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

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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-32P]dCTP (3000 Ci mmol⁻¹) was...
obtained from ICN, and [32P]ATP (5000 Ci mmol−1) and [35S]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 (Agnellulun 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 × 10−5 M-FeCl2+ while low-iron cultures were grown at 7.1 × 10−7 M-FeCl2+. 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 mM-NaCl, 4% (v/v) CTAB, 0-4% (v/v) mercaptoethanol, and incubating at 55 °C for 45 min. The DNA was spoiled 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 λM1149 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 (Nytran). 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).

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 λM1149 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-monomonucleotide-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 AG = −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
Iron stress genes in a marine cyanobacterium

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

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HindIII  bglI  SacI  AccI  HindIII  CiaI
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10  20  30  40  50  60  70  80
GACTTAGTTAATTTAGCTAGTTTTCGCGGCCAGGAATTCGACTTACCTAGGGCAGATCGGCCCTTTTTATTTGCGATGAACACCAA
100
CTGATAATTATTAGTAAAAATTCTGTGAATAATTATTGGGT TTCTTCATATCAAATCTGATTAGCAAAAAAGACGT
110
120  130  140  150  160
GATCGTTTCGCGGAGAATGTGGACAGAAATTGCTAAAATCTCTATTTTCTTGAGAGATGATTTTTGGCTCTCAGT
170  180  190  200  210  220  230  240
GAGTATGGCCAGGGCAACTTTTTTCAAGGTAACTAGTTACCTTTTACTGAGAATTGTAAT~GCCATACACGAAAACCGTGGCGA
250  260  270  280  290  300  310  320
TAATATCTGACGTTTTTAATCGACGCTCGGATGCTACCCGTCGTTTCTTGCAGTTTATTGGTGCCCACA
330  340  350  360  370  380  390  400
GACTTAGTTAATTTAGCTAGTTTTCGCGGCCAGGAATTCGACTTACCTAGGGCAGATCGGCCCTTTTTATTTGCGATGAACACCAA
410  420  430  440  450  460  470  480
CTGATAATTATTAGTAAAAATTCTGTGAATAATTATTGGGT TTCTTCATATCAAATCTGATTAGCAAAAAAGACGT
490  500  510  520  530  540  550  560
GATCGTTTCGCGGAGAATGTGGACAGAAATTGCTAAAATCTCTATTTTCTTGAGAGATGATTTTTGGCTCTCAGT
570  580  590  600  610  620  630  640
CATGGCCGAGCAAATATATCATTGCTAGCTGATTGTTTAACGACCACCTCTTTTAAATCTACGATGAGAACCCGATCAAT
730  740  750  760  770  780  790  800
CGTGGGCCCAAGATCTCAAAAACACGCAAGGCGCCGGCCGACGCCGCGCTTTTCTTCTTGTGGGAAGACCCGAGCATTGGG
810  820  830  840  850  860  870  880
TTAATTTTTTGGCCATCACTTATCTGGTTGCGACTTTTCTTGTCGATGCGAAGACGAGGTATACCTACGACTTTTGCGG
890  900  910  920  930  940  950  960
TGTACGACACACTGCCCTTTTTTTGAACCAGCCGCGGAGCTTTTGCGCTTTTCTTCTTGGCAGTTTATTGGTGCCCACA
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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.
Iron stress genes in a marine cyanobacterium

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 BglII/Sacl 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.

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 (BglII/Sacl 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 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
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 HindIII fragment containing all of psbDZ and the first half of psbC. The 2.7 kb transcript contains the messages from the dicistronic psbDZ-psbC operon, while the 1.3 kb transcript is the message from psbDZ. Lanes C and D represent Northern blots for RNA from normal and iron-stressed cells, respectively, probed with a 1.6 kb HindIII 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 $7.1 \times 10^{-3}$ M FeCl$_3$ 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 normal A medium and in A medium containing $7.1 \times 10^{-3}$ M FeCl$_3$ indicated that psbC is transcribed in both iron-replete and iron-stress conditions (Fig. 6, lanes A and B). The transcript length was 2.7 kb, which is long enough to encode both psbD1 and psbC, and is in agreement with the results of Gingrich et al. (1990).
Iron stress genes in a marine cyanobacterium

**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). 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).

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 *isiA* 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 iprA, 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 the chlorophyll-binding 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 PSII (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 α 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 (1988a, 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 (1988a, b), has yet to be determined.

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**References**


Iron stress genes in a marine cyanobacterium


