

# The *sufR* Gene (*sll0088* in *Synechocystis* sp. Strain PCC 6803) Functions as a Repressor of the *sufBCDS* Operon in Iron-Sulfur Cluster Biogenesis in Cyanobacteria

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Received 26 August 2003/Accepted 11 November 2003

The *suf* operon is composed of four genes (*sufB*, *sufC*, *sufD*, and *sufS*) and is highly conserved in the genomes of cyanobacteria. Open reading frame *sll0088* in *Synechocystis* sp. strain PCC 6803 is located near the 5' end of the *suf* operon but is transcribed in the direction opposite that of the *suf* operon. We previously reported the isolation of two independent suppressor strains of C14S<sub>PsaC</sub> that mapped to *sll0088* and restored photoautotrophic growth. The protein encoded by *sll0088* has two significant features: (i) a DNA-binding domain near the N terminus and (ii) four highly conserved cysteine residues near the C terminus. The protein has high sequence similarity to transcription regulatory proteins with a conserved DNA-binding domain and can be classified in the DeoR family of helix-loop-helix proteins. The protein falls into a further subclass that contains a C-X<sub>12</sub>-C-X<sub>13</sub>-C-X<sub>14</sub>-C motif near the C terminus, which may represent a metal-binding site. The expressed Sll0088 protein harbored an iron-sulfur cluster as shown by optical and electron paramagnetic resonance spectroscopy. Compared to the wild type, expression levels of the *sufBCDS* genes were elevated when cells were grown under conditions of oxidative and iron stress and were even higher in a null mutant of *Synechococcus* sp. strain PCC 7002 in which the *sll0088* homolog was insertionally inactivated. In agreement with the proposed role of the *sufBCDS* genes in iron metabolism, the growth rate of the null mutant was significantly higher than that of the wild type under iron-limiting conditions. We propose that the protein encoded by *sll0088* is a transcriptional repressor of the *suf* operon, and we name the gene *sufR*.

The biogenesis and assembly of fully functional photosystem I (PS I) require the assembly of F<sub>X</sub>, F<sub>A</sub>, and F<sub>B</sub>, which are [4Fe-4S] clusters, and soluble ferredoxin, which contains a [2Fe-2S] cluster. Iron-sulfur clusters can be assembled and inserted into proteins in vitro by incubating the apoprotein with iron, sulfide, and a thiol-containing reducing agent, such as 2-mercaptoethanol (22). This approach has been used in conjunction with bacterial expression systems to elucidate the structure and function of a variety of photosynthetic iron-sulfur proteins, including PsaC (reviewed in reference 11). In contrast, the in vivo biosynthesis of iron-sulfur clusters in photosynthetic complexes involves biochemical assembly processes that are poorly characterized. One known factor is a membrane-bound rubredoxin, which appears to be associated exclusively with the assembly of the F<sub>X</sub> iron-sulfur cluster (33, 34). Another known factor is the open reading frame *sll0088* in *Synechocystis* sp. strain PCC 6803, which appears to have a role in regulating the biogenesis of PS I (43) and is the topic of this study.

In nonphotosynthetic organisms, iron-sulfur cluster assembly is known to be a multistep process that involves cluster biosynthesis, insertion, and stabilization (9). The *isc* operon is implicated in generalized iron-sulfur cluster assembly in many

organisms, including *Azotobacter vinelandii* (45), *Escherichia coli* (25), and *Saccharomyces cerevisiae* (31). Homologs of several genes in the *isc* operon, including *iscS* and *iscA*, have been identified in the genome of the cyanobacterium *Synechocystis* sp. strain PCC 6803 (16, 17). In fact, three distinct *iscS*-like homologs have been identified in genomes of *Synechocystis* sp. strain PCC 6803 (16, 18), *Anabaena* sp. strain PCC 7120 (15, 26), and *Synechococcus* sp. strain PCC 7002 (J. Zhao, T. Li, J. Marquardt, and D. A. Bryant, unpublished data). The presence of multiple *iscS* homologs hints at different functions or regulatory mechanisms in the biosynthesis of iron-sulfur clusters. Two additional iron-sulfur cluster assembly systems that are more specialized in function, *nif* and *suf*, exist in bacteria. The *nif* operon in *A. vinelandii* is involved in the biosynthesis of the nitrogenase iron-molybdenum cofactor (8). The *suf* operon has been shown to function in the assembly of iron-sulfur clusters under conditions of oxidative stress (24, 41). It is known that at least two of these systems, *isc* and *suf*, exist in mitochondria (31, 39) and in the chloroplasts of higher plants (20, 28, 40), respectively. However, virtually nothing is known concerning their regulation.

As reported previously (43), a methodology of selecting suppressors to primary mutations in iron-sulfur proteins was used to isolate spontaneous suppressors from *psaC* site-directed mutants of *Synechocystis* sp. strain PCC 6803. The phenotypes of the C51D<sub>PsaC</sub> (F<sub>B</sub>) and C14S<sub>PsaC</sub> (F<sub>A</sub>) mutants of PsaC were such that the strains failed to grow photoautotrophically, yet electron throughput from cytochrome *c*<sub>6</sub> to flavodoxin in iso-

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lated PS I complexes was similar to that of the wild type (14, 44). The mutants were sensitive to high light intensities and could not grow photomixotrophically under white-light intensities in the range from 20 to 60  $\mu\text{E m}^{-2} \text{s}^{-1}$ . The mutants had two additional phenotypes: the amount of PS I, but not PS II, was lower in the mutants than in the wild type on a per cell basis, and the mutants were able to grow photomixotrophically at a light intensity of 20 to 60  $\mu\text{E m}^{-2} \text{s}^{-1}$  only in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of PS II. Thus, the failure to grow photoautotrophically was apparently due to the lowered ratio of PS I to PS II in the mutant cells rather than an inefficiency in forward electron transfer in any individual PS I complex. Screening the C51D<sub>PsaC</sub> and C14S<sub>PsaC</sub> mutants under high-light intensity resulted in the appearance of suppressor mutants. Two of the intergenic suppressor strains, C14S<sub>PsaC</sub>-R18 and C14S<sub>PsaC</sub>-R62, which were capable of photoautotrophic growth at normal light intensities, were selected for further study. These two suppressor strains retained the primary mutation, which was verified through amplification of the *psaC* gene fragment by PCR and sequencing. The suppressor mutations were mapped to a specific gene, *sll0088*, by phenotypic complementation and identified by DNA sequencing (43).

To elucidate the function of the *sll0088* gene homologs in cyanobacteria, we sought to characterize the physical properties of the protein encoded by this gene, to investigate the regulation of the *sufBCDS* operon, and to study the physiology of a *sll0088* null mutant. We show that the protein encoded by *sll0088* is an iron-sulfur protein that functions as a transcriptional repressor, regulating the expression of the *sufBCDS* operon. Thus, it is similar in function to IscR, which is an iron-sulfur protein that functions as a transcriptional repressor of the *isc* operon (32).

## MATERIALS AND METHODS

**Growth of the wild-type and mutant cells under optimal conditions.** *Synechococcus* sp. strain PCC 7002 was grown under photoautotrophic conditions in A medium supplemented with 1 mg of  $\text{NaNO}_3 \text{ ml}^{-1}$  (hereafter called A<sup>+</sup> medium) (37). Solid medium for maintaining the mutant strains of *Synechococcus* sp. strain PCC 7002 was supplemented with 0.3% (wt/vol) sodium thiosulfate and 100  $\mu\text{g}$  of kanamycin  $\text{ml}^{-1}$ . *Synechococcus* sp. strain PCC 7002 cells were grown under photoheterotrophic conditions in A<sup>+</sup> liquid medium containing 5 mM glycerol. *Synechocystis* sp. strain PCC 6803 cells were grown under photoautotrophic conditions in BG11 medium as described previously (36). All liquid cultures were bubbled with air supplemented with 1% (vol/vol)  $\text{CO}_2$ . Fluorescent bulbs provided continuous white-light illumination. The temperature was maintained at 30°C for *Synechocystis* sp. strain PCC 6803 and 38°C for *Synechococcus* sp. strain PCC 7002 by a water bath. We monitored growth of the wild-type and mutant strains by measuring the optical density at 730 nm ( $\text{OD}_{730}$ ) with a Cary-14 spectrophotometer modified for computerized data acquisition by On-Line Instruments, Inc. (Bogart, Ga.).

**Growth of the wild-type and mutant cells under stress conditions.** For growth studies under iron stress conditions, wild-type and mutant cells of *Synechococcus* sp. strain PCC 7002 were grown to mid-exponential phase, collected by centrifugation at 4,000  $\times g$  for 10 min, and resuspended to an  $\text{OD}_{730}$  of 0.1 in fresh medium containing 50  $\mu\text{M}$  2,2'-dipyridyl or 0.25  $\mu\text{M}$  streptonigrin. For growth studies under iron starvation conditions,  $\text{FeCl}_3$  was eliminated from the A<sup>+</sup> medium. For growth studies under oxidative stress conditions, wild-type and mutant cells of *Synechococcus* sp. strain PCC 7002 were grown to mid-exponential phase under standard growth condition with the addition of 5 mM glycerol in the medium. The cell cultures were diluted with fresh medium to an  $\text{OD}_{730}$  of 0.1 and grown to an  $\text{OD}_{730}$  of 0.5. A total of 0.5  $\mu\text{M}$  DCMU (an inhibitor of PS II), 10  $\mu\text{M}$  2,5-dibromo-6-isopropyl-3-methyl-1,4-benzoquinone (DBMIB) (an inhibitor of cytochrome *b<sub>6</sub>* or *f*), or 150  $\mu\text{M}$  hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was then

TABLE 1. Sequences of oligonucleotides used for RT-PCR analysis and cloning the *sll0088* gene

Primer	Sequence <sup>a</sup>
<i>sufRF</i> .....	5'-ACCTTTCTGACACCCGCGCCGAAAC
<i>sufRR</i> .....	5'-ATCTTTGGCTTGGATGATGTAGCCGC
<i>sufBF</i> .....	5'-AGTGCACCCGTCAAAACCCCTGTCAA
<i>sufBR</i> .....	5'-CAACAGTGCCTTCGAGTTTGAGGCTG
<i>sufCF</i> .....	5'-GAGTGAAGTTATCTTAGCGATTA
<i>sufCR</i> .....	5'-ACAACCCCGCAGCCAGTCTTGT
<i>sufDF</i> .....	5'-TCTAGCGGTACCATGACAGCGGCGATC
<i>sufDR</i> .....	5'-GTCAACGGTGCACAGGCAACACAAC
<i>sufSF</i> .....	5'-GATCACAACCAACCGAAACCCCTTG
<i>sufSR</i> .....	5'-CTACCCATGCTGGTAAAGAAATCAATG
<i>Esl0088F</i> .....	5'-CTTCTCTGTTGTTGCATATGACCCTCAGTTCT
<i>Esl0088R</i> .....	5'-CGTCAATTGATTA <del>AAAAAGCTT</del> TCTGCCGCCG

<sup>a</sup> Nucleotides of the restriction site sequence are underlined.

added to the medium. The cells were grown for 10 to 12 h, during which time they undergo no more than one division, and collected for total RNA isolation.

**Protein quantitation and absorption spectroscopy.** Protein concentrations were measured by using the Coomassie Plus protein assay with bovine serum albumin as the standard (Pierce, Rockford, Ill.). Absorption spectra were recorded on a Cary-14 spectrophotometer modified for computerized data acquisition by On-Line Instruments, Inc.

**DNA isolation and Southern blotting.** Chromosomal DNA from *Synechococcus* sp. strain PCC 7002 and *Synechocystis* sp. strain PCC 6803 was isolated as previously described (35). The DNA fragment containing the *sll0088* gene in *Synechocystis* sp. strain PCC 6803 or its homolog in *Synechococcus* sp. strain PCC 7002 was cloned and sequenced. DNA sequencing was performed at the Penn State Nucleic Acid Facility. Southern blot and PCR analyses were used to verify whether the gene interruption was homozygous. For Southern hybridization, genomic DNA of the wild-type and mutant strains of *Synechococcus* sp. strain PCC 7002 was digested with appropriate restriction endonucleases and transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, N.H.). The DNA probe fragments were labeled with [ $\alpha$ -<sup>32</sup>P]dATP (New England Nuclear, Boston, Mass.) using a random priming method.

**Expression and purification of the protein encoded by *sll0088*.** The *sll0088* gene from *Synechocystis* sp. strain PCC 6803 (17) was amplified by PCR. *Nde*I and *Hind*III restriction enzyme sites were incorporated via the *Esl0088F* and *Esl0088R* primers used for PCR as listed in Table 1. The amplified *sll0088* gene fragment was digested with *Nde*I and *Hind*III and cloned into expression vector pET24a (Novagen, Madison, Wis.), resulting in the pET24a/*sll0088* expression plasmid. The resulting construct was resequenced to ensure the correctness of the PCR amplification. Plasmid pET24a/*sll0088* was transformed into *E. coli* strain BL21(DE3). Overproduction of the Sll0088 protein in *E. coli* cells was induced by addition of 0.5 mM isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG). The cells were harvested and washed once in TS buffer (10 mM Tris-HCl [pH 8.0], 50 mM NaCl). The cell pellets were resuspended in TS buffer with 1 mM phenylmethylsulfonyl fluoride (PMSF) and 0.1 mg of DNase I (Sigma, St. Louis, Mo.)  $\text{ml}^{-1}$  and passed twice through a chilled French pressure cell. The overexpressed protein was present in inclusion bodies, which were pelleted by low-speed centrifugation at 7,000  $\times g$  for 20 min. The pellet was washed twice with TS buffer and solubilized in 7 M urea in TS buffer by addition of 2 mM dithiothreitol (DTT), 0.1% (wt/vol) Triton X-100, and 1 mM PMSF. After centrifugation at 12,000  $\times g$  to pellet the insoluble material, the supernatant was applied to a Sepharose G-100 column that was pre-equilibrated with TS buffer containing 5 mM DTT.

**Reconstitution of iron-sulfur cluster in the Sll0088 protein and EPR spectroscopy.** Solubilization of the inclusion bodies with urea under aerobic conditions leads to disassembly of any iron-sulfur clusters that may be present in the Sll0088 protein. To reconstitute an anticipated iron-sulfur cluster, the apoprotein was treated with ferric chloride, sodium sulfide, and 2-mercaptoethanol (22). The reconstituted holoprotein was resuspended in 50 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) buffer (pH 10.5) and purified from the inorganic reconstitution agents by repeated ultrafiltration. Samples for electron paramagnetic resonance (EPR) spectroscopy contained 5 mg of protein  $\text{ml}^{-1}$  and 20% (vol/vol) glycerol and were reduced with 20 mM sodium dithionite in an anaerobic chamber (Coy Products, Grass Lake, Mich.). The EPR spectrum was recorded using a Bruker ECS106 EPR X-band (9.2 GHz) spectrometer operating with an ER 4012 ST resonator and an Oxford liquid helium cryostat. The

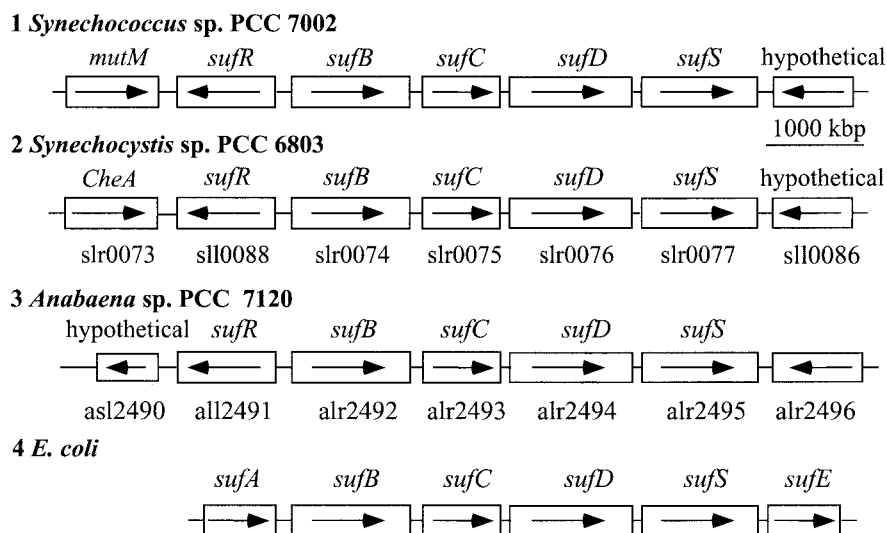


FIG. 1. Map of gene organization of *slI0088* (*sufR*) and the *sufBCDS* operon of three cyanobacteria compared to the *sufABCDE* operon in *E. coli*. Genomic sequences of DNA fragments containing the *sufBCDS* operon were compared for three available sequences of cyanobacteria, *Synechococcus* sp. strain PCC 7002, *Synechocystis* sp. strain PCC 6803 (17), and *Anabaena* sp. strain PCC 7120 (15), and the *E. coli* genome sequence (4). The direction of transcription is indicated by the arrows.

microwave frequency was determined with a Hewlett-Packard 5340A frequency counter.

**Production of antibodies.** To obtain polyclonal antibodies, the recombinant SII0088 protein was partially purified by Sepharose G-100 chromatography and subjected to preparative polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). An extra lane was loaded identically and used for Coomassie blue staining to locate the position of the protein band. The protein-containing region of the gel was excised, and the protein was eluted using a Bio-Rad electroelutor (Bio-Rad, Hercules, Calif.). Polyclonal antibodies against the SufR protein were generated in rabbits at the Centralized Biological Laboratory at Pennsylvania State University.

**Protein electrophoresis and Western immunoblotting.** Methods used for SDS-PAGE and immunoblotting were identical to those described previously (35). Samples, including uninduced cells, induced cells, inclusion bodies, and purified proteins, were solubilized in loading buffer and boiled for 2 min before they were loaded onto an SDS-polyacrylamide gel. A 12% acrylamide gel was used to resolve proteins, which were stained with Coomassie blue. To prepare cyanobacterial extracts, cells were harvested by centrifugation, suspended in 50 mM Tris-HCl buffer (pH 8), and broken by sonication. For immunoblots, proteins separated by electrophoresis were transferred electrophoretically to nitrocellulose membranes (Schleicher & Schuell) by using the Semi-Dry system (Bio-Rad). The nitrocellulose membranes were subjected to immunoreaction with polyclonal antibodies and developed.

**RNA isolation and RT-PCR analysis.** For RNA preparation, cells of the wild-type and mutant strains of *Synechococcus* sp. strain PCC 7002 were harvested during exponential growth. Total RNA was isolated using the High Pure RNA isolation kit according to the protocol provided by the manufacturer (Roche Diagnostics, Indianapolis, Ind.). Contaminating DNA was eliminated by incubating the sample with DNase I for 1 h at room temperature during the preparation procedure. The absence of DNA contamination in RNA samples was confirmed by PCR analysis. For Northern blot analysis, 10  $\mu$ g of total RNA from the wild-type and mutant strains was loaded per lane, and the samples were electrophoresed in 1.3% (wt/vol) denaturing agarose-formaldehyde gels. The RNA was transferred to nylon membranes by capillary transfer, fixed by UV illumination for 1 min, and baked for 1 h at 80°C in a vacuum. The *slI0088* homolog gene-specific probe was generated by PCR using *Synechococcus* sp. strain PCC 7002 genomic DNA as the template. The PCR product was purified by electrophoresis on an agarose gel, and the DNA was extracted and purified by using the QIAGEN Gel Extract kit (Valencia, Calif.). The probe was labeled by random priming as described previously (35). Hybridization with  $^{32}$ P-labeled DNA was performed overnight at 42°C. After the membranes were washed, they were incubated for fluorography at  $-80^{\circ}\text{C}$ . RNA concentrations were determined by absorption spectroscopy. Reverse transcriptase PCR (RT-PCR) experiments were performed by using the QIAGEN OneStep RT-PCR kit. The prim-

ers were designed specifically to amplify the *sufBCDS* genes of *Synechococcus* sp. strain PCC 7002 (Table 1). Total RNA (2.4 ng) was used as the template for the wild-type and mutant strains. The RT-PCRs were performed as follows: a 30-min reverse-transcription reaction at 50°C, a 15-min initial heating step at 95°C, and 32 three-step cycles (1 min at 94°C, 1 min at 59°C, and 1.5 min at 72°C).

## RESULTS

**The *slI0088* gene is adjacent to the *suf* operon in *Synechocystis* sp. strain PCC 6803.** The majority of functional studies described in this paper were performed in *Synechococcus* sp. strain PCC 7002, because we were able to construct a fully segregated, null mutation in the *slI0088* homolog of this cyanobacterium, which we could not do for *Synechocystis* sp. strain PCC 6803. Because the genome sequence is not yet available for *Synechococcus* sp. strain PCC 7002, we needed to determine the gene organization surrounding the *slI0088* homolog. A DNA fragment containing the *slI0088* homolog and its upstream flanking region were cloned and sequenced from the genome of *Synechococcus* sp. strain PCC 7002 (GenBank accession number AY375041). DNA sequence analysis of the *slI0088* homolog and the open reading frames in the region upstream from this gene indicate that the gene organization is identical to that in *Synechocystis* sp. strain PCC 6803 (16, 17) and *Anabaena* sp. strain PCC 7120 (15). As shown in Fig. 1, sequence comparisons show that four conserved genes in cyanobacterial genomes, *sufB*, *sufC*, *sufD*, and *sufS*, have homologs in the *suf* operon of *E. coli* (4, 27). The genes in the *suf* operon have been shown to function in iron-sulfur cluster biogenesis under conditions of oxidative stress (24, 41). In cyanobacteria, the genes homologous to *slI0088* are located near the 5' end of the presumed *suf* operons but are transcribed in the direction opposite that of the *sufBCDS* genes. The *sufA* and *sufE* genes also exist in cyanobacteria (*slr1417* and *slr1419*, respectively, in *Synechocystis* sp. strain PCC 6803), but they are not contiguous with the *sufBCDS* genes, as they are in *E. coli* and certain other bacteria.



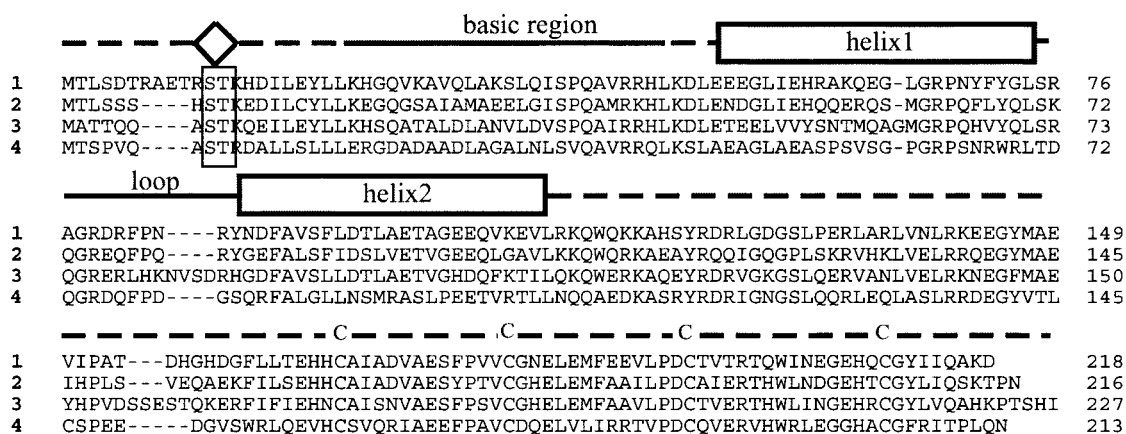


FIG. 2. Conserved domains in SufR proteins from four cyanobacterial strains. The sequences of four cyanobacterial strains are indicated in the figure as follows: 1, *Synechococcus* sp. strain PCC 7002; 2, *Synechocystis* sp. strain PCC 6803; 3, *Anabaena* sp. strain PCC 7120; and 4, *Synechococcus* sp. strain WH8102. Their amino acid sequences were aligned using the Clustal-W algorithm in the MacVector DNA analysis program (Accelrys, Madison, Wis.). The basic region, the helix-loop-helix domain, and the four conserved cysteines in the C-terminal region are indicated above the alignment. The diamond shape near the N terminus indicates the putative protein kinase C phosphorylation site. Gaps introduced to maximize alignment are indicated by dashes.

As shown in Fig. 2, the amino acid sequences of the proteins coded by the *sll0088* homologs are highly conserved in *Synechococcus* sp. strain PCC 7002, *Synechocystis* sp. strain PCC 6803, *Anabaena* sp. strain PCC 7120, and *Synechococcus* WH8102. A BLAST search shows that highly similar proteins also exist in the photosynthetic prokaryotes *Trichodesmium erythraeum* IMS101, *Nostoc punctiforme*, *Thermosynechococcus elongatus*, and *Prochlorococcus marinus* strain MIT9313. Three features can be identified in these proteins using PROSITE program analysis: (i) near the N terminus, a basic region preceded by the elements of a putative protein kinase C phosphorylation site (Ser-Thr-Lys); (ii) a DNA-binding domain that contains a basic region and a putative helix-loop-helix motif whose sequence is very similar to the sequences of DNA-binding proteins from other organisms; and (iii) near the C terminus four highly conserved cysteine residues in a C-X<sub>12</sub>-C-X<sub>13</sub>-C-X<sub>14</sub>-C motif with a possible function in metal binding. Comparative analyses show that the sequences of the *sll0088* homologs are very similar to the sequences of transcription regulatory proteins that bind DNA by a conserved helix-loop-helix motif near the N terminus. The C terminus, with similarly spaced cysteine residues, may confer functional specificity to this class of regulatory proteins.

**The purified Sll0088 protein binds an iron-sulfur cluster.**

To characterize the properties of the protein coded by *sll0088*, the gene from *Synechocystis* sp. strain PCC 6803 was cloned into the expression vector pET24a and expressed in *E. coli* strain BL21(DE3). Very little Sll0088 protein was present in the soluble fraction; rather, the majority was found in inclusion bodies, which were light brown in color, indicating that this protein might harbor an iron-sulfur cluster. The inclusion bodies were solubilized in a solution containing 7 M urea, 4 mM DTT, and 0.1% (wt/vol) Triton X-100, and the Sll0088 protein was purified by gel exclusion chromatography. The Sll0088 protein had an apparent mass of 24 kDa as shown by gel exclusion chromatography and SDS-PAGE (data not shown).

This measured mass is in close agreement with a calculated mass of 24.6 kDa determined from the DNA sequence. (A molecular mass of 27.4 kDa is calculated from the DNA sequence of the *sll0088* open reading frame in CyanoBase (<http://www.kazusa.or.jp/cyanobase/>). However, due to the presence of two closely spaced ATG start codons near the beginning of the open reading frame, this mass may be higher than that of the actual protein. Based on the results of a comparison with Sll0088 sequences from other cyanobacteria [Fig. 1], it is likely that the real start is the second ATG codon. Therefore, the Sll0088 protein from *Synechocystis* sp. strain PCC 6803 is probably made up of 217 amino acids.)

The presence of four highly conserved cysteines in the C-terminal region suggests that their side groups might play a role as metal-binding ligands. To test the possibility that they bind an iron-sulfur cluster, purified Sll0088 protein was incubated with ferric chloride, sodium sulfide, and 2-mercaptoethanol and repurified under anaerobic conditions by size exclusion chromatography (22, 42). The UV-visible spectrum of the reconstituted Sll0088 protein showed absorbance in the UV and visible regions, with maxima at wavelengths of 280, 320, and 411 nm (Fig. 3A). The peaks at 320 and 411 nm are typical of the broad S → Fe charge transfer transitions found in iron-sulfur proteins. The A<sub>411</sub>/A<sub>280</sub> ratio of 0.29 is within the usual range for a typical iron-sulfur protein (2, 13). The absence of absorption peaks in the 450- and 550-nm-wavelength regions is more characteristic of a [4Fe-4S] cluster than a [2Fe-2S] cluster (5, 42). Similar to other iron-sulfur proteins, the spectrum lost about one-half of its amplitude after reduction of the protein with sodium hydrosulfite. When the protein was oxidized in air, the spectrum lost most of its absorbance in the 320- to 420-nm-wavelength region (Fig. 3A, inset), suggesting that the iron-sulfur cluster is oxygen sensitive. Figure 3B shows the low-temperature, X-band EPR spectrum of the reconstituted Sll0088 protein after chemical reduction with dithionite. The axial spectrum of the reduced protein, with a low-field peak at g = 2.02 and a high-field trough at g = 1.89 is characteristic of

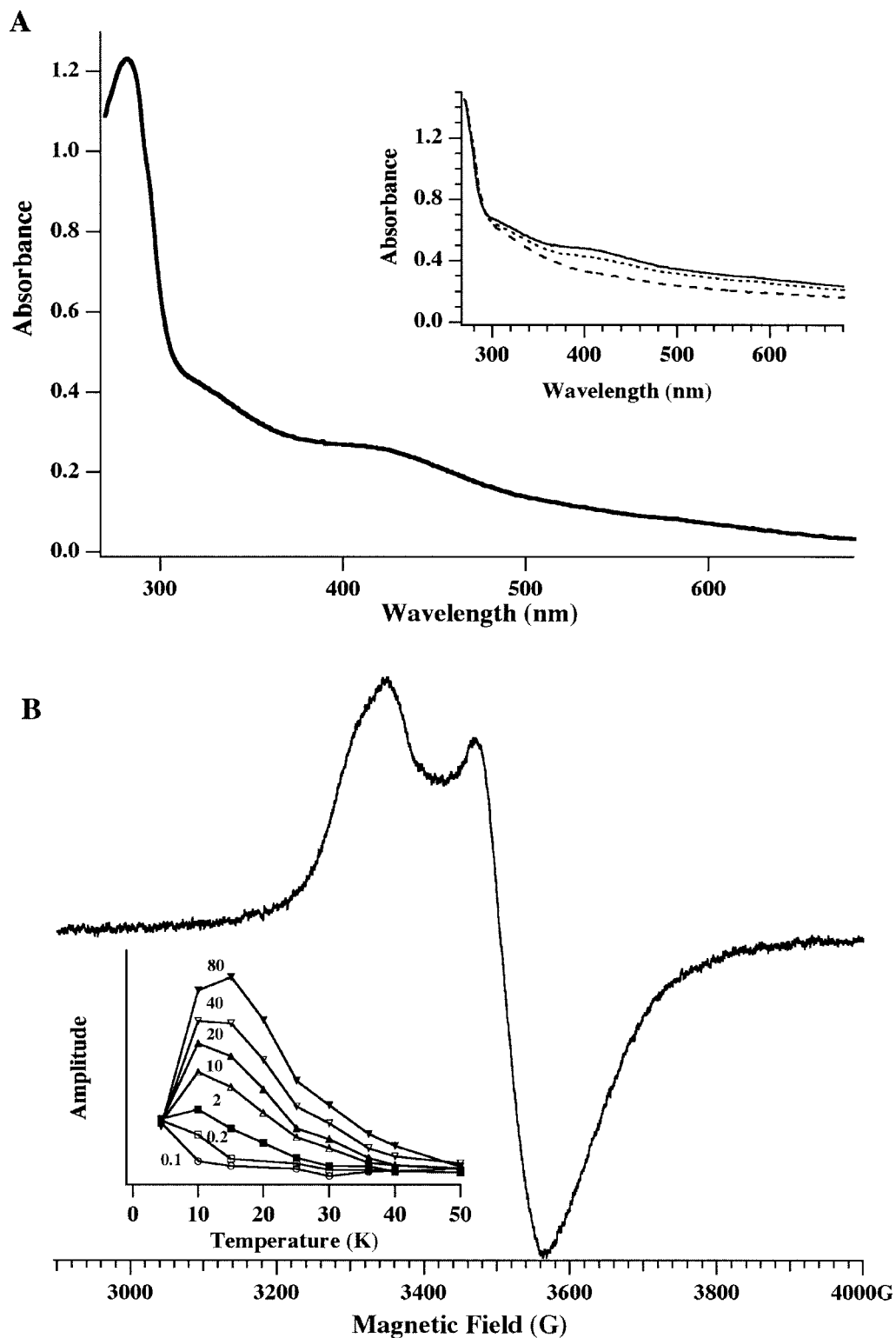


FIG. 3. UV-visible absorption spectrum (A) and EPR spectrum (B) of the recombinant SII0088 protein after reconstitution with iron, sulfide, and 2-mercaptoethanol. The UV-visible absorption spectrum shows maxima at wavelengths of 280, 320, and 411 nm. The inset shows the loss of the absorbance of the oxidized protein upon exposure to air for 30 min (solid line), 60 min (dotted line), and 90 min (dashed line). The EPR spectrum shows the axial line shape of an iron-sulfur cluster, with a low-field peak at  $g = 2.02$  and a high-field trough at  $g = 1.89$ . The inset shows the signal intensity as a function of temperature and microwave power (in milliwatts). EPR conditions follow: microwave frequency, 9.4709 GHz; microwave power, 40 mW; modulation amplitude, 10 G; receiver gain,  $6.3 \times 10^4$ ; temperature, 14 K. The spectrum represents an average of four scans.

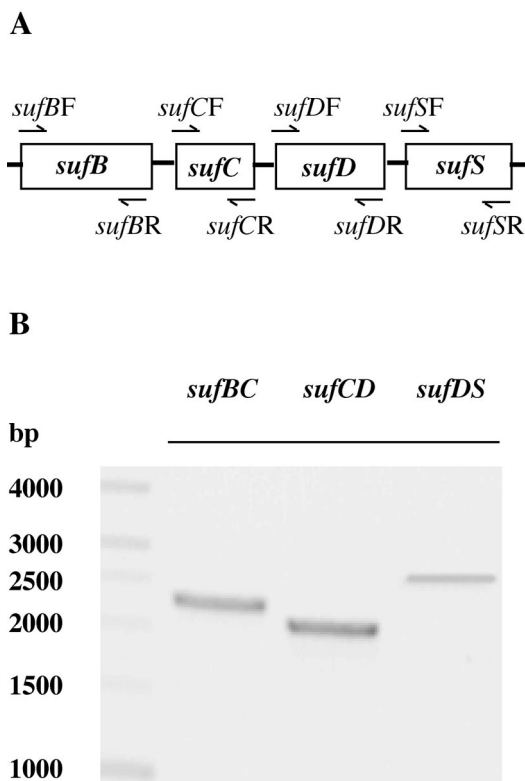


FIG. 4. The primer pairs used for the RT-PCR experiment (A) and the cotranscription of *suf* genes detected by RT-PCR (B). The *sufBF* and *sufCR* primers were used to amplify the *sufBC* fragment, the *sufCF* and *sufDR* primers were used to amplify the *sufCD* fragment, and the *sufDF* and *sufSR* primers were used to amplify the *sufDS* fragment. Products of the expected sizes for *sufB* and *sufC*, *sufC* and *sufD*, and *sufD* and *sufS* establish that the four genes were cotranscribed.

proteins that contain an iron-sulfur cluster. The spectrum and high spin relaxation rate implied from the microwave power dependence (Fig. 3B, inset) and the lower amplitude of the signal at temperatures above 20 K (Fig. 3B, inset) are consistent with the presence of a [4Fe-4S] cluster. The cluster is stable for days in either the oxidized or reduced state when the protein is maintained under anaerobic conditions.

**The *sufBCDS* genes compose an operon in cyanobacteria.** The *sufABCDS* genes in *E. coli* (41) and *Erwinia chrysanthemi* (24) are organized into operons. In cyanobacteria, the adjacent *sufBCDS* genes suggest that they also compose an operon (Fig. 1). To determine whether *sufB*, *sufC*, *sufD*, and *sufS* are cotranscribed in *Synechococcus* sp. strain PCC 7002, the 5' primers for *sufB*, *sufC*, and *sufD* were added to an RT-PCR mixture together with the 3' primers for *sufC*, *sufD*, and *sufS*, respectively, to form primer pairs (Fig. 4A). As shown in Fig. 4B, three fragments were amplified with sizes corresponding to *sufBC* (2.2 kbp), *sufCD* (2.1 kbp), and *sufDS* (2.6 kbp). The cotranscription of the *sufB* and *sufC*, *sufC* and *sufD*, and *sufD* and *sufS* genes clearly indicates that in cyanobacteria the four genes also compose an operon.

**Increased *sufBCDS* mRNA levels in a null mutant of the *sll0088* homolog.** The proximity of the *sll0088* homologs to the *sufBCDS* operon in several cyanobacteria and the identification of the product of the *sll0088* gene from *Synechocystis* sp.

strain PCC 6803 as a possible transcription regulatory protein suggested that Sll0088 might play a role in regulation of expression of the *sufBCDS* operon. To test this idea, we compared the mRNA levels for the *sufB*, *sufC*, *sufD*, and *sufS* genes in the wild type and a null strain of the *sll0088* homolog in *Synechococcus* sp. strain PCC 7002. As we have shown previously (43), null mutants were created by inserting the *aphII* gene into the *StuI* restriction site of a gene homologous to *sll0088* in *Synechococcus* sp. strain PCC 7002 either parallel or antiparallel to the transcription direction of the *sll0088* homolog. Both mutants were completely segregated, as verified by PCR and Southern blot analysis. Their growth rates under low ( $50 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) and normal ( $250 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) light intensities were very similar (data not shown), suggesting that the insertion direction of *aphII* gene has no effect on the mutant phenotype. The complete absence of the *sll0088* homolog in the null strains was confirmed by immunoblot analysis (Fig. 5). Given these results, all further studies reported here in which the wild type and the null mutant were compared used only the strain in which the *aphII* gene was inserted into the *sll0088* homolog parallel to its transcription orientation. We chose this orientation, because in principle, a parallel insertion is likely to have less effect on the other genes downstream of the *sll0088* homolog than an antiparallel insertion.

The transcript level for the *sufS* gene, probed by Northern blotting analysis, was found to be higher in the null strain than in the wild type (data not shown). The intensities of the hybridization signals for the *sufS* gene were quantified using the National Institutes of Health (NIH) Image Program, and the expression level was three to four times higher in the null strain. In addition, RT-PCR analysis was performed to probe more sensitively the transcript levels for the *sufB*, *sufC*, *sufD*, and *sufS* genes in cells of the wild-type and null strains. As shown in Fig. 6, mRNA levels for the *sufB*, *sufC*, *sufD*, and *sufS* genes were consistently higher in the *sll0088* null strain. These results therefore implicate the *sll0088* gene product in regulation of *suf* operon expression in *Synechococcus* sp. strain PCC 7002. We propose that the *sll0088* homolog be named *sufR* and that the protein coded by this gene be named SufR.

**Transcript levels of *sufB* and *sufS* are elevated in the *sufR* null strain under stress conditions.** Proteins encoded by the *suf* operon in *E. coli* are thought to be involved in the assembly of iron-sulfur clusters, particularly under conditions of oxida-

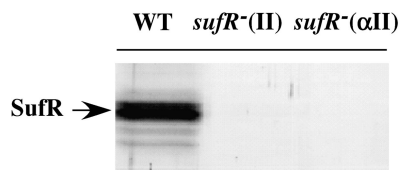


FIG. 5. Immunodetection of the SufR protein in the cells of the wild type (WT) and two *sufR* null mutants of *Synechococcus* sp. strain PCC 7002 by use of antibodies against the recombinant SufR protein of *Synechocystis* sp. strain PCC 6803. Proteins corresponding to 10  $\mu\text{g}$  of chlorophyll from the whole-cell lysates were loaded onto each lane. *sufR*<sup>-</sup>(II) and *sufR*<sup>-</sup>( $\alpha$ II) strains are mutants in which the inserted *aphII* gene is transcribed in the same direction (II) or in the direction opposite ( $\alpha$ II) that of the *sufR* gene. Only the *sufR*<sup>-</sup>(II) strain was used for subsequent work; it is referred to in the text as the *sufR*<sup>-</sup> strain and is indicated in the figures as the *sufR*<sup>-</sup> strain.

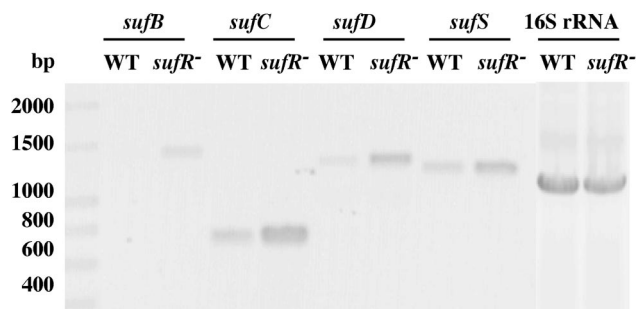


FIG. 6. RT-PCR analysis of the *suf* gene transcription levels in *Synechococcus* sp. strain PCC 7002 (wild type) and *sufR* null strain. Primers specific for the *sufB*, *sufC*, *sufD*, and *sufS* genes were used in RT-PCRs and are listed in Table 1. The 16S rRNA gene was used as a control to assure that the RNAs of the wild type (WT) and *sufR* mutant strain were added to the RT-PCR mixtures at equal concentrations and that both were equally competent for the RT-PCR.

tive stress (24, 41). To probe expression of the *suf* genes in cyanobacteria under stress conditions, the wild type and the *sufR* null strain were grown in normal media and shocked with DCMU (an inhibitor of PS II), DBMIB (an inhibitor of cytochrome *b<sub>6</sub>* or *f*),  $H_2O_2$ , and Fe deprivation for various lengths of time before being harvested and processed for RNA isolation. The transcription levels of the *sufB* and *sufS* genes under these conditions were compared with those under normal growth conditions. As shown in Fig. 7, the expression levels of *sufS* were higher in the presence of DCMU, DBMIB, and  $H_2O_2$  and in the absence of iron in the medium than the level in the wild type. In the *sufR* null strain (Fig. 5), the expression levels of *sufS* were higher than the level in the wild type, regardless of the stress imposed. Thus, the genes in the *sufBCDS* operon are constitutively expressed when the SufR repressor is absent. The expression levels of *sufB* were also higher in the presence of DCMU, DBMIB, and  $H_2O_2$  and in the absence of iron in the medium than in the wild type; in the *sufR* null strain, the *sufB* mRNA levels were even higher and independent of the stress imposed. Thus, the expression levels of the *sufB* and *sufS* genes in the *sufR* null mutant are constant and higher under all conditions than those in the wild type. This would be expected if the *sufBCDS* operon were no longer under SufR regulation. Therefore, these observations suggest that the iron-sulfur cluster assembly proteins encoded by the *sufBCDS* operon have increased functional importance under conditions of oxidative stress in cyanobacteria.

**Growth rates of the *sufR* null strain under stress conditions are higher than that of the wild type.** The increased mRNA levels of the *sufBCDS* operon in the *sufR* null strain would be expected to have physiological consequences if the proteins coded by these genes normally function in response to stress. As shown in Fig. 8A, the 4-h doubling time of the wild type at normal ( $250 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) light intensities increased to 6.5 h in the *sufR* null strain, suggesting that the overexpression of the Suf proteins is mildly inhibitory in cells grown under near-optimal nutrient and illumination conditions. To probe the relationship between *sufR* and iron metabolism, cells were transferred to fresh growth medium containing 2,2'-dipyridyl (an iron chelator) or streptonigrin (an iron-activated antibiotic) during mid-exponential phase, and the growth rates were

measured. In the presence of 2,2'-dipyridyl (Fig. 8B), the 15-h doubling time of the wild type decreased to 10.5 h in the *sufR* null strain. This might be expected if the iron-sulfur cluster biosynthetic enzymes in the *sufR* null mutant were up-regulated and were thereby able to scavenge low levels of iron more efficiently than the wild type. To test this idea further, growth rates of the wild type and *sufR* null strain of *Synechococcus* sp. strain PCC 7002 were compared when iron was eliminated from the growth medium. When the cells were transferred to fresh medium without added  $\text{FeCl}_3$  during mid-exponential phase, the 14-h doubling time of the wild type decreased to 11 h in the *sufR* null strain (Fig. 8C). When the cells were transferred to fresh growth medium in the presence of streptonigrin (Fig. 8D, open symbols), growth of the wild type and the mutant were inhibited for 3 and 7 days, respectively. The higher sensitivity of the mutant compared to the wild type could indicate that iron accumulated in the mutant cells, thereby providing the cells with a higher intracellular iron content than the wild type. The inhibiting effect of streptonigrin could be alleviated by iron starvation; the *sufR* mutant resumed growth after 2 days, and the wild type resumed growth after 3 days (Fig. 8D, filled symbols). Thus, the *sufR* null strain survived the presence of streptonigrin under iron starvation conditions better than in normal growth medium. This can be

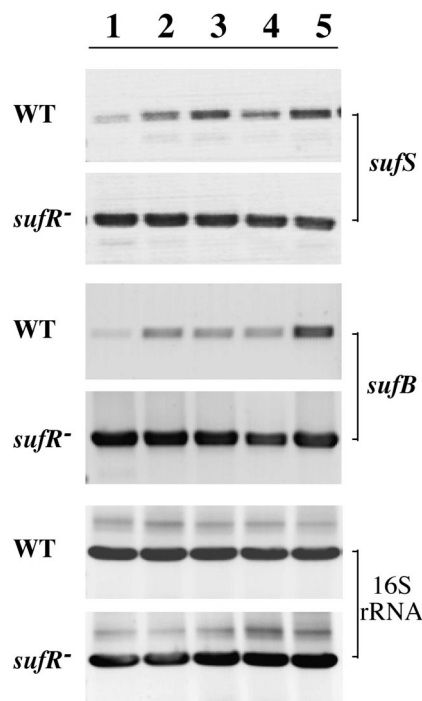


FIG. 7. RT-PCR analysis of the expression of the *sufS* and *sufB* genes in the wild-type (WT) and *sufR* mutant strains of *Synechococcus* sp. strain PCC 7002. Total RNA was isolated from cells grown under the following conditions: standard growth conditions (lane 1),  $A^+$  medium supplemented with 5 mM glycerol and 0.5  $\mu\text{M}$  DCMU (lane 2),  $A^+$  medium supplemented with 5 mM glycerol and 10  $\mu\text{M}$  DBMIB (lane 3),  $A^+$  medium supplemented with 150  $\mu\text{M}$   $H_2O_2$  (lane 4), and  $A^+$  medium with no iron salts added (lane 5). The gene for 16S rRNA was used as a control to assure that the RNAs of the wild type and *sufR* mutant strain were added to the RT-PCRs at equal concentrations and that both were equally competent for the RT-PCR.



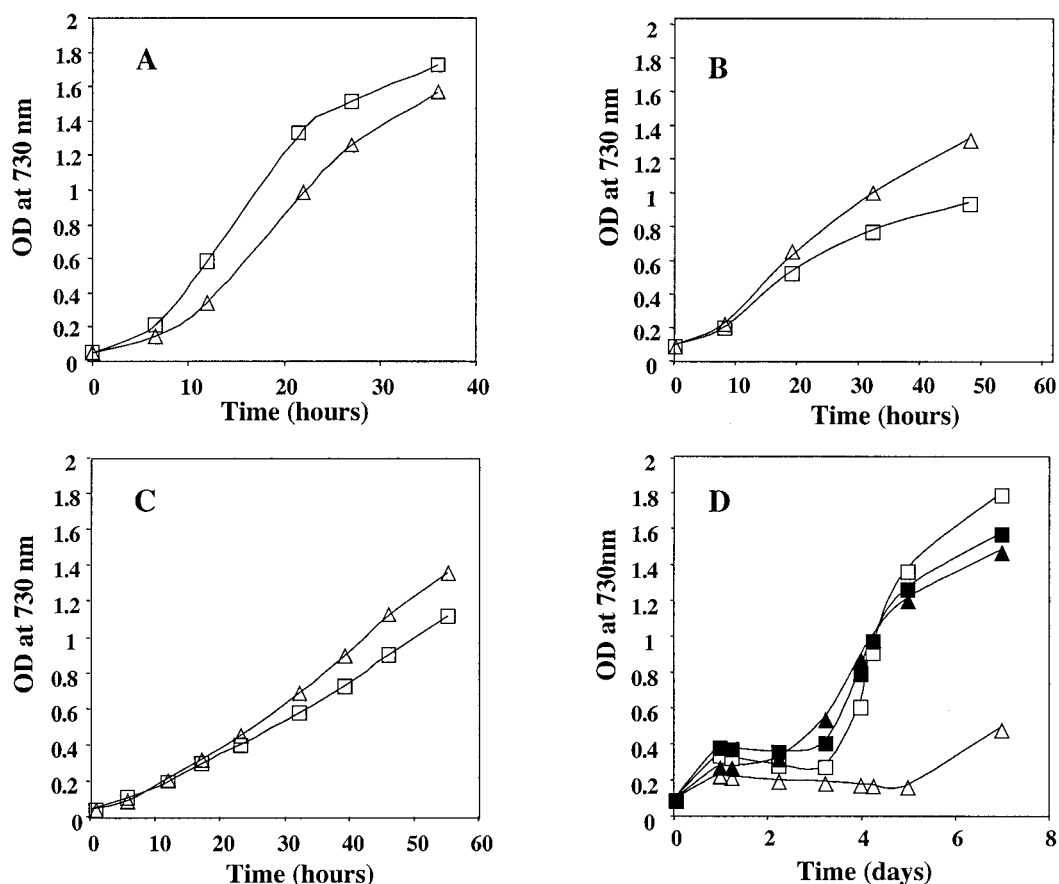


FIG. 8. Growth curves of *Synechococcus* sp. strain PCC 7002 under different conditions. The wild type (open square) and the *sufR* mutant (open triangle) under various conditions are shown. (A) Standard conditions ( $250 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ,  $38^\circ\text{C}$ ,  $\text{A}^+$  medium); (B) standard conditions in  $\text{A}^+$  medium with no  $\text{FeCl}_3$  added; (C) standard conditions in  $\text{A}^+$  medium with  $\text{FeCl}_3$  added; (D) standard conditions in the presence of streptonigrin for the wild type with (open symbols) or without (filled symbols) addition of  $\text{FeCl}_3$  and for the *sufR* mutant strain with (open symbols) or without (filled symbols) addition of  $\text{FeCl}_3$ . These experiments were repeated three times; all showed similar growth profiles. Data from only one experiment are depicted in this figure.

explained if most of the iron were tied up in iron-sulfur clusters due to the up-regulation of the *sufBCDS* operon. In contrast, growth of the wild type showed no significant differences under these conditions, probably because iron starvation exerted contradictory effects: on the one hand, it inhibited wild-type growth, and on the other hand, it alleviated the toxicity of streptonigrin.

These growth studies clearly show that the increased transcript levels for the *suf* genes in the *sufR* null mutant allow cyanobacterial cells to grow more efficiently in a low-iron environment. This result is in clear contrast to the inhibitory effect exerted by increased *suf* gene expression level on the growth of *sufR* null mutants under standard growth conditions. The inhibitory effect of the high expression of the *suf* genes is more obvious under iron-replete conditions, as shown by the streptonigrin-containing cultures. As shown previously in Fig. 7, the expression level of *suf* genes in the *sufR* null mutant always exceeds that of the wild type in response to oxidative stresses. High expression levels of the Suf proteins are also toxic in *E. coli* (41). These studies show that increased mRNA

levels for the *suf* genes in the *sufR* null mutant lead to the predicted physiological response of enhanced survivability under iron-limiting conditions.

## DISCUSSION

**The *sufR* gene is usually located adjacent to the *sufBCDS* operon in cyanobacteria.** The *sll0088* (*sufR*) gene is immediately upstream of the *sufBCDS* operon in *Synechocystis* sp. strain PCC 6803, and homologs occupy a similar position in other cyanobacteria, including *Synechococcus* sp. strain PCC 7002, *Anabaena* sp. strain PCC 7120, *Trichodesmium erythraeum* IMS101, and *Prochlorococcus marinus* MIT9313. In *Synechococcus* sp. strain WH8102, the ferredoxin-thioredoxin reductase catalytic chain, a subunit of heterodimeric ferredoxin-thioredoxin reductase (*firC*, which corresponds to open reading frame *sll0554* in *Synechocystis* sp. strain PCC 6803), is located between *sufR* and the *sufBCDS* operon. There is only a 7-bp-long spacer between *firC* and the *sufBCDS* operon, suggesting that the *firC* homolog is cotranscribed with the downstream



*sufBCDS* genes. Ferredoxin-thioredoxin reductase is a [4Fe-4S] protein through which thioredoxin undergoes redox regulation of protein function and signaling via thiol redox control. Thioredoxin has additional functions in defense against oxidative stress and is required by a number of transcription factors for DNA binding (1). The organization of the *sufR*, *ftrC*, and *sufBCDS* genes in *Synechococcus* sp. strain WH8102 implies that SufR may also be involved in regulation of the ferredoxin-thioredoxin system. A more pronounced exception to the gene organization exists in *Thermosynechococcus elongatus*, in which *sufB* is located immediately upstream of the *sl10088* homolog, and even though the *sufC* and *sufD* genes are adjacent to each other, they are located elsewhere in the genome, as is the *sufS* gene. This difference raises the possibility that SufR might act to control a regulon rather than simply the *sufBCDS* operon. An indication that this might be the case is that the mRNA level for the bacterioferritin gene of *Synechococcus* sp. strain PCC 7002 is also increased in the *sufR* mutant strain (data not shown).

**Homologs of the *sufR* gene may exist in nonphotosynthetic pathogenic bacteria.** Nonphotosynthetic bacteria, particularly pathogens and organisms that live under extreme conditions, also contain genes with strong similarity to *sufR*. Bacterial pathogens have developed efficient iron acquisition systems to counteract the defensive sequestration of iron by their hosts (6). These organisms must deal with oxidative stress exerted by either the host defense response or the environment. Although all four cysteines are conserved in *sufR* homologs of cyanobacteria, only three cysteines (the first, second, and fourth) are conserved in *sufR* homologs of nonphotosynthetic bacteria. The third position in the bacterial *sufR* homologs is occupied by either Ala (in *Bacillus anthracis* and *Thermoplasma acidophilum*), Thr (in *Mycobacterium leprae* and *Streptomyces coelicolor*), or Val (in *Yersinia pestis*), just to name a few possibilities. This group of homologs also includes *Chloroflexus aurantiacus*, a thermophilic photosynthetic green nonsulfur bacterium in which the SufR homolog contains only three cysteine residues. It should be noted that certain nonphotosynthetic bacteria have SufR homologs with additional cysteine residues near the C terminus. For example, *Vibrio cholerae* SufR contains two cysteines in addition to the four conserved cysteine residues that are found in cyanobacterial SufR. The iron-sulfur cluster coordinated by the common cysteine motif near the C terminus of these proteins may sense oxidative stress and thus control the expression of their target genes. Because there are only three conserved cysteine residues in the SufR homologs of nonphotosynthetic organisms, a noncysteine residue may be required as the fourth ligand to the iron-sulfur cluster. Indeed, IscR, a transcriptional repressor of the *iscSUA* operon, contains only three conserved cysteines, but EPR studies show that it binds a stable [2Fe-2S] cluster (32). Among the bacteria that contain *sufR* homologs, it is only in *Mycobacterium tuberculosis* strain H37RV and *Mycobacterium leprae* that four *suf* genes and one *iscU*-like gene (*sufBB'*-*iscU*) are located adjacent to the *sufR* homolog. In *Thermoplasma acidophilum*, only three *suf* genes (*sufCBD*) are adjacent to the *sufR* homolog, and in the other organisms that bear a *sufR* homolog, *sufR* and the other *suf* genes are separated by significant distances on the genome. This strengthens the notion suggested above that SufR may act to control a regulon rather

than only the *sufBCDS* operon. *E. coli* and *Erwinia chrysanthemi* also contain a *suf* operon that is composed of all six *suf* genes (*sufABCDS*), but no *sufR* homolog could be discerned.

**The SufR protein is a member of the DeoR family of helix-loop-helix proteins.** An amino acid alignment and BLAST search against the GenBank database shows that the proteins coded by the *sl10088* homologs contain three putative motifs: a protein kinase C phosphorylation site near the N terminus, a helix-loop-helix DNA-binding domain, and a motif of four cysteine residues separated by 12, 13, and 14 amino acids near the C terminus. The DNA-binding motif is highly conserved among *sufR* homologs of cyanobacteria and can be classified in the DeoR family of helix-loop-helix proteins. These proteins are typically highly diverse. However, because the helix-loop-helix motif is situated near the N terminus, SufR falls into the DeoR subfamily, which includes DeoR (23), LacR (30), and FucR (21). SufR appears to be a member of yet another DeoR subclass that contains a highly conserved C-X<sub>12</sub>-C-X<sub>13</sub>-C-X<sub>14</sub>-C motif near the C terminus.

**The SufR protein may utilize an iron-sulfur cluster to sense iron levels or stress.** The SufR protein harbors an iron-sulfur cluster, as shown by optical absorption and EPR spectroscopy, and the spectra and relaxation properties are consistent with its identification as a [4Fe-4S] cluster. In future work, we will verify its identity as a [4Fe-4S] cluster, determine which of the cysteines provide ligands to the cluster, and decide whether the reconstituted protein is a monomer or dimer. It should be kept in mind that the iron-sulfur cluster was inserted in vitro into the *E. coli*-expressed apoprotein; hence, it is possible that SufR does not harbor an iron-sulfur cluster in vivo or that it harbors a different type of cluster (i.e., a [2Fe-2S] cluster) when functioning within the cyanobacterium. Nevertheless, given the functions of the proteins encoded by the *sufBCDS* operon, it is reasonable that SufR would bind an iron-sulfur cluster and serve as a sensor of oxidative stress that is commonly encountered by this organism. We propose that SufR indirectly senses the levels of iron-sulfur clusters in the cells through its own unstable iron-sulfur cluster; when this cluster is present, SufR binds to its operator and functions as a regulatory repressor of the *sufBCDS* operon. The oxygen sensitivity of the iron-sulfur cluster on the SufR protein may limit the lifetime of the active state, thereby allowing the poise of a quasi-steady-state population that could respond rapidly as the level of oxidative stress rises or falls.

There are other regulatory proteins that are proposed to fill a similar role (3). In a study with *Erwinia chrysanthemi*, Nachin and colleagues found that the *suf* operon might participate in a SoxR-dependent response to oxidative stress (24). SoxR is a transcription activator and contains a helix-loop-helix motif that confers sequence-specific DNA-binding capability (reviewed in reference 29). SoxR in *E. coli* is a homodimer and contains one [2Fe-2S] cluster per monomer. It has been shown that the iron-sulfur cluster is not required for SoxR to bind to the promoter of the *soxS* gene and that only the form of SoxR that contains the 2+ state iron-sulfur cluster can lead to the initiation of transcription of the *soxS* gene (12). SoxR senses reversible oxidation/reduction of the iron-sulfur cluster, a one-electron redox system (10). A SoxR homolog, a MerR-like protein, can be identified from searches in genomic sequences of *Synechocystis* sp. strain PCC 6803 (17), *Synechococcus* sp.

strain PCC 7002 (Zhao et al., unpublished) and *Anabaena* sp. strain PCC 7120 (15). Whether this *merR*-like gene participates in redox regulation of the *suf* operon in cyanobacteria will be investigated in future studies. The IscR protein of *E. coli* also functions as a repressor of the *iscRSUA* operon, because deletion strains of *iscR* exhibit increased expression of this operon. IscR isolated anaerobically from a bacterial expression system contains a  $[2\text{Fe-2S}]^{1+}$  cluster that appears to be important for IscR function. It has been proposed that IscR may function in a autoregulatory mechanism that senses the iron-sulfur cluster assembly status of cells (32). The presence of a  $[4\text{Fe-4S}]$  cluster in SufR and the presence of a  $[2\text{Fe-2S}]$  cluster in IscR constitute significant differences between these two proteins, even though both function as iron-sensing transcriptional regulators. If the  $[4\text{Fe-4S}]$  cluster were indeed present in vivo, SufR would represent an entirely new subclass of regulator.

**The SufBCDS proteins function in iron-sulfur cluster biogenesis in cyanobacteria.** Proteins encoded by the *sufABCDS* genes have been proposed to be involved in the biogenesis and assembly of iron-sulfur clusters in bacteria (24, 41). However, detailed functions of most of these proteins are unknown. SufC is known to play a key role in iron metabolism and oxidative stress response (24); it contains an ATP-binding domain and may function as a versatile orphan ATPase in the iron-sulfur cluster assembly machinery. Reverse genetics has shown that the *sufB* and *sufC* genes are important in iron metabolism and oxidative stress in *Erwinia chrysanthemi* (24). Homologs of *sufB* and *sufC*, named *ycf24* and *ycf16*, respectively, have been located in the chloroplast of the red alga *Porphyra purpurea* (38) and the cyanelle of *Cyanophora paradoxa* (19). SufC is also encoded in the plastid genome of the cryptomonad *Guillardia theta*, whereas in *Arabidopsis thaliana*, the *sufC* gene is encoded in the nucleus. However, the existence of a transit peptide in *A. thaliana* SufC indicates that the SufC protein is targeted to plant chloroplasts. This suggests that SufC may play a role in the assembly of the photosynthetic apparatus in cyanobacteria and the chloroplasts of higher plants. SufB and SufD share a highly conserved UPF0051 domain, suggesting that they assume similar functions in iron metabolism. In a *sufD* deletion mutant in *E. coli*, no iron-sulfur clusters are assembled in the FhuF protein (27); therefore, SufD is thought to be necessary for the stability of the iron-sulfur cluster proteins. The *sufS* gene codes for a NifS homolog; NifS has been shown to be essential for the construction iron-sulfur clusters in the nitrogenase enzyme of *A. vinelandii* (46). In *E. coli*, the *iscS* gene codes for a NifS homolog that has been shown to be a pyridoxal phosphate-dependent cysteine desulfurase (7). The cyanobacterial *sufS* gene is most closely related to the chloroplast *nifS*-like gene of *A. thaliana*. The SufS homolog also has some similarities to a protein encoded by the *at1g08490* gene in *A. thaliana*, named AtNFS2 (20) or AtCpNIFS (28), which is a cysteine desulfurase targeted to the chloroplast. Indeed, cysteine desulfurase activity has been detected in the chloroplasts of *A. thaliana* (28). The SufS homolog is less similar to a protein encoded by the *at5g65720* gene in *A. thaliana*, which is a putative cysteine desulfurase targeted to the mitochondria (L. McIntosh, unpublished data). Both organelles contain high concentrations of iron-sulfur clusters due largely to the presence of NADH dehydrogenase and fumarase in the mitochon-

dria and PS I in the chloroplast. On the basis of these proposed functions, it is reasonable to propose that the *suf* operon in cyanobacteria is also involved in iron-sulfur cluster assembly for the photosynthetic complexes. Hence, the SufB, SufC, SufD, and SufS proteins probably work together to form iron-sulfur clusters in cyanobacteria.

**The C14<sub>PsaC</sub> suppressor mutations are functionally equivalent to *sufR* null mutants.** The relationship between *slr0088* (renamed here *sufR*) and PS I biogenesis and assembly was established by our earlier finding that a secondary mutation to *sufR* in the C14<sub>PsaC</sub> mutant of *Synechocystis* sp. strain PCC 6803 restored photoautotrophic growth (43). The C14<sub>PsaC</sub>-R62 and C14<sub>PsaC</sub>-R18 suppressor mutants exhibit an increased number of PS I reaction centers per cell (43). C14<sub>PsaC</sub>-R62 was found to substitute Pro for Arg at residue 161 as the result of a G<sub>482</sub>→C change, and C14<sub>PsaC</sub>-R18 was found to have a 3-amino-acid insertion of Gly-Tyr-Phe following Cys<sub>231</sub> as the result of a TGGTTATTT duplication at T690. These two mutations were proposed to disrupt the structure, and hence the function, of *sufR* in *Synechocystis* sp. strain PCC 6803. We propose that the C14<sub>PsaC</sub>-R62 and C14<sub>PsaC</sub>-R18 suppressor mutants are phenotypically identical to the *sufR* null mutants in that both result in the up-regulation of the *sufBCDS* genes that encode proteins involved in the synthesis of iron-sulfur clusters. PS I is the most abundant iron-sulfur protein complex in the cyanobacterial cell. The primary C14<sub>PsaC</sub> mutation results in the occurrence of a mixed-ligand (3 Cys · 1 Ser)  $[4\text{Fe-4S}]$  cluster in the F<sub>B</sub> site. We propose that these PS I complexes are targeted as faulty, resulting in the turnover of the entire PS I complex. The level of PS I in the C14<sub>PsaC</sub> mutant would then represent a balance between the rate of synthesis and the rate of turnover. If the rate-limiting step in the biosynthesis of PS I is indeed the formation of iron-sulfur clusters, then the overexpression of enzymes involved in iron-sulfur cluster biosynthesis would explain why the C14<sub>PsaC</sub>-R62 and C14<sub>PsaC</sub>-R18 mutants are able to increase the steady-state population of PS I reaction centers per cell in the secondary mutants. The nearly normal ratio of PS I to PS II would support photoautotrophic growth, which was the criterion by which the secondary suppressor mutants were initially selected. Thus, the mixed-ligand iron-sulfur cluster in the F<sub>B</sub> site of the C14<sub>PsaC</sub> mutant is fully functional in vivo, as was implied by the ability of isolated PS I complexes to support high rates of electron transfer from cytochrome *c*<sub>6</sub> to flavodoxin in vitro (43, 44).

**Conclusions.** Four *suf* genes (*sufBCDS*) in cyanobacteria constitute an operon, whose transcription is negatively regulated by the SufR protein. SufR is therefore similar in function to *iscR*, which is an iron-sulfur protein that functions as a transcriptional repressor of the *isc* operon. The transcript levels of the *suf* genes increase under conditions of oxidative stress and iron limitation. As an iron-sulfur protein, SufR may serve as a sensor of oxidative stress. As for the distribution of *suf* genes, all four *suf* gene homologs in *A. thaliana* contain a transit peptide sequence at the N terminus of the Suf proteins that targets them to the chloroplast. This makes it very likely that the Suf proteins are involved in iron-sulfur assembly and biogenesis in chloroplasts. We propose that SufR plays an indirect role in controlling PS I biogenesis by regulating *suf*

gene expression for the control and synthesis of iron-sulfur clusters.

#### ACKNOWLEDGMENTS

This work was supported in part by the U.S. Department of Agriculture award 2001-35318-10125 to J.H.G., by a Department of Energy contract to L.M., and by National Science Foundation grant MCB-0077586 and NIH grant GM-31625 to D.A.B.

We thank David Becker for critically reading the manuscript.

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