65	−7.19	−2.09	0.86	−1.86	−1.93	−2.53
*slr*1834 (*psaA*)	P700 apoprotein subunit Ia	−4.22	−5.62	−5.97	−6.98	−2.33	0.91	−1.76	−2.33	−2.27
*slr*1655 (*psaL*)	PS I subunit XI	−5.13	−5.02	−6.15	−4.18	−2.64	−1.55	−1.79	−1.74	−1.59
*slr*0533	Two-component sensor histidine kinase (*hik10*)	−3.59	−3.41	−2.96	−3.69	0.89	1.09	−2.13	−2.33	0.94
*slr*0083 (*crhR*)	RNA helicase light	−2.53	−3.05	−3.87	−5.29	−3.33	−1.50	−3.77	−4.35	**1.94**
*sll*1451 (*nrtB*)	Nitrate/nitrite transport system permease protein	1.09	1.24	−1.74	−3.76	0.88	−1.67	−1.47	−2.54	**2.07**
*slr*0750 (*chlN*)	Light-independent protochlorophyllide reductase subunit	−7.28	−7.68	−5.30	−7.64	0.87	0.86	−1.35	−1.64	−3.51
*slr*0749 (*chlL*)	Light-independent protochlorophyllide reductase subunit	−6.71	−5.98	−5.15	−6.86	0.79	0.85	−1.29	−1.49	−3.14
*slr*1634	Unknown	−10.12	−10.40	−9.02	−14.25	−7.72	−5.86	−8.50	−9.80	0.99
*sll*1184	Heme oxygenase	−5.92	−4.73	−3.10	−1.48	−2.79	−2.07	−1.66	−1.44	0.85
*sll*1330	Two-component system response regulator OmpR subfamily	−5.42	−4.87	−4.83	−4.78	−2.43	−1.94	−2.72	−2.33	0.86
*sll*0756	Unknown	−4.03	−3.14	−3.13	−4.01	−1.25	0.94	−1.38	−1.86	−1.46
*sll*0022	Unknown	−3.99	−2.92	−3.50	−3.53	−1.24	1.32	−2.20	−2.82	**1.49**
*sll*1783	Unknown	−3.83	−3.55	−2.16	−2.26	−2.35	−1.97	−1.96	−1.58	1.17
*sll*1306	Periplasmic protein, function unknown	−3.67	−3.52	−3.00	−3.51	−2.01	−1.65	−1.94	−1.98	1.00
*sll*1305	Probable hydrolase	−3.30	−3.43	−2.72	−3.27	−1.95	−1.69	−2.09	−2.08	1.05
*slr*1535	Unknown	−3.01	−2.96	−2.72	−3.17	−1.46	−1.37	−1.41	−1.68	0.99
*slr*0082	Unknown	−2.98	−3.04	−3.66	−4.57	−2.82	−1.37	−3.08	−3.66	**2.03**
*sll*1304	Unknown	−2.84	−2.65	−2.41	−2.77	−1.74	−1.52	−1.64	−1.66	**1.19**
*sll*1784	Periplasmic protein, function unknown	−2.75	−2.42	−2.16	−2.03	−1.69	−1.43	−1.31	−1.38	**1.33**
*sll*1307	Periplasmic protein, function unknown	−2.73	−2.59	−2.24	−2.60	−1.56	0.83	−1.46	−1.66	1.11
*slr*0374	Unknown	−1.62	−2.48	−3.36	−4.10	−2.42	0.90	−3.74	−4.84	**1.43**
*slr*1974	GTP binding protein	−1.60	−1.59	−1.69	−2.15	−1.81	−1.45	−1.98	−2.80	**2.88**
*slr*1403	Unknown	−1.36	−1.35	−1.30	−1.47	0.75	0.90	−1.47	−2.17	**5.14**
*slr*0287	Unknown	−1.33	−2.09	−2.88	−3.93	−2.44	0.89	−3.83	−5.86	1.08

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TABLE 2—*Continued*

Gene[b]	Function	Statistical significance of transcript levels after HL treatment (h)								
		Wt				*dspA*				*dspA*/Wt
		0.5	1	3	6	0.5	1	3	6	0
Group 4										
*sll*1626 (*lexA*)	LexA repressor	−5.42	−4.30	−2.55	0.99	0.89	1.16	**1.64**	**1.68**	1.09
*slr*0737 (*psaD*)	PS I subunit II	−5.30	−5.07	−3.96	−3.02	1.03	1.12	0.81	0.69	1.06
*sll*1695 (*hofG*)	Pilin polypeptide PilA2	−3.14	−3.38	−3.86	−4.11	**1.57**	**1.86**	1.30	1.14	−1.87
*sll*1694 (*hofG*)	Pilin polypeptide PilA1	−2.56	−3.43	−5.47	−5.14	**1.37**	**1.65**	1.13	0.90	−2.03
*sll*0517 (*rbpA*)	Putative RNA binding protein	−3.03	−3.72	−2.64	−2.14	0.86	**1.74**	0.84	0.70	**1.43**
*sll*1696	Unknown	−2.74	−3.12	−3.17	−3.83	**1.52**	**2.01**	**1.44**	**1.36**	−1.66
*sll*0177	Unknown	−3.68	−3.34	−3.88	−4.29	0.99	1.13	0.89	0.75	−2.11
Group 5										
*sll*1029 (*ccmK*)	Carbon dioxide concentrating mechanism protein	−1.31	−1.44	−1.67	−1.72	**3.93**	**5.97**	1.00	−1.73	**1.34**
*sll*1028 (*ccmK*)	Carbon dioxide concentrating mechanism protein	−1.36	−1.52	0.70	−1.61	**3.26**	**4.56**	0.89	−1.72	**1.29**
*sll*1031 (*ccmM*)	Carbon dioxide concentrating mechanism protein	−1.17	−1.43	−1.45	−1.83	**2.77**	**4.45**	−1.32	−2.03	1.19
*sll*1032 (*ccmN*)	Carbon dioxide concentrating mechanism protein	−1.19	−1.38	−1.48	−2.00	**2.76**	**4.51**	0.88	−1.78	**1.47**
*sll*1030 (*ccmL*)	Carbon dioxide concentrating mechanism protein	−1.34	−1.41	−1.41	−1.50	**2.87**	**4.24**	1.02	−1.58	**1.43**
*slr*0009 (*rbcL*)	Ribulose bisphosphate carboxylase large subunit	0.94	0.73	−1.47	0.91	**2.12**	**3.92**	0.93	−1.55	1.19
*slr*0012 (*rbcS*)	Ribulose bisphosphate carboxylase small subunit	−1.33	−1.79	0.70	−1.04	**2.82**	**3.60**	0.95	−1.59	1.09
*sll*1853	Unknown	0.94	0.98	0.88	−1.41	**2.51**	**3.32**	0.95	−1.37	**1.22**
*slr*0006	Unknown	−1.72	−1.71	−1.69	−1.59	**4.44**	**5.78**	**1.19**	0.79	1.02
Group 6										
*ssr*2595 (*hliB*)	HL-inducible polypeptide (*scpD*)	**8.85**	**10.13**	**3.47**	**2.18**	**2.17**	**3.69**	**2.60**	**1.43**	**1.86**
*ssl*1633 (*hliC*)	HL-inducible polypeptide (*scpB*)	**5.28**	**7.33**	**3.92**	**4.25**	**5.02**	**4.30**	**4.61**	**1.79**	**6.85**
*ssl*2542 (*hliA*)	HL-inducible polypeptide (*scpC*)	1.16	**1.73**	**1.49**	**1.55**	**3.10**	**2.81**	**2.90**	**1.65**	**1.92**
*sll*0416 (*groEL-2*)	60-kDa chaperonin 2	**4.89**	**6.04**	**2.21**	**1.89**	**5.59**	**3.93**	**3.10**	**1.38**	**1.51**
*slr*2076 (*groEL*)	60-kDa chaperonin	**4.45**	**7.38**	**2.66**	**2.40**	**7.65**	**5.28**	**5.93**	**2.21**	**1.62**
*slr*2075 (*groES*)	10-kDa chaperonin	**3.43**	**6.01**	**2.46**	**2.52**	**5.66**	**3.74**	**4.74**	**1.90**	**1.52**
*sll*0430 (*htpG*)	Heat shock protein 90	**3.13**	**2.24**	0.98	0.96	**2.66**	**3.35**	**1.89**	1.03	**1.66**
*sll*1514 (*hsp17*)	16.6-kDa small heat shock protein	**1.81**	**1.87**	1.12	**1.72**	1.32	1.30	1.12	1.34	**4.51**
*slr*1291 (*ndhD2*)	NADH dehydrogenase subunit 4	**3.36**	**3.80**	**1.89**	**1.78**	1.00	**2.37**	−1.35	−1.67	**1.82**
*sll*1732 (*ndhF*)	NADH dehydrogenase subunit 5	1.05	1.13	**1.51**	−1.36	**1.47**	**3.14**	1.12	−1.41	**1.40**
*sll*1733 (*ndhD3*)	NADH dehydrogenase subunit 4	1.03	1.04	**1.32**	0.80	**1.71**	**3.16**	1.08	−1.42	**1.45**
*sll*1867 (*psbA3*)	PS II D1 protein	**2.94**	**4.31**	**2.56**	**2.48**	1.38	**1.64**	1.15	0.70	**6.30**
*slr*1311 (*psbA2*)	PS II D1 protein	**2.81**	**4.33**	**2.42**	**2.46**	**1.47**	**1.59**	1.20	0.68	**5.80**
*slr*1908	Probable porin, major outer membrane protein	**2.86**	**4.00**	**1.86**	**2.05**	1.03	**1.38**	**1.25**	−0.53	**2.74**
*slr*1746 (*murI*)	Glutamate racemase	**1.61**	**1.93**	**1.72**	**1.85**	1.06	**1.78**	0.83	−1.83	**4.10**
*ssl*0020 (*petF*)	Ferredoxin I	**2.58**	**2.99**	**1.72**	**1.76**	**1.77**	**1.39**	**1.54**	−1.30	**1.32**
*sll*1363 (*ilvC*)	Ketol-acid reductoisomerase	**1.83**	**2.07**	**1.74**	**1.52**	**3.07**	**2.53**	**3.76**	**1.82**	0.98
*slr*0447 (*amiC*)	ABC-type urea transport system substrate-binding protein	**1.46**	**2.09**	**1.18**	1.09	**3.33**	**2.58**	**2.51**	1.12	−1.90
*sll*0330 (*fabG*)	Sepiapterine reductase	**1.44**	**1.66**	1.27	1.06	1.12	1.24	0.84	0.94	**5.50**
*slr*1469 (*rnpA*)	Protein subunit of ribonuclease P	**1.38**	**1.50**	1.09	0.97	**3.06**	**2.71**	**2.76**	**1.39**	1.14
*slr*0394 (*pgk*)	Phosphoglycerate kinase	**1.35**	**1.42**	1.16	1.01	1.23	**1.65**	**1.32**	−1.45	**9.08**
*slr*1251 (*cvp*)	Peptidyl-prolyl *cis-trans* isomerase	1.18	**1.43**	**1.89**	**2.22**	**1.99**	**1.59**	**3.48**	**2.03**	0.87
*sll*1799 (*rpl3*)	50S ribosomal protein L3	**2.31**	**2.41**	**1.51**	1.19	**5.76**	**6.25**	**4.58**	**2.04**	−1.60
*sll*1800 (*rpl4*)	50S ribosomal protein L4	**2.13**	**2.07**	**1.34**	1.08	**5.72**	**6.18**	**3.99**	**2.06**	−1.31
*sll*1801 (*rpl23*)	50S ribosomal protein L23	**2.13**	**2.08**	**1.33**	1.05	**6.51**	**6.77**	**4.35**	**1.94**	−1.33
*sll*1802 (*rpl2*)	50S ribosomal protein L2	**1.99**	**2.07**	1.12	0.87	**5.03**	**6.20**	**3.44**	**1.71**	−1.40
*ssl*3432 (*rps19*)	30S ribosomal protein S19	**1.90**	**1.90**	1.05	0.86	**6.33**	**6.48**	**3.94**	**1.89**	−1.38
*sll*1804 (*rps3*)	30S ribosomal protein S3	**1.85**	**1.79**	1.02	1.46	**4.51**	**4.95**	**3.12**	**1.52**	−1.73
*sll*1803 (*rpl22*)	50S ribosomal protein L22	**1.82**	**1.87**	1.13	0.83	**5.09**	**5.42**	**3.44**	**1.65**	−1.34
*sll*1805 (*rpl16*)	50S ribosomal protein L16	**1.73**	**1.78**	1.05	−1.34	**4.54**	**5.37**	**3.06**	**1.57**	−1.57
*sll*1806 (*rpl14*)	50S ribosomal protein L14	**1.57**	**1.55**	0.97	−1.40	**5.37**	**5.68**	**3.06**	**1.65**	−1.63
*ssl*3436 (*rpl29*)	50S ribosomal protein L29	**1.57**	**1.58**	1.06	−1.19	**5.14**	**5.70**	**3.18**	**1.55**	−1.64
*sll*1808 (*rpl5*)	50S ribosomal protein L5	**1.52**	**1.62**	0.95	−1.40	**4.63**	**5.60**	**2.59**	**1.55**	−1.68
*sll*1807 (*rpl24*)	50S ribosomal protein L24	**1.52**	**1.53**	0.96	−1.37	**5.35**	**6.11**	**3.37**	**1.62**	−1.66
*ssl*3437 (*rps17*)	30S ribosomal protein S17	**1.50**	**1.57**	1.10	−1.27	**4.73**	**5.29**	**2.85**	**1.61**	−1.59
*sll*1260 (*rps2*)	30S ribosomal protein S2	**1.45**	**1.65**	1.07	0.99	**3.36**	**3.19**	**3.18**	**1.71**	−1.39
*sll*1816 (*rps13*)	30S ribosomal protein S13	**1.40**	**1.49**	0.96	−1.30	**3.05**	**2.74**	**2.93**	**1.43**	−1.42
*sll*1744 (*rpl1*)	50S ribosomal protein L1	**1.38**	**1.56**	1.00	0.93	**3.85**	**3.81**	**2.94**	**1.58**	−1.31
*sll*1812 (*rps5*)	30S ribosomal protein S5	**1.34**	**1.34**	0.89	−1.65	**4.26**	**4.65**	**3.09**	**1.58**	−1.83
*slr*0628 (*rps14*)	30S ribosomal protein S14	**1.34**	**1.78**	**1.59**	**1.85**	**2.39**	**2.09**	**3.31**	**2.02**	1.25
*sll*1809 (*rps8*)	30S ribosomal protein S8	**1.34**	**1.44**	0.84	−1.49	**3.97**	**4.71**	**2.94**	**1.43**	−1.41
*sll*1810 (*rpl6*)	50S ribosomal protein L6	**1.33**	**1.41**	0.86	−1.69	**4.16**	**4.62**	**2.70**	1.36	−1.65
*slr*0469 (*rps4*)	30S ribosomal protein S4	**1.33**	**1.54**	1.09	1.15	**3.60**	**3.05**	**2.96**	**1.52**	0.94
*sll*1745 (*rpl10*)	50S ribosomal protein L10	**1.28**	**1.53**	1.05	0.97	**3.21**	**3.64**	**2.54**	**1.41**	−1.38
*sll*1811 (*rpl18*)	50S ribosomal protein L18	**1.27**	**1.36**	0.85	−1.57	**4.18**	**4.82**	**2.74**	**1.55**	−1.70
*sll*1813 (*rpl15*)	50S ribosomal protein L15	**1.24**	**1.40**	0.95	−1.46	**2.96**	**3.53**	**2.25**	**1.39**	−1.56
*sll*1743 (*rpl11*)	50S ribosomal protein L11	1.23	**1.46**	0.99	0.92	**3.76**	**3.38**	**2.94**	**1.49**	−1.33

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TABLE 2—*Continued*

Gene[b]	Function	Statistical significance of transcript levels after HL treatment (h)								
		Wt				*dspA*				*dspA*/Wt
		0.5	1	3	6	0.5	1	3	6	0
*sll*1746 (*rpl12*)	50S ribosomal protein L12	**1.22**	**1.56**	1.04	1.15	**3.31**	**3.51**	**2.54**	**1.43**	−1.31
*ssr*2016	Unknown	**12.06**	**16.02**	**5.58**	**2.41**	**7.90**	**10.08**	**5.37**	**2.14**	**1.67**
*sll*1483	Similar to transforming growth factor-induced protein	**5.59**	**8.75**	**7.00**	**9.22**	**4.14**	**2.48**	**5.39**	**3.99**	**9.00**
*slr*1544	Unknown	**4.54**	**4.70**	**2.26**	**1.45**	**1.48**	**2.27**	**1.60**	0.91	**1.48**
*sll*1911	Unknown	**4.46**	**6.09**	**3.85**	**3.66**	**1.45**	1.19	**1.81**	1.03	**3.04**
*slr*1963	Water-soluble carotenoid protein	**3.92**	**3.61**	**1.62**	1.14	**3.22**	**2.55**	**2.55**	1.33	**1.89**
*sll*0788	Unknown	**3.26**	**3.44**	**1.49**	**1.39**	**1.32**	**1.91**	0.87	0.74	**2.56**
*slr*0075 (*vcf16*)	ABC transporter ATP-binding protein	**3.02**	**3.03**	**1.62**	−1.53	**1.64**	**2.69**	**2.33**	−1.54	0.97
*slr*1992 (*gpx2*)	Glutathione peroxidase-like NADPH peroxidase	**2.96**	**3.58**	**2.73**	**2.54**	**1.36**	1.22	**1.78**	1.01	**3.33**
*slr*0074 (*ycf24*)	ABC transporter subunit	**2.90**	**3.01**	**1.61**	−1.47	1.25	**2.32**	**1.92**	−1.67	0.98
*sll*0661 (*ycf35*)	Unknown	**2.51**	**3.35**	**2.55**	**2.12**	1.27	1.29	**1.32**	0.97	**1.76**
*sll*0185	Unknown	**2.44**	**4.00**	**1.64**	1.28	**2.07**	**3.48**	**1.47**	0.99	**1.81**
*slr*1982	Two-component response regulator CheY subfamily	**2.31**	**3.07**	**2.04**	**2.13**	**1.39**	**1.60**	1.13	0.75	**4.78**
*sll*0982	Unknown	**2.00**	**3.92**	**2.43**	**1.56**	1.22	1.11	1.07	0.95	**2.11**
*sll*0905	Unknown	**1.71**	**2.07**	**1.34**	**1.58**	0.99	1.22	0.85	−1.56	**3.75**
*slr*1512 (*sbtA*)	Sodium-dependent bicarbonate transporter	**1.24**	1.29	**1.51**	1.05	**3.02**	**7.68**	1.20	−1.55	**2.05**
*slr*1513	Unknown	**1.19**	**1.24**	**1.38**	1.23	**2.47**	**3.56**	**1.51**	0.97	**1.97**
*sll*1219	Unknown	**1.19**	**1.19**	1.06	1.04	**1.91**	**2.93**	**1.37**	−1.36	**3.50**
*ssr*1853	Unknown	**1.15**	**1.49**	**1.83**	**2.53**	**4.02**	**2.17**	**4.83**	**3.16**	**1.83**
*slr*0581	Unknown	**1.11**	1.31	**1.21**	**1.91**	**1.51**	**1.34**	**1.56**	**1.41**	**3.25**
*slr*0821	Unknown	**1.07**	**1.58**	**1.69**	**1.70**	**2.93**	**1.80**	**3.41**	**1.74**	0.92
*sll*0219	Flavoprotein	**0.94**	0.97	0.88	0.77	1.20	**3.02**	0.91	−1.30	**1.82**

[a] Transcript levels of 143 genes were changed by at least threefold in either wild-type (Wt) cells or the *dspA* mutant in at least one of the time points following the transfer of cells to HL and the ratio of *dspA* to the wild type (*dspA*/Wt) at time zero are presented. Expression patterns with respect to both wild-type cells and the *dspA* mutants were divided into six groups based on similarity of hierarchical clustering analyses. The statistical significance of the expression patterns are indicated for each time point and for the *dspA*/Wt time zero ratio; boldface type indicates an increase in the transcript level, underlining indicates a decrease in the transcript level, and plain type indicates no significant change in the transcript level. The significance was computed by *t* tests (see Materials and Methods).

[b] For group 1, transcripts in wild-type cells increased while *dspA* transcripts declined; group 2, transcripts changed slightly in wild-type cells and declined in the *dspA* mutant; group 3, levels of transcripts in both wild-type cells and the *dspA* mutant strongly declined; group 4, levels of transcript markedly declined in wild-type cells but increased in *dspA;* group 5, transcripts declined slightly in wild-type cells but increased in the *dspA* mutant; group 6, transcripts increased in both wild-type cells and *dspA*.

previously (4). Interestingly, even in LL, the *dspA* strain showed very high levels (relative to wild-type cells) of *pstS* (both *sll*0680 and *slr*1247), *pstC* (*sll*0681 and *slr*1248, integral membrane protein), and *pstB* (*sll*0683 and *slr*1250, nucleotide binding protein) transcripts, as well as of transcripts encoding an alkaline phosphatase (*sll*0654) and the extracellular NucH nuclease (*sll*0656) (Table 3). Although nucleases have been associated with the *pho* regulon, it is not known whether the *nucH* transcript increases in response to phosphorus limitation. However, it is likely to be part of the *pho* regulon since it is immediately downstream of the alkaline phosphatase and the two genes may be part of the same operon. These data demonstrate the apparent derepression of several genes associated with phosphorus starvation in the *dspA* mutant in LL. There was no further increase in the levels of *pho* transcripts when the *dspA* mutant was exposed to HL, and the abundance of some of the transcripts declined significantly (Table 3).

Interestingly, while the transcript encoding phosphoglycolate phosphatase (CbbZp, *sll*1349) was not elevated significantly in wild-type cells during HL treatment, it was fivefold higher in the mutant relative to wild-type cells under LL growth conditions. This enzyme is part of the photorespiratory pathway, which can serve as an energy sink that prevents an overreduction of the photosynthetic electron transport chain (and consequent photoinhibition) and may be especially valuable under stress conditions that lead to reduced CO_2 fixation (79). Photorespiration also provides metabolites for the production of glutathione, a tripeptide that protects cells from oxidative damage. Other genes in this category included *htrA* (*slr*1204; 4.7-fold), encoding a serine protease; *rfbP* (*sll*1535), encoding a putative sugar transferase; and six genes encoding proteins of unknown function.

Group 3: levels of transcript in both wild-type cells and the *dspA* mutant strongly declined. Genes associated with photosynthetic function, especially those encoding phycobilisomes and PS I polypeptides, are in group 3. The levels of most *cpc* transcripts showed a marked decrease in both wild-type and *dspA* mutant cells following the imposition of HL conditions (Tables 2 and 3). The *cpcE* and *cpcF* transcripts were exceptions to this trend. These transcripts code for subunits of a lyase involved in chromophorylation of the α-PC subunit (16, 17). Interestingly, most *cpc* transcripts (*cpcA*, *cpcB*, *cpcC*, *cpcD*, and *cpcG*) were already 25 to 50% lower in the mutant than in wild-type cells during LL growth (Table 3). In contrast, most *apc* transcripts were equally abundant in LL-grown mutant and wild-type cells. There was a pronounced transient change in *apc* transcript levels following exposure of wild-type cells to HL, but by 6 h in HL, the transcript levels approached those observed for LL-grown cells.

The transcripts for most PS I polypeptides (including *psaA*, *psaB*, *psaF*, and *psaL*) were already 30 to 60% lower in the *dspA* mutant than in wild-type cells in LL. The most pronounced difference between the mutant and wild-type cells was for the *psaA*/*psaB* transcripts, which were 60% lower in LL-grown mutant cells (Tables 2 and 3). Furthermore, the levels of many transcripts declined in wild-type cells following exposure

TABLE 3. Influence of light on levels of transcripts encoding phycobilisomes, PS I, PS II, and polypeptides involved in HL acclimation and phosphate acquisition in wild-type cells and the *dspA* mutant[a]

Gene ID	Function	Statistical significance of transcript levels after HL treatment (h)								
		Wt				*dspA*				*dspA*/Wt
		0.5	1	3	6	0.5	1	3	6	0
Phycobilisome										
*slr*2067 (*apcA*)	Allophycocyanin a chain	−4.36	−3.21	−2.60	−1.40	−2.85	−2.05	−1.36	0.81	0.98
*slr*1986 (*apcB*)	Allophycocyanin b chain	−7.50	−5.09	−2.83	0.64	−5.03	−2.94	−1.93	−1.56	0.89
*ssr*3383 (*apcC*)	Phycobilisome LC linker polypeptide	−4.48	−3.41	−2.32	0.62	−2.76	−2.43	−1.81	−1.78	0.99
*sll*1471 (*cpcG*)	Phycobilisome rod-core linker polypeptide	−10.05	−8.42	−7.85	−11.58	−3.23	−3.14	−2.61	−3.27	−2.97
*slr*2051 (*cpcG*)	Phycobilisome rod-core linker polypeptide	−0.50	−2.01	−1.86	−1.56	−2.19	−1.76	−1.73	−1.72	**1.43**
*sll*1578 (*cpcA*)	Phycocyanin a subunit	−14.20	−15.06	−22.71	−9.60	−8.55	−4.06	−4.37	−3.14	−1.42
*sll*1577 (*cpcB*)	Phycocyanin b subunit	−13.59	−16.82	−24.57	−7.86	−9.60	−4.27	−4.07	−2.85	−1.33
*sll*1579 (*cpcC*)	Phycocyanin associated linker protein	−17.20	−14.42	−12.19	−10.64	−5.87	−3.45	−4.31	−3.79	−1.93
*sll*1580 (*cpcC*)	Phycocyanin associated linker protein	−14.16	−12.11	−16.11	−10.14	−5.19	−3.03	−3.99	−3.34	−2.00
*ssl*3093 (*cpcD*)	Phycocyanin associated linker protein	−8.26	−6.77	−5.41	−5.13	−2.48	−1.69	−2.06	−2.26	−1.58
HL-inducible protein										
*ssr*2595 (*hliB*)	HL-inducible protein	**8.85**	**10.13**	**3.47**	**2.18**	**2.17**	**3.69**	**2.60**	1.43	**1.86**
*ssl*1633 (*hliC*)	HL-inducible protein	**5.28**	**7.33**	**3.92**	**4.25**	**5.02**	**4.30**	**4.61**	1.79	**6.85**
*ssr*1789 (*hliD*)	HL-inducible protein	**1.68**	**1.79**	1.21	1.18	1.08	**1.36**	1.15	−1.36	**2.16**
*ssl*2542 (*hliA*)	HL-inducible protein	1.16	**1.73**	**1.49**	**1.55**	**3.10**	**2.81**	**2.90**	1.65	**1.92**
PS I										
*slr*1834 (*psaA*)	P700 apoprotein subunit la	−4.22	−5.62	−5.97	−6.98	−2.33	0.91	−1.76	−2.33	−2.27
*slr*1835 (*psaB*)	P700 apoprotein subunit lb	−3.83	−5.46	−5.65	−7.19	−2.09	0.86	−1.86	−1.93	−2.53
*slr*0737 (*psaD*)	PS I subunit II	−5.30	−5.07	−3.96	−3.02	0.97	1.12	0.81	0.69	1.06
*sll*0819 (*psaF*)	PS I subunit III	−5.20	−4.77	−4.81	−3.91	−1.97	−1.47	−1.68	−1.55	−1.35
*sml*0008 (*psaJ*)	PS I subunit IX	−3.45	−3.52	−3.55	−2.95	−1.50	0.80	−1.50	−1.51	0.83
*ssl*0563 (*psaC*)	PS I subunit VII	−2.07	−2.02	−1.93	−2.46	−1.52	0.87	−1.47	−1.53	0.84
*slr*1655 (*psaL*)	PS I subunit XI	−5.13	−5.02	−6.15	−4.18	−2.64	0.65	0.56	0.58	−1.59
PS II										
*sll*1194 (*psbU*)	PS II 12-kDa extrinsic protein	**1.19**	**1.64**	**1.37**	**1.49**	0.98	0.99	**1.70**	1.11	**2.00**
*sll*0851 (*psbC*)	PS II CP43 protein	0.96	1.30	0.77	−1.80	−2.43	−1.77	−1.99	−2.29	**1.48**
*slr*0906 (*psbB*)	PS II CP47 protein	−0.64	1.08	0.96	0.91	−2.85	−2.25	−1.59	−1.71	**1.52**
*sll*1867 (*psbA3*)	PS II D1 protein	**2.94**	**4.31**	**2.56**	**2.48**	1.38	**1.64**	1.15	0.70	**6.30**
*slr*1311 (*psbA2*)	PS II D1 protein	**2.81**	**4.33**	**2.42**	**2.46**	**1.47**	**1.59**	1.20	0.68	**5.80**
*slr*0927 (*psbD2*)	PS II D2 protein	**1.76**	**2.79**	**1.52**	1.02	−2.47	−1.79	−1.87	−2.39	**3.43**
*sll*0849 (*psbD*)	PS II D2 protein	**1.52**	**2.20**	1.21	0.85	−2.36	−1.63	−1.95	−2.40	**2.62**
*sml*0005 (*psbK*)	PS II PsbK protein	0.94	0.98	0.74	0.95	1.04	1.19	−1.32	−1.71	**2.09**
*smr*0007 (*psbL*)	PS II PsbL protein	−1.22	0.91	0.96	1.13	0.84	0.91	1.01	0.82	**2.18**
*smr*0001 (*psbT*)	PS II PsbT protein	**1.70**	**1.82**	1.09	0.87	**1.72**	**2.04**	**1.62**	1.03	1.18
*sml*0002 (*psbX*)	PS II PsbX protein	**1.75**	**1.76**	1.12	1.01	**1.97**	**2.08**	**1.72**	1.13	1.10
Pho regulon										
*sll*0680 (*pstS*)	Phosphate binding periplasmic protein precursor	**2.66**	**4.17**	**2.32**	**2.58**	−3.37	−3.19	**1.21**	0.77	**17.48**
*sll*0681 (*pstC*)	Phosphate transport system permease protein	**1.77**	**2.21**	1.24	1.14	−5.48	−4.60	−1.56	−2.45	**10.35**
*sll*0682 (*pstA*)	Phosphate transport system permease protein	**1.28**	**1.39**	1.04	0.84	−1.83	−1.59	0.79	−1.69	**2.63**
*slr*1248 (*pstC*)	Phosphate transport system permease protein	0.88	0.90	0.89	−1.42	−4.44	−4.38	−4.41	−4.78	**4.61**
*slr*1249 (*pstA*)	Phosphate transport system permease protein	0.85	0.85	0.87	0.74	−2.43	−2.27	−2.49	−3.14	**2.48**
*sll*0683 (*pstB*)	Phosphate transport ATP-binding protein	**1.46**	**1.63**	1.02	0.87	−3.39	−2.79	−1.68	−2.16	**5.91**
*sll*0684 (*pstB*)	Phosphate transport ATP-binding protein	**1.23**	**1.40**	0.96	−1.78	−3.15	−2.70	−1.72	−2.13	**4.71**
*slr*1250 (*pstB*)	Phosphate transport ATP-binding protein	0.96	1.00	1.01	0.92	−1.71	−1.73	−1.81	−2.19	**2.58**
*slr*1247 (*pstS*)	Periplasmic phosphate binding protein	0.92	1.00	0.95	0.95	−7.30	−6.98	−4.70	−5.76	**14.79**
*sll*0222 (*phoA*)	Alkaline phosphatase	0.99	1.15	−1.30	−1.65	−1.40	0.87	−1.31	−2.05	**2.02**
*sll*0654	Alkaline phosphatase	0.83	0.89	0.88	0.77	−6.82	−5.82	−5.60	−4.90	**9.19**

[a] For the genes included in this table, the transcripts changed by at least twofold in either wild-type (Wt) cells or the *dspA* mutant in at least one of the time points (0.5, 1, 3, and 6 h) following the transfer of cells to HL. The ratio of dspA to the wild-type (*dspA*/Wt) at time zero for each of the genes is also presented. Statistical significances of the expression patterns are indicated for each time point and for the *dspA*/Wt time zero ratio, boldface type indicates an increase in the transcript level, underlining indicates a decrease in the transcript level, and plain type indicates no significant change in the transcript level. The significance was computed by *t* tests (see Materials and Methods).

to HL and stayed low (*psaA*, *psaB*, *psaC*, *psaD*, *psaF*, *psaJ*, and *psaL*), while others didn't decline nearly as much or showed a transient decline (*psaE*, *psaI*, and *psaK*). For a number of PS I genes, the decline in the *dspA* mutant was not quite as severe as is it was in wild-type cells (e.g., *psaD*, *psaF*, and *psaJ*) (Table 3, compare the wild type and the *dspA* mutant).

Transcripts encoding polypeptides associated with chlorophyll biosynthesis are also in this category. Transcripts encoding two of the subunits of the light-independent prochlorophyllide reductase (*chlL* [*slr*0749] and *chlN* [*slr*0750]) were already reduced in the *dspA* mutant relative to wild-type cells (30 to 40%) in LL. While *chlL* and *chlN* transcripts declined markedly following the exposure of wild-type cells to HL (Table 2), they did not decline to nearly the same extent in the mutant (they were already low in the mutant strain in LL). The other genes in this category with characterized functions were *nrtB*

(*sll*1451), encoding a nitrate/nitrite permease; *crhR* (*slr*0083), encoding a RNA helicase; and *ho1* (*sll*1184), encoding a heme oxygenase.

Group 4: transcript that markedly declined in wild-type cells but increased in *dspA*. A small number of genes were clustered in group 4, which included genes encoding the pilin polypeptide (*hofG* or *pilA* [*sll*1694 and *sll*1695]), *psaD*, *rbpA* (*sll*0517), and *lexA* (*sll*1626) (Table 2).

Group 5: transcripts that declined slightly in wild-type cells but increased in *dspA*. The transcripts for most genes encoding constituents of the carbon-concentrating mechanism (CCM) (47) were slightly higher in the *dspA* mutant than in wild-type cells in LL conditions. Furthermore, the levels of most of these transcripts did not change much when wild-type cells were transferred to HL, although the *ccmM* and *ccmK* transcripts may have declined slightly. Interestingly, there was a relatively large transient increase in the levels of transcripts (four- to sixfold) from a number of the CCM genes (*ccmK* [*sll*1028 and *sll*0129], *ccmL*, *ccmM*, and *ccmN*) (Table 2) following exposure of the *dspA* mutant to HL, suggesting that the mutant cells were starved for CO_2 immediately following the transfer to HL. There was essentially no difference in the levels of *rbcL* and *rbcS* transcripts (and in transcripts encoding components of the reductive pentose phosphate pathway; data not shown) between wild-type cells and the *dspA* mutant grown in LL, and HL did not significantly affect the abundance of these transcripts in wild-type cells, although there may have been a small increase in *rbcL* and *rbcS* transcript levels following the exposure of *dspA* to HL. Furthermore, small but significant increases in the *prk* transcript (encoding phosphoribulokinase) were observed in both wild-type and mutant cells following HL exposure.

Group 6: transcripts that increased in both wild-type cells and *dspA*. There were a total of 69 genes whose transcript levels were increased significantly in both wild-type cells and the *dspA* mutant following the transfer from LL to HL. These genes included those with functions associated with photosynthesis (PS II, NADH dehydrogenase, and ATP synthase), HL and heat shock acclimation processes, and ribosome biosynthesis. Importantly, the transcripts from a number of these genes were already higher in the LL-grown *dspA* mutant relative to LL-grown wild-type cells. As presented in Tables 2 and 3, the level of the *psbA* transcripts (we were not able to distinguish between *psbAII* and *psbAIII* transcripts because they are nearly identical) was five- to sixfold higher in the mutant than in the wild-type strain grown under LL conditions. Similarly, the levels of *psbDI* and *psbDII* transcripts were 2.6- to 3.4-fold more in the *dspA* mutant than in wild-type cells grown in LL (Tables 2 and 3). Similar to the *psbA* genes, the *psbDI* and *psbDII* genes show strong sequence similarity, and substantial cross-hybridization of the cDNA probes to the *psbD* array elements almost surely occurred.

Upon the transfer of wild-type cells from LL to HL, the levels of transcripts for some PS II polypeptides showed a transient increase and then declined to near-LL levels (*psbDI* and *psbDII*) (Table 3), while others did not change very much. However, the *psbA* transcript levels went up sharply (approximately 5-fold) but declined to 2.5-fold of the LL level following a 6-h exposure to HL. Transient features of *psbA* transcript accumulation in cyanobacteria following HL exposure were previously described (24, 50). The changes in transcript levels for the *psbA* genes in the *dspA* mutant were not as pronounced as those in wild-type cells, and while there was some increase in *psbA* mRNA at early times following HL imposition (between 1.5- and 2-fold), the change was only transient, and the final level after 6 h in HL was similar to the level in LL (Table 3) (although, as already mentioned, the initial levels were much higher in the *dspA* mutant than in wild-type cells in LL).

Transcripts for all *hli* genes were also higher in the *dspA* mutant than in wild-type cells in LL, which had been previously observed from RNA blot hybridizations (42); the transcript for *hliC* showed the highest increase (approximately sevenfold) relative to wild-type cells (Table 3). Furthermore, there was a large transient increase in the levels of *hliB* and *hliC* transcripts in wild-type cells following 1 h of HL exposure (7- to 10-fold), with a subsequent decline; but the level still remained well above the LL level. The *dspA* mutant also exhibited elevated levels of *hli* transcripts following the exposure of cells to HL, although the relative increases were not as great as those observed for wild-type cells (Table 3).

Transcripts from many of the genes encoding subunits of the pyridine nucleotide dehydrogenase complex were elevated by 1.4- to 2.0-fold in the *dspA* mutant relative to wild-type cells grown in LL (Table 2). Furthermore, for most *ndh* genes, there was either no significant change in transcript levels or a transient change immediately following exposure of cells to HL (data not shown). The *ndhD2* (*slr*1291) transcript exhibited a relatively large transient change in wild-type cells at 30 min (3.6-fold) and 1 h (3.8-fold) after HL exposure (the transient change was greater in the mutant than in wild-type cells), but the level dropped to 1.78-fold the LL value after 6 h in HL (Table 2). The NdhD2 protein has been associated with the transfer of electrons between NADPH and the plastoquinone pool (61), which may be important for controlling cyclic electron flow and redox poise under HL conditions.

Transcripts encoding subunits of the ATP synthase and cytochrome b_6f complexes were generally slightly higher in the *dspA* mutant than in the wild-type strain in LL (data not shown). However, there were only small, transient effects of HL on levels of transcripts encoding polypeptides of these complexes, with the most significant influence on *atpD*, *atpG*, and *petF* transcripts, which exhibited approximately twofold transient increases.

There were pronounced changes in transcript levels for a number of stress-related genes following the exposure of both mutant and wild-type cells to HL; the transcripts for many of these increased in both mutant and wild-type cells exposed to HL (Table 2, group 6). The level of *groEL1* (*slr*2076), *groEL2* (*sll*0416), and *groES* (*slr*2075) transcripts were increased in both wild-type cells and the *dspA* mutant by six- to eightfold following HL imposition and were present at only slightly higher levels in the mutant strain during LL growth. The *hsp17* mRNA showed a twofold increase following HL exposure but was already high in the *dspA* mutant in LL (4.5-fold *dspA*/wild-type cell mRNA). One of the transcripts encoding glutathione peroxidase (*slr*1992) was also already elevated by about threefold in LL in the *dspA* mutant relative to the wild type. The mRNA for the heat shock protein HtpG (*sll*0430) increased three- to fourfold in both wild-type and mutant cells following HL exposure but was only slightly more abundant in the *dspA*

TABLE 4. Comparison of microarray and real-time RT-PCR data[a]

Gene	Test type	Fold change (SD) at time points following HL exposure							
		Wild type				*dspA* mutant			
		0.5 h	1 h	3 h	6 h	0.5 h	1 h	3 h	6 h
cpcB (*sll*1577)	Array	0.07 (0.01)	0.06 (0.013)	0.04 (0.07)	0.13 (0.023)	0.1 (0.04)	0.23 (0.09)	0.24 (0.06)	0.35 (0.144)
	PCR	0.02 (0)	0.017 (6E-04)	0.012 (0.001)	0.0431 (0.0055)	0.03 (0)	0.1 (0.01)	0.08 (0.01)	0.185 (0.063)
psbAII (*slr*1311)	Array	2.94 (0.44)	4.31 (1.295)	2.56 (0.062)	2.48 (0.948)	1.38 (0.58)	1.64 (0.75)	1.15 (0.26)	0.7 (0.24)
	PCR	5.79 (0.33)	9.278 (1.822)	7.476 (0.39)	3.4102 (0.6257)	0.79 (0.04)	1.35 (0.01)	0.62 (0.05)	0.388 (0.096)
pstS (*sll*0680)	Array	2.66 (0.03)	4.17 (0.53)	2.32 (0.07)	2.58 (0.36)	0.3 (0.04)	0.31 (0.04)	1.21 (0.19)	0.77 (0.23)
	PCR	6.66 (0.66)	11.04 (2.285)	9.257 (0.382)	6.8607 (1.9526)	0.18 (0.03)	0.18 (0.04)	0.7 (0.13)	0.794 (0.248)
groEl-1 (*slr*2076)	Array	4.45 (0.75)	7.38 (1.57)	2.66 (0.001)	2.4 (0.68)	7.65 (1.4)	5.28 (2.69)	5.93 (1.53)	2.21 (0.8)
	PCR	14.7 (1.58)	24.29 (7.31)	3.92 (0.72)	3.79 (0.05)	20.6 (5.55)	7.94 (1.58)	15.5 (4.82)	3.49 (0.243)
groEl-2 (*sll*0416)	Array	4.89 (0.15)	6.04 (0.68)	2.2 (0.33)	1.89 (0.42)	5.59 (2.17)	3.93 (1.06)	3.1 (1.5)	1.38 (0.8)
	PCR	12.2 (0.24)	15.96 (3.241)	3.42 (0.134)	4.48 (1.1331)	13.6 (3.27)	7.61 (0.12)	8.49 (3.12)	2.648 (0.293)
ccmK (*sll*1029)	Array	0.76 (0.02)	0.69 (0.048)	0.6 (0.011)	0.58 (0.075)	3.93 (0.94)	5.97 (1.83)	1.0 (0.2)	0.58 (0.19)
	PCR	0.92 (0.04)	0.378 (0.133)	0.357 (0.081)	0.2284 (0.0128)	1.66 (0.64)	5.57 (0.53)	0.19 (0.08)	0.103 (0.005)
psaA (*slr*1834)	Array	0.24 (0.02)	0.18 (0.034)	0.17 (0.007)	0.14 (0.016)	0.43 (0.03)	0.91 (0.12)	0.57 (0.09)	0.43 (0.139)
	PCR	0.05 (0.03)	0.005 (0.002)	0.002 (4E-04)	0.0054 (0.0023)	0.02 (0.01)	0.18 (0.07)	0.58 (0.58)	0.031 (0.003)
rps1B (*slr*1984)	PCR	0.9 (0.06)	1.142 (0.265)	1.149 (0.199)	1.0596 (0.2681)	0.96 (0.29)	0.97 (0.25)	1.43 (0.46)	1.128 (0.343)

[a] The levels of *cpcB*, *psbAII*, *pstS*, *groEL-1*, *groEL-2*, *ccmK*, and *psaA* transcripts in both wild-type cells and the *dspA* mutant were quantified by real-time RT-PCR (PCR) at each time point following HL exposure and compared to spot intensities generated from microarray (Array) analysis. For the real-time RT-PCR quantification, the *rps1b* (*slr*1984) transcript was used as an external standard since the level of this transcript did not change in either wild-type cells or the *dspA* mutant following HL exposure (under all conditions that we used). Quantitative real-time RT-PCR analysis of the *rps1b* transcript was repeated 10 times at each of the 5 time points (0, 0.5, 1, 3, and 6 h) following HL exposure, while the target genes were analyzed two times using independently isolated RNA samples. SD indicates the standard deviation from the mean.

mutant in LL. Most transcripts for the other *hsp* genes (including the *dnak*, *dnaJ*, and *clp* genes) were not dramatically affected under the conditions used for these experiments (data not shown).

A number of other transcripts increased in both wild-type cells and the *dspA* mutant following exposure to HL (Table 2, group 6); some of these transcripts exhibited large transient increases. These included transcripts encoding a glucanase (*slr*0897; 12-fold), phosphoglycerate kinase; phosphoribulokinase (*prk* [*slr*0394]; 9-fold), which is involved in both photosynthetic and respiratory metabolism; FabG (*sll*0330; 5.5-fold), which is involved in lipid metabolism; CheY (*slr*1982; 4.8-fold), a chemosensory protein; HlyD (*sll*1181; 6-fold), which is involved in secretory processes; and a number of hypothetical proteins encoded by genes of unknown function. Analyzing the activities and functions of the genes encoding hypothetical proteins are likely to unveil novel aspects of acclimation processes.

(iii) Analysis of transcript levels by real-time RT-PCR. We have used real-time RT-PCR to examine transcript levels in wild-type cells and the *dspA* mutant following the imposition of HL to help evaluate the microarray data. These analyses were performed for some genes whose transcripts increased and others whose transcripts declined following HL exposure. The results of these analyses are presented in Table 4. Similar to the microarray results, real-time RT-PCR showed that HL stimulated an increase in the levels of *groEL-1* and *groEL-2* transcripts; the highest levels in wild-type cells were observed 1 h after the imposition of HL. Similar trends were noted for the *dspA* mutant, although peak transcript levels were at 30 min (for both the microarray and real-time RT PCR analysis). Furthermore, similar to the *groEL* results, both microarray and real-time RT-PCR results showed that the *psbAIII* and *pstS* transcripts in wild-type cells accumulated to maximal levels after 1 h in HL, although the increase measured by real-time RT-PCR was two to three times that determined by microarray analysis. In contrast, the levels of these transcripts did not increase much in the *dspA* strain following the imposition of HL; this result probably reflects the fact that they are already high in the mutant strain prior to HL exposure. Finally, the *cpcB* and *psaA* transcripts generally declined in both wild-type and mutant cells (as measured by both methods), and the *ccmK* transcript declined in wild-type cells but showed a strong transient increase in the *dspA* mutant. Overall, the comparisons of real-time RT-PCR and microarray results were in excellent agreement with respect to the direction of change in transcript abundance for any given gene, while the extent of change determined by these two methods was often significantly different (note that the standard deviations for the real-time RT-PCR are relatively high).

DISCUSSION

Analysis of wild-type cells. Cells grow in environments that may rapidly fluctuate with respect to light, temperature, and nutrient availability. Changing environmental conditions will cause changes in the flux of electrons through the photosynthetic electron transport system and may also cause an imbalance in electron flow between the two photosystems, which either directly or indirectly stimulates a change in gene activity and the activities of specific protein complexes.

A number of groups have begun to examine the effects of light on levels of mRNAs in *Synechocystis* sp. strain PCC6803 (36, 43), as well as in other photosynthetic organisms such as *Chlamydomonas reinhardtii* (44) and *Arabidopsis thaliana* (49, 64), using both cDNA and oligonucleotide-based arrays. This "semiquantitative" technology has been validated with RNA blot hybridizations and real-time RT-PCR, as presented in this paper (and the companion paper [42]). We have been especially interested in how the photosynthetic machinery changes in response to changing light conditions. In cyanobacteria, the PS II/PS I ratio generally increases upon a shift from LL to HL

(37, 48, 57). It was suggested that diminished PS I content would be expected to lower the susceptibility of cells to HL damage, particularly during prolonged exposure. Many of the results obtained with wild-type cells in this study are congruent with this idea as well as with results obtained in other studies with *Synechocystis* sp. strain PCC6803, in which levels of various transcripts were analyzed following a transfer of cells from LL to HL. For example, both we an others have demonstrated that levels of transcripts encoding *psbAII*/*psbAIII* and *psbD* genes increase following the exposure of *Synechocystis* sp. strain PCC6803 to HL (13, 36, 42). Increased transcript production may reflect the need for more rapid synthesis of these proteins, especially D1, which is known to rapidly turn over under HL conditions. Levels of the *cpc*, *psaA*, and *psaB* transcripts declined, and levels of *apc* transcripts remained relatively constant following the transfer of cultures to HL conditions. This result suggests that both light-harvesting capacity and PS I activity are declining under the light conditions used in this experiment, which agrees with physiological and biochemical experiments (19, 20) as well as with molecular experiments (58) performed by others. Transcripts from other genes such as those belonging to the *pho* regulon, and especially those involved in phosphate transport, were shown to increase following the exposure of cells to HL. This was first observed in studies in which differential display was used to identify genes responsive to HL conditions (4). Differences in values and precise sets of genes modulated by HL conditions are likely to reflect differences in conditions (and strains) used for the experiments. For example, such differences are observed when our data are compared with that of Hihara et al. (36), with respect to some of the *ndh* genes (*ndhF3* and *ndhD3*), some of the *ccm* genes (*ccmM* and *ccmK*), and the *rbcS* and *rbcL* genes. The experiments presented in this paper result from a transfer of cells from 30 to 500 μmol of photon m^{-2} s^{-1}, while Hihara et al. transferred cells from 50 to 200 μmol of photon m^{-2} s^{-1} (36). It should be noted that even the HL levels used in the experiments presented in this study are not detrimental to cell growth (the cells maintain an optimal doubling time of ~6 h), which suggests that modulating components and activities of the photosynthetic apparatus (and other metabolic processes in the cell) compensates for the relatively high-intensity light conditions, limiting photodamage and its potential deleterious effects on cell growth. Higher light levels might result in more extreme changes in transcript levels and the activation of additional genes encoding chaperones and oxidative stress-related polypeptides.

Chaperones and heat shock proteins are also affected by light conditions. Heat shock proteins have been shown to bind unfolded polypeptides and to release them in an ATP-dependent reaction under stress condition such as those of high temperature and HL (32). These proteins may assist in the proper folding of nascent polypeptide chains as they emerge from ribosomes, participate in transmembrane protein transport, prevent aggregation of denatured proteins, and disassemble multiprotein complexes (32, 39). There are a number of genes in *Synechocystis* sp. strain PCC6803 that encode proteins homologous to heat shock proteins from other organisms (23, 46). Many of these genes were shown to be induced during both heat shock and HL exposure (36, 52a); these genes include *clpB*, *htpG*, *dnaK* (*sll*0170), *groEL-1*, *groEL-2*, *groES*, and *hsp17*. In our experiments, the genes for the chaperones *groEL-1*, *groEL-2*, and *groES* showed strong induction under HL stress; the transcripts for many of the other *hsp* genes did not increase to very high levels following the exposure of cells to HL. Although the actual function of GroEL or Hsp60 in *Synechocystis* sp. strain PCC6803 is not clear, recent studies have shown that Hsp70 of *C. reinhardtii* helps protect cells from photoinhibition; the overexpression of Hsp70 reduced photoinactivation of PS II and enhanced recovery from photoinhibition, while reduced expression caused the opposite effect (67). The role of the Hsp proteins in protection against photoinhibition had already been proposed based on the finding that the exposure of *C. reinhardtii* to heat shock resulted in enhanced resistance to light stress (68).

Analysis of mutant cells. We recently identified the *nblS* gene (nonbleaching mutant sensor), which is important for the acclimation of cyanobacteria to both HL and nutrient limitation conditions (77). This gene encodes a sensor molecule predicted to be integral to the membranes and contains a PAS domain that might bind a redox active molecule such as a flavin (this still needs to be clearly established). The homolog to *nblS* in *Synechocystis* sp. strain PCC6803, which is *dspA* or *hik33*, is important for *Synechocystis* sp. strain PCC6803 to acclimate to HL, nutrient limitation, cold, and osmotic conditions (42, 55, 58, 73, 77). NblS and DspA polypeptides may be global regulators that tune cellular metabolism to the growth potential (77).

In our initial studies of a *dspA* null mutant, we demonstrated abnormal regulation of several genes that respond to light intensity (42), and it was therefore important to understand how wild-type *Synechocystis* sp. strain PCC6803 responded to HL and the relationship between the HL responses observed in the mutant and wild-type strains. In this study, we have demonstrated that numerous transcripts were markedly different in abundance in wild-type *Synechocystis* sp. strain PCC6803 and the *dspA* mutant, even under our LL conditions. Importantly, many transcripts that accumulated in wild-type cells in HL were already high in the *dspA* strain in LL. Furthermore, a number of transcripts that declined in wild-type cells following exposure to HL were already low in LL-grown *dspA*. Such changes include increases in levels of transcripts encoding PsbAII/PsbAIII, PsbD, the Hli polypeptides, the HtpG and to some extent the GroEL/GroES chaperones and decreases in transcripts encoding the PC subunits and the associated linker proteins (CpcA, CpcB, CpcC, CpcD, and CpcG), the reaction center polypeptides of PS I (PsaA and PsaB), and polypeptides involved in chlorophyll biosynthesis (ChlL and ChlN); many of these changes were subsequently verified by real-time RT-PCR. Additionally, the *dspA* mutant showed increased levels of transcripts for some genes encoding polypeptides associated with oxidative stress, such as glutathione peroxidase. Finally, additional increases in at least some of these transcripts (e.g., *psbAII*/*III* and *hliA*, *hliB*, *hliC*, and *hliD*) were observed upon exposure of the *dspA* mutant to HL. The transcript accumulation data presented in this paper, along with the phenotypes determined for the *dspA* mutant (42, 58, 73, 77), demonstrate that the mutant strain is constitutively derepressed for many genes associated with stress conditions. The DspA protein may be directly or indirectly involved in negative regulation of stress-responsive genes or the *dspA* mutant may be experienc-

ing stress even under normal growth conditions. The responses of at least some of these genes that appear to be derepressed in the *dspA* mutant still show some response to HL (although the extent of the response might be reduced), suggesting that other mechanisms may also function to control the expression levels of these genes. However, the results from the studies in this paper do suggest that the DspA protein is likely to be a global regulatory element that either directly or indirectly controls numerous genes in the cell and may in fact be key for maintaining an appropriate homeostatic cellular milieu as environmental conditions fluctuate. Of course, there are transcripts whose abundances in wild-type cells and the *dspA* mutant cannot be easily explained by the hypothesis that the mutant is more sensitive to light levels. Therefore, it is likely that the DspA regulatory activity integrates with other regulatory systems to generate a final homeostatic output. It is important to understand how DspA might be sensing changing environmental conditions; the most obvious possibilities are that DspA monitors intracellular redox levels, the concentration of reactive oxygen species, and possibly even levels of blue or UV-A light (or a combinations of these factors) (77). Other sensors thought to participate in redox control of gene expression in *Synechocystis* sp. strain PCC6803 have recently been identified by Li and Sherman (52).

The *dspA* mutant appeared to be constitutively activated for many genes that are thought to be constituents of the *pho* regulon. These components include *pstS*, *pstC*, a gene encoding an alkaline phosphatase, and *nucH*. However, many of these genes are also activated under HL conditions. Interestingly, the sensory polypeptide SphS (*sll*0337) of *Synechocystis* sp. strain PCC6803 may contain a PAS domain (4), which has the potential to bind a chromophore and sense light and/or redox levels and coordinate light availability with the acquisition and assimilation of phosphate. Alternatively, it is possible that a major function of DspA is to manage the phosphate budget of the cell, and when this is not properly achieved in the mutant strain, the *pho* regulon is activated and many genes that are generally associated with stress and the absorption of excess excitation energy become active. Secondary effects may also cause the activation of some of the *ccm* genes when the mutant is exposed to HL (the mutant cannot take up enough for optimal CO_2 fixation). All of these results raise the question, what are the primary and secondary effects of deleting *dspA* in *Synechocystis* sp. strain PCC6803? It is clear that the phenotype of the mutant and its pattern of transcript accumulation strongly suggest that it is experiencing generalized stress responses that occur even under LL and high-nutrient conditions; these responses in many cases match the responses observed for wild-type cells transferred to HL. However, it is still not clear which genes are under the direct control of DspA as a consequence of its interactions with specific response regulators and exactly what factor(s) in the intracellular environment is being monitored by this sensory polypeptide.

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