An iron stress operon involved in photosynthetic electron transport in the marine cyanobacterium *Synechococcus* sp. PCC 7002

KARIN LEONHARDT and NEIL A. STRAUS*

Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 3B2

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The iron-stress-induced genes *isiA* and *isiB* have been cloned and sequenced from the marine unicellular cyanobacterium *Synechococcus* sp. PCC 7002. These genes code for a photosystem II chlorophyll-binding protein and flavodoxin respectively. The genes form a dicistronic operon that is transcriptionally activated under iron-stress conditions to produce an abundant monocistronic message containing *isiA* and a much less abundant dicistronic message that also contains *isiB*. The arrangement of these genes, their transcriptional control and the relative abundance of the monocistronic and dicistronic messages produced under iron stress parallels the pattern shown by the freshwater cyanobacterium *Synechococcus* sp. PCC 7942. The genes for the corresponding proteins found under iron-replete conditions, CP-43 and ferredoxin, have also been cloned and sequenced. Northern blot analysis indicates that both of these genes are constitutively expressed under both iron-stress and iron-replete conditions.

Introduction

Although iron is normally abundant in aqueous, oxic ecosystems, its low solubility above neutral pH may limit its biological availability. This is a crucial problem to aquatic organisms since iron is an essential component of cytochromes, and of ferredoxins and other iron-sulphur proteins. It is particularly critical for the growth of microbial populations because each round of cell division necessitates a doubling of the iron-containing molecules. When faced with conditions of iron deprivation, cyanobacteria pursue three strategies for survival. First, like other bacteria, they improve their iron acquisition capabilities by producing siderophores to scavenge any available iron from the environment (Crosa, 1989; Bagg & Neilands, 1987; Boyer *et al.*, 1987). Second, they decrease the amount of photosynthetic and respiratory redox proteins that contain iron (Pardo *et al.*, 1990; Sandmann, 1985). Third, they replace iron-containing proteins with non-iron-containing functional equivalents; for example, Fe-S-containing ferredoxin is replaced with flavodoxin as the terminal electron acceptor of photosynthesis (Hutber *et al.*, 1977).

In the freshwater cyanobacterium *Synechococcus* sp. PCC 7942, the gene for flavodoxin, *isiB*, is the second open reading frame of a dicistronic message whose appearance is tightly regulated by iron concentration (Laudenbach *et al.*, 1988). The first open reading frame of this message contains a gene, *isiA*, that is very similar to *psbC* (Laudenbach & Straus, 1988). To investigate whether this iron stress response is a more general feature of cyanobacteria, we have studied the organization and transcriptional regulation of these genes in the marine cyanobacterium *Synechococcus* sp. PCC 7002. In this paper, we report on the cloning and sequencing of *isiA*, *isiB*, *psbC* and *petF* from *Synechococcus* sp. PCC 7002. Northern blot analysis of RNA from cells in different iron regimes shows that *isiA* and *isiB* form a dicistronic operon that is transcriptionally regulated to express under iron stress in the same way as in *Synechococcus* sp. PCC 7942. We also show that *petF* and *psbC* are constitutively expressed under all iron regimes tested.

Methods

Materials. All chemicals were reagent grade. Restriction endonucleases were obtained from Pharmacia, Bethesda Research Laboratories and New England Biolabs. Alkaline phosphatase was obtained from Boehringer Mannheim; DNA polymerase I Klenow fragment from Pharmacia; T4 polynucleotide kinase and AMV reverse transcriptase from New England Biolabs; and T4 DNA ligase from Bethesda Research Laboratories. [α^{32} P]dCTP (3000 Ci mmol⁻¹) was

* Author for correspondence. Tel. (416) 978 3532; fax (416) 978 5878; Email: strausna@gpu.uts.utoronto.ca.

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obtained from ICN, and [γ - 32 P]ATP (5000 Ci mmol $^{-1}$) and [35 S]dATP (1000 Ci mmol $^{-1}$) were obtained from Amersham (1 Ci = 37 GBq). 'Gene Clean' glass milk was obtained from Bio/Can Scientific.

Culture conditions. *Synechococcus* sp. PCC 7002 (*Agmenellum quadruplicatum* PR-6) was grown in A medium (Stevens & Porter, 1980) at 29 °C in liquid culture or on plates containing 1% (w/v) agar (Difco). For growth under controlled iron conditions, citric acid was omitted and ferric ammonium citrate was replaced with the desired amount of ferric chloride. Normal iron conditions constitute 1.4×10^{-5} M-Fe $^{3+}$ while low-iron cultures were grown at 7.1×10^{-7} M-Fe $^{3+}$. All media were made in acid-washed glassware with deionized, ultrapure water from a Milli-Q Reagent Water System (Millipore Canada).

Library construction and screening. Genomic DNA of *Synechococcus* sp. PCC 7002 was isolated using a modification of the CTAB (cetyltrimethylammonium bromide) technique developed for chloroplast DNA (Milligan, 1989). The cell pellet was resuspended in 100 mM-Tris, 20 mM-EDTA, 3 mg lysozyme ml $^{-1}$ and incubated at 37 °C for 1 h with intermittent shaking. Lysis was achieved by adding an equal volume of 100 mM-Tris, 20 mM-EDTA, 2.8 M-NaCl, 4% (w/v) CTAB, 0.4% (v/v) mercaptoethanol, and incubating at 55 °C for 45 min. The DNA was spooled on a glass rod at each alcohol precipitation step.

Partial plasmid libraries were constructed using restriction-enzyme-cut genomic DNA separated by size on a low-melting-temperature agarose gel. Regions containing DNA of the appropriate insert size were cut out of the gel and the purified DNA was cloned. Transformations into *Escherichia coli* JM109 were carried out by the method of Hanahan (1985). The libraries were screened by using the original cloning enzyme to digest the DNA from pooled groups of 25 clones and performing Southern hybridization to identify clones of interest. Phage λ NM1149 libraries were constructed as described by Pirrotta (1986) using the Packagene packaging system (Promega). Screening of λ libraries in *E. coli* LE392 and C600hfl via plaque lifts, purification of λ DNA incorporating a glycerol step gradient, and plasmid DNA isolation were carried out as described by Sambrook *et al.* (1989). Hybridization probes were respective genes cloned from *Synechococcus* sp. PCC 7942 (see Results).

RNA isolation and Northern blot analysis. Total RNA from exponentially growing cells was isolated as described by Golden *et al.* (1987). RNA was denatured with glyoxal and dimethyl sulphoxide, loaded onto a 1.1% (w/v) agarose gel and electrophoresed according to Sambrook *et al.* (1989). RNA was transferred to nylon membranes (Nytran; Schleicher and Schuell) using the Vacugene transfer system (Pharmacia) and a transfer solution of 7.5 mM-NaOH. Northern hybridization was carried out at 65 °C as described by Sambrook *et al.* (1989).

Nucleotide sequencing, sequence analysis and primer extension analysis. Single-stranded M13mp18/19 DNA was isolated by the method of Dale *et al.* (1985) and sequenced using the commercial Sequenase kit (United States Biochemical). Oligonucleotide primers were prepared on a Cyclone DNA synthesizer (Biosearch). DNA sequence analyses, protein sequence alignments and protein similarity comparisons were performed using Microgenie version 6.0 (Beckman Instruments).

Primer extension was carried out using the method of Kjems & Garrett (1988) developed for reverse transcriptase sequencing, omitting the dideoxynucleotides from the extension mixture.

Results

Cloning and sequence analysis of the *isi* operon

In order to see whether *Synechococcus* sp. PCC 7002 contains *isiA* and whether the arrangement of *isiA* and

isiB that was observed in PCC 7942 is conserved in *Synechococcus* sp. PCC 7002, both genes were cloned and sequenced for PCC 7002. The DNA fragment containing the flavodoxin gene of *Synechococcus* sp. PCC 7942 was used as a probe to identify a 0.8 kb *SacI/HindIII* fragment that contains the corresponding gene in *Synechococcus* sp. PCC 7002. This fragment was cloned into M13mp18 and mp19 and completely sequenced. The sequence data indicated that the clone contained part of *isiA* and terminated just before the end of *isiB*. The 0.8 kb *SacI/HindIII* fragment was then used as a probe to clone the remainder of the gene for flavodoxin as a 1.1 kb *SacI/ClaI* fragment into pDPL13 (Gendel *et al.*, 1983) for double-stranded sequencing. To clone the remainder of *isiA*, an *EcoRI* library in λ NM1149 was probed with a DNA fragment containing the *isiA* gene from *Synechococcus* sp. PCC 7942. The hybridizing λ clone contained a 3.6 kb *EcoRI* fragment. Since repeated attempts to subclone the 3.6 kb fragment into pUC19 were unsuccessful, a 2.0 kb *HindIII/SacI* fragment was subcloned into M13mp18 and mp19 and sequenced. Fig. 1 contains the map of the *isiAB* operon. To verify the continuity of the 2.0 kb *HindIII/SacI* fragment and the 0.8 kb *SacI/HindIII* fragment, the 3.6 kb *EcoRI* fragment was isolated from a low-melting-temperature electrophoretic gel and further purified with 'Gene Clean' glass milk. Double-stranded sequencing across the *SacI* site indicated that these fragments were adjacent. Fig. 2 contains the nucleotide sequence for the *isiAB* operon including surrounding regions.

The operon contains two open reading frames. The first open reading frame codes for a putative polypeptide containing 342 amino acids; this polypeptide shares a similarity of 75.4% with the putative *isiA* protein of *Synechococcus* sp. PCC 7942. The second open reading frame codes for a mature polypeptide with 169 amino acids (assuming proteolytic cleavage of the initial methionine residue; Drummond, 1985). This polypeptide shares a similarity of 69.0% with flavodoxin of *Synechococcus* sp. PCC 7942 and a similarity of 67% with that of *Anabaena* sp. PCC 7120 (Leonhardt & Straus, 1989); the flavin-mononucleotide-binding regions (Smith *et al.*, 1983; Laudenbach *et al.*, 1988) are highly conserved in all three species.

Potential transcriptional termination structures are found within the sequence shown in Fig. 2. One occurs 55 bp downstream of the *isiA* open reading frame with an 8-base inverted repeat structure, a loop of 6 bases and a thermal stability of $\Delta G = -9.6$ kcal mol $^{-1}$ (40.2 kJ mol $^{-1}$). The stem structure is followed by a string of 6 T residues, as is common in rho-independent prokaryotic transcription terminators (Rosenberg & Court, 1979). The second palindromic sequence, which is not followed by a string of Ts, occurs 19 bp after the flavodoxin gene sequence. It consists of a 9-base stem and a loop of

Fig. 1. Partial restriction map of the *isiAB* operon of *Synechococcus* sp. PCC 7002.

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      10      20      30      40      50      60      70      80
GACTTAGTTAATTTAGCGTAGTTTGCGGCCAGGAATCCGACTGATACCTAGGCGATCGCCTTTTATTGCATGAACACCAA

      90     100     110     120     130     140     150     160
CTGATAATTAATAGTGATAAACTTGTCTGAAAATAATTATTGGGTTTCTTCATATCAAAACTGATTAGCAAAAAAGACGT

      170     180     190     200     210     220     230     240
GATCGTTGCGGCGAGAAGTGGAGCGAGAAATTCGTAAAACTCCTATTTTCTTGCAGAGATAAGGTTTTGGTCTCATG

      250     260     270     280     290     300     310     320
GAGTGATTGGCCAGGGCAACTTTTTACAAAAGTTAACTTGAACTTTACTGAGAATAATTGTAGGATAAAGCCAATCCTTT

      330     340     350     360     370     380     390     400
ATTGCCAAATATATGCGATAAGTGATTAGCCTGATTAGTTTAAACGACCACTCTTTTTTAAATCACGATGCAAACCTACG
      M   Q   T   Y

      410     420     430     440     450     460     470     480
ATAATCCAGACGTTAAATACGAATGGTGGGCAGGCAATGCCCGGTTTGCTGACCTTCCGGTCAGTTTATTGGTGCCAC
D N P D V K Y E W W A G N A R F A D L S G Q F I G A H

      490     500     510     520     530     540     550     560
GTGGCCCATGCTGCCTTAATTGTTTTCTGGGCAGGGGCCTTCACGTTGTTTCAAATTTCTTACTTTGACCCGACCCCTACC
V A H A A L I V F W A G A F T L F E I S Y F D P T L P

      570     580     590     600     610     620     630     640
CATGGGCGAGCAAAATTTAATCCTCTTGCCCCATTTAGCCACCCTTGGCCTGGGCATCGAAGGAAACGGTGCGATCAATA
M G E Q N L I L L P H L A T L G L G I E G N G A I N

      650     660     670     680     690     700     710     720
CAGAGCCTTATTTTGTGATTGGTGAATTCACCTAATTTCTTCAGCGGTACTGGCGGCAGGGGGATTGTTCCATGTGTTG
T E P Y F V I G A I H L I S S A V L A A G G L F H V L

      730     740     750     760     770     780     790     800
CGTGGCCCCAAGATCTCAAAACAGCCACCGGCCAGCCGTCGTTTCCATTTTGATTGGGAAGACCCGAAGCAATTGGG
R G P Q D L K T A T G P A R R F H F D W E D P K Q L G

      810     820     830     840     850     860     870     880
TTTAATTTTGGGCCATCACTTACTGTTGCTGGGACTGGGTGCTTTCTTGCTCGTAGCGAAAGCGATGTACTTTGGCGGTT
L I L G H H L L L L G L G A F L L V A K A M Y F G G

      890     900     910     920     930     940     950     960
TGTACGACACAGCGACCCAAACCGTGCGCTTGGTTACAGAGCCGACCCTCGATCCGGCGGTGATCTACGGGTATCAAACC
L Y D T A T Q T V R L V T E P T L D P A V I Y G Y Q T

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Fig. 2 (continued on following page). Nucleotide sequence of *isiA*, *isiB* and flanking regions. The one-letter abbreviations of the deduced amino acid sequences for the products of each gene are shown below the nucleotide sequence; the open reading frames for *isiA* and *isiB* occupy nucleotide positions 388 to 1413 and 1583 to 2092 respectively. Putative transcriptional stop sites are underlined with inverted arrows. The $-10'$, $-35'$ and ribosome-binding sequences are boxed. The start site of transcription is indicated by a vertical arrow. The potential Fur regulatory sequences are underlined with heavy lines.

970 980 990 1000 1010 1020 1030 1040
 CACTTCGCCACCGTTGATAATCTCGAAGATATCGTTGGTGGCCATATTTATGTGGGTGTACTGCTTGTGGCTGGGGGAAT
 H F A T V D N L E D I V G G H I Y V G V L L V A G G I

1050 1060 1070 1080 1090 1100 1110 1120
 TTGGCACATTCTCGTGCCGCCACTCCAGTGGGCAAAAAAGGTGCTCCTGTTCTCTGCGGAAGCGATTTTGTCTTATTTCC
 W H I L V P P L Q W A K K V L L F S G E A I L S Y S

1130 1140 1150 1160 1170 1180 1190 1200
 TGGGGGCGATCGCCTTGGCGGGATTTGTGGCCGCTACTTCTGCGCAGTGAATACCACGGCCTATCCCGTTGAATTTTAT
 L G A I A L A G F V A A Y F C A V N T T A Y P V E F Y

1210 1220 1230 1240 1250 1260 1270 1280
 GGGCCTGTTCTCGATGTCAAACCTCAGCATTGTGCCTTACTTTGCCGACACCATCGAGCTCCCCATGAACGAACACACTTC
 G P V L D V K L S I V P Y F A D T I E L P M N E H T S

1290 1300 1310 1320 1330 1340 1350 1360
 CCGTGCCTGGTTAGCCAATGCCATTTCTTCTTTGCCTTTTCTTCTCCAGGGGCACCTCTGGCAGCCCTCCGCGCCA
 R A W L A N A H F F F A F F F L Q G H L W H A L R A

1370 1380 1390 1400 1410 1420 1430 1440
 TGGGCTTTGATTTCCGTCGAGTCGAAAAAGTCCTTAGCGATCCACTGGATGCCTAGGTTTTTTTAACCGCTACACATCAAC
 M G F D F R R V E K V L S D P L D A

1450 1460 1470 1480 1490 1500 1510 1520
 TTAGTAAAAGTTTCTCTCATAGACAATCGGGTTCATGCAGTGATTGGAACCTTTTTTCTTCTCTTAACGCGACTC

1530 1540 1550 1560 1570 1580 1590 1600
 TTTTGTAGAGTAATTGATTTTTCAATTCAAACAACCTTCTAAAGCAAGATTACGGTTTTTAATCATGTCAAAAATTGGTTTA
 M S K I G L

1610 1620 1630 1640 1650 1660 1670 1680
 TTTTTCGGCACACAAACAGGCAACACAGAAGAACTTGCCCAAGCGATCCAAGCGGCCTTTGGTGGTTCAGATATTGTCTGA
 F F G T Q T G N T E E L A Q A I Q A A F G G S D I V E

1690 1700 1710 1720 1730 1740 1750 1760
 ACTTTTTGATGTGGCTGAGGTTGATATCGAAGCACTACGGGATTTTGATCAGCTGATCATTGGTTGTCCAACCTGGAATG
 L F D V A E V D I E A L R D F D Q L I I G C P T W N

1770 1780 1790 1800 1810 1820 1830 1840
 TGGGAGAACTACAGTCAGACTGGGAAGCCCTCTATGATGATCTCGATGACGTAGACTTTTTCAGGAAAAACCATTGCCTAT
 V G E L Q S D W E A L Y D D L D D V D F S G K T I A Y

1850 1860 1870 1880 1890 1900 1910 1920
 TTTGGGGCTGGGGATCAAGTGGGCTATGCCGACAATTTTCAGGATGCCATGGGCGTTTTAGAGAAAAAATTACCAGCCT
 F G A G D Q V G Y A D N F Q D A M G V L E E K I T S L

1930 1940 1950 1960 1970 1980 1990 2000
 TGGCGGGAAAACCGTAGGCCAATGGCCCACCGCTGGTTATGACCACAGCGAATCCAAAGCGGAACGGGACGGTAAATTTG
 G G K T V G Q W P T A G Y D H S E S K A E R D G K F

2010 2020 2030 2040 2050 2060 2070 2080
 TCGGTTTGGCGATCGATGAAGATAATCAACCGGAATTGACGGCAGAACGTATTCAAGCTTGGGTTGCCCAACTTAAACCA
 V G L A I D E D N Q P E L T A E R I Q A W V A Q L K P

2090 2100 2110 2120 2130 2140 2150 2160
 GCCTTTGGTCTGTAGAACGCTTGATTAATGTCAATTCAGGGGAGGAGCTTGTTGTACTCTTCTCTGGTGGAATCAAAAGG
 A F G L

2170 2180 2190 2200 2210 2220
 AATTCGGTAAAGGGATTTTTCCAAATAAATTTTTTTTAAATCAATAACAATAAAAAAT

Fig. 3. Transcriptional regulation of the *isiAB* operon in response to iron stress. Lanes A, B and C are the Northern blots for RNA from cells under iron stress. The *SacI/HindIII* fragment (Fig. 1) that was used as the probe for lane A contains sequences from both *isiA* and *isiB*, the *AccI/ClaI* fragment that was used as the probe for lane B contains only *isiB* sequences, and the *BglI/SacI* fragment that was used as the probe for lane C contains only *isiA* sequences. Lane B has been exposed much longer than the other lanes to enhance the 1.8 kb band; this exposure also enhances a lower band that may correspond to the apparent breakdown products of lane A. Lane D is the Northern blot for RNA extracted from cells under the normal iron concentration found in A medium; the probe for this reaction is the same as in lane C.

9 bases, and has a thermal stability of $\Delta G = -11.0$ kcal mol⁻¹ (46.0 kJ mol⁻¹).

Transcriptional analysis of the *isi* operon

To examine how these genes are expressed in *Synechococcus* PCC 7002, probes containing *isiAB* (*SacI/HindIII*, Fig. 1), *isiA* (*BglI/SacI* fragments, Fig. 1) and *isiB* (*AccI/ClaI* fragment, Fig. 1) were reacted to Northern blots of RNA extracted from cells grown in A medium and in A medium containing 7.1×10^{-7} M-ferric chloride. No transcripts were detected for cells grown in standard A medium. Under iron-stress conditions the probes containing *isiA* hybridized to messenger RNA molecules of about 1.8 kb and 1.1 kb; the probe for *isiB* only hybridized to the larger messenger molecule (Fig. 3).

Primer extension experiments using a complementary oligonucleotide sequence that is 70 nucleotides downstream from the start codon of *isiA* indicated a

Fig. 4. Primer extension studies to determine the transcriptional start site of the *isiAB* operon. The sequence ladder was constructed using the same primer that was used in the primer extension reaction. The lanes of the sequencing ladder are labelled with the bases that complement the dideoxynucleotides of each reaction, so that the sequence read from the ladder corresponds to the sequence of Fig. 2. The numbers to the right of the figure correspond to the actual nucleotide position of the sequence in Fig. 2.

transcriptional start site 72 bp upstream of the translation initiation codon for *isiA* (Fig. 4). This, along with the putative transcription terminators mentioned previously, predicts transcripts of lengths in close agreement (roughly 1185 and 1830 bases) with those estimated from the Northern hybridization analysis.

Cloning, sequencing and transcript analysis of *petF* and *psbC*

Under iron stress, flavodoxin replaces ferredoxin as the terminal electron acceptor of photosynthesis. Northern blot analysis indicated that the expression of the gene for flavodoxin is regulated at the transcriptional level. To see how the gene for ferredoxin (*petF*) is regulated in *Synechococcus* PCC 7002, a DNA fragment from *Synechococcus* sp. PCC 7942 containing the gene for ferredoxin was used to clone this gene from *Synechococcus* sp. PCC 7002 as a 1.6 kb *HindIII* fragment in

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      10      20      30      40      50      60      70      80
AAGCTTAGGCCCAGGCACACTCTTGAATGGCCGGATGAAATCCTTGTAACACCCTGTTACACCAGAGGAAACACTAATG
M

      90      100     110     120     130     140     150     160
GCTACATATAAGGTTACACTCATCACTCCCGACGGCGAAGTATCCTACGACGCTCCCGATGATGAATACATTCTAGACTC
A T Y K V T L I T P D G E V S Y D A P D D E Y I L D S

      170     180     190     200     210     220     230     240
CGCCGGTGATGCTGGTTATGACCTTCCTGCATCCTGCCGTGCCGGTGCTTGTCTCCACCTGTGCTGGTAAGATCGTTTCCG
A G D A G Y D L P A S C R A G A C S T C A G K I V S

      250     260     270     280     290     300     310     320
GTACTGTTGACCAATCCGAACAGTCCTTCCTCGATGATGATCAAATTGAAGCGGGTTACGTTCTGACCTGTATTGCTTAT
G T V D Q S E Q S F L D D D Q I E A G Y V L T C I A Y

      330     340     350     360     370     380     390     400
CCTCAATCTGATGTCACCATCGAACTAACAAAGAGGAAGAACTCTACTAGAATTTCTCGGCCACCCCTGGCAACTAGA
P Q S D V T I E T N K E E E L Y

      410     420     430     440     450     460     470     480
TAAATAGAACATTTAGAAAGTGTTCCTTGGGAAGCGCTTCTATTTTTTTCGCCACGGGGGTGCAAGTACAAAAAATCC

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Fig. 5. Nucleotide sequence of *petF* and its flanking regions. The one-letter abbreviations for the deduced amino acid sequence are shown below the nucleotide sequence. The putative transcriptional stop site is underlined with inverted arrows. The potential ribosome-binding site is boxed.

Fig. 6. Transcript analysis of the genes for ferredoxin and CP-43 in normal and iron-stressed cells. Lanes A and B represent Northern blots for RNA from normal and iron-stressed cells respectively. These were probed with a 3 kb *Hind*III fragment containing all of *psbDI* and the first half of *psbC*. The 2.7 kb transcript contains the messages from the dicistronic *psbDI-psbC* operon, while the 1.3 kb transcript is the message from *psbDII*. Lanes C and D represent Northern blots for RNA from normal and iron-stressed cells, respectively, probed with a 1.6 kb *Hind*III fragment containing all of the gene for ferredoxin I (*petF*).

M13mp19. Sequencing of this fragment revealed a 291 bp open reading frame corresponding to a mature polypeptide of 96 amino acids (assuming proteolytic cleavage of the first methionine; Matsubara *et al.*, 1978).

Fig. 5 contains the sequence of the *petF* gene of *Synechococcus* sp. PCC 7002. The decoded protein sequence shares a similarity of 75% with ferredoxin I of *Anabaena* sp. PCC 7120 (Alam *et al.*, 1986) and of 73% with that of *Synechococcus* sp. PCC 7942 (Reith *et al.*, 1986). A potential transcriptional terminator is found 42 bases after the stop codon. It consists of a 12-base stem and a loop of 2 bases followed by 6 Ts with a predicted thermal stability of $\Delta G = -13.0$ kcal mol⁻¹ (54.4 kJ mol⁻¹). A potential ribosome-binding site (AGGA) was found 7 bases upstream of the initiation codon.

Northern blot analysis of RNA from cells grown in normal A medium or in A medium containing 7.1×10^{-7} M FeCl₃ indicated that *petF* is transcribed in both iron-replete and iron-stress conditions (Fig. 6, lanes C and D). The cloned probe of *petF* reacted to a message of about 570 bases, indicating that *petF* is located in a monocistronic operon.

The sequence for *psbC* of *Synechococcus* sp. PCC 7002 can be found in Gingrich *et al.* (1990). This gene was also cloned and sequenced in our laboratory. The sequence generated from these clones was identical to that reported by Gingrich *et al.* (1990).

Northern hybridization experiments using RNA from cells grown in A medium and in A medium containing 7.1×10^{-7} M FeCl₃ indicated that *psbC* is transcribed in both the iron-replete and the iron-stressed conditions of these experiments (Fig. 6, lanes A and B). The transcript length was 2.7 kb, which is long enough to encode both *psbDI* and *psbC*, and is in agreement with the results of Gingrich *et al.* (1990).

<i>E. coli</i>					
		*	*	*	***
<i>iucA</i> (primary)	TGA	TAATGAGAA	TCATT	ATT	
(secondary)	CA	TAATTGTTA	TTATT	TTA	
<i>fhuA</i>	TCT	TTATAATAA	TCATT	CTC	
<i>fepA</i>	TA	TTATGATAA	CTATT	GCA	
<i>fur</i> (i)	CTA	TAATGAT	ACGCATT	ATC	
(ii)	TTT	TTATTAACA	ATATT	TGC	
<i>Synechococcus</i> sp. PCC 7942					
<i>isiAB</i> (i)	AAC	TTATTGAGAATTATT	GTA		
(ii)	ACT	TAATATCAACTTATT	GAG		
(iii)	TCT	TTATTGAGAGTAATT	TCT		
<i>irpA</i>	TGA	TTATTATT	CTCATT	TTT	
<i>Synechococcus</i> sp. PCC 7002					
<i>isiAB</i> (+5)	CCT	TTATTGCCAAATATA	TGC		
(-29)	ACT	TTACTGAGAATAATT	GTA		
(-183)	TTC	ATATCAAACTGATT	AGC		
(-229)	TGA	TAATTAATAGTGATA	AAA		

Fig. 7. Comparison of inferred Fur regulatory sequences with the Fur-binding site in the aerobactin promoter. The Fur-binding sequences of the *iucA* promoter have been used to line up published sequences with inferred iron regulatory functions. The *Synechococcus* sp. PCC 7002 sequences have been selected as those sequences best fitting the nucleotides that are invariant (*) in the published *E. coli* iron-regulated promoters (de Lorenzo *et al.*, 1987).

Discussion

When cyanobacteria are exposed to iron stress, a dramatic decrease in phycocyanin occurs, the chlorophyll content and organization within the thylakoid membrane changes, and flavodoxin replaces ferredoxin as the terminal electron acceptor of photosystem I (Hutber *et al.*, 1977; Guikema & Sherman, 1983; Sandmann, 1985; Riethman & Sherman, 1988*b*; Pardo *et al.*, 1990). In iron-stressed *Synechococcus* sp. PCC 7942 a new photosystem II chlorophyll-binding complex CPVI-4 appears that contains an iron-stress-induced 36 kDa chlorophyll-binding protein (Riethman & Sherman, 1988*a, b*).

In *Synechococcus* sp. PCC 7942 the ferredoxin gene, *petF*, is transcribed into a monocistronic message that is constitutively expressed under all iron regimes; thus, ferredoxin expression appears to be regulated in a post-transcriptional manner (Reith *et al.*, 1986; Laudenbach *et al.*, 1988). The appearance of flavodoxin, however, is tightly regulated at the transcriptional level (Laudenbach *et al.*, 1988). The flavodoxin message, which is not detectable in iron-replete conditions, appears under iron-stress conditions and rapidly disappears when iron is added back to the medium. In *Synechococcus* sp. PCC 7942 flavodoxin is the second open reading frame of an iron-stress-induced operon (*isiAB*) which yields two messages when transcribed. The larger message contains both *isiA* and *isiB*; the smaller message contains only *isiA* and is about nine times more abundant than the dicistronic message (Laudenbach *et al.*, 1988). The putative protein deduced from the sequence of *isiA* is very similar to CP-43 (Laudenbach & Straus, 1988).

We have studied the organization and expression of the genes for flavodoxin, ferredoxin and CP-43 in the

marine cyanobacterium *Synechococcus* sp. strain PCC 7002. We found that, as in *Synechococcus* sp. PCC 7942, the gene for ferredoxin is constitutively transcribed in both iron-replete and iron-stress conditions. We also showed that *psbC* is constitutively expressed under iron-stress and iron-sufficient conditions. The gene for flavodoxin is part of an iron-stress-regulated dicistronic operon with similar arrangement and pattern of expression to that of the freshwater *Synechococcus* sp. PCC 7942. The first gene of this operon (*isiA*) is similar to *psbC* and under iron-stress conditions is found on a dicistronic message of about 1800 nucleotides and a more abundant message of 1100 nucleotides. As in *Synechococcus* sp. PCC 7942, *isiA* and *isiB* are separated by a palindromic structure; however, unlike *Synechococcus* sp. PCC 7942, which contained no obvious termination structure downstream from the flavodoxin gene, *Synechococcus* sp. PCC 7002 has a potential transcriptional termination signal 19 nucleotides downstream of *isiB*.

Regulatory sequences upstream of the *isiAB* operon

Primer extension analysis indicated a 72-base leader sequence for the *isi* transcripts of *Synechococcus* sp. PCC 7002. Based on this, other potential regulatory sequences have been identified (Fig. 2). A GATAAA sequence in the -10 region bears similarity to the consensus *E. coli* promoter -10 region sequence and the TTGAAC sequence in the -35 region is similar to the *E. coli* consensus of TTGACA (Rosenberg & Court, 1979).

In *E. coli* the Fur repressor binds to the *fur* regulatory sequences in the aerobactin promoter to repress transcription during iron-replete conditions (de Lorenzo *et al.*, 1987). Similar sequences are found in the promoter regions of other *E. coli* iron-regulated genes: *fur*, *fhuA*,

and *fepA* (Bagg & Neilands, 1987; de Lorenzo *et al.*, 1987). In *Synechococcus* sp. PCC 7942 there are three 17-base sequences, approximately 15, 25, and 150 bp upstream of the transcription start site of the *isiAB* message, which resemble the Fur-binding sequence of the aerobactin promoter (de Lorenzo *et al.*, 1987; Laudenbach & Straus, 1988). *Synechococcus* sp. PCC 7942 also contains *fur* consensus sequences upstream to *irpA*, which is thought to transcribe a product involved in the acquisition or storage of iron (Reddy *et al.*, 1988). In *Synechococcus* sp. PCC 7002, potential *fur* consensus sequences have been located 5 nucleotides downstream, and 29, 183 and 229 nucleotides upstream from the transcriptional start site. Fig. 7 compares the identified *fur* consensus sequences of *Synechococcus* sp. PCC 7002 to those that were identified for the aerobactin promoter and those that have been inferred for other iron-regulated genes.

The putative protein product of *isiA*

In *Synechococcus* sp. PCC 7002, the *isiA* gene encodes a putative polypeptide (IsiA) of 342 amino acids that is 40% similar to the amino acid sequence deduced for CP-43 (Gingrich *et al.*, 1990). The most striking difference between CP-43 and IsiA is a deletion of about 120 largely hydrophilic amino acids between the last two hydrophobic domains of CP-43. Thus, the similarity between CP-43 and IsiA is enhanced to 48% when only the first 286 amino acids are compared. Characteristics shared with other chlorophyll-binding polypeptides reported for the *isiA* gene product of *Synechococcus* sp. PCC 7942 (Laudenbach & Straus, 1988) hold true for that of *Synechococcus* sp. PCC 7002. The *psbB* (CP-47) and *psbC* (CP-43) (Alt *et al.*, 1984), and the *isiA* gene products possess seven major hydrophobic regions, at least some of which could form membrane-spanning helices. In photosynthetic bacteria, histidine residues are believed to play an important role by binding bacteriochlorophyll via the Mg atom in pigment-protein complexes (Zuber, 1986). In CP-47 of *Synechococcus* sp. PCC 6803, histidine residues separated by 13 or 14 amino acids are located in five of the putative membrane-spanning regions (Vermaas *et al.*, 1987). The same spacing of histidine residues has also been found in chlorophyll-protein complexes of PSI (Fish *et al.*, 1985). Five sets of histidines separated by 13 or 14 amino acids, three of which occur within putative membrane-spanning segments reported for the *Synechococcus* sp. PCC 7942 *isiA* gene product, are conserved in *Synechococcus* sp. PCC 7002. The chlorophyll-binding sequence Ala-X-X-X-His (Youvan & Ismail, 1985) is also found in residues 124 to 128 of the *isiA* gene product of *Synechococcus* sp. PCC 7002.

Pakrasi *et al.* (1985) identified an iron-stress-induced

photosystem II chlorophyll-protein complex that contained a chlorophyll *a* binding protein with a molecular mass of 36 kDa. This polypeptide formed the major chlorophyll-protein complex in *Synechococcus* sp. PCC 7942 cells grown in media not containing iron. These authors postulated that this 36 kDa polypeptide forms part of an intermediate light-harvesting antenna for photosystem II that acts as a replacement for phycobilisomes during iron depletion. Interestingly, the proposed function of CP-43 is also that of interior chlorophyll *a* light-harvesting antennae for PSII (Bricker, 1990; Vermaas & Ikeuchi, 1991). Riethman & Sherman (1988*a, b*) suggested that this protein may also act as a chlorophyll *a* reservoir and be involved with membrane development during recovery from iron stress.

Since *isiA* is induced by iron-stress conditions, it is reasonable to assume that its CP-43-like product might compensate in some way for the lack of available iron. Iron is associated with photosystem II as the nonhaem iron bound between QA and QB, and in the haems of cytochrome *b559*. Whether the product of *isiA* replaces a photosystem II protein (CP-43 for example) under iron stress in a way that compensates for the lack of iron, or acts as a reservoir for chlorophyll as suggested by Riethman & Sherman (1988*a, b*), has yet to be determined.

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