Unusual Regulatory Elements for Iron Deficiency Induction of the *idiA* Gene of *Synechococcus elongatus* PCC 7942

KLAUS-PETER MICHEL,[1,2] ELFRIEDE K. PISTORIUS,[2] AND SUSAN S. GOLDEN[1]*

Department of Biology, Texas A&M University, College Station, Texas 77843-3258,[1] and Biologie VIII: Zellphysiologie, Universitaet Bielefeld, D-33615 Bielefeld, Germany[2]

Expression of a thylakoid membrane-associated protein called IdiA (iron-deficiency-induced protein A) is highly elevated and tightly regulated by iron limitation in *Synechococcus elongatus* PCC 6301 and PCC 7942. Although this protein is not essential for photosystem II (PSII) activity, it plays an important role in protecting the acceptor side of PSII against oxidative damage, especially under iron-limiting growth conditions, by an unknown mechanism. We defined the iron-responsive *idiA* promoter by using insertional inactivation mutagenesis and reporter gene assays. A 67-bp DNA region was sufficient for full iron deficiency-inducible *idiA* promoter activity. Within this fragment is a palindromic sequence 4 bp upstream of a putative −35 promoter element, which resembles the binding site of FNR/CAP-type helix-turn-helix transcription factors. The absence of this palindromic sequence or a 3-bp mutation in a putative −10 region eliminated promoter activity completely. A previously identified candidate for a positively acting transcription factor is the IdiB protein, whose gene lies immediately downstream of *idiA*. IdiB shows strong similarity to helix-turn-helix transcription factors of the FNR/CAP family. A His$_{6x}$-tagged IdiB that was overexpressed in *Escherichia coli* bound to a 59-bp fragment of the *idiA* regulatory region that included the palindrome. Although the *idiA* promoter lacks a consensus binding site for the iron-sensing regulator Fur, we attempted to inactivate *fur* in order to investigate the potential role of this factor. The resulting merodiploid mutants showed constitutive partial derepression of IdiA expression under iron-sufficient growth conditions. We concluded that IdiB is a specific iron-responsive regulator of *idiA* and that Fur has an indirect role in influencing *idiA* expression.

Numerous cyanobacterial genes that are tightly regulated in response to iron availability have been identified in *Synechococcus* species and other cyanobacteria. These genes include *irpA*, *isiA*, *isiB*, *mapA*, *cpcG*, *slr0374*, *nblA*, and *idiA* (21–23, 29, 32, 35, 38). The fact that upstream DNA regions of *isiAB*, *irpA*, and *mapA* contain operator sequences resembling Fur (ferric iron uptake repressor) boxes of gram-negative bacteria led to the assumption that expression of these genes under iron-limiting growth conditions is mediated via the Fur system (36). Fur was first discovered in the gram-negative bacteria *Salmonella* and *Escherichia coli* (5, 13), in which it is responsible for specific regulation of many genes involved in iron metabolism. The *E. coli* Fur protein is a dimeric DNA binding protein related to the CAP family (18). Each 17-kDa monomer contains an N-terminal DNA binding motif and a C-terminal metal binding domain. Fur can act as a transcription repressor only in the presence of its corepressor, Fe^{2+}. Under iron-sufficient growth conditions it binds to specific DNA sequences, known as Fur boxes, and inhibits transcription of virtually all genes and operons that are repressed by iron (14). When iron becomes a limiting resource, the dimeric Fur complex releases its bound Fe^{2+}, can no longer bind to its repressor site, and, therefore, allows transcription of iron-regulated genes. Fur boxes consist of a 19-bp sequence with dyad symmetry (two 9-bp inverted repeats), but they can also be interpreted as three 5-bp direct repeats (6).

Only one of the cyanobacterial genes mentioned above, *isiA*, has been shown to exhibit iron-regulated expression facilitated by a cyanobacterial Fur homolog (10). This cyanobacterial Fur homolog exhibits only moderate sequence similarity to the *E. coli* protein. A typical Fe^{2+} binding site shared with other gram-negative Fur proteins (HHXHXXCXXC) justifies classification of this protein as a Fur homolog and not a DtxR homolog, the major iron-dependent regulation system of gram-positive organisms (10, 15). However, some features of the cyanobacterial Fur are unusual, and the *Synechococcus elongatus* gene cannot complement an *E. coli fur* mutant (10). Additionally, insertional inactivation of *fur* in *S. elongatus* results in merodiploid mutants, whereas in *E. coli* mutants there is complete segregation of the mutant allele. Despite the fact that no fully *fur*-inactivated strain has been obtained, the merodiploid cyanobacterial mutant does exhibit derepression of several iron-regulated genes and proteins, probably due to a significantly lower number of intracellular Fur molecules (10).

Another prominent iron-regulated protein of *S. elongatus* PCC 7942 and PCC 6301 is IdiA (iron-deficiency-induced protein A) (29). The amount of this 35-kDa protein, which is attached to the cytoplasmic side of thylakoid membranes, is elevated under iron-deficient growth conditions and to some extent under manganese-deficient growth conditions (26). Although biochemical assays revealed that IdiA is most likely involved in protection of photosystem II against oxidative stress, especially under iron-limiting growth conditions, its precise function is unknown (7). Expression analyses using Western and Northern blot techniques in the presence of protein and RNA biosynthesis inhibitors, respectively, have indicated that *idiA* is monocistronically transcribed and that expression of IdiA is most likely regulated at the transcriptional level (27).

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* Corresponding author. Mailing address: Department of Biology, Texas A&M University, College Station, TX 77843-3258. Phone: (979) 845-9824. Fax: (979) 862-7659. E-mail: sgolden@tamu.edu.

A 5′ transcript end for *idiA* has been mapped to 193 bp (bp 1929 in the *idiA* EMBL database accession no. Z48754 entry) upstream of the first of at least three potential translational start codons (bp 2121, 2148, and 2160). Biochemically purified IdiA is processed at a procaryotic AA cleavage site (RRRAE AAEGEV), which is consistent with the *idiA* mRNA size determined from Northern blots. A putative Fur box was identified upstream of the mapped 5′ end of the *idiA* mRNA. However, the 19-bp sequence only poorly resembles the Fur box consensus sequence from *E. coli* (11 of 19 possible matches), and it lacks the typical dyad symmetry. Additional sequencing of the DNA regions flanking the *idiA* gene and interposon mutagenesis led to identification of two downstream genes, *idiB* and *dpsA*, which encode a putative helix-turn-helix transcriptional activator of the FNR/CAP family and a Dps-PexB homolog, respectively. Loss of either IdiB or DpsA by gene inactivation led to a drastic decrease in IdiA content and loss of the ability to increase expression under inducing conditions (27). These data suggest that *idiA* is inducible by iron deficiency rather than repressed under iron-sufficient conditions via Fur.

Because *idiA* is one of the most prominent cyanobacterial genes regulated by iron availability (7, 35) and/or oxidative stress and because available data suggested regulatory involvement of DNA binding proteins other than Fur, we performed a detailed analysis of the *idiA* promoter in *S. elongatus* PCC 7942. Functional analysis of the *idiA* promoter was required because although *idiA* transcription is tightly regulated in response to manganese and iron limitation, no typical promoter structures were evident upstream of the previously mapped 5′ end of the *idiA* transcript.

We determined that the mapped mRNA 5′ end lies upstream of the iron-regulated promoter of *idiA* and, therefore, could not represent the transcription start site. The functional *idiA* promoter is located in a 67-bp region which includes the first of three potential start codons and requires a 14-bp region of dyad symmetry, which was bound by the IdiB protein in mobility shift assays. Despite the absence of a consensus Fur box in the *idiA* regulatory region, expression of *idiA* is partially derepressed in merodiploid Δ*fur* mutants.

MATERIALS AND METHODS

Culture conditions. *S. elongatus* wild-type strain PCC 7942 (formerly *Anacystis nidulans* R2 or *Synechococcus* sp. strain PCC 7942) (17) and mutant strains were grown in BG-11M medium (4) in 100-ml cultures in shaking flasks with 1% CO_2-enriched air at a light intensity (photosynthetic photon flux density) of 150 microeinsteins $m^{-2} s^{-1}$ (standard fluorescent light bulbs). For reporter gene experiments starter cultures that had optical densities at 750 nm (OD_{750}) of 0.9 to 1.2 were used to inoculate 40-ml tubes at an OD_{750} of 0.25. The cultures were placed in an aquarium, incubated at 30°C with a light intensity of 250 microeinsteins $m^{-2} s^{-1}$, bubbled with 1% CO_2 in air, and sampled after 24, 48, and 72 h. For growth under iron-deficient conditions, cells from starter cultures were harvested, washed twice with distilled water, and then transferred to medium from which iron was omitted. All cyanobacterial strains were grown on BG-11M agar plates containing 1.5% agar (Difco Bacto Agar). As indicated below, antibiotics were added at the following concentrations: ampicillin, 10 mg liter^{-1}; chloramphenicol, 10 mg liter^{-1}; gentamicin, 2 mg liter^{-1}; kanamycin, 25 mg liter^{-1}; and spectinomycin, 20 mg liter^{-1}.

E. coli. *E. coli* DH10B and JM107 were hosts for all plasmids. Cells were cultivated in liquid Luria-Bertani medium or on Luria-Bertani medium containing 1.5% agar. As indicated below, antibiotics were added at the following concentrations: ampicillin, 200 mg liter^{-1}; chloramphenicol, 50 mg liter^{-1}; gentamicin, 10 mg liter^{-1}; kanamycin, 50 mg liter^{-1}; and spectinomycin, 50 mg liter^{-1}.

Construction of mutant strains and DNA manipulations. Plasmid clone analysis, cleavage with restriction endonucleases, agarose electrophoresis, ligation, Southern blotting, and transformation of *E. coli* strains were performed by using standard procedures (33). The plasmids, strains, and oligonucleotides used for PCR amplification of genomic template DNA with Pwo polymerase (Roche Molecular Biochemicals, Indianapolis, Ind.) are listed in Table 1. All plasmids used in β-galactosidase assays were derivatives of neutral site I (NS1) (GenBank accession no. U30252) targeting vector pAM990 (25). Promoter fragments of *idiA* generated by PCR were cloned into a unique *Sma*I site of pAM990 to produce out-of-frame transcriptional fusions with a promoterless *lacZ* gene. The constructs used to create strains for bioluminescence assays were derivatives of neutral site II (NS2) (GenBank accession no. U44761) targeting vector pAM1580 (1). PCR-derived *idiA* promoter fragments were cloned into *Stu*I- or *Sma*I-*Sal*I-digested pAM1580 to create out-of-frame transcriptional fusions to promoterless *luxAB* genes. All fragments made with primers AMO524 (EMBL database accession no. Z48754 entry starting at bp 2299) and AMO536 (EMBL database accession no. Z48754 entry starting at bp 2129) were cut with *Sal*I prior to cloning into pAM1580. Transformation of the *S. elongatus* wild-type strain with pAM990 derivatives and transformation of strain AMC395 (*psbAI::luxCDE* background in NS1) with pAM1580 derivatives occurred through homologous recombination with the chromosome (11). This method was also used to generate the interposon mutants in the upstream *idiA* region and the sigma factor genes.

Insertional inactivation of the *fur* locus in *S. elongatus* PCC 7942. Primers AMO534 (5′-AAGTTTTGAGGCTCCGACTGCTG-3′; database accession no. L41065 entry starting at bp 1) and AMO535 (5′-GATCGCCTCGAACAGCTC TATCA-3′; database accession no. L41065 entry starting at bp 864) were used to PCR amplify an 864-bp fragment encoding the entire *fur* gene and flanking DNA regions (10). This fragment was cloned into *Sac*I-cut and T4 DNA polymerase-blunted pUC19. To interrupt the *fur* gene, a Km^r cassette and a Cm^r cassette were individually cloned into a unique *Ppu*MI site of *fur,* leaving flanking regions of the same size on both sides to allow efficient homologous recombination with the *S. elongatus* chromosome.

In vivo luciferase measurements. For in vivo bioluminescence measurements whole cells of *lux* reporter strains grown under iron-sufficient, -deficient, or -replete growth conditions were taken from a liquid culture and diluted in the corresponding medium to an OD_{750} of 0.2. Aliquots (10 μl) were transferred to a scintillation vial and dark adapted for 3 min to allow decay of chlorophyll fluorescence. Luciferase expression was measured as light production in counts per minute with a Beckman LS5301 scintillation counter with coincidence disabled. In vivo bioluminescence measurements were also obtained for cyanobacterial cultures streaked onto BG-11M agar pads in 96-well microtiter plates by using a modified Packard Instrument Co. TopCount luminometer as previously described (19). Prior to these measurements a 12-h dark pulse was administered to all cultures to reset the circadian clock.

Protein determination and β-galactosidase assays. The methods used for quantification of soluble proteins by a modified Lowry technique and for determination of specific β-galactosidase activity (in nanomoles per minute per milligram of protein) by a colorimetric assay with the substrate *o*-nitrophenyl-β-D-galactopyranoside have been described previously (34).

Preparation of French press extracts, SDS-PAGE, and immunoblotting. To prepare soluble protein extracts for Western blot experiments, 35-ml cultures of *S. elongatus* cells were harvested by centrifugation, washed once with 10 mM sodium phosphate buffer (pH 7), and then resuspended in the same buffer to a final volume of 4 ml. Each cell suspension was passed twice through a prechilled French press at 137.9 MPa (20,000 lb/in^2). Unbroken cells were removed by centrifugation at 4,000 × *g* for 5 min at 4°C. Protein samples from the supernatant fraction were denatured for 30 min at 65°C by using a dithiothreitol-containing buffer. *E. coli* cells were lysed and extracts were denatured by heating them at 100°C for 5 min in a β-mercaptoethanol-containing denaturing buffer system. Protein samples (20 μg) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (20) and transferred to a nitrocellulose membrane (BA85S; Schleicher & Schuell, Keene, N.H.) as previously described (28). Proteins were immunostained with an anti-IdiA antiserum (28) at the dilutions indicated in the figure legends and were detected with an ECL detection kit (Amersham Pharmacia Biotech, Piscataway, N.J.).

Overexpression of IdiB and electrophoretic mobility shift assays with partially purified IdiB protein. IdiB was overexpressed by using the Qiaexpress overexpression system (Qiagen, Valencia, Calif.) for synthesis of N-terminal His-tagged proteins (His$_6$). A PCR-derived 629-bp fragment obtained by using primers AMO532 (5′-PATGATTGCCAGTCACGTAACC-3′; EMBL database accession no. Z48754 entry starting at bp 4090) and AMO533 (5′-PGCTTAGGCAT CTATGGCATC-3′; EMBL database accession no. Z48754 entry starting at bp

TABLE 1. *S. elongatus* strains and plasmids

Strain	Parent strain	Plasmid[a]	Characteristics	Primer combination	Source or reference(s)
PCC 7942					
PCC 7942			Wild type		Laboratory collection
AMC520	Wild type	pAM1997	*psbAII::luxAB* in NS2, *psbAI::luxCDE* in NS1, Cmʳ Spʳ		Laboratory collection
AMC539	Wild type	pAM1518[b]	*conII::luxAB* in NS2, *psbAI::luxCDE* in NS1, Cmʳ Spʳ	19	Laboratory collection
AMC777	Wild type	pAM2338	−54 to +43 *psbAI::luxAB* in NS2, *psbAI::luxCDE* in NS1, Cmʳ Spʳ	30	
Interposon mutagenesis[c]					
AMC395	Wild type	pAM1518	*psbAI::luxCDE* in NS1, Spʳ		Laboratory collection
AMC838	Wild type	pKPM226	Kmʳ in *Nru*I site at bp 582[d]		27
AMC839	Wild type	pKPM228	Spʳ in deletion of *Nae*I sites at bp 1586 to 1777[d]		27
AMC840	Wild type	pKPM227	Spʳ in *Kpn*I site at bp 1397[d]		27
AMC843	Wild type	pKPM231	Spʳ in *Ava*I site at bp 1901[d]		This study
AMC844	Wild type	pKPM234	Spʳ in *Sca*I site at bp 1829[d]		This study
AMC808	Wild type	pAM2389	Spʳ in *Nsi*I site at bp 2121[d]		This study
AMC809	Wild type	pAM2390	Spʳ in *Apo*I site at bp 1974[d]		This study
AMC810	Wild type	pAM2392	Spʳ in *Sfo*I site at bp 2060[d]		This study
***luxABCDE* reporter strains**					
AMC811	AMC395	pAM1580	Promoterless *luxAB*, Cmʳ Spʳ		Laboratory collection
AMC812	AMC395	pAM2393	1,419-bp PCR fragment in *Sma*I-*Sal*I sites in pAM1580, Cmʳ Spʳ	AMO537-AMO536[e]	This study
AMC814	AMC395	pAM2395	1,589-bp PCR fragment in *Sma*I-*Sal*I sites in pAM1580, Cmʳ Spʳ	AMO537-AMO536[e]	This study
AMC815	AMC395	pAM2396	902-bp PCR fragment in *Sma*I-*Sal*I sites in pAM1580, Cmʳ Spʳ	AMO538-AMO524[e]	This study
AMC816	AMC395	pAM2397	747-bp PCR fragment in *Sma*I-*Sal*I sites in pAM1580, Cmʳ Spʳ	AMO539-AMO524[e]	This study
AMC817	AMC395	pAM2398	480-bp PCR fragment in *Sma*I-*Sal*I sites in pAM1580, Cmʳ Spʳ	AMO540-AMO524[e]	This study
AMC818	AMC395	pAM2399	357-bp PCR fragment in *Sma*I-*Sal*I sites in pAM1580, Cmʳ Spʳ	AMO541-AMO524[e]	This study
AMC819	AMC395	pAM2400	623-bp PCR fragment in *Stu*I site in pAM1580, Cmʳ Spʳ	AMO539-AMO525	This study
AMC820	AMC395	pAM2401	611-bp PCR fragment in *Stu*I site in pAM1580, Cmʳ Spʳ	AMO539-AMO526	This study
AMC821	AMC395	pAM2402	357-bp PCR fragment in *Stu*I site in pAM1580, Cmʳ Spʳ	AMO540-AMO526	This study
AMC822	AMC395	pAM2403	345-bp PCR fragment in *Stu*I site in pAM1580, Cmʳ Spʳ	AMO540-AMO525	This study
AMC823	AMC395	pAM2404	259-bp PCR fragment in *Stu*I site in pAM1580, Cmʳ Spʳ	AMO529-AMO524[e]	This study
AMC824	AMC395	pAM2405	222-bp PCR fragment in *Stu*I site in pAM1580, Cmʳ Spʳ	AMO530-AMO525	This study
AMC899	AMC395	pAM2534	192-bp PCR fragment in *Sma*I-*Sal*I sites in pAM1580, Cmʳ Spʳ	AMO531-AMO524[e]	This study
AMC911	AMC395	pAM2559	211-bp PCR fragment in *Stu*I site in pAM1580, Cmʳ Spʳ	AMO541-AMO524[e]	This study
AMC912	AMC395	pAM2558	201-bp PCR fragment in *Stu*I site in pAM1580, Cmʳ Spʳ	AMO541-AMO527	This study
***lacZ* reporter strains**					
AMC801	Wild type	pAM990	Promoterless *lacZ* in NS1 Spʳ		Laboratory collection
AMC802	Wild type	pAM2406	1,422-bp PCR fragment in *Sma*I site in pAM990, Spʳ	AMO537-AMO536[e]	This study
AMC805	Wild type	pAM2409	761-bp PCR fragment in *Sma*I site in pAM990, Spʳ	AMO539-AMO524[e]	This study
AMC807	Wild type	pAM2411	371-bp PCR fragment in *Sma*I site in pAM990, Spʳ	AMO541-AMO524[e]	This study
Sigma factor mutant strains					
AMC851	Wild type	pAM1344	Insertional inactivation of *rpoD2*, Kmʳ		30; this study
AMC852	Wild type	pAM2414	Insertional inactivation of *rpoD3*, Kmʳ		30; this study
AMC853	Wild type	pAM2413	Insertional inactivation of *rpoD4*, Kmʳ		30; this study
AMC854	Wild type	pAM2331	Insertional inactivation of *sigC*, Gmʳ		30; this study
PCC 7942 ΔFur strains					
AMC915	Wild type	pAM2571	864-bp Fur fragment in pUC19, Kmʳ in *Ppu*MI site		This study
AMC930	Wild type	pAM2571	864-bp Fur fragment in pUC19, Cmʳ in *Ppu*MI site		This study
AMC916	Wild type	pAM2572	864-bp Fur fragment in *Ppu*MI site		This study

[a] Homologous recombination substrate for integration of genes into the chromosome.

[b] *conII::luxAB* reporter plasmid not archived.

[c] Mutagenesis by insertion of antibiotic resistance genes into the chromosome was mediated by homologous recombination.

[d] EMBL database accession no. Z48754 entry.

[e] AMO536 and AMO524 create a *Sal*I site that is not present in the EMBL database accession no. Z48754 entry. All pAM990 derivatives carrying a PCR-amplified fragment obtained with AMO536 have three mutated bases near the right end of the PCR fragment, and all fragments used for *luxAB* reporter experiments were cut with *Sal*I prior to cloning.

3463), encoding the entire *idiB* gene, was cloned into *Bam*HI-digested and T4 DNA polymerase-blunted overexpression vector pQE32. After induction with 50 μM isopropyl-β-D-thiogalactopyranoside (IPTG) at an OD_{600} of 0.5 and growth for an additional 1 h at 25°C to minimize the formation of insoluble protein, cells were harvested and passed twice through a prechilled French press at 137.9 MPa. The extract, containing at least 50% soluble IdiB, was centrifuged for 30 min at $12,000 \times g$ to prepare a cleared lysate for affinity purification on an Ni-nitrilotriacetic acid (NTA) matrix (Qiagen). The partially purified IdiB was used for electrophoretic mobility shift assays as previously described (24). The 222-, 163-, and 59-bp *idiA* promoter fragments used for binding and competition assays were released from pAM2405 by digestion with *Hin*dIII, *Xho*I, and *Nsi*I, gel purified, and end labeled as described previously (24), except that the binding buffer did not contain KCl. After electrophoresis (5% polyacrylamide) at 4°C, the gels were dried and images were captured with a Fujix BAS 2000 phosphor imaging system.

RESULTS

Interposon mutagenesis of upstream *idiA* gene regions located the *idiA* promoter downstream of the previously mapped 5′ end. Interposon cassettes bearing spectinomycin or kanamycin resistance genes and Ω terminators (8) were introduced at eight different locations on the *S. elongatus* chromosome, approaching the putative start codon of *idiA* at bp 2121 (Fig. 1B). All recombination events were verified by Southern analysis (data not shown). Only one of the mutant strains, AMC808, was affected in terms of IdiA expression compared to the wild type under iron-sufficient or iron-deficient growth conditions. Strain AMC808, carrying a Sp^r cassette in the putative start codon of *idiA* at bp 2121, did not show any detectable IdiA expression under either type of growth conditions. These results show that neither ORF1 nor ORF2 (both of which are upstream of *idiA*) is necessary for *idiA* expression and that *idiA* is transcribed monocistronically, as previously concluded from Northern analysis (27). Furthermore, there is no autonomously functional promoter activity downstream of bp 2121. Surprisingly, neither AMC809 (Sp^r in *Apo*I at bp 1974) nor AMC810 (Sp^r in *Sfo*I at bp 2060) showed modified expression of IdiA under iron-sufficient or -deficient growth conditions, although the interposons in those strains are between the only detectable 5′ end of *idiA* mRNA at bp 1929 and the *idiA* gene (Fig. 1B). Thus, the functional promoter and the actual transcription start site of *idiA* are downstream of bp 1929.

Initial mapping of the *idiA* promoter by *idiA*::*lacZ* reporter assays. In order to define promoter elements of the *idiA* gene, we constructed transcriptional fusions between different *idiA* upstream DNA fragments and a promoterless *E. coli lacZ* gene in a recombinational vector that targets the reporter gene to a neutral site (NS1) in the *S. elongatus* genome. Three PCR-derived fragments of the upstream DNA region of *idiA*, with two different downstream ends and three different upstream ends, were cloned into pAM990 to create reporter strains AMC802 (bp 687 to 2129), AMC805 (bp 1539 to 2299), and AMC807 (bp 1929 to 2299). *S. elongatus* reporter strains were tested for β-galactosidase activity after growth for 2 days under iron-sufficient and -deficient growth conditions. Strain AMC801, carrying a promoterless *lacZ* gene, served as a negative control. Fragments with a right endpoint (orientated as shown in Fig. 1B) at bp 2299, located in the coding region of *idiA* and downstream of a protein cleavage site of IdiA at bp 2255, showed promoter activity, whereas strain AMC802, which carried a fragment with a downstream end at bp 2129, showed no activity (Fig. 2). Strains AMC805 and AMC807 exhibited up to 20-fold increases in β-galactosidase activity
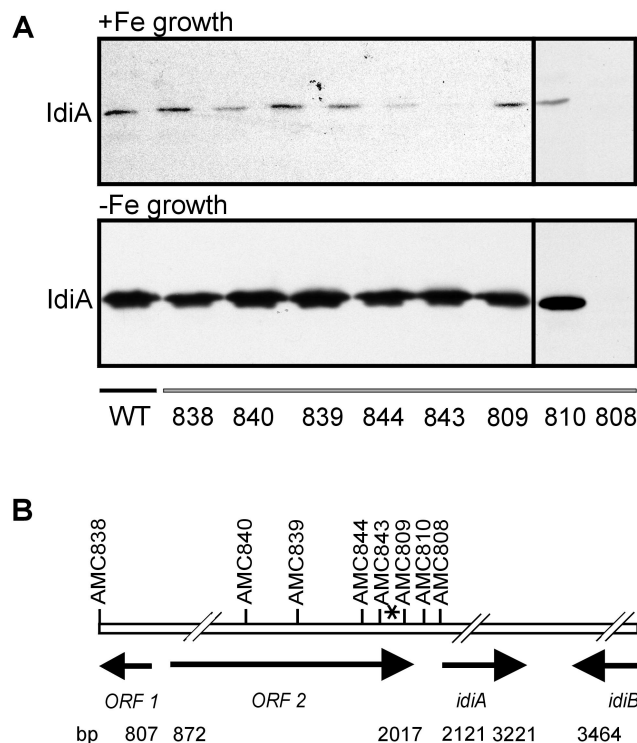
FIG. 1. (A) Comparative immunoblot with French press extracts from *S. elongatus* wild-type (WT) and mutant strains carrying antibiotic resistance cassettes in the chromosomal DNA region upstream of *idiA*. Cells were grown for 3 days under iron-sufficient conditions (+Fe growth) or iron-deficient conditions (-Fe growth). After preparation of French press extracts, samples containing 25 μg of protein were subjected to SDS-PAGE, transferred to nitrocellulose, and immunostained with an anti-IdiA antiserum (dilution, 1:20,000). (B) Location of antibiotic resistance cassettes inserted near the *idiA* locus. The AMC strains are mutants that carry insertions at different positions. The hash marks indicate breaks in the scale required to include several loci on the map. The numbers are positions in the EMBL database accession no. Z48754 entry. The asterisk indicates the position of the previously mapped 5′ transcript end.

after 2 days of growth in iron-deficient medium compared to the values obtained for iron-sufficient cultures. The fact that strain AMC807 showed inducible reporter activity indicates that the previously mapped 5′ end of the *idiA* mRNA is not the transcription start site. The uninduced level of *idiA*::*lacZ* expression in inducible strains AMC805 and AMC807 was only twice the level in strain AMC801, which carried a promoterless *lacZ* gene, indicating that the *idiA* promoter is nearly shut off under regular growth conditions. The fact that AMC802 exhibited levels of activity that are about 10% of the AMC801 background level is rather surprising, because the fragment tested comprised bp 697 to 2129 and, therefore, included the previously suggested start codon at bp 2121. However, the right endpoint of this fragment carried a 3-bp mutation, which did not include the ATG potential start codon. The sequence change was introduced to create a *Sal*I site for directional cloning into pAM1580 for finer mapping of the *idiA* promoter with *luxAB*-based reporter assays.

Fine mapping of the *idiA* promoter and identification of distinctive regulatory elements with *idiA*::*luxAB*-based re-
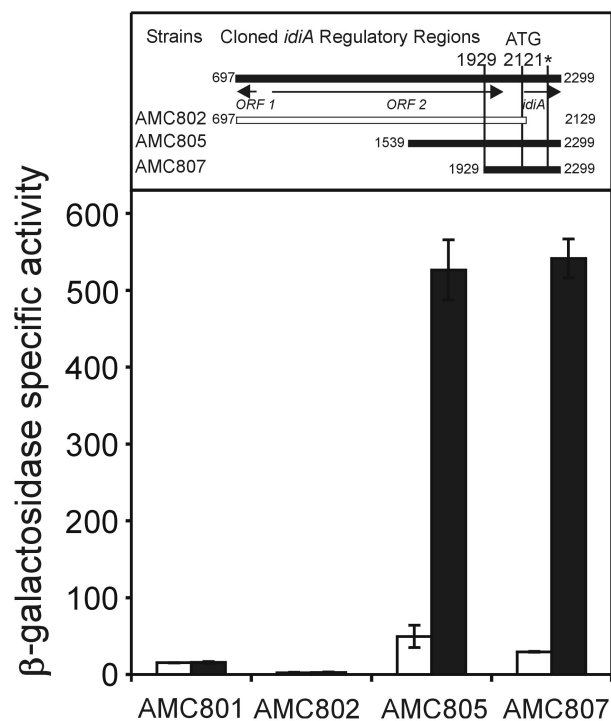
FIG. 2. β-Galactosidase activities from *idiA*::*lacZ* fusions in *S. elongatus* reporter strains after 48 h of growth under iron-sufficient (open bars) and iron-deficient (solid bars) growth conditions. At least three experiments were carried out to calculate the average activity and the standard error of the means. Strain AMC801, which carries a promoterless *lacZ* gene, was used as a negative control. The numbers given for cloned *idiA* regulatory regions are the positions in the EMBL database accession no. Z48754 entry.

porter gene assays. To narrow down the *idiA* regulatory region to a minimal functional promoter and to identify distinctive structural elements, we constructed transcriptional fusions between different *idiA* upstream fragments and promoterless *luxAB* genes from *Vibrio harveyi* in recombinational vector pAM1580, which targets the reporter gene to a specific locus (NS2) in the *S. elongatus* genome. Altogether, we created 15 PCR-derived fragments of the *idiA* DNA region with different left and right endpoints (between bp 697 and 2285). Each fragment was cloned into pAM1580 and then used to transform *S. elongatus* AMC395 (with *psbAI*::*luxCDE* genes providing aldehyde as a substrate for LuxAB bioluminescence) (Table 1). After the ability to express native IdiA in immunoblots with the anti-IdiA antiserum (data not shown) was verified, reporter strains were tested for bioluminescence under iron-sufficient and -deficient growth conditions at two time points (Fig. 3). Strain AMC811, carrying promoterless *luxAB* genes, served as a negative control, whereas AMC539 (*E. coli* consensus promoter, *conII*::*luxAB* in NS2, *psbAI*::*luxCDE* in NS1), AMC520 (*psbAII*::*luxAB* in NS2, *psbAI*::*luxCDE* in NS1), and AMC777 (−54 to +43 *psbAI*::*luxAB* in NS2, *psbAI*::*luxCDE* in NS1) were used to define the transcriptional characteristics of known *S. elongatus* promoters under iron-deficient conditions and to measure *idiA* promoter strength. As our data illustrate, none of the control strains exhibited an increase in bioluminescence with time under either iron-sufficient or iron-deficient growth conditions. Whereas AMC811 exhibited a con-

stant very low level of bioluminescence, strain AMC539 had a 50% decrease in the initial level of bioluminescence and strains AMC520 and AMC777 showed more than 75% decreases in the initial levels of bioluminescence after 48 h of growth under both types of conditions, which may have reflected physiological changes in the cultures as light penetration decreased with increasing cell density. To track down the *idiA* promoter region from the left end, we constructed reporter strains AMC814, AMC818, AMC823, and AMC824 by using a right-end primer in the coding region of *idiA* that worked in β-galactosidase assays, except that the fragment was 14 bp shorter due to *Sal*I digestion for cloning into pAM1580. All strains exhibited low initial levels of bioluminescence similar to that of AMC811 under both iron-sufficient and iron-deficient conditions 2 h after inoculation of cultures ($t_{2 h}$), verifying results obtained with the *lacZ* reporter strains and showing that transcription of *idiA* under regular growth conditions is nearly shut off independent of the growth phase. Whereas the bioluminescence at $t_{48 h}$ for all test strains grown in iron-sufficient medium remained constant or increased slightly (up to 2.5-fold), the levels of bioluminescence of these strains grown in iron-deficient medium increased tremendously. With strain AMC818 (bp 1929 to 2285) we detected a 170-fold increase in bioluminescence (when $t_{48 h}$ values obtained in the presence and in the absence of Fe were compared). The levels of induction in the inducible strains varied between 81- and 170-fold.

To narrow down the location of the *idiA* promoter from the right end, we constructed reporter strains AMC812, AMC819, AMC820, AMC821, AMC822, and AMC911 by using left-end primers that were previously shown to be sufficient for promoter activity. All strains showed a low initial rate of bioluminescence 2 h after inoculation under both iron-sufficient and iron-deficient conditions. The level of bioluminescence at $t_{48 h}$ in all strains grown in regular medium was the same as the $t_{2 h}$ value or was up to 7.5-fold greater than the $t_{2 h}$ value. After 48 h of growth under iron-deficient conditions the bioluminescence of all strains increased dramatically (when $t_{48 h}$ values obtained in the presence and in the absence of Fe were compared); the increases ranged from 44-fold for AMC822 to 74-fold for AMC911. The only exception was AMC812, which exhibited only background (promoterless) levels of bioluminescence. These findings reveal that a DNA region between bp 2115 and 2139 is essential for *idiA* promoter activity. Thus, a minimal, fully iron deficiency-inducible *idiA* promoter comprises bp 2063 (e.g., AMC824) to bp 2139 (e.g., AMC911). A closer examination of this DNA region revealed a 14-bp palindromic sequence (GTGTGCTGGCACAC) at bp 2076 that resembles binding sites of helix-turn-helix transcriptional activators, followed by a 6-bp sequence (TTGGCC) at bp 2094 that is similar to the *E. coli* −35 consensus sequence (Fig. 4). A region at bp 2118, TAAATG, may represent a −10 box. This segment carries a 3-bp mutation in the reporter fusion in AMC802, which exhibited very low noninducible β-galactosidase activity (Fig. 2). To test whether a wild-type promoter fragment with a right end at bp 2129 (as in AMC802, but without mutations) would create a fully functional *idiA* promoter fragment and to test the influence of the palindromic sequence on transcriptional activity, we created reporter strains AMC912 (bp 1929 to 2129) and AMC899 (bp 2094 to 2285, without palindrome). As strain AMC912 exhibited all of
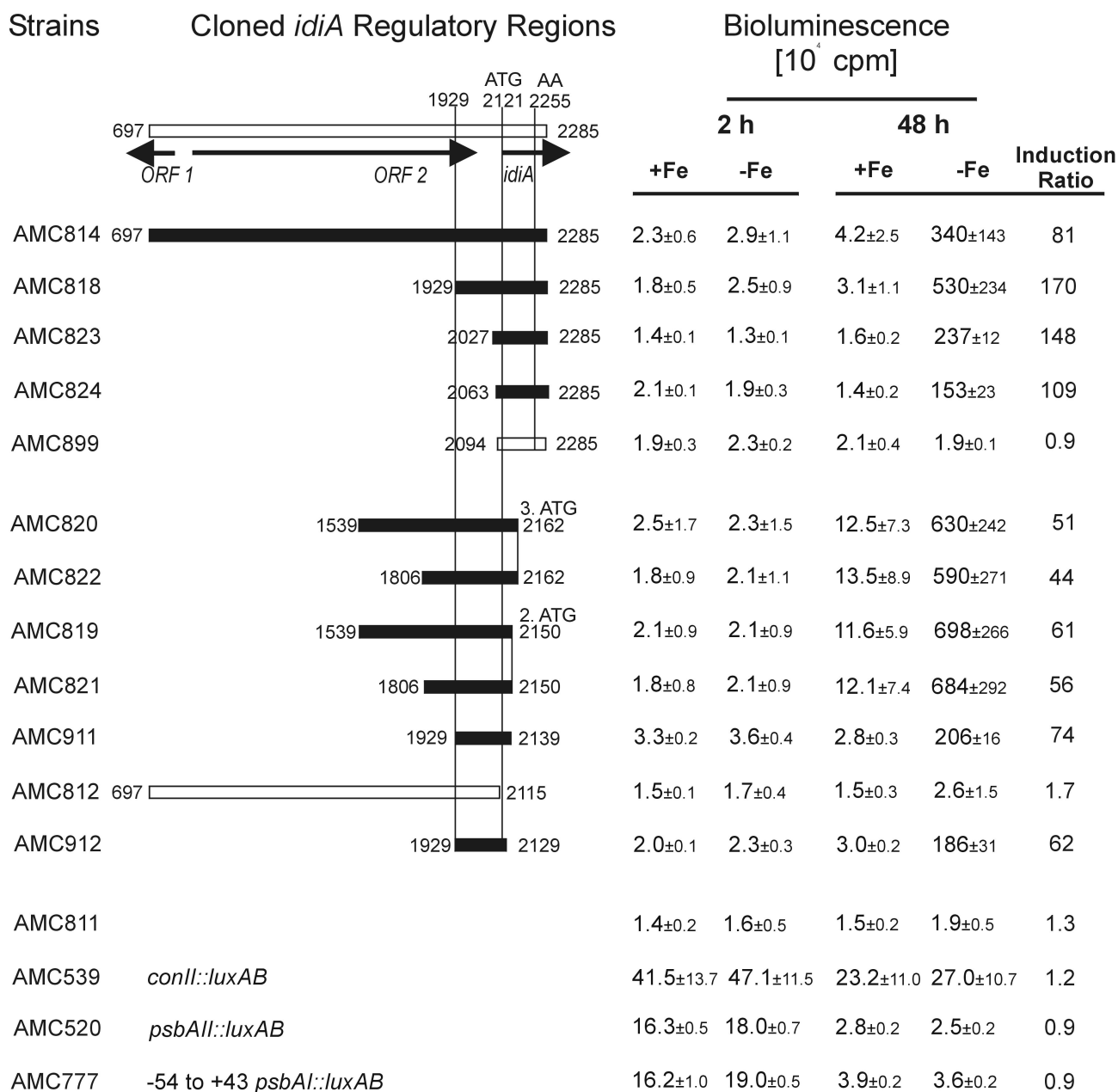
FIG. 3. Expression of *idiA*::*luxAB* fusions in *S. elongatus* reporter strains, as determined by measuring bioluminescence after growth for 2 and 48 h under iron-sufficient and -deficient conditions. The numbers above and to the left and right of bars are the positions of the ends of cloned PCR fragments with respect to the positions in the EMBL database accession no. Z48754 entry. The map at the top shows the positions of relevant open reading frames. AA indicates the position of the IdiA protein cleavage site. Strains AMC520, AMC539, and AMC777 were used as well-characterized controls for general transcriptional and translational activity. The average values and standard deviations were calculated on the basis of at least three independent growth experiments; 2 and 48 h refer to the duration of growth under iron-sufficient or -deficient conditions prior to sampling. The induction ratios are the ratios of the values obtained with iron-deficient samples to the values obtained with iron-sufficient samples after 48 h of growth.

Strains	2 h +Fe	2 h -Fe	48 h +Fe	48 h -Fe	Induction Ratio
AMC814	2.3±0.6	2.9±1.1	4.2±2.5	340±143	81
AMC818	1.8±0.5	2.5±0.9	3.1±1.1	530±234	170
AMC823	1.4±0.1	1.3±0.1	1.6±0.2	237±12	148
AMC824	2.1±0.1	1.9±0.3	1.4±0.2	153±23	109
AMC899	1.9±0.3	2.3±0.2	2.1±0.4	1.9±0.1	0.9
AMC820	2.5±1.7	2.3±1.5	12.5±7.3	630±242	51
AMC822	1.8±0.9	2.1±1.1	13.5±8.9	590±271	44
AMC819	2.1±0.9	2.1±0.9	11.6±5.9	698±266	61
AMC821	1.8±0.8	2.1±0.9	12.1±7.4	684±292	56
AMC911	3.3±0.2	3.6±0.4	2.8±0.3	206±16	74
AMC812	1.5±0.1	1.7±0.4	1.5±0.3	2.6±1.5	1.7
AMC912	2.0±0.1	2.3±0.3	3.0±0.2	186±31	62
AMC811	1.4±0.2	1.6±0.5	1.5±0.2	1.9±0.5	1.3
AMC539 conII::luxAB	41.5±13.7	47.1±11.5	23.2±11.0	27.0±10.7	1.2
AMC520 psbAII::luxAB	16.3±0.5	18.0±0.7	2.8±0.2	2.5±0.2	0.9
AMC777 -54 to +43 psbAI::luxAB	16.2±1.0	19.0±0.5	3.9±0.2	3.6±0.2	0.9

the features of inducible *idiA*::*luxAB* transcriptional activity, the mutations in AMC802 at bp 2020, 2018, and 2017 eliminated inducible promoter activity. In contrast, promoter activity was barely detectable in AMC899. Therefore, the absence of the palindromic sequences eliminated promoter activity even though the putative −35 region was still present and intact. These data are consistent with the presence of a promoter having the −35 and −10 elements shown in Fig. 4 and requiring the palindromic sequence for induction.

His-tagged IdiB binds to the upstream region of *idiA*. A gene designated *idiB* (bp 4090 to 3464), which lies downstream of *idiA* and is transcribed in the opposite direction (Fig. 1B) (27), encodes a putative helix-turn-helix DNA binding protein that is similar to another *S. elongatus* transcriptional activator,
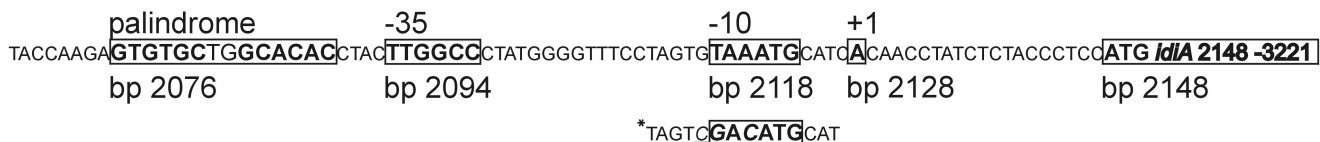
palindrome   −35   −10   +1

TACCAAGA**GTGTGC**T**GGCACAC**CTAC**TTGGCC**CTATGGGGTTTCCTAGTG**TAAATG**CATC**A**CAACCTATCTCTACCCTCC**ATG** *IdiA* **2148 -3221**

bp 2076   bp 2094   bp 2118   bp 2128   bp 2148

*TAGTC**GA**C**ATG**CAT

FIG. 4. Structural elements of the *idiA* promoter. Proposed functional elements are enclosed in boxes and labeled. The proposed start codon, the −35 and −10 sites, and the palindrome are indicated by boldface type. The bp numbers are the positions in the EMBL database accession no. Z48754 entry. Mutated bases in the AMC802 sequence compared to the wild-type sequence are italicized and underlined.

CysR, which is involved in sulfur metabolism (31). Both IdiB and CysR belong to the FNR/CAP family of transcriptional activator proteins (37). Because of the predicted function of IdiB and the limited ability of an IdiB-free mutant to express IdiA (27), we overexpressed and partially purified a His_{6x}-tagged IdiB molecule to test its DNA binding capacities in gel mobility shift experiments with *idiA* probes. After the IPTG concentration was reduced to 50 μM and the growth temperature (25°C) and time of induction (1.25 h) were reduced to prevent the formation of insoluble protein, 50% of the induced His_{6x}-tagged IdiB remained soluble in the cleared lysate and was affinity purified to homogeneity (Fig. 5A). Three DNA probes corresponding to the following regions of the *idiA* gene were prepared: (i) a 222-bp probe from bp 2063 to 2285 (including the minimal promoter and parts of the translated region), (ii) a 163-bp probe from bp 2121 to 2285 (lacking the minimal promoter but with parts of the translated region), and (iii) a 59-bp probe from bp 2063 to 2121 (including the minimal promoter fragment only). Greater amounts of IdiB bound greater amounts of the 222-bp probe (Fig. 5B). IdiB also bound to the 59-bp probe containing the minimal promoter (Fig. 5D, lane 2). Unlabeled 222-bp (Fig. 5D, lane 3) and 59-bp (Fig. 5C, lane 3) probes competed for binding, but the unla-

beled 163-bp probe did not compete (Fig. 5C and D, lanes 4). This shows that binding of IdiB to the *idiA* promoter region is specific. Moreover, IdiB never bound to the radiolabeled 163-bp probe (data not shown).

Comparative time courses of *idiA*::*luxAB* gene expression under iron-sufficient, -depleted, and -replete conditions. We predicted that if *idiA* transcription were regulated via the Fur system, *idiA*::*luxAB* transcription would stop as soon as iron entered the cells and formed stable Fe^{2+}-Fur transcription repressor complexes. To test whether *idiA* is regulated by iron in this way, we investigated the kinetic characteristics of *idiA* expression by monitoring bioluminescence over a 72-h period under standard growth conditions, under iron-depleted conditions, and after iron-replete conditions were restored (Fig. 6). As stated above, transcription of *idiA*::*luxAB* in AMC824 (bp 2063 to 2285), measured as bioluminescence, seemed to stop under iron-sufficient growth conditions. In two cultures of AMC824 grown in parallel in iron-deficient medium, the bioluminescence values increased 13- to 14-fold by 24 h and 93-fold by 47 h (compared to $t_{2 h}$ values). A linear increase in the levels of bioluminescence in the iron-depleted strains up to 48 h was verified with additional time points by growing the reporter strains on solid BG-11M medium lacking iron and
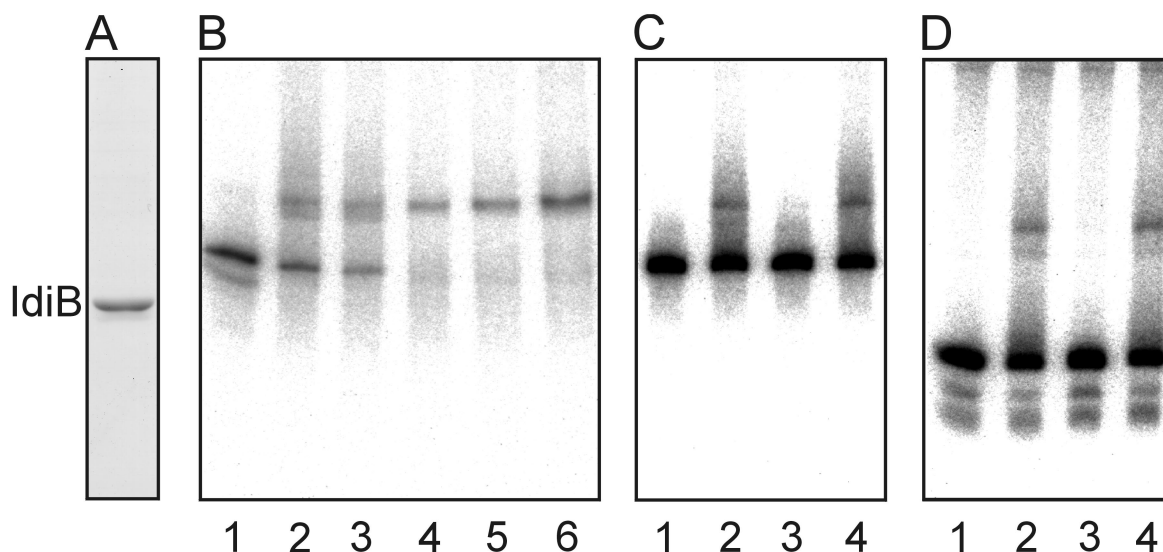

FIG. 5. Electrophoretic mobility shift assay with *idiA*-specific probes and purified His_{6x}-tagged IdiB protein. (A) Coomassie blue-stained SDS-PAGE gel of IdiB representing 15 μl of eluate from Ni-NTA affinity matrix. (B) Different amounts of IdiB eluate (0.1, 0.5, 1, 2, and 5 μl in lanes 2 to 6, respectively) were incubated with 10 to 20 pg of the radiolabeled 222-bp fragment (bp 2063 to 2285 of the accession no. Z48754 sequence). Lane 1 contained only radiolabeled probe. (C) Radiolabeled 222-bp probe was incubated with 0.5 μl of IdiB eluate (lane 2) and in the presence of unlabeled 59-bp (bp 2063 to 2121) (lane 3) and 163-bp (bp 2122 to 2285) (lane 4) competitor fragments. (D) Radiolabeled 59-bp probe was incubated with 0.5 μl of purified IdiB eluate (lane 2) and in the presence of unlabeled 222-bp (lane 3) and 163-bp (lane 4) competitor fragments. In panels B to D lane 1 contained the probe fragment with no IdiB extract added. Competitor fragments were added at a 50-fold molar excess to binding assay mixtures. All assay mixtures contained 0.5 μg of poly(dI-dC) as a nonspecific competitor.
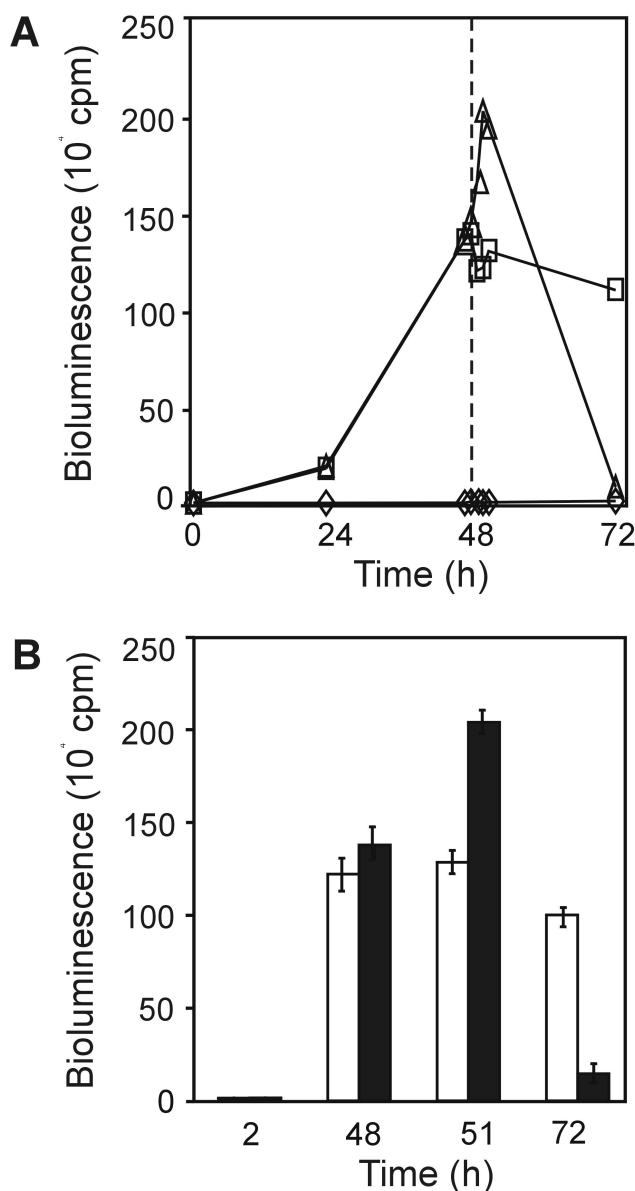
FIG. 6. Time course of *idiA*::*luxAB* gene expression in AMC824 under iron-sufficient, -depleted, and -replete conditions. Strains were grown under iron-sufficient or -deficient conditions. (A) Single time course assay with AMC824. The dashed line indicates when the regular amount of iron components in BG-11M medium (30 μM ferric ammonium citrate) was added to the iron-replete culture. Symbols: ◇, iron-sufficient conditions; □, iron-deficient conditions; △, iron-deficient and -replete conditions. (B) Average values (with error bars) for the key time points in three experiments like those whose results are shown in panel A. Open bars, iron-deficient growth conditions; solid bars, iron-deficient and -replete growth conditions.

measuring activity every 1.61 h with a cycling luminometer (TopCount; Packard Instrument Co.) (data not shown). After bioluminescence was measured at $t_{48\ h}$, we added 30 μM ferric ammonium citrate (the regular iron content of BG-11M medium) to one of the iron-depleted parallel cultures. While the strain which remained under iron-deficient growth conditions bioluminesced at a nearly constant level for at least another 24 h, the strain grown under iron-replete conditions exhibited

a burst of bioluminescence that seemed to peak at $t_{51\ h}$ (the value was 137-fold higher than the $t_{2\ h}$ value). This represented a 48% increase in bioluminescence within 3 h after repletion of iron. This increase cannot be attributed to a growth phenomenon because all values were normalized to cell number and the increase was consistently observed in multiple experiments (Fig. 6B). Despite the initial induction after iron addition, by $t_{72\ h}$ bioluminescence had decreased to almost noninduced levels. These results are inconsistent with direct regulation via the Fur system.

Insertional inactivation of *fur* in *S. elongatus* PCC 7942 creates a merodiploid mutant that shows partial derepression of *idiA* expression under iron-sufficient growth conditions. Because Fur is known to act as a repressor, we would have predicted that if *idiA* were under Fur control, the promoter analysis of *idiA* would have identified a derepressed promoter that lacked the Fur binding site. However, the only two classes of promoter fragments were the fragments that were iron inducible and the fragments that did not activate reporter expression at all. The kinetics of reporter gene expression also did not fit criteria for Fur regulation. However, because Fur is known to be involved in iron-responsive expression of at least one gene in cyanobacterial strain PCC 7942, we tested the possibility that Fur affects *idiA* expression. Attempts to construct insertionally inactivated *S. elongatus* mutants with plasmids pAM2571 (Km^r Ap^r) and pAM2572 (Cm^r Ap^r) resulted in an unusually high frequency of single-crossover mutants (11). Although double-crossover transformants (Ap^s) were obtained with both donor plasmids, Southern blot analysis and colony PCR revealed a merodiploid phenotype (unsegregated mutant and wild-type chromosomes). This result is consistent with previously published data showing that *fur* is an essential gene in *S. elongatus* PCC 7942 (10). While *fur* mutant strains AMC915 (Km^r), AMC930 (Km^r), and AMC916 (Cm^r) grew slowly on solid BG-11M agar plates, the growth rates in liquid cultures were comparable to wild-type growth rates (approximately ≥80%). Immunoblot analysis performed with an anti-IdiA antiserum and French press extracts from wild-type and mutant strains revealed that the Fur mutants have higher IdiA contents under iron-sufficient growth conditions than wild-type strains have (Fig. 7). However, the amount of detectable IdiA was only 25% of the amount in wild-type cells grown under iron-deficient conditions.

Insertional inactivation of four group II sigma factor genes (*rpoD2*, *rpoD3*, *rpoD4*, and *sigC*) in *S. elongatus* PCC 7942 has no effect on IdiA expression. We tested the hypothesis that one of four known class 2 sigma factors in *S. elongatus* PCC 7942 (12, 30) is responsible for, or at least affects, regulation of *idiA* transcription, as is true for FecI regulation of genes involved in uptake of ferric iron citrate in *E. coli* (2). We insertionally inactivated each of the group 2 sigma factor genes (GenBank accession no. AB006910, AB024709, AB024710, and AF288784) by using previously described constructs (30) and tested the corresponding mutants, AMC851 (Δ*rpoD2*), AMC852 (Δ*rpoD3*), AMC853 (Δ*rpoD4*), and AMC854(Δ*sigC*), for the ability to express IdiA under iron-sufficient and -deficient growth conditions. Immunoblot analysis showed that none of the mutants was affected in terms of its ability to express IdiA or to significantly enhance its expression under iron-limiting conditions (data not shown). We concluded that either the *idiA* promoter is redundantly
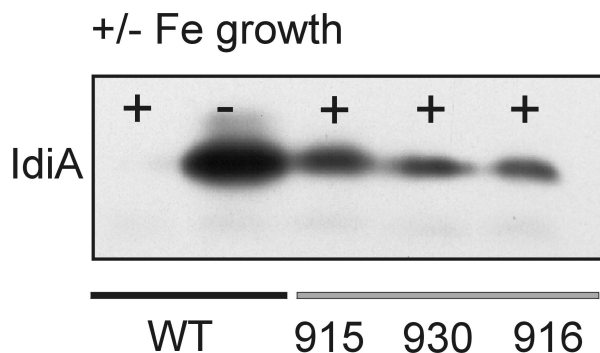
+/- Fe growth

FIG. 7. Analysis of IdiA expression in *S. elongatus* PCC 7942 and three merodiploid Δ*fur* mutants. Wild-type (WT) or *fur* mutant cultures (AMC915, AMC930, AMC916) were grown for 3 days in iron-sufficient (+) or iron-deficient (−) medium. After preparation of French press extracts, samples containing 25 μg of protein were subjected to SDS-PAGE, transferred to nitrocellulose, and immunostained with an anti-IdiA antiserum (dilution, 1:15,000).

recognized by sigma factors or expression of this promoter specifically requires a sigma factor that has not been tested yet.

DISCUSSION

Functional analysis of the *idiA* regulatory region revealed promoter elements that were not predicted by in vitro transcript mapping. Despite the consistent data obtained with four primers, a previous primer extension analysis revealed an mRNA end that cannot represent the *idiA* transcription start site; polar insertions downstream of the mapped site still allowed normal *idiA* expression (Fig. 1), and the DNA fragment that drives reporter gene expression with the characteristic properties of *idiA* regulation is located much closer to the *idiA* open reading frame (Fig. 2). Deletion analysis also indicated that unlike iron-regulated genes of the Fur regulon in many gram-negative bacteria, *idiA* is positively regulated. No deletions were capable of derepressing expression, and regulation by iron was not separable from promoter activity per se. If the −35 and −10 elements shown in Fig. 4 are the actual RNA polymerase promoter recognition sites, they seem to require the upstream palindrome for initiation of transcription. In the presence of iron or in the absence of either the palindrome or *idiB* function, *idiA* promoter activity is negligible. The data are consistent with the hypothesis that IdiB plays a role in binding to the palindrome to induce expression under iron-limiting conditions.

The start codon for *idiA* is ambiguous, as three in-frame ATG triplets precede the sequence that gives rise to the mature, processed N terminus of IdiA in vivo. Our data indicate that the first possible ATG is unlikely to be the start codon but rather is part of the −10 element of the promoter. The results obtained with reporter strains AMC802 and AMC812 showed that a fragment with a right endpoint just beyond the first ATG can drive *luxAB* expression in a transcriptional fusion when the sequence is the wild-type sequence but not when base substitutions are incorporated that would alter the proposed −10 element immediately adjacent to the ATG in question. It is unlikely that these nucleotide alterations would completely abolish transcription by modifying the context of the transla-

tion signals. Moreover, the left endpoint deletions of the *idiA* promoter leave few other options for placement of a promoter element upstream of the *idiA* open reading frame, unless the transcript has no untranslated leader, which has not been demonstrated previously in cyanobacteria. We propose that either the second or third ATG, at bp 2148 or bp 2160, is the genuine start codon for the open reading frame. However, neither of these potential start codons is preceded by a recognizable ribosome binding site.

The position of the *idiA* palindrome (<u>GTGTGCTGGCACAC</u>), 4 bp in front of a −35 box, and its position relative to the −10 box meet the expectations for the binding site for a transcriptional activator that positively interacts with the C-terminal domain of the α subunit of RNA polymerase (3, 9, 16, 39). IdiB, a putative helix-turn-helix transcription activator of the FNR/CAP family, is encoded next to *idiA* and bound specifically to the minimal *idiA* promoter comprising the palindromic sequence. Binding experiments with IdiB and a 30-bp minimal probe derived from overlapping oligonucleotides (bp 2068 to 2097) that included the palindromic sequence and a minimal flanking DNA region failed, perhaps merely because of poor stability of binding with such a small fragment of DNA (24). We believe that IdiB is the transcriptional activator of *idiA,* a hypothesis supported by the inability of an IdiB-free mutant to express IdiA at wild-type levels (27).

A search for potential IdiB binding sites of other iron-regulated genes in *S. elongatus* identified a sequence nearly identical to the *idiA* palindrome, <u>GTGTG</u>ATGC<u>CACAC</u>, upstream of the *irpA* gene, which encodes a protein that is localized at the cytoplasmic membrane and might be involved in iron acquisition (32). Located 4 bp downstream of this palindrome is a putative −35 box, TTGCCC, which is very similar in terms of sequence and spacing to the proposed −35 element of *idiA* (Fig. 4). However, neither the transcriptional start of *irpA* nor the functional promoter of *irpA* has been mapped. Binding of IdiB as an activator of *irpA* contrasts with the previous model, which suggested that *irpA* is regulated via the Fur system (32, 36). The proposed Fur box in front of *irpA*, however, is very unusual as it comprises 28 bp instead of the 19-bp consensus sequence of *E. coli*. Another *idiA* type of palindromic sequence is found upstream of *mapA* (38) but is more degenerate (<u>GTG</u>N$_9$<u>CAC</u>), and previous work placed it in the 5′ untranslated region (UTR) of the *mapA* transcript (38). However, we note that physical mapping would have placed the IdiB binding palindrome in the 5′ UTR before functional mapping revised the position of the promoter. Examination of the *idiA* homologs *slr1295, slr0513,* and *sll0237* in the *Synechocystis* sp. strain PCC 6803 genome did not reveal any *idiA* type of palindromic sequences, suggesting that IdiB is not a universal cyanobacterial iron-responsive regulatory factor.

Despite arguments against Fur regulation of *idiA,* the known role of Fur as a key iron response regulator and evidence for involvement of Fur in regulation of *isiA* led us to examine *idiA* expression in a Fur mutant. As previously shown in the *isiA* study, an attempt to insertionally inactivate *fur* in *S. elongatus* resulted in merodiploid mutants (10). Neither repeated selection on antibiotic-containing medium nor the use of different antibiotics allowed total segregation of the mutant allele. Therefore, the cyanobacterial Fur protein plays a role in me-

tabolism different from that of the *E. coli* protein, which is not essential (13). However, even without complete segregation, *fur* merodiploid mutants showed derepression of IdiA expression under iron-sufficient growth conditions. We concluded that the number of wild-type alleles and thus the number of Fur molecules per cell are reduced in the merodiploids. The effect of Fur reduction on *idiA* was surprising, especially since neither IdiA nor a protein of its size was among the proteins previously identified as derepressed in a merodiploid *S. elongatus* Fur mutant (10).

The tight regulation of reporter genes by iron suggests that the *idiA* promoter has great potential as a tool for conditional expression of heterologous genes in *S. elongatus*. When the promoter is induced by transfer to iron-deficient medium, in which *S. elongatus* cells grow robustly, the promoter strength is in the range of the promoter strength of the *psbAI* promoter, the strongest promoter characterized so far for this organism (1, 24, 30). Reporter expression remained high for at least 48 h after induction. Because *lacI*-based repression in *S. elongatus* is leaky, *idiA* may prove to be superior for conditional expression when low constitutive expression is harmful or compromises experimental design.

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