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ORIGINAL PAPER

The heat shock response in the cyanobacterium *Synechocystis* sp. Strain PCC 6803 and regulation of gene expression by HrcA and SigB

Abhay K. Singh · Tina C. Summerfield · Hong Li · Louis A. Sherman

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Abstract We report on the genome-wide response, based on DNA microarrays, of the cyanobacterium *Synechocystis* sp. PCC 6803 wild type and Δ*sigB* to a 15 min heat shock. Approximately 9% of the genes in wild type and Δ*sigB* were significantly regulated ($P < 0.001$) following this treatment, with chaperones induced the most. The absence of *sigB* had no dramatic effect on specific genes induced by heat shock, but did affect the level of transcription of the chaperones. In addition, *sigE* was induced in Δ*sigB*. Comparison of global gene expression of the wild type and the *hrcA* mutant at 30°C enabled us to examine the HrcA regulon, which included *groESL* and *groEL2*. Several genes belonging to specific functional groups (e.g., pilus biogenesis/assembly and phototaxis, biosynthesis of aromatic amino acids, murien sacculus and peptidoglycan, surface polysaccharides, and the Sec pathway) were differentially regulated following heat shock. We used results from knock-out mutants in *sigB, sigD* and *sigE* to construct a model of the network of group 2 sigma factor regulation upon each other. In this network, SigB represented the major node and SigE a secondary node. Overall, we determined that transcription of the heat-shock genes are regulated to various degrees by SigB, SigE and HrcA.

Keywords Heat shock · Cyanobacteria · Sigma factors · HrcA · Microarrays · Differential gene expression · Chaperones

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A. K. Singh · T. C. Summerfield · H. Li · L. A. Sherman (✉)
Department of Biological Sciences, Purdue University,
1392 Lilly Hall of Life Sciences,
West Lafayette, IN, 47907, USA
e-mail: lsherman@bilbo.bio.purdue.edu

A. K. Singh
e-mail: asingh@biology2.wustl.edu

H. Li
e-mail: hli710@yahoo.com

Present Address:
A. K. Singh
Department of Biology, Washington University,
St. Louis, MO 63130, USA

Present Address:
H. Li
UMDNJ-Robert Wood Johnson Medical School,
Piscataway, NJ 08854, USA

Introduction

Exposure of organisms to a sudden increase in temperature triggers a ubiquitous and homeostatic cellular response (Webb and Sherman 1994; Gross 1996; Yura et al. 2000). This heat shock response is characterized by the rapid induction of specific sets of genes that encode heat shock proteins (HSP), many of which function as molecular chaperones or ATP-dependent proteases (Webb and Sherman 1994, Gross 1996; Yura et al. 2000). Molecular chaperones play important roles in protein folding and the assembly of oligomers and, help to stabilize protein structure and solubilize protein aggregates. Heat shock inducible proteases are required to degrade irreversibly damaged proteins (Gross 1996).

The heat shock response in bacteria is best characterized in *Eschericia coli* and *Bacillus subtilis*. In *E. coli*,

expression of HSPs is controlled by two sigma factors, σ^{32} and σ^{24} (Gross 1996; Yura et al. 2000; Gruber and Gross 2003). The σ^{32} regulon consists of at least 30 heat inducible proteins, including HSPs that are essential for growth at the high temperature and most of the ATP-dependent proteases. The σ^{24} regulon includes σ^{32}, σ^{24}, DegP and FkpA (Yura et al. 2000). σ^{24} autogenously regulated its own expression as well as that of σ^{32}. The other two members of the regulon include a periplasmic protease (DegP) and a periplasmic peptidyl prolyl isomerase (FkpA) that can assist in protein turnover or folding, respectively (Danese and Silhavy 1997). There are also additional heat-inducible proteins that are not regulated by these two sigma factors. *B. subtilis* uses entirely different mechanisms for the heat shock response. The three known regulons in *B. subtilis* are the controlling inverted repeat of chaperone expression (CIRCE)/heat shock regulation at CIRCE elements (HrcA), the σ^B-dependent and the CtsR regulons (Helmann et al. 2001). CIRCE consists of a 9 bp inverted repeat separated by 9 bp. The HrcA acts as a repressor and prevents the expression of HSPs by binding directly to CIRCE. Additionally, there are numerous genes expressed in response to heat shock that are not controlled by these three systems, suggesting the presence of additional regulons (Helmann et al. 2001).

Cyanobacteria are oxygenic photosynthetic microbes that are closely related to chloroplasts of higher plants and they synthesize a number of HSPs when subjected to heat shock (Webb et al. 1990; Webb and Sherman 1994). Interestingly, expression of some of these HSPs is also light dependent (Asadulghani et al. 2003). A specific contribution of these HSPs in thermotolerance in cyanobacteria has been shown using mutants of ClpB, HtpG, and HspA (Eriksson and Clarke 1996; Tanaka and Nakamoto 1999; Lee et al. 2000; Nakamoto et al. 2000). It was demonstrated that the absence of these genes led to comparatively more sensitivity to high temperature than those of *E. coli* or other organisms. More recently, the physiological role of HspA has become better defined in *Synechococcus* strain ECT16-1 (Nitta et al. 2005). These authors concluded that HspA stabilized nucleoid morphology and is involved in stabilizing both thylakoid and cytoplasmic proteins (Nitta et al. 2005). HspA was also shown to play a role in providing thylakoid stability in high light (Nitta et al. 2005), as well as helping the cell respond to salt stress (Asadulghani et al. 2004).

Such studies suggest that HSPs play an important role in cyanobacteria, yet the mechanism involved in the regulation of HSPs in cyanobacteria is largely unknown. Two *cis*-acting elements, CIRCE and an AT-rich region found in the 5′-untranslated region, have been implicated (Kojima and Nakamoto 2002; Nakamoto et al. 2003). However, deletion of *hrcA* did not have a major effect on the expression of many of the HSPs; this led to the suggestion that both negative and positive regulation may contribute to the induction of these genes during heat shock (Nakamoto et al. 2003). The possible involvement of sigma factors in the regulation of HSPs is also mostly unknown. *Synechocystis* sp. PCC 6803 has nine sigma factors and *sigB* was shown to be the only highly inducible sigma factor following heat shock treatment (Imamura et al. 2003; Inaba et al. 2003, Li et al. 2004). Recently a histidine kinase, Hik34 (slr1285), was shown to play a role in the thermotolerance of *Synechocystis* sp. PCC 6803 by acting as a negative regulator of some heat shock genes (Suzuki et al. 2005). However, the absence of Hik34 reduced the induction of heat shock genes to varying extents, suggesting regulation by additional mechanisms (Suzuki et al. 2005).

We have utilized microarrays to analyze gene regulation in *Synechocystis* sp. PCC 6803 under many physiological conditions and with specific mutants (Singh et al. 2003; Li et al. 2004). In this paper, we will detail the global response to heat shock in the wild-type strain and the role of SigB and HrcA in the regulation of heat-shock induced gene expression in *Synechocystis* sp. PCC 6803. We also describe experiments using mutants in SigD and SigE that permit us to construct a regulatory network among the group 2 sigma factors.

Materials and methods

Strains, growth conditions and stress induction

The standard growth condition was at 30 ± 2°C with a light intensity of ~30 $\mu E\, m^{-2}\, s^{-1}$ with shaking at 125 rpm in BG-11 medium. The non-buffered BG-11 medium was used for cell growth and high temperature treatments. The cell density of the cultures was determined by optical density at 730 nm as previously described (Colon-Lopez et al. 1997; Meunier et al. 1997) and the doubling times of the wild type and the mutants were virtually identical under these growth conditions (data not shown). For the heat shock experiment, cultures of wild-type and mutant strains of *Synechocystis* sp. PCC 6803 were grown to exponential phase (1×10^8 cells ml^{-1}) and cells were harvested and resuspended in fresh BG-11 medium to 1×10^9 cells ml^{-1}. These washed cells were then added to a pre-warmed BG-11 medium that was kept in a water bath maintained at temperatures ranging from 40 to 50°C to obtain 5×10^7 cells ml^{-1}. Cultures were illuminated

during treatments at 25 μmol photons $m^{-2} s^{-1}$. The differential absorption spectra at room temperature and Northern blots were used to determine the optimum condition for the heat shock treatment. The determining factors for optimum heat shock time and temperature were the induction of the expression of previously identified HSPs, but with no photobleaching. Treatment of the cultures at 45°C for 15 min met these conditions (data not shown). For growth in BG-11 ($-NO_3$), the cells were spun down as above and resuspended in fresh BG-11 ($-NO_3$) medium at a cell density of 1×10^8 cells ml^{-1} and grown for 24 h under the normal growth conditions. Cells were treated with hydrogen peroxide as previously described (Li et al. 2004).

Construction of mutants

Insertion or deletion mutations of the following genes were constructed: *hrcA* (sll1670), *sigB* (sll0306), *sigD* (sll2012) and *sigE* (sll1689). Primers were used to amplify regions of the *Synechocystis* sp. PCC 6803 genome containing these genes, the amplified fragments were cloned into pGEM-T (Promega) and the open reading frames disrupted by the addition of an antibiotic resistance cassette. For *hrcA* the primers: 5′-CAG GGA GAT GAA GCA AGA CC-3′; 5′-TCG CAT GGA AGC TCT GTA CG-3′ were used, giving a 2,184 bp product, *BstEII* digestion deleted 717 bp from the gene. The primers used to amplify *sigB* were 5′-CCT GTG ATT ATG ACT ACA TCG AGC-3′ and 5′-CAT AAT GAA CCG TTC CGG CTG GTC-3′ resulting in a 3,123 bp product in which *sigB* was interrupted by digestion with *SmaI*. A 2,430 bp PCR product containing *sigD* was obtained using the primers 5′-GGA GCC AAA CGA CCT TTT AAT TGC-3′ and 5′-GTT AAC TGT GTC CTA TCC CAT TGC-3′, *XbaI* was used to remove 264 bp of this gene. In the case of *sigE* 5′-CAA GGA ATT GGT CCA ACG CTA TCG-3′ and 5′-GCG TCA ACG ATG CCG AAA TTA TCG-3′ were used to produce a 2,929 bp fragment which was digested *BamHI* to remove a 448 bp region of *sigE*. A spectinomycin resistance cassette (from pRL453) was used for *hrcA*, *sigB* and *sigE* and a kanamycin resistance cassette (from pRL448) was used for *sigD*. Complete segregation of the mutants was obtained as confirmed by PCR and southern blotting (data not shown).

RNA isolation and northern analysis

Total RNA was extracted and purified using phenol–chloroform extraction and $CsCl_2$ gradient purification as previously described (Reddy et al. 1990; Singh and Sherman 2002). Five microgram of total RNA was fractionated by electrophoresis on a 1.0% agarose gel with 0.6 M formaldehyde. RNA was transferred to a nylon membrane and fixed by baking at 80°C for 2 h in a vacuum oven. The blots were hybridized with α-^{32}P-labeled DNA probes prepared by random primer labeling using a Ready-To-Go kit (Pharmacia Biotech, Piscataway, NJ, USA). Hybridization was performed at 42°C with 50% formamide. Staining the ribosomal RNA with ethidium bromide standardized the equal loading of total RNA.

Microarray experimental design and statistical analysis

The complete description of array construction is described in Postier et al. (2003). The cDNA labeling, glass treatment, prehybridization and hybridization protocols are described in detail in Singh et al. (2003). The biological variation was sampled by extracting RNA from three separate experiments and pooling them prior to hybridization. This strategy has been used in many microarray experiments (Arfin et al. 2000; Peng et al. 2003) and simplifies the final analysis. Microarray experiments involved a loop design that allowed comparison of all conditions by using an ANOVA model (Singh et al. 2003; Li et al. 2004). We could analyze the effect of treatment on the wild type, on the various mutants, and on the relationship between the wild type and the mutant. We report on three separate loop experiments: heat shock, by changing temperature from 30 to 45°C for 15 min (wild type and Δ*sigB*); effect of 5 mM H_2O_2 for 15 min on wild type and Δ*sigD*; and, growth of wild type and Δ*sigE* in regular BG-11 and BG-11 ($-NO_3$) for 24 h. In these later two experiments, we were only interested in the comparison between wild type and the mutant and we will not analyze the effects of the treatments further in this report. In addition, wild type and Δ*hrcA* were compared at 30°C in a dye swap experiment. Since HrcA acts as a repressor, we only compared wild type (containing a functional HrcA repressor) versus Δ*hrcA* (no repressor) at 30°C to identify the genes controlled by HrcA.

Spot intensities of the images were quantified by using Quantarray 3.0 (Packard BioChip Technologies, Boston, Mass). In the case of the loop design, data for the slides used in each experiment were then collated into two data sets (one for each experiment) by using SAS (version 8.02; SAS Institute, Cary, NC, USA). The local background was subtracted from each spot. For each replicate block on a slide, there were 422 empty spots, and there were three replicates per slide. We examined the distribution of spot 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 (703 genes in the wild type vs. Δ*sigB* heat shock 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). Each experiment contained two genotypes (mutant and wild-type for controls) and two stimuli (mutant and wild-type for heat shock) for a total of four treatment combinations. The effects of the mutant and the temperature stimulus were examined in an analysis of variance (ANOVA) essentially as described (Kerr and Churchill 2001; Singh et al. 2003; Li et al. 2004). Once the analysis was completed, we focused our attention on statistically significant and interesting genes (P value < 0.001) that exhibited a change of at least 1.4-fold (Singh et al. 2003; Li et al. 2004). In some cases, we included genes with a P value of 0.05 because they augmented or completed a functional category. Our objective was to identify genes that exhibited differential expression for further experimentation. Thus, we bracketed our interpretation of the results with a conservative (Bonferroni) threshold (2×10^{-5}) and a liberal 0.001 criterion, and we used a fold change filter to focus our efforts. The entire data set for the Δ*sigB* heat shock experiment is shown in Table S1 in the supplemental material, which include the fold changes and the various p values from the ANOVA analysis. For the microarray comparison of the wild-type and Δ*hrcA* strains, the local background was subtracted from each spot and the spots were normalized using the total signal intensity of the appropriate dye for that slide. The data were analyzed using the software Cyber-T (http://www.visitor.ics.uci.edu/cgi-bin/genex/cybert/CyberT-8.0.form.pl?DATATYPE=CE); this procedure employs a Bayesian probabilistic framework where the variance of each gene is combined with the local background variance of neighboring genes to determine the strength of background variance (Baldi and Long 2001; Long et al. 2001).

Results

Overview of Microarray results for heat shock in wild type and Δ*sigB*

The effect of heat shock on gene expression in wild type and Δ*sigB*, based on the functional categories, is shown in Table 1. In both strains, approximately 9% of all genes demonstrated changes in transcript levels that were at least 1.4-fold ($P < 0.001$). The use of a liberal filter, along with the requirement for similar significance in both experiments, provided a comprehensive overview of the impact that heat shock had on gene expression for the different functional categories. As seen in Table 1, the total number of genes differentially regulated in response to heat shock was similar in both wild type and Δ*sigB*. The functional groups with significantly differentially regulated genes are listed in Table 2 and we will discuss these specific functional groups in detail. All of the genes in Table 2 met the Bonferonni threshold ($P < 2 \times 10^{-5}$).

Cellular processes

Genes coding for proteins involved in the cellular processes (chaperones, chemotaxis, and protein and peptide secretion) were the most highly regulated following the heat shock treatment (Table 2). The transcript levels of eight chaperones (*groEL1*, *groES*, *groEL2*, *htpG*, *hspA*, the two copies of *dnaJ* and *dnaK*) increased significantly, with induction varying from 2.4- to 10.3-fold in the wild type (Table 2). The fold induction of *htpG* was significantly reduced in the absence of SigB (four fold in Δ*sigB* vs. 10.3-fold in wild type), whereas *dnaJ* (slr0093) was induced slightly more in Δ*sigB*. To a lesser extent, the fold induction of *hspA*, *dnaK*, *groEL1* and *groES* was also reduced in Δ*sigB*. Recently, Tuominen et al. (2006) also showed lesser accumulation of *hspA* transcript in the Δ*sigB* mutant compared to wild type, following 60 min heat shock. The modest effect of SigB on the heat shock response led us to investigate other strategies by which *Synechocystis* sp. PCC 6803 could regulate distinct sets of HSPs and the effect of a mutation in *hrcA* will be discussed shortly.

Cyanobacteria flourish under environmental conditions in which nutrients, light, metals and temperature can fluctuate and one possible survival mechanism is to move towards a favorable condition. Several structural and regulatory genes involved in pilus biogenesis/assembly and phototaxis have been identified in *Synechocystis* sp. PCC 6803 (Bhaya 2004). One of the photoreceptor genes involved in phototaxis is encoded by sll0041 (Bhaya et al. 2001). This gene was significantly induced in response to heat shock in wild type and somewhat less so in Δ*sigB* (Table 2). Other possible phototaxis genes that were up regulated by heat shock included sll1107, a type IV pilus biogenesis protein PilI homolog, and slr1274, a putative fimbrial assembly protein, PilM. Interestingly, certain putative phototaxis genes were somewhat down regulated (−1.5 to −1.8-fold), including genes annotated as *pilA1* and *pilA2*

Table 1 Functional categories of heat shock-responsive genes in wild- type and Δ*sigB* *Synechocystis* sp. PCC 6803

General pathways	Number of genes	Differentially regulated genes in	
		WT (Up)[b]	Δ*sigB* (Up)[b]
Amino acid biosynthesis	97	16 (14)	20 (17)
Biosynthesis of cofactors, prosthetic groups, and carriers	124	10 (6)	10 (7)
Cell envelope	67	9 (7)	9 (8)
Cellular processes	76	22 (16)	19 (16)
Central intermediary metabolism	31	1 (1)	2 (2)
DNA replication, restriction, recombination, and repair	60	1 (1)	3 (3)
Energy metabolism	132	11 (4)	8 (7)
Hypothetical	1,076	88 (60)	101 (64)
Other categories	306	23 (14)	26 (18)
Photosynthesis and respiration	141	10 (2)	6 (1)
Purines, pyrimidines, nucleosides, and nucleotides	41	1 (0)	1 (1)
Regulatory functions	146	12 (9)	16 (5)
Transcription	30	9 (3)	7 (3)
Translation	168	29 (14)	22 (16)
Transport and binding proteins	196	8 (7)	10 (6)
Unknown	474	26 (12)	37 (18)
Total number	3,165[a]	276 (170)	297 (191)

Genes were considered differentially regulated when $P < 0.001$ and fold change > 1.4-fold

[a] Total number of genes based on Kazusa annotation prior to May 2002

[b] Number of genes up-regulated in a functional category

(sll1694 and sll1695). Although PilA1 appeared to be involved in pilin production and motility, PilA2 did not (Bhaya et al. 2001).

Transcription

Genes encoding transcriptional proteins demonstrated significant differential expression, including the strong induction of a group 2 sigma factor in wild type and a second group 2 sigma factor in Δ*sigB*. *Synechocystis* sp. PCC 6803 contains nine different sigma factors that have been categorized into four groups (Gruber and Gross 2003): group1 (*sigA*); group 2 (*sigB*, *sigC*, *sigD* and *sigE*); group 3 (*sigF*), and group 4 (*sigG*, *sigH*, and *sigI*). Our microarray results showed that *sigB* was the most up-regulated sigma factor in wild-type following heat shock, as shown previously (Imamura et al. 2003). In addition, two other group 2 sigma factors, *sigD* and *sigE*, were also up-regulated approximately two fold (Table 2). On the other hand, genes coding for the core RNA polymerase subunits (*rpoA*, *rpoB* and *rpoC2*) were somewhat down-regulated (−1.6 to −2.7-fold). Deletion of *sigB* led to a significant decrease in the fold induction of its own transcript, likely due to the accumulation of the truncated transcript of *sigB*. Interestingly, induction of *sigE* was higher in Δ*sigB* (4.6-fold in Δ*sigB* vs. 2.0-fold in wild-type).

Translation

Genes under the sub-functional category of 'degradation of proteins, peptides, and glycopeptides' were highly regulated, including *htrA*, *ctpA*, *clpB1* and *clpB2*. Interestingly, the two genes (slr0164 and slr0165) encoding ATP-dependent *clp* protease proteolytic subunits were somewhat down-regulated (−1.4 to −2.3-fold). Expression of *clpB* genes in response to heat shock has been shown previously in *Synechocystis* sp. PCC 6803 (Nakamoto et al. 2003, Inaba et al. 2003). Both reports showed that *clpB1* was induced in response to heat shock, whereas the heat shock induction of *clpB2* was observed by Inaba et al. (2003), but not by Nakamoto et al. (2003). Our microarray data show that both these genes were induced approximately 2–3-fold in both strains in response to heat shock.

Aromatic amino acid biosynthesis

Several genes in the functional category 'biosynthesis of aromatic amino acids', especially those involved in histidine and tryptophan biosynthesis, were induced significantly. Three genes (*hisA*, *hisB* and *hisC*) that code for proteins involved in histidine biosynthesis were up regulated by 1.6- to 3.8-fold in both wild type

Table 2 Selected differentially regulated genes following heat shock in *Synechocystis* sp. PCC 6803 wild type and Δ*sigB*

Gene	Gene function	WT(45C/30C)[a]	Δ*sigB*(45/30°C)[a]	*P*-treatment
Amino acid biosynthesis				
Aromatic amino acid family				
sll0356	*N*-(5′-phosphoribosyl)anthranilate isomerase (*trpF*)	3.1	3.1	2.4E-08
sll1669	Shikimate kinase (*aroK*)	3.3	3.2	1.6E-16
sll1958	Histidinol phosphate aminotransferase (*hisC*)	1.6	2.4	8.0E-06
slr0500	Imidazoleglycerol-phosphate dehydratase (*hisB*)	3.4	2.7	3.4E-13
slr0546	Indole-3-glycerol phosphate synthase (*trpC*)	2.2	2.8	6.1E-07
slr0652	Phosphorybosilformimino-5-amino-phosphorybosil-4-imidazolecarboxamideisomerase (*hisA*)	3.6	3.8	1.7E-12
slr0738	Anthranilate synthetase alpha-subunit (*trpE*)	2.6	2.8	3.1E-10
Cell envelope				
Murein sacculus and peptidoglycan				
slr0017	UDP-*N*-acetylglucosamine 1-carboxyvinyltransferase	1.8	2.3	9.0E-06
slr0646	Probable D-alanyl-D-alanine carboxypeptidase	2.0	3.0	6.3E-07
slr1656	*N*-acetylglucosamine transferase (*murG*)	3.3	2.5	1.6E-09
slr1708	Probable peptidase	−2.1	−1.5	5.3E-07
slr1744	*N*-acetylmuramoyl-L-alanine amidase	2.4	1.6	8.0E-07
Surface polysaccharides, lipopolysaccharides and antigens				
Sll1395	dTDP-6-deoxy-L-mannose-dehydrogenase (*rfbD*)	1.8	1.6	5.7E-06
Slr0985	dTDP-4-dehydrorhamnose 3,5-epimerase (*rfbC*)	2.6	2.6	6.2E-07
slr1072	GDP-D-mannose dehydratase	2.3	1.7	1.0E-05
Cellular processes				
Chaperones				
sll0170	DnaK protein 2, heat shock protein 70	6.1	3.1	5.5E-10
sll0416	60 kDa chaperonin 2 (*groEL2*)	4.2	4.1	6.3E-10
sll0430	Heat shock protein 90 (*htpG*)	10.3	4.0	5.7E-06
sll0897	DnaJ protein, heat shock protein 40	2.4	2.5	1.1E-12
sll1514	16.6 kDa small heat shock protein (*hspA*)	5.4	3.7	1.3E-07
slr0093	DnaJ protein, heat shock protein 40	4.3	6.6	6.3E-12
slr2075	10 kD chaperonin (*groES*)	9.3	5.8	2.5E-12
slr2076	60 kD chaperonin (*groEL1*)	8.8	6.0	7.1E-13
Chemotaxis				
sll0041	Photoreceptor for positive phototaxis (*pixJ1, taxD1*)	4.4	2.5	1.9E-14
sll1107	Type IV pilus biogenesis protein PilI homolog	1.8	3.8	4.1E-10
sll1694	Pilin polypeptide PilA1 (*pilA1*)	−1.8	−1.6	2.4E-04
sll1695	Pilin polypeptide PilA2 (*pilA2*)	−1.5	−1.0	6.5E-07
slr0162	A part of pilC, pilin biogenesis protein	−1.6	−1.4	6.5E-06
slr1274	Probable fimbrial assembly protein (*pilM*)	1.9	1.8	6.8E-06
slr1929	Type 4 pilin-like protein (*pilA6*)	−1.5	−1.8	3.8E-06
Protein and peptide secretion				
slr0774	Protein-export membrane protein (*secD*)	4.2	4.0	5.1E-16
slr0775	Protein-export membrane protein (*secF*)	4.1	4.2	2.5E-10
slr1377	Leader peptidase I (signal peptidase I)	3.4	2.9	1.3E-09
Transcription				
RNA synthesis, modification, and DNA transcription				
sll0306	RNA polymerase group 2 sigma factor (*sigB*)	8.2	2.9	7.8E-09
sll0856	RNA polymerase ECF-type (group 3) (*sigH*)	1.0	-1.7	3.0E-06
sll1689	Group2 RNA polymerase sigma factor (*sigE*)	2.0	4.6	5.7E-08
sll1787	RNA polymerase beta subunit (*rpoB*)	−1.8	−2.2	1.0E-09
sll1789	RNA polymerase beta prime subunit (*rpoC*2)	−2.7	−1.9	1.6E-06
sll1818	RNA polymerase alpha subunit (*rpoA*)	−1.6	1.3	3.8E-08
sll2012	Group2 RNA polymerase sigma factor (*sigD*)	2.6	1.6	2.9E-06
slr0083	RNA helicase Light (*crhR*)	−2.5	−1.9	2.2E-15
Translation				
Degradation of proteins, peptides, and glycopeptides				
slr0008	Carboxyl-terminal processing protease (*ctpA*)	3.8	2.0	7.0E-12
slr0156	ClpB protein (*clpB2*)	2.1	2.4	7.5E-09
slr0164	ATP-dependent Clp protease proteolytic subunit	−1.6	−2.3	7.7E-09
slr0165	ATP-dependent Clp protease proteolytic subunit	−1.7	−1.4	1.1E-04
slr1204	Protease	6.7	4.6	1.0E-14
slr1331	periplasmic processing protease	2.4	4.3	1.3E-07
slr1641	ClpB protein (*clpB1*)	2.9	3.5	3.9E-10

Table 2 Selected differentially regulated genes following heat shock in *Synechocystis* sp. PCC 6803 wild type and Δ*sigB*

Gene	Gene function	WT(45C/30C)[a]	Δ*sigB*(45/30°C)[a]	*P*-treatment
Miscellaneous				
sll0408	Peptidyl-prolyl *cis–trans* isomerase	2.6	2.0	3.2E-07
sll0541	Acyl-lipid desaturase (delta 9) (*desC*)	−2.5	−1.3	2.0E-10
sll1110	Peptide chain release factor 1 (*prfA*)	1.8	1.8	1.9E-06
sll1621	AhpC/TSA family protein (*ahpC*)	2.9	3.5	3.3E-09
sll1742	Transcription antitermination protein (*nusG*)	1.9	2.4	1.9E-04
slr0095	*O*-methyltransferase	2.8	2.8	4.6E-07
slr0473	Cyanobacterial phytochrome 1(*cph1,hik35*)	−1.2	−1.5	9.4E-03
slr0474	Regulator for phytochrome 1 (*rcp1*)	−2.4	−2.9	6.4E-07
slr0946	Arsenate reductase (*arsC*)	7.1	6.1	9.7E-16
slr0974	Initiation factor IF-3 (*infC*)	1.9	2.6	6.2E-08
slr1192	Probable alcohol dehydrogenase	2.1	2.9	3.8E-10
slr1205	Similar to chlorobenzene dioxygenase	2.6	2.9	1.9E-11
slr1516	Superoxide dismutase (*sodB*)	4.1	2.4	2.7E-08
slr1597	Chromosome partitioning ATPase, (*parA*)	2.7	2.1	2.6E-08
slr1675	Putat. hydrogenase formation protein (*hypA1*)	3.5	3.6	6.8E-09
slr1738	Peroxide regulator (*perR*)	2.8	3.1	4.2E-07
slr1884	Tryptophanyl-tRNA synthetase (*trpS*)	2.2	1.9	3.6E-04
slr1916	Probable esterase	5.2	4.9	4.4E-08

Genes were considered differentially regulated when $P < 0.05$ and fold change > 1.4-fold

[a] The fold changes for wild-type and Δ*sigB* were calculated by dividing the normalized mean intensities of heat shock-treated strains with non-treated strains

and Δ*sigB* (Table 2). Similarly, four genes (*aroK*, *trpC*, *trpE* and *trpF)* involved in tryptophan biosynthesis were up regulated by 2.2- to 3.3-fold in both wild type and Δ*sigB* (Table 2). A tryptophanyl-tRNA synthetase (*trpS*) was also induced over two fold (Table 2). Amino acid starvation during heat shock has been observed in other bacteria; e,g., Wiberg et al. (1988) found that exposure of *E. coli* to 44°C for 8 min led to tryptophan deficiency. Similarly, Helmann et al. (2001) found that there was a strong induction of genes coding for arginine biosynthesis in *B. subtilis* and it was suggested that heat shock might induce a transient arginine starvation.

Cell envelope

Genes of two sub-functional categories 'murien sacculus and peptidoglycan', and 'surface polysaccharides' were differentially up regulated. Murein (or peptidoglycan) is located in the periplasm and is closely associated with the inner surface of the outer membrane and is responsible for the maintenance of cell shape and the preservation of cell integrity (Heijenoort 1996). As shown in Table 2, several murien sacculus and peptidoglycan genes were induced up to 3.3-fold in both wild type and Δ*sigB*. The induction of these genes suggested that heat shock treatment in cyanobacteria leads to the increased synthesis of peptidoglycan, which may help in the synthesis of multilayered murein used to protect cells during this stress. It is known that multilayered murein found in *staphylococci* can undergo considerable swelling and shrinking in response to the osmotic strength of the medium (Labischinski and Johannsen 1986).

Three genes coding for surface polysaccharides were also induced in both strains. A similar induction of genes involved in surface structure biosynthesis and modification was also demonstrated in *C. jejuni* (Stintzi 2003). This differential expression suggests that cells protect membrane structure during heat shock treatment by reinforcing important constituents of the cell wall.

Other important genes

In addition to the major changes in some functional groups described above, several genes with important functions required for cyanobacterial survival under various conditions, were differentially regulated by heat shock treatment (Table 2; e.g., *cph1*, *rcp1*, *perR*, peptidyl-prolyl *cis–trans* isomerase, acyl-lipid desaturase, arsenate reductase and superoxide dismutase). We have observed high expression of *arsC* under several other stress conditions (Singh et al. 2003; Li et al. 2004). The cyanobacterial phytochrome (Cph1) and its response regulator (Rcp1) are co-transcribed in *Synechocystis* sp. PCC 6803 and are differentially expressed during light–dark transitions. We have shown that this operon is under the control of a two-component regulatory system in which Hik8 is the sensor (Singh and Sherman 2005).

Another interesting finding was the induction of *sodB* and *ahpC*, thus suggesting that heat shock treatment led to the production of reactive oxygen species.

Indeed, it has been shown that high temperature can induce photoinhibition at light intensities otherwise normal at ambient temperature (Singh and Singhal 1999). This can lead to the generation of various reactive oxygen species and to the induction of lipid peroxidation (Mishra and Singhal 1992). In this regard, the up-regulation of PerR (slr1738) by heat shock treatment is very interesting (Table 2). We have recently shown that *perR* and the divergently transcribed *ahpC* were highly up-regulated by hydrogen peroxide and these genes appear to be regulated by *perR* (Li et al. 2004). Our microarray results demonstrated that both *ahpC* and *perR* were induced approximately 2.9-fold by heat shock and that the absence of *sigB* had no effect on its induction. An important gene that was down regulated following heat shock treatment codes for an acyl-lipid desaturase (*desC*) that is involved in unsaturation of the fatty acids of membrane lipids. Growth at high temperature generally results in the decrease in the abundance of unsaturated fatty acids (Murakami et al. 2000). It was suggested that an increase in saturation of the membrane lipids at high temperature might be important for maintaining an optimum fluidity necessary for effective functional activities. Lastly, peptidyl-prolyl *cis–trans* isomerases are important for protein folding and one such gene (sll0408) was up regulated by heat shock. Similar genes, *ppiD* and *fkpA*, coding for peptidyl-prolyl isomerases, has been shown to be heat-inducible and a part of σ^{32} and σ^{24} regulon, respectively, in *E. coli* (Yura 2000).

Regulation of gene clusters

We identified several gene clusters that were differentially expressed (either up- or down-regulation) in both wild type and Δ*sigB* during heat shock (data not shown). Most or all of the genes in such a cluster were differentially regulated in response to other stress treatments (Hihara et al. 2001; Kanesaki et al. 2002; Hihara et al. 2003; Singh et al. 2003; Li et al. 2004). For example, one cluster containing nine genes (slr0144–slr0152) was down regulated following heat shock (see Supplemental Table 1). This cluster was also down regulated following treatment with hydrogen peroxide and low-iron growth (Singh et al. 2003; Li et al. 2004), but up-regulated following high light treatment (Hihara et al. 2001). Figure 1 shows examples of two clusters containing genes that were either up- or down-regulated during heat shock. The cluster which was up-regulated (slr1674–slr1677) was also up-regulated during treatment with DCMU, DBMIB, high light, high salt and osmotic stress (Hihara et al. 2001; Kanesaki et al. 2002; Hihara et al. 2003; Li et al. 2004). Four genes in this cluster were strongly up regulated by heat shock (Fig. 1a). The first gene in this cluster codes for a protein with unknown function that is present in several cyanobacteria. The second gene of this cluster codes for a putative hydrogenase expression/formation protein HypA1. The next two genes, slr1676 and slr1677, also code for hypothetical proteins with putative functions as amino acid transporter and lipid A disaccharide synthetase, respectively. The genes in the second cluster (Fig 1b) were down regulated following heat shock treatment. These genes are designated in Cyanobase as hypothetical with putative function as Pil genes. It is interesting that two other putative pilin genes, *pilA1* and *pilA2*, were also down regulated and these results suggest common or related functions.

Microarray results for Δ*hrcA* compared to wild type

We also investigated the role of HrcA in the regulation of heat shock induced gene expression in *Synechocystis* sp. PCC 6803. Gene expression of an Δ*hrcA* mutant strain was compared to wild type under normal growth conditions (see Table 3 and Supplemental Table S2 for proteins annotated as hypothetical and unknown in the Kazusa database). The chaperone-encoding genes *groEL1*, *groES* and *groEL2* were up-regulated (3.8- to 3.1-fold) in the mutant compared to wild type, similar to the results of Nakamoto et al. (2003). No significant increase in transcript was observed for heat shock genes *dnaK2*, *htpG*, or *hspA*, consistent with the data of Nakamoto et al. (2003). Interestingly, the transcript level of the gene cluster sll1694–sll1696 (encoding PilA1, PilA2 and a hypothetical protein) also increased by 2.4–3.5-fold in Δ*hrcA* although no CIRCE element was identified in the upstream region of these three genes. Figure 2 shows Northern hybridization confirmation of the microarray results for *groEL1*, *pilA1* and

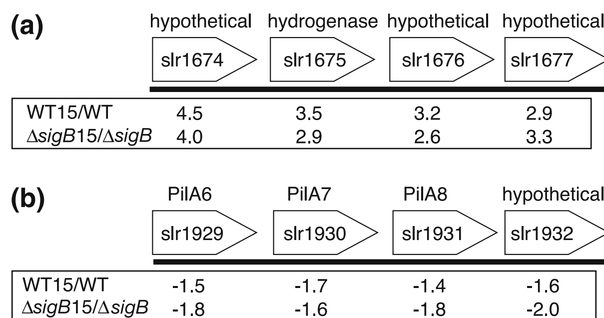

Fig. 1 Two gene clusters in which all genes were differentially regulated in both *Synechocystis* sp. PCC 6803 wild type and Δ*sigB* following heat-shock. **a** an up-regulated cluster; **b** a down-regulated cluster

dnaK2. Following heat shock, the *groEL1* and *dnaK2* transcripts increased in the wild type and the mutant, similar to Nakamoto et al. (2003). In contrast, the *pilA1* transcript decreased in the both strains following heat shock. This decrease in the *pilA1* transcript had previously been observed for both the wild-type and Δ*sigB* strains following heat shock (Table 2). Other genes up-regulated at least two fold in the Δ*hrcA* mutant strain were: a probable lipopolysaccharide ABC transporter ATP binding subunit (sll0575); an ATP-binding protein of ABC transporter (sll0415), and succinyl-CoA synthetase beta chain (sll1023), none of which contain the CIRCE element in the upstream region. Genes that were down regulated two fold or more in the Δ*hrcA* mutant cells compared to wild type included the transcript of the response regulator *rre34* and the neighboring hypothetical gene sll0788. Interestingly, these transcripts also were decreased in the Δ*hik34* mutant (Suzuki et al. 2005).

Effect of SigD and SigE Mutations on the transcription of other sigma factors

Our results with Δ*sigB* and the concomitant changes in the transcript levels of SigE and SigD, encouraged us to look at mutants in *sigE* and *sigD*. SigD is highly induced in response to hydrogen peroxide (Li et al. 2004), whereas SigE has been suggested to play role in nitrogen availability (Muro-Pastor et al. 2001). We performed loop experiments similar to the heat shock with wild type and Δ*sigB*, but using different treatments—H_2O_2 treatment for comparing wild type with SigD and growth in the absence of nitrate for comparison of wild type with SigE (see Materials and methods for details). The differential expression of the RNA polymerase genes, the group 1 and group 2 sigma factors and selected chaperone genes is shown in Table 4. It is obvious that the deletion of SigE has a major negative impact on the transcription of the *rpo* genes, and *sigA*, *sigB* and *sigC* in both the presence and absence of nitrate in the growth medium. The lack of SigE in cells grown in normal media also led to a significant decrease in the transcript levels of the key chaperone genes—*dnaK2, groEL2*, *groESL*, *hspA* and especially *htpG* (decreased 5.2-fold). The changes were less severe in the nitrate-deprived medium, although all transcript levels decreased. Lemeille et al. (2005) also observed lesser transcript level of *sigA*, *sigB* and *sigC* in the absence of SigE. The lack of SigD led to modest increases in transcript levels of *sigB*, *sigC* and *sigD* and a slight decrease in *sigE* levels. In contrast, Lemeille et al. (2005) report a small decrease in the levels of *sigB* and *sigC* in a Δ*sigD* mutant compared to wild type. This may be due to differences in culture conditions, as the level of transcripts of these genes have been shown to fluctuate with growth stage and conditions (Lemeille et al. 2005, Foster, Singh and Sherman, unpublished observations). As we have indicated previously, the further addition of 5 mM H_2O_2 for 15 min strongly down regulated most of the group 2 sigma factors. This stress has little impact on the chaperones, except for further increases in *groESL* (Table 4).

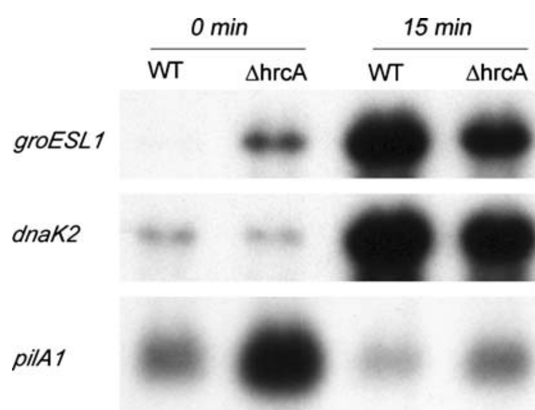

Fig. 2. Northern blots showing the effect of heat treatment on wild type and Δ*hrcA*. RNA was isolated from the cells before (0 min) and after (15 min) a 45°C, incubation and the same northern blot was probed for *groEL1*, *dnaK2* and *pilA1*. This is representative of results obtained from three independent heat treatments. Transcript sizes were approximately 2.3, 2.2 and 0.6 kb for *groEL1*, *dnaK2* and *pilA1*, respectively

Discussion

The primary objective of the present study was to identify the heat shock regulated genes in *Synechocystis* sp. PCC 6803 and the role of SigB and HrcA in heat shock dependent gene expression. We have demonstrated that: (a) the chaperones represented the gene category induced the most by a brief heat shock; (b) the key chaperone genes were under complex regulation; e.g., some genes (*groESL* and *groEL2*) were positively regulated by SigB and SigE and negatively regulated by HrcA. Other heat shock genes, such as *hspA* and *htpG* were positively regulated by SigB and SigE, but not controlled by HrcA; (c) the heat shock also induced genes coding for biosynthesis of aromatic amino acids (especially histidine and tryptophan), murien sacculus and peptidoglycan, and surface polysaccharides.

In most cases the induction of genes encoding chaperones was less in the Δ*sigB* than the wild type, although the pattern of induction was similar for both strains. The Δ*sigB* mutant also showed induction of *sigE*. This is significant, since the promoter recognition

Table 3 Differentially regulated genes in *Synechocystis* sp. PCC 6803 Δ*hrcA* mutant compared to wild type

Gene	Gene function	Δ*hrcA*/WT (30°C)[a]	*P*-value
Amino acid biosynthesis			
sll1499	Ferredoxin-dependent glutamate synthase	1.5	3.4E-03
Biosynthesis of cofactors, prosthetic groups, and carriers			
sll1875	Heme oxygenase	−1.5	1.5E-02
slr0749	Light-independent protochlorophyllide reductase iron protein subunit	1.5	1.7E-02
Cellular processes			
sll0170	**DnaK protein 2, heat shock protein 70**[b]	**1.4**	**2.0E-02**
sll0416	**60 kDa chaperonin 2 (*groEL2***	**3.8**	**2.3E-08**
slr2075	**10 kD chaperonin (*groES*)**	**3.1**	**6.3E-07**
slr2076	**60 kD chaperonin (*groEL1*)**	**3.1**	**2.3E-06**
sll1694	**Pilin polypeptide PilA1 (*pilA1*)**	**2.4**	**1.0E-05**
sll1695	**Pilin polypeptide PilA2 (*pilA2*)**	**3.5**	**3.3E-07**
slr0079	**Probable general secretion pathway protein E**	**1.7**	**8.6E-03**
Energy metabolism			
sll1077	Agmatinase	1.6	6.3E-03
sll1023	Succinyl-CoA synthetase beta chain	2.2	3.1E-05
Other			
ssl2542	High light-inducible polypeptide (*hliA*)	−2.1	4.0E-03
ssr2595	High light-inducible polypeptide (*hliB*)	−2.0	1.6E-04
sll0086	Putative arsenical pump-driving ATPase	−1.6	6.4E-03
ssl2250	Bacterioferritin-associated ferredoxin	1.5	1.3E-02
sll1078	**Putative hydrogenase expression/formation protein**	**1.6**	**6.2E-03**
sll1621	AhpC/TSA family protein	1.8	3.5E-03
slr0078	Putative 6-pyruvoyl tetrahydrobiopterin synthase	−1.5	3.0E-02
slr1501	Probable acetyltransferase	−1.5	2.0E-02
ssl0769	Putative transposase	−1.6	2.4E-02
Photosynthesis and respiration			
sll1322	ATP synthase A chain of CF(0)	1.4	2.6E-02
sll0819	Photosystem I reaction center subunit III precursor (PSI-F), plastocyanin (cyt c553) docking protein	1.4	3.2E-02
slr1655	Photosystem I subunit XI	1.4	1.8E-02
slr1739	Photosystem II 13 kDa protein homolog	−1.4	1.5E-02
slr1986	Allophycocyanin beta subunit	1.5	1.7E-02
slr2067	Allophycocyanin alpha subunit	1.4	4.1E-02
ssl0453	Phycobilisome degradation protein (*nblA*)	1.5	5.4E-03
ssl3093	Phycobilisome small rod linker polypeptide	1.4	4.0E-02
sll1382	Ferredoxin, petF-like protein	−1.6	1.3E-02
Regulatory functions			
sll0789	Two-component response regulator OmpR subfamily	−2.3	4.9E-05
Translation			
slr0033	Glutamyl-tRNA(Gln) amidotransferase subunit C	−1.7	6.9E-03
slr0918	Methionine aminopeptidase	−1.5	4.5E-02
Transport and binding proteins			
sll0415	ATP-binding protein of ABC transporter	2.1	1.5E-05
sll0575	Probable lipopolysaccharide ABC transporter ATP binding subunit	2.5	9.8E-06
sll1598	Mn transporter MntC	−1.9	3.2E-02
slr0074	ABC transporter subunit	1.5	1.4E-02
slr1336	H^+/Ca^{2+} exchanger	1.5	2.1E-02

Genes were considered differentially regulated when $P < 0.05$ and fold change > 1.4-fold

Genes encoding hypothetical and unknown proteins are in Supplemental Table2

[a] The fold changes were calculated by dividing the normalized mean intensities obtained for the Δ*hrcA* strain by those for the wildtype

[b] Genes indicated in bold exhibited differential expression in Δ*sigB* compared to wild type following heat shock

in cyanobacteria is thought to have some redundancy among sigma factors. Thus, we postulate that *sigE* might have some involvement in the expression of HSPs, especially in the absence of *sigB*. In *E. coli*, extra-cytoplasmic accumulation of unfolded or immature outer membrane protein precursors are directly involved in the expression of σ^{32} (Mecsas et al. 1993; Danese and Silhavy 1997) and σ^{24} is involved in the

Table 4 Differential transcription of *Synechocystis* sp. PCC 6803 sigma factors and chaperones in the absence of SigD or SigE

Gene function	Δ*sigE*/WT ($+NO_3$)	Δ*sigE*/WT ($-NO_3$)	Δ*sigD*/WT Control	Δ*sigD*/WT ($+H_2O_2$)
Sigma factors				
sll1818 *rpoA*	−3.3[a]	−1.6	–	−1.2
sll1787 *rpoB*	−1.8	−2.3	–	−2.1
sll1789 *rpoC2*	–	–	–	–
slr0653 *sigA*	−2.9	−1.6	–	−1.5
sll0306 *sigB*	−3.6	−2.5	+ 1.6	−1.5
sll0184 *sigC*	−2.1	−1.8	+1.4	−1.4
sll2012 *sigD*	–	–	+2.1	−3.4
sll1689 *sigE*	–	–	−1.6	–
sll0856 *sigH*	–	–	–	+3.9
Chaperones				
sll0170 *dnaK2*	−2.5	–	–	–
sll0416 *groEL2*	−1.6	−1.4	–	–
sll0430 *htpG*	−5.2	−2.3	–	–
sll1514 *hspA*	−3.1	−1.9	+2.4	+1.8
slr2075 *groES*	−2.4	−1.4	+1.5	+3.1
slr2076 *groEL*	−2.8	–	+1.6	+1.8
Chemotaxis				
sll1694 *pilA1*	−1.5	−1.9	–	−1.6
sll1695 *pilA2*	−2.4	−1.9	–	−1.9

[a] All values have *P*-value < 0.05. Fold changes listed only if the value is ≥ ± 1.4-fold

expression of σ^{32}, the primary sigma factor involved in the expression of HSPs (Wang and Kaguni 1989). Our results suggest that, in *Synechocystis* sp. PCC 6803, *sigB* and *sigE* have inter-related functions in gene expression similar to those found in *E. coli* for σ^{32} and σ^{24}.

In addition to induction of the *groESL* operon and the *groEL2* gene, both of which contain the CIRCE element, the Δ*hrcA* mutant showed altered transcript levels for a number of genes lacking this element. This included genes differentially expressed in the Δ*hik34* strain, a strain that also exhibited increased expression of chaperone genes (Suzuki et al. 2005). Nakamoto et al. (2003) reported both inhibition and accumulation of some heat shock genes without CIRCE in the Δ*hrcA* mutant following heat treatment. Such differential regulation of genes lacking CIRCE in the Δ*hrcA* may result from a secondary effect caused by the increased levels of GroEL1, GroES and GroEL2 in the pre-stressed cells. The case of the *pilA1*, *pilA2*, sll1696 gene cluster appears more complex. These genes were induced in the Δ*hrcA* mutant where levels of *groEL1*, *groES* and *groEL2* are elevated, and were also induced in the mutant over-expressing *hik34*, where *groEL1*, *groES* and *groEL2* are decreased (Suzuki et al. 2005) and in a Δ*isiA* mutant (Singh et al. 2005).

The relationship among the group 2 sigma factors and other regulatory elements is just coming into focus, as discussed in a series of recent papers (Suzuki et al. 2005; Lemeille et al. 2005; Imamura et al. 2005). Overall, the coordinated regulation pathways among sigma factors is similar to those reported by other workers, although we noticed some minor differences in the transcription pattern of some sigma factors in the Δ*sig* strains. Such small differences can be caused by subtle differences in the physiological conditions of the cells, the way that heat shock was generated and by differences in the light intensity used in the various studies. The results presented herein, as well as in recent reports (Singh et al. 2003; Li et al. 2004 and Singh and Sherman 2005), shed new light on what can only be described as a pulsating web of interactions. Figure 3 highlights the mutual regulation pathways among the principal and group 2 sigma factors, as we now understand it in *Synechocystis* sp. PCC 6803. It is evident that SigB represents a major node in this network. SigB positively controls the transcription of *sigE* and itself and negatively regulates *sigE* under heat shock conditions. We also show the positive control of *sigB* on *sigC* and on the group 4 sigma factor *sigH*, based on comparison of results from the transition between exponential growth to the stationary phase for wild type and Δ*sigB* (Foster et al., unpublished observations). SigB positively regulates SigD during the heat shock, but SigD represses the transcription of SigB under normal growth conditions. Both SigB and SigE positively regulate transcription of the heat shock chaperones and thus work with HrcA to fine tune the transcript level of

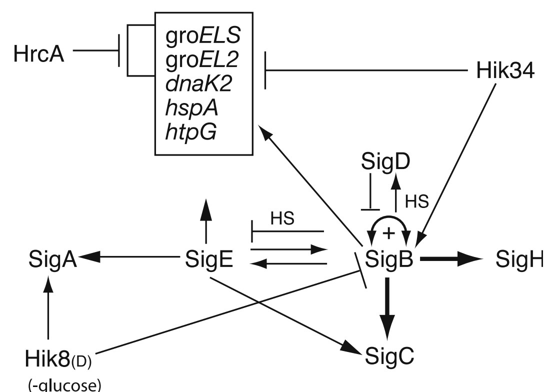

Fig. 3 Schematic model of transcriptional regulation among sigma factors in the cyanobacterium *Synechocystis* sp. PCC 6803. The model is based on microarray experiments involving heat shock and exponential versus stationary phase (Foster et al., unpublished observations) for wild type and Δ*sigB*. In addition, the effects of mutations in *sigD* and *sigE* are included, as are the results of experiments that described related results for Δ*hik8* (Singh and Sherman 2005) and Δ*hik34* (Suzuki et al. 2005). The *thick arrows* from SigB represent results obtained during the exponential growth to stationary phase transition. *HS* heat shock. *Hik8 (D)* results with Δ*hik8* during growth in the dark phase

genes such as *groES* and *groEL2*. SigE appears to represent a secondary node in the network and positively controls *sigA, sigB* and *sigC* transcription (see Fig. 3 for details).

The control by two histidine kinases (Hik8 and Hik34) is also depicted in Fig. 3. When Δ*hik8* cells were grown in the dark on glucose or under short day (less than 8 h light) conditions on glucose, we found significant decreases in the transcript levels of both *sigA* and *sigB* (Singh and Sherman 2005). Similarly, Suzuki et al. (2005) demonstrated that Hik34 positively regulated *sigB* and negatively regulated heat shock genes, as diagrammed in Fig. 3. The lack of this two-component regulatory protein led to enhanced thermotolerance and this phenotype may be due to enhanced chaperone production (Suzuki et al. 2005). We consider Fig. 3 to be the most detailed descriptive model of the sigma factor network in *Synechocystis* sp. PCC 6803 built on the transcriptional patterns, but it is still nowhere near complete. For example, Imamura et al. (2005) have shown that SigB and SigC also have a mutual regulatory loop—SigB induces *sigC* under exponential growth and stationary phase conditions, whereas SigC induces *sigB* under stationary conditions, but represses *sigB* during exponential growth. There are likely many such interactions still to be uncovered.

In general, many aspects of the response to heat shock are similar in *Synechocystis* sp. PCC 6803 and other prokaryotes. For example, a similar effect on the expression of chemotaxis genes in response to heat shock has also been demonstrated in *E. coli* (Richmond et al. 1999) and *C. jejuni* (Stintzi 2003). The regulation of chemotaxis genes by heat shock suggests that *Synechocystis* sp. PCC 6803 tries to avoid the unfavorable temperature by moving away from the stress. The induction of the *secDF* genes (induced ~four fold in both wild type and Δ*sigB*) also has a parallel in other bacteria. In vitro studies have shown that protein translocation by the Sec translocase does not require SecDF, although cells lacking SecD and/or SecF are severely defective in protein export and barely viable (Driessen and van der Does 2002). Several possible functions of SecDF has been proposed, including its involvement in protein release at the periplasmic side of the membrane, the control of the ATP-driven catalytic cycle of SecA, the slowdown, reverse and forward movement of preprotein, and the removal of the signal peptide or phospholipids from the aqueous protein-conducting pore formed by the translocase (Driessen and van der Does 2002). In addition to *secDF*, a gene coding for a leader peptidase I (slr1377) was also induced by approximately three fold in both wild type and Δ*sigB* strains. Leader peptidase is required to cleave the signal sequence in the pre-protein involved in the translocation of proteins across membrane. These results suggest that *Synechocystis* sp. PCC 6803 responds to heat shock by increasing its ability to transport proteins across the cytoplasmic membrane to the periplasm or to the cell wall.

A number of periplasmic proteases were also induced by the heat shock, including Slr1204 that showed strong similarity with HtrA of *E. coli*. HtrA in *E. coli* is regulated by σ^{24} and plays an important role in degradation of abnormal proteins in the periplasm (Bass et al. 1996). CtpA was another interesting protease that was up regulated by heat shock treatment. CtpA is a member of a novel family of endoproteases that includes a tail-specific protease from *E. coli*. CtpA catalyzes C-terminal processing of the D1 protein of photosystem II (Anbudurai et al. 1994), an essential event for the assembly of the manganese cluster to PS II and consequent light-mediated water oxidation in *Synechocystis* sp. PCC 6803 (Haussuhl et al. 2001). These results have important ramifications as to the relationship of photosynthetic complex assembly in the cytoplasmic and photosynthetic membranes.

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