Expression of two alternative sigma factors of *Synechococcus* sp. strain PCC 7002 is modulated by carbon and nitrogen stress

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The *sigB* and *sigC* genes, encoding two alternative sigma factors of the unicellular marine cyanobacterium *Synechococcus* sp. PCC 7002, were cloned and characterized. Strains in which the *sigB* and *sigC* genes were insertionally inactivated were viable under standard laboratory conditions, indicating that SigB and SigC are group 2 sigma factors. Starvation for either nitrogen or carbon caused an increase in *sigB* mRNA levels. Transcripts for the *sigC* gene initially increased but then decreased during nitrogen and carbon starvation. The SigC protein could not be identified in cyanobacterial extracts using antisera to *Synechococcus* sp. PCC 7002 SigA or RpoD from *Bacillus subtilis*. The ratio of the principal vegetative sigma factor, SigA, to SigB decreased during either nitrogen starvation or carbon starvation, and the levels of SigB also increased in the *sigC* mutant strain. These results imply that SigB and SigC play roles in modifying transcription in response to changes in carbon and nitrogen availability in this cyanobacterium.

Keywords: RNA polymerase, sigma factors, transcription, cyanobacteria, nutrient stress

INTRODUCTION

The major control point for gene expression is transcription, and DNA-dependent RNA polymerase (EC 2.7.7.6), through its interactions with the promoter regions of genes and operons as well as its catalytic activities in RNA synthesis, is the key enzyme of transcription. In contrast to the apparent complexity of the transcription initiation process in eukaryotes (Goodrich & Tjian, 1994) and archaea (Bult *et al.*, 1996), eubacteria require a single polypeptide, known as the sigma factor, to confer promoter-specific transcription initiation capability on RNA polymerase (Burgess *et al.*, 1969). Two major families of sigma factors are known to occur in eubacteria: the σ^{70} family (Helmann & Chamberlin, 1988) and the σ^{54} (RpoN) family (Kustu *et al.*, 1989).

During exponential-phase growth of eubacteria, the primary, or group 1 (Lonetto *et al.*, 1992), sigma factor

directs transcription. In the case of *Escherichia coli*, RpoD (or σ^{70}) recognizes promoters with the consensus DNA sequence motif 5' TATAAT centred at about -10 with respect to the transcription start at +1, and 5' TTGACA centred at about -35; these two elements are typically separated by 17 bp of nonconserved sequence (Harley & Reynolds, 1987). Functional studies of RpoD (σ^{43} or σ^A) of *Bacillus subtilis* (Moran, 1993; Haldenwang, 1995) suggest that the promoters of this Gram-positive eubacterium are similar in sequence and recognized in a manner similar to those of *E. coli*. The X-ray structure of a significant portion of the σ^{70} sigma factor of *E. coli*, including the region of the protein that interacts with the -10 motif of the promoter, has recently been solved at 2.6 Å resolution (Malhotra *et al.*, 1996). The structure suggests that the -10 promoter motif is recognized by an exposed, amphipathic α -helix within region 2.4 of the σ^{70} protein; an adjacent region of the same α -helix, derived from region 2.3, contains four conserved aromatic residues that are probably involved in melting of the DNA duplex at the promoter. The structure of the carboxy-terminal portion of σ^{70} , that is known to interact with the -35 region of the promoter (Helmann & Chamberlin, 1988; Siegele *et al.*, 1989), is not known.

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The GenBank accession numbers for the nucleotide sequences reported in this paper are U82435 (*sigB*) and U82436 (*sigC*).

Although eubacteria use a single polypeptide to confer promoter-specific transcription initiation capability on RNA polymerase, most eubacteria have the ability to synthesize several different sigma factors (Helmann & Chamberlin, 1988; Lonetto *et al.*, 1992). For example, *E. coli* can utilize seven sigma factors: RpoD (σ^{70}), RpoH, RpoS, RpoE, FliA, FecI and RpoN (Mulvey & Loewen, 1989; Kustu *et al.*, 1989; Lonetto *et al.*, 1992, 1994; Liu & Matsumura, 1995). Sigma factors that are highly similar in sequence to group 1 sigma factors but which are not required for cell viability have been classified as group 2 sigma factors (Lonetto *et al.*, 1992). A few organisms, including cyanobacteria, *Streptomyces* sp. and *Chloroflexus aurantiacus*, are unusual among eubacteria in having several group 2 sigma factors (Tanaka *et al.*, 1991; Curtis & Martin, 1994; Gruber & Bryant, 1997). A much studied member of the group 2 subgroup is RpoS, which is responsible for specific transcription of genes during the post-exponential growth phase (McCann *et al.*, 1991; Kolter *et al.*, 1993). Finally, still more divergent members of the σ^{70} protein family include the group 3 sigma factors such as RpoH, which plays a key role in the heat-shock response of *E. coli* (Grossman *et al.*, 1984; Bukau, 1993; Mager & de Kruijff, 1995), sigma factors that play important roles in sporulation of *B. subtilis* (Stragier, 1991; Haldenwang, 1995), the extracellular factor (ECF) family (including RpoE and FecI; Lonetto *et al.*, 1994), and sigma factors involved in flagellar synthesis (e.g., FliA; Liu & Matsumura, 1995).

Although cyanobacteria may have as many as four group 2 sigma factors (Curtis & Martin, 1994; Kaneko *et al.*, 1996a, b; Gruber & Bryant, 1997), the roles of these sigma factors in gene regulation and cellular physiology are just beginning to be investigated. Two genes encoding type 2 sigma factors, *sigB* and *sigC*, have been isolated from *Anabaena* sp. PCC 7120 (Brahamsha & Haselkorn, 1992). These genes were only transcribed in the period from 6 h to 24 h after removal of combined nitrogen from the growth medium. Strains with null mutations in *sigB* or *sigC* were viable under normal photoautotrophic conditions and were unaffected in their ability to perform nitrogen fixation and to differentiate heterocysts. In a more recent study, it has been suggested that the RpoD2 sigma factor, which appears to be the homologue of SigB of *Anabaena* sp. PCC 7120, modifies the circadian expression of a subset of genes in the cyanobacterium *Synechococcus* sp. PCC 7942 (Tsinoremas *et al.*, 1996).

We are interested in the regulation of gene expression in the unicellular marine cyanobacterium *Synechococcus* sp. PCC 7002. The *sigA* gene, encoding the group 1 sigma factor of this organism, was previously cloned and its product characterized (Caslake & Bryant, 1996). To further understanding of the role of group 2 sigma factors in gene expression in cyanobacteria, in the present study we have characterized two alternative sigma factors, denoted SigB and SigC, of *Synechococcus* sp. PCC 7002. Changes in the relative abundances of these sigma factors during carbon and nitrogen limi-

tation provide insights into their possible biological functions.

METHODS

Bacterial strains, plasmids, and growth conditions. The unicellular marine cyanobacterium *Synechococcus* sp. PCC 7002 strain PR6000 (formerly *Agmenellum quadruplicatum* strain PR-6) was grown in medium A supplemented with sodium nitrate (designated medium A⁺) as described by Caslake & Bryant (1996). For growth of kanamycin-resistant transformants, medium A⁺ was supplemented with 200 mg kanamycin l⁻¹. Cells for nitrogen starvation experiments were grown as described by Caslake & Bryant (1996). For carbon starvation experiments, cells were initially grown in replete medium to an average OD₅₅₀ of 0.5; cultures were then bubbled with nitrogen gas for the specified time period.

E. coli HB101 was used as the host for cosmid pHC79, and *E. coli* DH5 α (Bethesda Research Laboratories) was used for all other recombinant DNA manipulations except protein overproduction. Plasmid pUC19 (Yanisch-Perron *et al.*, 1985) was used for all routine cloning and sequencing procedures. *E. coli* strains were grown on Luria-Bertani (LB) medium, supplemented, when appropriate, with ampicillin (100 mg l⁻¹ in LB), chloramphenicol (25 mg l⁻¹ in LB) or kanamycin (40 mg l⁻¹ in LB). *E. coli* BL21(DE3) was used for overproduction of the SigB protein, and BL21(DE3)pLysS was used for overproduction of the SigC protein (Studier *et al.*, 1990).

DNA and RNA isolation, DNA sequence analysis, and Southern and Northern hybridization analyses. Procedures for the preparation of plasmid DNA from *E. coli*, the isolation of DNA and RNA from *Synechococcus* sp. PCC 7002 strain PR6000, DNA sequence analysis, Southern and Northern (RNA) blot hybridization, the isolation and radioisotopic labelling of DNA fragments, and primer extension mapping of 5' endpoints of mRNA, have been described elsewhere (Caslake & Bryant, 1996). Oligonucleotides were synthesized on a model 380A Applied Biosystems automated DNA synthesizer at the Biotechnology Institute, Pennsylvania State University.

Cloning, insertional inactivation, and transformation procedures. The *sigB* gene (encoded on a 6.0 kbp *EcoRI* genomic DNA fragment; see Caslake & Bryant, 1996) and the *sigC* gene (encoded on a 3.4 kbp *EcoRI* fragment; see Caslake & Bryant, 1996) were isolated by screening a cosmid library of *EcoRI* partial digestion products of *Synechococcus* sp. PCC 7002 strain PR6000 total DNA cloned in cosmid vector pHC79. An 820 bp *KpnI*-*BglII* fragment containing the *sigA* gene was the hybridization probe. A 1.62 kbp region encoding *sigB* and a 2.25 kbp region encoding *sigC* were sequenced (Fig. 1). *Synechococcus* sp. strain PR6501, in which the *sigB* gene is insertionally inactivated with a DNA fragment encoding kanamycin resistance, was constructed by transformation (Buzby *et al.*, 1983) with a DNA construct (Fig. 1a) in which a 1.32 kbp *SmaI* fragment encoding the *aphII* gene of Tn5 was inserted into the unique *SmaI* site of the *sigB* gene (*aphII* encodes aminoglycoside 3'-phosphotransferase II). *Synechococcus* sp. strain PR6502, in which the *sigC* gene is insertionally inactivated with the *aphII* gene, was constructed by transformation with a DNA construct (Fig. 1b) in which a 1.32 kbp *EcoRI* fragment encoding the *aphII* gene of Tn5 was inserted into the unique *StyI* site of the *sigC* gene.

Overproduction of SigB and SigC. The *sigB* gene was amplified by PCR using primers designed to create an *NdeI* site overlapping the start codon of the gene and a *BglIII* site

Fig. 1. Physical maps of (a) the 1.62 kbp *HincII* fragment containing the *sigB* gene and (b) the 2.25 kbp *EcoRV*–*BclI* fragment containing the *sigC* gene, encoding alternative sigma factors of the RNA polymerase of *Synechococcus* sp. PCC 7002. Arrows indicate the direction of transcription. The vertical lines in the box representing the *sigC* gene indicate the position of alternative translation initiation codons. The *aphII* gene, which encodes aminoglycoside 3'-phosphotransferase II and confers resistance to kanamycin, was inserted into the *SmaI* site of the *sigB* gene (in both orientations as indicated by the arrows) and the *StyI* site of the *sigC* gene in mutational studies. Restriction enzyme sites are: E, *EcoRI*; C, *HincII*; X, *XbaI*; S, *SmaI*; L, *SspI*; R, *EcoRV*; U, *Sau3AI*; H, *HindIII*; J, *SstI*; Y, *StyI*; O, *BclI*; M, *XmnI*; Q, *BsmI*; V, *AvaI*.

downstream from the stop codon. The *sigC* gene was amplified by PCR using primers designed to create an *NcoI* site overlapping the start codon of the gene and a *BglII* site downstream from the stop codon. The PCR-amplified products were extracted with phenol, purified by gel electrophoresis, and digested with *BglII* and either *NdeI* or *NcoI*. The digestion products were ligated into pET11a digested with *NdeI* and *BamHI* (for *sigB*) or pET3d digested with *NcoI* and *BamHI* (for *sigC*). The pET11a/*SigB* plasmid was transformed into *E. coli* BL21(DE3). The pET3d/*SigC* plasmid was transformed into *E. coli* BL21(DE3)pLysS. *E. coli* BL21(DE3) cells harbouring the pET11a/*SigB* plasmid and BL21(DE3)pLysS cells harbouring the pET3d/*SigC* plasmid were grown in NZCYM medium supplemented with the appropriate antibiotics at 30 °C with shaking. When the OD₆₀₀ of the cell culture reached 0.6, IPTG was added to 0.5 mM. The cells were harvested 5 h after induction.

SDS-PAGE, immunoblot analysis, and protein sequencing. RNA polymerase of *Synechococcus* sp. PCC 7002 was partially purified as previously described (Caslake & Bryant, 1996). SDS-PAGE was performed as described by Laemmli (1970), and immunoblotting of proteins on nitrocellulose membranes (Schleicher & Schuell) was performed as described by Caslake & Bryant (1996). Rabbit antibodies against the *Synechococcus* sp. PCC 7002 SigA protein were produced as previously described (Caslake & Bryant, 1996). Rabbit antibodies against the *Bacillus subtilis* RpoD/ σ^{43} protein were kindly provided by Dr Roy Doi (University of California, Davis). Amino-terminal and carboxy-terminal protein sequencing was performed by Dr Jindong Zhao (Applied Biosystems).

RESULTS

Cloning and sequence analysis of the *sigB* and *sigC* genes

Previous Southern hybridization results had demonstrated the presence of at least five sigma factor homologues in *Synechococcus* sp. PCC 7002 (Caslake &

Bryant, 1996). The *sigB* and *sigC* genes were isolated from an *EcoRI* partial-digestion cosmid library in vector pHC79, and the nucleotide sequences of these genes were determined. These sequences have been deposited in GenBank under the accession numbers U82435 (*sigB*) and U82436 (*sigC*). A physical map of the 1.623 kbp *HincII* fragment encoding *sigB* is shown in Fig. 1(a). The *sigB* gene predicts a polypeptide of 328 amino acids (38176 Da) (Fig. 2), and no additional genes or open reading frames were identified in the region sequenced through searches of the databases. The ATG start codon for the *sigB* gene is preceded by a weak ribosome-binding sequence (5'-AAAG-3') at a typical distance (-9 to -6) from the start codon, but a much better ribosome-binding sequence (5'-AAGGAG-3') is observed further upstream at -17 to -12 relative to the start codon. An inverted repeat with the potential to form a GC-rich stem-loop followed by a run of T residues occurs immediately downstream from the *sigB* gene. This sequence resembles typical rho-independent transcription termination signals in *E. coli* (Richardson & Greenblatt, 1996) and could play a role in transcription termination, mRNA stabilization, or both processes.

A physical map of the 2.257 kbp *EcoRV*–*BclI* fragment encoding *sigC* is shown in Fig. 1(b). Three in-frame methionine codons, which could serve as translational start sites for the *sigC* gene, are indicated by vertical lines within the box representing the *sigC* gene in Fig. 1(b). The longest potential SigC polypeptide would contain 365 amino acids (42245 Da), the mid-length polypeptide would contain 336 amino acids (39073 Da), and the shortest polypeptide would contain 305 amino acids (35528 Da) (see Fig. 2). Each of these start codons is preceded by potential ribosome-binding sequences. An inverted repeat with the potential to form a GC-rich stem-loop followed by a run of T residues occurs immediately downstream from the *sigC* gene. This sequence strongly resembles rho-independent transcription termination signals in *E. coli* and could play a role in transcription termination, mRNA stabilization, or both processes (Richardson & Greenblatt, 1996). The 3' end of the *moeB* (or *chlN*) gene, encoding a protein that plays a role in molybdenum insertion into the molybdopterin cofactor of molybdo-enzymes (Nohn et al., 1988), was found on the complement strand from nucleotides 2257 to 2079. No open reading frames with significant similarity to sequences in the databases were identified in the more than 700 nucleotides that were sequenced upstream from the *sigC* gene.

An alignment of the deduced amino acid sequences of the SigA, SigB and SigC sigma factors of *Synechococcus* sp. strain PR6000 is shown in Fig. 2. The two group 2 or alternative sigma factors, SigB and SigC, were about 46–47% identical in sequence to the group 1 or essential vegetative sigma factor, SigA. However, in the region implicated in binding the -10 hexamer (region 2.3/2.4), these three proteins are approximately 80% identical. In region 4.2, which is implicated in binding the -35 promoter motif, the pairwise percentage identity values

region 2

DSIRIYLQEIGRIRLLRAEEETIELARKIADLLELERMREQLTEHESRVPTDKWEAAAGMP-LKDFRRRLFHGRRAKDKMVQSNLRLVVSIAKKYMNRLGSFQDL
DMVRTYLREIGRVPPLLTHEEETIVYGKQVQRIMALQEKRQTLEESLDRERTREEWAAEAQLT-PKQLQRDLQIGERAKRKMIEANLRLVVAIARNIKKRNLEFLDL
DMVRSYLHBIGRVPLLTHEQEIIIGKQVQMMALLHEKKALDLGREPSPDEWAAEADLS-VTKLHRYLGQGERAKRKMIEANLRLVVAIAKKYQKRNMFEFLDL
DAVSIYHLKIGRVPLLSHEQEIFFAQVQVQMMVMTFAATEELAEKLGHREPITLQEWADKMQLK-EDVLLQQLSGQGAIAQKQMIQANLRLVVSIAKKYQKRNLEFLDL
DLVRYALYHIGRVRLLTAAEELCFGRQVRLMMLLDAQTELDRDLGHEPSKEEWAADVLDN-LEDLDREIEQGQRAKRKMIEANLRLVVSIAKKYQKRHMEFLDL
DTVGAFFKEMARYPLLPDEETQLAHSVFLNDAEERLEIQEQLGRSPNTNEEWASVLGLETVKQLNSALYKGRVAKRKMIRSNLRLVVSIAKRYLNRGVPFPLDL
DTVGAFFKEMARYPLLSAAEEVELARQIRLLVSAEDVRQQLTQQLERTPSLQEWGQALEFPQVRQFEIWL YQLRAAKRRMIRSNLRLVVSIAKRYLNRGVPFPLDL
* * * * *

region 3

IQEGLSLIRAAEKFDHEKGYKFSTYAIWWIRQAITRAIADQSRITIRLPVHLVETISRIKKTIKLSQELGRKPTEEEIAERMEMTEIKLRFIAKSAQLPISLET
IQEGLSLGERGVEKFDPTKGYKFSTYAYVWIRQAITRAIAQQGRITIRLPITHITEKLNKIKKTQRQLAQDLGRAATIAETAEALELEPAQIREYFVRSRHPISLDDV
IQEGLSLGERGVEKFDPTKGYKFSTYAYVWIRQAITRAIAQQGRITIRLPITHITEKLNKIKKTQRELSQQLGRSATPAEVAKALEIDPSQIREYLSLSRQPISLDV
IQEGALGLERGVEKFDPTLKGYKFSTYAYVWIRQISITRAIAQQSRITIRLPIMHADKLNKIKCVORELSQKLGIYAGTVETIAQALNLEPSQIREYQLVRFVPSLDDV
IQEGLTLGERGVEKFDPSKGYKFSYAYVWIRQAITRAIAQQSRITIRLPITHITEKLNKIKKTQRELSQQLGRSATASELAEVLEPLEQVREYIQMNRQFVPSLDDV
IQEGAIGLNRAAEKFDPNKGYKFSTYAYVWIRQAITRTIANDARTIRLPITHITEKLNKLKKAQRTLKQTQRNPTAEALAEQELDITADQLHQLQLRRQSLSLNH
IQEGAIGLNRAAEKFDPDKGYKFSTYAYVWIRQAITRTIANDARTIRLPITHIVEKLNKIKKAQRSRLKQELKRNPNEGELAAALDITPAQLRQLQLRRQSLSLNH

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----- region 4.2 -----

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PIGKEEDSRLGDFTEAD-GETPEDQVSKSLREDLENVLDTLISA-RERDVLRRLRYGLDGRMKTLEEIGQIFNVTRERIRQTEAKARLKLHPNRNSILKEYIR	375
RVGDNQDQTELMDLLEDGSGESPDNNVTYELLKQDLRLDIADLTP-QQEVIIILRFGLNDGKELSLAKIGKRMNLSRERVRQLERQALNHLRR--RRSQIREYLVAS	328
RVGDNQDTELSLELLED--GVSPDAYITQESMRQDLQDLNLAELTP-QQAVLITRMFGLNGQELSLAKIQGHLNISRERVRQLENQALQALRR--RRANMAYETIAS	345
RIGFQDQTLQLLKDD--GMSPERYAEERLLQYDQHNLAKLTP-QQEVILIRFGLAGGCELTIVQISQRMNISRERVRQVEQVALTLRLR--YGYDSRSYFLAD	331
KVGDSQDTELCLELLED--QSSPSDYVEQESLRDLRLNLMAELTP-QQAVIALRYGLDEGDSLAKVGERLNISSRERVRKLERQAMDHLRR--RSRLLAETIYAS	320
RVGKGEDTELVDLLEDGLQLPEERMSEAMMRQELWDVLDVLTDRKEVIALRYGLSSEQPHITLVEVGGMFNLSRERVRQIQSKAMRKLRRPQVARRLKSPLY	365
RVGKGEDTELVDLLEDQQLQLPEDLMNESMLRREIVEVLAELVLTREMEVICRLRYGIASHQSYTLEEVGMFNLSRERVRQIQSKAMRKLRRPQVARRLKGWL	369

Fig. 2. Alignment of the predicted amino acid sequences of RNA polymerase sigma factors SigA, SigB and SigC from *Synechococcus* sp. PCC 7002 strain PR600 (Caslake & Bryant, 1996; this work); RpoD2 from *Synechococcus* sp. PCC 7942 (Tsinoremas *et al.*, 1996); *Anabaena* sp. PCC 7120 SigB (Brahamsha & Haselkorn, 1992); and *Synechocystis* sp. PCC 6803 RpoD [GenBank accession numbers D63999 and (Kaneko *et al.*, 1996a) D90908]. The overlined regions correspond to conserved regions 1, 2, 3 and 4 of sigma factors as defined by Lonetto *et al.* (1992). Amino acid identities between proteins are indicated with an asterisk; conserved amino acids are indicated by a period; gaps introduced to align the protein sequences optimally are indicated by dashes. The alternative methionines in SigC that could serve as translation initiation starts, corresponding to codons 30 and 61 of *sigC*, are indicated by boldface type. The length of the respective proteins is indicated by the number of amino acids at the end of each respective sequence.

similar to SigE of *Synechococcus* sp. PCC 7002 (Gruber & Bryant, 1997).

Total RNA was isolated from *Synechococcus* sp. strain PR6000 cells grown in replete medium and from cells deprived of nitrogen (Fig. 3) or carbon (Fig. 4) for increasing periods of time. The RNA samples were separated by electrophoresis, transferred to a nylon membrane and hybridized with the 917 bp *HincII*-*BsmI* fragment of the *sigB* gene (Fig. 3a, 4a) or the 792 bp

Fig. 3. Northern blot hybridization analyses of the *Synechococcus* sp. strain PR6000 *sigB* (a) and *sigC* (b) transcripts from cells undergoing nitrogen starvation. (a) The Northern blot was probed with a radiolabelled 917 bp *HincII*-*BsmI* fragment of the *sigB* gene (see Fig. 1a). (b) The Northern blot was probed with a radiolabelled 792 bp *XmnI*-*AvaI* fragment of the *sigC* gene (see Fig. 1b). Lanes 1, RNA extracted from cells grown under standard conditions. Lanes 2–6, RNAs from cells deprived of nitrogen for increasing periods of time: lanes 2, 1 h; lanes 3, 2 h; lanes 4, 4 h; lanes 5, 6 h; lanes 6, 8 h. Transcript sizes were estimated by comparison with 23S (Kumano *et al.*, 1983) and 16S (Tomioka & Sugiura, 1983) rRNAs and their breakdown products (Doolittle, 1972).

XmnI-*AvaI* fragment of the *sigC* gene (Fig. 3b, 4b). The major *sigB* transcript class was approximately 1400 bases in length, and the relative abundance of the *sigB*

Fig. 4. Northern blot hybridization analyses of the *Synechococcus* sp. strain PR6000 *sigB* (a) and *sigC* (b) transcripts from cells undergoing carbon starvation. (a) The Northern blot was probed with a radiolabelled 917 bp *HincII*-*BsmI* fragment of the *sigB* gene (see Fig. 1a). (b) The Northern blot was probed with a radiolabelled 792 bp *XmnI*-*AvaI* fragment of the *sigC* gene (see Fig. 1b). Lanes 1, RNA extracted from cells grown under standard conditions. Lanes 2–7, RNAs from cells deprived of CO₂ for increasing periods of time: lanes 2, 1 h; lanes 3, 2 h; lanes 4, 4 h; lanes 5, 6 h; lanes 6, 8 h; lanes 7, 16 h. Transcript sizes were estimated as described in the legend to Fig. 3.

transcripts increased after 4–8 h of nitrogen starvation. Transcripts larger than the most abundant species were also detected in RNA samples from cells grown in replete medium or from cells that had not been nitrogen-starved for long periods of time (Fig. 3a, lanes 1–3). The *sigB* transcript levels initially decreased in response to carbon starvation, but increased dramatically after 16 h of starvation (Fig. 4a). Furthermore, *sigB* transcript sizes decreased from approximately 1400 bases to about 1000 bases for the 16 h starvation time point (Fig. 4a), which was more apparent in a lighter exposure of the fluorogram (data not shown). No change in *sigB* transcript levels was detected in RNA isolated from cells grown under low light intensity or from cells subjected to a mild heat shock treatment (45 °C for 60 min; data not shown). The major *sigC* transcripts were approximately 1500 bases long, and *sigC* transcripts were less abundant than *sigB* transcripts in cells grown in replete medium. The *sigC* transcripts increased significantly during the first 2 h of nitrogen starvation and then decreased back to levels similar to those found in cells grown in replete medium. Likewise, during carbon starvation, *sigC* transcript levels increased to a maximum at approximately 2 h of starvation, and subsequently decreased until no transcripts were detected after 16 h of starvation (Fig. 4b). No obvious change in the size distribution of the *sigC* transcripts occurred during this time-course.

Fig. 5. Primer extension analyses of the *Synechococcus* sp. strain PR6000 *sigB* (a) and *sigC* (b) transcript. Lanes 1, RNA extracted from cells grown under standard conditions. (a) Lanes 2–4, RNA from cells deprived of nitrogen for increasing periods of time: lane 2, 2 h; lane 3, 4 h; lane 4, 8 h. Primer extension products were denatured and subjected to electrophoresis alongside a DNA sequencing ladder generated using a universal primer on single-stranded M13 phage DNA (US Biochemicals). The two mapped 5' endpoints are 256 and 179 nt upstream of the translation start site for the *sigB* gene. (b) Primer extension products from the *sigC* gene were denatured and subjected to electrophoresis alongside a DNA sequencing ladder generated using the same primer on the *EcoRV*–*StyI* fragment. The mapped 5' endpoints are 255, 141 and 114 nt upstream of the translation start site for the longest SigC polypeptide.

Transcripts hybridizing to *sigC* probes appeared reproducibly as a smear, suggesting that this mRNA is relatively unstable; control hybridization experiments showed that this was not due to artifactual degradation of the RNA (data not shown).

Primer extension analyses to map the 5' endpoints of the *sigB* transcripts were performed with RNA extracted from *Synechococcus* sp. strain PR6000 cells grown under standard growth conditions and with RNA extracted from cells deprived of nitrogen for 2 h, 4 h, and 8 h (Fig. 5a). The longest primer extension product detected, corresponding to a 5' endpoint 256 bases upstream of

the translation start site (denoted –256 in Fig. 5a), was not significantly affected by nitrogen starvation. However, transcripts with 5' endpoints 179 bases upstream of the translation start site (denoted –179 in Fig. 5a) increased as the length of the starvation period increased. Additional potential endpoints were detected, but all of these increased equally in intensity as the duration nitrogen starvation increased. The predicted size of transcripts beginning 256 bases upstream of the translation start site and ending at the last T of the inverted repeat is 1400 bases, a value that agrees well with the estimated size of the transcripts observed by Northern blot hybridization (Figs 3a, 4a).

To map the 5' endpoints of the *sigC* transcripts, primer extension analysis was performed with RNA extracted from *Synechococcus* sp. strain PR6000 cells grown in replete medium under standard conditions (Fig. 4b). Three major 5' endpoints were detected, at 255, 141 and 114 nucleotides upstream of the translation start site for the longest SigC polypeptide. The predicted size of transcripts beginning 255 bases upstream of the translation start site for the longest SigC polypeptide and ending at the last nucleotide of the inverted repeat is 1450 bases. This value is in excellent agreement with the size estimated for these transcripts from the Northern blot hybridization experiments (see Figs 3b, 4b).

Interposon mutagenesis of the *sigB* and *sigC* genes

To determine whether the *sigB* and *sigC* gene products are required for viability of *Synechococcus* sp. PCC 7002 and to learn more about the roles of these sigma factors in cellular physiology, each gene was insertionally inactivated by interposon mutagenesis. The *aphII* gene of Tn5, encoding aminoglycoside 3'-phosphotransferase II and conferring resistance to kanamycin, was inserted into a unique restriction site within the coding sequence of each gene (see Fig. 1a, b). After repeated streaking to allow segregation of alleles to occur, Southern-blot hybridization analyses of chromosomal DNA isolated from selected transformants confirmed that complete segregation had occurred and that both the *sigB* and *sigC* genes were insertionally inactivated as anticipated (data not shown). Thus, neither *sigB* nor *sigC* is essential for viability of *Synechococcus* sp. PCC 7002, and SigB and SigC can be classified as group 2 sigma factors (Lonetto *et al.*, 1992). The *Synechococcus* sp. PCC 7002 strain in which the *sigB* gene has been insertionally inactivated has been designated PR6501. The *Synechococcus* sp. PCC 7002 strain in which the *sigC* gene has been insertionally mutagenized has been designated PR6502. The growth rates of the two mutant strains were identical to that of the wild-type strain under nutrient-replete, standard growth conditions. The growth rates of the mutants were also similar to that of the wild-type under steady-state conditions of reduced CO₂ (air-levels), iron, sulfate or nitrate concentrations, and low light intensity (approximately 60 $\mu\text{E m}^{-2} \text{s}^{-1}$). However, the *sigC* mutant grew noticeably more slowly after a CO₂ shift-down from 1% to 0.03%, suggesting

Fig. 6. SDS-PAGE (a) and immunoblot (b) analyses of the overproduction of *Synechococcus* sp. strain PR6000 SigB and SigC polypeptides. (a) Coomassie-blue-stained SDS-PAGE 10% acrylamide gel. Lanes 1–4, whole-cell extracts of: lane 1, *E. coli* BL21(DE3) containing the pET11aSigB plasmid; lane 2, *E. coli* BL21(DE3) containing the pET11aSigB plasmid after 4 h induction with IPTG; lane 3, *E. coli* BL21(DE3)pLysS containing the pET3dSigC plasmid; lane 4, *E. coli* BL21(DE3)pLysS containing the pET3dSigC plasmid after 4 h induction with IPTG. Lane 5, low-molecular-mass prestained standards. (b) Immunoblot analysis of whole-cell extracts from (a) using the *Synechococcus* sp. strain PR6000 SigA antibody. Lane 6, low-molecular-mass prestained protein standards; lane 7, *E. coli* BL21(DE3) containing the pET11aSigA plasmid after 4 h induction with IPTG; lane 8, *E. coli* BL21(DE3) containing the pET11aSigB plasmid after 4 h induction with IPTG; lane 9, *E. coli* BL21(DE3)pLysS containing the pET3dSigC plasmid after 4 h induction with IPTG.

that SigC might play a role in the ability to adapt to changes in this critical nutrient. A full description of these effects will be described elsewhere (T. Gruber & D. A. Bryant, unpublished).

Overproduction and analyses of recombinant SigB and SigC

SigB and SigC were overproduced in *E. coli* using using a T7 RNA polymerase based expression system (Studier *et al.*, 1990) as described in Methods. As shown in Fig. 6(a), large amounts of both SigB (41 kDa; Fig. 6a, lane 2) and SigC (39 kDa; Fig. 6a, lane 4) could be produced after induction of the T7 RNA polymerase; the over-produced proteins were largely recovered as inclusion bodies (data not shown). Recombinant SigB and SigC were electrophoretically transferred to PVDF membrane filters and subjected to both amino- and carboxy-terminal sequence analysis. The carboxy-terminal sequences determined for SigB and SigC exactly matched the predicted amino acid sequence. Recombinant SigB had proline at its amino-terminus, and thus the amino-terminal methionine had been post-translationally removed (see Fig. 2). Since the actual start codon for the

sigC gene was not known, and since at the time these experiments were performed no other closely related sequences were available in the databases, the over-production clone for SigC was arbitrarily constructed using the second potential methionine initiation codon (see Figs 1 and 2). The amino-terminal sequence of recombinant SigC had a mixture of methionine and valine at the amino terminus (see Fig. 2). The sequence (M)ALQE ... was not detected during sequencing, and thus the third potential methionine initiation codon is not recognized as a start codon in *E. coli*. These results indicate that, in spite of their relative mobilities upon SDS-PAGE (Fig. 6a), the actual molecular mass of recombinant SigB was 38 kDa while that of recombinant SigC was 39 kDa. Anomalous electrophoretic mobilities have been noted for several other sigma factors and have been attributed to the large percentage of charged residues that occur in these proteins (Burton *et al.*, 1981; Chang & Doi, 1990; Brahamsha & Haselkorn, 1991).

In previous work polyclonal rabbit antibodies to *Synechococcus* sp. PCC 7002 SigA were produced (Caslake & Bryant, 1996). Fig. 6(b) shows an immunoblot prepared with proteins from whole-cell extracts of *E. coli* strains induced to overproduce SigA (Fig. 6b, lane 7), SigB (Fig. 6b, lane 8) and SigC (Fig. 6b, lane 9). Although antibodies to SigA cross-react with SigB and SigC, this heterologous cross-reaction was much weaker than the homologous reaction with SigA.

Immunoblot analyses of sigma factors in extracts of *Synechococcus* sp. PCC 7002 cells grown under different conditions

Immunoblot analyses were employed to follow the relative levels of SigA, SigB and SigC in cells grown under nutrient-replete conditions and after nutrient starvation. Crude RNA polymerase fractions were prepared from *Synechococcus* sp. strains PR6501 and PR6502 grown under standard growth conditions in replete medium and from wild-type strain PR6000 grown in replete medium or subjected to nitrogen starvation for 12 h or carbon starvation for 16 h. Fig. 7(a) shows the Coomassie-blue-stained proteins separated during SDS-PAGE analysis of these crude RNA polymerase fractions. Although it is clearly not possible to detect directly polypeptides that can be identified as the sigma factor subunits, this figure demonstrates that approximately equal amounts of protein were loaded for each sample. Fig. 7(b) shows an immunoblot analysis of the RNA polymerase preparations shown in Fig. 7(a) using antibodies to *Synechococcus* sp. PCC 7002 SigA. The major cross-reacting protein in lanes 2–6 (apparent mass 49 kDa) is presumed to be the SigA polypeptide synthesized *in vivo*. Because an alternative start codon was used for production of the (larger) recombinant SigA protein (Fig. 7b, lane 7), the naturally occurring SigA protein comigrates with a minor component of the recombinant SigA protein (for complete details, see Caslake & Bryant, 1996). For cells grown under standard growth conditions, the relative abundances of

Fig. 7. SDS-PAGE (a) and immunoblot (b, c) analyses of partially purified RNA polymerase from *Synechococcus* sp. strain PR6000 and mutants. Lanes 1, low-molecular-mass prestained protein standards. Lanes 2–6, partially purified RNA polymerase from: lane 2, *Synechococcus* sp. strain PR6000 grown in replete medium; lane 3, *Synechococcus* sp. strain PR6000 grown in replete medium and then starved for nitrogen for 12 h; lane 4, *Synechococcus* sp. strain PR6000 grown in replete medium and then starved for carbon for 16 h; lane 5, *Synechococcus* sp. strain PR6501 grown in replete medium; lane 6, *Synechococcus* sp. strain PR6502 grown in replete medium. Lane 7, a mixture of whole-cell extracts from *E. coli* BL21(DE3) containing the pET11aSigA, pET11aSigB plasmids, and BL21(DE3)pLysS cells containing the pET3dSigC plasmid after 4 h induction with IPTG. (a) Coomassie-blue-stained SDS-PAGE 10% acrylamide gel. (b, c) Immunoblot analyses of partially purified RNA polymerase preparations from (a) probed with the *Synechococcus* sp. strain PR6000 SigA antibodies (b) or with the *B. subtilis* σ^{43} antibodies (c). Recombinant SigA, SigB, and SigC are indicated on the right side of each panel.

SigA in the crude RNA polymerase fraction of *sigB* mutant strain PR6501 (Fig. 7b, lane 5) and *sigC* mutant strain PR6502 (Fig. 7b, lane 6) were similar to the level observed in the wild-type strain pPR6000 (Fig. 7b, lane 2). However, the relative abundance of SigA in extracts of the wild-type strain after starvation for nitrogen (Fig. 7b, lane 3) or carbon (Fig. 7b, lane 4) was significantly lower than the level found in the control cells grown in replete medium (Fig. 7b, lane 2). Antibodies to the *Synechococcus* sp. PCC 7002 SigA protein cross-reacted very weakly with a polypeptide with the apparent mass of recombinant SigB, but no cross-reacting species with a mass similar to that of the recombinant SigC protein could be detected in any extract.

Fig. 7(c) shows an immunoblot analysis of the same cell extracts shown in panels (a) and (b) but using antibodies raised against the RpoD (or σ^{43}) protein of *B. subtilis* (Chang & Doi, 1990). As can be seen from the intensity of the cross-reactions to the control recombinant protein mixture in lane 7, these antibodies recognized the three sigma factor proteins of *Synechococcus* sp. PCC 7002 more or less equivalently. As also shown in Fig. 7(b) with antibodies to SigA, SigA levels decreased during nitrogen (Fig. 7c, lane 3) and carbon starvation (Fig. 7c, lane 4) and appeared to be of the same abundance in RNA polymerase preparations isolated from cells grown under standard growth conditions from the wild-type strain PR6000 (Fig. 7c, lane 2), the *sigB* mutant strain

PR6501 (Fig. 7c, lane 5) and *sigC* mutant strain PR6502 (Fig. 7c, lane 6). The antibodies to the σ^{43} protein of *B. subtilis* also recognized a protein in the crude polymerase fractions with an apparent mass of 41 kDa; this protein co-migrated with the overproduced SigB protein (Fig. 7c, lane 7), but no polypeptide corresponding to SigC was detected with this antibody. Strongly supporting the identification of this cross-reacting protein as SigB, this polypeptide was not detected in extracts from the *sigB* mutant strain PR6501 (Fig. 7c, lane 5). The relative abundances of the SigA and SigB polypeptides changed significantly in response to nitrogen and carbon starvation (compare Fig. 7c, lane 2 to lanes 3 and 4). Low levels of the SigB polypeptide were detected in strain PR6000 grown under nutrient-replete conditions (Fig. 7c, lane 2), and similar levels of the SigB polypeptide were detected after nitrogen starvation for 12 h (Fig. 7c, lane 3). Thus, the ratio of SigB to SigA increased significantly after nitrogen limitation. An even greater increase in the level of the SigB polypeptide was observed after carbon starvation for 16 h (Fig. 7c, lane 4); since the SigA level also decreased under this growth condition, the SigB:SigA ratio was even larger under these conditions. Since our RNA polymerase enrichment procedure involves heparin-agarose chromatography, the immunoblot analyses suggest that the number of RNA polymerase holoenzymes containing the SigA polypeptide decreases during nitrogen and carbon starvation, while those associated with the SigB polypeptide

increase during the same treatments. Interestingly, SigB levels were as high in the *sigC* mutant strain PR6502 (Fig. 7c, lane 6) as in the carbon-starved cells of the wild-type strain (Fig. 7c, lane 4); however, SigA levels under nutrient-replete conditions were not detectably affected by the absence of SigC in strain PR6502. These observations strongly suggest that the ratio of SigB to SigA is important in determining the cellular response to changes in carbon and nitrogen availability in the environment.

DISCUSSION

In this work the *sigB* and *sigC* genes, encoding two alternative sigma factors, were cloned and characterized from *Synechococcus* sp. PCC 7002. Since strains harbouring null mutations in *sigB* and *sigC* were viable under standard laboratory growth conditions, the products of these genes can be categorized as group 2 sigma factors (Lonetto *et al.*, 1992; Gruber & Bryant, 1997). Multiple group 2 sigma factors have now been identified in several cyanobacteria, including *Synechococcus* sp. PCC 7002 (Caslake & Bryant, 1996; Gruber & Bryant, 1997), *Synechococcus* sp. PCC 7942 (Tanaka *et al.*, 1992a, b; Tsinoemas *et al.*, 1996), *Anabaena* sp. PCC 7120 (Brahamsha & Haselkorn, 1991, 1992), and *Synechocystis* sp. PCC 6803 (Kaneko *et al.*, 1996a, b). Analysis of the complete genome of *Synechocystis* sp. PCC 6803 (Kaneko *et al.*, 1996a, b) has revealed that this cyanobacterium can produce up to eight sigma factors: one group 1 sigma factor; four group 2 sigma factors; one group 3 sigma factor with strong sequence similarity to SigB of *B. subtilis* (Binnie *et al.*, 1986) and homologues in *Staphylococcus aureus* (Wu *et al.*, 1996) and *Streptomyces* sp. (Kato *et al.*, 1995); and two members of the group 3-RpoE subfamily (Lonetto *et al.*, 1994). Homologues of each of these eight sigma factors have been identified in the genome of *Synechococcus* sp. PCC 7002 (Caslake & Bryant, 1996; Gruber & Bryant, 1997; T. Gruber & D. A. Bryant, unpublished results).

Functional studies of RNA polymerase and group 1 sigma factors from *Anabaena* sp. PCC 7120 (Schneider *et al.*, 1991) and *Calothrix* sp. PCC 7601 (Schyns *et al.*, 1994) strongly suggest that the cyanobacterial promoters recognized by these principal sigma factors will have strong sequence similarity to the -10 and -35 promoter motifs of *E. coli*, *B. subtilis*, and other eubacteria (Harley & Reynolds, 1987; Moran, 1993; Haldenwang, 1995; Record *et al.*, 1996). Conserved region 2.4 is involved in recognition of the -10 motif of promoters (Helmann & Chamberlin, 1988; Lonetto *et al.*, 1992; Malhotra *et al.*, 1996). Since region 2.4 of all group 1 and 2 sigma factors, including the SigB and SigC proteins described here, is virtually invariant in amino acid sequence (Gruber & Bryant, 1997), it can be surmised that most eubacterial promoters recognized by group 1+2 sigma factors will contain -10 motifs similar to the *E. coli* consensus. Conserved region 4.2 recognizes the -35 motif of promoters (Helmann & Chamberlin, 1988; Lonetto *et al.*, 1992). Since some residues of region

4.2 that are known to be involved in -35 motif recognition are also invariant, it can be assumed that significant sequence conservation in the -35 recognition motif will also occur. However, there is greater sequence variation in region 4.2 of group 1+2 sigma factors, and it is probable that some variation in the preferred -35 motifs of promoter regions may occur. Nitrocellulose filter binding assays have shown that the *E. coli* σ^{70} is highly sensitive to the interhexamer spacing (Dombroski *et al.*, 1996). The strong collinearity of all group 1+2 sigma factor sequences between regions 2.1 and 4.2 strongly implies that the preferred spacing for the -10 and -35 motifs will be close to 17 nt for all promoters (Gruber & Bryant, 1997).

The high degree of sequence similarity among all group 1 and group 2 sigma factors in the regions expected to interact with the -10 and -35 promoter motifs raises the question of how these sigma factors might differ functionally from the primary sigma factors. One possibility is that these alternative sigma factors could exhibit significantly different responses to activators or repressors. A number of subunits of the RNA polymerase, including α , β' and σ , have been shown to interact with activators and repressors (reviewed by Ptashne & Gann, 1997). Region 4 of RpoD in *E. coli* has been shown to interact specifically with activators (Busby & Ebright, 1994). The bacteriophage λ cI protein binds to a site centred at position -42 and overlaps the -35 element of the λP_{RM} promoter (Li *et al.*, 1994). An allele-specific intergenic suppressor of the cI mutation (Li *et al.*, 1994) was isolated in the RpoD protein (R596H); this suppressor substitution occurs just after the helix-turn-helix motif that binds the -35 motif of the promoter. Hu & Gross (1985) also found that residue R596 of RpoD affects activation of transcription by AraC and MalT. The corresponding regions from the carboxy-terminal portion of region 4.2 to the carboxy-terminus of the SigA, SigB and SigC proteins differ significantly and hence might interact in very different ways with activators or repressors. A second domain, highly variable both in length and sequence, extends from the amino-terminus to region 2.1 of the group 1+2 sigma factors. For SigA, SigB and SigC of *Synechococcus* sp. PCC 7002, this domain is 146, 99 and 133 amino acids, respectively, and there is very little sequence similarity among the three proteins in this region (Fig. 2). As with other sigma factors which have been studied (Dombroski *et al.*, 1992, 1993), the amino-terminal amino acids may inhibit or modulate the DNA-binding activity of each sigma factor. These highly variable regions could also be responsible for specific functions of the various group 1+2 sigma factors and could confer significant differences in the ability to be modulated by transcription activators or repressors, without necessarily requiring differences in promoter recognition properties.

The effects of various environmental conditions on the expression of the *sigB* and *sigC* genes were examined in *Synechococcus* sp. PCC 7002. Northern blot hybridization and primer extension analyses indicated that the

relative abundance of *sigB* transcripts increased significantly when cells were starved for nitrogen or carbon. This observation is similar to that reported for nitrogen-starved cells of *Anabaena* sp. PCC 7120 (Brahamsha & Haselkorn, 1992). A change in the predominant 5' endpoint of the transcripts occurred as well, suggesting that a new promoter was used for transcription of this gene under these conditions. In this study SigB protein levels seem to correlate rather well with the transcript levels. For example, the levels of both mRNA and protein are elevated in cells that have been starved for carbon for 16 h, although the protein levels did not increase to the same degree as transcript levels. Protein turnover, or other post-transcriptional events, may also be involved in regulating the levels of SigB protein in cells. Nonetheless, the ratio of SigA to SigB changed significantly, since the levels of SigA associated with RNA polymerase were much lower after nitrogen starvation. It was previously shown that *sigA* transcripts decrease in abundance when cells are starved for nitrogen for increasing periods of time (Caslake & Bryant, 1996), and it has also been found that carbon limitation causes a decrease in *sigA* transcript levels (data not shown). Interestingly, the highest levels of SigB protein were detected in cells that had been starved for carbon (Fig. 7b, c). These results strongly suggest that SigB levels are altered in response to changes in carbon and nitrogen metabolism in these cells. A change in the SigA : SigB ratio could, in combination with interactions with additional activators or repressors, significantly alter the rate of transcription of specific operons during nutrient stress conditions. The increased levels of SigB in the *sigC* mutant could indicate that both the regulation and functions of these two sigma factors overlap such that loss of one protein can be compensated for by increased expression of the other. Alternatively, the loss of SigC may cause an increase in transcription of the *sigB* gene. Studies are now being conducted to distinguish among these possibilities.

Transcripts for the *sigC* gene initially increased and then decreased during nitrogen and carbon starvation, and *sigC* levels were much higher in cells grown at 1% CO₂ than in cells grown on air. In cells grown under nutrient-replete conditions, transcripts for the *sigC* gene were less abundant than those of either the *sigA* (Caslake & Bryant, 1996) or *sigB* genes (see Fig. 3). Using antibodies to SigA of *Synechococcus* sp. PCC 7002 and RpoD of *B. subtilis*, we were unable to detect the SigC protein in either whole-cell extracts or RNA-polymerase-enriched fractions, although both antibodies weakly cross-reacted with recombinant SigC produced in *E. coli*. To overproduce the SigC protein in *E. coli*, the second potential methionine start codon was arbitrarily selected and re-engineered as the start codon for the expression construct used in *E. coli*. At the time these studies were completed, no other cyanobacterial sequences with strong similarity to SigC were available. However, since the entire *Synechocystis* sp. PCC 6803 genome has subsequently been sequenced (Kaneko *et al.*, 1996a, b) and encodes a homologue of SigC, this decision can now

be re-evaluated. The length and limited sequence similarity between the amino-terminal regions of the two SigC proteins suggest that the first potential methionine start codon may actually be used in *Synechococcus* sp. PCC 7002. If the first methionine were to be used as the start codon, the predicted protein product would have a mass of 42.2 kDa and would be 3.2 kDa larger than the product that was overproduced in these studies in *E. coli* (see Fig. 6). Since the SigB protein migrates anomalously slowly upon SDS-PAGE, a larger SigC protein might have co-migrated with SigB in the RNA-polymerase-enriched fractions from *Synechococcus* sp. PCC 7002. Nevertheless, SigC should have been detectable in the *sigB* mutant strain PR6501, but only SigA was detected in extracts of this strain. Resolution of this issue will require antibodies with much higher specificity for the SigC protein.

The observed changes in the SigA : SigB ratio provoked a variety of experiments to search for phenotypic differences between the wild-type strain and the *sigB* and *sigC* mutant strains in response to nitrogen starvation, nitrogen readdition and growth properties on different nitrogen sources and CO₂ levels. Under conditions of nitrogen or carbon starvation, *Synechococcus* sp. can degrade up to 90% of the phycobiliproteins for maintenance metabolism (Tandeau de Marsac & Houmard, 1993; Grossman *et al.*, 1994). After readdition of nitrogen or carbon, the phycobiliproteins are resynthesized. It has been shown that genes for the major phycobiliproteins phycocyanin and allophycocyanin are not transcribed during nitrogen or carbon starvation (Gasparich *et al.*, 1987; Wealand *et al.*, 1989; Zhou *et al.*, 1992). To test whether SigB was involved in the degradation or resynthesis of phycobiliproteins, nitrogen was removed from cultures of *Synechococcus* sp. strains PR6000 and PR6501 in the mid-exponential growth phase. At increasing times after nitrogen removal, up to 24 h, nitrogen was readded and phycobiliprotein contents of the cells were determined. No difference between *Synechococcus* sp. strains PR6000 and PR6501 was detectable (data not shown). This may mean that SigB is not involved in this response to nitrogen starvation, or may mean that SigB is part of a redundant system and can be replaced by another sigma factor. Recent experiments involving carbon limitation have been more revealing. Growth of the *sigC* mutant strain was noticeably slower immediately after a step-down from 1% CO₂ to 0.03% CO₂, although steady-state growth on air-levels of CO₂ was identical for the wild-type and the *sigC* mutant strain (data not shown). Given the central importance of carbon metabolism in autotrophs, it is possible that redundant regulatory responses also exist to allow cells to respond to changes in the availability of inorganic carbon. Additional physiological studies of the *sigB* and *sigC* mutant strains will be published elsewhere.

In conclusion, the results presented here suggest that the alternative sigma factors SigB and SigC of *Synechococcus* sp. PCC 7002 play a role in adaptive responses to a cellular imbalance in carbon and nitrogen metab-

olism. In this regard, it is interesting to note that the *Synechocystis* sp. PCC 6803 genome does not contain any genes with significant homology to the *rpoN* gene, encoding the σ^{54} sigma factor that plays important roles in nitrogen-regulated gene expression in enteric bacteria (Kustu *et al.*, 1989). With the clues to function identified in these studies, future studies will focus on changes in expression patterns of genes whose expression is known to be strongly modulated by the cellular balance of carbon and nitrogen metabolism in cyanobacteria (Tandeau de Marsac & Houmard, 1993; Bryant, 1994).

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