IdiA, a 34 kDa protein in the cyanobacteria *Synechococcus* sp. strains PCC 6301 and PCC 7942, is required for growth under iron and manganese limitations

Klaus-Peter Michel,¹ Hubert H. Thole² and Elfriede K. Pistorius¹

Author for correspondence: Elfriede K. Pistorius. Tel: +49 521 1065601. Fax: +49 521 1065626.
e-mail: E.Pistorius@Biologie.Uni-Bielefeld.DE

¹ Biologie VIII:
Zellphysiologie, Universität
Bielefeld, 33501 Bielefeld,
Germany

² Max-Planck-Institut für
experimentelle
Endokrinologie, 30603
Hannover, Germany

In the cyanobacteria *Synechococcus* PCC 6301 and PCC 7942 a protein with an apparent molecular mass of about 34 kDa (called IdiA for iron-deficiency-induced protein A) accumulates under iron and manganese limitation. IdiA from *Synechococcus* PCC 6301 was partially sequenced, showing that the N-terminal amino acid is an alanine. Moreover, the gene encoding this protein in *Synechococcus* PCC 6301 has been identified and completely sequenced. The *idiA* gene codes for a protein starting with valine and consisting of 330 amino acid residues. Thus, IdiA is apparently synthesized as a precursor protein of 36.17 kDa and cleaved to its mature form of 35.01 kDa between two alanine residues at positions 9 and 10. IdiA is a highly basic protein having an isoelectric point of 10.55 (mature protein). Comparison of the amino acid sequence of IdiA with protein sequences in the database revealed that IdiA has similarities to two basic bacterial iron-binding proteins, SfuA from *Serratia marcescens* and Fbp from *Neisseria gonorrhoeae*. Insertional inactivation of the *idiA* gene in *Synechococcus* PCC 7942 resulted in a mutant which was unable to grow under iron- or manganese-limiting conditions. Manganese limitation of the mutant strain led to a drastic reduction of photosystem II activity (O₂ evolution) within less than 48 h, while wild-type cells required a prolonged cultivation in Mn-deficient medium before an effect on photosystem II was observed. Thus, IdiA is a protein involved in the process of providing photosystem II with manganese.

Keywords: *Synechococcus* PCC 6301 and PCC 7942, IdiA, *idiA* gene, iron and manganese limitations, photosystem I and II

INTRODUCTION

Iron is an essential redox component in a number of enzymes and in protein complexes of respiratory and photosynthetic electron transport. Although iron is the fourth most abundant element by weight in the earth's crust, its biological availability is strongly reduced because at physiological pH Fe³⁺ forms insoluble hydroxides. This limitation in iron availability has led to several strategies for iron acquisition involving iron-binding

molecules (siderophores) and the capability of storing iron during times of relative abundance. As Straus (1994) recently stated, the responses of many micro-organisms, including cyanobacteria, to limited iron availability can be divided into three categories: retrenchment (alterations and reduction in cellular structures and physiological activities), compensation (production of new proteins) and acquisition (enhancement of ability to scavenge iron from the environment) (see recent reviews by Straus, 1994; Ferreira & Straus, 1994; Carr & Mann, 1994).

Iron deficiency causes substantial changes in the ultrastructure of thylakoid membranes including a decrease of phycobilisomes, as well as of the chlorophyll protein complexes of PS I and PS II in *Synechococcus* PCC 7942 (Guikema & Sherman, 1984; Pakrasi *et al.*, 1985a, b). Parallel to the reduction of the major chlorophyll protein

Abbreviations: Ap, ampicillin; Chl, chlorophyll; IdiA, iron deficiency induced protein A; Km, kanamycin; MSP, manganese stabilizing protein; PS I, photosystem I; PS II, photosystem II.

The GenBank accession number for the sequence reported in this paper is Z48754.

complexes, a substantial increase in a new chlorophyll protein complex called CPVI-4 was observed. This complex, which copurifies with PS II, consists of polypeptides of 36, 34 and 12 kDa, and is the major pigment-protein complex in *Synechococcus* PCC 7942 cells under conditions of iron limitation (Riethman & Sherman, 1988a, b). It has been suggested that CPVI-4 may function as an auxiliary light-harvesting complex that compensates for the loss of phycobilisomes during stress and may also function as a chlorophyll reservoir that contributes to the assembly of reaction centre complexes in the early stages of recovery from iron deficiency (Riethman & Sherman, 1988b). Further investigations with *Synechococcus* PCC 7942 resulted in the identification of two iron-regulated genes: *irpA*, encoding a protein located in the cytoplasmic membrane and presumably involved in iron acquisition or storage (Reddy *et al.*, 1988); and *mapA*, encoding a protein of unknown function (Webb *et al.*, 1994). *IrpA* was shown to be essential for growth under Fe-deficient conditions, while *MapA* was shown to be not essential. In the marine cyanobacterium *Synechococcus* PCC 7002 an iron-repressible operon, *isiAB*, was identified that contains a gene predicted to encode a chlorophyll-binding protein and a flavodoxin (Leonhardt & Strauss, 1992). *IsiA* was also shown to be expressed under iron deficiency in the freshwater cyanobacterium *Synechococcus* PCC 7942 (the calculated molecular mass of the gene product was given as 36.8 kDa) (Laudenbach & Straus, 1988) and in the nitrogen-fixing cyanobacterium *Anabaena* sp. PCC 7120 (Leonhardt & Straus, 1994). Insertional inactivation of the *isiAB* operon in *Synechococcus* PCC 7942 resulted in loss of CPVI-4 expression, implying that *isiA* encodes a component of the CPVI-4 complex (Burnap *et al.*, 1993).

Although iron uptake has been extensively studied, especially in *Escherichia coli* (Braun & Hantke, 1991; Braun *et al.*, 1987) and to some extent also in cyanobacteria (Straus, 1994), very little information exists in the literature about storage and transport of iron within the cyanobacterial cell and about Fe exchange between Fe storage proteins and the 'functional' Fe proteins/enzymes. Storage and buffering of iron in cells is mainly achieved by ferritins, which are widely distributed. A recent report (Laulhère *et al.*, 1992) has shown their presence in the cyanobacterium *Synechocystis* PCC 6803 together with a pool of low-molecular-mass acidic proteins involved in Fe binding.

We have previously isolated a 34 kDa polypeptide from *Synechococcus* PCC 6301, which is closely related to *Synechococcus* PCC 7942 (Golden *et al.*, 1989). After raising a polyclonal antiserum against this protein, it was shown that in *Synechococcus* PCC 6301 the 34 kDa protein is only weakly expressed under iron-sufficient conditions, but its expression is strongly enhanced under iron-deficient conditions (Michel & Pistorius, 1992). In the present work we have extended our investigations of the conditions leading to expression of this 34 kDa protein (called *IdiA* for iron deficiency induced protein A) and also investigated its location in the cell. Partial sequencing of *IdiA* from *Synechococcus* PCC 6301 allowed isolation and

DNA sequencing of the gene encoding *IdiA* (*idiA*) and construction of an *IdiA* mutant of *Synechococcus* PCC 7942.

METHODS

Bacterial strains and growth conditions. *Synechococcus* sp. strain PCC 6301 (SAUG B 1402-1) and *Synechococcus* sp. strain PCC 7942 were obtained from the Sammlung von Algenkulturen der Universität Göttingen, Göttingen, Germany, and from the Pasteur Culture Collection of Cyanobacterial Strains, Paris, France, respectively.

For regular growth conditions, cyanobacteria were grown in gas wash bottles containing 250 ml medium in a stream of 2% (v/v) CO₂ in air (inoculum 60 µl cells). *Synechococcus* PCC 6301 was grown in the medium of Kratz & Myers (1955) with slight modifications as given by Pistorius *et al.* (1989) at 30 °C, and *Synechococcus* PCC 7942 was grown in BG11 medium (Rippka, 1988) at 30 °C. When cells were grown with ammonium as nitrogen source, nitrate was omitted and 7.5 mM NH₄Cl and 50 mM HEPES/NaOH, pH 7.6, were added to the medium. In micro-element solution Co(NO₃)₂ was replaced by CoSO₄. Growth of both *Synechococcus* strains on agar plates was in BG11 medium containing 1% (w/v) agar (Gibco BRL). Cells were cultivated under Fe-sufficient conditions (Kratz & Myers medium: 16 µM FeCl₃ and 14 µM FeSO₄; BG11 medium: 30 µM Fe-citrate) or Fe-deficient conditions (the medium contained one tenth of the regular Fe concentration or no Fe, as indicated in the legends to the figures). Other stress conditions were achieved by the following modifications: Mg²⁺/Ca²⁺ deficiency: the medium contained one tenth of the regular MgCl₂ concentration and one half of the regular CaCl₂ concentration (Wälzlein & Pistorius, 1991); Mn deficiency: the medium contained no manganese; nitrogen deficiency: the medium contained no nitrogen source; salt stress: the medium contained 0.68 M NaCl; heat stress: cells were grown in the regular medium for 2 d at 40 °C or for 2 d at 42 °C or 6 h at 45 °C after 2 d of regular growth at 30 °C. Where indicated, antibiotics were added at the following concentrations: 10 mg ampicillin l⁻¹ or 25 mg kanamycin l⁻¹.

Cyanobacterial growth was either determined as µl packed cell volume or as increase in optical density at 750 nm.

Escherichia coli DH5αMCR (Grant *et al.*, 1990) was cultivated at 37 °C in LB medium (Miller, 1972) or on PA plates containing, in 1 l distilled water, 17.5 g antibiotic medium no. 3 (assay broth, Oxoid) and 16.0 g bacteriological agar (Oxoid). Where indicated, antibiotics were added at the following concentrations: 150 mg ampicillin l⁻¹ and 50 mg kanamycin l⁻¹.

Preparation of French press extracts, isolation of membranes, periplasm and spheroplast fraction. Cells were grown for 2 d under regular growth conditions (nitrate as nitrogen source, cultivated in gas wash bottles containing 250 ml medium, see above), harvested by centrifugation, washed once either with phosphate buffer or with sucrose-containing medium (see below) and then resuspended to give a cell density of 100 µl cells ml⁻¹. Subsequently the cells were broken by treatment in a French press at 138 MPa. Unbroken cells were removed by centrifugation for 5 min at 4000 g (Sorvall SS34), and the supernatant was referred to as French press extract. When photosynthetic activities were measured, the cells were washed with 50 mM HEPES/NaOH, pH 6.5, containing 50 mM CaCl₂ and 400 mM sucrose, and resuspended in the same medium. Otherwise the cells were washed and resuspended in 10 mM sodium phosphate buffer, pH 7.0.

Isolation of the outer cell membrane, cytoplasmic membrane and thylakoid membrane of *Synechococcus* PCC 6301 was ac-

cording to Omata & Murata (1984), and extraction of the soluble protein fraction from the periplasmic space and isolation of the spheroplast fraction was according to Block & Grossman (1988). The spheroplast fraction separated from the periplasmic fraction by centrifugation was broken by a French press treatment for the experiments shown in Fig. 2.

Isolation of IdiA from *Synechococcus* PCC 6301. IdiA used for sequencing was isolated as described previously (Michel & Pistorius, 1992) with minor modifications. As starting material, the thylakoid membrane fraction was used, which was obtained from Fe-starved *Synechococcus* PCC 6301 cells grown in medium containing one tenth of the regular Fe concentration (harvested after the second transfer into Fe-deficient medium). IdiA was solubilized from thylakoid membrane preparations by treatment with 1% sulfobetaine 12 (*N*-dodecyl-*N,N*-dimethylammonio-3-propanesulfonate) for 1 h on ice. Other conditions were as described by Michel & Pistorius (1992). Subsequently IdiA was purified on a DEAE-Sephacel column and on a MonoS column coupled to FPLC (Pharmacia) as described previously (Michel & Pistorius, 1992).

Since IdiA in *Synechococcus* PCC 6301 is present in the membranous fraction as well as in the soluble protein fraction of total French press extracts, IdiA was also isolated from the latter fraction to allow comparison of the N-terminal amino acid sequence. For this purpose *Synechococcus* PCC 6301 cells were grown for 2 d in Fe-deficient medium, harvested by centrifugation, washed and resuspended in 10 mM sodium phosphate buffer, pH 7.0. Cells were broken by treatment in a French press at 138 MPa (twice) and the membranous fraction was subsequently removed by centrifugation for 3 h at 160 000 *g* (Beckman, Ti60). The soluble protein fraction obtained was dialysed against 25 mM Tris/HCl, pH 8.2, containing 10 mM NaCl and chromatographed on a DEAE Sephacel column. Elution of IdiA was achieved with a linear gradient of 10–500 mM NaCl in the equilibration buffer. After dialysing the fractions containing IdiA (detected by immunoblotting) against 25 mM MES/NaOH, pH 6.0, containing 10 mM NaCl, the sample was chromatographed on a MonoS column coupled to FPLC (Pharmacia). Elution of IdiA was achieved with a linear gradient of 10–500 mM NaCl in the equilibration buffer. IdiA eluted from this column at an NaCl concentration of 250 mM.

Measurements of PS I and PS II activities. PS I and PS II activities were determined by O₂ exchange in French press extracts using a Clark-type electrode as described previously (Michel & Pistorius, 1992). For PS I measurements the reaction mixture contained, in a total volume of 1.9 ml, 54 mM HEPES/NaOH, pH 7.0, 0.4 mM KCN, 0.17 mM methylviologen, 3.3 mM sodium ascorbate, 80 µM 2,6-dichlorophenol-indophenol, 10 µM 3-(3,4-dichloro-phenyl)-1,1-dimethylurea and French press extract corresponding to 10 µg Chl. For PS II measurements the reaction mixture contained, in a total volume of 1.9 ml, 54 mM HEPES/NaOH, pH 7.0, 33 mM CaCl₂, 16.7 mM sucrose, 1.7 mM potassium ferricyanide and French press extract corresponding to 10 µg Chl.

Protein determination, SDS-PAGE and immunoblotting. Protein was determined according to Smith *et al.* (1985). SDS-PAGE was performed according to Laemmli (1970) or Schägger & von Jagow (1987). Protein samples for SDS-PAGE were denatured either at 70 °C for 15 min (Laemmli system) or at 100 °C for 5 min (Schägger & von Jagow system). Immunoblotting was done as described by Michel & Pistorius (1992) by transferring proteins to nitrocellulose (Schleicher and Schüll, BA85) and with the following previously raised antisera: anti-IdiA antiserum (Michel & Pistorius, 1992), anti-D1 and anti-MSP antiserum (Engels *et al.*, 1992). As the second antiserum,

peroxidase-coupled anti-rabbit immunoglobulins/HRP (DAKO Chemicals; dilution 1:500), was used.

Protein sequencing. For N-terminal sequencing, IdiA was concentrated in a Centricon 3 tube (Amicon) at 4 °C (coated with 0.1% Tween 20; Pierce, sequencing grade). Subsequently, 0.2 nmol of the protein were sequenced on a pulsed liquid phase sequenator model 477 A with HPLC system 120A (Applied Biosystems) as described by Bökenkamp *et al.* (1994). To obtain additional endogenous amino acid sequences, IdiA was treated with endopeptidase Lys-C (*Achromobacter lyticus*, Wako Chemicals) or with endopeptidase Glu-C (*Staphylococcus aureus* V8, sequencing grade, Boehringer Mannheim). For Lys-C cleavage, 2.75 µg Lys-C and 55 µg IdiA were incubated in 50 µl 0.1 M Tris/HCl, pH 9.0, 1 mM EDTA and 0.1% Triton X-100 (hydrogenated, Calbiochem). After incubation for 15 h at 30 °C, 400 µl 10 mM Tris/HCl, pH 8.0, containing 6 M guanidino-hydrochloride, 6 µl 88% (v/v) formic acid and 2.5 µl 1 M dithiothreitol were added, and the sample was chromatographed on an Aquapore PR300 (C8) reverse-phase column (30 × 2.1 mm, Brownlee/Applied Biosystems) using a micro-bore 140A HPLC system (Applied Biosystems) with on-line UV detection at 215 nm. Peptides were separated at 100 µl min⁻¹ using (argon-purged) solvents A (0.1% trifluoroacetic acid in H₂O) and B (60% acetonitrile, 20% 2-propanol and 0.085% trifluoroacetic acid in H₂O) as follows: linear gradient 1–10% B for 1 min, linear gradient 10–20% B for 9 min, linear gradient 20–50% B for 45 min, linear gradient 50–80% B for 15 min, linear gradient 80–99% B for 5 min, and finally 99% B for 10 min. Peptides were sampled manually, frozen and stored at –80 °C. For Glu-C cleavage, 1 nmol IdiA protein was buffer-exchanged with 100 mM (NH₄)₂CO₃, pH 7.8, and concentrated to 50 µl in a Centricon 3. The sample was made 0.1% in SDS and heated to 95 °C for 5 min. Endopeptidase Glu-C was added at a ratio of 1:20 (Glu-C sample), and the sample was digested at room temperature for 20 h. SDS-PAGE and immunoblotting to ProBlott PVDF membranes, as well as sequencing from the membranes were performed as described by Bökenkamp *et al.* (1994).

Oligonucleotide synthesis. The following oligonucleotide was designed on the basis of a partial amino acid sequence of IdiA from the N-terminal region utilizing a codon usage dictionary of *Synechococcus* PCC 6301/7942: 5'-TG TA(T/C) TCG GG(T/C) CG(C/G) CAC TA(T/C) AAC AC(G/C) GA-3'. The oligonucleotide was synthesized with an Applied Biosystems DNA synthesizer model 380B using the phosphoramidite method (Beaucage & Caruthers, 1981; Matteucci & Caruthers, 1981) and purified as recommended by the manufacturer. The oligonucleotide was labelled with the 'DIG oligonucleotide 3' end labeling kit' and detected with the 'DIG DNA labeling and detection kit' (Boehringer).

DNA isolation, cloning, sequencing and analysis. Plasmid pSVB30 was used in this study (Arnold & Pühler, 1988). The details of the pSVB30 derivatives constructed in this work are given below and in the legend to Fig. 5.

Total DNA of *Synechococcus* PCC 6301 was isolated by the Sarkosyl method and purified by the phenol extraction procedure (William, 1988; Sambrook *et al.*, 1989). DNA was digested with *Hind*III and size fractionated on agarose gels. The fragments were recovered by using the 'Jetsoorb gel extraction kit' from Genomed. A 0.7 kb *Eco*RI *Pst*I fragment and a 2.2 kb *Bgl*II fragment of the 5.4 kb *Hind*III fragment were cloned into the linearized vector plasmid pSVB30 (Arnold & Pühler, 1988).

Cloning procedures, Southern blotting, and other recombinant DNA methods were performed using established techniques (Sambrook *et al.*, 1989). Subclones of the resulting hybrid

plasmid were made by using the Pharmacia Double stranded nested deletion kit. Overlapping subclones were used for sequencing on an ALF Sequencer (Pharmacia). The enzymes used in this study were purchased from Boehringer, Bethesda Research Laboratories or Pharmacia. All reactions were performed following the recommendations of the corresponding manufacturer.

Nucleic acid sequences were analysed using the Staden Software package (Staden, 1986). Analysis of the deduced protein sequence was performed with the program 'PC/Gene' (Rel. 6.80, 1993, IntelliGenetics). Related protein sequences were searched in the SwissProt and GenBank databases using the 'BLAST' software (Altschul *et al.*, 1990).

Construction of an *IdiA*-free *Synechococcus* PCC 7942 mutant. A 5.4 kb *Hind*III DNA fragment of *Synechococcus* PCC 6301 carrying the *idiA* gene cloned into vector plasmid pSVB30 (plasmid pKPM24) was chosen for gene interruption with a kanamycin resistance gene. A 2.2 kb *Bam*HI fragment from plasmid pHP45 Ω Km^R (Fellay *et al.*, 1987) carrying the kanamycin resistance gene was cloned into the unique *Bam*HI site of the *idiA* gene 309 bp downstream from the putative starting codon. Insertion of the kanamycin resistance gene into the *idiA* gene resulted in two additional *Hind*III cleavage sites. This construction was transferred to *E. coli* DH5 α MCR, and clones with the interrupted *idiA* gene were identified by screening for double resistance (Ap resistance encoded by the vector, Km resistance encoded by the Tn5 fragment) and subsequent restriction analysis. Detection was achieved with the synthetic oligonucleotide. The protocol for construction of the *IdiA*-free mutant of *Synechococcus* PCC 7942 was basically the same as described for construction of an MSP-free *Synechococcus* PCC 7942 mutant (Bockholt *et al.*, 1991).

RESULTS AND DISCUSSION

Growth conditions leading to expression of *IdiA*

For these experiments *Synechococcus* PCC 6301 was grown under regular growth conditions for 2 d, then washed with distilled water, and transferred into either a medium depleted of Fe or Mn, containing a reduced Mg²⁺/Ca²⁺ concentration, or containing ammonium instead of nitrate as nitrogen source, or no nitrogen source. The cells were also exposed to elevated temperatures or elevated NaCl concentrations (salt stress) (see Methods). After a growth period of 2 d or several 2 d periods under the respective conditions (see legend of Fig. 1), the cells were harvested, washed and resuspended in 10 mM sodium phosphate buffer, pH 7, broken by treatment in a French press, and the extract was submitted to SDS-PAGE and immunoblotting. *IdiA* was hardly detectable when cells were grown on nitrate under regular growth conditions (Fig. 1, lane A). These cells were taken from stock cultures growing slowly in test tubes under low light intensities. When these cells were grown over a prolonged period under our regular experimental growth conditions, a small amount of *IdiA* was observed (lane B). The highest expression of *IdiA* was observed under iron deficiency (Fig. 1, lane C; see also Fig. 4 of Michel & Pistorius, 1992). Besides Fe deficiency, substantial accumulation of *IdiA* was obtained when manganese was omitted from the medium or when the Mg²⁺/Ca²⁺ concentration in the medium was reduced (lanes D, E, F). Growth of the cells

Fig. 1. Accumulation of *IdiA* in *Synechococcus* PCC 6301 cultivated in medium with nitrate or ammonium as nitrogen source and in medium deficient in Fe, Mn or Mg²⁺/Ca²⁺. *Synechococcus* PCC 6301 cells were grown for 2 d under regular growth conditions (nitrate as nitrogen source) (lane A), or for several 2 d periods under regular growth conditions (lane B). Subsequently cells were transferred to various deficient media: lane C, growth for 2 d in medium lacking Fe; lane D, growth for 1 d in medium containing one tenth of the regular MgCl₂ and one half of the regular CaCl₂ concentration; lane E, growth for 2 d in medium as in D; lane F, growth for six 2 d periods in medium lacking MnCl₂; lane G, growth in medium containing 7.5 mM NH₄Cl instead of nitrate as nitrogen source under iron-sufficient conditions. French press extracts (100 μ g protein each) of the various samples were submitted to SDS-PAGE and, after transfer of proteins to nitrocellulose, immunostaining was performed with the anti-*IdiA* antiserum (dilution 1:4000).

under iron-sufficient conditions with ammonium replacing nitrate as nitrogen source also caused accumulation of *IdiA* (lane G), while no accumulation was observed under nitrogen-deficient conditions (not shown). Heat or salt stress did not, or only very slightly, increase the expression of *IdiA* (data not shown), indicating that *IdiA* is not a general heat or salt stress protein. The results indicate that *IdiA* mainly accumulates under iron limitation and other conditions leading to damage of PS II, such as manganese limitation (Mn is a cofactor of photosynthetic water oxidation; see Debus, 1992) or Mg²⁺/Ca²⁺ deficiency, which also leads to substantial reduction of PS II activity (Wälzlein & Pistorius, 1991). Small amounts of *IdiA* also accumulate in fast-growing cultures (with nitrate or ammonium as nitrogen source) under iron-sufficient conditions (Fig. 1, lanes B and G). This is most likely due to a kinetically limited Fe uptake from BG 11 medium in fast growing cultures resulting in a lowered internal level of Fe and thus causing partial derepression of *idiA*.

Localization of *IdiA* in the *Synechococcus* PCC 6301 cell

To obtain information about the localization of *IdiA* in the Fe-depleted cell, extensive subcellular fractionations were performed. The soluble periplasmic proteins were

Fig. 2. Investigation of the presence or absence of IdiA in the soluble protein fraction extracted from the periplasmic space of *Synechococcus* PCC 6301. Cells were grown for 2 d under Fe sufficient conditions (lanes A and B) and then cultivated for 2 d in a medium containing one tenth of the regular Fe concentration (lanes C and D) or lacking Fe (lanes E and F). Lanes A, C and E show the French press extracts of the spheroplast fraction (acting as controls to document IdiA accumulation in the cell) and lanes B, D and F show the corresponding soluble protein fractions extracted from the periplasmic space. Samples were submitted to SDS-PAGE (40 μ g protein in French press extract and 20 μ g protein in the protein fraction from periplasm) and, after transfer to nitrocellulose, immunostaining was performed with the anti-IdiA antiserum (dilution 1:4000).

extracted by using an osmotic shock procedure which disrupts the outer cell-membrane in Gram-negative bacteria (Block & Grossman, 1988). No IdiA protein could be detected in the soluble protein fraction after osmotic shock in spheroplasts which still had an intact inner cell membrane (cells cultivated for 1 or 2 d in Fe-deficient medium), indicating that IdiA is not a soluble protein in the periplasm (Fig. 2, lanes C and D). However, when cells were cultivated over a prolonged period under iron starvation (≥ 3 d), IdiA was partially released after osmotic shock (Fig. 2, lanes E and F). Under these conditions release of phycobiliproteins was also detected (by measuring absorbance spectra) in the soluble protein fraction, indicating that after prolonged Fe starvation the osmotic shock procedure damaged the cytoplasmic membrane.

Examination of subcellular fractions in immunoblot experiments revealed IdiA in the thylakoid fraction and in a fraction containing thylakoid membranes as well as phycobilisomes (Fig. 3, lanes 2 and 3), but no IdiA was detected in fractions corresponding to the cytoplasmic or outer cell membrane. The fractions containing IdiA also contained the PS II polypeptides D1 and MSP.

Partial amino acid sequence of the IdiA protein from *Synechococcus* PCC 6301

IdiA was purified from thylakoid membrane preparations of *Synechococcus* PCC 6301 grown under Fe-deficient conditions. N-terminal sequencing yielded an amino acid sequence of 18 amino acid residues with alanine as the N-

Fig. 3. Subcellular fractionation for isolation of various membranes from *Synechococcus* PCC 6301 grown under Fe limiting conditions. Membranes from *Synechococcus* PCC 6301 cells grown for 2 d in medium without Fe were separated on a sucrose gradient according to the procedure described by Omata & Murata (1984). Lanes 1, cytoplasmic membrane; lanes 2, thylakoid membrane; lanes 3, phycobilisomes contaminated with thylakoid membranes; lanes 4, outer membrane. Samples were submitted to SDS-PAGE and, after transfer to nitrocellulose, immunostained with (a) the anti-IdiA antiserum (dilution 1:5000), (b) anti-D1 antiserum (dilution 1:2000) or (c) anti-MSP antiserum (dilution 1:2000).

terminal amino acid residue (Table 1). In addition, IdiA was submitted to proteolysis with the endopeptidases Lys-C and Glu-C. The N-terminal amino acid sequence of these proteolytic fragments of IdiA are summarized in Table 1. N-terminal sequencing of IdiA isolated from the soluble protein fraction gave a contiguous amino acid sequence of 12 residues which was identical to that for the thylakoid-membrane-associated IdiA. Thus, both purifications gave a protein which starts with an alanine.

Characterization of the gene encoding IdiA from *Synechococcus* PCC 6301

The presence of a single copy of the putative *idiA* gene of *Synechococcus* PCC 6301 was detected using an oligonucleotide designed on the basis of an amino acid sequence from the N-terminal region of IdiA (see Methods) (Fig. 4). The oligonucleotide hybridized with a 5.4 kb *Hind*III fragment, which was isolated from a size-fractionated gene bank based on vector plasmid pSVB30. The physical map of this 5.4 kb fragment in plasmid pKPM24 is shown in Fig. 5(a).

Fragments of pKPM24 obtained by partial *Eco*RI/*Pst*I and *Bgl*II digestion were subcloned and used for DNA sequence analysis (see Methods). The sequenced 2273 bp

Table 1. Partial amino acid sequence of several proteolytic peptides of IdiA isolated from thylakoid membrane preparations of *Synechococcus* PCC 6301 cells grown under iron-deficient conditions

Details of experimental procedures are given in Methods.

N-terminal sequence of IdiA AEGEVNLVSGRHYNTDNQ	
Amino acid sequences of proteolytic peptides of IdiA	
Lys-C cleavage	Glu-C cleavage
TEAWARGLVQNFARQ	AEGEVNLVSGRHYNTDNQ
SRSPADVFTITVDAGR	MIAADGAAKTEA
RARVIMYNRDRVNASQLSTY	FTQKTGIKVNLIERGEATALL
ISASVFGANNAQALQLMDRAGW	VNLYSGRHYNTDNQIYREF
LYQAVPANLRDPQGR	AARKIKVFFPNQKGRGTHVN
VNLIERGEATALLARLK	MIAADGAAKTEAWAR
AEGEVNLVSGRHYNTDNQIYREF	

Fig. 4. Southern blot of total DNA from *Synechococcus* PCC 6301 digested with various endonucleases. Genomic DNA from *Synechococcus* PCC 6301 was digested with *Bam*HI (lane A), *Eco*RI (lane B), *Hind*III (lane C), *Pst*I (lane D), *Kpn*I (lane E), *Xba*I (lane F) and *Xho*I (lane G). Detection of the *idiA*-carrying DNA fragment was performed with a DIG-labelled synthetic oligonucleotide synthesized according to the amino acid sequence of the N-terminal region of the isolated IdiA from *Synechococcus* PCC 6301.

fragment contains three ORFs (one being incomplete) and the gene encoding IdiA (Fig. 5b). The *idiA* gene (GC content 54.08 mol%) has a putative translational start at base position 723 with a GTG codon and terminates at base position 1712 with a TAA stop codon, encoding a protein of 330 amino acids in length with a predicted molecular mass of 36 167 Da (Fig. 6). The amino acid sequence derived from the DNA sequence includes all of the partial amino acid sequences obtained from proteolytic peptides of IdiA (Table 1, Fig. 6). There were no differences, thus allowing the conclusion that the correct gene encoding IdiA had been identified.

Comparison of the N-terminal sequence of IdiA obtained by protein sequencing with that deduced from the DNA sequence of the *idiA* gene showed that the mature protein (starting with alanine) is nine amino acid residues shorter than the protein deduced from the DNA sequence data (starting with valine). IdiA is most likely processed between two alanine residues at positions 9 and 10 (resulting in a calculated molecular mass of 35 009 Da; the apparent molecular mass obtained by SDS-PAGE corresponds to 34 kDa). Since IdiA isolated from thylakoid membrane preparations and from the soluble protein fraction starts with alanine and not with valine, we conclude that the IdiA in the cell is present primarily in the processed form. However, preliminary results indicate that a minor amount of an unprocessed form of IdiA also seems to be present in the cell, since a protein with an apparent molecular mass of about 35 kDa was N-terminal blocked and could be detected with the anti-IdiA anti-serum.

IdiA has no typical prokaryotic signal sequence, although it is apparently synthesized as a precursor protein. The isoelectric point is 10.68 and 10.55 for the unprocessed and processed forms, respectively, which is consistent with the behaviour of IdiA during purification; it bound more tightly to cation- than to anion-exchange columns. IdiA is classified as a peripheral protein having no

Fig. 5. Physical map of the *idiA* gene region from *Synechococcus* PCC 6301 and insertional inactivation of the *Synechococcus* PCC 7942 *idiA* gene. (a) The pSVB30 derivative pKPM24 contains a 5.4 kb *Hind*III fragment of *Synechococcus* PCC 6301 DNA carrying the *idiA* gene. Size and orientation of the *idiA* gene is shown by the arrow. The position of the oligonucleotide probe is shown by the black box. (b) The partially sequenced *Eco*RI-BglII fragment contains three ORFs (one being incomplete) and the complete sequence of the *idiA* gene. (c) pKPM224 is a derivative of pSVB30 lacking the native *Bam*HI site in its multiple cloning site. The 5.4 kb *Hind*III fragment from pKPM24 was subcloned into the *Hind*III site of pSVB30 Δ *Bam*HI (pKPM124). The kanamycin resistance gene (Km^R) was cloned into the single *Bam*HI site 309 bp downstream from the putative start codon of *idiA*. Size and orientation of the *idiA* gene and the Km^R gene are indicated by the arrows. This construct was then used to transform *Synechococcus* PCC 7942.

transmembrane helices according to the criteria of Kyte & Doolittle (1982), Klein *et al.* (1985) and Eisenberg *et al.* (1984), while one potential transmembrane helix is predicted (in position 195–210 of the unprocessed protein) according to Rhao & Argos (1986).

Analysis of the DNA sequence upstream of the *idiA* gene (Fig. 6) revealed two sequences with similarity to sequences upstream of *irpA* and the operon *isiAB*. The sequence 5'-cAtgtcTGAATCAaTgTTc-3' between nucleotides -65 and -83 corresponds in 11 of 19 bases (nucleotides in capital letters) to a sequence upstream of the coding region of the *irpA* gene of *Synechococcus* PCC 7942 (Reddy *et al.*, 1988) and the sequence 5'-AgTcgTcGgGAcTTtTGTt-3' between nucleotides -45 and -64 corresponds in 12 of 20 bases (nucleotides in capital letters) to a sequence upstream of the coding

TCAGGTGACCTGACTAAGTCGACTGCGAGCAATGATGATCTGCTCTACACAGCGTT	60	ORF 1
TCGAATGAGCAGCAGGCGCGCCTATGCTGCGGATAGTGATCATCCGCGCAGAAAT	120	bp 38-208
TGTCAGTATTCTGATGCGCGGCTGTGCAATGAGCGATGCGGCTATCGGCTGGGAC	180	
AAATCAGTGTCTGCTGCGCAUCCGGCTGATCAGCTTTTCCTACGGCTAGCGAGCAAT	240	
CGATTCTGTTGCGCCCTGCGCCCTGTTGCGCGCGCTCAGGCTCTTACCGCGCGGATCG	300	
CCATCAGTCTGCTGCTCAGCTTCACTACTGACGCTTACCGCTCAGCGCAATACCGAG	360	ORF 2
ACATCCGCAATCTATTGGTTTACCGCGCTCAGTACCGCTTTGCGTGGCTTCTGGAGT	420	bp 312-605
CCTGCGGCTTATGCTCAGCTTGGCTGCGAGCGCGCGAGGACAGCAATTTGGTCAGCA	480	
CTCTCAGCGCAGCGCTGGATGTCGCCCTGAGTTATGCGGCTTGGAGTTTCTGGGTTT	540	
AGCAGCTCAAAAGCGCGCGATACCAAGAGTGTGCTGGCGACACTACTTGGGCTTATGGG	600	
TTTCTAGTGTAAATGCATCACAACCTATCTTACCTCTCTCTGCGCGCGGAGTTCTG	660	
TCGTCGCGGACTTTTGTGGGCGGACAGCTCTGCGCGGACGCTATTACTCGATATTT	720	
TGCTGACTGGGCGCGCTGGGCGAGAGCTGCTGAAGTGAATCAATTTCTACTCGGCTC	780	<i>idiA</i>
V T G R R R A E A A E G E V N L Y S G	bp 723-1712	
GGCAGTCAACACCAATCAGATCTATCGGGAATTCACCAAAAGCAGGATTAAAG	840	
R H Y N T D N O I Y R E F T O K T G I K		
TCAATCTAATGAGGCGAGGCGACTGCACTTTTACCGCGCTCAGAGTGAAGCGAGCG	900	
V N L I T G E A T A L L A R L K S E G S		
CGAGCGCGCGAGATGTTTCACTACGCTGATGCGGCGCGCTTGGCAAGCGACTCAAG	960	
R S P A D V F I T V D A G E L W Q A T Q		
CGAACCTGCTCAGACCACTGACTCAAGCGCAAGCTCGCAAACTGATCAAGCAGTTCGG	1020	
A N L L R P L T Q A Q A P K L Y O A V P		
CGAACTGCTCGGATTCAGCGAGCTGTGTTTGGCTTGGCAAGCGCGCGAGTCAATTA	1080	
A N L R D F Q G R W F A L S C T K V F F N V I		
TGTACACCGGAGTCGGGCAATGCTAGCGAGTGTCTACCTACGAGGATTGGCGAATC	1140	
N Y N R D R V N A S Q L S T Y E D L A N		
CAAAATGGCGCAATCAATCTCTGCGCGAGTTCAGCAAGCTCATAACCTCTCTCTGA	1200	
F K W R N Q I L V R S S S N V L S L		
CUGGAGAGATGATGCTGCGGATGCTGCGCGCAAACTGAGGCTTGGGCGCGGGGCTTG	1260	
T G S M T A A D G A A K T E A W A R Q L		
TCGAATCTTGGCGCTCAGCGCGCAAGGTGGAGATACCGCGCAATTTGCTGCTGCGCG	1320	
V Q N F A R Q P O G G D T F Q I A A C A		
CGGCTGCTGCGCTCTGCGCAATGCGCAACCTACTATTGCTGGGCTCTCTCAAGTCGA	1380	
A G V G S L A I A N T Y Y L V R L F K S		
AAAGAGCAGAGAGCGGCGAGCTGCAAGAAAGATTAAGTCTTCTTCCCAACCAAAAG	1440	
K K A E R E A A R K I K I V F F F N Q K		
GACGGGCGACCGCACTCAACATCAGCGGTGCTGGCATCTGCGCGCGCGCGCAATCAC	1500	
G R G T H V N I S G A G I V R T A P N P		
GCGCTGCTCAACTGTTACTGGAGTACCTGCTCAGTAGCGCAAGCGCGCTGTGTCGCTA	1560	
R A A Q L L L E Y L L S S N V L S L		
GAGGCAATGGTGAATACCACTCTGCGTGGGCTCTGCTGATCGGATTTGGCAGGCT	1620	
R G N G E Y P V L R G V S L D P I L A G		
TGCTCAATTCAAGAAATCCAAATCAGTCCCTCAGCTCTGCGCGCGCAATGATGCTCAAG	1680	
F G Q P K E S K I S A S V F G A N A Q		
CCCTCAGTTGATGGATCGTGGCTGGAATAAGACGTTATGGATCACTTCACTCC	1740	
A L Q L M D R A G N K *		
TCTCAGCAGCAAGCGCGCTGTAGAGGAGTTTATTTGATATTGATATTGTTTGG	1800	
TCGAAGAACTAGGACATTTTATTTGGCACTCATCAGCTAAAGTCGATGATCAATATCG	1860	
CTAACITTTACTCAGATTGACTCAGATTGAGCTTTAAAGAGCTCCAGAACCCCT	1920	
GCGCGCTTCTGTTTGAATCTGTTGCGCGGATGCTTAGGATCTATGCGCAATAG	1980	
GTAGGAGCTGATGATGTTGGTGAATCAGCAGTCTGACCACTAAGTCGAGTCACTG	2040	
CCTTCGTTTCAACTGATGATGATGATGTTGATGTTGATGTTGATGTTGATGTTGAT	2100	
TCGCGCGGAGCTGATGTTGATGATGATGATGATGATGATGATGATGATGATGATGAT	2160	ORF 3
CGGAATGAAATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT	2200	bp 2148 →
CGAACAATCTAGGAGTCTCTCACTTCTGCTGAGTGCAGCAGCAGAGTCTCT	2273	

Fig. 6. Complete nucleotide sequence of the *idiA* gene from *Synechococcus* PCC 6301 and its derived amino acid sequence and the nucleotide sequence of three additional ORFs: ORF 1, bp 38–208; ORF 2, bp 312–605; *idiA*, bp 723–1712; and ORF 3, bp 2148– (incomplete). The amino acid residues verified by sequencing the *IdiA* protein are underlined. The position of the oligonucleotide binding site is double-underlined. The unique *Bam*HI site in the *idiA* coding region used for cassette mutagenesis is boxed. Two sequences (bp -65 to -83 and bp -45 to -64) with homologies to upstream regions of iron-regulated cyanobacterial genes are also boxed.

region of the *isiAB* operon (Laudenbach & Straus, 1988; Straus, 1994). This might imply that *idiA*, *irpA* and *isiAB* have regulatory sites in common although belonging to different regulons.

Comparison of the IdiA sequence with sequences in the PIR database (not shown) revealed similarities to two basic proteins with iron-binding properties located in the periplasm: SfuA from *Serratia marcescens* (molecular mass of precursor and mature protein 36.15 and 33.5 kDa, respectively, and isoelectric point of the precursor protein 9.52; Mietzner *et al.*, 1987; Angerer *et al.*, 1990); and Fbp from *Neisseria gonorrhoeae* (molecular mass 33.5 kDa and isoelectric point 10.1; Berish *et al.*, 1990a) or from *N. meningitidis* (Berish *et al.*, 1990b). These two bacterial proteins belong to a class of basic proteins which can bind iron reversibly similar to the basic iron-binding protein lactoferrin (Montreuil *et al.*, 1960; Lippard & Berg, 1994). In addition to sequence homology SfuA and IdiA are both synthesized as precursors and are processed between two alanine residues (Angerer *et al.*, 1990). However, SfuA has a typical prokaryotic signal sequence, which is presumably cleaved when the protein is transported into the periplasm, while IdiA has no typical signal sequence (only nine amino acid residues are removed), and it is located intracellularly, being mainly associated with the thylakoid membrane. Whether IdiA might be transported into the lumen of the thylakoid, cannot be answered at present.

Besides having sequence homology to SfuA and Fbp, IdiA has a slight similarity to a membrane-associated

Fig. 7. Southern blot of DNA from *Synechococcus* PCC 7942, from *Synechococcus* PCC 6301, and from *Synechococcus* PCC 7942 mutant I4 digested with *Xba*I, *Bam*HI or *Hind*III. Detection of the *idiA*-carrying DNA fragments was achieved with the synthetic oligonucleotide. Lane A, DNA markers; lanes B, C, D, DNA digested with *Xba*I; lanes E, F, G, DNA digested with *Bam*HI; and lanes H, I, J, DNA digested with *Hind*III. DNA from *Synechococcus* PCC 7942 mutant I4 is present in lanes B, E, H; DNA from *Synechococcus* PCC 7942 is present in lanes C, F, I; and DNA from *Synechococcus* PCC 6301 is present in lanes D, G and J.

Fig. 8. Immunoblot with the anti-IdiA antiserum. SDS-PAGE of French press extracts of *Synechococcus* PCC 7942 (a) or mutant I4 (b) cells which were grown for 2 d under Fe sufficient conditions (lanes 1), with one tenth of the regular Fe concentration (lanes 2) and with no Fe in the medium (lanes 3). After transfer of proteins to nitrocellulose, samples were immunostained with the anti-IdiA antiserum (antiserum dilution 1:5000). In all lanes 30 µg protein was applied.

protein, MapA, of unknown function from *Synechococcus* PCC 7942 (Webb *et al.*, 1994) (data not shown).

Insertional inactivation of the *idiA* gene from *Synechococcus* PCC 7942

To inactivate the *idiA* gene, plasmid pKPM24 was used for mutational gene interruption with a 2.2 kb *Bam*HI fragment taken from plasmid pHP45ΩKm^R (Fellay *et al.*, 1987). The resulting plasmid, pKPM224, is shown in Fig. 5(c). The Km^R gene in this plasmid is orientated in the same direction as the *idiA* gene.

Synechococcus PCC 7942 is closely related to *Synechococcus* PCC 6301 (Golden *et al.*, 1989), but is more readily transformed than *Synechococcus* PCC 6301. Therefore, *Synechococcus* PCC 7942 was transformed with pKPM224 (Fig. 5c). More than 80% of the Km-resistant colonies had lost the vector-encoded Ap resistance, implying that the wild-type *idiA* gene was replaced by the inactivated gene. The replacement of the *idiA* gene in PCC 7942 was verified by Southern analysis of a Km-resistant and Ap-sensitive clone (Fig. 7) which was called *Synechococcus* PCC 7942 I4 and used in the subsequent experiments.

Immunoblot analysis of *Synechococcus* PCC 7942 and mutant I4 cells with the anti-IdiA antiserum showed that the mutant cells contained no detectable IdiA either under Fe-sufficient or under Fe-deficient growth conditions, clearly demonstrating that the mutant I4 is indeed an IdiA-free mutant (Fig. 8).

Fig. 9. Growth of *Synechococcus* PCC 7942 and mutant I4 under Fe- and Mn-sufficient or deficient conditions. After 2 d growth under regular growth conditions *Synechococcus* PCC 7942 (●, ■, ▲) and mutant I4 (○, □, △) cells were transferred into media containing the regular Fe and Mn (●, ○) concentration or into medium containing either no Fe (■, □) or no Mn (▲, △).

Comparative analysis of *Synechococcus* PCC 7942 and mutant I4

The IdiA mutant I4 of *Synechococcus* PCC 7942 was able to grow photoautotrophically. The growth rate in Fe- and Mn-sufficient medium was approximately 50–75 % the rate of the wild-type (Fig. 9). The reduced growth rate of the mutant was observed in the presence or absence of kanamycin, indicating that the growth defect of the mutant under iron-sufficient conditions was not a consequence of the added antibiotic. When cells were transferred to a medium containing either no Fe or no Mn, wild-type cells grew only slightly slower for the first 3 d than cells grown in Fe- and Mn-sufficient medium. Omission of Fe from the medium affected wild-type cells after the second transfer in Fe deficient medium, while omission of Mn required several transfers (at least 6×2 d) before a reduction in the growth rate was detectable. In contrast, growth of mutant cells was immediately affected by omission of Fe or Mn, indicating that mutant cells lacking IdiA were unable to cope with a reduced level of either Fe or Mn.

A comparative analysis of PS I and PS II activities in *Synechococcus* PCC 7942 wild-type and IdiA mutant cells grown in Fe- and Mn-sufficient or -deficient medium is summarized in Fig. 10. Wild-type cells grown under Fe-deficient conditions have an approximately 30 % lower PS I activity, while the PS II activity remained unchanged or even increased slightly within the first 2 d of Fe deficiency. When comparable measurements were performed with the IdiA mutant strain I4, PS I and PS II activity were only slightly lower in the mutant (10–20 % for PS I activity and 15–30 % for PS II activity) under Fe- and Mn-sufficient conditions as compared to wild-type. Under either Fe- or Mn-limiting growth conditions, PS I activity in the mutant I4 dropped to about the same level as in wild-type. Thus, the absence of IdiA does not greatly

Fig. 10. PS I and PS II activities of *Synechococcus* PCC 7942 and mutant I4 cells grown under regular conditions or under Fe- or Mn-limiting growth conditions. Cells were grown for 2 d under regular conditions and then transferred into medium containing the regular Fe and Mn concentration or into medium containing either no Fe or no Mn. After 2 d cells were harvested and broken by a French press treatment and subsequently PS I and PS II activities were determined (see Methods). For each experimental series at least six replicates were performed. The reduction of PS II activity in the mutant I4 cells, when grown under Fe or Mn limitation, was at least 50 %, and in five out of the six experiments the reduction was 80–90 %. In all other cases the deviation from the activity values given in the figure was never more than 10–15 %.

affect PS I activity within the first 2 d of Fe or Mn deficiency. However, a substantial difference was observed in PS II activity in mutant cells grown either under Fe or Mn deficiency compared to wild-type. In the IdiA mutant I4, Fe as well as Mn limitation caused a 50–90 % reduction of photosynthetic O_2 evolution within 48 h. Thus, it can be concluded that IdiA is essential under iron as well as manganese limitation and that absence of IdiA affects activity of PS II significantly more than that of PS I, implying that IdiA has an essential role in the process of providing PS II with manganese.

So far only limited information exists in the literature about manganese metabolism in photosynthetic organisms (for a general review on Mn metabolism see Fráusto da Silva & Williams, 1993) and of how PS II is supplied with manganese (Debus, 1992). The only information about a manganese transporter comes from a very recent report on an ABC transporter complex for manganese which has been identified in the cyanobacterium *Synechocystis* PCC 6803 (Bartsevich & Pakrasi, 1995). Whether IdiA is a general iron- and/or manganese-binding protein in *Synechococcus* PCC 6301 and PCC 7942, which is either loosely associated with thylakoid membranes or located in the lumen of the thylakoid remains to be determined. However, the results clearly show that IdiA is essential under iron as well as manganese limitation and that its absence affects PS II activity more than PS I activity suggesting that its main role is in manganese metabolism.

ACKNOWLEDGEMENTS

The financial support of the Friedrich-Naumann-Stiftung is gratefully acknowledged (fellowship for K.-P.M.). We thank Professor Pühler for a kind gift of plasmid pSVB30 and of plasmid pHP45ΩKm^R as well as for allowing the DNA sequencing in his laboratory. The synthesis of the oligonucleotide by Ms M. Bellanco-Garcia is gratefully acknowledged. Moreover, we thank Professor Pühler, Dr R. Bockholt, Dr B. Nußbaumer and Dr A. Perlick for many stimulating and helpful discussions.

REFERENCES

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Angerer, A., Gaisser, S. & Braun, V. (1990). Nucleotide sequences of the *sfuA*, *sfuB*, and *sfuC* genes of *Serratia marcescens* suggest a periplasmic-binding-protein-dependent iron transport mechanism. *J Bacteriol* **172**, 572–578.
- Arnold, W. & Pühler, A. (1988). A family of high-copy-number plasmid vectors with single end-label sites for rapid nucleotide sequencing. *Gene* **70**, 171–179.
- Bartsevich, V. V. & Pakrasi, H. B. (1995). Molecular identification of an ABC transporter complex for manganese: analysis of a cyanobacterial mutant strain impaired in the photosynthetic oxygen evolution process. *EMBO J* **14**, 1845–1853.
- Beaucage, S. L. & Caruthers, M. H. (1981). Deoxynucleoside phosphoramidites – a new class of key intermediates for deoxy-polynucleotide synthesis. *Tetrahedron Lett* **22**, 1859–1862.
- Berish, S. A., Mietzner, T. A., Mayer, L. W., Genco, C. A., Holloway, B. P. & Morse, S. A. (1990a). Molecular cloning and characterization of the structural gene for the major iron-regulated protein expressed by *Neisseria gonorrhoeae*. *J Exp Med* **171**, 1535–1546.
- Berish, S. A., Kapczynski, D. R. & Morse, S. A. (1990b). Nucleotide sequence of the Fbp gene from *Neisseria meningitidis*. *Nucleic Acids Res* **18**, 4596.
- Block, M. A. & Grossman, A. R. (1988). Identification and purification of a derepressible alkaline phosphatase from *Anacystis nidulans* R2. *Plant Physiol* **86**, 1179–1184.
- Bockholt, R., Masepohl, B. & Pistorius, E. K. (1991). Insertional inactivation of the *psbO* gene encoding the manganese stabilizing protein of photosystem II in the cyanobacterium *Synechococcus* PCC 7942: effect on photosynthetic water oxidation and L-amino acid oxidase activity. *FEBS Lett* **294**, 59–63.
- Bökenkamp, D., Jungblut, P. W. & Thole, H. H. (1994). The C-terminal half of the porcine estradiol receptor contains no post-translational modification: determination of the primary structure. *Mol Cell Endocrinol* **104**, 163–172.
- Braun, V. & Hantke, K. (1991). Genetics of bacterial iron transport. In *CRC Handbook of Microbial Iron Chelates*, pp. 107–138. Edited by G. Winkelmann. Boca Raton: CRC Press.
- Braun, V., Hantke, K., Erik-Helmerich, K., Koster, W., Pressler, U., Sauer, M., Schaffer, S., Schoffler, H., Staudenmaier, H. & Zimmermann, L. (1987). Iron transport systems in *Escherichia coli*. In *Iron Transport in Microbes, Plants and Animals*, pp. 35–51. Edited by G. Winkelmann, D. van der Helm & J. B. Neilands. Weinheim: VCH Verlagsgesellschaft.
- Burnap, R. L., Troyan, T. & Sherman, L. A. (1993). The highly abundant chlorophyll-protein complex of iron-deficient *Synechococcus* sp. PCC 7942 (CP43') is encoded by the *isiA* gene. *Plant Physiol* **103**, 893–902.
- Carr, N. G. & Mann, N. H. (1994). The oceanic cyanobacterial picoplankton. In *The Molecular Biology of Cyanobacteria*, pp. 27–48. Edited by D. A. Bryant. Dordrecht, Boston & London: Kluwer Academic Publishers.
- Debus, R. J. (1992). The manganese and calcium ions of photosynthetic oxygen evolution. *Biochim Biophys Acta* **1102**, 269–352.
- Eisenberg, D., Schwan, E., Komaromy, M. & Wall, R. (1984). Analysis of membrane and surface protein sequences with the hydrophobic moment plot. *J Mol Biol* **179**, 125–142.
- Engels, D. H., Engels, A. & Pistorius, E. K. (1992). Isolation and partial characterization of an L-amino acid oxidase and of photosystem II complexes from the cyanobacterium *Synechococcus* PCC 7942. *Z Naturforsch* **47c**, 859–866.
- Fellay, R., Frey, J. & Krisch, H. (1987). Interposon mutagenesis of soil and water bacteria: a family of DNA fragments designed for *in vitro* insertional mutagenesis of Gram-negative bacteria. *Gene* **52**, 147–154.
- Ferreira, F. & Straus, N. A. (1994). Iron deprivation in cyanobacteria. *J Appl Phycol* **6**, 199–210.
- Fraústo da Silva, J. J. R. & Williams, R. J. P. (1993). Manganese: dioxygen evolution and glycosylation. In *The Biological Chemistry of the Elements. The Inorganic Chemistry of Life*, pp. 370–387. Oxford: Clarendon Press.
- Golden, S. S., Nalty, M. S. & Cho, D.-S. C. (1989). Genetic relationship of two highly studied *Synechococcus* strains designated *Anacystis nidulans*. *J Bacteriol* **171**, 24–29.
- Grant, S. G. N., Jessee, J., Bloom, F. G. & Hanahan, D. (1990). Differential plasmid rescue from transgenic mouse DNAs in *Escherichia coli* methylation restriction mutants. *Proc Natl Acad Sci USA* **87**, 4645–4649.
- Guikema, J. A. & Sherman, L. A. (1984). Influence of iron deprivation on the membrane composition of *Anacystis nidulans*. *Plant Physiol* **74**, 90–95.
- Klein, P., Kanihisa, M. & DeLisi, C. (1985). The detection and classification of membrane-spanning proteins. *Biochem Biophys Acta* **815**, 468–476.
- Kratz, W. A. & Myers, J. (1955). Nutrition and growth of several blue-green algae. *Am J Bot* **42**, 282–287.
- Kyte, J. & Doolittle, R. F. (1982). A simple method for displaying the hydrophobic character of a protein. *J Mol Biol* **157**, 105–132.
- Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
- Laudenbach, D. E. & Straus, N. A. (1988). Characterization of a cyanobacterial iron stress-induced gene similar to *psbC*. *J Bacteriol* **170**, 5018–5026.
- Laulhère, J.-P., Labouré, A.-M., van Wuytswinkel, O., Gagnon, J. & Briat, J.-F. (1992). Purification, characterization and function of bacterioferritin from the cyanobacterium *Synechocystis* PCC 6803. *Biochem J* **281**, 785–793.
- Leonhardt, K. & Straus, N. A. (1992). An iron stress operon involved in photosynthetic electron transport in the marine cyanobacterium *Synechococcus* sp. PCC 7002. *J Gen Microbiol* **138**, 1613–1621.
- Leonhardt, K. & Straus, N. A. (1994). Photosystem II genes *isiA*, *psbDI* and *psbC* in *Anabaena* sp. PCC 7120: cloning, sequencing and the transcriptional regulation in iron-stressed and iron-replete cells. *Plant Mol Biol* **24**, 63–73.
- Lippard, S. J. & Berg, J. M. (1994). Control and utilization of metal-ion concentration in cells. In *Principles of Bioinorganic Chemistry*, pp. 139–173. Mill Valley, CA: University Science Books.
- Matteucci, M. D. & Caruthers, M. H. (1981). Synthesis of deoxy-

oligonucleotides on a polymer support. *J Am Chem Soc* **103**, 3185–3191.

Michel, K.-P. & Pistorius, E. K. (1992). Isolation of a photosystem II associated 36 kDa polypeptide and an iron-stress 34 kDa polypeptide from thylakoid membranes of the cyanobacterium *Synechococcus* PCC 6301 grown under mild iron deficiency. *Z Naturforsch* **47c**, 867–874.

Mietzner, T. A., Bolan, G., Schoolnik, G. K. & Morse, S. A. (1987). Purification and characterization of the major iron-regulated protein expressed by pathogenic *Neisseriae*. *J Exp Med* **165**, 1041–1057.

Miller, J. H. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Montreuil, J., Tonnelat, J. & Mullet, S. (1960). Préparation et propriétés de la lactosidérophiline (lactotransferrine) du lait de femme. *Biochim Biophys Acta* **45**, 413–421.

Omata, T. & Murata, N. (1984). Isolation and characterization of three types of membranes from cyanobacterium (blue-green alga) *Synechocystis* PCC 6717. *Arch Microbiol* **139**, 113–116.

Pakrasi, H. B., Goldenberg, A. & Sherman, L. A. (1985a). Membrane development in the cyanobacterium, *Anacystis nidulans*, during recovery from iron starvation. *Plant Physiol* **79**, 290–295.

Pakrasi, H. B., Riethman, H. C. & Sherman, L. A. (1985b). Organization of pigment proteins in the photosystem II complex of the cyanobacterium *Anacystis nidulans* R2. *Proc Natl Acad Sci USA* **82**, 6903–6907.

Pistorius, E. K., Kertsch, R. & Faby, S. (1989). Investigations about various possible functions of the L-amino acid oxidase in the cyanobacterium *Anacystis nidulans*. *Z Naturforsch* **44c**, 370–377.

Reddy, K. J., Bullerjahn, G. S., Sherman, D. M. & Sherman, L. A. (1988). Cloning, nucleotide sequence, and mutagenesis of a gene (*irpA*) involved in iron deficient growth of the cyanobacterium *Synechococcus* sp. strain PCC 7942. *J Bacteriol* **170**, 4466–4476.

Rhao, M.-J.-K. & Argos, P. (1986). A conformational preference parameter to predict helices in integral membrane proteins. *Biochim Biophys Acta* **869**, 197–214.

Riethman, H. C. & Sherman, L. A. (1988a). Immunological charac-

terization of iron-regulated membrane proteins in the cyanobacterium *Anacystis nidulans* R2. *Plant Physiol* **88**, 497–505.

Riethman, H. C. & Sherman, L. A. (1988b). Purification and characterization of an iron stress-induced chlorophyll-protein from the cyanobacterium *Anacystis nidulans* R2. *Biochim Biophys Acta* **935**, 141–151.

Rippka, R. (1988). Isolation and purification of cyanobacteria. *Methods Enzymol* **167**, 3–27.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Schägger, H. & von Jagow, G. (1987). Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* **166**, 368–379.

Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goecke, N. M., Olson, B. J. & Klenk, D. C. (1985). Measurement of protein using bicinchoninic acid. *Anal Biochem* **150**, 76–85.

Staden, R. (1986). The current status and portability of our sequence handling software. *Nucleic Acids Res* **14**, 217–232.

Straus, N. A. (1994). Iron deprivation: physiology and gene regulation. In *The Molecular Biology of Cyanobacteria*, pp. 731–750. Edited by D. A. Bryant. Dordrecht, Boston & London: Kluwer Academic Publishers.

Wälzlein, G. & Pistorius, E. K. (1991). Inactivation of photosynthetic O₂ evolution in the cyanobacterium *Anacystis nidulans* PCC 6301: influence of nitrogen metabolites and divalent cation concentration. *Z Naturforsch* **46c**, 1024–1032.

Webb, R., Troyan, T., Sherman, D. & Sherman, L. A. (1994). MapA, an iron-regulated, cytoplasmic membrane protein in the cyanobacterium *Synechococcus* sp. strain PCC 7942. *J Bacteriol* **176**, 4906–4913.

William, J. G. K. (1988). Construction of specific mutations in photosystem II photosynthetic reaction centre by genetic engineering methods in *Synechocystis* 6803. *Methods Enzymol* **167**, 766–778.

Received 7 February 1996; revised 22 April 1996; accepted 13 May 1996.