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Control of Photosynthetic and High-Light-Responsive Genes by the Histidine Kinase DspA: Negative and Positive Regulation and Interactions between Signal Transduction Pathways

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We have deleted a gene for a sensor histidine kinase, *dspA* (or *hik33*), in the cyanobacterium *Synechocystis* sp. strain PCC6803. In low and moderate light, the mutant grew slowly under photoautotrophic conditions, with a doubling time of ~40 h, and had severely reduced photosynthetic oxygen evolution. When the mutant was maintained in low or moderate light in the presence of glucose, its growth rate was only somewhat lower than that of wild-type cells. However, the mutant was light sensitive and rapidly died in high light. Furthermore, levels of many transcripts encoding genes associated with photosynthesis were altered in the mutant relative to wild-type *Synechocystis* sp. strain PCC6803 both in low light and following exposure to high light. There was constitutive expression of several high-light-inducible genes, including *hli*, *psbAIII*, and *gpx2*; there was little increased accumulation of *sodB* mRNA in high light; and the cells failed to accumulate *cpcBA* and *psaAB* mRNAs in low light in the presence of glucose, although a normal decline in the levels of these mRNAs was observed during exposure to high light. These results suggest that DspA is involved in controlling sets of photosynthetic and high-light-responsive genes, either directly or indirectly. These and other results, some of which are presented in a companion paper (C.-J. Tu, J. Shrager, R. Burnap, B. L. Postier, and A. R. Grossman, J. Bacteriol. 186:3889–3902, 2004), suggest that DspA acts as a global regulator that helps coordinate cellular metabolism with growth limitations imposed by environmental conditions.

Photosynthetic organisms adjust their photosynthetic activity to balance the absorption of light energy with the energy and growth requirements of the cell. This coordination reduces the accumulation of potentially damaging reactive oxygen species that may result from the excitation of pigment molecules; reactive oxygen species may interact with proteins, lipids, and nucleic acids, ultimately causing a loss of cell viability, but they may also have a signaling role (3, 10, 20, 21, 25).

Both microalgae and vascular plants have evolved mechanisms for photoacclimation that enable them to tolerate the absorption of excess excitation energy (10, 24, 25, 31, 32). Acclimation mechanisms include, but are not limited to, changes in the composition of light-harvesting and/or reaction center pigment-protein complexes (4, 6, 7, 38), dissipation of excess absorbed excitation energy as heat, and synthesis of enzymes with antioxidant function, such as superoxide dismutase (26, 35), catalase (27, 29, 42), and peroxidases (11, 16, 40). Efficient degradation and repair of photodamaged polypeptides also occur when photosynthetic organisms are exposed to high light (HL). The D1 polypeptide of photosystem II (PS II), encoded by members of the *psbA* gene family, carries the reaction center P680 chlorophyll molecules. This protein represents the primary site of photodamage during photoinhibition (2, 18), and its turnover rate increases in HL. In *Synechocystis* sp. strain PCC6803, there are two active *psbA* genes, termed *psbAII* and *psbAIII*. The *psbAII* mRNA predominates in low light (LL), but there is a dramatic increase in the level of *psbAIII* mRNA and a slight increase in *psbAII* mRNA when *Synechocystis* sp. strain PCC6803 is exposed to HL (8).

Other cyanobacterial genes have been shown to be sensitive to light conditions and important for acclimation of cells to HL. The HliA protein of *Synechococcus* sp. strain PCC7942 is a small thylakoid membrane-associated polypeptide (72 amino acids) with sequence similarity to members of the chlorophyll *a*/*b*-binding family of proteins (9; N. A. Dolganov, Q. He, and A. R. Grossman, unpublished data). There are four *hli* genes present on the *Synechocystis* sp. strain PCC6803 genome, with a fifth sequence fused to the ferrochelatase gene (12, 15). The levels of Hli polypeptides increase in response to HL, low temperature, and nutrient limitation and are necessary for the survival of cells in HL. Strains unable to synthesize specific Hli polypeptides could not cope with HL as effectively as wild-type cells, and a mutant in which all four of the *hli* genes were deleted rapidly dies in HL (15). The Hli polypeptides may enable cyanobacterial cells to cope with excess absorbed excitation energy, possibly by promoting its dissipation as heat (14), or they may be important in controlling tetrapyrrole biosynthesis (and perhaps binding intermediates in this pathway) (39).

In photosynthetic microbes, acclimation responses are generally mediated by signaling systems comprised of transmembrane sensory kinases that sense extra- or intracellular cues and transmit the information to response regulators that may directly control the transcriptional activity of specific sets of genes. Recently, a sensory histidine kinase termed NblS, identified in *Synechococcus* sp. strain PCC7942, was shown to be

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critical for both acclimation to HL and nutrient limitation. This sensory kinase controls HL-regulated and blue or UV-A light-regulated expression from a number of genes whose polypeptide products are involved in photosynthetic function. NblS appears to influence light-dependent modulation of *hliA* gene expression, HL regulation of the *psbA* genes, and the biosynthesis and degradation of light-harvesting phycobilisome polypeptides. The deduced polypeptide sequence of NblS revealed the presence of a PAS domain that may bind a flavin (37). The association of NblS with a pigmented electron carrier (the flavin) may allow for direct monitoring of both light and intracellular redox conditions.

NblS of *Synechococcus* sp. strain PCC7942 has strong sequence similarity to DspA (also called Hik33) of *Synechocystis* sp. strain PCC 6803 (5, 34). Studies of the nonhomoplasmic *hik33* mutant showed that this polypeptide is involved in sensing and controlling gene expression in response to low-temperature conditions (33). Low-temperature treatment reduces the anabolic activity of cells, resulting in the absorption of excess excitation energy and elevated cellular redox, even under moderate light conditions. Recent studies suggest that Hik33 also controls the expression of osmostress-regulated genes (19). These findings add support to the hypothesized role of NblS as a global regulator that integrates redox and light signals and suggest that this regulatory polypeptide may influence other signaling pathways involved in acclimation responses (37).

Recently, under heterotrophic growth conditions, we have been able to obtain a homoplasmic strain disrupted for *dspA* or *hik33*. The data in this report suggest that DspA acts as a global regulator that helps coordinate cellular metabolism with growth limitations imposed by environmental conditions. Additional support for a global regulatory role for NblS/DspA/Hik33 comes from microarray analyses (36), which demonstrate pronounced changes in the levels of numerous transcripts in the *dspA* mutant, even under LL conditions.

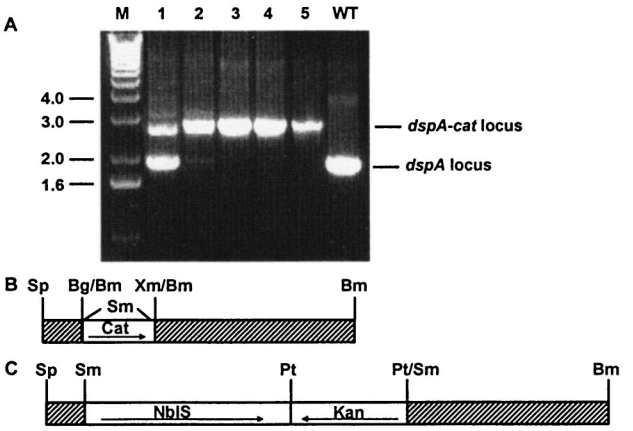

FIG. 1. Disruption of the *dspA* gene. (A) PCR analysis of the *dspA* gene in potential mutants in which the *dspA* gene was insertionally inactivated with the *cat* gene. WT, wild type; M, DNA size marker. (B and C) Depiction of the constructs used to generate the *dspA-cat* strain (B) and the *nblS*$^+$ *dspA* mutant strain (C). To generate the *dspA-cat* disruption, an internal 29-bp *dspA* fragment from the *dspA* gene (from Sp to Bm; shaded area) was deleted and replaced by a 0.85-kbp chloramphenicol resistance cartridge (Cat); in panel C, the *cat* gene shown in panel B was replaced by a 2.2-kb *nblS* gene linked with a 1.2-kb kanamycin resistance cartridge (Kan); the *nblS* and *kan*^{r} genes are transcribed in opposite directions. In panel A, both the intact *dspA* (~2.0 kbp) and the *dspA-cat* (~2.8 kbp) loci were efficiently amplified from genomic DNA preparations by PCR. A total of 5 chloramphenicol-resistant transformants were isolated (lanes 1 to 5) and analyzed for a disruption of the *dspA* gene; transformants 3 and 4 showed no detectable wild-type *dspA* PCR product, and transformant 4 was used in all subsequent analyses. Sp, SphI; Bg, BglII; Bm, BamHI; Xm, XmnI; Sm, SmaI; Pt, PstI.

MATERIALS AND METHODS

Culture conditions. *Synechocystis* sp. strain PCC6803 was cultivated in BG-11 medium (1) buffered with 10 mM TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid], pH 8.2, at 30°C. The cultures were bubbled with 3% CO_2 in air under both LL (40 μmol of photon m^{-2} s^{-1}) and HL (500 μmol of photon m^{-2} s^{-1}) conditions. For HL treatments, cells grown to mid-logarithmic growth phase (optical density at 730 nm [OD_{730}] of ~0.8) were diluted with fresh medium to an OD_{730} of ~0.3, and then the cultures (~35 ml in 50-ml culture tubes) were placed in a temperature-controlled glass chamber (maintained at 30°C) and exposed to HL (incandescent bulbs) for various lengths of time, as indicated in the text.

DNA manipulation and mutant construction. To inactivate the *dspA* (*hik33*) gene *sll*0698 in CyanoBase (34), the entire coding region was amplified by PCR to yield a fragment of 1992 bp. The two primers used for the amplification were 5′-gc(TT)ATGc(G)GGACTTCTGTGTCCAATCCA-3′ and 5′-gg(CT)At(G)C CCACCACCATCAACATGGATTG-3′. Lowercase letters indicate mutations introduced into the sequence, with the original nucleotides given in parentheses to the left of those nucleotides that were modified. The PCR product was cloned into pGEM-T vector (TA ligation), and a 0.85-kbp DNA fragment with the *cat* gene (chloramphenicol acetyltransferase), under the control of the *Synechocystis* sp. strain PCC6803 *psbAII* promoter, was exchanged with a 29-bp fragment (335 to 364 bp downstream of the *dspA* start codon, from a BglII site to an XmnI site) of the *dspA* gene, generating the plasmid pDsp-cat. The direction of transcription of the *cat* gene was opposite of that of *dspA*. The plasmid was partially sequenced to determine the relative orientation of the *dspA* and *cat* genes and to ensure that no modifications in the nucleotide sequence occurred during cloning.

The pDsp-cat plasmid was used to transform *Synechocystis* sp. strain PCC6803, and transformants were selected by screening for resistance to 20 μg of chloramphenicol/ml in BG-11 medium supplemented with 10 mM glucose. Transformants were restreaked into the same medium, and segregation of the inactivated *dspA* gene was monitored by PCR using genomic DNA of transformants used as the template and primers that recognize sequences upstream or downstream of the inserted *cat* gene. The homoplasmic mutant obtained was designated *dspA-cat*.

To test complementation of the *Synechocystis* sp. strain PCC6803 *dspA-cat* mutant with the *nblS* gene from *Synechococcus* sp. strain PCC7942, the *cat* cartridge of the plasmid pDsp-cat was replaced by the *nblS* gene (under the control of its own promoter) linked to a kanamycin resistance cassette, generating a plasmid that we designated pNblSrDspA. The *nblS* gene was in the same reading direction as that of *dspA*. pNblSrDspA was transformed into the *dspA-cat* mutant, and transformants were repeatedly streaked onto BG-11 medium containing 25 μg of kanamycin/ml until the chloramphenicol resistance phenotype was lost. In the new strain (*nblS*$^+$ *dspA* mutant), the *nblS* gene was nested within the *dspA* gene (Fig. 1).

For PCR analysis, *Synechocystis* sp. strain PCC6803 genomic DNA was prepared by a mini preparation procedure. Briefly, a loopful of cyanobacterial cells was suspended in 200 μl of Tris-EDTA buffer (pH 8.0) and transferred to a microcentrifuge tube with 200 μl of glass beads (0.1-mm diameter; Sigma). The cells were broken in a MiniBead Beater (Biospec Products, Bartlesville, Okla.) by two cycles of agitation at the low speed setting for 30 s; between each cycle, the cells were cooled on ice for 2 to 3 min. Lysates were extracted with phenol-chloroform, and the DNA was precipitated, washed, and dried according to standard procedures (28). The dry DNA pellet was dissolved in 40 μl of H_2O, of which 1 μl was used for each PCR.

Photosynthetic O_2 evolution. O_2 exchange in cell suspensions was measured at 30°C with a Clark-type O_2 electrode (Hansatech DW2/2). Cells were illuminated with white light produced by a Schott KL1500 lamp equipped with a flexible light guide. The intensity of illumination was adjusted with combinations of neutral density filters. Cell viability was monitored by using the vital stain TO-PRO-1 iodine (see the legend to Fig. 2).

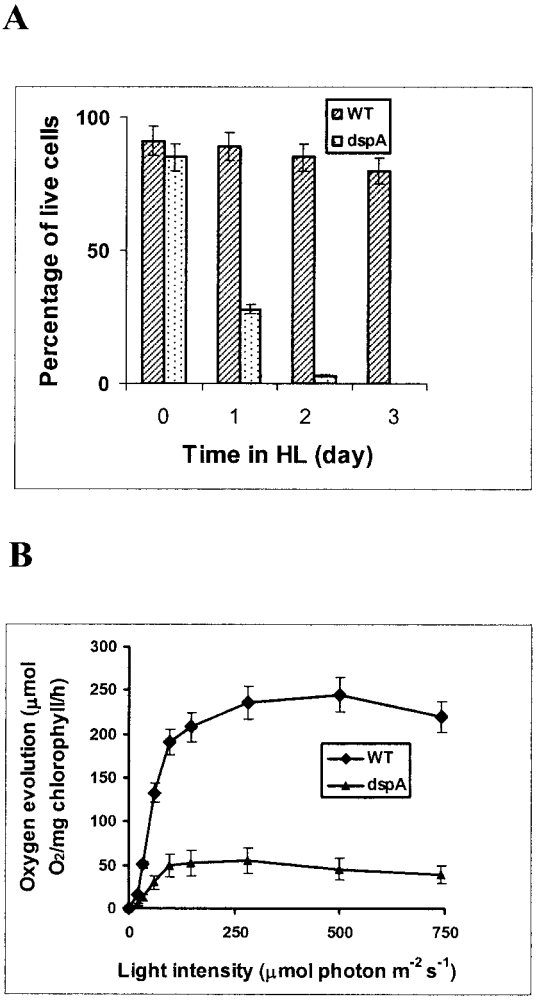

FIG. 2. Light sensitivity of the *dspA* mutant. (A) Cells were grown in BG-11 medium with 10 mM glucose in LL to an OD_{730} of ~0.3 (day 0) before the cultures were transferred and incubated in HL (days 1 to 3). Cell viability was determined by staining with TO-PRO-1 iodine, a nonpermeable fluorescence dye that stains nucleic acid (only in dead cells). Curves were generated by averaging the data obtained from three representative experiments. (B) Light-response curves of photosynthetic O_2 evolution in wild-type *Synechocystis* sp. strain PCC6803 (WT) and *dspA-cat* mutant (dspA). Cells were grown in LL in the presence of 10 mM glucose to an OD_{730} of ~0.6. Cultures were concentrated to a OD_{730} of 0.9 for O_2 evolution measurements under various actinic light intensities.

RNA isolation and Northern hybridizations. RNA was isolated from cells as previously described (22). For RNA blot hybridizations, equal amounts of RNA (determined spectroscopically) were resolved by electrophoresis in formaldehyde gels; ethidium bromide was included in the loading buffer, allowing for visualization of the rRNA bands and confirmation of equal loading of RNA samples. The DNA probes used for hybridization were prepared by PCR in a 15-μl reaction mixture containing PCR buffer, 2 U of *Taq* DNA polymerase (Roche, Palo Alto, Calif.), 0.1 μg each of the two PCR primers, 30 μCi of [^{32}P]dCTP (3,000 Ci/mmol, 10 μCi/μl; NEN-DuPont, Boston, Mass.), and ~10 ng of gene-specific PCR products as a template. The gene-specific PCR products were originally generated from plasmid clones or from genomic DNA by two cycles of PCR. In the first cycle, a larger fragment was amplified, which was purified from agarose gels and used as the template for a second PCR to generate gene-specific probes. The second PCR cycle employed either one or two new internal primers, and the product was purified from agarose gels. The different probes generated and primers used for amplification of these probes are given in Table 1.

RESULTS

Impacts on cell viability and photosynthetic function. To investigate the functions of DspA, we inactivated the *dspA* gene in *Synechocystis* sp. strain PCC6803 (see Materials and Methods). The construct (pDsp-cat) used to generate the *dspA*-disrupted strain is shown in Fig. 1B. A homoplasmic mutant was obtained, as evaluated by PCR analysis (Fig. 1A, lanes 3 to 5). The growth of the *dspA* mutant under photoautotrophic and photomixotrophic conditions was monitored in LL and HL (data not shown). The *dspA* mutant grew very slowly, with a doubling time of ~40 h under photoautotrophic growth conditions (in contrast to ~8 h for wild-type cells); however, it grew well, although not as well as wild-type cells, in medium supplemented with glucose; the doubling time of wild-type cells under our photomixotrophic growth conditions was 6 h, while that of the mutant was 8 h. In contrast, when the *dspA* mutant was placed in HL for more than 1 day in either the presence (Fig. 2A) or absence (data not shown) of glucose, it stopped dividing and lost viability, as measured by both the replating of cells onto solid BG-11 medium with 10 mM glucose (unpublished data) and cell viability assays with vital stains (Fig. 2A); by 48 h in HL, nearly all of the cells were dead. These results demonstrated that glucose cannot sustain the *dspA* mutant in HL and that photodamage in HL resulted in a loss of cell viability. The cells also died much more rapidly than wild-type cells following exposure to nutrient deprivation (unpublished data).

Photosynthetic O_2 evolution in the wild-type and mutant strains was measured as a function of light intensity (Fig. 2B). For wild-type cells, O_2 evolution saturated at ~250 μmol of photon m^{-2} s^{-1} with a peak activity of ~250 μmol of O_2/mg of chlorophyll/h. In contrast, the photosynthetic efficiency in LL was reduced in the mutant relative to the wild-type strain, and the maximum rate of O_2 evolution attained by the mutant was 50 μmol of O_2/mg of chlorophyll/h. These results demonstrate that the *dspA* lesion caused a marked decline in photosynthetic performance and explain why a homoplasmic strain disrupted for *dspA* was difficult to obtain under photoautotrophic growth conditions, even using antibiotic selection (34). In our study, complete segregation was made possible by the supplementation of the antibiotic-containing growth medium with 10 mM glucose.

Analyses of transcripts for photosynthetic and stress-responsive genes. The growth data and measurements of O_2 evolution demonstrated that the *dspA* mutant was severely compromised in photosynthetic function and was unable to acclimate to HL conditions. To further characterize responses of the mutant to HL, we measured levels of transcripts from the various genes involved in photosynthesis and in the amelioration of oxidative stress. Total RNA from cells grown in LL or 30 min following the transfer of cells to HL was hybridized to specific fragments of genes encoding polypeptides of the photosynthetic apparatus or associated with oxidative stress responses. As shown in Fig. 3 (columns 1 and 2), in wild-type cells, the transcripts for all four of the *hli* genes and for one of the two *gpx* genes (encoding glutathione peroxidase) accumulated to high levels following the transfer of cells to HL. Furthermore, total *psbA* mRNA increased by more than threefold

TABLE 1. Sequences of primers used for generating gene-specific probes[a]

Gene	Template	Primer	Position on genome
hliA	Plasmid	TAATCCAATTATGACCACCCG	701360–701340
		CTCGGCCTATTCTACAGGCTA	701127–701147
hliB	Plasmid	CAACACTATGACTAGCCGC	982961–982979
		ACCCAGCCAATTAGAGAGAG	983190–983171
hliC	Plasmid	ACAGACTTGCCATGGGCGCAA	1142026–1142006
		CTATGGAAAAATTACAGAATGC	1141792–1141813
hliD	Plasmid	GGAAATCCCATGAGTGAAGAAC	398179–398200
		ACTCCCTAGCGCAGTCCCAACC	398366–398345
psbA	Plasmid	ACCATTGCCGTTGCCGGTAAAG	943222–943243
		GGTGGTAACAATACCTCCGAGG	943902–943881
gpx2	PCR	ATGCCATTACCCACTTCCCTGAC	1436699–1436721
		TTAGCCCAAGGCTTTTTCGATCG	1437163–1437141
		CGGCTTAACCCCCCAATAC	1436800–1436818
		TTAGCCCAAGGCTTTTTCGATCG	1437163–1437141
sodB	PCR	ATGGCTTACGCACTAC	1607353–1607373
		CTAACCTAGGCCGCTGCTAAGTTAGC	1607952–1607932
		CAAACACCATGCCGCCTAC	1607439–1607457
		AGCATGCTCCCATACGTCC	1607850–1607832
cpcBA	Plasmid	GTTTCCACCGCTGACTCTCAAG	726813–726792
		CTAGCTCAGAGCATTGATGGCG	726349–726370
psaAB	Plasmid	CCATTGCCGTTGCCGGTAAAGTC	943223–943245
		GTGGTAACAATACCTCCGAGGAG	943901–943879

[a] The nucleotide sequences of the primers used for amplification of the gene-specific probes are given, and the positions of these primer sequences on the *Synechocystis* sp. strain PCC6803 genome are defined by the base numbers provided by CyanoBase (http://www.kazusa.or.jp/cyanobase/) (right-hand column). The templates used for amplification were either plasmids containing full-length genes or second-round PCR products purified from agarose gels. The *hliA* and *hliB* DNA fragments used as probes are 95% identical. Transcripts from these two genes were distinguishable based on their size difference. The *psbA* probe was prepared by using a plasmid containing the full-length *psbAII* gene; it could hybridize to all three of the potential *psbA* transcripts of *Synechocystis* sp. strain PCC6803.

following 30 min of HL treatment, consistent with results previously reported (13). The transcript for the *sodB* gene, encoding superoxide dismutase, increased by two- to threefold following HL treatment, with the generation of a high-molecular-mass transcript (the ends of these transcripts have not been characterized). In contrast, levels of *cpcBA* and *psaAB* transcripts declined, in agreement with the observations of Muramatsu and Hihara (23).

In the *dspA* mutant, as in wild-type cells, transcripts from *hli*, *psbA*, and *gpx2* genes accumulated to high levels following 30 min of HL treatment. However, levels of these mRNAs were already relatively high under LL conditions. In addition, the *sodB* transcript in the mutant strain no longer accumulated to very high levels in HL, while the HL-induced decline of *cpcBA* and *psaAB* transcripts was similar to that observed for wild-type cells.

Interestingly, wild-type cells grown for 1 to 2 days in LL in medium supplemented with 10 mM glucose showed a marked accumulation of *cpcBA* and *psaAB* transcripts; this elevated accumulation was not observed in the *dspA* mutant. The transcript levels of other genes shown in Fig. 3 were not strongly affected by the addition of glucose to the growth medium.

Complementation of the *dspA* mutant with the *nblS* gene from *Synechococcus* sp. strain PCC7942. Expression of *hli* genes in the *dspA* mutant (as shown in Fig. 3) was different from that observed in the *nblS1-1* mutant of *Synechococcus* sp. strain PCC7942, although it should be remembered that the latter strain contains two point mutations in *nblS*, and we were never able to generate a null mutation. To investigate whether DspA is functionally related to NblS, the full-length *nblS* gene of *Synechococcus* sp. strain PCC7942 was transformed into the *dspA* mutant of *Synechocystis* sp. strain PCC6803 (see Materials and Methods for details); in this strain, the *nblS* gene was nested within the *dspA* gene containing an internal deletion of 29 bp (Fig. 1C). As shown in Fig. 4, the *nblS*-harboring transformant regained the ability to suppress accumulation of *hli* transcripts under LL conditions (just like wild-type cells), although the levels of mRNA accumulation in HL appeared to be slightly lower than those observed for wild-type cells. Furthermore, the rescued mutant survived HL conditions and grew at a rate similar to that of wild-type cells in both LL and HL (data not shown). These results strongly suggest that DspA and NblS are functional orthologs.

Stability of transcripts. Since the absolute levels of specific transcripts are governed by a combination of the rates of transcription and transcript degradation, we investigated whether mRNA stability contributed to differential accumulation of transcripts observed in the *dspA* mutant under LL and HL conditions. LL-grown cells were exposed to HL for 30 min before rifampin (150 μg/ml) was added to cultures to block transcription. Cultures were then divided and incubated in either LL or HL for an additional 60 min. Aliquots were removed at various time points following the administration of rifampin, and RNA levels were monitored by RNA blot hybridizations. As shown in Fig. 5, a disruption of the *dspA* gene did not strongly influence the rate of transcript degradation under LL or HL conditions. The *hliA* messages appeared to be slightly more stable in the *dspA* mutant than in wild-type cells, but the differences were not very large. Furthermore, glucose did not significantly influence the stability of any of the transcripts examined (data not shown). The half-lives of the transcripts deduced from Fig. 5, under both LL and HL conditions, are presented in parentheses in the figure.

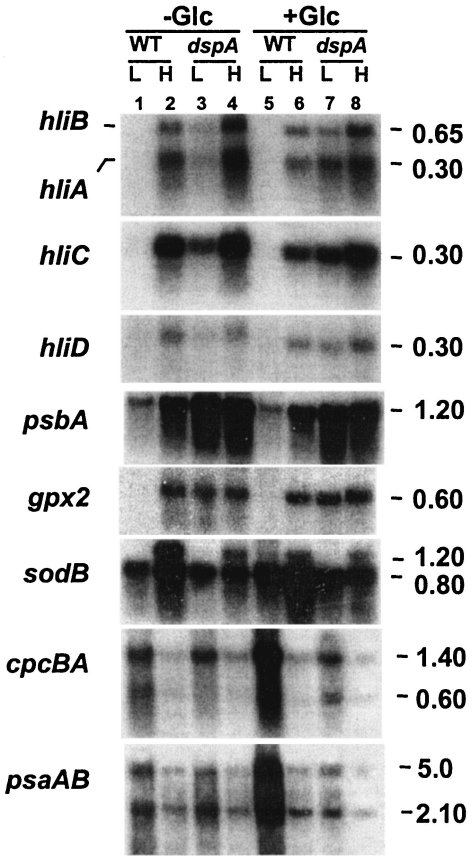

FIG. 3. RNA blot hybridizations with specific probes for genes involved in photosynthesis and stress responses. Total RNA was isolated from cells grown in LL (L) or exposed to HL (H) for 30 min. The RNA was resolved by denaturing electrophoresis on 1.3% agarose gels, transferred to nylon membranes, and hybridized with the labeled probes indicated. The three *psbA* genes were not distinguished in these analyses. The *sodB* gene fragment consistently hybridized to two transcripts (bands), the lower band having the size predicted from the CyanoBase gene sequence. The upper band may have a different transcription start site or represent a read-through product, but this possibility was not investigated. The *psaAB* probe hybridized to two transcripts as previously reported (30). The *cpcBA* fragment also hybridized to two transcripts; the signal from the 1.4-kb transcript represents the full-length transcript of *cpcBA*. The size of each transcript is indicated. *gpx2* (*slr*1992), glutathione peroxidase; −Glc, without glucose in the growth medium; +Glc, with 10 mM glucose in the growth medium; WT, wild-type cells.

DISCUSSION

Our results demonstrate that the loss of DspA function strongly impacts photosynthetic performance and survival of the cells in HL. This finding may be a consequence of aberrant assembly of the photosynthetic apparatus in the mutant strain, which leads to increased photodamage and the production of reactive oxygen species, especially under HL conditions. Furthermore, there was a high level of accumulation of the *hli*, *psbA*, and *gpx2* transcripts in the *dspA* mutant but not in wild-type cells grown in LL, regardless of glucose supplementation. Constitutive expression of *hli*, *psbA*, and *gpx2* genes suggests that these genes are repressed by DspA activity in LL and that HL activation involves removing the repression imposed by DspA (either by direct or indirect effects on gene activity). While this is the first report that suggests that the HL activation of bacterial genes may involve, at least in part, derepression, negative regulation of stress-responsive genes has been reported for both prokaryotic and eukaryotic systems. For example, the catalase gene (*katG*) of *Mycobacterium tuberculosis* is negatively regulated by the global ferric uptake regulator FurA (41), and ethylene responses in vascular plants are negatively regulated by a family of receptors encoded by *ETR* genes (17).

Interestingly, in the *dspA* mutant, the *hli* transcripts still accumulated to a significantly higher level in HL than in LL (Fig. 3). This enhanced accumulation may be due to differential mRNA stability, as the *hli* transcripts are slightly more stable in HL than in LL, regardless of whether or not *dspA* is functional (Fig. 5). However, it is also possible that the *hli* genes are subject to a positive regulation in addition to the negative control imposed by DspA. The convergence of a potential negative and a positive regulation in controlling *hli* genes resembles what has been observed in the regulation of the *katG* gene in *M. tuberculosis* (41). Furthermore, in contrast to the negative regulation of the *hli* and *psbA* genes by DspA, *sodB* expression appears to be positively regulated by DspA. These results demonstrate that DspA has both a positive and a negative impact on the accumulation of different transcripts.

Another interesting finding of this study is that the transcript levels of *cpcBA* and *psaAB* dramatically increase if glucose is added to the growth medium of wild-type cells growing in LL

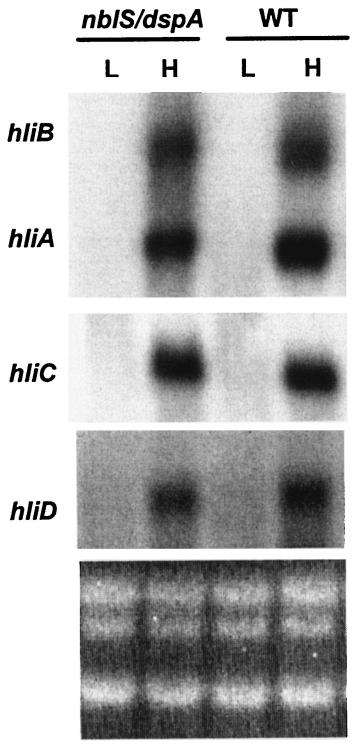

FIG. 4. Expression of *hli* genes in the *dspA-cat* mutant complemented with the *nblS* gene (*nblS*/*dspA*). Total RNA was isolated from cells grown in LL (L) or exposed to HL (H) for 30 min. The RNA was hybridized with the fragments from the four *hli* genes, as indicated. WT, wild type.

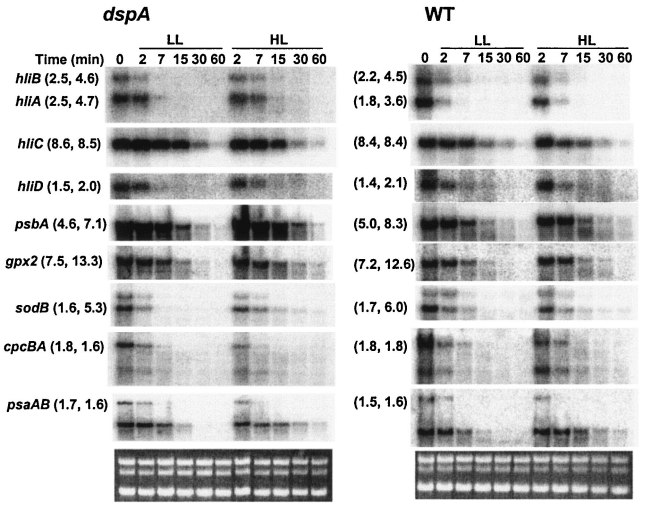

FIG. 5. Transcript stability in wild-type cells (WT) and the *dspA-cat* mutant (*dspA*). The cells were exposed to HL for 30 min followed by the addition of 150 μg of rifampin/ml (transcription inhibitor) immediately after placing half of the culture in LL and the other half in HL; the addition of rifampin occurred at time zero. Aliquots of the cultures were withdrawn at the times indicated at the top of the figure, and the mRNA levels were determined by RNA blot hybridizations. The number pairs in the parentheses indicate the estimated mRNA half-life of each gene under LL (numbers at left within each set of parentheses) and HL (numbers at right within each set of parentheses) in the *dspA-cat* mutant (parentheses at the left side of the figure) and in the wild type (parentheses in the center of the figure). To estimate the mRNA half-life of a messenger, the intensities of the specific hybridization signals on the autoradiograms were measured by densitometry, and the half-life of each transcript was calculated. The half-lives of the *cpcBA* and *psaAB* transcripts were determined from the loss of full-length transcripts following the addition of rifampin, while the half-life of *sodB* was estimated from the loss of the transcript, represented by the lower hybridization signal (which is the expected size of the transcript based on the gene sequences; the upper transcript might be a consequence of a second transcription initiation or termination site).

and that this effect is dependent on DspA. The increase was sustained for 1 to 2 days after adding glucose to the medium and resulted in a ~25% increase in the corresponding polypeptides (data not shown) over the time period. These findings suggest an interaction (either direct or indirect) between the sugar and DspA signaling pathways.

In sum, the results presented in this paper demonstrate that DspA profoundly influences the growth characteristics and photosynthetic capacity of *Synechocystis* sp. strain PCC6803, that it is required for survival of cyanobacterial cells in HL, and that it appears to be functionally equivalent to NblS of *Synechococcus* sp. strain PCC7942 (which is critical for the acclimation of cells to both HL and nutrient limitation conditions). The results also suggest that DspA or NblS polypeptides may elicit both negative and positive regulatory responses under a variety of conditions and that they may also influence glucose signaling and, potentially, the integration of respiratory and photosynthetic function. Combined with previous results (34, 37), the data reported here suggest that DspA or NblS plays a central role in integrating cellular metabolism with environmental cues (and the potential for cell growth). While a direct regulatory relationship between DspA and potential target genes cannot be clearly established, the inability of the *nblS1-1* allele to derepress (or activate) *hli* or *psbA* genes suggests a primary role for DspA in controlling the expression of at least some of the HL-activated genes. The precise environmental cues that NblS or DspA senses are not clear; however, it is likely that these polypeptides are able to perceive light, redox, and/or reactive oxygen species and that their signaling outputs are integrated into a regulatory web that controls the activities of numerous cyanobacterial genes (36).

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