Transcript Profiling Reveals New Insights into the Acclimation of the Mesophilic Fresh-Water Cyanobacterium *Synechococcus elongatus* PCC 7942 to Iron Starvation[1][W]

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The regulatory network for acclimation of the obligate photoautotrophic fresh water cyanobacterium *Synechococcus elongatus* PCC 7942 to iron (Fe) limitation was studied by transcript profiling with an oligonucleotide whole genome DNA microarray. Six regions on the chromosome with several Fe-regulated genes each were identified. The *irpAB* and *fut* region encode putative Fe uptake systems, the *suf* region participates in [Fe-sulfur] cluster assembly under oxidative stress and Fe limitation, the *isiAB* region encodes CP43′ and flavodoxin, the *idiCB* region encodes the NuoE-like electron transport associated protein IdiC and the transcriptional activator IdiB, and the *ackA/pgam* region encodes an acetate kinase and a phosphoglycerate mutase. We also investigated the response of two *S. elongatus* PCC 7942 mutants to Fe starvation. These were mutant *K10*, lacking IdiB but containing IdiC, and mutant *MuD*, representing a *idiC*-merodiploid mutant with a strongly reduced amount of IdiC as well as IdiB. The absence of IdiB in mutant *K10* or the strongly reduced amount of IdiB in mutant *MuD* allowed for the identification of additional members of the Fe-responsive IdiB regulon. Besides *idiA* and the *irpAB* operon *somB(1)*, *somA(2)*, *ftr1*, *ackA*, *pgam*, and *nat* also seem to be regulated by IdiB. In addition to the reduced amount of IdiB in *MuD*, the low concentration of IdiC may be responsible for a number of additional changes in the abundance of mainly photosynthesis-related transcripts as compared to the wild type and mutant *K10*. This fact may explain why it has been impossible to obtain a fully segregated IdiC-free mutant, whereas it was possible to obtain a fully segregated IdiB-free mutant.

Iron (Fe) starvation frequently occurs in aquatic habitats and severely limits biomass production of photosynthetic organisms (Geider and La Roche, 1994; Martin et al., 1994; Behrenfeld and Kolber, 1999; Tortell et al., 1999). During recent years substantial knowledge has accumulated on how cyanobacteria adapt to Fe starvation (Straus, 1994; Michel and Pistorius, 2004). Among other metabolic processes, photosynthetic/respiratory electron transport chain with its high number of Fe cofactors is especially susceptible to the deleterious effects of Fe limitation. To counteract concomitant metabolic limitations, cyanobacteria have developed highly sophisticated modifications of their electron transport chains, which allow them to maintain their photosynthetic lifestyle (Straus, 1994; Sandström et al., 2002; Michel and Pistorius, 2004). The acclimation to Fe limitation results in a reduction of the photosynthetic linear electron transport activity from water to $NADP^+$ and an increase of the photosynthetic cyclic and the respiratory electron transport activity (Michel et al., 2003). In this respect, the functions of IsiA and IsiB as well as IdiA, IdiB, and IdiC under Fe limitation have been investigated in greater detail during the recent years.

IsiA (Riethman and Sherman, 1988; Burnap et al., 1993) has been assigned several functions (Nield et al., 2003; Barber et al., 2006). It can form an additional membrane-integral light-harvesting antenna around trimeric PSI complexes (Bibby et al., 2001; Boekema et al., 2001; Melkozernov et al., 2003; Nield et al., 2003), and it can also interact with PSI monomers, forming single or double rings with multiple copies of IsiA (Kouril et al., 2005). In addition, IsiA has been suggested to act as a chlorophyll (Chl) sink (Burnap et al., 1993) to prevent high quantities of unbound potentially hazardous ^{3}Chl. IsiA also protects PSII and PSI against the damage caused by excessive light through a nonradiative type of energy dissipation (Ivanov et al., 2000, 2006; Sandström et al., 2001, 2002; Ihalainen et al., 2005). Besides its expression under low Fe concentrations and during conditions of oxidative stress

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(Jeanjean et al., 2003; Yousef et al., 2003; Li et al., 2004; Michel and Pistorius, 2004; Singh et al., 2004), IsiA is also expressed in high amounts during sodium chloride stress (Vinnemeier et al., 1998; Havaux et al., 2005), heat stress (Fulda et al., 2006), and during the stationary growth phase (Singh and Sherman, 2006). The expression of IsiA in *Synechococcus elongatus* PCC 7942 is thought to be regulated by the transcriptional repressor Fur (Ghassemian and Straus, 1996). An additional transcription factor of yet unknown nature is involved in the regulation of IsiA expression in *Synechocystis* sp. PCC 6803 (Kunert et al., 2003), and the steady-state *isiA* mRNA pool has been shown to be regulated by the small internal cis-type RNA *isrR* in *Synechocystis* sp. PCC 6803 (Duhring et al., 2006). IsiB is a flavodoxin that partially replaces ferredoxin under Fe limitation in several cyanobacteria (Straus, 1994), and it has been shown that accumulation of flavodoxin contributes to enhanced cyclic electron flow activities around PSI (Hagemann et al., 1999).

Another major stress responsive protein of *S. elongatus* PCC 7942 is IdiA (Michel and Pistorius, 2004), which becomes strongly expressed under Fe starvation (Michel et al., 1996). For *S. elongatus* PCC 7942 IdiA has been shown to be a predominantly thylakoid membrane-associated protein (Michel et al., 1998), which protects and shields the acceptor side of PSII that becomes progressively exposed toward the cytoplasm in the cause of ongoing Fe starvation-induced partial degradation of phycobilisomes (Exss-Sonne et al., 2000). The protective function of IdiA for PSII has recently gained further support, as IdiA has been detected in highly purified PSII complexes from Fe-starved *Thermosynechococcus elongatus* BP-1 cells (Lax et al., 2007). This conclusion is in agreement with results of previous comparative investigations of *S. elongatus* PCC 7942 wild type and an IdiA-free mutant (Exss-Sonne et al., 2000), and the fact that Fe-starved IdiA-expressing *S. elongatus* PCC 7942 cells show a higher resistance toward deleterious effects of the herbicide bentazone (Bagchi et al., 2003). The transcription of *idiA* is controlled by the transcriptional activator IdiB (Michel et al., 2001). *IdiB* is located adjacent to *idiA* and is part of an operon consisting of *orf6*, *idiC*, and *idiB* that is transcribed in the opposite direction to *idiA*. Transcription of the *idiB* operon itself is facilitated by a yet unknown Fe-dependent transcriptional control mechanism (Yousef et al., 2003; Pietsch et al., 2007).

In addition to IdiA and IdiB, we have recently identified the novel Fe-regulated protein IdiC, which may also contribute to the Fe starvation-induced modification of the electron transport chain. The gene *idiC* is part of the Fe-responsive *idiCB* operon of *S. elongatus* PCC 7942 and encodes a 20.5-kD protein (Pietsch et al., 2007). IdiC belongs to the superfamily of thioredoxin-like (TRX-like) [2Fe-2sulfur (2S)] ferredoxins and has similarity to the peripheral subunit NuoE of the *Escherichia coli* NAD(P)H dehydrogenase (NDH)-1 complex. We have shown that IdiC expression increases under Fe starvation as well as during the late growth phase (Pietsch et al., 2007). Because attempts to insertionally inactivate *idiC* merely generated merodiploid mutants with a strongly reduced IdiC content but no IdiC-free mutant strain, IdiC is considered to be an essential protein for the viability of *S. elongatus* PCC 7942. The results of a comparative analysis of *S. elongatus* PCC 7942 wild type and the *idiC*-merodiploid mutant *MuD* suggest a function of IdiC in photosynthetic cyclic electron transport around PSI and/or in respiratory electron transport (Pietsch et al., 2007).

To obtain a profound view on the complex regulatory network involved in acclimation to Fe limitation in *S. elongatus* PCC 7942 wild type, we performed DNA microarray analyses with cells grown in the presence or absence of Fe in BG11 medium. Such microarray analyses of the genome-wide transcriptional response to Fe starvation had previously been performed for *Synechocystis* sp. PCC 6803 (Singh et al., 2003). In contrast to *Synechocystis* sp. PCC 6803 with a genome size of 3.6 Mbps and either photoautotrophically or photoheterotrophically growth capability, *S. elongatus* PCC 7942 has a smaller genome size of 2.7 Mbps and represents an obligate photoautotrophic strain. Because *S. elongatus* PCC 7942 is only capable of this growth mode, it might have developed more effective mechanisms to maintain its oxygenic photosynthetic lifestyle under Fe starvation than the metabolically more versatile *Synechocystis* sp. PCC 6803 strain. To identify Fe starvation-induced gene transcription, we used a novel whole-genome microarray for *S. elongatus* PCC 7942 wild type, which consisted of a total of 2,898 spotted 70-mer oligonucleotides. Moreover, we investigated an IdiB-free *S. elongatus* PCC 7942 mutant *K10* to unravel novel members of an IdiB regulon and an *idiC*-merodiploid *S. elongatus* PCC 7942 mutant *MuD* with a very low content of IdiC as well as of IdiB. The latter strain was included in the investigation because both genes, *idiB* and *idiC*, are located next to each other in an operon (Pietsch et al., 2007) and because it was possible to successfully insertionally inactivate the gene encoding the transcription factor IdiB, whereas the *idiC*-insertionally inactivated mutant never showed full segregation. Because of the suggested essential function of IdiC for the viability of *S. elongatus* PCC 7942, we included mutant *MuD* in the investigations to uncover the effects of a highly reduced amount of IdiC in addition to a low content of IdiB on the overall transcriptome during acclimation to Fe-deplete conditions.

RESULTS AND DISCUSSION

S. elongatus PCC 7942 wild type, the IdiB-free mutant *K10*, and *idiC*-merodiploid mutant *MuD* were cultivated in Fe-sufficient or Fe-deficient BG11 medium. Mutant *K10* lacks IdiB completely but contains regular or slightly elevated amounts of IdiC as compared to wild type (Pietsch et al., 2007). Mutant *MuD* is an *idiC*-merodiploid mutant with a very low amount of IdiC as

well as IdiB because the genes encoding these two proteins constitute an operon and *idiC* lies upstream of *idiB* (Pietsch et al., 2007). The corresponding growth curves and the phenotypical appearance are illustrated in Figure 1. In microarray experiments we compared the transcriptome of *S. elongatus* PCC 7942 wild type when grown under Fe-deficient conditions for 24 and 72 h to that of wild type when grown under Fe-sufficient conditions for 24 and 72 h, respectively. Moreover, we compared the transcriptomes of mutant *K10* and mutant *MuD* when grown for 72 h under Fe-deficient conditions to that of mutant *K10* and mutant *MuD* when grown under Fe-sufficient conditions, respectively. The diagrams in Figure 2 give an overview on the number of differentially regulated genes, and the major changes in transcript abundance of selected genes are given in Table I. Table I in combination with Supplemental Table S1 list the entire number of significantly transcriptionally regulated genes.

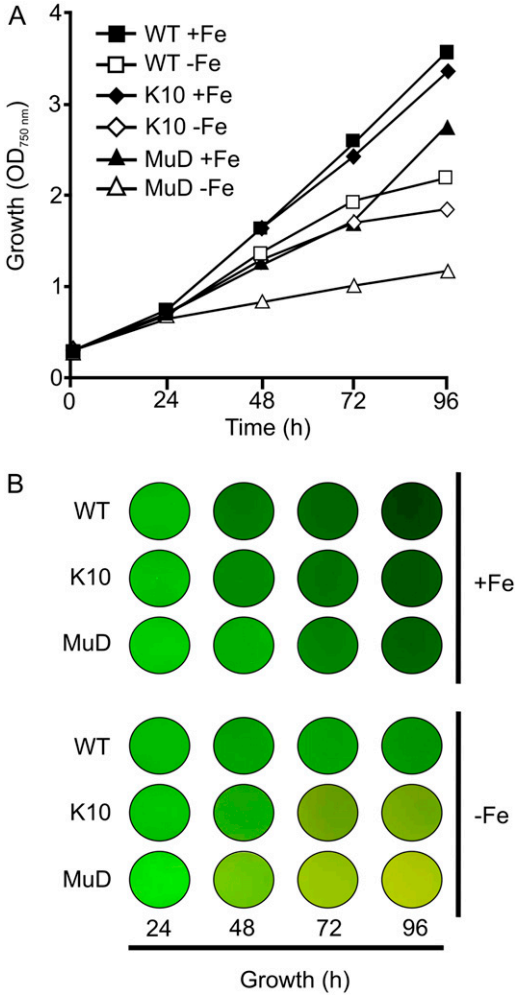

Figure 1. Growth and appearance of *S. elongatus* PCC 7942 wild type, the IdiB-free mutant *K10*, and the *idiC*-merodiploid mutant *MuD* grown in the presence (+Fe) or absence (−Fe) of Fe for 24, 48, 72, and 96 h.

Changes of the Transcriptome of *S. elongatus* PCC 7942 Wild Type, the IdiB-Free Mutant *K10*, and the *idiC*-Merodiploid Mutant *MuD* in Response to Fe Availability

In *S. elongatus* PCC 7942 wild type grown for 24 h under Fe-limited conditions, the steady-state transcript level of 50 genes was increased due to Fe limitation, while the steady-state transcript level of 10 genes was down-regulated. After 72 h of Fe depletion, 64 transcripts were found at increased levels, while the steady-state transcript level of 24 genes diminished significantly at the same time. In mutants *K10* and *MuD*, we identified 42 and 60 increased transcript levels and 21 and 60 decreased transcript levels after 72 h of Fe-deficient growth, respectively (Fig. 2; Table I; Supplemental Table S1).

Detection of Transcripts of Major Fe-Regulated Clustered Genes

In total, we identified six regions on the chromosome of *S. elongatus* PCC 7942 with clusters of genes, whose transcripts significantly accumulated due to Fe-deficient growth conditions (Table I). These gene clusters contain the genes *irpA* and *irpB*, the *fut* genes, the *suf genes*, the *isiABC* genes, the genes *idiB* and *idiC*, and the *ackA/pgam* genes. The structure of these gene regions is shown in Figure 3.

1. The highest increase in transcript abundance during Fe limitation was observed for *irpA* and the transcript of gene *1461*, which we named *irpB* (Table I). The gene *irpB* is located immediately down-stream of *irpA* and is transcribed in the same direction. Both genes overlap by 5 bp. Thus, we assume that *irpA* and *irpB* constitute a dicistronic operon (Fig. 3). Previously, it has already been suggested that IrpA is located in an operon and that the genes of this operon encode proteins of an Fe acquisition system (Reddy et al., 1988). IrpA is a protein of 38.6 kD and is located in the cytoplasmic membrane (Reddy et al., 1988). The gene *irpB* encodes a protein of 49.3 kD and belongs to the multiheme Cyt *c*-type cytochrome family with two CXXC heme-binding sites (InterProScan). A role of IrpAB in Fe acquisition is supported by the fact that immediately downstream of *irpA* the genes *1463* and *1464* are located that are transcribed in opposite direction to *irpAB.* These genes encode proteins with similarity to SomB(1) and SomA(1). SomA(1) and SomB(1) are outer membrane proteins that form porin-like β-barrel structures (Hansel et al., 1998), which may change the permeability and selectivity of the outer membrane as a diffusion barrier. The transcript of *somB(1)*, but not of *somA(1)*, was also found at increased concentration in the transcriptome of Fe-depleted wild-type cells. In addition, the transcript of gene *1607*, which is located in a different region on the chromosome, and which encodes a SomA(2)-similar protein as well as the transcript of gene *2421* for a Ftr1-similar

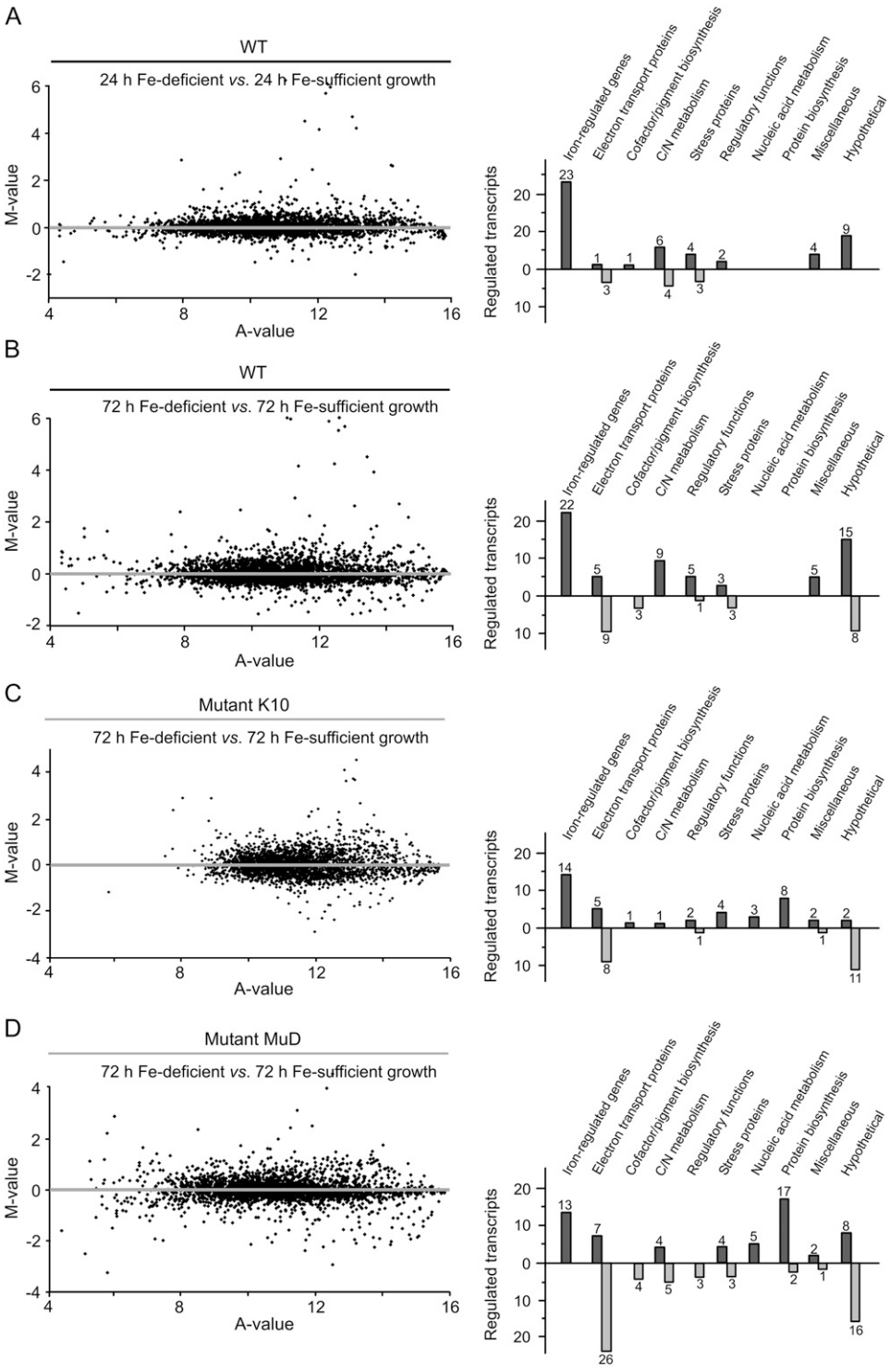

Figure 2. Scattered plot of differentially regulated transcripts (left) and distribution of the genes in different metabolic categories (right) from *S. elongatus* PCC 7942 wild type, the IdiB-free mutant *K10*, and the *idiC*-merodiploid mutant *MuD* grown for 24 or 72 h with BG11 medium in the presence or absence of Fe. Increased transcript levels are given in dark gray, whereas decreased transcript levels are given in light gray. A, *S. elongatus* PCC 7942 wild type 24-h Fe-deficient growth (−Fe) versus *S. elongatus* PCC 7942 wild type 24-h Fe-sufficient growth (+Fe). B, *S. elongatus* PCC 7942 wild type 72-h Fe-deficient growth (−Fe) versus *S. elongatus* PCC 7942 wild type 72-h Fe-sufficient growth (+Fe). C, IdiB-free mutant *K10* 72-h Fe-deficient growth (−Fe) versus IdiB-free mutant *K10* 72-h Fe-sufficient growth (+Fe). D, *idiC*-merodiploid mutant *MuD* 72-h Fe-deficient growth (−Fe) versus *idiC*-merodiploid mutant *MuD* 72-h Fe-sufficient growth (+Fe).

protein, were found to be substantially increased during Fe limitation. Ftr1 functions as a permease of a high-affinity Fe uptake system first identified in yeast (Stearman et al., 1996; Larrondo et al., 2007). Ftr1 lies adjacent to the *ackA/pgam operon* and is transcribed in opposite direction to this operon (Fig. 3, sixth operon). We suggest that the proteins IrpA, IrpB, SomB(1), SomA(2), and Ftr1 represent a novel Fe acquisition system in *S. elongatus* PCC 7942, whose expression is regulated by the transcriptional activator IdiB (see later). IrpA- or IrpB-similar proteins are not present in all, so far sequenced cyanobacterial genomes (National Center for Biotechnology Information database; November, 2007), whereas

Table I. *List of major Fe-regulated genes in S. elongatus PCC 7942 wild type, the IdiB-free mutant K10, and the idiC-merodiploid mutant MuD in response to growth for 24 h (wild type only) or 72 h (wild type, K10, and MuD) with Fe-deficient versus Fe-sufficient BG11 medium*

The table contains the evaluated data of three biological and two technical replicates and includes a dye-swap experiment. The fold-change value is calculated as $\log_2^{M\ value}$ of *M* values with corresponding *P* value ≤0.051. *M* values >−0.90 and <+0.90 indicate no significant change in the transcriptional levels (corresponding to a fold change of ≤1.87 and ≥0.53). Significantly increased or decreased transcript levels are printed in bold letters. JGI open reading frames correspond to the JGI annotation. Common gene names are given in the column to the right. Not annotated means the gene is not annotated in JGI annotation. ND, Not determined.

JGI Open Reading Frame	Gene	Annotated Protein Function	Fold Change			
			Growth −Fe versus +Fe		Growth −Fe versus +Fe	
			Wild Type 24 h	Wild Type 72 h	*K10* 72 h	*MuD* 72 h
Transcripts of major Fe-regulated and clustered genes						
1462	*irpA*	Fe-regulated protein A function	**75.58**	**62.68**	0.82	1.21
1461	*irpB*	Multiheme *c*-type cytochrome family protein with two heme-binding sites	**51.63**	**46.21**	1.04	0.89
1463	*somB(1)*	Major outer membrane protein probably forming porin-like β-barrel structure and which might also connect to the S-layer	**22.78**	**17.87**	1.38	0.95
1607	*somA(2)*	Major outer membrane protein; see above	**6.11**	**4.53**	0.61	0.89
2421	*ftr1*	Ftr1-similar protein, part of a high-affinity Fe^{2+} uptake system	**2.85**	**3.27**	1.08	1.22
1406	*futC*	Fe (III) transport ATP-binding protein	**2.07**	1.65	1.44	1.42
1407	*futB*	Fe (III) ABC transporter permease	**5.46**	**3.63**	**2.55**	**5.35**
1408	*mapA*	Membrane-associated protein A, partly resembles type 12 methyltransferases and periplasmic solute-binding proteins	1.37	1.44	1.50	**2.19**
1409	*futA2*	Fe (III) transport substrate-binding protein	**2.50**	**2.08**	**2.55**	1.50
1733	*sufR*	Repressor of the *suf* regulon	**2.01**	**2.27**	**2.79**	**1.91**
1734	*ftrC*	Ferredoxin:thioredoxin reductase catalytic subunit β-chain	**1.86**	**2.00**	**2.19**	**2.07**
1736	*sufC*	[Fe-S]-assembly ATPase SufC	**2.23**	**2.55**	**5.43**	**3.95**
1737	*sufD*	[Fe-S]-assembly protein SufD	**2.00**	**2.30**	**4.41**	**3.05**
1738	*sufS*	Cys desulfatase, NifS-similar, involved in formation of [Fe-S] centers	**2.30**	**2.57**	**4.99**	**3.02**
1739	*merR*	MerR-like protein that contains HTH DNA-binding motif	**1.92**	**2.04**	**4.93**	**2.33**
Not annotated	*0017*	Hypothetical 4.2-kD protein with signal peptide	**7.52**	**7.62**	**3.39**	**1.95**
1542	*isiA*	Fe stress-induced protein A or CP43′, formation of membrane-integral light harvesting antenna around trimeric photosystem I	**25.99**	**22.79**	**23.59**	**11.00**
1541	*isiB*	Flavodoxin, soluble electron transport protein, in part replaces ferredoxin under Fe starvation	**61.82**	**51.63**	**13.45**	**5.66**
1540	*isiC*	Putative hydrolase with typical $\alpha\beta$-fold of hydrolases	**68.60**	**63.35**	**17.39**	**8.64**
2175	*idiA*	Fe deficiency-induced protein A, modifies and protects photosystem II against selected stresses	**6.24**	**6.50**	0.68	1.00
2174	*idiB*	Fe deficiency-induced protein B, positively acting transcription factor of IdiA and the IdiB regulon	**17.88**	**18.89**	**6.41**[a]	1.18
2173	*idiC*	Fe deficiency-induced protein C, suggested to participate in photosynthetic cyclic electron transport	**18.50**	**15.24**	**12.91**	**16.34**[a]
2172	*orf6*	Gene immediately upstream of *idiC*, encodes a protein of unknown function	ND	1.26	**12.55**	2.27
2079	*ackA*	Acetate kinase, production of acetate from acetyl-phosphate with synthesis of ATP	**5.03**	**3.14**	1.06	0.97
2078	*pgam*	Pgam, transfers phosphate groups within glycerate and converts 3-PGA to 2-PGA	**3.05**	**2.43**	1.38	1.06
Transcripts of genes encoding electron transport-related proteins (photosynthesis and respiration)						
0679	*psbB*	CP47 light-harvesting antenna protein of photosystem II	0.91	0.87	0.71	**0.54**
0656	*psbC*	CP43 light-harvesting antenna protein of photosystem II	0.88	1.15	0.84	**0.46**
0294	*psbO*	Manganese- and calcium-stabilizing protein of photosystem II	0.63	**0.52**	**0.42**	**0.42**
0696	*psbT*	Small PSII protein; involved in stabilization of photosystem II dimers and recovery from photodamage	0.94	1.02	0.84	**0.46**
2049	*psaA*	PSI reaction center core protein A	0.80	0.92	0.62	**0.43**
2048	*psaB*	PSI reaction center core protein B	0.75	0.79	0.61	**0.38**

(*Table continues on following page.*)

Table I. (*Continued from previous page.*)

JGI Open Reading Frame	Gene	Annotated Protein Function	Fold Change			
			Growth −Fe versus +Fe		Growth −Fe versus +Fe	
			Wild Type 24 h	Wild Type 72 h	*K10* 72 h	*MuD* 72 h
0535	*psaC*	PSI reaction center subunit C, F_A, and F_B [4Fe-4S]-containing protein on the stromal surface of photosystem I	0.93	0.91	0.75	**0.35**
0407	*psaK(1)*	PSI reaction center subunit X, hydrophobic subunit of unknown function	0.91	**0.49**	**0.30**	**0.34**
0920	*psaK(2)*	PSI reaction center subunit X, hydrophobic subunit of unknown function	1.04	**0.53**	**0.31**	**0.34**
2342	*psaL*	PSI reaction center subunit L, trimerization of photosystem I monomers	0.97	0.56	**0.34**	**0.30**
1249	*psaJ*	PSI reaction center subunit IX, hydrophobic subunit close to PsaF	0.90	0.86	0.57	**0.29**
2343	*psaI*	PSI reaction center subunit VIII, crucial role in aiding normal structural organization of PsaL	0.99	**0.50**	**0.35**	**0.27**
1250	*psaF*	PSI reaction center subunit III, small subunit of unknown function	1.00	0.65	**0.37**	**0.26**
1231	*petA*	Apocytochrome *f*	**0.47**	**0.50**	0.95	0.86
2331	*petB*	Cytochrome b_6	0.86	1.24	0.79	0.68
1232	*petC*	Rieske protein	**0.48**	**0.49**	0.98	0.82
1630	*petJ*	Apocytochrome c_6 precursor (Cyt c_{553})	0.57	**0.48**	0.56	0.61
2332	*petD*	Cytochrome b_6/f complex subunit IV	0.63	0.76	0.71	**0.50**
1439	*ndhD*	NADH dehydrogenase subunit IV (NdhD2)	**2.11**	**6.15**	**3.48**	**2.79**
0609	*ndhD*	NADH dehydrogenase subunit IV (NdhD4)	1.01	0.93	1.07	1.40
1767	*cydA*	Cytochrome *bd* oxidase subunit I	1.27	**2.08**	**2.04**	**1.89**
2601	*ctaA*	Cytochrome oxidase assembly protein, required for assembly of Cyt aa_3 oxidase	1.55	1.36	1.61	**2.04**
2602	*ctaC*	Cytochrome aa_3 oxidase subunit II	1.52	1.55	**2.64**	**2.17**
2604	*ctaE*	Cytochrome aa_3 oxidase subunit III	1.82	**2.20**	**4.29**	**2.20**
0201	*ccoO*	Cytochrome oxidase cytochrome *c* subunit	1.26	1.73	**1.97**	1.44
0202	*ccoN*	Cytochrome oxidase, *cb*-type cytochrome oxidase subunit I	1.24	**1.92**	1.82	1.39
0814	*2124*	Putative 7× Fe ferredoxin with [3Fe-4S] and [4Fe-4S] cofactors	**0.51**	**0.48**	0.87	0.94
1649	*0140*	Ruberythrin and rubredoxin-type [4Fe-4S]-like protein, putatively involved in electron transfer reactions, sometimes replacing ferredoxins in electron transport	0.83	0.93	1.80	**2.04**
0327	*apcA*	Allophycocyanin α-subunit	0.77	0.87	0.63	**0.26**
2158	*apcB(1)*	Allophycocyanin β-subunit (1)	1.05	0.82	0.48	**0.48**
0326	*apcB(2)*	Allophycocyanin β-subunit (2)	0.75	0.99	0.84	**0.37**
0328	*apcE*	Phycobilisome anchor protein	0.66	**0.50**	**0.43**	**0.21**
0325	*apcI*	Allophycocyanin linker protein	0.89	**0.42**	**0.29**	**0.23**
1053	*cpcA*	Phycocyanin α-subunit (1)	0.77	0.99	0.95	**0.46**
1048	*cpcA*	Phycocyanin α-subunit (2)	1.31	**3.14**	0.92	**4.63**
1052	*cpcB(1)*	Phycocyanin β-subunit (1)	0.76	1.03	0.82	**0.28**
1047	*cpcB(2)*	Phycocyanin β-subunit (2)	0.76	1.05	0.80	**0.29**
1050	*cpcI(1)*	33-kD Phycocyanin linker protein	0.83	0.81	0.72	**0.23**
1051	*cpcI(2)*	33-kD Phycocyanin linker protein	0.81	0.63	0.61	**0.36**
1049	*cpcH*	Rod-rod linker protein	0.75	0.78	0.64	**0.25**
2030	*cpcG*	Phycobilisome rod-core linker polypeptide	0.93	0.93	0.72	**0.48**
Transcripts of genes encoding proteins of C and N metabolism						
2079	*ackA*	Acetate kinase, production of acetate from acetyl-phosphate with synthesis of ATP	**5.03**	**3.14**	1.06	0.97
0650	*nat*	*N*-Acetyltransferase	**4.74**	**4.72**	1.36	0.50
2078	*pgam*	Pgam, transfers phosphate groups within glycerate molecules, converts 3-PGA to 2-PGA	**3.05**	**2.43**	1.38	1.06
1608	*0094*	Man-1-P guanylyltransferase/Man-6-P isomerase	**2.04**	**2.17**	1.30	0.85
1609	*nrdJ*	Ribonucleoside triphosphate reductase, adenosyl-cobalamine-dependent enzyme	1.78	**2.36**	1.79	1.06
1072	*cobO*	Cobalamine adenosyl transferase, involved in adenosyl cobalamine biosynthesis	1.46	**2.06**	1.22	1.26
1585	*0069*	*N*-Acetylmuramoyl L-Ala amidase	1.39	**2.00**	1.22	1.35
2388	*oxdC*	Oxalate decarboxylase with cupin-like β-barrels	1.11	0.79	0.58	**0.47**

(*Table continues on following page.*)

Table I. (*Continued from previous page.*)

JGI Open Reading Frame	Gene	Annotated Protein Function	Fold Change			
			Growth −Fe versus +Fe		Growth −Fe versus +Fe	
			Wild Type 24 h	Wild Type 72 h	*K10* 72 h	*MuD* 72 h
1240	*nirA*	Ferredoxin-nitrite reductase	0.79	0.82	0.59	**0.45**
1239	*nrtA*	ABC-type nitrate transporter subunit A	0.57	0.88	0.60	**0.44**
1237	*nrtC*	ABC-type nitrate transporter subunit C	0.63	1.02	0.76	**0.54**
2529	*gifB*	Hypothetical 11.6-kD protein, similar to Sll1515 from *Synechocystis* sp. PCC 6803 to Gln synthetase inactivating factor IF17	**3.29**	**2.27**	1.42	**2.36**
2150	*0687*	Linear amide C-N hydrolase, choloyl-Gly hydrolase, member of the NTN hydrolase family	**2.17**	**1.95**	1.23	1.19
1513	*dxr*	1-Deoxy-D-xylulose 5-P reductoisomerase, involved in terpenoid orisoprenoid biosynthesis	0.75	0.74	0.85	**0.48**
1562	*draG*	ADP-ribosylglycohydrolase dinitrogenase reductase activating glycohydrolase	0.95	1.80	**2.99**	**3.29**
1713	*mocD*	Hydrocarbon oxygenase-similar protein	0.81	1.21	1.14	**2.00**
1438	*pmgA*	Photomixotrophic growth-related protein A homolog	1.60	**1.99**	1.32	1.39
2043	*speH*	*S*-Adenosyl Met decarboxylase, involved in spermidine biosynthesis	0.86	1.45	1.84	**2.77**
0510	*serB*	Haloacid dehalogenase-like hydrolase	0.84	1.02	1.59	**2.17**
2107	*cynA*	Periplasmic-binding protein	**0.39**	0.84	1.43	1.16
2106	*cynB*	Sulfonate transport system permease protein	**0.40**	0.89	1.41	1.55
2105	*cynD*	ATP-binding protein of sulfonate transport system	**0.49**	0.93	1.49	1.29
2104	*cynS*	Cyanase, detoxification of cyanate ($N \equiv C\text{-}O^-$)	**0.24**	0.78	1.20	1.09
Transcripts of genes encoding general stress proteins						
1813	*htpG*	Heat shock protein HSP90	**2.33**	**2.12**	1.56	0.90
2313	*groL*	GroL chaperonine HSP60	**2.03**	1.23	**2.04**	1.01
2314	*groS*	GroS chaperonine HSP10	**2.27**	1.27	**2.04**	1.13
2306	*dnaJ*-like	Protein similar to the C terminus of DnaJ (HSP40) lacking three conserved domains of DnaJ proteins	0.88	0.89	0.74	**0.45**
2401	*hspA*	Molecular chaperone of the HSP20 family	1.13	1.77	**2.00**	**2.02**
0801	*sodB*	Fe superoxide dismutase	**4.00**	**0.37**	0.53	**0.34**
1656	*katG*	Catalase peroxidase	**0.84**	**0.43**	0.51	**0.30**
2309	*aphC*	Alkyl hydroperoxide reductase C, 2-Cys peroxiredoxin-type protein	1.24	1.78	1.88	**2.03**
1290	*2659*	Metallothionine-similar protein	**0.21**	**0.34**	0.66	1.11
0243	*hliC*	High light-induced protein C, LHC-like protein Lhl4	1.25	**3.61**	**4.53**	**2.83**
2127	*nblA*	Nonbleaching protein A, phycobilisome-degradation protein	1.58	**2.66**	1.83	**2.07**
1635	*somB(2)*	Major outer membrane protein probably forming porin-like barrel structure and putative connection to S-layer	**0.50**	0.57	1.32	1.25

[a]These oligonucleotides are placed upstream of the site used for insertional inactivation of the *idiC* and the *idiB* gene, and thus detects wild-type allele messages as well as *idiC* and *idiB* mutant allele transcripts.

several Som-like proteins and a Ftr1-similar protein (e.g. Slr0964 in *Synechocystis* sp. PCC 6803) are present.

2. Transcripts for a second Fe acquisition system, the Fut system (Katoh et al., 2001a, 2001b), were also found to be transcribed in elevated levels in *S. elongatus* PCC 7942 (*futA2*, *futB*, and *futC*; Table I; Fig. 3). Among these transcripts, the highest increase was seen for the steady-state level of *futB* mRNA, encoding a putative Fe-(III)-transporter permease. Because the gene *mapA*, encoding a 34-kD protein (Webb et al., 1994), is located between *futB* and *futA2*, MapA may also have a role in Fe acquisition. Although *futB*, *mapA*, and *futA2* are arranged in line on the chromosome and transcribed in the same direction, the increase in their steady-state mRNA levels were found to be substantially different under Fe-limiting growth conditions suggesting that their transcript stability is rather different. The gene *futC* is located upstream of *futB*, but is transcribed in the opposite direction (Fig. 3). As for *futB*, the steady-state transcript pool for this mRNA was up-regulated under conditions of Fe starvation. The cyanobacterial Fut system is closely related to the well-characterized bacterial Sfu-, Hit-, and Fbp-Fe uptake systems (Angerer et al., 1990; Chen et al., 1993; Nowalk et al., 1994; Sanders et al., 1994). Because the increase in transcript level for the four *fut* genes was different, it remained unclear whether three of these genes

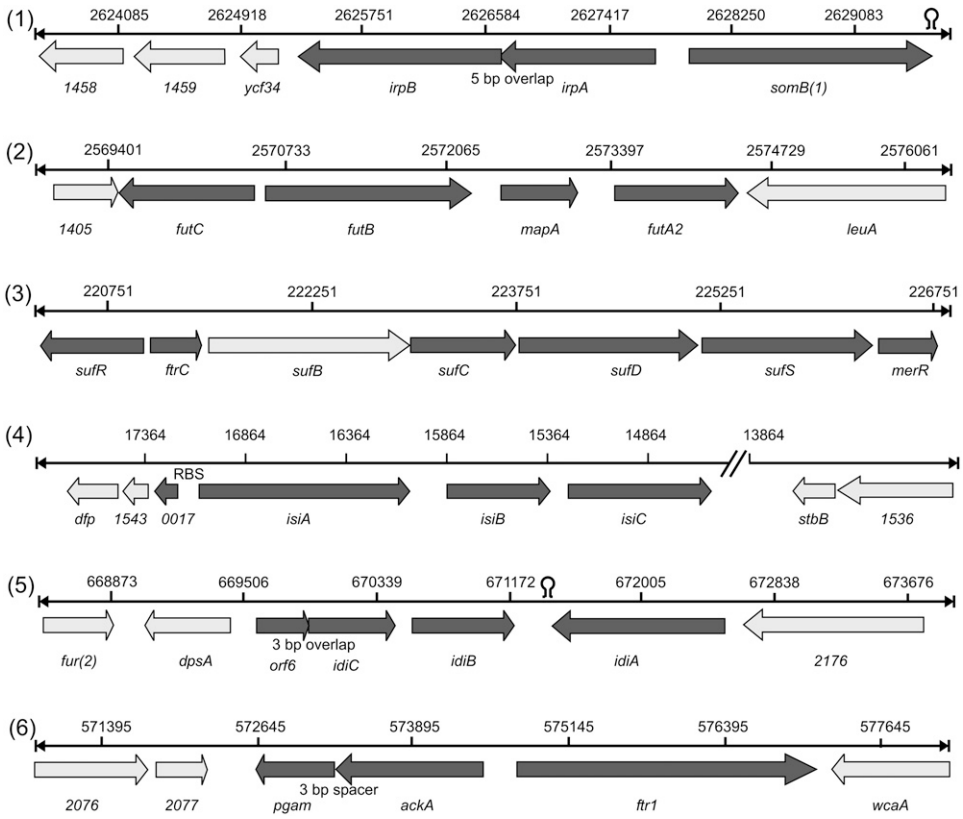

Figure 3. Partial map of the *S. elongatus* PCC 7942 chromosome with genes arranged in sequence that transcriptionally respond to the Fe status of the cell: (1) *irpAB* region, (2) *fut* region, (3) *suf*-region, (4) *isiAB*-region, (5) *idiCB* region, and (6) the *ackA* region. Transcription of *idiA* has previously been shown to be regulated by IdiB (Michel et al., 2001). The results suggest that the *irpAB* regulon and the acetate kinase regulon are also regulated by IdiB. The *isiAB* operon is regulated by the transcriptional repressor Fur (Ghassemian and Straus, 1996). The *suf* operon is assumed to be regulated by the repressor SufR (Wang et al., 2004). The transcriptional regulator(s) for the *idiBC* and the *fut* operon are still unknown. The genes given in dark gray color were up-regulated in *S. elongatus* PCC 7942 wild type upon Fe starvation.

indeed form an operon like in other eubacteria or represent a regulon. A transcriptional regulator for the Fut system of *S. elongatus* PCC 7942 has so far not been identified. In *Synechocystis* sp. PCC 6803, a function in Fe acquisition has been proven for FutA1 (Slr1295), FutA2 (Slr0513), FutB (Slr0327), and FutC (Sll1878; Katoh et al., 2001a, 2001b). These proteins represent an ATP-binding cassette (ABC)-type ferric Fe transporter. FutA1 (Koropatkin et al., 2007) and FutA2 are Fe-binding proteins, and FutB and FutC contain nucleotide-binding motifs and belong to the ABC-transporter family of inner-membrane-bound and membrane-associated proteins, respectively (Katoh et al., 2001a, 2001b). In *Synechocystis* sp. PCC 6803, FutA2 is predominantly located in the periplasm (Fulda et al., 1999, 2000), while FutA1 is mainly detected in the thylakoid membrane fraction copurifying with PSII (Tölle et al., 2002). The localization of FutA2 in *S. elongatus* PCC 7942 has not yet been investigated. The protein MapA has been shown to be predominantly located in the cytoplasmic membrane, but has also been detected in the thylakoid membrane of Fe-depleted *S. elongatus* PCC 7942 cells (Webb et al., 1994). The N-terminal part of MapA has similarity to chloroplast envelope protein E37, while the C-terminal part resembles bacterial Fe acquisition proteins. Like IrpA and IrpB (see above), MapA has no counterpart in any of the so far sequenced and annotated cyanobacterial genomes (National Center for Biotechnology Information database; October, 2007).

3. The Suf [Fe-S] assembly system of *S. elongatus* PCC 7942 is assumed to function as an auxiliary [Fe-S] assembly system besides the housekeeping Isc system and most likely facilitates the assembly and/or repair of the oxygen-labile [Fe-S] clusters under conditions of oxidative stress and under Fe limitation (Nachin et al., 2003; Wang et al., 2004; Balasubramanian et al., 2006). Selected transcripts of this system were up-regulated in *S. elongatus* PCC 7942 during Fe limitation. In detail, an increase in the steady-steady transcript level was seen for the transcripts of *sufC*, *sufD*, and *sufS* but not for *sufB*. An increase was also observed for the transcript of *sufR* (*1733*) as well as for *ftrC*, and for a gene encoding a MerR-like helix-turn-helix (HTH)-type transcription factor. In total, this gene cluster comprises six genes that are arranged in line and are transcribed in the same direction (genes *ftrC*, *sufB*, *sufC*, *sufD*, *sufS*, and *merR*), and one gene (*sufR*) that lies adjacent but is transcribed in the opposite direction (Fig. 3). The transcripts/proteins of this system have been shown to be expressed at elevated levels in several cyanobacteria during Fe-limiting growth conditions. The structure of the *suf* operon found in *S. elongatus* PCC 7942 is similar to those of several other cyanobacterial strains, such as *Synechocystis* sp. PCC 6803, *Synechococcus* sp. PCC 7002, and *Anabaena* sp. PCC 7120 (Wang et al., 2004).

Because the *ftrC* gene is located between *sufR* and the *sufBCDS* operon in *S. elongatus* PCC 7942, the *suf* region resembles most that of *Synechococcus* sp. strain WH8102. Gene *1734* encodes a protein with similarity to the catalytic subunit of a [4Fe-4S] ferredoxin:thioredoxin reductase FtrC that may function in thioredoxin-mediated redox regulation of protein function and signaling via a thiol redox control (Wang et al., 2004). SufR has been shown to be a [4Fe-4S] protein, which acts as the transcriptional repressor of the *suf* operon in *Synechococcus* sp. PCC 7002. The *suf* operon in *S. elongatus* PCC 7942 contains an additional gene for a MerR-type transcriptional regulator protein. MerR belongs to the family of HTH transcription factors including SoxR of *Escherichia coli*, which e.g. activates the transcription of flavodoxin (Brown et al., 2003). This MerR protein of *S. elongatus* PCC 7942 is somewhat atypical because it lacks two of four invariant Cys residues, which have been shown to bind the [4Fe-4S] cofactor of the repressor. Whether MerR is involved in the regulation of the *suf* genes, in addition to SufR in *S. elongatus* PCC 7942, is still unknown.

4. The fourth operon in *S. elongatus* PCC 7942, whose steady-state transcript level was found to be highly increased as of consequence of Fe depletion, contains the genes *isiA*, *isiB*, and *isiC* (Fig. 3). The gene *isiA* encodes CP43′, and *isiB* encodes flavodoxin. The function of these two proteins is discussed in the introduction. Moreover, this operon also contains a gene that we named *isiC* and that encodes a hydrolase-like protein. This putative hydrolase (26.9 kD) is 54.3% similar to the esterase Fes (18.2% identical amino acid residues, 22.1% strongly similar amino acid residues, 14% weakly similar amino acid residues) from *E. coli.* FesA hydrolyzes ester bonds of internalized ferri-enterobactin siderophores (Andrews et al., 2003). The increased transcript abundance of *isiB* and *isiC* was substantially higher than that of *isiA*. Differences in the *isiA* and *isiB* steady-state transcript levels under Fe-limiting growth conditions have already been described for *S. elongatus* PCC 7942 (Bagchi et al., 2003, 2007; Pietsch et al., 2007). In addition, it has also been observed that a monocistronic *isiA* message was more abundant than a dicistronic *isiAB* message under Fe limitation (Straus, 1994). The expression of this operon is under the control of the transcriptional repressor Fur in *S. elongatus* PCC 7942 (Ghassemian and Straus, 1996). Gene *0017* is located upstream of *isiA* and encodes a putative 4.2-kD polypeptide of unknown function. This gene is transcribed in the opposite direction, and its transcript abundance was also strongly increased under Fe limitation. Thus, this particular genome region contains four genes in total, whose transcript levels increased during Fe limitation. For *Synechocystis* sp. PCC 6803, it has previously been reported that the *isiAB* region contains three more genes in addition to *isiA* and *isiB* that were transcribed at elevated levels during Fe-limited growth conditions (Singh et al., 2003).
5. A fifth group of transcripts, which accumulated under Fe starvation, was that of *idiB* and *idiC*. The function of the corresponding proteins is explained in the introduction (Yousef et al., 2003; Michel and Pistorius, 2004; Pietsch et al., 2007). The *idiCB* genes localize in an operon (Fig. 3). Whether the third gene, named *orf6*, of this operon encodes a protein, remains to be investigated (Yousef et al., 2003). The gene *idiA* separates from the neighboring *idiCB* operon by a strong terminator sequence and is transcribed in the opposite direction. The increase in the steady-state pool of *idiB* and *idiC* mRNA was almost as high as in the case of *isiA*, while the increase of the *idiA* mRNA pool was about one-third of the *idiB* level. IdiB is an HTH-type transcription factor and regulates transcription of *idiA* (Michel et al., 2001).The Fe-responsive transcriptional regulator of the *idiCB* operon is still unknown. *Synechocystis* sp. PCC 6803 also contains an IdiA-similar protein Slr1295 (Tölle et al., 2002) named FutA1 (Katoh et al., 2001a, 2001b), but lacks an IdiB-similar and IdiC-similar protein. Upstream of the *idiCB* operon locates the gene *dpsA*. The corresponding transcript was not found at increased levels under Fe-deplete growth conditions (Michel et al., 2003). DpsA is a DNA-binding heme protein and confers resistance to oxidative stress to genomic DNA (Dwivedi et al., 1997).
6. The sixth region with Fe-regulated genes arranged in sequence comprises two genes that are separated by only 3 bp and that are transcribed in the same direction. The genes encode an acetate kinase (AckA) and a phosphoglycerate mutase (Pgam). An increase of the steady-state mRNA pool of these two genes was observed during Fe starvation in wild type but not in mutant *K10* and mutant *MuD*.

The observed increase of the Pgam transcript concentration implies that during Fe limitation 3-phosphoglycerate (3-PGA) is in part withdrawn from the Calvin cycle to increase the rate of glycolysis, and the elevated transcript level for acetate kinase further supports the assumption that pyruvate in part becomes metabolized to acetate. This fact would imply that glycogen fermentation and increased utilization of the Calvin cycle intermediates in catabolism result in an enhanced production of acetate (van der Oost et al., 1989; Moezelaar and Stal, 1994; Moezelaar et al., 1996; Steunou et al., 2006) and an additional synthesis of ATP. For verification of the microarray results related to the increased transcript level of the genes under Fe starvation being located in an operon or regulon, we performed northern blots with gene-specific digoxygenin-dUTP (Dig-dUTP) labeled DNA probes and total RNA isolated from *S. elongatus* PCC 7942 grown either with Fe-sufficient or Fe-deficient BG11 medium for 24 or 72 h (Fig. 4). An increased mRNA level under Fe limitation was verified for all genes that

are listed in Table I. The highest increase was detected for *irpA*, *isiB*, and *isiC*, which is in good agreement with the microarray results. A lower increase was observed for the transcripts of the *suf* operon, which again agrees quite well with the obtained microarray data.

Transcripts of Genes Encoding Electron Transport-Related Proteins (Photosynthesis and Respiration)

The steady-state *psbO* transcript level for the Mn- and Ca^{2+}-stabilizing protein of PSII (De Las Rivas et al., 2004), which has been assigned a regulatory function for the photosynthetic oxygen evolving activity at least in some cyanobacteria (Sherman et al., 1998; Tucker et al., 2001), showed the strongest decrease among the PSII-related transcripts (Ke, 2001). In addition, the transcript concentrations for proximal antenna proteins CP47 and CP43, and also the transcript pool of PsbT, which probably contributes to stabilization of PSII dimers, were slightly decreased.

Among the PSI-encoding genes (Ke, 2001), the steady-state transcript level for the reaction center proteins PsaA, PsaB, and PsaC as well as the transcripts for the auxiliary PSI proteins PsaK, PsaL, PsaJ, PsaI, and PsaF were diminished under Fe depletion. PsaL participates in trimerization of PSI monomers (Chitnis and Chitnis, 1993) and PsaI aids the structural organization of PsaL. This result indicates that prolonged Fe limitation results in a reduction of PSI reaction center proteins and in a decreased percentage of trimerized PSI relative to the total pool of PSI. The latter finding supports previous results showing that monomeric PSI is favored over trimeric PSI in *S. elongatus* PCC 7942 during Fe-deficient growth conditions (Ivanov et al., 2006). A similar result was also obtained from *Synechocystis* sp. PCC 6803 under prolonged Fe limitation (Yeremenko et al., 2004).

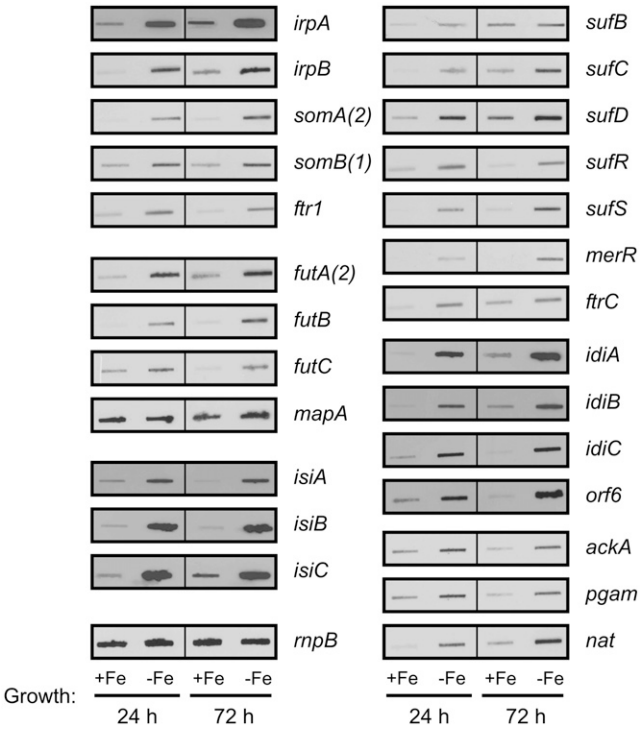

Figure 4. Transcript analysis of selected Fe-regulated genes from *S. elongatus* PCC 7942 wild type. Total RNA was isolated from *S. elongatus* PCC 7942 wild-type cultures grown either in the presence or absence of Fe for 24 and 72 h. Steady-state transcript pools were detected with gene-specific Dig-dUTP-labeled DNA probes. An *rnpB*-specific probe was used to assure equal loading.

Transcript levels of the four major proteins of the Cyt b_6/f complex, *petA*, *petB*, *petC*, and *petD* as well as the transcript for the mobile electron carrier Cyt c_{553} (PetJ) were also reduced during Fe depletion. Further, the transcript level for gene *0814*, encoding a protein with a putative [3Fe-4S] and a [4Fe-4S] cofactor, was detected at decreased concentrations.

In the course of Fe depletion, the amount of a number of transcripts encoding subunits of the phycobilisome antenna and transcripts for enzymes involved in pigment biosynthesis substantially decreased in the transcriptome of *S. elongatus* PCC 7942 wild type.

A change in the transcript level was also detected for a specific subunit of the cyanobacterial NDH-1 complex (Kaplan and Reinhold, 1999; Ohkawa et al., 2001, 2002; Badger et al., 2002; Badger and Price, 2003) due to Fe depletion. The amount of the *ndhD2* transcript, encoding a protein, which has a function in the NDH-1-type A complex-mediated respiration, was found at an elevated level. This finding is in agreement with the observation that Fe limitation results in an enhanced respiratory and photosynthetic cyclic electron transport (Michel and Pistorius, 2004) and a reduced linear electron transport activity (Ivanov et al., 2000). An increased mRNA level for subunits of various terminal oxidases of the respiratory electron transport chain (Schmetterer, 1994; Vermaas, 2001) was also detected; e.g. the steady-state mRNA level of *ctaA*, *ctaC*, and *ctaE*, encoding three subunits of the Cyt oxidase aa_3 (similar to the mitochondrial complex IV), was measured at elevated levels. In addition, the transcript level for *cydA* (cyanide-sensitive alternative Cyt *bd*-type quinol oxidase *bd* subunit), *ccoO*, and *ccoN* (subunits of Cyt *cb*-type oxidase) was detected at increased levels. Concomitantly with an increased transcript level, an increase in the capacity of the terminal oxidase(s) would lead to an enhanced electron transfer capacity to molecular oxygen under conditions where PSII and PSI activities decline.

Transcripts of Genes Encoding Carbon Metabolism-Related Proteins

A few transcript levels for carbon (C) metabolism-related proteins were significantly increased in the transcriptome of Fe-depleted *S. elongatus* PCC 7942 wild-type cells. The transcript level for the Pgam, an

enzyme of glycolysis, increased due to Fe starvation, whereas no substantial changes were measured for transcripts of other glycolytic enzymes. In addition, a significant increase of the acetate kinase transcript (*ackA*) concentration, an *N*-acetyltransferase transcript (*nat*), and a Man-1-P guanylyltransferase/Man-6-P isomerase transcript *nrdJ* were measured. The genes encoding Pgam and AckA are located next to each other and are transcribed in the same direction (see Fig. 3).

Transcripts of Genes Encoding Nitrogen Metabolism-Related Proteins

Transcript levels of mRNAs for proteins involved in nitrate/nitrite assimilation were slightly reduced in the transcriptome of Fe-starved wild-type cells; e.g. the *nirA* transcript that encodes the ferredoxin:nitrite reductase (NIR) as well as transcripts for proteins of the nitrate/nitrite uptake system were detected at lower amounts. An increase of the steady-state mRNA level for a putative Gln synthetase-inactivating factor similar to Sll1515 of *Synechocystis* sp. PCC 6803 was detected, suggesting that nitrogen (N) assimilation was reduced most likely due to the lower photosynthetic activity under Fe limitation.

Transcripts of Genes Encoding General Stress Proteins

The transcripts for several chaperones and/or heat shock proteins such as GroS, GroL, and HtpG were detected at significantly increased concentrations under Fe-limiting growth conditions. Especially, the steady-state *hspA* transcript pool was increased after 72 h of Fe-limited growth. In contrast, the mRNA level for a DnaJ-like protein of the HSP40 family was substantially decreased. The transcript concentration for the Fe superoxide dismutase (Herbert et al., 1992; Samson et al., 1994) was found to be lower in Fe-depleted cells than in Fe-sufficient cells. Altogether, these findings reveal that Fe-independent detoxification systems compensate in part the Fe-dependent parts of the cellular detoxification system. It could also suggest that the rate of superoxide anion formation was lower in PSII and PSI of Fe-starved cells when the protective proteins IdiA and IsiA were expressed at highly elevated concentrations. Moreover, the transcript pool for the heme-containing catalase peroxidase KatG (Tichy and Vermaas, 1999) was reduced under Fe limitation, while the transcript abundance for *aphC* (gene *2309*), encoding a 2-Cys peroxiredoxin (Tichy and Vermaas, 1999; Dietz et al., 2002; Stork et al., 2005), was increased. This increase only occurred under prolonged Fe limitation. Under these conditions KatG may in part be replaced by a peroxiredoxin that does not require a catalytic Fe cofactor. Among six so far identified peroxiredoxins of *S. elongatus* PCC 7942 (Stork et al., 2005), the 2-Cys peroxiredoxin is the one with the highest hydrogen peroxide-decomposing activity (T. Stork, unpublished data). Such a compensatory role of catalase and a peroxiredoxin has previously been suggested, e.g. for *Synechocystis* sp. PCC 6803 and *Staphylococcus aureus* (Tichy and Vermaas, 1999; Cosgrove et al., 2007). Probably as a consequence of the reduced Fe concentration in the cell, a metallothionine-related transcript was also down-regulated in *S. elongatus* PCC 7942, especially in the early phase of Fe limitation. As expected, levels of the high light-induced protein C transcript (Huang et al., 2002), and of the *nblA* transcript, encoding a protein that is involved in phycobilisome degradation (Collier and Grossman, 1994; van Waasbergen et al., 2002), were found to be significantly increased in Fe-depleted cells.

Transcripts of Genes Encoding Regulatory Proteins

Transcripts of genes *2466* and *1316*, encoding a CheY-like response regulator similar to Rre37 of *Synechocystis* sp. PCC 6803 and Ycf27 of *Guillardia theta*, and a transcription factor similar to Tlr1758 of *Thermosynechococcus elongatus* BP-1 were found at increased concentrations in the transcriptome of Fe-starved *S. elongatus* PCC 7942 wild-type cells. Furthermore, the transcript levels for three alternative σ factors, *rpoD4* (group II σ factor), *rpoD3* (group II σ factor), and *sigF2* (group III σ factor), were changed as a result of prolonged Fe depletion. Moreover, the mRNA level for an anti-σ factor antagonist-similar protein was increased, while the transcripts for the light-repressed transcript A protein (LrtA), which is suggested to either modulate cellular transcription and/or translation activity (Samartzidou and Widger, 1998) in response to illumination, was found to be decreased in the transcriptome of Fe-depleted cultures.

Major Differences between *S. elongatus* PCC 7942 Wild Type, Mutant *K10*, and Mutant *MuD* in the Acclimation to Fe Limitation

The *S. elongatus* PCC 7942 mutant *K10* lacks IdiB but contains IdiC, while the *idiC*-merodiploid mutant *MuD* has a strongly reduced amount of IdiC and IdiB (Pietsch et al., 2007). IdiB is an HTH transcriptional activator (Michel et al., 1999), and IdiC has been suggested to have a function in the modification of the electron transport chain under Fe limitation and in the late growth phase (Pietsch et al., 2007). In mutant *K10* as well as in mutant *MuD*, the steady-state concentration of the *idiA* mRNA level as shown previously (Yousef et al., 2003), and the mRNA level for IrpA/IrpB, Som(A2), Som(B1), a Ftr1-similar protein as well as for a putative acetate kinase (AckA), an *N*-acetyltransferase (Nat), and the Pgam was substantially lower than in wild type. This suggests that the Fe-responsive transcriptional activator IdiB regulates the expression of: (1) a putative high-affinity Fe uptake system consisting of IrpA/B and Som(A2), Som(B1), and possibly Ftr1; (2) the protein IdiA having a function in protecting PSII (Exss-Sonne et al., 2000); (3)

enzymes having a function in acetate metabolism (AckA and Nat); and (4) Pgam suggesting that intermediates of glycogen fermentation and of the Calvin cycle such as 3-PGA might in part be used for synthesis of acetyl-phosphate resulting in an additional substrate-level phosphorylation site for ATP synthesis. An alignment of the putative IdiB-binding sites for the genes *irpA*, *ftr1*, *somB(1)*, *ackA*, and *pgam* is given in Figure 5. The binding of IdiB to the upstream DNA region of *idiA* was previously proven experimentally (Michel et al., 2001), while the interaction of IdiB with the other five upstream DNA regions of the genes await experimental proof. Although *nat* and *somA(2)* also seem to be regulated by IdiB, these genes lack the characteristic IdiB-binding site.

The above-discussed observation implies that in the mutants *K10* and *MuD* the putative high-affinity Fe uptake system IrpA/IrpB is not as efficiently working as in wild type and that as a consequence, the cells suffer more rapidly from the consequences of Fe limitation than wild type under Fe depletion. Moreover, the lack of IdiA prevents the protection of PSII by this protein and thus, Fe limitation causes a faster proceeding damage of PSII. This is in line with the observed lower O_2 evolving activity in the two mutants as compared to wild type (Pietsch et al., 2007). The reduced transcript level for enzymes in favor of acetate-phosphate biosynthesis (Pgam and AckA) indicates that the mutant cells cannot benefit to the same extent as wild type from an additional site of substrate-level phosphorylation via the conversion of acetyl-phosphate to acetate with concomitant synthesis of ATP (van der Oost et al., 1989; Moezelaar and Stal, 1994; Moezelaar et al., 1996; Steunou et al., 2006). The latter difference suggests that the strategies to minimize imbalances in the C-N ratio and/or the NADPH/ATP ratio under Fe limitation are slightly different in wild type as compared to the two mutants due to the absence or reduced concentration of IdiB and IdiC. Presently, the regulatory protein(s) for the *idiCB* operon remain(s) unidentified.

The detected transcript levels for regulatory proteins reveal a major difference between the transcriptome of Fe-depleted wild type and mutant cells with respect to a transcript for a CheY-like two-component response regulator. The steady-state transcript level was found to be elevated in wild type, but was decreased in both mutants. Further, the transcript for the group II σ factor RpoD4 was found at a higher level in wild type, while the corresponding transcript level diminished in mutants *K10* and *MuD* grown for 72 h in Fe-depleted medium.

Due to the strongly reduced amount of IdiC and the reduced amount of IdiB in *MuD*, a number of additional changes in transcript abundance relative to wild type and mutant *K10* were observed for mutant *MuD*—especially with respect to photosynthesis-related transcripts (Table I). The results suggest that the modification of the electron transport chain due to Fe starvation from a preferentially photosynthetic linear transport to a preferentially photosynthetic cyclic and respiratory electron transport in mutant *MuD* did not proceed equally well in mutant *MuD* as in wild type. This became particularly obvious, when the mRNA levels for *isiA*, *isiB*, and *isiC* were compared in the three strains—suggesting differences in the redox signals mediated by the electron transport chain. This fact might also explain why it has been impossible to obtain a fully segregated IdiC-free mutant, while it was possible to obtain a fully segregated IdiB-free mutant.

Comparison of the Changes in the Transcriptomes of *S. elongatus* PCC 7942 and *Synechocystis* sp. PCC 6803 under Fe-Deficient Growth Conditions

A comparison of the DNA microarray results of *S. elongatus* PCC 7942 with results for *Synechocystis* sp. PCC 6803 (Singh et al., 2003) showed that in both strains the amounts of *isiA* and *isiB* mRNAs were highly increased in the course of Fe depletion. Moreover, several mRNAs for proteins of the PSII complex, the PSI complex, the phycobilisomes, and the Cyt b_6/f

Figure 5. Comparison of the IdiB-binding site in the upstream DNA region of *idiA* with putative IdiB-binding sites upstream of the genes *irpA*, *somB(1)*, *ftr1*, *ackA*, and *pgam* of *S. elongatus* PCC 7942. The binding site of the transcription factor IdiB in the upstream DNA region of *idiA* has been verified experimentally (Michel et al., 2001). The transcripts of the other above-mentioned five genes accumulated under Fe starvation in wild type but not in mutants *K10* and *MuD*, which have either no or only a very low amount of IdiB protein. Putative IdiB-binding sites are boxed in light gray, putative ribosome-binding sites are boxed in black with white lettering, and the annotated start codons are boxed. For *idiA* the transcriptional start site was experimentally determined and is indicated as +1 (K.-P. Michel, unpublished data).

Table II. *Oligonucleotides used in the work*

Primer	Amplified Product	DNA Sequence, 5′→3′ Direction
Primers for amplification of DNA probes for slot-blot RNA-DNA hybridization		
ackA	419 bp	GAGCAGATGGAGCAGTTGTT
		TGCTGATTCAGGACCGTGCT
ftrC	358 bp	GACCCAGACGACCAGCCCAG
		AGCAGGCTGGGTCGTGGCAC
futA2	476 bp	AATCATCCGTGCTCACCGCT
		AACGCCGGCACCACTGACAT
futB	502 bp	GGATGGCCTTGGCGTTGATG
		CGCACCGCTAGCTGAATTGG
futC	468 bp	CACTGTTTCCGCATCTAACG
		GATACTCTGTGGCCAAGAAG
idiA	986 bp	GCTGAAGGTGAAGTCAA
		TGAAGTGATCCAATAAC
idiB	623 bp	TGATTGCCAGTCACGTAACC
		GGCATCTATGGCATCAATCG
idiC	576 bp	CAAGGATCCAACTGCCGTTCTA
		TCGAAGCTTTTTAGCCCACGGC
irpA	586 bp	ACAGGCTCTCAGGTCAGGCA
		AGGCAGGCTGATCACTCGCT
irpB	507 bp	GGCTGCTGAACATCGACAGG
		TCGTGATTGCGATCCTCTGG
isiA	486 bp	ACACCACTTGCTGTTCCTCG
		TTGGATAGCCAAGCACGAGG
isiB	389 bp	CTGACTTTAGCTGGCTGACC
		TGCGAATGCTGATGCCAGTG
isiC	541 bp	TGTTGGCTAACTTGCTCGGG
		AAGCGGCTTCTCTGGATACA
mapA	429 bp	ATCATCCTACGATCGCCGCT
		TGCAACCATCGATCTAGGAG
merR	410 bp	GACCGCTACACTGCTCAAAA
		TGGTTGCAATTAACGGACAG
nat	326 bp	TGATTCGAGAGTTGGCCAGC
		CCGTTGATTCCAGTCGAGCA
pgam	521 bp	AACGACCGCTAGCCGTGAAG
		AAGACAGGATGGAGAGCGAG
somB(1)	512 bp	CGTTCCAACAGCATCACCTC
		TTCCGGTAAGCGTAGGCCAG
sufB	471 bp	AAATACGGCTTCGTCACGGA
		CACACTGCCGAGGTACTGCT
sufC	429 bp	AATACGGCTTCGTCACGGAT
		GCACGGCTTCGGAGATTGAG
sufD	457 bp	CTGTGCTGGAGCAAGTCAAC
		ACGCGAATCACGGCTGCTTG
sufS	422 bp	TACGCTAGGTTGCTGCAATC
		GCCGTTGGAAGAGGTGTTGG

complex declined in both strains in the course of Fe limitation. However, a decrease of mRNAs encoding subunits of the ATP synthase and the CO_2 concentration mechanism was only detected for *Synechocystis* sp. PCC 6803. In the latter strain, six ferredoxin transcripts (four PetF-type ferredoxins) were found either at elevated or at reduced concentrations, and the transcript for the ferredoxin:NADP oxidoreductase (PetH) was found to be down-regulated. In contrast, *S. elongatus* PCC 7942 only revealed a single decreasing ferredoxin transcript (gene *2124*). No substantial differences were seen for the steady-state transcript pools of *petF* and *petH*. Transcripts for proteins involved in N assimilation were slightly down-regulated in *S. elongatus* PCC 7942 but not in *Synechocystis* sp. PCC 6803. In *Synechocystis* sp. PCC 6803 transcripts for glycolysis enzymes were down-regulated, e.g. glucokinase, phosphofructokinase, Fru-1,6-bisphosphate aldolase, and Glc-6-P isomerase, while in *S. elongatus* PCC 7942 the mRNA concentrations for the Pgam and also for enzymes of the acetate metabolism were up-regulated.

With respect to Fe acquisition and Fe uptake systems of *S. elongatus* PCC 7942, the strongest increase upon Fe limitation was detected for the transcripts of the putative IrpAB Fe acquisition system. Additionally, an increased level of Fut transcripts was observed, but this increase was lower than that for the *irpA* and *irpB*. This implies that *S. elongatus* PCC 7942 has two major Fe uptake systems. The IrpAB system most likely represents a high-affinity system because its transcripts accumulated to a very high extent under conditions of Fe starvation. This system obviously requires outer membrane porins of the Som-type, while the Fut system most likely does not necessarily require outer membrane receptors, which has also been suggested for the bacterial Sfu and Fbp systems (Andrews et al., 2003). In *Synechocystis* sp. PCC 6803, the ferrichrome Fe receptor transcripts of the FhuA-type and transcripts encoding proteins of the Fut system (as e.g. Slr0513), were up-regulated (Katoh et al., 2001a, 2001b; Singh et al., 2003).

CONCLUSION

Our DNA microarray analyses identified six regions on the *S. elongatus* PCC 7942 chromosome with clusters of genes, whose transcripts increased in the course of Fe-limited growth conditions. These were the *irpAB* region and the *fut* region, encoding proteins of two Fe acquisition systems of which the IrpAB system is substantially higher up-regulated than the Fut system under Fe limitation. Moreover, transcripts of the Suf system, having a function in assembly and/or repair of the oxygen-labile [Fe-S] clusters under oxidative stress and Fe limitation, were detected at higher steady-state levels. Two gene clusters with genes encoding proteins, which modify the electron transport chain (IsiA, IsiB, IdiC, and IdiA) as well as the transcriptional regulator IdiB, were found to be up-regulated. The sixth cluster contains the genes encoding an acetate kinase and a Pgam. The increased transcript level for these enzymes suggests that under Fe limitation acetate synthesis is up-regulated, which provides an additional substrate-level ATP synthesis reaction. An increased activity of the latter pathway might contribute to minimize imbalances in the NADPH-ATP ratio and/or the C-N ratio under Fe limitation.

As expected due to previous investigations showing a reduction of the linear photosynthetic electron transport and an increase in respiratory electron transport under Fe starvation (Michel et al., 2003; Pietsch et al., 2007), steady-state mRNA levels of several transcripts for proteins of the photosynthetic electron transport

chain were down-regulated, while mainly transcripts encoding subunits of three types of cytochrome oxidases were up-regulated. The up- or down-regulation was higher in the IdiB-free mutant *K10* and the *idiC*-merodiploid mutant *MuD* than in wild type. This is likely due to the extremely low concentration of the IrpAB Fe uptake system in both mutants as compared to wild type and the reduced amount of IdiC, preventing the IdiC-mediated adaptation of the electron transport chain.

Previously, it has been proven that the HTH-type transcription activator IdiB regulates the expression of the *idiA* mRNA. The genome-wide microarray analysis provided evidence that IdiB has additional regulatory function. IdiB is a regulator of the *irpAB* regulon and of the *ackA/pgam* operon. This suggests that IdiB has a major function in the signal transduction leading to acclimation *S. elongatus* PCC 7942 to Fe deficiency.

MATERIALS AND METHODS

Cyanobacterial Strains, Growth Conditions, and Cell Harvesting

Synechococcus elongatus PCC 7942 was obtained from the Institut Pasteur, Collection Nationale de Cultures de Microorganismes, Paris. Construction of the IdiB-free *S. elongatus* PCC 7942 mutant *K10* and the *idiC*-merodiploid mutant *MuD* have been described earlier (Michel et al., 1999; Pietsch et al., 2007). *S. elongatus* PCC 7942 wild type and both mutants were cultivated in 250-mL gas wash bottles with BG11 medium and were continuously bubbled with 2% CO_2-enriched air. The IdiB-free mutant and the *idiC*-merodiploid mutant were grown in the presence of 25 and 50 μg mL^{-1} spectinomycin, respectively. The culture bottles were placed in a water bath set at 30°C and illuminated with fluorescent tubes (Lumilux Plus Eco, L 18W/31–830, Warm White; Osram) with an intensity of 100 μmol photons m^{-2} s^{-1}. Prior to inoculation, the cells were centrifuged for 10 min at 3,000*g*, washed with the BG11 medium as used later on for growth, and centrifuged again for 10 min at 3,000*g*. The cells were resuspended and were inoculated with Fe-sufficient or iron-deficient BG11 medium with a cell density corresponding to an $OD_{750\ nm}$ of 0.3. The Fe-sufficient BG11 medium contained 30 μM Fe-III-citrate, while Fe-III-citrate was completely omitted from the Fe-deficient medium. After 24 h or 72 h of growth (for both mutants only 72 h), cells were harvested and transferred into 50-mL Falcon tubes (Sarstedt) together with crushed ice. The tubes were centrifuged at 4,000*g* (Multifuge 1 LR; Heraeus) for 5 min at 4°C, and the cell pellets were frozen in liquid N to be stored at −80°C for further use. Each experiment was performed either in three (wild type and *MuD*) or two (*K10*) biological and two technical replicates.

Design of Oligonucleotides and Preparation of Se3kOligo Microarrays

The sequencing of the *S. elongatus* PCC 7942 genome has been performed by the *S. elongatus* PCC 7942 Functional Genome Project Initiative at Texas A&M University headed by Professor Susan Golden. The results are available at http://genome.ornl.gov/microbiol/synPCC7942/ (NC_007604). The genome of *S. elongatus* PCC 7942 consists of 2.7 Mbps with a total of 2,612 annotated chromosomally localized protein-encoding genes, 53 tRNA genes, and 50 plasmid-localized protein-encoding genes (Joint Genome Institute [JGI] annotation).

The overall GC content corresponds to 55.4%. On the basis of the annotated protein-encoding genes and 183 additionally predicted genes, in particular small polypeptide-encoding genes, a total of 2,898 70-mer oligonucleotides were designed using the Oligo Designer software (Bioinformatics Resource Facility, Center for Biotechnology, Bielefeld University).

The oligonucleotides were synthesized by Operon Biotechnologies GmbH. Oligonucleotide probes were printed in four replicates.

In addition, several control spots were applied to the slides: Five 70-mer oligonucleotides directed against NT03SE0857 (*rpsO*), NT03SE0747 (*rpsI*), NT03SE2644 (*rpmI*), NT03SE0278 (*rpsP*), NT03SE0452 (*gap*; 70%, 80%, and 90% identity) were spotted in four replicates to function as a stringency control. As a negative control four alien 70-mer oligonucleotides against *Sinorhizobium meliloti* alien-SMb20957, -SMb20959, -SMb20961, -SMb21008 were spotted in four replicates and eight alien 70-mer oligonucleotides against *Medicago truncatula* alien-MT000016, -MT000017, -MT000018, -MT000019 were spotted two times in four replicates. Five alien spikes 1 to 5 (Stratagene) in four replicates, 96 empty spots in four replicates, and spotting buffer in 292 replicates were also applied.

Microarrays were produced and processed as described previously (Brune et al., 2006). Oligonucleotides (40 μM) in 1.5 M betaine, 3× SSC (1× SSC is 0.15 M sodium chloride, 0.015 M sodium citrate) were printed onto Nexterion Slide E (Schott AG) using the MicroGrid II 610 spotter (BioRobotics) equipped with 48 SMP3 stealth pins (TeleChem International). DNA was cross-linked to the surface by incubation of the slides for 2 h at 85°C.

Isolation of Total RNA from *S. elongatus* PCC 7942 Wild Type, Mutant *K10*, and Mutant *MuD*

Total RNA was isolated from cell pellets (harvested as described above). Frozen cells were resuspended in 200 μL Tris-HCl, pH 8.0, 700 μL RLT buffer, provided with RNeasy Mini Kit (QIAGEN), and 7 μL mercaptoethanol. The cell suspension was transferred to Fast Protein Tubes (Lysing Matrix B; Q Biogene), and cells were disrupted with a ribolyzer (30 s at level 6.5; Hybaid). Total RNA was isolated with the RNeasy Mini Kit (QIAGEN) and subjected to an on-column DNase treatment (RNase-free DNase set; QIAGEN). The isolated total RNA was concentrated with a Microcon-30 filter (Millipore) and the RNA concentration was measured with a Nanodrop ND-1000 spectrophotometer (PeqLab).

Complementary DNA Synthesis and Dye-Labeling Protocol

Fluorescent-labeled complementary DNA (cDNA) was prepared according to DeRisi et al. (1997). Starting from 10 μg total RNA, aminoallyl-modified first-strand cDNA was synthesized by reverse transcription using random hexamer primers (Operon), Superscript III RT (Stratagene), and 0.5 mM dNTP, dTTP:aminoallyl-dUTP (1:4; dNTPs; PeqLab, aa-dUTP; Sigma-Aldrich). After hydrolysis and clean-up using CyScribe GFX purification columns (GE Healthcare), Cy3- and Cy5-*N*-hydroxysuccinimidyl ester dyes (GE Healthcare) were coupled to the aminoallyl-labeled first-strand cDNA. Uncoupled dye was removed using the CyScribe GFX Purification kit.

Microarray Hybridization and Image Acquisition

Processing of microarrays prior to hybridization included the following washes: once in 0.1% Triton-X100 (5 min, 20°C); twice in 0.032% (w/v) HCl (2 min, 20°C); once in 0.1 M KCl (10 min, 20°C); once in H_2O (1 min, 20°C); once in 0.064% (w/v) HCl, 1× Nexterion blocking solution (Schott AG; 15 min, 50°C); once in H_2O (1 min, 20°C). Microarrays were dried by centrifugation (3 min, 185*g*, 20°C).

Hybridization was performed in EasyHyb hybridization solution (Roche) supplemented with sonicated salmon sperm DNA at 50 μg/mL in a final volume of 100 μl for 90 min at 45°C using the HS 4800 hybridization station (Tecan Trading AG). Before application to the microarrays, labeled samples were denatured for 5 min at 65°C. After hybridization microarrays were washed once in 2× SSC, 0.2% SDS (w/v; 5 min, 42°C), twice in 0.2× SSC, 0.1% SDS (w/v; 1 min, 21°C), twice in 0.2× SSC (1 min, 21°C), and once in 0.05× SSC (1 min, 21°C). Following the washes, slides were dried by centrifugation (3 min, 185*g*, 20°C) and scanned with a pixel size of 10 μm using the LS Reloaded microarray scanner (Tecan Trading AG).

Microarray Data Analysis

Mean signal and mean background intensities were obtained for each spot of the microarray images using the ImaGene Software 6.0 software (Bio Discovery) for spot detection, image segmentation, and signal quantification.

Spots were flagged as "empty" if $R \leq 0.5$ in both channels, where R = (signal mean − background mean) / background SD. The remaining spots were considered for further analysis. After subtractions of the local background intensities from the signal intensities and introduction of a floor value of 20, the $\log_2$ value of the ratio of intensities was calculated for each spot using the formula $M_i = \log_2(R_i/G_i)$. $R_i = I_{ch1(i)} - Bg_{ch1(i)}$ and $G_i = I_{ch2(i)} - Bg_{ch2(i)}$, where $I_{ch1(i)}$ or $I_{ch2(i)}$ is the intensity of a spot in channel 1 or channel 2 and $Bg_{ch1(i)}$ or $Bg_{ch2(i)}$ is the background intensity of a spot in channel 1 or channel 2, respectively. The mean intensity was calculated for each spot, $A_i = \log_2(R_iG_i)^{0.5}$ (Dudoit et al., 2002). A normalization method based on local regression was applied according to Yang et al. (2002), $M_i = \log_2(R_i/G_i) \rightarrow \log_2(R_i/G_i) - c(A) = \log_2(R_i/[k_i(A)G_i])$, where $c(A)$ is the locally weighted scatter plot smoothing fit to the MA plot. Normalization and t statistics were carried out using the *EMMA* 2.2 microarray data analysis software developed at the Bioinformatics Resource Facility, Center for Biotechnology, Bielefeld University (Dondrup et al., 2003; http://www.cebitec.uni-bielefeld.de/groups/brf/software/emma_info/). Significant up- or down-regulation of genes was identified by t statistics (Dudoit et al., 2002). Genes were classified differentially expressed, if $P \leq 0.051$ and $M \geq 0.90$ or $M \leq -0.90$. Each experiment was performed with three biological replicates, two technical replicates, and one dye swap.

Northern Hybridization

For slot-blot RNA-DNA hybridization experiments 5 μg of total RNA were denatured for 10 min at 68°C in a formaldehyde/formamide-containing buffer and transferred to HybondN$^+$ membranes (GE Healthcare Life Sciences) using the Bio-Rad dot-blot SF microfiltration apparatus (Bio-Rad) as described in the corresponding manual. RNA was UV cross-linked to the membrane and samples were probed with different PCR-derived Dig-dUTP labeled gene-specific DNA probes. Detection was performed using the CDP-Star ready-to-use system (Roche) according to the manufacturer's recommendation. The *rnpB* probe was used to ensure equal loading. All used primers are listed in Table II.

All experimental array data including the array layout were submitted to Array Express at EMBL (submission A–MEXP–1115 pending; release date June 1, 2008).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Table S1. List of further differentially regulated genes encoding regulatory proteins, cofactor and pigment biosynthesis-related proteins, nucleic acid metabolism-related proteins, as well as proteins of unknown function or hypothetical proteins from *S. elongatus* PCC 7942 wild type, the IdiB-free mutant *K10*, and the *idiC*-merodiploid mutant *MuD* in response to growth for 24 (wild type) or 72 h (wild type, *K10*, and *MuD*) with iron-deficient versus iron-sufficient BG11 medium.

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