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Isolation and Nucleotide Sequence Analysis of the Ferredoxin I Gene from the Cyanobacterium *Anacystis nidulans* R2

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Two mixed oligonucleotide probes derived from conserved regions of the *Synechocystis* sp. strain PCC 6714 ferredoxin amino acid sequence were utilized to isolate an *Anacystis nidulans* R2 clone containing the ferredoxin I gene. Nucleotide sequence analysis revealed a 297-base-pair (bp) open reading frame with a deduced amino acid sequence having high homology to other cyanobacterial ferredoxins. Assuming proteolytic cleavage of the initial methionine residue, the molecular weight of the mature *A. nidulans* R2 ferredoxin was 10,370. The initial methionine residue was preceded by a probable ribosome-binding site sequence, AGGA. Northern hybridization analysis with the cloned ferredoxin gene indicated an RNA transcript of approximately 450 bp. S1 nuclease mapping localized the transcription start site to a position 64 bases upstream from the initial methionine residue. The nucleotide sequence 14 to 8 bp preceding the transcription start site resembled a typical *Escherichia coli* promoter, but the sequence in the −35 region did not. Southern hybridization detected only a single copy of the ferredoxin sequence in the *A. nidulans* R2 genome.

The nonheme, iron-sulfur protein ferredoxin is the terminal constitutent of the photosynthetic electron transport chain. In addition to its role in the photoreduction of NADP and cyclic photophosphorylation, ferredoxin has been implicated in a number of other cyanobacterial processes, including nitrogen fixation (34) and the reduction of nitrate and nitrite (21, 23). In higher plants, ferredoxin plays a role in glutamate synthesis (20), the reductive carboxylic acid cycle (4), fatty acid metabolism (4), pseudocyclic electron flow (18), sulfite reduction (30), and the light regulation of photosynthesis via the ferredoxin-thioredoxin system (3). Thus, ferredoxin plays a central role in the distribution of light-generated reducing power.

In some cyanobacteria and plants, two distinct ferredoxins are present (13, 17, 25). Amino acid sequence data (11, 12, 14) have confirmed that these two ferredoxins (designated I and II) are encoded by distinct structural genes. Gene duplication early in evolution is probably responsible for the presence of two ferredoxin genes within a single organism (13, 14). While it has been proposed that the two ferredoxins may differ in function (31), no differences have been detected to date.

In eucaryotic algae, clostridia, and cyanobacteria, ferredoxin levels appear to be regulated by iron availability (16). In the cyanobacterium *Nostoc* sp. strain MAC, both types of ferredoxin decrease dramatically as the iron concentration falls below 2 μM, with type II ferredoxin disappearing more rapidly than type I. The flavoprotein flavodoxin substitutes for ferredoxin under these conditions. Natural isolates of the cyanobacterium *Microcystis aeruginosa* have been found which contain both types of ferredoxin, only type I, or only flavodoxin, apparently because of differences in growth conditions (5). At present, little is known regarding how these genes are regulated by the available concentration of iron.

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In this communication we report the isolation and nucleotide sequence of the gene (*petF*) encoding the ferredoxin I protein from the unicellular, transformable cyanobacterium *Anacystis nidulans* R2. The nucleotide sequence data contain the coding information for a mature polypeptide of 10,370 daltons which is highly homologous to other cyanobacterial ferredoxin I proteins. Southern hybridization data indicated the presence of only one copy of this ferredoxin sequence in the *A. nidulans* R2 genome.

MATERIALS AND METHODS

Culture conditions and cyanobacterial-DNA purification. *A. nidulans* R2 was grown in BG-11 medium (1) at 29°C, either in liquid media or on plates containing 1% agar. Cells in the exponential phase of growth were harvested, and chromosomal DNA was isolated essentially as described in reference 35.

Hybridization with ^{32}P-labeled probes. Restricted genomic DNAs were electrophoresed in 0.7% agarose gels containing 80 mM Tris hydrochloride (pH 8.0), 10 mM sodium acetate, and 2 mM EDTA. Southern transfer was carried out as described in reference 22. Conditions for hybridization were as described previously (19), except that synthetic oligonucleotide probes were hybridized at 37°C. Oligonucleotide probes (generously supplied by R. Barnett, Allelix Inc.) were end labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (22). The ferredoxin clone was labeled by nick translation (19) or the random oligonucleotide priming method (8).

Library construction and DNA cloning. Plasmid clone banks were constructed by standard techniques (22) in the *Escherichia coli* vector pDPL13 (19). Ligated DNA was used to transform *E. coli* HB101 by the RbCl method (22). Large-scale *E. coli* plasmid DNA isolation was carried out as described elsewhere (19).

DNA from plasmid clone banks was electrophoresed on low-melting-temperature agarose gels and manually fractionated into several size categories. DNA was recovered from each fraction (22), and a portion was reelectrophoresed and transferred to nitrocellulose filters. The filters were then

hybridized to the oligonucleotide probes, and the remaining DNA from positive fractions was used to transform HB101. DNA was isolated from individual colonies (19) and rescreened by another round of electrophoresis, blotting, and hybridization.

Nucleotide sequence analysis. A 1.1-kilobase (kb) *Pst*I fragment was cloned into M13mp19 for DNA sequence analysis. Sequencing was carried out by the dideoxy chain termination method (29) with a universal primer (P-L Biochemicals Inc.) and ^{35}S-dATP (Amersham Corp.). Subclones for further sequencing were generated either by the sequential deletion method of Dale et al. (7) or by restricting and religating the original clones with selected restriction enzymes. Single-stranded M13 DNA was isolated as described elsewhere (7). Sequencing reactions were electrophoresed on 0.4-mm-thick 8.3 M urea–8% polyacrylamide gels, and the data were analyzed with a series of computer programs created by J. Pustell (27).

RNA isolation and transcript analysis. *A. nidulans* R2 RNA was isolated by centrifugation (4,000 × *g*, 15 min) of cells from exponentially growing cultures (approximately 1 liter) and suspension in 20 ml of 0.1 M Tris hydrochloride (pH 6.5), 6 M guanidine hydrochloride, 10 mM dithiothreitol, and 1% N-lauryl sarcosine (6). After incubation at room temperature for 15 min the suspension was passed through a French pressure cell at 16,000 lb/in^2. The suspension was centrifuged (12,000 × *g*, 10 min, 0°C) to remove insoluble material, and the supernatant was adjusted to 0.11 M sodium acetate (pH 5.5). The RNA was precipitated at −20°C overnight after the addition of 0.75 volume of ethanol. RNA was gently pelleted (3,000 × *g*, 1 h, 0°C), suspended in 8 ml of sterile distilled water, and adjusted to 0.2 g/ml of CsCl (40). This solution was layered over a 1.2-ml cushion of 5.7 M CsCl and centrifuged in a Beckman SW41 rotor at 30,000 rpm for approximately 15 h. The RNA pellet was dissolved in 0.5 ml of sterile 10 mM Tris hydrochloride (pH 8.0)–1 mM EDTA, extracted once with 2 volumes of 1:1 phenol-chloroform and once with 2 volumes of chloroform, and precipitated with 2 volumes of ethanol.

RNA was electrophoresed on 1.1% agarose gels after denaturation with glyoxal and dimethyl sulfoxide (27). Northern blotting and hybridization were done by the method of Thomas (37). For mapping the 5′ end of the ferredoxin transcript, the 1.1-kb *Pst*I fragment was restricted with *Asp*718 (a *Kpn*I isoschizomer which leaves 5′ overhanging ends), treated with phosphatase (22), and purified on a low-melting-temperature agarose gel (22). The recovered fragment was end labeled with T4 polynucleotide kinase, and 0.1 μg was mixed with 80 μg of *A. nidulans* R2 RNA. Hybridization and S1 nuclease digestion were carried out as described elsewhere (22). Samples were extracted with phenol-chloroform, precipitated with ethanol, and suspended in 90% formamide–0.1% xylene cyanol–0.1% bromophenol blue for analysis on either 8 M urea–5% polyacrylamide gels or sequencing gels. End-labeled fragments of a *Taq*I digest of pBR322 or a nucleotide sequence ladder of M13mp19, respectively, were used as size markers.

RESULTS

Cloning and sequence analysis of the *A. nidulans* ferredoxin gene. The amino acid sequences of ferredoxins from a wide range of cyanobacteria are relatively homologous, with approximately 40 invariant residues (10). To isolate the ferredoxin gene from *A. nidulans* R2, two conserved regions from the *Synechocystis* sp. strain 6714 amino acid sequence

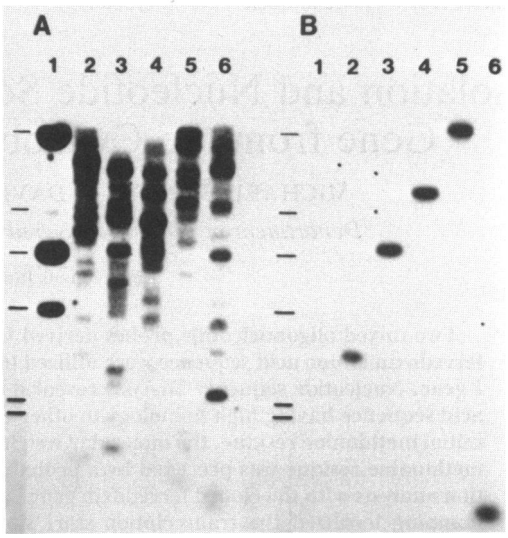

FIG. 1. Hybridization of oligonucleotide probes to *A. nidulans* R2 DNA. (A) Hybridization with probe I (see text). (B) Hybridization with probe II. Lanes in both panels are as follows: 1, *Hin*dIII digest of lambda DNA; 2 through 6, *A. nidulans* R2 DNA digested with *Eco*RI, *Hin*dIII, *Bam*HI, *Xho*I, and *Cla*I, respectively. Molecular weight markers (*Hin*dIII-digested lambda DNA) are indicated by the horizontal marks and correspond to sizes of 23.1, 9.4, 6.7, 4.3, 2.3, and 2.0 kb.

(amino acids 65 to 71 and 88 to 94) (10) were selected as the basis for oligonucleotide probes. The sequences for probe I were

```
5′ GAC GAC GAC CAA ATC GAA GC 3′
     T   T   T   G   T
   Asp Asp Asp Gln Ile Glu Ala
```

The sequences for probe II were

```
5′ GAA ACC CAC AAA GAA GAA GA 3′
       T       G
   Glu Thr His Lys Glu Glu Asp
```

For each codon, base composition was predicted on the basis of codon usage information from the nucleotide sequence analysis of other cyanobacterial genes (reference 36 and references therein). Both probes are 20 bases long and contain a mixture of oligonucleotides. Probe I has either of two bases incorporated at five positions, while probe II incorporates two bases at two positions.

The two oligonucleotide mixtures were used to probe Southern blots of restricted *A. nidulans* R2 genomic DNA. A large number of hybridization signals were obtained with probe I, while a single hybridization band was obtained for each restriction enzyme digest with the second ferredoxin probe (Fig. 1). The lack of specificity of probe I is probably due to the large number (32) of different oligonucleotides present in the mixture. Increased hybridization stringency resulted in an overall decrease in signal intensity rather than a significant decrease in the number of signals (results not shown). However, one band in each of the probe I lanes comigrated with the bands hybridized by probe II.

To clone the sequence homologous to ferredoxin probe II, a *Hin*dIII plasmid library of *A. nidulans* R2 genomic DNA was constructed and screened as described in Materials and Methods. Several plasmid recombinants gave strongly hybridizing signals, and one of them, pFAN1, was studied further. This clone contained a total insert of 9.8 kb consist-

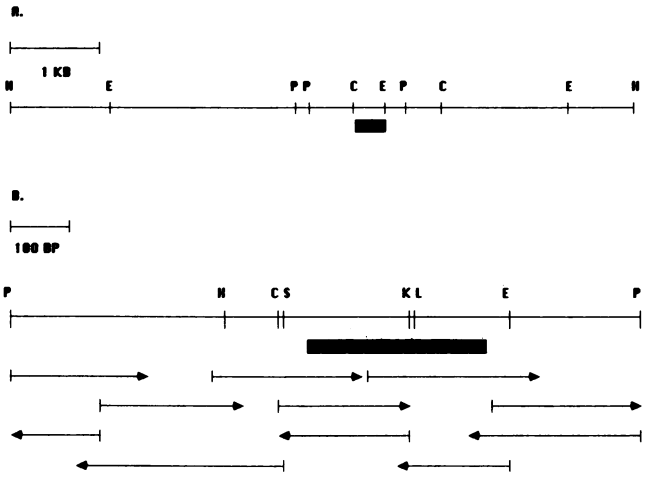

FIG. 2. (A) Restriction map of the large *Hin*dIII fragment of pFAN1. Only sites for *Cla*I, *Eco*RI, *Hin*dIII, and *Pst*I are indicated. (B) Restriction map and sequencing strategy of the *A. nidulans* R2 *Pst*I fragment containing the ferredoxin gene. Arrows below the restriction map indicate the direction and extent of DNA regions analyzed by the chain termination sequencing procedure. Letters indicate restriction enzymes as follows: C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; *K*, *Kpn*I; P, *Pst*I; S, *Sac*I; L, *Sal*I; X, *Xba*I. The coding regions of the ferredoxin gene are indicated by the black boxes.

ing of three *Hin*dIII fragments. Further subcloning localized the hybridization signal to a 1.1-kb *Pst*I fragment which was contained entirely within the largest *Hin*dIII fragment (7.0 kb) of pFAN1 (Fig. 2A). The *Pst*I fragment was subcloned into M13mp19 and sequenced. The sequencing strategy and a detailed restriction map of this fragment are shown in Fig. 2B.

The nucleotide sequence of the *A. nidulans* R2 ferredoxin gene is shown in Fig. 3. Based on the amino acid sequences of other cyanobacterial ferredoxins, the mature protein begins at the alanine residue (25). The deduced amino acid sequence codes for a protein containing 98 amino acids with a calculated molecular weight of 10,370 and an estimated pI of 4.6. These values are typical of plant ferredoxins. The protein encoded by this gene appeared to be more similar to cyanobacterial type I ferredoxins than to type II (24). This gene was approximately 65% homologous to the *Silene pratensis* ferredoxin gene at the nucleotide level (33). The two oligonucleotide probes had 95 and 100% homology, respectively, with the *A. nidulans* R2 ferredoxin gene sequence.

Several cyanobacteria have been shown to contain two forms of ferredoxin which differ in amino acid sequence (11, 14). Southern hybridization (at low stringency conditions: hybridization in 6× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate] at 60°C, washing in 2× SSC, 50°C) with a 0.95-kb *Cla*I fragment containing the *A. nidulans* R2 ferredoxin gene (Fig. 2A) indicated that only one copy of the *petF* gene was present in the genome (Fig. 4). The sizes of the faint higher-molecular-weight bands in Fig. 4A, lanes 1 and 2, were consistent with their being partial digestion products. However, given the substantial differences between the amino acid sequences of cyanobacterial type I and II ferredoxins (*Nostoc* sp. strain MAC has 34 amino acid differences between types I and II; *Aphanothece sacrum* has 38 such differences), it is possible that the two types of ferredoxin genes might not hybridize under these conditions.

Transcript and promoter analysis. The length of the ferredoxin transcript was determined by hybridizing a ferredoxin gene probe to Northern blots of *A. nidulans* R2 RNA. The ferredoxin transcript was approximately 450 bases long (Fig. 5). Given the length of the ferredoxin gene, it was the only gene encoded on this transcript.

The 5′ end of the transcript was determined by S1 nuclease protection experiments (Fig. 6). In these experiments, the 5′ end of the transcript occurred approximately 237 bases from the first C in the *Asp*718 restriction site. This placed the transcript start at position −64 (Fig. 3). Consistent with these data was the presence of the sequence TAGTAT 8 bases upstream of the start site. This sequence was homologous to the −10 region of the consensus *E. coli* promoter (28). However, the sequence from the region in which one would expect to find the −35 region of the promoter (GCAGTT) was in no way similar to the *E. coli* consensus sequence (TTGACA). These promoter regions were also very different from the consensus *nif* type promoters which regulate cyanobacterial genes involved in nitrogen fixation (15).

Approximately 50 base pairs downstream from the *petF*

```
-240        -230        -220        -210        -200        -190
ACT AAT GCT TAG CTT AGA GGG CTT ACT GGG AGC GGG CCG AGT TTG AGC CGT GAT TAC CCC

-180        -170        -160        -150        -140        -130
TAC GAA CTT TCC GGC CAC GCT CCA TTG CTT AGA CAT AAA ATT CCC TTA TGT CTA GAC TGG

-120        -110        -100        -90         -80         -70
CGA TTG ATA GCA TTT CTC GCG GCG CAG TTC GCC CTT TGG CAA CCC ATA GTA TCA ATG GGA

-60         -50         -40         -30         -20         -10
AAG GTA CGG GCA GGC TGT CAA TCG ATG AGC TCT GCC ACC CCA AAA GCG ATA GAG GAC ACG

 1          10          20          30          40          50
CTC ATG GCA ACC TAC AAG GTT ACG CTC GTC AAT GCT GCC GAA GGC TTG AAC ACC ACG ATC
 Met Ala Thr Tyr Lys Val Thr Leu Val Asn Ala Ala Glu Gly Leu Asn Thr Thr Ile

 60          70          80          90          100         110
GAC GTG GCT GAC GAT ACC TAC ATC TTG GAC GCC GCT GAA GAG CAA GGC ATT GAC CTG CCT
Asp Val Ala Asp Asp Thr Tyr Ile Leu Asp Ala Ala Glu Glu Gln Gly Ile Asp Leu Pro

 120         130         140         150         160         170
TAC TCC TGC CGT GCT GGT GCT TGC TCG ACC TGT GCT GGC AAA GTC GTC TCT GGT ACC GTC
Tyr Ser Cys Arg Ala Gly Ala Cys Ser Thr Cys Ala Gly Lys Val Val Ser Gly Thr Val

 180         190         200         210         220         230
GAC CAA TCG GAT CAA TCC TTC TTG GAT GAC GAC CAA ATT GCA GCA GGC TTT GTC CTG ACC
Asp Gln Ser Asp Gln Ser Phe Leu Asp Asp Asp Gln Ile Ala Ala Gly Phe Val Leu Thr

 240         250         260         270         280         290
TGC GTC GCC TAT CCG ACC TCC GAT GTG ACG ATC GAA ACC CAC AAA GAA GAA GAC CTC TAC
Cys Val Ala Tyr Pro Thr Ser Asp Val Thr Ile Glu Thr His Lys Glu Glu Asp Leu Tyr

 300         310         320         330         340         350
TAA GTC TTG CTT CGA CTG CTT CAA TCC TTA GAA TTC AAA TCA AAT TGC GGC TTC CAA ATT
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 360         370         380         390         400         410
GGG AGT CGC TTT TTT ATC GCC GTA GTC AGC AGC GAT CGC TCA CTA CCG TCC AGA AAC AGC
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 420         430         440         450         460         470
CTG CGA TCG CCC TGA CTT AAC GCC CCT CCC CTA GCA ATC CTG ACT CGG TAG AGT TCA GGC

 480         490         500         510         520         530
CAA GGA CTC AAG GTT GAG GCG ATC GTT CAG CAA CGT CTT GCT CTA GGC GTC CGG CGT GAC

 540         550         560         570
TGT TGT GGG GAA GAG CGA TCG CCT TCC TGC AG
```

FIG. 3. Nucleotide sequence of the *A. nidulans* ferredoxin gene. The sequence presented begins approximately 110 base pairs upstream from the *Xba*I site and ends at the 3′ *Pst*I site. Promoter regions are underlined. The transcription start site is indicated by the arrowhead. The ribosome-binding-site sequence is double underlined. The transcription termination stem-and-loop structure is indicated by the arrows.

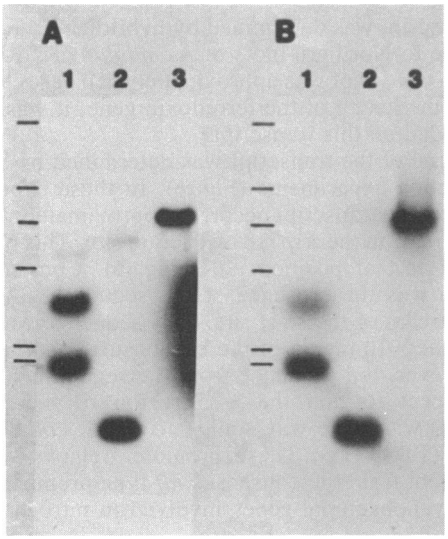

FIG. 4. Hybridization of the 0.95-kb *Cla*I fragment containing the ferredoxin gene to (A) *A. nidulans* R2 DNA and (B) pFAN1 DNA. In both panels DNAs were digested with *Eco*RI (lane 1), *Cla*I (lane 2), and *Hin*dIII (lane 3). Molecular weight markers are as described in the legend to Fig. 1.

termination codon was a palindromic sequence (Fig. 3) which appears to be a typical procaryotic transcription terminator (28). The palindromic structure (nucleotides 344 to 366) had a 9-base inverted repeat sequence (7 of which were complementary) and a loop of 5 bases. The stem structure was G-C rich (14 of 18 bases) and was followed by a series of six T residues. Both of these features are common in procaryotic transcription terminators (28). If one assumes that the 3′ end of the ferredoxin transcript occurred at the end of this stem-and-loop structure (position 366), the length of the transcript was then 430 bases. This length is in close agreement with the estimate from the Northern hybridization analysis.

Within the ferredoxin mRNA, there were two possible methionine codons at which translation could be initiated (Fig. 3, positions −39 and 1). However, only one of these methionine codons (position 1) was associated with a ribosome binding sequence. The sequence AGGA, which is complementary to the 3′ end of the *A. nidulans* 16S rRNA sequence (2, 38) was found 7 bases upstream from the initiator codon. The observation that all ferredoxin amino acid sequences begin with an alanine residue (25) suggests that the methionine is proteolytically removed after translation, as has been reported for several cyanobacterial proteins (36, 39).

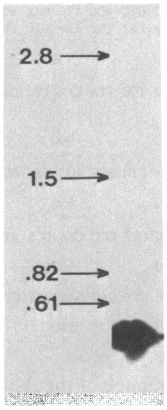

FIG. 5. Northern hybridization analysis. Total *A. nidulans* RNA (20 μg) was denatured, electrophoresed, blotted, and hybridized to a 0.61-kb *Sac*I fragment containing the ferredoxin gene. Size markers (in kilobases) are the rRNAs (two largest markers) and two DNA restriction fragments from pFAN1.

DISCUSSION

DNA sequence analysis of the *A. nidulans* R2 ferredoxin I gene revealed the presence of a 297-base-pair open reading frame with a deduced amino acid sequence having high homology to other cyanobacterial ferredoxins. This gene was transcribed as an approximately 430-base-pair monocistronic mRNA. The initial methionine of the primary translation product appeared to be posttranslationally processed to produce a mature protein of 98 amino acids.

Several regulatory sequences from the *A. nidulans* ferredoxin gene conformed to the features of typical *E. coli* control regions, including the transcription terminator and ribosome-binding site. However, the apparent promoter region of the *petF* gene resembled the *E. coli* promoter only in the −10 region. The −35 region (GCAGTT) showed no homology to either the *E. coli* consensus promoter or to other cyanobacterial promoters (15, 26, 32). The only other *A. nidulans* promoter sequence published, that for the large and small subunits of ribulose bisphosphate carboxylase (32), also has an *E. coli*-like −10 region (TAGGTT) but an unusual −35 region (CAATAA). A similar promoter is found for these same genes in the heterocyst-forming cyanobacterium, *Anabaena* sp. strain 7120 (26). Isolated *Anabaena* RNA polymerase transcribes efficiently from this promoter, suggesting that it may not necessarily recognize

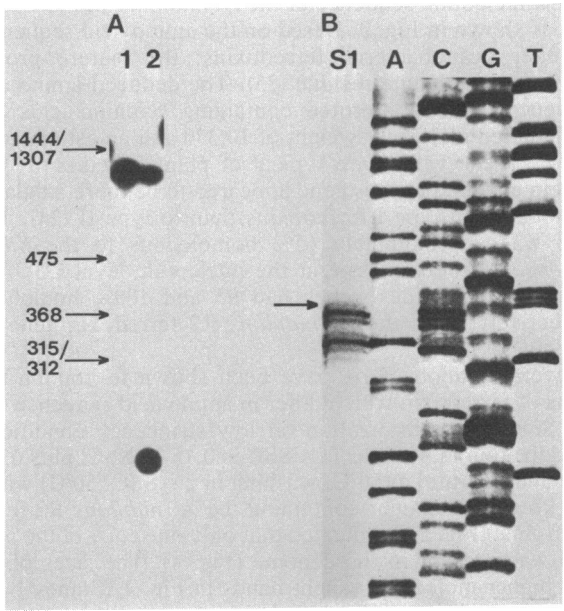

FIG. 6. Determination of the ferredoxin transcription start site. (A) S1 nuclease-protected fragments were electrophoresed on an 8 M urea–5% polyacrylamide gel. Lane 1, Undigested, end-labeled *Asp*718 fragment; lane 2, S1 protected fragments. Size markers (in base pairs) were an end-labeled *Taq*I digest of pBR322. (B) High-resolution S1 mapping. S1 nuclease-protected fragments were electrophoresed next to a DNA sequencing ladder of M13mp19. The largest protected fragment (arrow) is 237 base pairs.

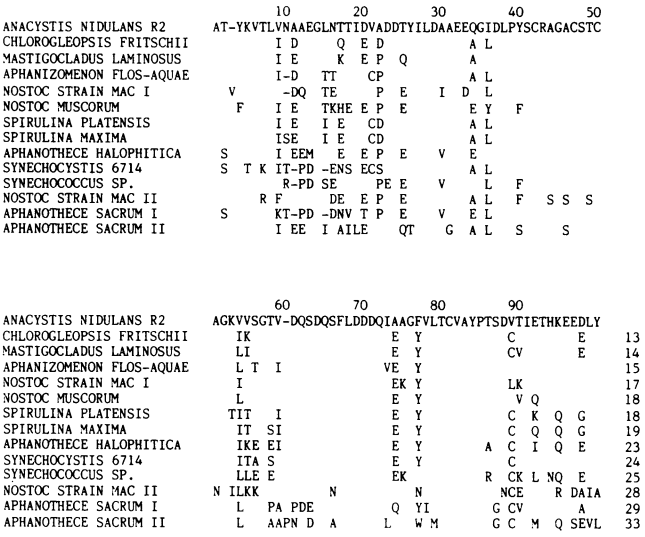

FIG. 7. Comparison of cyanobacterial ferredoxin amino acid sequences. All sequences other than *A. nidulans* R2 are from reference 24. A, Alanine; D, aspartic acid; E, glutamic acid; G, glycine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan. Dashes denote gaps introduced to maximize homology. Numbers at the end of each sequence indicate the number of amino acid differences relative to *A. nidulans* R2.

the consensus *E. coli* promoter (15). However, the *A. nidulans* ferredoxin gene promoter differs substantially from the ribulose bisphosphate carboxylase promoter. As this gene may be regulated by iron availability, it is possible that an activating protein which binds near the promoter is required for expression. Further investigation of the ferredoxin promoter regions will allow an understanding of the regulation of this gene.

When the deduced amino acid sequence of the *A. nidulans* R2 ferredoxin protein was compared with those of other cyanobacterial ferredoxins, the number of amino acid differences ranged from 13 to 33 (Fig. 7). The *A. nidulans* ferredoxin appeared to be more homologous to ferredoxins from filamentous cyanobacteria than to those from the two unicellular cyanobacteria (*Aphanothece sacrum* and *Synechocystis* sp. strain 6714) investigated. Previous comparisons (10) between the ferredoxins of unicellular and filamentous cyanobacteria have noted a similar overall lack of homology among unicellular species. To reconcile this difference with the traditional taxonomic divisions of the cyanobacteria (9), Hase et al. (10) have proposed that the presence of two gaps (positions 11 and 15) in the amino acid sequence of ferredoxins from unicellular species is the characteristic that distinguishes these species from filamentous ones. However, the ferredoxin from *A. nidulans*, a unicellular species, did not conform to this proposal. On the basis of overall amino acid homology, one must conclude that either cyanobacterial taxonomy requires massive revision or that plant ferredoxins are evolving too quickly to be useful taxonomic tools. Indeed, exceptionally high evolutionary mutation rates have been calculated for plant ferredoxins (24), and phylogenetic trees constructed for higher plant ferredoxins show little resemblance to traditional taxonomy, suggesting that ferredoxins are not a good general taxonomic indicator.

The isolation and sequencing of the *A. nidulans* R2 *petF* gene is the first step in understanding the gene regulation and expression of the ferredoxin polypeptide. Since ferredoxin is involved in a large number of metabolic processes because of its role as a distributor of light-generated reducing power, it is critical to understand the effects of environmental influences on the expression of this important protein.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Allen, M.** 1968. Simple conditions for growth of unicellular blue-green algae on plates. J. Phycol. **4**:1–3.
2. **Borbely, G., and A. Simoncsits.** 1981. 3′-Terminal conserved loops of 16S rRNAs from the cyanobacterium *Synechococcus* AN PCC 6301 and maize chloroplast differ only in two bases. Biochem. Biophys. Res. Commun. **101**:846–852.
3. **Buchanan, B. B.** 1984. The ferredoxin/thioredoxin system: a key element in the regulatory function of light in photosynthesis. BioScience **34**:378–383.
4. **Cammack, R., K. K. Rao, and D. O. Hall.** 1981. Metalloproteins in the evolution of photosynthesis. Biosystems **14**:57–80.
5. **Cohn, C. L., J. Alam, and D. W. Krogmann.** 1985. Multiple ferredoxins from cyanobacteria. Physiol. Veg. **23**:659–667.
6. **Conley, P. B., P. G. Lemaux, and A. R. Grossman.** 1985. Cyanobacterial light-harvesting complex subunits encoded in two red light-induced transcripts. Science **230**:550–553.
7. **Dale, R. M. K., B. A. McClure, and J. P. Houchins.** 1985. A rapid single-stranded cloning strategy for producing a sequential series of overlapping clones for use in DNA sequencing. Application to sequencing the corn mitochondrial 18S rRNA. Plasmid **13**:31–40.
8. **Feinberg, A. P., and B. Vogelstein.** 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **137**:266–267.
9. **Fogg, G. E., W. D. P. Stewart, P. Fay, and A. E. Walsby.** 1973. The blue-green algae. Academic Press, Inc., New York.
10. **Hase, T., K. Inoue, H. Matsubara, M. M. Williams, and L. J. Rogers.** 1982. Amino acid sequence of *Synechocystis* 6714 ferredoxin: a unique structural feature of unicellular blue-green algal ferredoxin. J. Biochem. **92**:1357–1362.
11. **Hase, T., H. Matsubara, G. N. Hutber, and L. J. Rogers.** 1982. Amino acid sequences of *Nostoc* strain MAC ferredoxins I and II. J. Biochem. **92**:1347–1355.
12. **Hase, T., K. Wada, and H. Matsubara.** 1976. Amino acid sequence of the major component of *Aphanothece sacrum* ferredoxin. J. Biochem. **79**:329–343.
13. **Hase, T., K. Wada, and H. Matsubara.** 1977. Horsetail (*Equisetum arvense*) ferredoxins I and II. Amino acid sequences and gene duplication. J. Biochem. **82**:277–287.
14. **Hase, T., S. Wakabayashi, K. Wada, and H. Matsubara.** 1978. Amino acid sequence of *Aphanothece sacrum* ferredoxin II (minor component). J. Biochem. **83**:761–770.
15. **Haselkorn, R., S. E. Curtis, J. W. Golden, S. A. Nierzwicki-Bauer, S. J. Robinson, and N. E. Tumer.** 1985. Organization and transcription of *Anabaena* genes regulated during heterocyst differentiation, p. 443–453. *In* L. van Vlotten Doting, G. S. T. Groot, and T. C. Hall (ed.), Molecular form and function of the plant genome. Plenum Publishing Corp., New York.
16. **Hutber, G. N., K. G. Hutson, and L. J. Rogers.** 1977. Effect of iron deficiency on levels of two ferredoxins and flavodoxin in a cyanobacterium. FEMS Microbiol. Lett. **1**:193–196.
17. **Hutson, K. G., L. J. Rogers, B. G. Haslett, D. Boulter, and R. Cammack.** 1978. Comparative studies on two ferredoxins from the cyanobacterium *Nostoc* strain MAC. Biochem. **172**: 465–477.

18. **Ivanov, B. N., T. P. Red'ko, V. L. Shmeleva, and E. N. Mukhim.** 1980. Participation of ferredoxin in pseudo-cyclic electron-transport in isolated pea chloroplasts. Biokhimiya **45**:1078–1084.
19. **Laudenbach, D. E., N. A. Straus, and J. P. Williams.** 1985. Evidence for two distinct origins of replication in the large endogenous plasmid of *Anacystis nidulans* R2. Mol. Gen. Genet. **199**:300–305.
20. **Lea, P. J., and B. Miflin.** 1974. Alternative route for nitrogen assimilation in higher plants. Nature (London) **251**:614–616.
21. **Losada, M., and A. Paneque.** 1971. Nitrite reductase. Methods Enzymol. **23A**:487–491.
22. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Manzano, C., P. Cardau, C. Gomez-Morano, A. M. Relimpio, and M. Losada.** 1976. Ferredoxin-dependent photosynthesis reduction of nitrate and nitrite by particles of *Anacystis nidulans*. Mol. Cell. Biochem. **10**:161–169.
24. **Matsubara, H., and T. Hase.** 1983. Phylogenetic consideration of ferredoxin sequences in plants, particularly algae, p. 168–181. *In* U. Jensen and D. E. Fairbrothers (ed.), Proteins and nucleic acids in plant systematics. Springer-Verlag, Berlin.
25. **Matsubara, H., T. Hase, S. Wakabayashi, and K. Wada.** 1978. Gene duplications during evolution of chloroplast-type ferredoxins, p. 209–219. *In* H. Matsubara and T. Yamada (ed.), The evolution of protein molecules. Japan Scientific Societies Press, Tokyo.
26. **Nierzwicki-Bauer, S. A., S. E. Curtis, and R. Haselkorn.** 1984. Cotranscription of genes encoding the small and large subunits of ribulose-1,5-bisphosphate carboxylase in the cyanobacterium *Anabaena* 7120. Proc. Natl. Acad. Sci. USA **81**:5961–5965.
27. **Pustell, J., and F. C. Kafatos.** 1984. A convenient and adaptable package of computer programs for DNA and protein sequence management, analysis and homology determinations. Nucleic Acids Res. **12**:643–655.
28. **Rosenberg, M., and D. Court.** 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13**:319–353.
29. **Sanger, F., S. Nicklen, and A. R. Coulsen.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
30. **Schmidt, A., and A. Trebst.** 1969. The mechanism of photosynthetic sulfate reduction by isolated chloroplasts. Biochim. Biophys. Acta **180**:529–535.
31. **Shin, M., M. Sukenobu, R. Oshino, and Y. Kitazume.** 1977. Two plant-type ferredoxins from a blue-green alga, *Nostoc verrucosum*. Biochim. Biophys. Acta **460**:85–93.
32. **Shinozaki, K., and M. Suguira.** 1985. Genes for the large and small subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase constitute a single operon in a cyanobacterium, *Anacystis nidulans* 6301. Mol. Gen. Genetic. **200**:27–32.
33. **Smeekens, S., J. van Binsbergen, and P. Weisbeek.** 1985. The plant ferredoxin precursor: nucleotide sequence of a full length cDNA clone. Nucleic Acids Res. **9**:3179–3194.
34. **Smith, R. V., R. J. Noy, and M. C. Evans.** 1971. Physiological electron donor systems to the nitrogenase of the blue-green alga *Anabaena cylindrica*. Biochim. Biophys. Acta **253**:104–109.
35. **Tandeau de Marsac, N., W. E. Borrias, C. J. Kuhlemeier, A. M. Castets, G. A. van Arkel, and C. A. M. J. J. van de Hondel.** 1982. A new approach for molecular cloning in cyanobacteria: cloning of an *Anacystis nidulans met* gene using a Tn*901*-induced mutant. Gene **20**:111–119.
36. **Tandeau de Marsac, N., D. Mazel, D. A. Bryant, and J. Houmard.** 1985. Molecular cloning and nucleotide sequence of a developmentally regulated gene from the cyanobacterium *Calothrix* PCC 7601: a gas vacuole protein gene. Nucleic Acids Res. **20**:7223–7236.
37. **Thomas, P. S.** 1983. Hybridization of denatured RNA transferred or dotted to nitrocellulose paper. Methods Enzymol. **100**:255–266.
38. **Tomioka, N., and M. Suguira.** 1983. The complete nucleotide sequence of a 16S ribosomal RNA gene from a blue-green alga, *Anacystis nidulans*. Mol. Gen. Genet. **191**:46–50.
39. **Tumer, N. E., S. J. Robinson, and R. Haselkorn.** 1983. Different promoters for the *Anabaena* glutamine synthetase gene during growth using molecular or fixed nitrogen. Nature (London) **306**:337–342.
40. **Turpen, T. H., and O. M. Griffith.** 1986. Rapid isolation of RNA by a guanidium thiocyanate/cesium chloride gradient method. BioTechniques **4**:11–15.