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Role of Sigma Factors in Controlling Global Gene Expression in Light/Dark Transitions in the Cyanobacterium *Synechocystis* sp. Strain PCC 6803▽†

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We report on differential gene expression in the cyanobacterium *Synechocystis* sp. strain PCC 6803 after light-dark transitions in wild-type, Δ*sigB*, and Δ*sigD* strains. We also studied the effect of day length in the presence of glucose on a Δ*sigB* Δ*sigE* mutant. Our results indicated that the absence of SigB or SigD predominately altered gene expression in the dark or in the light, respectively. In the light, approximately 350 genes displayed transcript levels in the Δ*sigD* strain that were different from those of the wild type, with over 200 of these up-regulated in the mutant. In the dark, removal of SigB altered more than 150 genes, and the levels of 136 of these were increased in the mutant compared to those in the wild type. The removal of both SigB and SigE had a major impact on gene expression under mixotrophic growth conditions and resulted in the inability of cells to grow in the presence of glucose with 8-h light and 16-h dark cycles. Our results indicated the importance of group II σ factors in the global regulation of transcription in this organism and are best explained by using the σ cycle paradigm with the stochastic release model described previously (R. A. Mooney, S. A. Darst, and R. Landick, Mol. Cell 20:335–345, 2005). We combined our results with the total protein levels of the σ factors in the light and dark as calculated previously (S. Imamura, S. Yoshihara, S. Nakano, N. Shiozaki, A. Yamada, K. Tanaka, H. Takahashi, M. Asayama, and M. Shirai, J. Mol. Biol. 325:857–872, 2003; S. Imamura, M. Asayama, H. Takahashi, K. Tanaka, H. Takahashi, and M. Shirai, FEBS Lett. 554:357–362, 2003). Thus, we concluded that the control of global transcription is based on the amount of the various σ factors present and able to bind RNA polymerase.

Cyanobacteria represent a diverse group of organisms that occupy habitats from oceans to lakes to soil. They are forced to deal with many environmental changes, and the impact of many such stresses has been investigated for various strains. One environmental factor that affects cyanobacteria each day in every habitat is the changing light conditions. The growth characteristics of cyanobacteria and the response of the transcriptional machinery to different light conditions have been studied in some detail (1–4, 10, 14, 24).

The effort to understand how light affects the transcription of cyanobacterial genes, especially those encoding photosynthesis proteins, includes a number of microarray studies that described differential transcription based on acclimation to high levels of light (14, 15), response to UV-B and white light (16), and light-to-dark transitions (10). More recently, Muramatsu and Hihara (33, 34) have studied the high-light-responsive promoter of the *psaAB* operon as a follow up to a high-light microarray experiment (14). They concluded that the coordinated high-light response of the photosystem I (PSI) genes is achieved by AT-rich upstream sequences in *Synechocystis* sp. strain PCC 6803 (34). The experiments performed by Gill et al. (10) were closest to those to be discussed in this paper, since they also put wild-type cultures through light-to-dark and dark-to-light transitions. However, that study did not include mutants in any of the key transcriptional factors, as will be described herein.

The presence of multiple sigma factors represents one mechanism of regulating gene expression. Similar to *Escherichia coli*, cyanobacteria such as *Synechocystis* sp. strain PCC 6803 have an RNA polymerase (RNAP) that is able to bind different σ factors; these subunits specify transcriptional initiation at appropriate promoters. *Synechocystis* sp. strain PCC 6803 has an essential group 1 principal σ factor and four group 2 σ factors (see the Cyanobase at http://www.kazusa.or.jp/cyano/cyano.html) (21). The group 2 σ factors (SigB, SigC, SigD, and SigE) are similar to the principal SigA in sequence and structure but are nonessential for any known condition (12, 32). Different group 2 σ factors have been shown to modulate gene expression under different conditions. For example, the σ factor, SigB, mediates transcriptional responses after exposure to heat shock and upon entering stationary phase (9, 19, 44). Both SigB and SigC are up-regulated in stationary phase and exhibit regulation of gene expression under low-nitrogen conditions (3, 18). SigE is involved in the response to nitrogen depletion as well as being a positive regulator of sugar catabolism (35). The group 2 σ factors also respond to light/dark stimuli; e.g., SigB and SigD have demonstrated antagonistic dark/light-induced expression via changes in redox potential and SigE was shown to accumulate in the light but did so more slowly than SigD (17).

Studies have indicated extensive regulation between the σ factors that are specific to particular growth conditions. For example, SigB and SigC were able to regulate each other's

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transcriptional levels but with somewhat different effects in exponential- or stationary-phase conditions (18). Additionally, Imamura et al. (17) showed that SigC down-regulated SigB on transition from dark to light. Recently, Yoshimura et al. (50) indicated that SigD and SigE also negatively regulated SigB in the light. Cross-talk among group 2 σ factors has been shown at the transcript level. For example, the work of Lemeille et al. (25) included nitrogen deprivation and long-term growth as experimental conditions, and they suggested a network of transcriptional interactions between the group 2 σ factors. The recent work of Matsui et al. (27) indicated that the group 3 sigma factors may play a role in these interactions. Importantly, Imamura et al. (19) purified all group 1 and group 2 σ factors and determined their intracellular levels under steady-state growth conditions, as well as their growth-phase-dependent changes. We will relate the levels of the σ factor proteins to the changes we find in global gene expression when individual σ factors are no longer present in the cell.

The process of transcriptional initiation and elongation was described as a σ cycle in which σ associates with the RNAP to initiate transcription and then dissociates after the formation of a stable elongation complex (7, 45). The polymerase can bind another σ factor and may be reprogrammed by different σ factors in each round of transcription. There is a great deal of evidence to support this σ cycle paradigm, but the initial model did not take into account interactions with multiple σ factors involved with global regulation. Thus, a number of versions of the σ cycle model have been proposed that try to account for the binding-dissociation-rebinding of σ factors to RNAP (6, 11, 31, 37, 40). Six different models were reviewed by Mooney et al. (30), and they compared the models using recent experimental evidence. They concluded that the stochastic release model best conforms to both the in vitro and in vivo data. In this model, the affinity of the σ factor for the RNAP decreases significantly after promoter release, rather than σ release at a specific stage of elongation, as suggested by the obligate release model. Furthermore, in the stochastic release model, the dissociation of the σ factor from the RNAP may be altered by factors including concentration of the RNAP core and other σ factors. The authors suggest that the main postulate of the σ cycle, wherein σ factors compete for binding to the polymerase after each round of mRNA synthesis, is still the operative model for transcriptional initiation in bacteria. This is consistent with competition between the σ factors, such that elevated levels of alternative σ factors are able to direct transcription to specific promoters and maintain the cell's ability to respond rapidly to environmental fluctuations.

In this report, we describe the differential transcription of *Synechocystis* sp. strain PCC 6803 as cells transition from dark to light in the morning and from light to dark in the evening. We also performed similar experiments using mutations in *sigB* and *sigD* to investigate the roles of these σ factors at light/dark transitions. In addition, we examined autotrophic and mixotrophic growth of strains lacking combinations of SigB, SigD, and SigE to determine the physiological importance of these subunits. Finally, we analyzed the impact of light/dark transitions when cells are growing mixotrophically in the presence of glucose using the wild-type strain and a strain lacking SigB and SigE. We present a hypothesis that combines our data on transcriptional regulation with the relative amounts of σ factors as determined by Imamura et al. (17, 19) and that provides a simple, yet comprehensive, model of gene regulation as cells progress through their diurnal cycle.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The glucose-tolerant strain *Synechocystis* sp. strain PCC 6803 was used throughout this study (48). Cultures were grown at 30 ± 2°C using cool white fluorescent light at an intensity of ~30 $\mu E \cdot m^{-2} \cdot s^{-1}$, with shaking at 125 rpm in BG-11 medium. The cell density of the cultures was determined by the optical density at 750 nm as previously described (8, 28). The antibiotic concentrations used for the mutant strains were the following: 25 μg/ml kanamycin, 25 μg/ml spectinomycin, and 15 μg/ml chloramphenicol.

Construction of mutants. Mutations of *sigB* (sll0306), *sigD* (sll2012), and *sigE* (sll1689) were constructed by interruption or deletion of part of each gene and insertion of an antibiotic resistance cassette, as described by Singh et al. (44). A spectinomycin resistance cassette was used to create Δ*sigB* and Δ*sigE* mutants, and a kanamycin resistance cassette was used to produce the Δ*sigD* strain. Double mutants lacking SigB and SigD as well as SigD and SigE were produced using the same constructs as those for the single mutants. In the case of the Δ*sigB* Δ*sigE* and Δ*sigB* Δ*sigD* Δ*sigE* strains, the *sigE* gene contained a kanamycin resistance cassette and a chloramphenicol resistance cassette, respectively. Complete segregation of the mutants was obtained as confirmed by PCR and Southern blotting (data not shown).

RNA isolation. Total RNA was extracted and purified using phenol-chloroform extraction and CsCl gradient purification as previously described (38, 42).

Microarray design. The microarray platform and construction were as described by Postier et al. (36). The cDNA labeling, glass treatment, prehybridization, and hybridization protocols were described in detail previously (41). Microarray experiments involved a loop design that allowed comparison of all conditions by using an analysis of variance (ANOVA) model (26, 41). This work is comprised of four separate loop experiments. In the first two experiments, we analyzed the effect of light or dark treatment on the wild type, on each of two mutant strains, and on the relationship between the wild type and the mutants. The third experiment examined the impact of the length of dark incubation on mixotrophically grown wild-type cells, and the fourth experiment compared a mutant strain to the wild-type strain as described for the first two experiments.

The first loop compared the effects of light or dark treatment of the wild-type strain to that of the Δ*sigB* strain, and the second compared the light or dark treatment of the wild-type strain to that of the Δ*sigD* strain. In both experiments, cells were grown under photoautotrophic conditions with constant light for 72 h and then were transferred to a 12-h dark and 12-h light regimen. Cells were harvested at 1 h following transition to light (L1) and 1 h following transition to dark (D1), as shown in Fig. 1A. The third loop experiment allowed examination of the wild type at different time points following a transition to darkness. Cells were grown photoautotrophically in constant light for 48 h before being transferred to a 16-h dark and 8-h light regimen; 5 mM glucose was added at 40 h of this regimen. Samples were collected at 7 h light (GL7), 1 h dark (GD1), 8 h dark (GD8), and 16 h dark (GD16), as shown in Fig. 1D. The fourth loop compared the light or dark treatment of the wild-type strain to that of the Δ*sigB* Δ*sigE* strain grown under the same mixotrophic conditions as those used for the third loop. Samples were collected at 1 h following transition to dark (GD1) and at 1 h following transition to light (GL1) (Fig. 1D). Biological variation was sampled by pooling RNA extracted from three experiments prior to labeling and hybridization.

Data analysis. Spot intensities of the images were quantified by using Quantarray 3.0 (Packard BioChip Technologies, Boston, MA). Data for the slides used in each experiment then were collated into four data sets (one for each experiment) by using SAS (version 8.02; SAS Institute, Cary, NC). The local background was subtracted from each spot. For each replicate block on a slide, there were 480 empty spots, and there were three replicates per slide. We examined the intensities for these empty spots and declared data from a nonempty spot to be detected if the background-corrected intensity of the spot was greater than that for 95% of the empty spots. If all the spots for a given gene were not detected on all the slides in an experiment, then the gene was considered to be off and was not analyzed further (282 genes in the Δ*sigB* experiment, 652 genes in the Δ*sigD* experiment, 326 in the wild-type mixotrophic growth experiment, and 414 genes in the Δ*sigB* Δ*sigE* experiment). We then calculated the log of the background-corrected signals that were normalized to the slide median (the median for all non-control spots detected). Three of the experiments contained two genotypes (mutant and wild type) and two stimuli (mutant and wild type for light and dark

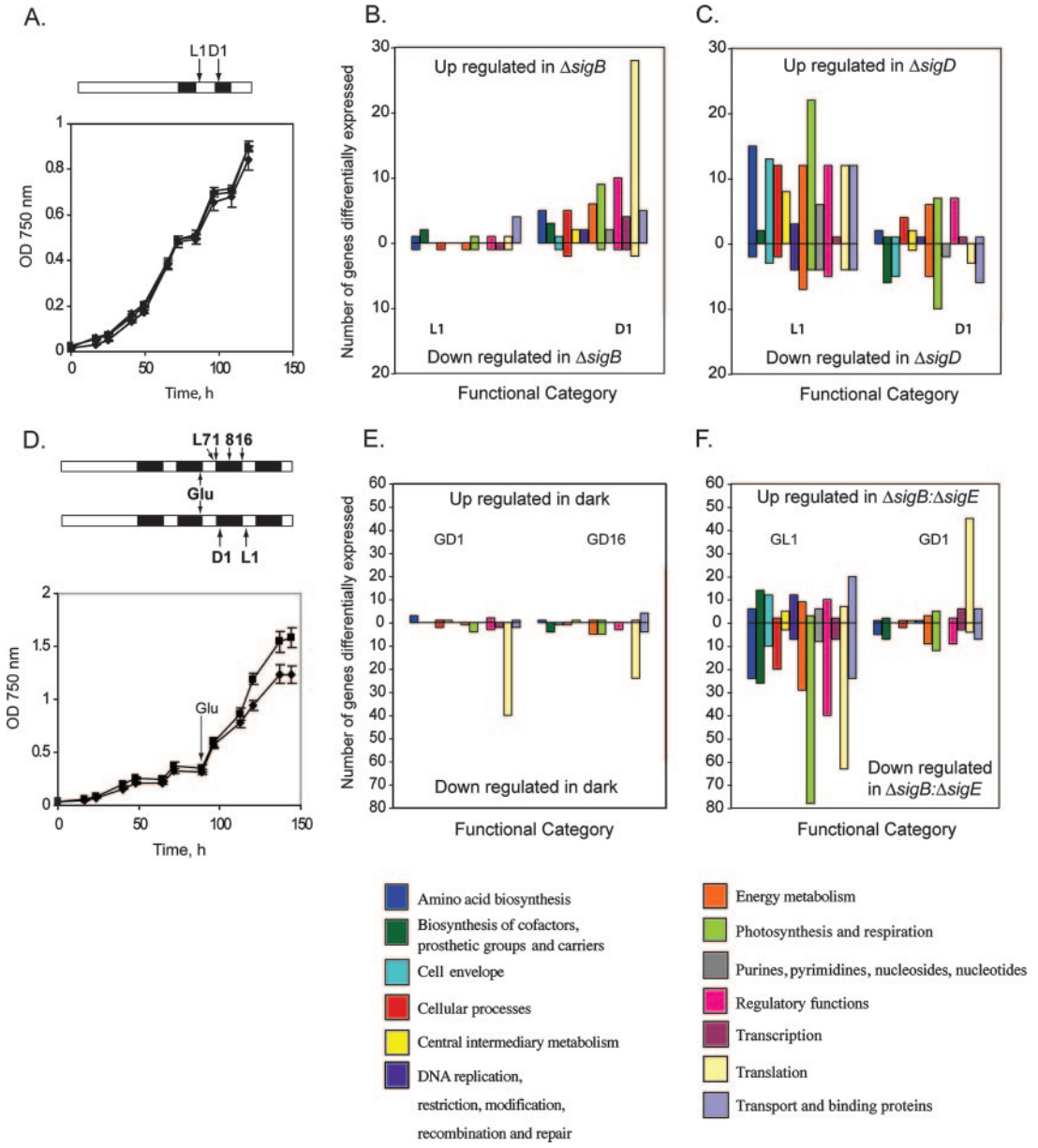

FIG. 1. (A) Photoautotrophic growth of cultures from the Δ*sigB* and Δ*sigD* experiments. The bar (top) represents the light-dark regimen used in these experiments. Cells were grown for 72 h in continuous light before beginning the 12-h light/12-h dark cycles. The growth curves for the wild-type (square), Δ*sigB* (triangle), and Δ*sigD* (diamond) strains, as measured by the optical density at 750 nm (OD_{750}), are shown to scale beneath the bar. The data are the averages ± standard errors of three independent measurements. The arrows represent the time points L1 and D1, at which cells were harvested and RNA was extracted and used for microarray analysis. (B and C) Histogram of genes differentially expressed 1.5-fold or more (FDR = 0.05) at L1 and D1. The numbers of differentially expressed genes for different functional categories are plotted on the *x* axis, with genes up-regulated in the mutant shown above the zero line on the *y* axis and genes down-regulated shown beneath zero line. (B) Comparison of the Δ*sigB* strain to the wild type. (C) Comparison of the Δ*sigD* strain to the wild type. Genes are presented as Cyanobase functional categories, as defined in the key beneath the graphs. (D) The light-dark regimen for mixotrophic growth of wild-type and Δ*sigB* Δ*sigE* strains (top). The cells were grown for 72 h in the light before transfer to a 16-h dark/8-h light cycle. The addition of glucose (Glu) to a final concentration of 5 mM is indicated. The arrows on the top bar represent time points L7, D1, D8, and D16, the points at which wild-type cells were harvested and RNA was extracted and used for microarray analysis. The arrows on the second bar represent time points D1 and L1, the points at which wild-type and Δ*sigB* Δ*sigE* cells were harvested and RNA was extracted and used for microarray analysis. The growth curves for the wild type (square) and Δ*sigB* Δ*sigE* (diamond), as measured by the OD_{750}, are shown to scale beneath the bar. The data are the averages ± standard errors of three independent measurements. (E and F) Histogram of genes differentially expressed 1.5-fold or more (FDR = 0.05). (E) Genes differentially regulated in the wild type at L7 compared to D1 and D16. (F) Genes differentially regulated in the Δ*sigB* Δ*sigE* strain compared to the wild type at L1 and D1. The genes were divided into functional categories as described for panels B and C.

TABLE 1. Differentially regulated genes organized by functional category[a]

General pathway(s)	No. of genes	No. of genes differentially regulated (no. of genes up-regulated) under:								
		Photoautotrophic conditions (12 h of light/12 h of dark)							Mixotrophic conditions (8 h of light/16 h of dark)	
		L1/D1			Δ*sigB*/WT		Δ*sigD*/WT		Δ*sigB* Δ*sigE*/WT	
		WT	Δ*sigB*	Δ*sigD*	L1	D1	L1	D1	L1	D1
Amino acid biosynthesis	97	14 (7)	13 (5)	27 (18)	2 (1)	5 (5)	17 (15)	2 (2)	30 (6)	6 (1)
Biosynthesis of cofactors, prosthetic groups, and carriers	124	27 (15)	24 (13)	35 (16)	2 (2)	3 (3)	2 (2)	7 (1)	40 (14)	9 (2)
Cell envelope	67	15 (13)	10 (9)	21 (20)		2 (1)	16 (13)	6 (1)	22 (12)	
Cellular processes	76	18 (15)	18 (12)	27 (18)	1 (0)	7 (5)	14 (12)	4 (4)	22 (2)	3 (1)
Central intermediary metabolism	31	5 (2)	6 (1)	11 (7)		2 (2)	8 (8)	3 (2)	8 (5)	1 (1)
DNA replication, restriction, recombination, and repair	60	6 (2)	6 (2)	11 (6)		2 (2)	7 (3)	1 (1)	19 (12)	1 (1)
Energy metabolism	132	30 (15)	32 (11)	55 (34)	1 (0)	6 (6)	19 (12)	11 (6)	38 (9)	12 (3)
Hypothetical	1076	164 (82)	170 (71)	255 (110)	21 (14)	44 (33)	112 (52)	50 (29)	317 (165)	81 (26)
Other categories	306	52 (38)	38 (20)	57 (36)	2 (2)	8 (7)	21 (12)	9 (1)	82 (51)	25 (18)
Photosynthesis and respiration	141	67 (62)	58 (52)	83 (72)	2 (1)	10 (9)	26 (22)	17 (7)	81 (3)	17 (5)
Purines, pyrimidines, nucleosides, and nucleotides	41	5 (4)	6 (5)	11 (8)		2 (2)	10 (6)	2 (0)	14 (6)	
Regulatory functions	146	22 (10)	22 (7)	47 (16)	2 (1)	11 (10)	17 (12)	7 (7)	50 (10)	11 (2)
Transcription	30	11 (10)	6 (5)	11 (10)	1 (0)	5 (4)	1 (1)	1 (1)	9 (2)	9 (6)
Translation	168	67 (64)	59 (51)	63 (54)	2 (1)	30 (28)	16 (12)	3 (0)	70 (7)	49 (45)
Transport and binding proteins	196	32 (19)	26 (11)	41 (30)	4 (4)	5 (5)	16 (12)	7 (1)	44 (20)	13 (6)
Unknown	474	70 (41)	87 (36)	117 (49)	8 (3)	18 (14)	43 (20)	19 (16)	152 (56)	30 (11)
Total	3,165[b]	605 (399)	581 (311)	872 (504)	48 (29)	160 (136)	345 (214)	149 (79)	998 (380)	267 (128)

[a] Genes were considered differentially regulated when the FDR was 0.05 and the fold change was >1.5-fold. WT, wild type.
[b] Total number of genes based on Cyanobase annotation prior to May 2002.

transitions) for a total of four treatment combinations. The effects of the mutant and the light/dark stimuli were examined in an ANOVA essentially as described previously (22, 23, 26, 41). Our normalization and overall statistical approach enabled us to make comparisons among our experiments without the need for renormalization (26, 41).

The above analysis included the use of the very stringent Bonferroni correction, which emphasizes very strong effects when many tests are performed (47). Many such analyses now use the false discovery rate (FDR), which controls the proportion of significant results that are type I errors (false rejection of the null hypothesis) (5, 47). Once our initial analysis was completed, we used the FDR of 5% to control the number of false positives in gene lists. Genes with such FDRs (corresponding to 5% expected false positives) and that exhibited a change of at least 1.5-fold were considered interesting and were retained for further analysis. The entire data set for the Δ*sigB*, Δ*sigD* experiments, the wild-type time course, and the Δ*sigB* Δ*sigE* experiment are shown in Table S1 in the supplemental material. These data include the fold changes and the various *P* values from the ANOVA analysis.

qPCR. RNA was treated with DNase I (Invitrogen, Carlsbad, CA) for 1 h at 37°C, and successful DNase treatment was confirmed by quantitative PCR (qPCR) on each DNase-treated RNA sample. Reverse transcription was performed using Superscript II (Invitrogen) and random primers by following the manufacturer's instructions. Primer Express software (Applied Biosystems, Foster City, CA) was used to design primers for the following genes: slr2072 (forward, 5′-GTTGGGTACCAGGGCGATTA-3′; reverse, 5′-CTTTGACCGCCTCCACTTTC-3′), sll0329 (forward, 5′-CGGCTTGAGCAACGAACA-3′; reverse, 5′-TCATCGGTTTGATTCCATTGG-3′), slr0474 (forward, 5′-CCTTGTCCAGGAGGTGTTGAA-3′; reverse, 5′-CCCGGAGAATGATCAGTTCGT-3′), slr1311 (forward, 5′-GGTTGGTTCGGTACCTTGATGA-3′; reverse, 5′-CGGCGATGAAGGCAATG-3′), and slr1667 (forward, 5′-GATTGTTCTGATGCTACTGGTTGTG-3′; reverse, 5′-AACTTGCTCTTCTGCGATTGC-3′). qPCR was performed on the ABI 7300 real-time PCR system (Applied Biosystems) using a SYBR green master mix and 50 nM primers (Applied Biosystems) at 95°C for 10 min and then 40 cycles of 95°C for 15 s and 60°C for 1 min. The slr2072 transcript abundance was used as an internal control for normalization, as microarray data from this study and previous studies (9, 41, 44) indicated that the transcript level for slr2072 was unchanged under a number of growth conditions. Standard curves used for each primer set indicated similar efficiencies for the slr2072 primers and all other primer pairs in the range of 1 ng to 10 pg cDNA. The results represent the means of triplicate technical replicates for duplicate biological samples.

RESULTS

Differentially regulated genes in the wild type at L1 compared to D1. The design of the experiment is presented in Fig. 1A. The cultures were grown in continuous light for 3 days and then in 12-h dark and 12-h light periods. Samples were taken after 1 h in the light (L1) and after 1 h in the dark (D1). As indicated in Materials and Methods, we considered genes to be differentially regulated if they showed a fold change of ≥1.5 with an FDR of 0.05. For the wild type, the expression of 19% of the chromosomal genes met these criteria (605/3,165 genes) at L1 compared to D1, and about two-thirds of these transcripts (399/605 genes) were up-regulated at L1 (Table 1). This is similar to the number of genes (387) positively regulated following 30 to 90 min of light in the experiment of Gill et al. (10). It should be noted that Gill et al. (10) compared light-exposed samples to 24-h dark-incubated samples, not 1-h-dark samples, as in our experiment.

The differentially expressed genes were divided into functional categories according to the Cyanobase designation, and the number of genes in each category is shown in Table 1. The functional categories (excluding hypothetical genes) with the largest percentage of differentially regulated genes at L1 relative to D1 were photosynthesis and respiration (67/141 genes) and translation (67/168), and these were mostly up-regulated. Other categories containing elevated levels of transcripts after 1 h of growth in the light included biosynthesis of cofactors,

prosthetic groups, and carriers (15 genes); cell envelope (13 genes); energy metabolism (15 genes); regulatory functions (10 genes); and transport and binding proteins (19 genes). Many up-regulated genes belonged to the hypothetical (82) or unknown (41) functional categories, and more than 50% (111/206 genes) of the transcripts down-regulated also belonged to these categories (Table 1). Other down-regulated genes encoded products that are involved in energy metabolism (15), regulatory functions (12), transport and binding proteins (13), and biosynthesis of cofactors, prosthetic groups, and carriers (12). These data indicated that genes in many functional categories were affected when cells sensed light.

The absence of SigB predominately alters gene expression in the dark. One objective of this study was to determine the impact on global transcription of removing various σ factors. The growth curves for the wild type, Δ*sigB*, and Δ*sigD* under photoautotrophic growth conditions are presented in Fig. 1A, and it can be seen that growth was virtually identical among the three cultures. Removal of SigB led to very little difference in transcript levels from those of the wild type at L1, and only 48 genes (31 hypothetical or unknown) were differentially regulated in the mutant compared to the wild type (Table 1 and Fig. 1B). Selected genes that demonstrated significant transcriptional changes at L1 in Δ*sigB* relative to the wild type are shown in Table S2 in the supplemental material. In contrast, 160 genes were differentially regulated at D1 in Δ*sigB* relative to the wild type (Table 1), and the majority of these (136 genes) were increased in the mutant. Genes up-regulated in the mutant included 28 that encoded proteins involved in translation: 10 with regulatory functions, 9 encoding photosynthesis and respiration proteins, and 6 others involving energy metabolism (Table 1; also see Table S2 in the supplemental material).

Absence of SigD alters expression of more genes in the light than in the dark. The removal of SigD resulted in considerably more changes in the global transcription pattern (Table 1 and Fig. 1C). In the light, some 345 genes displayed transcript levels in Δ*sigD* different from those of the wild type, with 214 of these genes up-regulated in the mutant. The genes with elevated transcript levels included genes encoding proteins involved in photosynthesis and respiration (22 genes), amino acid biosynthesis (15 genes), energy metabolism (12 genes), regulation (12 genes), and translation (12 genes). In the dark, fewer genes showed differential expression, although 149 genes in the Δ*sigD* strain were regulated differently than those of the wild type. It was evident that the removal of SigD had a more profound effect on transcription in the light than removal of SigB. On the other hand, although they both affected a similar number of genes in the dark, the removal of SigB resulted in the up-regulation of the genes affected, whereas SigD affected transcription in both directions (Table 1; Fig. 1B and C).

Differential expression of selected genes. Transcription of genes encoding photosynthesis and respiration proteins demonstrated light-responsive regulation during dark-light transitions, with the photosynthesis genes mostly up-regulated in the light. This pattern can be seen clearly in Table 2, where we list select groups of differentially regulated genes involved in photosynthesis or with central metabolism. Similar to the results of Gill et al. (10), there was up-regulation of genes that encode proteins involved with the structure and assembly of the photosynthetic membrane. This included phycobilisome genes encoding components of both the peripheral rods (phycocyanin) and core (allophycocyanin), six PSI genes, genes encoding a number of core components of PSII (also observed in reference 13), and both ATP synthase operons. Table 2 displays the transcriptional changes in the light compared to the dark for the wild type for both the light-dark experiments as well as for the respective mutant in each experiment. The comparison of the changes in the wild type is informative and provides an idea of the close correspondence of the results between such repetitions.

In our experiments, the photosynthesis genes were always represented among the genes with the highest transcript levels. Thus, photosynthesis genes represented 20 to 25% of the top 200 transcript levels in both the light and the dark (based on the data prior to normalization). Light-responsive expression in many photosynthesis genes that has been reported previously (10) included the up-regulation of *psbA* transcripts encoding the PSII protein D1 (39). The fold changes we observed were attenuated in two ways. First, the two *psbA* transcripts, *psbA2* (slr1311) and *psbA3* (sll1867), could not be distinguished by the microarray, as the coding regions differ by only 4 nucleotides. In addition, in comparisons of L1 to D1, the fold changes in the *psbA2* and *psbA3* transcripts often were less than 1.5-fold due to the high transcript levels under both light and dark conditions. This was not unexpected, as the *psbA* transcript levels have been shown to remain elevated following at least 1 h of dark incubation (29), and our dark incubation time was 1 h. In addition, the *psbA* transcript levels were so abundant in the dark that further elevation of expression in the light may be outside the dynamic range of our detection system. This also may explain why we do not observe the ~1.5-fold reduction of both *psbA2* and *psbA3* transcripts in the absence of SigD compared to the wild type, which were reported by Imamura et al. (17).

The complete data set for all genes in all four experiments is provided in Table S1 in the supplemental material. This table includes the *P* values obtained from the ANOVA and indicates how individual genes were affected after cells went between dark and light growth conditions, either in a wild-type background or in the absence of SigB, SigD, or SigB and SigE. A few genes from important functional categories are shown in Table 2 and in Table S2 in the supplemental material. Certain genes that clearly were light regulated were altered by the absence of SigB or SigD; these are shown in Table 2. This included photosynthesis genes, such as ATPase genes, that were enhanced by the light in the wild type and to a similar level in Δ*sigB*, but most were further up-regulated in Δ*sigD*. Similarly, genes encoding many phycobilisome components were up-regulated by the light in the wild-type and Δ*sigB* strains. Many of these genes were further increased in the Δ*sigD* strain in the light, and some also were elevated in the dark in the Δ*sigD* strain compared to wild-type levels (e.g., *cpcC2*, *cpcC1*, and *cpcD*). Similarly, the removal of SigD had a greater impact than the removal of SigB on PSI and PSII genes (Table 2). Key chaperone genes had previously been shown to be up-regulated in the light (10), and these were up-regulated in this study in the wild type and in the Δ*sigB* and Δ*sigD* strains. The enhancement in transcript level was virtually identical between the wild type and Δ*sigB*, whereas the genes *groES* and

TABLE 2. Relative transcript abundance of selected differentially regulated genes[a]

Gene and function	Gene name	Fold change between regulation at L1 and D1			
		WT (B)[c]	WT (D)[c]	Δ*sigB*	Δ*sigD*
Biosynthesis of cofactors, prosthetic groups, and carriers					
slr0506	*por*	2.3	2.8	2.6	4.6
slr0749	*chlL*	−2.4	−2.7	−1.3	−1.7
slr0750	*chlN*	−3.4	−2.8	−2.2	−2.6
slr0772	*chlB*	1.6	1.8	1.8	3.7
Cellular processes					
sll0170	*dnaK2*	2.3	2.4	1.5	2.3
sll0416	*groEL2*	3.4	3.4	3.4	5.0
sll0430	*htpG*	2.3	2.6	1.9	2.6
slr2075	*groES*	3.9	4.7	3.2	6.6
slr2076	*groEL1*	4.9	4.4	3.6	4.6
Energy metabolism					
Glycolysis					
sll0018	*fbaA*	2.4	2.3	1.9	4.1
slr1349	*pgi*	1.1	1.0	−1.2	1.6
slr0884	*gap1*	−1.1	−1.4	−1.4	−2.2
slr0752	*eno*	−1.1	1.0	−1.4	−1.9
slr1945	*pgm*	ND[b]	1.4	ND	3.2
sll1196	*pfkB1*	−1.5	−1.5	−1.8	−1.9
sll0745	*pfkB2*	−2.0	−2.3	−3.0	−4.1
slr0394	*pgk*	1.9	2.1	1.4	5.0
sll0587	*pyk1*	−1.5	−1.9	−2.0	−1.7
OPP pathway					
sll0329	*gnd*	−3.7	−3.4	−6.4	−3.2
slr1843	*zwf*	−2.9	−3.1	−4.0	−2.2
slr1734	*opcA*	−2.9	−2.6	−4.4	−2.4
sll0807	*rpe*	2.2	1.9	1.9	3.0
slr1793	*tal*	−1.9	−2.6	−2.9	−2.3
sll1070	*tktA*	1.7	2.0	1.4	3.1
Pyruvate and acetyl-CoA metabolism					
sll0920	*ppc*	−2.4	−2.2	−1.7	−3.3
slr0301	*pps*	−1.7	−2.0	−2.4	−5.7
slr2132	*pta*	−1.6	−2.1	−2.4	−3.2
Photosynthesis and respiration					
ATP synthase					
sll1321	*Prot1*	4.7	4.3	4.1	4.7
sll1322	*atpI*	3.3	3.2	3.3	4.2
sll1323	*atpG*	3.9	3.1	4.0	4.9
sll1324	*atpF*	4.4	4.9	4.4	8.8
sll1325	*atpD*	5.5	5.7	4.9	9.3
sll1326	*atpA*	4.6	5.2	4.2	8.7
sll1327	*atpC*	6.3	6.5	4.6	6.9
ssl2615	*atpH*	4.4	3.8	4.6	4.9
slr1329	*atpB*	2.2	2.2	1.9	4.4
slr1330	*atpE*	ND	3.4	ND	5.8
Phycobilisomes					
sll0928	*apcD*	2.0	1.8	1.5	1.6
slr1986	*apcB*	ND	2.1	ND	4.1
slr2067	*apcA*	2.1	1.9	1.8	3.8
sll1577	*cpcB*	3.3	2.7	3.1	3.9
sll1579	*cpcC2*	3.6	2.8	4.1	2.7
sll1580	*cpcC1*	5.0	2.1	5.0	1.8
slr1459	*apcF*	2.2	2.0	2.0	4.1
ssr3383	*apcC*	3.9	3.5	3.7	5.0
ssl3093	*cpcD*	5.5	2.9	6.2	3.2
PSI					
ssl0563	*psaC*	3.1	1.8	2.8	2.1
slr1655	*psaL*	2.4	1.7	2.1	2.4
PSII					
sll0427	*psbO*	3.0	2.3	3.0	4.4
slr0927	*psbD2*	2.9	1.9	2.0	1.2
slr1739	*psb28-2*	−2.3	−1.9	−2.0	−2.6
sml0001	*psbI*	2.4	2.2	2.3	3.1
sml0002	*psbX*	1.7	1.6	1.7	1.6
ssl2598	*psbH*	1.6	1.4	1.1	1.7
smr0008	*psbJ*	1.7	1.7	1.7	2.5

[a] Genes were considered differentially regulated if the FDR was 0.05 and the fold change was >1.5-fold. WT, wild type.
[b] ND, not determined.
[c] WT (B) and WT (D) data are from the Δ*sigB* and Δ*sigD* experiments, respectively.

TABLE 3. Mixotrophic growth characteristics of *Synechocystis* sp. strain PCC 6803 wild type and sigma factor mutants under different light regimens

Strain	Doubling time (h)[a] by growth condition		
	24 h of light	12 h of light/ 12 h of dark	8 h of light/ 16 h of dark
Wild type	9 ± 0	16 ± 1	19 ± 1
Δ*sigB*	9 ± 0	16 ± 1	18 ± 1
Δ*sigD*	9 ± 0	17 ± 1	19 ± 1
Δ*sigE*	9 ± 0	20 ± 1	37 ± 1
Δ*sigB* Δ*sigD*	10 ± 1	18 ± 1	21 ± 1
Δ*sigB* Δ*sigE*	10 ± 0	44 ± 4[b]	NG
Δ*sigD* Δ*sigE*	10 ± 0	36 ± 2	50 ± 4[b]
Δ*sigB* Δ*sigD* Δ*sigE*	12 ± 0	NG[c]	NG[c]

[a] Doubling times for strains are means ± standard errors for $n \geq 3$.
[b] Cells reached an optical density at 750 nm of <0.2.
[c] NG, no growth following initial dark period.

groEL2 were further up-regulated in Δ*sigD* (Table 2). Importantly, most of the genes encoding ribosomal proteins had ~2-fold more abundant transcripts in the light than in the dark for the wild-type, Δ*sigD*, and, to a lesser extent, Δ*sigB* strain (see Table S1 in the supplemental material).

The transition from light to dark resulted in a shift from photosynthesis and the accumulation of carbohydrates to sugar catabolism through glycolysis and the oxidative pentose phosphate pathway (OPP pathway). A number of genes that are involved in sugar catabolism and that are regulated by a histidine kinase (Hik8) or by SigE (35, 43) exhibited light-sensitive transcription. In the wild type, four genes involved in glycolysis were differentially regulated at L1 compared to their regulation at D1 (see Table S2 in the supplemental material). This included *fbaA* and *pgk*, which were up-regulated in the light, whereas the levels of transcripts of both phosphofructokinase genes (*pfkB1* [sll1196] and *pfkB2* [sll0745]) were elevated in the dark (Table 2) (43). The strain Δ*sigD* had decreased levels of the *fbaA* transcript in the dark and elevated *pgk* transcript in the light, and these findings are shown in Table 2 as increased fold changes between light and dark gene expression. Other glycolysis genes that were unchanged in the wild type but were up-regulated in Δ*sigD* in the light included *eno*, *fbaI*, *pgi*, and *pgm*. In addition, the transcript encoding *gap1* was unchanged in the wild type at L1 compared to its status at D1, as previously reported (43), but was down-regulated in the Δ*sigD* mutant in the light. In general, the transcription of these genes was affected more by the lack of SigD than the lack of SigB (Table 2).

The OPP pathway plays an important role in glucose breakdown and generation of reducing power (43). In the wild type, down-regulated transcripts in the light included the genes encoding two key enzymes in the OPP pathway, *gnd* and *zwf*, as well as the glucose 6-phosphate dehydrogenase assembly protein (*opcA* [slr1734]) and transaldolase (*tal* [slr1793]). Changes in transcript abundance agreed with previous reports of dark-responsive regulation of these four genes (35, 43). Two genes up-regulated by light in the wild type were *rpe* and *tktA* (Table 2). Overall, more of the genes encoding energy metabolism enzymes were differentially regulated in the Δ*sigD* strain compared to the wild type than in the Δ*sigB* strain compared to the wild type. The numbers of differentially regulated genes are shown in Table 1.

Photoautotrophic, mixotrophic, and heterotrophic growth of σ factor mutants. We next studied the effect of removing SigB, SigD, and SigE on growth under photoautotrophic, mixotrophic, and heterotrophic conditions. The Δ*sigB*, Δ*sigD*, and Δ*sigB* Δ*sigD* strains had doubling times comparable to those of the wild type under photoautotrophic growth conditions (Fig. 1A). The absence of either SigB or SigD altered some of the transcripts encoding components of sugar metabolism following transition from light to dark (Table 2; also see Table S1 in the supplemental material). Nonetheless, the Δ*sigB*, Δ*sigD*, and Δ*sigB* Δ*sigD* strains had doubling times similar to those of the wild type in mixotrophic conditions, including under different light regimens (Table 3). A number of genes involved in sugar catabolism exhibited light-sensitive transcription and were shown to be regulated by SigE (35), and we observed that Δ*sigE* exhibited decreased mixotrophic growth with 12-h/12-h or 8-h/16-h light/dark regimens (Table 3). Under these conditions, the removal of SigB or SigD from the Δ*sigE* strain reduced growth, such that the Δ*sigB* Δ*sigE* strain grew less than the Δ*sigD* Δ*sigE* strain, and the Δ*sigB* Δ*sigD* Δ*sigE* strain was unable to grow in 12-h/12-h light/dark or 8-h/16-h light/dark regimens (Table 3). Similarly, the transfer of cultures grown on BG-11 plates (supplemented with 5 mM glucose) from continuous light to continuous dark resulted in decreased growth of the Δ*sigE* strain compared to that of the wild type and a further decrease in growth of the Δ*sigB* Δ*sigE*, Δ*sigD* Δ*sigE*, and Δ*sigB* Δ*sigD* Δ*sigE* strains (Fig. 2). However, under autotrophic conditions with different light regimens and mixotrophic conditions with 24 h of light, growth of these strains was similar to that of the wild type (Fig. 2). These results indicated that whereas SigE is the principal group 2 σ factor involved in sugar catabolism, SigB and SigD do play at least a secondary role in regulating gene expression at light/dark transitions and, thus, affect cell viability when SigE is absent.

Previous microarray analyses indicated that during long-term growth in continuous light, the presence or absence of glucose had little impact on gene expression in *Synechocystis* sp. strain PCC 6803 (20, 46). Furthermore, Yang et al. (49) reported that cultures grown with glucose in the light and dark showed altered expression of only 2 out of 13 transcripts examined, but the expression level of many proteins was altered.

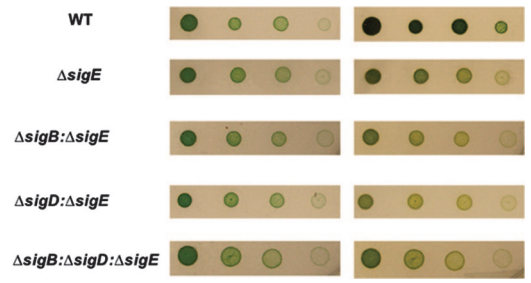

FIG. 2. Effect of sigma mutations on heterotrophic growth. Cultures of the wild type (WT) and sigma mutants were grown photoautotrophically and were spotted (at the optical density at 750 nm indicated) onto BG-11 plates containing 5 mM glucose. Plates were incubated in the light for 96 h (left gels) and then were transferred to the dark for 96 h (right gels).

TABLE 4. Fold change of transcript abundance for selected differentially regulated genes under different growth conditions at L1 and D1[a]

Gene	Gene regulatory function	Fold change of transcript abundance between WT and mutant:					
		Photoautotrophic				Mixotrophic	
		Δ*sigB*		Δ*sigD*		Δ*sigB* Δ*sigE*	
		L1	D1	L1	D1	L1	D1
slr0473	Cyanobacterial phytochrome 1, two-component sensor histidine kinase (*cph1*)		1.5		1.5	ND[b]	ND
slr0474	Two-component response regulator (*rcp1*)		1.6	−1.8		−1.7	−1.9
sll0789	Two-component response regulator OmpR subfamily (*rre34*)		3.2	−1.5		−1.8	−1.5
sll0790	Two-component sensor histidine kinase (*hik31*)		3.2	−2.5		−1.6	
slr1805	Two-component sensor histidine kinase (*hik16*)			2.4	4.2		
sll0750	Two-component sensor histidine kinase (*hik8*)					−1.9	−6.1
slr0947	Response regulator for energy transfer from phycobilisomes to photosystems (*rpaB*)		−1.6				−1.6
sll1672	Two-component hybrid sensor and regulator (*hik12*)					−3.7	−2.3
slr1285	Two-component sensor histidine kinase (*hik34*)	−1.5			2.0	−2.3	

[a] Genes were considered differentially regulated if the FDR was 0.05 and the fold change was >1.5-fold. WT, wild type.
[b] ND, not determined.

The phenotype of the SigE mutants was seen following periods of heterotrophic growth, indicating the importance of σ-factor-mediated transcriptional regulation under these conditions. Therefore, we examined changes in gene expression in the presence of glucose on transfer from light to dark under conditions that resulted in impaired growth of the strains that lacked SigE in combination with SigB and/or SigD.

Differential gene expression of the wild type during mixotrophic growth. In wild-type cells grown under mixotrophic conditions (8 h of light/16 h of dark), only ~3 to 5% of genes were differentially expressed in the light (7 h) compared to expression in the dark (1 or 16 h) (Fig. 1D). A large number of transcripts encoding the ribosomal protein genes were down-regulated (1.5- to 4.4-fold) on transition from light to dark, and the levels of many of these transcripts also were decreased following 16 h in the dark compared to their levels after 7 h in light (Fig. 1E; also see Table S1 in the supplemental material). A small number of genes from several functional categories, including photosynthesis, energy metabolism, and regulatory proteins, were differentially regulated following transition from light to dark (see Table S1 in the supplemental material).

The fourth microarray experiment compared gene expression of the wild type to that of the Δ*sigB* Δ*sigE* mutant, as represented by the lower bar in Fig. 1D. This experiment included comparison of gene expression in wild-type cells incubated for 1 h in the dark to expression in wild-type cells incubated for 1 h in the light (Fig. 1D), which showed that ~7% of transcripts were differentially regulated, of which about 61% were up-regulated (Table 1). More than half of these genes belonged to the unknown, hypothetical, or other functional categories. Genes encoding many of the ribosomal proteins were up-regulated in the light compared to the dark (1.7- to 6.7-fold), consistent with results of the previous microarray experiments in which there was down-regulation of ribosomal protein genes following transfer from light to dark. Genes up-regulated in the light included photosynthesis and respiration genes (e.g., ATP synthase and CO_2-concentrating mechanism genes) and genes encoding regulatory proteins. Differentially regulated genes involved in energy metabolism included five in glycolysis and five in the OPP pathway (see Table S3 in the supplemental material).

The absence of SigB and SigE has a major impact on gene expression under mixotrophic growth conditions. Levels of gene expression of the wild type and the Δ*sigB* Δ*sigE* mutant were compared for cells grown in the dark (1 h) and the light (1 h) under mixotrophic conditions with 8 h of light and 16 h of dark. Figure 1D shows the experimental design as well as the finding that the Δ*sigB* Δ*sigE* mutant grows more slowly than the wild type after the addition of glucose under these short-day conditions. At D1, 883 genes were regulated differently than they were at L1 in the Δ*sigB* Δ*sigE* strain, almost double the number of genes that were differentially expressed in the wild type. In the dark, 267 genes were differentially regulated in the mutant compared to the wild type, of which almost half encoded hypothetical, unknown, or other proteins. As shown in Fig. 1F, many of the genes (45) encoding ribosomal proteins were up-regulated in the Δ*sigB* Δ*sigE* mutant in the dark (1.5- to 3.5-fold), consistent with the up-regulation of genes encoding ribosomal proteins that was observed with the Δ*sigB* mutant in the dark under photoautotrophic conditions. Glycolysis and OPP pathway genes were down-regulated in the dark in the Δ*sigB* Δ*sigE* strain compared to their expression in the wild type (see Table S3 in the supplemental material). A number of these genes were reported to be down-regulated in the absence of SigE in the dark (35).

In the light, more than 30% of the genes (998) were differentially regulated in the Δ*sigB* Δ*sigE* strain compared to the wild type, with 618 of these genes down-regulated in the mutant. Functional categories containing down-regulated genes included photosynthesis and respiration (78 genes), translation (63 genes, mostly ribosomal protein genes), regulatory function (40 genes), and energy metabolism (29 genes). This large number of differentially expressed genes reflects the significant change in growth status brought about by the lack of both SigB and SigE under these growth conditions.

The regulatory relationship of SigB, SigD, and SigE to histidine kinases. There is reciprocal regulation between the group 2 σ factors and specific histidine kinases (35, 43). As

TABLE 5. Comparison of transcript abundance at L1 to that at D1 using qPCR and microarray data for wild-type, Δ*sigB*, and Δ*sigD* *Synechocystis* sp. strain PCC 6803 strains[a]

Gene	WT			Δ*sigB*			Δ*sigD*		
	qPCR[b]		Microarray	qPCR		Microarray	qPCR		Microarray
	A	B		A	B		A	B	
gnd (sll0329)	0.10	0.08	0.27	0.04	0.03	0.16	0.24	0.14	0.31
rcp1 (slr0474)	0.15	0.10	0.32	0.12	0.04	0.17	0.08	0.04	0.08
psbA2 (slr1311)	1.56	1.13	1.28	1.18	0.78	1.14	0.47	0.43	1.10
hyp (slr1667)	17.00	16.00	11.11	7.59	5.00	6.41	2.28	1.48	1.94

[a] Transcript level at L1/D1.
[b] qPCR data are from three technical replicates for each of two biological replicates, A and B. The standard deviation of technical replicates was ≥15% of the fold change.

shown in Table 4, the removal of SigB and, to a lesser extent, the removal of SigD, but especially the removal of both SigB and SigE, had a dramatic effect on the transcription levels of a number of histidine kinases. In the case of *hik31* (and its possible response regulator, *rre34*), the absence of SigB increased the transcript level in the dark. However, the absence of both SigB and SigE decreased the *hik31* transcript level in Δ*sigB* Δ*sigE* in the light and, to a lesser extent, in the dark, and the removal of SigD decreased transcript levels in the light. The *hik8* gene was affected only when both SigB and SigE were missing, and the transcript level then was decreased in the light and was decreased significantly in the dark (Table 4). The *hik12* gene was affected in a similar fashion. On the other hand, SigD regulated *hik16*, as seen by the increased *hik16* transcript level in the Δ*sigD* strain in both the light and the dark.

Validation of microarray results by qPCR. The microarray data were validated by qPCR using two biological replicates (Table 5). The comparisons demonstrated excellent correspondence for genes that were up-regulated in the light (slr1667) and up-regulated in the dark (sll0329 and slr0474) and for *psbA2*, which showed relatively small fold changes during the 1-h transition. From a quantitative perspective, the ratios determined by microarray analysis appeared to be underestimated compared to those from qPCR. The qPCR data probably are more accurate for genes with particularly high or low transcript levels; this includes the *psbA* transcript, among the most abundant transcripts, and slr1667, which has low transcript levels. However, in the case of the Δ*sigD* strain, the *psbA* transcript levels from the microarray experiment appeared to be consistent with data of Yoshimura et al. (50), who reported little change in transcript levels on transfer from dark to light. They also are in agreement with our Northern blot analysis with an aliquot of the same total RNA samples as those used for the microarray experiments (data not shown). For all other samples and genes, there was quantitative agreement between the qPCR and microarray data.

DISCUSSION

Our results indicate the importance of the group 2 σ factors SigB, SigD, and SigE in the global regulation of transcription in *Synechocystis* sp. strain PCC 6803. We then needed to determine why so many genes in so many functional categories were affected by the deletion of a specific sigma factor. The results can best be explained by using the σ cycle paradigm (45) and particularly the stochastic release model described previously (30). Mooney et al. (30) have summarized the evidence for and against a half dozen different possible models of σ action and concluded that data support stochastic release over the obligate and nonrelease models. In addition, the authors discuss more nuanced modes of σ action, including models referred to as promoter-proximal σ pause, rebinding σ pause, and the hypothetical role of σ as an antitermination factor. Each model is consistent with results from specific promoter systems, and there is currently no way to compare such details in our system. Thus, we will refer only to the basic stochastic release model. In this model, a pool of σ factors competes for binding to the core RNAP to form an open complex. The affinity of σ for the RNAP decreases in the elongation complex, but release of the σ factor occurs stochastically after the RNAP has initiated transcription. The release is affected by factors such as RNAP and σ factor concentration and occurs during each transcription cycle, allowing σ competition for rebinding of the RNAP. Therefore, control of global transcription will be based on the amount of the various sigma factors present and able to bind to the RNAP.

The model depicted in Fig. 3 is based on our transcription data as well as the levels of the σ factors in the light and dark as calculated by Imamura et al. (19). In general, the group 3 σ factors were below the detectable level. In the wild type, the transition from growth in the light to growth in the dark generated an increase in SigB and a decrease in SigE. In the wild type, the major impact of this transition on global regulation was a decrease of the transcript levels of genes encoding ribosomal proteins and photosynthesis proteins. At the same time, genes encoding enzymes involved in energy metabolism, biosynthesis of various cofactors and prosthetic groups, regulatory functions, and transport and binding proteins tended to increase. In addition, many genes annotated as hypothetical or unknown also increased as cells went into the dark phase. This pattern was reversed as the cells were exposed to the light, where SigB decreased twofold and SigD and SigE increased twofold.

When SigB was absent, there was very little change from the wild-type levels in the light, as shown in Fig. 1B and Table 1. This is consistent with the low level of SigB in the light, when SigB represented less than 4% of the total sigma factors, as reported by Imamura et al. (17, 19) (Fig. 3). However, in the dark, SigB expression increased twofold and many more genes showed enhanced transcript levels relative to those of the wild

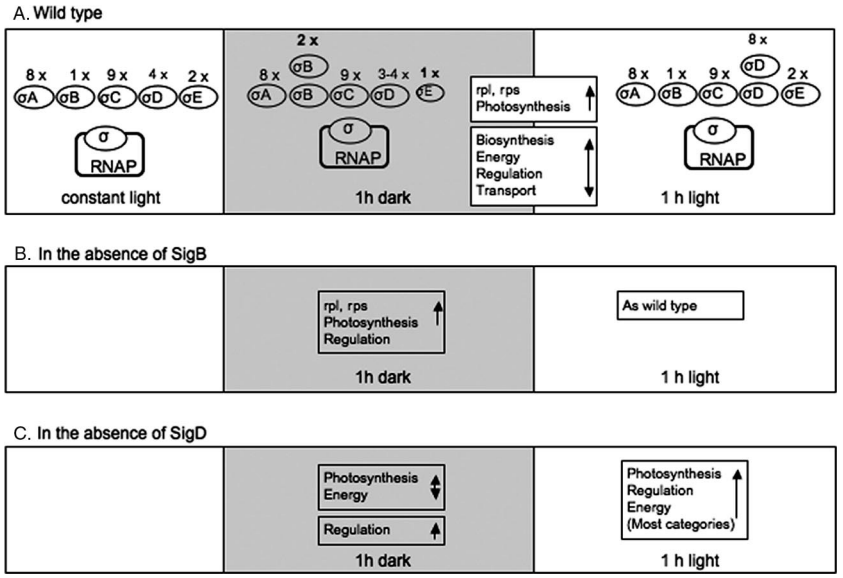

FIG. 3. Relationship between sigma factor abundance and the impact of SigB or SigD removal on global regulation of gene expression in light-to-dark and dark-to-light transitions. (A) Wild-type *Synechocystis* sp. strain PCC 6803. The relative amounts of the sigma factor proteins are represented by the numbers above the sigma factors, based on the calculations of Imamura et al. (19). A twofold increase in SigB and a twofold decrease in SigE were observed following transfer to the dark for 1 h. Functional groups containing differentially regulated genes at 1 h of dark and 1 h of light are shown in boxes; the up arrow indicates that transcripts were up-regulated in the light compared to their status in the dark, and the two-headed arrow indicates that there were both up- and down-regulated genes in these groups. The *rpl* and *rps* genes encode ribosome protein large subunits and small subunits, respectively. (B) Differentially regulated genes in Δ*sigB* compared to regulation in the wild type at 1 h of dark and 1 h of light. The up arrow indicates functional categories that contained up-regulated transcripts in the mutant compared to the wild type. (C) Differentially regulated genes in the Δ*sigD* strain compared to the wild type at 1 h of dark and 1 h of light. The up arrow indicates functional categories that contain up-regulated transcripts in the mutant, and the two-headed arrow indicates both increased and decreased transcript abundance in genes belonging to these functional categories.

type, especially in genes encoding ribosomal proteins and many hypothetical proteins. It is striking that the major effect of removing SigB was to up-regulate at least some genes in many different categories. Thus, according to the stochastic release model, the absence of SigB permitted increased binding of other σ factors to the RNAP and altered transcription of a select number of genes, especially the major operon that encodes ribosomal proteins. As noted in Fig. 1A and Table 3, the absence of SigB alone has little impact on cell-doubling times under either photoautotrophic or mixotrophic conditions. The role of SigB as a positive regulator of gene expression under heat shock conditions is consistent with this model. Following heat shock, SigB becomes the most abundant σ factor in the cell, increasing by 13-fold (19). The enhanced transcription of genes such as those encoding heat shock proteins may be explained by increased transcription from SigB promoters due to increased amounts of the SigB/RNAP holoenzyme.

The absence of SigD has a more profound impact on global regulation of transcription. Compared to SigB, SigD represented a larger proportion of σ factors available in both the light and the dark; during growth in the light, SigD represented more than one-quarter of the total number of sigma factors. The results shown in Fig. 1C clearly demonstrated a much greater influence of SigD removal in the light than in the dark. In the light, many genes exhibited transcription in Δ*sigD* that was different compared to that in the wild type, and many of them were up-regulated; this included numerous changes in photosynthesis genes. In the absence of SigD, approximately two-thirds of the genes differentially regulated in the light were up-regulated, whereas only approximately half of the genes differentially regulated in the dark were up-regulated. In accordance with the stochastic release model, we suggest that, as well as abolishing SigD promoter specific transcription, the absence of SigD allowed increased transcription from other sigma factors binding to RNAP.

Taken together, the results for both mutants indicated that SigB is much more important in the dark, whereas SigD has a greater influence on global transcription in the light. The stochastic release model explains the gene expression changes we observed but does not preclude other models of σ factor action. In fact, the stochastic release model may represent the basic σ-factor-regulatory mechanism that may be modified under specific conditions by more complex interactions.

The results with the Δ*sigB* Δ*sigE* double mutant can be interpreted in a similar fashion. The alteration in transcription in the double mutant in the presence of glucose is most pronounced in the light, and in this case, many more genes were down-regulated in the mutant than in the wild type. These changes probably reflect different growth states of the wild type and mutant (Fig. 1D). In the dark, the single greatest impact was on the transcript levels of genes encoding ribosomal proteins (Fig. 1F). In Δ*sigB*, the transcript level of the ribosomal proteins was not decreased, as occurs in the wild type, such that there was typically little difference between transcript levels in the light and the dark (the transcript levels of some genes decreased from 1.5- to 2.0-fold). Similarly, for Δ*sigB* Δ*sigE*, the transcript levels of the ribosomal protein genes were two- to

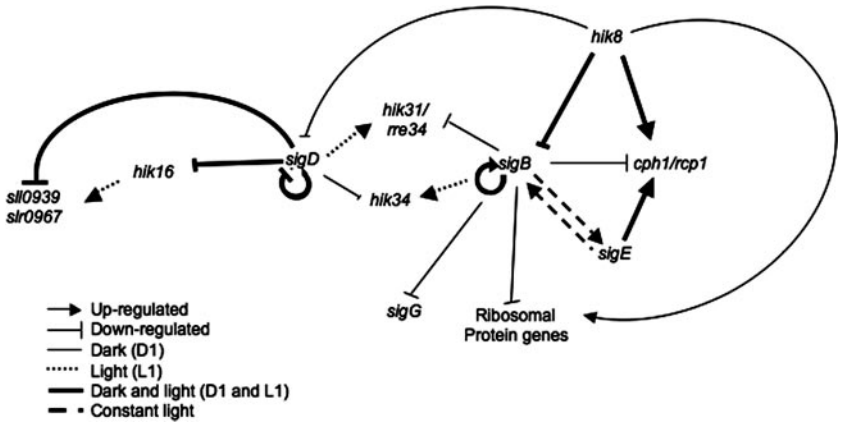

FIG. 4. Model of sigma factor and histidine kinase interactions in the light and the dark. Interactions are based on transcript abundance in the Δ*hik8*, Δ*sigB*, Δ*sigD*, and Δ*sigE* mutants compared to that of the wild type. The data are from Sherman and Singh (43), this study, and Osanai et al. (35) for the Δ*hik8* strain, Δ*sigB* and Δ*sigD* strains, and Δ*sigE* strain, respectively. The light/dark conditions at which the results were obtained are shown in the key.

fourfold higher in the dark than they were for the wild type. Indeed, the ribosomal protein genes represented 32 out of the top 50 genes that showed increases in Δ*sigB* Δ*sigE* relative to the levels for the wild type. Therefore, as cultures are grown over long periods with short days (8 h of light/16 h of dark), the cells retained high levels of ribosomal protein transcripts. This, combined with the down-regulation of genes involved in glycolysis and the OPP pathway in the dark, may adversely affect many components of cell growth and metabolism and cause the impaired mixotrophic growth under different light regimes. In the Δ*hik8* mutant, we showed down-regulation of ribosome protein genes, indicating that *hik8* is involved in positive regulation of these genes (43). This is consistent with the negative regulation of *sigB* by *hik8* shown in Fig. 4, as the absence of SigB resulted in increased levels of ribosome protein gene transcripts (Fig. 1B). Therefore, an increase in SigB in Δ*hik8* may be involved in down-regulation of the ribosomal protein genes in this strain. Based on our data, we propose that the cell down-regulated the level of ribosomal proteins in the dark in order to conserve resources, and this may be a major reason why growth of cells grown in light-dark cycles is significantly slower than that in continuous light.

Our results indicated that ribosomal protein genes represent one of the key regulated targets for the σ factors, as diagrammed in Fig. 4. In this figure, we summarize data from this study and from recent papers (35, 43) and demonstrate the relationship between the σ factors and select two-component histidine kinases. A recent paper developed a more complex network of sigma factor interactions (50), but we feel that our hypothesis provides a more comprehensive understanding of this regulation. We suggest that SigB is central to this regulatory network, especially in the dark. In the light, the absence of SigE affected SigB and *cph1*/*rcp1*, although the SigE level is relatively low and was changed only twofold between the light and the dark (Fig. 3). Removal of SigD altered transcription of a number of histidine kinases, including *hik31*. This histidine kinase, together with Hik8 and SigE, is known to play a role in sugar metabolism. Thus, mutants lacking *sigE*, *hik8*, and *hik31* demonstrated impaired growth under mixotrophic conditions and altered transcription of genes involved in glycolysis and/or the OPP pathway (20, 35, 43) This model illustrates the complex balance between the many regulatory components involved in fine-tuning the metabolic response of the cyanobacterium to changes in its light environment.

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REFERENCES

1. **Aoki, S., T. Kondo, and M. Ishiura.** 2002. A promoter-trap vector for clock-controlled genes in the cyanobacterium *Synechocystis* sp. PCC 6803. J. Microbiol. Methods **49**:265–274.
2. **Aoki, S., T. Kondo, H. Wada, and M. Ishiura.** 1997. Circadian rhythm of the cyanobacterium *Synechocystis* sp. strain PCC 6803 in the dark. J. Bacteriol. **179**:5751–5755.
3. **Asayama, M.** 2006. Regulatory system for light-responsive gene expression in photosynthesizing bacteria: *cis*-elements and *trans*-acting factors in transcription and post-transcription. Biosci. Biotechnol. Biochem. **70:**565–573.
4. **Asayama, M., S. Imamura, S. Yoshihara, A. Miyazaki, N. Yoshida, T. Sazuka, T. Kaneko, O. Ohara, S. Tabata, T. Osanai, K. Tanaka, H. Takahashi, and M. Shirai.** 2004. SigC, the group 2 sigma factor of RNA polymerase, contributes to the late-stage gene expression and nitrogen promoter recognition in the cyanobacterium *Synechocystis* sp. strain PCC 6803. Biosci. Biotechnol. Biochem. **68:**477–487.
5. **Benjamini, Y., and Y. Hochberg.** 1995. Controlling the false discovery rate—a practical and powerful approach to multiple testing. J. R. Stat. Soc. B. **57:**289–300.
6. **Campbell, E., and S. Darst.** 2005. Regulation of bacterial transcription by anti-σ factors, p. 1–15. *In* G. Waksman, M. Caparon, and S. Hultgren (ed.), Structural biology of bacterial pathogenesis. American Society for Microbiology, Washington, DC.
7. **Chamberlin, M.** 1976. RNA polymerase–an overview, p. 17–68. *In* R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
8. **Colón-López, M. S., D. M. Sherman, and L. A. Sherman.** 1997. Transcriptional and translational regulation of nitrogenase in light-dark- and continuous-light-grown cultures of the unicellular cyanobacterium *Cyanothece* sp. strain ATCC 51142. J. Bacteriol. **179:**4319–4327.
9. **Foster, J. S., A. K. Singh, L. J. Rothschild, and L. A. Sherman.** 2007. Growth-phase dependent differential gene expression in *Synechocystis* sp. strain PCC 6803 and regulation by a group 2 sigma factor. Arch. Microbiol. **187:**265–279.
10. **Gill, R. T., E. Katsoulakis, W. Schmitt, G. Taroncher-Oldenburg, J. Misra, and G. Stephanopoulos.** 2002. Genome-wide dynamic transcriptional profiling of the light-to-dark transition in *Synechocystis* sp. strain PCC 6803. J. Bacteriol. **184:**3671–3681.
11. **Gill, S. C., S. E. Weitzel, and P. H. von Hippel.** 1991. *Escherichia coli* σ^{70} and NusA proteins. I. Binding interactions with core RNA polymerase in solution and within the transcription complex. J. Mol. Biol. **220:**307–324.
12. **Gruber, T. M., and C. A. Gross.** 2003. Multiple sigma subunits and the

partitioning of bacterial transcription space. Annu. Rev. Microbiol. **57:**441–466.
13. **Herranen, M., E. M. Aro, and T. Tyystjarvi.** 2001. Two distinct mechanisms regulate the transcription of photosystem II genes in *Synechocystis* sp. PCC 6803. Physiol. Plant **112:**531–539.
14. **Hihara, Y., A. Kamei, M. Kanehisa, A. Kaplan, and M. Ikeuchi.** 2001. DNA microarray analysis of cyanobacterial gene expression during acclimation to high light. Plant Cell **13:**793–806.
15. **Hihara, Y., K. Sonoike, M. Kanehisa, and M. Ikeuchi.** 2003. DNA microarray analysis of redox-responsive genes in the genome of the cyanobacterium *Synechocystis* sp. strain PCC 6803. J. Bacteriol. **185:**1719–1725.
16. **Huang, L., M. P. McCluskey, H. Ni, and R. A. LaRossa.** 2002. Global gene expression profiles of the cyanobacterium *Synechocystis* sp. strain PCC 6803 in response to irradiation with UV-B and white light. J. Bacteriol. **184:**6845–6858.
17. **Imamura, S., M. Asayama, H. Takahashi, K. Tanaka, H. Takahashi, and M. Shirai.** 2003a. Antagonistic dark/light-induced SigB/SigD, group 2 sigma factors, expression through redox potential and their roles in cyanobacteria. FEBS Lett. **554:**357–362.
18. **Imamura, S., K. Tanaka, M. Shirai, and M. Asayama.** 2006. Growth phase-dependent activation of nitrogen-related genes by a control network of group 1 and group 2 σ factors in a cyanobacterium. J. Biol. Chem. **281:**2668–2675.
19. **Imamura, S., S. Yoshihara, S. Nakano, N. Shiozaki, A. Yamada, K. Tanaka, H. Takahashi, M. Asayama, and M. Shirai.** 2003. Purification, characterization, and gene expression of all sigma factors of RNA polymerase in a cyanobacterium. J. Mol. Biol. **325:**857–872.
20. **Kahlon, S., K. Beeri, H. Ohkawa, Y. Hihara, O. Murik, I. Suzuki, T. Ogawa, and A. Kaplan.** 2006. A putative sensor kinase, Hik31, is involved in the response of *Synechocystis* sp. strain PCC 6803 to the presence of glucose. Microbiology **152:**647–655.
21. **Kaneko, T., S. Sato, H. Kotani, A. Tanaka, E. Asamizu, Y. Nakamura, N. Miyajima, M. Hirosawa, M. Sugiura, S. Sasamoto, T. Kimura, T. Hosouchi, A. Matsuno, A. Muraki, N. Nakazaki, K. Naruo, S. Okumura, S. Shimpo, C. Takeuchi, T. Wada, A. Watanabe, M. Yamada, M. Yasuda, and S. Tabata.** 1996. Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. DNA Res. **3:**109–136.
22. **Kerr, M. K., and G. A. Churchill.** 2001. Experimental design for gene expression microarrays. Biostatistics **2:**183–201.
23. **Kerr, M. K., and G. A. Churchill.** 2001. Statistical design and the analysis of gene expression microarray data. Genet. Res. **77:**123–128.
24. **Kucho, K., K. Okamoto, Y. Tsuchiya, S. Nomura, M. Nango, M. Kanehisa, and M. Ishiura.** 2005. Global analysis of circadian expression in the cyanobacterium *Synechocystis* sp. strain PCC 6803. J. Bacteriol. **187:**2190–2199.
25. **Lemeille, S., J. Geiselmann, and A. Latifi.** 2005. Crosstalk regulation among group 2-sigma factors in *Synechocystis* PCC6803. BMC Microbiol. **5:**18.
26. **Li, H., A. K. Singh, L. M. McIntyre, and L. A. Sherman.** 2004. Differential gene expression in response to hydrogen peroxide and the putative PerR regulon of *Synechocystis* sp. strain PCC 6803. J. Bacteriol. **186:**3331–3345.
27. **Matsui, M., T. Yoshimura, Y. Wakabayashi, S. Imamura, A. Tanaka, H. Takahashi, M. Asayama, and M. Shirai.** 2007. Interference expression at levels of the transcript and protein among group 1, 2, and 3 sigma factor genes in a cyanobacterium. Microbes Environ. **22:**32–43.
28. **Meunier, P. C., M. S. Colon-Lopez, and L. A. Sherman.** 1997. Temporal changes in state transitions and photosystem organization in the unicellular, diazotrophic cyanobacterium *Cyanothece* sp. ATCC 51142. Plant Physiol. **115:**991–1000.
29. **Mohamed, A., J. Eriksson, H. D. Osiewacz, and C. Jansson.** 1993. Differential expression of the *psbA* genes in the cyanobacterium *Synechocystis* 6803. Mol. Gen. Genet. **238:**161–168.
30. **Mooney, R. A., S. A. Darst, and R. Landick.** 2005. Sigma and RNA polymerase: an on-again, off-again relationship? Mol. Cell **20:**335–345.
31. **Mostertz, J., and M. Hecker.** 2003. Patterns of protein carbonylation following oxidative stress in wild-type and *sigB Bacillus subtilis* cells. Mol. Genet. Genomics **269:**640–648.
32. **Murakami, K. S., and S. A. Darst.** 2003. Bacterial RNA polymerases: the whole story. Curr. Opin. Struct. Biol. **13:**31–39.
33. **Muramatsu, M., and Y. Hihara.** 2006. Characterization of high-light-responsive promoters of the *psaAB* genes in *Synechocystis* sp. PCC 6803. Plant Cell Physiol. **47:**878–890.
34. **Muramatsu, M., and Y. Hihara.** 2007. Coordinated high-light response of genes encoding subunits of photosystem I is achieved by AT-rich upstream sequences in the cyanobacterium *Synechocystis* sp. strain PCC 6803. J. Bacteriol. **189:**2750–2758.
35. **Osanai, T., Y. Kanesaki, T. Nakano, H. Takahashi, M. Asayama, M. Shirai, M. Kanehisa, I. Suzuki, N. Murata, and K. Tanaka.** 2005. Positive regulation of sugar catabolic pathways in the cyanobacterium *Synechocystis* sp. PCC 6803 by the group 2 sigma factor SigE. J. Biol. Chem. **280:**30653–30659.
36. **Postier, B. L., H. L. Wang, A. Singh, L. Impson, H. L. Andrews, J. Klahn, H. Li, G. Risinger, D. Pesta, M. Deyholos, D. W. Galbraith, L. A. Sherman, and R. L. Burnap.** 2003. The construction and use of bacterial DNA microarrays based on an optimized two-stage PCR strategy. BMC Genomics **4:**23.
37. **Raffaelle, M., E. I. Kanin, J. Vogt, R. R. Burgess, and A. Z. Ansari.** 2005. Holoenzyme switching and stochastic release of sigma factors from RNA polymerase in vivo. Mol. Cell **20:**357–366.
38. **Reddy, K. J., R. Webb, and L. A. Sherman.** 1990. Bacterial RNA isolation with one hour centrifugation in a table-top ultracentrifuge. BioTechniques **8:**250–251.
39. **Schaefer, M. R., and S. S. Golden.** 1989. Differential expression of members of a cyanobacterial *psbA* gene family in response to light. J. Bacteriol. **171:**3973–3981.
40. **Shimamoto, N., T. Kamigochi, and H. Utiyama.** 1986. Release of the sigma subunit of *Escherichia coli* DNA-dependent RNA polymerase depends mainly on time elapsed after the start of initiation, not on length of product RNA. J. Biol. Chem. **261:**11859–11865.
41. **Singh, A. K., L. M. McIntyre, and L. A. Sherman.** 2003. Microarray analysis of the genome-wide response to iron deficiency and iron reconstitution in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Physiol. **132:**1825–1839.
42. **Singh, A. K., and L. A. Sherman.** 2002. Characterization of a stress-responsive operon in the cyanobacterium *Synechocystis* sp. strain PCC 6803. Gene **297:**11–19.
43. **Singh, A. K., and L. A. Sherman.** 2005. Pleiotropic effect of a histidine kinase on carbohydrate metabolism in *Synechocystis* sp. strain PCC 6803 and its requirement for heterotrophic growth. J. Bacteriol. **187:**2368–2376.
44. **Singh, A. K., T. C. Summerfield, H. Li, and L. A. Sherman.** 2006. The heat shock response in the cyanobacterium *Synechocystis* sp. strain PCC 6803 and regulation of gene expression by HrcA and SigB. Arch. Microbiol. **186:**273–286.
45. **Travers, A. A., and R. R. Burgess.** 1969. Cyclic re-use of the RNA polymerase sigma factor. Nature **222:**537–540.
46. **Tu, C. J., J. Shrager, R. L. Burnap, B. L. Postier, and A. R. Grossman.** 2004. Consequences of a deletion in *dspA* on transcript accumulation in *Synechocystis* sp. strain PCC6803. J. Bacteriol. **186:**3889–3902.
47. **Verhoeven, K. J. F., K. L. Simonsen, and L. M. McIntyre.** 2005. Implementing false discovery rate control: increasing your power. Oikos **108:**643–647.
48. **Williams, J. G. K.** 1988. Construction of specific mutations in photosystem II photosynthetic reaction center by genetic engineering methods in *Synechocystis* 6803 Methods Enzymol. **167:**766–778.
49. **Yang, C., Q. Hua, and K. Shimizu.** 2002. Integration of the information from gene expression and metabolic fluxes for the analysis of the regulatory mechanisms in *Synechocystis*. Appl. Microbiol. Biotechnol. **58:**813–822.
50. **Yoshimura, T., S. Imamura, K. Tanaka, M. Shirai, and M. Asayama.** 2007. Cooperation of group 2 σ factors, SigD and SigE for light-induced transcription in the cyanobacterium *Synechocystis* sp. PCC 6803. FEBS Lett. **581:**1495–1500.