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Group 3 sigma factors in the marine cyanobacterium *Synechococcus* sp. strain PCC 7002 are required for growth at low temperature

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Three genes, *sigF*, *sigG* and *sigH*, encoding group 3 sigma factors have been cloned and characterized in the marine cyanobacterium *Synechococcus* sp. strain PCC 7002. The *sigF* gene product was similar to sigma factors involved in general stress response and sporulation in other organisms, and the *sigG* and *sigH* gene products were similar to extracytoplasmic function (ECF) sigma factors. The *sigG* and *sigH* genes were associated with the putative regulatory genes and the sizes of transcripts for *sigG* and *sigH* genes were large enough to be cotranscribed with the associated downstream genes. The *sigG* downstream gene was designated *sapG* (*sigG*-associated protein), and yeast two-hybrid analysis demonstrated that SigG and SapG interact when produced in yeast cells. Null mutants of these three group 3 sigma factor genes were created by interposon mutagenesis. The growth of the *sigF* mutant strain was much slower than the wild-type strain at 15°C, although the growth rates at 22°C and 38°C were identical to those of the wild-type strain. The *sigG* mutant could not grow continuously at 22°C, and no growth occurred at 15°C. Since SigG and SapG interact in yeast cells and the *sigG* and *sapG* mutants showed a similar growth phenotype, SapG is likely to be a regulatory protein for SigG involved in the same pathway in transcriptional regulation in this cyanobacterium.

Key Words—cyanobacterium; interposon mutagenesis; low-temperature acclimation; sigma factor; *Synechococcus* sp. strain PCC 7002; transcription initiation; yeast two-hybrid analysis

Introduction

Sigma factors are a family of proteins that associate

with the procaryotic DNA-dependent RNA polymerase in a reversible way to direct transcription initiation by recognizing specific promoter sequences. Many eubacteria contain multiple sigma factors, which regulate gene expression in response to changes in environmental conditions and during differentiation of the cells (Gruber and Gross, 2003).

Based on sequence similarities, the eubacterial sigma factors are divided into the σ^{70} (RpoD) and σ^{54} (RpoN) families (Lonetto et al., 1992; Merrick et al., 1987). Most sigma factors belong to the σ^{70} family that is divided into three structurally and functionally related groups (Lonetto et al., 1992). Group 1 comprises the primary sigma factors that are essential for cell viability

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and vegetative growth of organisms. Group 2 sigma factors are alternative sigma factors that are not essential for cell viability under optimal growth conditions but that possess different promoter-recognition properties (Gruber and Gross, 2003). The sigma factors of this group are highly similar in the amino acid sequences of their DNA-binding regions to the group 1 sigma factors. Group 3 sigma factors are much less similar to group 1 sigma factors and comprise alternative sigma factors that control the transcription of sets of genes under specific physiological and developmental conditions. According to their various functions, the group 3 sigma factors can be divided into several subgroups that include heat shock (Yura et al., 1993), sporulation (Haldenwang, 1995), extracytoplasmic function (ECF) (Hermann, 2002; Lonetto et al., 1994) and flagellar (Hermann, 1991) sigma factors. Sigma factors of the ECF subfamily are conserved in their structures, and their roles are often but not always related to extracytoplasmic functions (Lonetto et al., 1994). The ECF subfamily includes *Escherichia coli* RpoE, *Pseudomonas aeruginosa* CarQ, *Myxococcus xanthus* AlgT, *Photobacterium* sp. strain SS9 RpoE and *Mycobacterium* species SigE. These sigma factor genes are characterized by the presence of downstream regulatory genes, called anti-sigma factors (Hughes and Mathee, 1998; Missiakas and Raina, 1998). Recently it is becoming apparent that proteins with broader functions are also members of this group, such as SigR of *Streptomyces coelicolor* (Hermann, 2002). SigR combats oxidative stress and is regulated by its cytoplasmic anti-sigma factor RsrA (Kang et al., 1999).

In cyanobacteria, the *sigA* gene encodes a group 1 sigma factor that is essential for cell viability (Brahamsha and Haselkorn, 1991; Caslake and Bryant, 1996; Sugimoto et al., 1997). The roles of group 2 sigma factors have been extensively studied in *Anabaena* sp. strain PCC 7120 (Brahamsha and Haselkorn, 1992; Khudyakov and Golden, 2001), *Synechococcus* sp. strain PCC 7942 (Tsinoremas et al., 1996) and *Synechocystis* sp. strain PCC 6803 (Asayama et al., 2004; Muro-Pastor et al., 2001) by interposon mutagenesis, and these alternative sigma factors are not essential but their transcription levels are responsive to environmental conditions to alter the gene expression patterns in response to stress (Asayama et al., 2004; Goto-Seki et al., 1999; Imamura et al., 2003a, 2006; Muro-Pastor et al., 2001; Osa-

nai et al., 2005; Tuominen et al., 2003). The characterization of group 3 sigma factors is limited so far to the freshwater cyanobacterium *Synechocystis* sp. strain PCC 6803, in which four group 3 sigma factor genes have been found based upon the complete genome sequencing analysis (Kaneko et al., 1996). The *sigF* (*slr1564*) gene is shown to be required for biosynthesis of pili and motility of the cells (Bhaya et al., 1999) and the induction of salt stress proteins (Huckauf et al., 2000). The *sigG* (*slr1545*) gene is apparently essential for cell viability in this cyanobacterium (Huckauf et al., 2000). Although transcripts of these group 3 sigma factor genes are detectable, the amounts of the proteins in cells are less than the detection limits by immunoblot analysis (Imamura et al., 2003b).

Since cyanobacteria are a widely distributed group of phototrophic eubacteria and occupy highly diverse ecological niches (Stanier and Cohen-Bazire, 1977), each cyanobacterium presumably must have unique molecular mechanisms of environmental adaptation and acclimation. We have studied multiple sigma factors that belong to the σ^{70} family in the unicellular marine cyanobacterium *Synechococcus* sp. strain PCC 7002 and five genes encoding group 1 and 2 sigma factors, designated *sigA*, *sigB*, *sigC*, *sigD* and *sigE* have been isolated and characterized in this marine cyanobacterium. Two group 2 sigma factors, SigB and SigC, have roles in modifying transcription in response to changes in carbon and nitrogen availability (Caslake et al., 1997), while SigE is involved in the transcription of post-exponential-phase-specific gene expression in *Synechococcus* sp. strain PCC 7002 (Gruber and Bryant, 1998). To investigate the roles of group 3 sigma factors in *Synechococcus* sp. strain PCC 7002, three genes encoding group 3 sigma factors, *sigF*, *sigG* and *sigH*, were isolated and inactivated by interposon mutagenesis. To discover roles of these group 3 sigma factors in environmental stress response(s), low-temperature acclimation was studied using these mutants.

Materials and Methods

Bacterial strains and growth conditions. A laboratory wild-type strain of the unicellular marine cyanobacterium *Synechococcus* sp. strain PCC 7002, denoted PR6000, has been maintained at the Pennsylvania State University since 1981. Cells were grown photoautotrophically in medium A⁺ containing 1 mg

NaNO₃ ml⁻¹ as a nitrogen source (Stevens et al., 1973). Aeration and mixing was provided by bubbling with 1% (v/v) CO₂ in air under continuous illumination by cool-white fluorescent lamps at approximately 250 $\mu\text{E m}^{-2} \text{s}^{-1}$ at 38°C, 22°C or 15°C. For the selection of kanamycin- or streptomycin/spectinomycin-resistant mutants, kanamycin (50 $\mu\text{g ml}^{-1}$) or spectinomycin (100 $\mu\text{g ml}^{-1}$) were added to liquid media and agar plates. Growth of cyanobacterial cells was monitored by changes in the optical density at 550 nm by a Spectronic 20 spectrophotometer (Bausch and Lomb, now Milton Roy, Rochester, NY). For high-light intensity treatments, cells were illuminated with halogen bulbs, and light intensities were measured using a model QSL-100 quantum scalar irradiance meter (Biospherical Instruments, Inc., San Diego, CA) (Sakamoto and Bryant, 2002). For oxidative stress growth conditions, methyl viologen (also called paraquat; Sigma Chemicals, St. Louis, MO) was added to final concentrations of 1 μM to 0.5 mM (Sakamoto et al., 1998a). *E. coli* strains DH5 α and DH10B were used for recombinant DNA manipulations. For yeast two-hybrid analysis, a *Saccharomyces cerevisiae* strain, PJ69-4A (*MATa trp1-901 leu2-3, 112 ura3-52 his3-200 gal4 Δ gal80 Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*), and vectors, pGAD-C1, pGBD-C1 and pGBD-C3, were kindly donated by Drs. P. James and E. Craig (University of Wisconsin, Madison, WI). Yeast cells were grown on synthetic complete (SC) medium (Rose et al., 1990). Tryptophan, leucine, histidine and/or adenine were eliminated for the selection of transformants when appropriate.

Cloning of *sigF*, *sigG* and *sigH* genes. Three pairs of primers, 5'-AAT TCC AAC GCA AGC TAG TTG CCC-3' and 5'-GGT TAG TTG GCG ACT GTG TTG CAA-3' for *sigF*, 5'-GGA GTC AGC CGT GTT GGG-3' and 5'-GGG TGG GCT TAG GAA GCC-3' for *sigG*, and 5'-CCA GCG AAG TCA GCA GGG TGA-3' and 5'-TGA ACT CCC TGT TGC TGC AAG-3' for *sigH*, were designed and synthesized based on the nucleotide sequences of these genes in the freshwater cyanobacterium *Synechocystis* sp. strain PCC 6803 (Kaneko et al., 1996). Using these primers, the *sigF* (*slr1564*), *sigG* (*slr1545*) and *sigH* (*slr0856*) gene probes were amplified by PCR with genomic DNA template of *Synechocystis* sp. strain PCC 6803, and used as probes to screen a genomic DNA library of *Synechococcus* sp. strain PCC 7002. The probes were la-

beled with [α -³²P]dATP (New England Nuclear, Beverly, MA) using a random-primed DNA labeling kit (Boehringer Mannheim, Indianapolis, IN). Heterologous hybridization was performed according to the method described by Bryant and Tandeau de Marsac (1988) using stringent washing conditions at 62°C. A positive clone containing the *sigF* gene was isolated from an ordered cosmid library of *Synechococcus* sp. strain PCC 7002 genomic DNA which was constructed by *Sau3AI* partially digested DNA inserted into a vector SuperCos I (Stratagene, La Jolla, CA). The library was kindly provided by Dr. William R. Widger (University of Houston). Positive clones containing the *sigG* and *sigH* genes were isolated from a cosmid library which was constructed by genomic DNA partially digested by *EcoRI* and inserted into a vector pH79 (Wagner et al., 1993).

DNA fragments containing the *sigF* and *sigG* genes were subcloned into plasmid pUC19 (Yanisch-Perron et al., 1985). For subcloning of *sigH* gene, a low-copy number plasmid pHSG575 (Takeshita et al., 1987) was used. The multiple cloning site of pHSG575 was replaced by an *EcoRI-HindIII* fragment derived from Superlinker 2 (Brosius, 1989) to subclone a 2.1-kb *SacI-BglII* fragment containing *sigH* gene.

The nucleotide sequences of the cloned DNA fragments encoding sigma factors were determined at the Nucleic Acid Facility of the Biotechnology Institute of the Pennsylvania State University using *Taq* DNA polymerase, fluorescent dye terminators and gene-specific primers. All nucleotide sequences were verified by sequencing both strands completely. Protein sequence alignments and sequence similarity calculations were generated with the ClustalW program (Thompson et al., 1994). The phylogenetic analysis was performed using PHYLIP (Phylogeny Interference Package) version 3.6 (Felsenstein, 2004). The hydrophobic index was calculated according to the algorithm of Kyte and Doolittle (1982).

The nucleotide sequences of the sigma factor genes in *Synechococcus* sp. strain PCC 7002 have been submitted to GenBank with the accession numbers AF121848 (*sigF*), AF121849 (*sigG* and *sapG*) and AF121850 (*sigH*).

Interposon mutagenesis of the *sigF*, *sigG*, *sapG* and *sigH* genes. The *sigF*, *sigG*, *sapG* and *sigH* genes were inactivated by inserting antibiotics resistance gene cartridges into unique restriction sites within the coding regions (Fig. 1). The *aphII* gene encoding

Fig. 1. Disruption of *sigF*, *sigG*, *sapG* and *sigH* genes by interposon mutagenesis in *Synechococcus* sp. strain PCC 7002.

Physical maps of the regions around *sigF*, *sigG*, *sapG* and *sigH* genes with the positions and directions of antibiotic resistance cartridges are shown. A. For inactivation of the *sigF* gene, the *aphII* gene encoding aminoglycoside 3'-phosphotransferase, which confers resistance to kanamycin, was inserted into the *SacII* site of a subclone of a 2.7-kb *HindIII* fragment containing the partial *sigF* gene in parallel orientation. The putative 5' end of the transcript is shown by an arrow. The *tilS* gene encoding tRNA(Ile)-lysine synthase is found upstream of *sigF* in parallel orientation, and *dnaJ*, encoding a heat-shock protein is found downstream in antiparallel orientation. B. For inactivation of the *sigG* gene, the Ω fragment of the *aadA* gene, which confers streptomycin and spectinomycin resistance, was inserted into the *XmnI* site in anti-parallel orientation. The *ndhD* gene encoding a subunit of NADPH dehydrogenase is found downstream of the *sigG* gene in parallel orientation. C. For inactivation of the *sapG* gene, *aphII* was inserted into the *HindIII* site in parallel orientation. D. For inactivation of the *sigH* gene, *aphII* was inserted into the *HindIII* site in parallel orientation. *orf140*, similar to a hypothetical gene *sl0857* in *Synechocystis* sp. strain PCC 6803 is found downstream of *sigH* in parallel orientation.

aminoglycoside 3'-phosphotransferase II confers resistance to kanamycin and the *aadA* gene encoding aminoglycoside 3'-adenyl transferase confers resistance to spectinomycin and streptomycin. To inactivate the *sigF* gene (Fig. 1A), plasmid pUC19 with an insert of a 2.7-kbp *HindIII* fragment containing a partial *sigF* gene was digested with *SacII*, filled in by T4 DNA polymerase and ligated with a 1.3-kbp *HincII* fragment containing the *aphII* gene derived from plasmid pRL161 (Elhai and Wolk, 1988). To inactivate the *sigG* gene (Fig. 1B), plasmid pUC19 with an insert of a 3.5-kbp *XbaI* fragment containing the *sigG* gene was di-

gested with *XmnI* and ligated with a 2.0-kbp fragment with *SmaI* ends containing the *aadA* gene derived from plasmid pHP45 Ω (Prentki and Krisch, 1984). For inactivation of the *sapG* gene (Fig. 1C), the 3.5-kb *XbaI* insert was transferred to the *BamHI*-*SaI* site of pBlue-script KS(-) to create a unique *HindIII* site in the coding region, digested with *HindIII* and ligated with a 2.0-kbp *HindIII* fragment containing the *aadA* gene with transcriptional terminators at both ends or a 1.6-kbp *HindIII* fragment containing the *aphII* gene from plasmid pUC4-KIXX (Pharmacia, Uppsala, Sweden). For inactivation of *sigH* (Fig. 1D), plasmid pHSG575 with

an insert of a 2.1-kbp *SacI*-*Bgl*II fragment containing *sigH* gene was digested with *Hind*III and ligated with a 1.3-kbp *Hind*III fragment encoding the *aphII* gene from pRL161. The *sigF*, *sapG* and *sigH* constructs containing the *aphII* gene with parallel orientation and the *sigG* and *sapG* constructs containing the *aadA* gene with antiparallel orientation were used for transformation (Fig. 1).

Wild-type cells of *Synechococcus* sp. strain PCC 7002 (PR6000) were transformed with plasmid DNA essentially as described by Stevens and Porter (1980). To verify segregation of the mutant alleles of the *sigF*, *sigG*, *sapG* and *sigH* genes, total genomic DNA was isolated from these mutant strains by phenol-chloroform extraction and ethanol precipitation, and Southern blot hybridization analyses were performed using specific probes for the *sigF* (the 0.5-kb *Bst*XI fragment), *sigG* (the 0.8-kb *Xba*I-*Xmn*I fragment) or *sapG* (sequence 890–1495 of AF121849) genes. Alternatively, the insertion was confirmed by PCR for *sigH* gene with *sigH*-specific primers, 5'-AGG GCC ATG GCC GAT CAA GCT GTT GAC C-3' and 5'-TTT AGG ATC CGA ATT AAG TTG TCA TGG T-3'.

RNA analyses. RNA blot hybridization analysis was performed as described by Sakamoto and Bryant (1997) after LiCl precipitation for separation of RNA (Ausubel et al., 1987). Specific probes for each gene, *sigF* (sequence 635–1451 of AF121848), *sigG* (sequence 229–908 of AF121849), *sigH* (sequence 171–803 of AF121850) and *sapG* (sequence 890–1495 of AF121849) were amplified by PCR using specific primers.

RT-PCR analysis to detect transcripts was performed according to the method described by Sakamoto et al. (1999). A cDNA template was synthesized by the *sapG*-specific primer, 5'-CCC GTT GAC CGA GAT ATT-3' using total RNA and Moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega, Madison, WI). PCR primers located at the inside of the coding regions of *sigG* and *sapG* genes, 5'-ATG AAC CGA TCC CTT TCT AT-3' and 5'-CCC GTT GAC CGA GAT ATT-3', were used to detect the transcript containing both *sigG* and *sapG* genes.

A 5'-rapid amplification of cDNA ends (5'-RACE) analysis with poly(C) tailing was performed to determine the 5' end of *sigF* transcript according to the method described by Sakamoto et al. (1999). A *sigF*-specific primer, 5'-CGC GGG TAT AGG GAT T-3', was used for cDNA synthesis with total RNA as template

and M-MLV reverse transcriptase, and poly(C) tail was added to the 3' end of the cDNA using terminal transferase (New England Biolabs, Beverly, MA). A second *sigF*-specific primer, 5'-GAC TGC CGA TAT TCC TGT A-3', and a poly(G)-anchored primer, 5'-ACC TGC AGG CAT GCA AGC TTG (G)₁₅-3', were used for PCR. The secondary PCR was performed using an internal *sigF*-specific primer, 5'-GAG ACT AAG GGA GGA TTT-3', and a primer, 5'-ACC TGC AGG CAT GCA AGC TTG-3'. The sizes of the PCR products were determined by agarose gel electrophoresis and ethidium bromide staining.

Yeast two-hybrid analysis. A 0.7-kbp DNA fragment containing the *sigG* gene was subcloned into a vector pGBD-C1 (James et al., 1996) to allow a fusion of SigG with Gal4p DNA-binding domain. Restriction sites *Bam*HI and *Bgl*II were introduced into the ends of the 0.7-kbp *sigG* fragment by PCR using a set of primers, 5'-AGC ATG AAC GGA TCC CTT TC-3' and 5'-TCA TTC AAG ATC TAG TCC GCC AAA-3'. The resultant 0.7-kbp DNA fragment was digested with *Bam*HI and *Bgl*II and ligated into the *Bgl*II site of the vector pGBD-C1. The resultant plasmid, designated pGBD-SigG, was then transformed into the yeast strain PJ69-4A by a method using lithium acetate (Gietz et al., 1995).

To determine the concentration of 3-amino triazole (3-AT, Sigma Chemicals) to suppress the auto-activation of *GAL1-HIS3* gene in PJ-69-4A by pGBD-SigG, cell growth of transformants with pGBD-SigG was tested on SC minus histidine plates containing 0, 1, 5, 10 and 25 mM of 3-AT (Durfee et al., 1993). On the basis of the results from this assay, 1 mM 3-AT was added to the media throughout the study to suppress background expression of the *HIS3* gene.

A 0.6-kbp DNA fragment containing full length *SapG* was amplified by PCR with primers, 5'-CGG AAT TCA TAA TGA ATG AAA GCT-3' and 5'-TAA GAT CTG CCG AAA CAT TAG-3', from a plasmid that contains the *sapG* gene as a template. The amplified 0.6-kbp DNA fragment of *sapG* was subcloned into the *Eco*RV site of pBluescript KS(–) by the A-T overhang method to verify the nucleotide sequence of the insert by sequencing. The plasmid was then digested with *Eco*RI and *Bgl*II, and the resultant 0.6-kbp DNA fragment was ligated into the *Eco*RI and *Bgl*II sites of the vector pGAD-C1 to allow a fusion of *SapG* with the Gal4p transcription activation domain. The resultant plasmid was designated pGAD-*SapG*. A 0.28-kbp DNA frag-

ment of the N-terminal domain of SapG was amplified by PCR with primers, 5'-CGG AAT TCA TAA TGA ATG AAA GCT-3' and 5'-TTG AGG ATC CGT AGC TGT TGC TAT TGC TGT-3', and a plasmid that contains the *sapG* gene as a template. The resultant 0.28-kbp DNA fragment was digested with *EcoRI* and *BamHI* and ligated into the *EcoRI* and *BamHI* sites of the vector pGAD-C1 to allow a fusion of the N-terminal hydrophilic domain of SapG with the Gal4p transcription activation domain. The resultant plasmid was designated pGAD-SapGN. A 0.19-kbp DNA fragment for the C-terminal domain of SapG was amplified by PCR with primers, 5'-GTT ATC GAT CAG ATG CCG CG-3' and 5'-TAA GAT CTG CCG AAA CAT TAG-3', and a plasmid that contains the *sapG* gene as a template. The resultant 0.19-kbp DNA fragment was subcloned into the *EcoRV* site of pBluescript KS(-) by A-T overhang to verify the nucleotide sequence of the insert by sequencing. The plasmid was then digested with *Clal* and *BglII*, and the resultant 0.2-kbp DNA fragment was ligated into the *Clal* and *BglII* sites of the vector pGAD-C1. After sequencing of the plasmid DNA, the *Clal* site introduced into the 0.2-kbp fragment was found to be resistant to *Clal* digestion and the 0.2-kbp fragment generated by *Clal* and *BglII* digestion contained a 10-bp piece derived from pBluescript. To allow an in-frame fusion of C-terminal hydrophilic domain of SapG with Gal4p transcription activation domain, the 0.2-kbp insert in pGAD-C1 was moved to pGAD-C3. After the digestion with *BamHI* and *BglII*, the resultant 0.2-kbp fragment was subcloned into pGAD-C3. The resultant plasmid was designated pGAD-SapGC. The plasmids pGAD-SapG, pGAD-SapGN and pGAD-SapGC were transformed into the yeast strain that harbors the plasmid pGBD-SigG encoding a fusion product of the Gal4p DNA-binding domain with SigG.

Transformants harboring pGBD-C1 with a selection marker of *TRP1* gene was selected by tryptophan autotrophy on SC minus tryptophan medium, and transformants harboring pGAD-C1 or pGAD-C3 with a selection marker of the *LEU2* gene were selected by leucine autotrophy on SC minus leucine medium. For the two-hybrid analysis, adenine or histidine autotrophy of transformants was analyzed by growth test on SC minus tryptophan, leucine and adenine plates or SC minus tryptophan, leucine and histidine plates in the presence of 1 mM 3-AT.

Fatty acid analysis. Fatty acid composition of total lipids in wild-type and the mutant strains of *Syne-*

chococcus sp. strain PCC 7002 was analyzed by the method described by Sakamoto and Bryant (2002). Lyophilized cell pellets were directly methanolized with 10% (v/v) acetyl chloride in anhydrous methanol at 85°C for 2.5 h and products of methanolysis were extracted by *n*-hexane. The resultant fatty acid methyl esters were analyzed by a gas-liquid chromatograph (Model 8410, Perkin Elmer, Foster City, CA) equipped with a column (10% SP-2330 on 100/120 Chromasorb W AW, 6ft, catalogue number 1-3778, Supelco, Bellefonte, PA).

Results

Isolation and sequence analysis of the sigF gene

The *sigF* gene of *Synechococcus* sp. strain PCC 7002 was isolated by cross-hybridization with a 1.1-kbp DNA probe derived from the *sigF* gene (*slr1564*) of *Synechocystis* sp. strain PCC 6803. The 1,744-bp genomic region of the *sigF* gene of *Synechococcus* sp. strain PCC 7002 was sequenced, and an open reading frame (ORF) encoding a polypeptide of 260 amino acids with significant sequence similarity to the *sigF* gene product (Slr1564) of *Synechocystis* sp. strain PCC 6803 was found. Two other ORFs were found in the sequenced region (Fig. 1A); one ORF, located upstream of *sigF* and transcribed from the same DNA strand, was similar to ORF *slr1278* of *Synechocystis* sp. strain PCC 6803, and the other located downstream from *sigF* and encoded on the opposite strand showed sequence similarity to ORF *slr0093* of *Synechocystis* sp. strain PCC 6803, which encodes a protein of the DnaJ family. These adjacent genes were not found in the adjacent regions of the *sigF* gene (*slr1564*) in *Synechocystis* sp. strain PCC 6803 (Kaneko et al., 1996), indicating that the gene organization around the *sigF* locus is different in these two cyanobacterial strains. The deduced amino acid sequence of the *sigF* gene of *Synechococcus* sp. strain PCC 7002 showed 55% similarity to SigF of *Synechocystis* sp. strain PCC 6803 (Slr1564; GenBank accession number BA000022) and 24–27% similarity to the amino acid sequences of *Bacillus subtilis* RpoF/SigB (M34995/P06574), SigF/SpoIIAC (M15744/P07860), RpsG/SigG (P19940/X57547), *Streptomyces coelicolor* SigF (L11648/P37971), SigG (AF036131) and *Mycobacterium tuberculosis* SigF (Z92771).

Isolation and sequence analysis of the ECF sigma factors SigG and SigH

The *sigG* gene of *Synechococcus* sp. strain PCC 7002 was isolated by cross-hybridization with a 0.7-kbp probe derived from the *sigG* gene (*slr1545*) of *Synechocystis* sp. strain PCC 6803. The 1,580-bp genomic region of the *sigG* gene of *Synechococcus* sp. strain PCC 7002 was sequenced (Fig. 1B, C). This region encoded an ORF for a polypeptide of 218 amino acids showing 69% similarity to the *sigG* gene product (*Slr1545*) of *Synechocystis* sp. strain PCC 6803 and an ORF located downstream of SigG encoded on the same DNA strand encoded a polypeptide of 191 amino acids with sequence identity of 22% to the gene product of *slr1546*, which is similarly located downstream from the *sigG* gene in *Synechocystis* sp. strain PCC 6803. This downstream gene was designated *sapG* (*sigG*-associated protein) and characterized further by yeast two-hybrid analysis as described below.

The *sigH* gene of *Synechococcus* sp. strain PCC 7002 was isolated by cross-hybridization with a 0.5-kbp probe derived from the *sigH* gene (*slI0856*) of *Synechocystis* sp. strain PCC 6803. A 1,366-bp genomic region encoding the *sigH* gene of *Synechococcus* sp. strain PCC 7002 was sequenced, and two overlapping ORFs encoded on the same DNA strand were found (Fig. 1D). The upstream ORF encoded a polypeptide of 199 amino acids showing 51% similarity to the *sigH* gene product (*SlI0856*) of *Synechocystis* sp. strain PCC 6803, and the downstream ORF encoded a polypeptide of 140 amino acids with sequence similarity (21% identity) to the product of *slI0857* that is located downstream of the *sigH* gene in *Synechocystis* sp. strain PCC 6803. SigG and SigH of *Synechococcus* sp. strain PCC 7002 showed 24% similarity to one another, and these proteins also had similarity values of 22–31% to proteins that belong to other group 3 sigma factors of the extracytoplasmic function (ECF) subfamily including the RpoE proteins of *E. coli* (P34086), *Haemophilus influenzae* (P44790), *M. tuberculosis* (O05843), YbbL of *B. subtilis* (BAA19507) and SigE of *M. tuberculosis* (U87242/AAC45268; Wösten, 1998).

A 625-bp fragment derived from the *sigI* (*slI0687*) gene of *Synechocystis* sp. strain PCC 6803 was used as a probe in attempts to isolate a gene homologous to *sigI* in *Synechococcus* sp. strain PCC 7002. However, no positive cross-hybridization signal was detected with genomic DNA blots or with indexed cosmid

DNA libraries of *Synechococcus* sp. strain PCC 7002 (data not shown). This result indicates that either there is no *sigI* gene present in *Synechococcus* sp. strain PCC 7002 or the similarity of *slI0687* to any *sigI* gene homolog of *Synechococcus* sp. strain PCC 7002 is too low to allow detection of the gene by cross-hybridization.

Sequences showing similarity to SigF, SigG and SigH of *Synechococcus* sp. strain PCC 7002 were retrieved from the DDBJ/EMBL/GenBank database, and these similar sequences were annotated as sigma factors. Figure 2 shows the result of a phylogenetic analysis of the group 3 sigma factors in cyanobacteria compared to bacterial sigma factors whose functions have been characterized. These sigma factors appear to form two distinct groups. The first group at the bottom of Fig. 2 contains cyanobacterial SigF and the sigma factors of *B. subtilis* with known functions in stress responses (SigB, Hecker et al., 1996) and sporulation (SigF, Haldenwang, 1995 and SigG, Karmazyn-Campelli et al., 1989). The other grouping at the top of Fig. 2 contains cyanobacterial sigma factors SigG and SigH. We have tried to find the functions of the putative sigma factors in cyanobacteria, but the bacterial sigma factors with known functions do not group together with the cyanobacterial sigma factors. This suggests that the apparent similarity of the primary structures of these group 3 sigma factors is not correlated with their functions.

The genes encoding the ECF sigma factors have regulatory genes immediately downstream, which are cotranscribed with the sigma factor genes, and the order of genes encoding the ECF sigma factors and these regulatory factors are conserved in these eubacteria (Hughes and Mathee, 1998). However, the primary structures of the downstream gene products are much less similar to one another than the corresponding ECF sigma factors. The deduced amino acid sequence of the *sapG* gene of *Synechococcus* sp. strain PCC 7002 showed similarity to *slr1546* gene product of *Synechocystis* sp. strain PCC 6803 (22% identity). Figure 3 shows an alignment of the sequences of these putative cyanobacterial regulatory proteins. SapG was not detectably similar to the products of genes located downstream from the ECF sigma factor genes of *E. coli* (P38106), *P. aeruginosa* (L36379), *M. xanthus* (Q06910), *Photobacterium* sp. strain SS9 (L41688) and *Mycobacterium* species (U87242, U87307, U87308) or to product of the gene down-

Fig. 2. Unrooted neighbor-joining tree for group 3 sigma factors.

The amino-acid sequences of SigF (AF121848), SigG (AF121849) and SigH (AF121850) of *Synechococcus* sp. PCC 7002 were compared to SigF of *Synechococcus elongatus* PCC 7942 (YP_400527), *Thermosynechococcus elongatus* BP-1 (BAC08289), *Synechococcus* sp. JA-2-3B'a (ABD03422),

stream from *sigH* of *Synechococcus* sp. strain PCC 7002. Although the primary structures were not highly conserved, the hydropathic profile of the *sapG* gene product was similar to those of other regulatory proteins, including the product of the gene downstream from *sigH* in *Synechococcus* sp. strain PCC 7002. These regulatory proteins for the ECF sigma factors contain a hydrophobic region in the central domain of the polypeptides, which is a putative cytoplasmic membrane-spanning region, and this feature is very highly conserved (data not shown).

Synechococcus sp. JA-3-3Ab (YP_474076), *Nostoc punctiforme* PCC 73102 (ZP_00111455), *Synechocystis* sp. PCC 6803 (*slr1564*; BAA18703), *Gloeobacter violaceus* PCC 7421 (*glr2668*; BAC90609) and *Synechococcus elongatus* PCC 6301 (BAC80499); Sigma-70 of *Nostoc* sp. PCC 7120 (BAB75552), *Trichodesmium erythraeum* IMS101 (ZP_00671562) and *Crocospaera watsonii* WH 8501 (ZP_00516377); Sigma-37 of *Nostoc* sp. PCC 7120 (BAB77801) and *Nostoc punctiforme* ATCC 29133 (ZP_00109707); SigB (P06574), SigF (CAB14277) and SigG (CAB13407) of *Bacillus subtilis*; SigB of *Streptomyces coelicolor* (AAF23070); SigG of *Thermosynechococcus elongatus* BP-1 (BAC09661), *Synechococcus elongatus* PCC 6301 (BAC80361), *Gloeobacter violaceus* PCC 7421 (*glr1739*; BAC89680), *Synechocystis* sp. PCC 6803 (*slr1545*; BAA17471) and *Nostoc* sp. PCC 7120 (BAB74979); Sigma-70 of *Synechococcus* sp. JA-3-3Ab (YP_474920), *Synechococcus* sp. JA-2-3B'a (YP_477688) and *Crocospaera watsonii* WH 8501 (ZP_00514080); Sigma-24 of *Nostoc punctiforme* PCC 73102 (ZP_00111817); SigH of *Synechocystis* sp. PCC 6803 (*slr0856*; BAA17793), *Thermosynechococcus elongatus* BP-1 (BAC07869), *Synechococcus* sp. JA-2-3B'a (YP_476494) and *Gloeobacter violaceus* PCC 7421 (*glr3584*; BAC9525); Sigma-70 of *Crocospaera watsonii* WH 8501 (ZP_00514655); ECF sigma factors of gram-negative bacteria including Sigma-24 of *Thiobacillus denitrificans* (AAZ98048), AlgU of *Pseudomonas aeruginosa* (AAG04151), Sigma-E of *Haemophilus influenzae* (AAX87635), RpoE of *Photobacterium profundum* SS9 (AAA65226) and *E. coli* (AAC45314); ECF sigma factors of gram-positive bacteria including YbbL of *Bacillus subtilis* (BAA19507), RpoE of *Desulfotomaculum reducens* (ZP_01150491), SigE of *Mycobacterium tuberculosis* (CAB10918), SigR (CAA09088) and two Sigma-70 (NP_629295; NP_628323) of *Streptomyces coelicolor*, and sigma factors of phototrophic bacteria including RpoE1 (ZP_00767455) and RpoE2 (EAO058945) of *Chloroflexus aurantiacus*, RpoE of *Chlorobium phaeobacteroides* (EAM64381) and Sigma-70 of *Photobacterium profundum* SS9 (YP_129173). The phylogenetic tree was generated by PHYLIP (Phylogeny Interference Package).

Transcripts of the *sigF*, *sigG* and *sigH* genes

The sizes and abundance of the transcripts for *sigF*, *sigG* and *sigH* genes were analyzed in wild-type cells of *Synechococcus* sp. strain PCC 7002 by RNA-blot hybridization analysis (Fig. 4). The *sigF* transcript gave an intense hybridization signal with a size of approximately 1.5 kb (Fig. 4, lane 1), which was much larger than the size of the *sigF* coding region (0.8 kbp). Using *sigF* specific primers, 5'-RACE was performed to estimate the position of the 5' end of the transcript; a 0.85-kbp fragment was amplified with a primer located

7002	MNESFSQRQTDGDRRRGGDLEMKKNHTSHQHHTDSETPMGHN----	FE	44	
8501	MMSNFEHKQG-----	LFPTDSSDMDNQ----	FE	24
6803	MSNFRSESARVNNKQLIHYYLSVEIPKRFMTNSKFDGDCPRQPWDGAEPGNEDLLFDE		60	
29413	MTTDSNFDNRCR-----	RQFSRDLPPQKMARHTNEATGAMDVMKRD	40	
7120	MTTDSNFDNPCR-----	RQFSRDLPPQKMARHTNEATGAMDVMKRD	40	
73102	MTTDSQFYDRSS-----	LQLPQDLSDGMAKHTNESTGLMDVMKRD	40	
	...:	:		
7002	QFQRLSAYFDGEATPAERKEIQHLLDTPQVKQYQQLRQLKQALQLLPIP-TSISAQYL		103	
8501	RFELLSAYMDGELTAERKQVQWLDIDPEFHLYKQLLRVQRETPTLKVPESISVDDL		84	
6803	KFELLSAYIDGEVTPQEKQVQGWIDSDPEFKQTYLGLIRLQNALPQVPVP-PAIDADVL		119	
29413	RFELLSAYLDGEVTAERRQVEDWLANDVAVQRLYSRLLKLKLRQGIPTAQQSPETT		100	
7120	RFELLSAYLDGEVTAERRQVEDWLANDVAVQRLYSRLLKLKLRQGIPTAQQSPETT		100	
73102	RFELLSAYLDGEVTAPERRQVEEWLANDASVQCLYARLLKLRQGLRTLPIPTAQKSPEAT		100	
	:*: ****:*** *. *: : : : : * :. * * : : : : : * . . :			
TM				
7002	GQVRLARLRSLRSLWGSAGIAALFVAGVMQMPLRLN--FDRFAKNPDQQPTAALV		161	
8501	SQGVFDITDRQDGRKRGLIASALGIAAIVIGSIHISLQDGLFPRYARTNGDNQ-----		138	
6803	AQRVFAKLE-QENRWRIWFGSVTAAAVVVAAYSSILGKPLVPSLNQANLDPALVAMD-		177	
29413	AEQVFAKVN-RRSRLAWKLGGAAVAACVIGAVTNWLPGRQTGIPQLAQQPQEPTQAVTT		159	
7120	AEQVFAKVN-RRSRLAWKLGGAAVAACVIGAVTNWLPGRQTGIPQLAQQPQEPTQAVTT		159	
73102	VQQVFTLR-RRSRLNWMAGGAAVAACVIGAVSSSLVPGGLN-VPQLAQRPPKEPIQTSSA		158	
	: *: : *			
7002	ETSPQGEELVVALNRPVLQIPKLATTESP		191	
8501	-----ALTIALNRPVEIPPTLR		156	
6803	-----EDPLMVAIHEPMFEVLTKVDPGHGPFENEGAEQGLH		212	
29413	P--DALP---MIALNPNVIEIPKAASPTKFIQPQ-PQLGEIIPPDIN		201	
7120	P--DALP---MIALNPNVIEIPKAASPTKFIQPQ-PQLGEIIPPDIN		201	
73102	S--IIPSPMLVMGLNPNVIEIPKAASPKNPIYPVQPQRHDSKQDIN		204	
	:...:*			

Fig. 3. Multiple alignment of SapG regulatory proteins in cyanobacteria.

The deduced amino-acid sequence of SapG of *Synechococcus* sp. strain PCC 7002 was compared to orthologs in the sequenced genomes of the following cyanobacteria: *Crocospaera watsonii* WH 8501 (EAM53282), *Synechocystis* sp. PCC 6803 (BAA17472), *Anabaena variabilis* ATCC 29413 (ABA24531), *Nostoc* sp. PCC 7120 (BAB74980) and *Nostoc punctiforme* PCC 73102: (ZP_00111818). The multiple alignment was generated by Clustal W. A putative trans-membrane domain (TM) is shown.

105-bp upstream of *sigF* coding region and a poly(G)-anchored primer (data not shown), suggesting that the 5'-end of the transcripts was located inside the ORF found upstream of the *sigF* gene and that the estimated size of the 5' non-coding region of the *sigF* transcripts was approximately 0.95 kb (Fig. 1A).

An intense, smeary hybridization signal with sizes ranging from 0.7 to 1.6 kb was detected when a *sigG*-specific hybridization probe was hybridized to total RNA (Fig. 4, lane 2). This hybridization signal was similar to that obtained with a *sapG*-specific probe (Fig. 4, lane 3). A 1.6-kb transcript is large enough to encode both *sigG* (657 bases) and *sapG* (576 bases). RT-PCR analysis was performed using the *sigG*- and *sapG*-specific primers (positions 240–259 and 1201–1218 of accession AF121849) to detect the RT-PCR product containing both *sigG* and *sapG* regions, and the expected 0.97-kbp DNA fragment was amplified (data not shown). These results clearly establish that the *sigG* and *sapG* genes are cotranscribed.

A weak hybridization signal of 1.5 kb was detected when a *sigH*-specific probe was used (Fig. 4, lane 4).

Fig. 4. Accumulation of transcripts for *sigF*, *sigG*, *sapG* and *sigH* genes in *Synechococcus* sp. strain PCC 7002 cells grown under optimal growth conditions.

Total RNA (10 µg) isolated from wild-type cells grown at 38°C was separated on an agarose gel and transferred to nylon membrane filters. The transcripts of each gene were detected with the probes specific for the *sigF* (lane 1), *sigG* (lane 2), *sapG* (lane 3) or *sigH* (lane 4) genes. The 23S (2.8 kb) and 16S (1.5 kb) rRNA bands and the in vivo cleavage products of the 23S rRNA (2.3 and 0.5 kb) were detected by ethidium bromide staining of the gel and the sizes are indicated on the left in kilonucleotides.

The size of this transcript is larger than that expected for the *sigH* ORF (600 bases) plus the downstream ORF (423 bases). Although the *sigH* transcript levels were low, this result suggests that the *sigH* gene is probably cotranscribed with the immediately adjacent, downstream gene as found for *sigG* and *sapG*.

Interaction between sigG and sapG gene products in yeast two-hybrid analysis

In *E. coli*, *P. aeruginosa* and *M. xanthus*, it has been shown that the products of downstream genes associated with the ECF sigma factor genes are anti-sigma factors that bind directly to the sigma factors to inhibit their activities (reviewed by Hughes and Mathee, 1998). As described above, the *sigG* and *sapG* genes are cotranscribed as a dicistronic unit (Fig. 4), and these gene products are likely to participate in the same cellular function by interacting with each other. Thus, as in the case of RpoE and RseA, the *rpoE* downstream gene product of *E. coli* (Missiakas et al., 1997), the interaction of SigG and SapG was analyzed by the yeast two-hybrid system (James et al., 1996). In this system, a yeast strain with three marker genes, *HIS3*, *Ade2* and *lacZ*, was used to allow the Gal4-driven transcription of those genes (James et al., 1996). The *sigG* gene was expressed as a fusion protein with the DNA-binding domain of the Gal4 protein, and the *sapG* gene was expressed as a fusion protein with the transcription activation domain of the Gal4 protein, respectively (referred as Gal4BD-SigG and Gal4AD-SapG thereafter). The interaction of the gene products was examined by histidine and adenine autotrophy of the yeast transformants (Fig. 5). When the strains with both Gal4BD-SigG and Gal4AD-SapG were plated on media devoid of histidine or adenine, they recovered histidine and adenine autotrophy and grew (Fig. 5B and C). On the other hand, strains with Gal4BD-SigG alone or with Gal4BD-SigG and Gal4AD did not grow when cells were plated on media lacking histidine or adenine (Fig. 5B and C). These results indicate that both SigG and SapG are required for the activation of the *gal* promoters to turn on the *HIS3* and *ADE2* genes, demonstrating that the *sigG* and *sapG* gene products interact when expressed as fusion proteins with Gal4p domains in yeast cells. The strains which expressed Gal4BD-SigG and Gal4AD with only the N-terminal or C-terminal domains of SapG did not rescue histidine and adenine autotrophy in the yeast transformants, suggesting that the N-terminal or C-terminal

Fig. 5. Phenotype of the two-hybrid strain PJ69-4A transformed with the pGAD and pGBD constructs.

Plasmid pGBD-SigG expresses a fusion protein of SigG with Gal4 DNA-binding domain fusion; plasmid pGAD expresses a Gal4 activator domain alone; plasmid pGAD-SapG expresses a fusion protein of SapG with Gal4 activator domain; plasmid pGAD-SapGN expresses a fusion protein of the N-terminal hydrophilic domain of SapG with Gal4 activator domain. Transformants were grown on SC minus tryptophan and leucine medium (A), on SC minus histidine, tryptophan and leucine (B), and on SC minus adenine, tryptophan and leucine medium (C).

domains of SapG alone are not sufficient to activate the *gal* promoter in this yeast two-hybrid system (Fig. 5).

Insertional inactivation of the sigF, sigG, sapG and sigH genes

To examine the functions of the *sigF*, *sigG*, *sapG* and *sigH* genes in *Synechococcus* sp. strain PCC 7002 cells, each of the genes was insertionally inactivated with an antibiotic resistance gene cartridge (Fig. 1). The replacement of the wild-type genes by the mutant genes with the inserted cartridges was examined by Southern blot-hybridization analyses for *sigF* and *sigG*, and by PCR for *sigH* (data not shown). The results indicated that these transformants were homozygous for the mutant alleles, and that the targeted genes had been successfully mutated by the insertion of the antibiotic resistance genes.

For the disruption of the *sapG* gene, two constructs

were used for transformation as described in MATERIALS AND METHODS. In one the *aphII* gene was transcribed in the same orientation as *sigG* and *sapG* while in the other the *aadA* gene was transcribed opposite to the direction of *sigG* and *sapG*, and transcriptional terminators were located at both ends of the insertion. The transformants with these constructs were analyzed by Southern blot-hybridization analysis. In the *sapG* strain transformed with the *aphII* insertion, a 0.5-kb band hybridizing with a *sapG* specific probe was detected, and a 1.2-kb band expected for the wild-type *sapG* gene was absent. These results indicate that the wild-type and mutated alleles had completely segregated in the transformant (data not shown). On the other hand, in the *sapG* strain transformed with the *aadA* insertion, a 1.0-kb band derived from the mutant gene with the insertion and a 1.4-kb band derived from the wild-type *sapG* gene were detected with the same probe (data not shown), indicating that the transformant with the *aadA* gene insertion was heterozygous for the mutant alleles. Based upon these results, the *sapG* strain with the *aphII* insertion was selected for further physiological characterization.

Growth of the *sigF*, *sigG*, *sapG* and *sigH* mutant strains at low temperature

The wild type and the *sigF* and *sigG* mutant strains had identical doubling times of 4 h when grown at 38°C under illumination at $250 \mu\text{E m}^{-2} \text{s}^{-1}$ and nutrient-replete conditions (Fig. 6A). These results indicate that the *sigF* and *sigG* genes are not required for cell growth under optimal conditions. When cells grown at 38°C were shifted to 15°C, wild-type cells exhibited arithmetic growth (Fig. 6B) and chlorosis occurred (Sakamoto and Bryant, 1998). However, both the *sigF* and *sigG* mutant cells had more severe growth defects at 15°C. The *sigF* mutant cells had a much slower growth rate compared to wild-type cells, and these cells completely stopped growing after one or two doublings (Fig. 6B). When grown at 22°C, the *sigF* mutant cells had the same growth rate as wild-type cells with a doubling time of approximately 10 h (data not shown). These results suggest that the *sigF* gene is required for the cells to grow at 15°C but not at 22°C. The *sigG* mutant cells barely grew at 15°C and showed a very severe sensitivity to low temperature (Fig. 6B). However, the *sapG* mutant strain grew as well as the wild-type strain at 38°C, indicating that the *sapG* gene is not required for cell growth under opti-

Fig. 6. Growth curves for the wild-type, the *sigF* and *sigG* mutant strains of *Synechococcus* sp. strain PCC 7002 at 38°C and 15°C.

A. Cells were grown photoautotrophically at 38°C on medium A⁺ containing nitrate as the nitrogen source under $250 \mu\text{E m}^{-2} \text{s}^{-1}$ with aeration by 1% (v/v) CO₂ in air. B. Cells grown at 38°C were inoculated (OD_{550 nm} ≈ 0.1) into fresh medium A⁺ and transferred to 15°C under otherwise identical growth conditions.

Fig. 7. Growth of the wild-type, *sigG* and *sapG* mutant strains of *Synechococcus* sp. strain PCC 7002 at 22°C.

A. Cells grown and acclimated at 38°C were inoculated (OD_{550 nm} ≈ 0.1) into fresh medium A⁺ containing nitrate as the nitrogen source and transferred to 22°C under $250 \mu\text{E m}^{-2} \text{s}^{-1}$ with aeration of 1% (v/v) CO₂ in air. B. After acclimation at 22°C for 72 h, cells were inoculated (OD_{550 nm} ≈ 0.05) into fresh medium A⁺ and grown at 22°C under identical growth conditions. Wild-type strain (open circles), the *sigG* mutant strain (closed triangles) and the *sapG* mutant strain (closed squares).

mal growth conditions (data not shown).

The growth phenotype of *sigG* and *sapG* mutant strains was further analyzed at 22°C (Fig. 7). When cells grown at 38°C were inoculated to grow at 22°C,

the *sigG* and *sapG* mutant cells grew similarly to wild-type cells during the first subculture (Fig. 7A). However, neither the *sigG* nor the *sapG* cells grew at all in a second subculture when the cells grown at 22°C were inoculated to fresh media for continuous growth at 22°C (Fig. 7B). This low-temperature growth defect was reversible; both *sigG* and *sapG* mutant cells grew again when the cells treated at 22°C for 72 h were shifted back to 38°C (data not shown). These results indicate that the *sigG* and *sapG* gene products are required for acclimation to low temperature in *Synechococcus* sp. strain PCC 7002. The *sigH* mutant cells had the same growth rate as wild-type cells at 38°C, 22°C or 15°C, suggesting that the *sigH* gene product does not play a role in cell growth at optimal or low-temperature conditions (data not shown).

A defect in fatty acid unsaturation leads to a low-temperature-sensitive phenotype in *Synechococcus* sp. strain PCC 7002; growth of the *desA* mutant cells lacking polyunsaturated fatty acids is slower than that of wild-type cells at 22°C, and the *desB* mutant cells lacking ω 3 unsaturation cannot grow continuously at 15°C (Sakamoto and Bryant, 2002; Sakamoto et al., 1998b). The fatty acid composition of total lipids of the *sigF* and *sigG* mutant cells grown at 38°C and 22°C was examined, and the fatty acid compositions of both mutant strains were identical to that of the wild-type strain at the respective temperature (data not shown). These results indicate that the low-temperature-sensitive phenotype caused by mutations in the *sigF* and *sigG* genes is not due to changes in the fatty acid compositions of the lipids of these mutants. These results also suggest that the desaturase genes responsible for producing polyunsaturated fatty acids are not among the target genes whose transcription is controlled by these sigma factors.

High light intensity has a synergistic effect on the low temperature phenotype of the *desA* mutant; permissive temperature for cell growth shifts under high light intensity ($2.5 \text{ mE m}^{-2} \text{ s}^{-1}$) in the *desA* mutant cells (Sakamoto and Bryant, 2002). The *sigG* mutant cells could grow as well as wild-type cells under $2.5 \text{ mE m}^{-2} \text{ s}^{-1}$ of light at 38°C and 30°C (data not shown), suggesting that high light intensity does not have a synergistic effect on the low temperature phenotype of the *sigG* mutant.

Recently, it has been reported that a *sigF* null strain of *Caulobacter crescentus* shows an oxidative stress-sensitive phenotype in stationary phase (Alvarez-Mar-

tinez et al., 2006). To investigate the possible involvement of sigma factors in the responses to oxidative stress in the cyanobacterium, the sensitivity to methyl viologen (paraquat), which is a herbicide to produce the superoxide radical anion, was tested using the sigma factor mutants of *Synechococcus* sp. strain PCC 7002. The sigma factor mutants showed no greater sensitivity to methyl viologen than the wild type (data not shown). These results suggest that the group 2 and group 3 sigma factors are not likely to be involved in responses to oxidative stress in this cyanobacterium.

Discussion

Three sigma factor genes, *sigF*, *sigG* and *sigH*, have been isolated and characterized in the marine cyanobacterium *Synechococcus* sp. strain PCC 7002. The *sigF* gene product showed sequence similarities to the group 3 sigma factors involved in general stress response and sporulation (Fig. 2). The *sigG* and *sigH* gene products belong to the sigma factors of the ECF subfamily based on sequence comparison (Fig. 2). These two genes were associated with open-reading frames immediately downstream that encode putative membrane-spanning proteins, which is a feature typical of ECF sigma factors (Fig. 1, Fig. 3). The product of the gene downstream from *sigG*, designated SapG, interacts with SigG when produced in yeast cells (Fig. 5). Two important questions remain to be answered by future studies: identification of the target genes controlled by SigG for the acclimation to low-temperature growth conditions, and the regulatory mechanism(s) that alter gene expression patterns through the interactions of SigG and SapG in vivo during the acclimation process.

It is well known that multiple group 2 sigma factors are present in cyanobacteria (Brahamsha and Haselkorn, 1992; Campbell et al., 1998; Caslake et al., 1997; Gruber and Bryant, 1998; Tanaka et al., 1992). These non-essential sigma factors are thought to modulate the transcription genes, including the sigma factor genes themselves, whose expression levels change upon changing nutrient conditions for nitrogen, carbon or sulfur (Asayama et al. 2004; Brahamsha and Haselkorn, 1992; Caslake et al., 1997; Gruber and Bryant, 1998; Imamura et al., 2006; Khudyakov and Golden, 2001; Muro-Pastor et al., 2001; Osanai et al., 2005). In other cases, these sigma factors are required

for circadian expression of genes including *psbA1* (Tsiremas et al., 1996).

The functions of group 3 sigma factors of cyanobacteria appear to be more variable, since mutation of the *sigF* gene in *Synechocystis* sp. strain PCC 6803 caused defects in motility and surface structure of the cells (Bhaya et al., 1999), whereas mutation of the same gene in *Synechococcus* sp. strain PCC 7002 caused a low-temperature sensitivity phenotype (Fig. 6). The *sigG* gene product may also be involved in different functions in different cyanobacterial species because the gene is not essential under optimal growth conditions in *Synechococcus* sp. strain PCC 7002 (Fig. 6), but since the *sigG* gene cannot be inactivated by interposon mutagenesis (Huckauf et al., 2000), it has been concluded to be essential in *Synechocystis* sp. strain PCC 6803 under optimal growth conditions. Similarly to the non-segregation phenotype of the *Synechocystis* sp. strain PCC 6803 (Huckauf et al., 2000), we could not inactivate both genes of *sigF* and *sigG* simultaneously in *Synechococcus* sp. strain PCC 7002 (data not shown), suggesting that some genes essential to growth of *Synechococcus* sp. strain PCC 7002 are recognized by either SigF or SigG, and that the target genes of SigF and SigG may be overlapping in *Synechococcus* sp. strain PCC 7002, although SigF and SigG belong to sigma factors of different subfamilies and are supposed to have different promoter specificity.

In the *sigG* mutant of *Synechococcus* sp. strain PCC 7002, the *aadA* gene was inserted into the *sigG* gene with the anti-parallel orientation so that the *aadA* gene is transcribed in a direction opposite to *sigG*. The presence of strong transcriptional terminators at both ends of the cartridge (Prentki and Krisch, 1984) may also interfere with the expression of the *sapG* downstream of the *sigG* in this construct. In fact, the accumulation of transcripts of *sigG* and *sapG* significantly decreased in the *sigG* mutant cells. When the same cartridge containing the *aadA* gene and transcriptional terminators was inserted into the *sapG* gene in an anti-parallel orientation, the mutant allele could not replace the wild-type one in *Synechococcus* sp. strain PCC 7002. This suggests that the accumulation of SigG without SapG might be toxic for cells of *Synechococcus* sp. strain PCC 7002. This result is apparently similar to previous reports that the mutation of the *yhdL* gene encoding anti-sigma factor against SigM or the mutation of the *yxjC* gene encoding anti-sigma factor against SigY are

lethal in *B. subtilis* (Horsburgh and Moir, 1999; Yoshimura et al., 2004).

The *sapG* gene, which is located immediately downstream of the *sigG* gene in *Synechococcus* sp. strain PCC 7002, showed sequence similarity to the products of the downstream genes of the *sigG* in cyanobacteria (Fig. 3), suggesting that these downstream gene products are likely to be anti-sigma factors against SigG. In *E. coli*, the product of the *rseA* gene, which is located immediately downstream of *rpoE*, was shown to bind to RpoE and inhibit RpoE activity (De Las Peñas et al., 1997; Missiakas et al., 1997). Likewise, the product of *mucA*, located immediately downstream of *algT*, binds to AlgT and negatively affects AlgT activity in *P. aeruginosa* (Schurr et al., 1996; Xie et al., 1996). These anti-sigma factors of *E. coli* and *P. aeruginosa* are inner membrane proteins, in which the N-terminal domain is located in the cytoplasm and the C-terminal domain is located in the periplasm (De Las Peñas et al., 1997; Mathee et al., 1997; Missiakas et al., 1997). In many other bacteria, the ECF sigma factor genes are also associated with putative downstream anti-sigma factor genes, and these putative anti-sigma factors possess a hydrophobic region in the middle of the polypeptides (reviewed by Hughes and Mathee, 1998). SapG also has a hydrophobic region at the central portion of the polypeptide that can serve as a membrane-spanning region, and thus, although the cellular location of SapG in *Synechococcus* sp. strain PCC 7002 is yet to be determined, it is quite likely that SapG is localized to the membrane. Unlike the high sequence similarity of SigG to the other ECF sigma factors, the polypeptide sequence of SapG is much less similar to those of the putative anti-sigma factors of the other bacteria or to that of the *sigH* downstream gene product of *Synechococcus* sp. strain PCC 7002. This diversity in primary sequences of these regulatory proteins may result from different binding interactions of these proteins with the ECF sigma factors and/or may result from differences in sensing and responding to environmental signals. Determination of membrane topology of SapG in *Synechococcus* sp. strain PCC 7002, analysis of the effects of SapG on SigG activity and identification of a protein(s) that binds to SapG in *Synechococcus* sp. strain PCC 7002 will be necessary to determine whether SapG alone acts as the anti-SigG factor or whether additional factors are involved in the regulation of SigG in this cyanobacterium.

The *sigF* and *sigG* mutants showed a low-tempera-

ture-sensitive phenotype (Figs. 6, 7). The *sigA*, *sigB*, *sigC*, *sigD* or *sigE* mutants of *Synechococcus* sp. strain PCC 7002 did not show any growth defect at 15°C (data not shown), indicating that the low-temperature-sensitive phenotype is specifically associated with the *sigF* and *sigG* mutations. The low-temperature sensitivity of the *sigF* and *sigG* mutant strains may result from an alteration of the cell surface structure, as is observed in the *sigF* mutant strain of *Synechocystis* sp. strain PCC 6803 (Bhaya et al., 1999). Similarly to these observations in cyanobacteria, the *rpoE* gene is required for the synthesis of outer membrane proteins and for cell viability at cold temperatures in *Photobacterium* sp. strain SS9 (Chi and Bartlett, 1995). Isolation of genes required for cell surface structure and cell division, analysis of the promoter sequences of these genes and studies on the expression of these genes in the wild-type and *sigG* mutant strains of *Synechococcus* sp. PCC 7002 will be interesting in order to test whether those genes are members of the SigG regulon.

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References

- Alvarez-Martinez, C. E., Baldini, R. L., and Gomes, S. L. (2006) A *Caulobacter crescentus* extracytoplasmic function sigma factor mediating the response to oxidative stress in stationary phase. *J. Bacteriol.*, **188**, 1835–1846.
- Asayama, M., Imamura, S., Yoshihara, S., Miyazaki, A., Yoshida, N., Sazuka, T., Kaneko, T., Ohara, O., Tabata, S., Osanai, T., Tanaka, K., Takahashi, H., and Shirai, M. (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.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1987) Current Protocols in Molecular Biology, Wiley, New York, NY.
- Bhaya, D., Watanabe, N., Ogawa, T., and Grossman, A. R. (1999) The role of an alternative sigma factor in motility and pilus formation in the cyanobacterium *Synechocystis* sp. strain PCC6803. *Proc. Natl. Acad. Sci. USA*, **96**, 3188–3193.
- Brahamsha, B. and Haselkorn, R. (1991) Isolation and characterization of the gene encoding the principal sigma factor of the vegetative cell RNA polymerase from the cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Bacteriol.*, **173**, 2442–2450.
- Brahamsha, B. and Haselkorn, R. (1992) Identification of multiple RNA polymerase sigma factor homologs in the cyanobacterium *Anabaena* sp. strain PCC 7120: Cloning, expression, and inactivation of the sigB and sigC genes. *J. Bacteriol.*, **174**, 7273–7282.
- Brosius, J. (1989) Laboratory methods: Superpolylinkers in cloning and expression vectors. *DNA*, **8**, 759–777.
- Bryant, D. A. and Tandeau de Marsac, N. (1988) Isolation of genes encoding components of photosynthetic apparatus. *Methods Enzymol.*, **167**, 755–765.
- Campbell, E. L., Brahamsha, B., and Meeks, J. C. (1998) Mutation of an alternative sigma factor in the cyanobacterium *Nostoc punctiforme* results in increased infection of its symbiotic plant partner, *Anthoceros punctatus*. *J. Bacteriol.*, **180**, 4938–4941.
- Caslake, L. F. and Bryant, D. A. (1996) The *sigA* gene encoding the major σ factor of RNA polymerase from the marine cyanobacterium *Synechococcus* sp. PCC 7002: Cloning and characterization. *Microbiology*, **142**, 347–357.
- Caslake, L. F., Gruber, T. M., and Bryant, D. A. (1997) Expression of two alternative sigma factors of *Synechococcus* sp. strain PCC 7002 is modulated by carbon and nitrogen stress. *Microbiology*, **143**, 3807–3818.
- Chi, E. and Bartlett, D. H. (1995) An *rpoE*-like locus controls outer membrane protein synthesis and growth at cold temperatures and high pressures in the deep-sea bacterium *Photobacterium* sp. strain SS9. *Mol. Microbiol.*, **17**, 713–726.
- De Las Peñas, A., Connolly, L., and Gross, C. A. (1997) The σ^E -mediated response to extracytoplasmic stress in *Escherichia coli* is transduced by RseA and RseB, two negative regulators of σ^E . *Mol. Microbiol.*, **24**, 373–385.
- Durfee, T., Becherer, K., Chen, P. L., Yeh, S. H., Yang, Y., Kilburn, A. E., Lee, W. H., and Elledge, S. J. (1993) The retinoblastoma protein associates with the protein phosphatase type 1 catalytic subunit. *Genes Dev.*, **7**, 555–569.
- Elhai, J. and Wolk, C. P. (1988) A versatile class of positive-selection vectors based on the nonviability of palindrome-containing plasmids that allows cloning into long polylinkers. *Gene*, **68**, 119–138.
- Felsenstein, J. (2004) PHYLIP (Phylogeny Inference Package) Version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.
- Gietz, R. D., Schiestl, R. H., Willems, A. R., and Woods, R. A.

- (1995) Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. *Yeast*, **11**, 355–360.
- Goto-Seki, A., Shirokane, M., Masuda, S., Tanaka, K., and Takahashi, H. (1999) Specificity crosstalk among group 1 and group 2 sigma factors in the cyanobacterium *Synechococcus* sp. PCC7942: In vitro specificity and a phylogenetic analysis. *Mol. Microbiol.*, **34**, 473–484.
- Gruber, T. M. and Bryant, D. A. (1998) Characterization of the alternative σ -factors SigD and SigE in *Synechococcus* sp. strain PCC 7002. SigE is implicated in transcription of post-exponential-phase-specific genes. *Arch. Microbiol.*, **169**, 211–219.
- Gruber, T. M. and Gross, C. A. (2003) Multiple sigma subunits and the partitioning of bacterial transcription space. *Annu. Rev. Microbiol.*, **57**, 441–466.
- Haldenwang, W. G. (1995) The sigma factors of *Bacillus subtilis*. *Microbiol. Rev.*, **59**, 1–30.
- Hecker, M., Schumann, W., and Volker, U. (1996) Heat-shock and general stress response in *Bacillus subtilis*. *Mol. Microbiol.*, **19**, 417–428.
- Helmann, J. D. (1991) Alternative sigma factors and the regulation of flagellar gene expression. *Mol. Microbiol.*, **5**, 2875–2882.
- Helmann, J. D. (2002) The extracytoplasmic function (ECF) sigma factors. *Adv. Microbiol. Physiol.*, **46**, 47–110.
- Horsburgh, M. J. and Moir, A. (1999) Sigma M, an ECF RNA polymerase sigma factor of *Bacillus subtilis* 168, is essential for growth and survival in high concentrations of salt. *Mol. Microbiol.*, **32**, 41–50.
- Huckauf, J., Nomura, C., Forchhammer, K., and Hagemann, M. (2000) Stress responses of *Synechocystis* sp. strain PCC 6803 mutants impaired in genes encoding putative alternative sigma factors. *Microbiology*, **146**, 2877–2889.
- Hughes, K. T. and Mathee, K. (1998) The anti-sigma factors. *Annu. Rev. Microbiol.*, **52**, 231–286.
- Imamura, S., Asayama, M., Takahashi, H., Tanaka, K., Takahashi, H., and Shirai, M. (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.
- Imamura, S., Tanaka, K., Shirai, M., and Asayama, M. (2006) Growth phase-dependent activation of nitrogen-related genes by a control network of group 1 and group 2 sigma factors in a cyanobacterium. *J. Biol. Chem.*, **281**, 2668–2675.
- Imamura, S., Yoshihara, S., Nakano, S., Shiozaki, N., Yamada, A., Tanaka, K., Takahashi, H., Asayama, M., and Shirai, M. (2003b) Purification, characterization, and gene expression of all sigma factors of RNA polymerase in a cyanobacterium. *J. Mol. Biol.*, **325**, 857–872.
- James, P., Halladay, J., and Craig, E. A. (1996) Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics*, **144**, 1425–1436.
- Kaneko, T., Sato, S., Kotani, H., Tanaka, A., Asamizu, E., Nakamura, Y., Miyajima, N., Hirose, M., Sugiura, M., Sasamoto, S., Kimura, T., Hosouchi, T., Matsuno, A., Muraki, A., Nakazaki, N., Naruo, K., Okumura, S., Shimpo, S., Takeuchi, C., Wada, T., Watanabe, A., Yamada, M., Yasuda, M., and Tabata, S. (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.
- Kang, J. G., Paget, M. S., Seok, Y. J., Hahn, M. Y., Bae, J. B., Hahn, J. S., Kleanthous, C., Buttner, M. J., and Roe, J. H. (1999) RsrA, an anti-sigma factor regulated by redox change. *EMBO J.*, **18**, 4292–4298.
- Karmazyn-Campelli, C., Bonamy, C., Savelli, B., and Stragier, P. (1989) Tandem genes encoding sigma-factors for consecutive steps of development in *Bacillus subtilis*. *Genes Dev.*, **3**, 150–157.
- Khudyakov, I. Y. and Golden, J. W. (2001) Identification and inactivation of three group 2 sigma factor genes in *Anabaena* sp. strain PCC 7120. *J. Bacteriol.*, **183**, 6667–6675.
- Kyte, J. and Doolittle, R. F. (1982) A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.*, **157**, 105–132.
- Lonetto, M. A., Brown, K. L., Rudd, K. E., and Buttner, M. J. (1994) Analysis of the *Streptomyces coelicolor* sigE gene reveals the existence of a subfamily of eubacterial RNA polymerase σ factors involved in the regulation of extracytoplasmic functions. *Proc. Natl. Acad. Sci. USA*, **91**, 7573–7577.
- Lonetto, M., Gribskov, M., and Gross, C. A. (1992) The σ^{70} family: Sequence conservation and evolutionary relationships. *J. Bacteriol.*, **174**, 3843–3849.
- Mathee, K., McPherson, C. J., and Ohman, D. E. (1997) Post-translational control of the *algT* (*algU*)-encoded sigma 22 for expression of the alginate regulon in *Pseudomonas aeruginosa* and localization of its antagonist proteins MucA and MucB (AlgN). *J. Bacteriol.*, **179**, 3711–3720.
- Merrick, M., Gibbins, J., and Toukdarian, A. (1987) The nucleotide sequence of the sigma factor gene *ntrA* (*rpoN*) of *Azotobacter vinelandii*: Analysis of conserved sequences in NtrA proteins. *Mol. Gen. Genet.*, **210**, 323–330.
- Missiakas, D., Mayer, M. P., Lemaire, M., Georgopoulos, C., and Raina, S. (1997) Modulation of the *Escherichia coli* σ^E (RpoE) heat-shock transcription-factor activity by the RseA, RseB and RseC proteins. *Mol. Microbiol.*, **24**, 355–371.
- Missiakas, D. and Raina, S. (1998) The extracytoplasmic function sigma factors: Role and regulation. *Mol. Microbiol.*, **28**, 1059–1066.
- Muro-Pastor, A. M., Herrero, A., and Flores, E. (2001) Nitrogen-regulated group 2 sigma factor from *Synechocystis* sp. strain PCC 6803 involved in survival under nitrogen stress. *J. Bacteriol.*, **183**, 1090–1095.
- Osanai, T., Kanesaki, Y., Nakano, T., Takahashi, H., Asayama, M., Shirai, M., Kanehisa, M., Suzuki, I., Murata, N., and Tanaka, K. (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.
- Prentki, P. and Krisch, H. M. (1984) In vitro insertional mutagenesis with a selectable DNA fragment. *Gene*, **29**, 303–313.
- Rose, M. D., Winston, F., and Hieter, P. (1990) Methods in Yeast Genetics: A Laboratory Course Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Sakamoto, T. and Bryant, D. A. (1997) Temperature-regulated mRNA accumulation and stabilization for fatty acid desaturase genes in the cyanobacterium *Synechococcus* sp. strain PCC 7002. *Mol. Microbiol.*, **23**, 1281–1292.
- Sakamoto, T. and Bryant, D. A. (1998) Growth at low temperature causes nitrogen limitation in the cyanobacterium *Synechococcus* sp. PCC 7002. *Arch. Microbiol.*, **169**, 10–19.
- Sakamoto, T. and Bryant, D. A. (2002) Synergistic effect of high-light and low temperature on cell growth of the $\Delta 12$ fatty acid desaturase mutant in *Synechococcus* sp. PCC 7002. *Photosynthesis Res.*, **72**, 231–242.
- Sakamoto, T., Delgaizo, V. B., and Bryant, D. A. (1998a) Growth on urea can trigger death and peroxidation of the cyanobacterium *Synechococcus* sp. strain PCC 7002. *Appl. Environ. Microbiol.*, **64**, 2361–2366.
- Sakamoto, T., Inoue-Sakamoto, K., and Bryant, D. A. (1999) A novel nitrate/nitrite permease in the marine cyanobacterium *Synechococcus* sp. strain PCC 7002. *J. Bacteriol.*, **181**, 7363–7372.
- Sakamoto, T., Shen, G., Higashi, S., Murata, N., and Bryant, D. A. (1998b) Alteration of low-temperature susceptibility of the cyanobacterium *Synechococcus* sp. PCC 7002 by genetic manipulation of membrane lipid unsaturation. *Arch. Microbiol.*, **169**, 20–28.
- Schurr, M. J., Yu, H., Martinez-Salazar, J. M., Boucher, J. C., and Deretic, V. (1996) Control of AlgU, a member of the σ^E -like family of stress sigma factors, by the negative regulators MucA and MucB and *Pseudomonas aeruginosa* conversion to mucoidy in cystic fibrosis. *J. Bacteriol.*, **178**, 4997–5004.
- Stanier, R. Y. and Cohen-Bazire, G. (1977) Phototrophic prokaryotes: The cyanobacteria. *Annu. Rev. Microbiol.*, **31**, 225–274.
- Stevens, S. E., Patterson, C. O. P., and Myers, J. (1973) The production of hydrogen peroxide by blue-green algae: A survey. *J. Phycol.*, **9**, 427–430.
- Stevens, S. E. and Porter, R. D. (1980) Transformation in *Agmenellum quadruplicatum*. *Proc. Natl. Acad. Sci. USA*, **77**, 6052–6056.
- Sugimoto, Y., Tanaka, K., Masuda, S., and Takahashi, H. (1997) The *rpoD1* gene of *Synechococcus* sp. strain PCC 7942 encodes the principal sigma factor of RNA polymerase. *J. Gen. Appl. Microbiol.*, **43**, 17–21.
- Takeshita, S., Sato, M., Toba, M., Masahashi, W., and Hashimoto-Gotoh, T. (1987) High-copy-number and low-copy-number plasmid vectors for lacZ alpha-complementation and chloramphenicol- or kanamycin-resistance selection. *Gene*, **61**, 63–74.
- Tanaka, K., Masuda, S., and Takahashi, H. (1992) Multiple *rpoD*-related genes of cyanobacteria. *Biosci. Biotechnol. Biochem.*, **56**, 1113–1117.
- Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**, 4673–4680.
- Tsinoremas, N. F., Ishiura, M., Kondo, T., Andersson, C. R., Tanaka, K., Takahashi, H., Johnson, C. H., and Golden, S. S. (1996) A sigma factor that modifies the circadian expression of a subset of genes in cyanobacteria. *EMBO J.*, **15**, 2488–2495.
- Tuominen, I., Tyystjarvi, E., and Tyystjarvi, T. (2003) Expression of primary sigma factor (PSF) and PSF-like sigma factors in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *J. Bacteriol.*, **185**, 1116–1119.
- Wagner, S. J., Thomas, S. P., Kaufman, R. I., Nixon, B. T., and Stevens, S. E., Jr. (1993) The *glnA* gene of the cyanobacterium *Agmenellum quadruplicatum* PR-6 is nonessential for ammonium assimilation. *J. Bacteriol.*, **175**, 604–612.
- Wösten, M. M. S. M. (1998) Eubacterial sigma-factors. *FEMS Microbiol. Rev.*, **22**, 127–150.
- Xie, Z. D., Hershberger, C. D., Shankar, S., Ye, R. W., and Chakrabarty, A. M. (1996) Sigma factor-anti-sigma factor interaction in alginate synthesis: Inhibition of AlgT by MucA. *J. Bacteriol.*, **178**, 4990–4996.
- Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) Improved M13 phage cloning vectors and host strains: Nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene*, **33**, 103–119.
- Yoshimura, M., Asai, K., Sadaie, Y., and Yoshikawa, H. (2004) Interaction of *Bacillus subtilis* extracytoplasmic function (ECF) sigma factors with the N-terminal regions of their potential anti-sigma factors. *Microbiology*, **150**, 591–599.
- Yura, T., Nagai, H., and Mori, H. (1993) Regulation of the heat-shock response in bacteria. *Annu. Rev. Microbiol.*, **47**, 321–350.