

**Plant Gene Register**

# Molecular Analysis of the *psaC* Gene Encoding the F<sub>A</sub>/F<sub>B</sub> Apoprotein of Photosystem I in the Filamentous Cyanobacterium *Anabaena variabilis* ATCC 29413<sup>1</sup>

R. Mannar Mannan and Himadri B. Pakrasi\*

Department of Biology, Box 1137, Washington University, St. Louis, Missouri 63130–4899

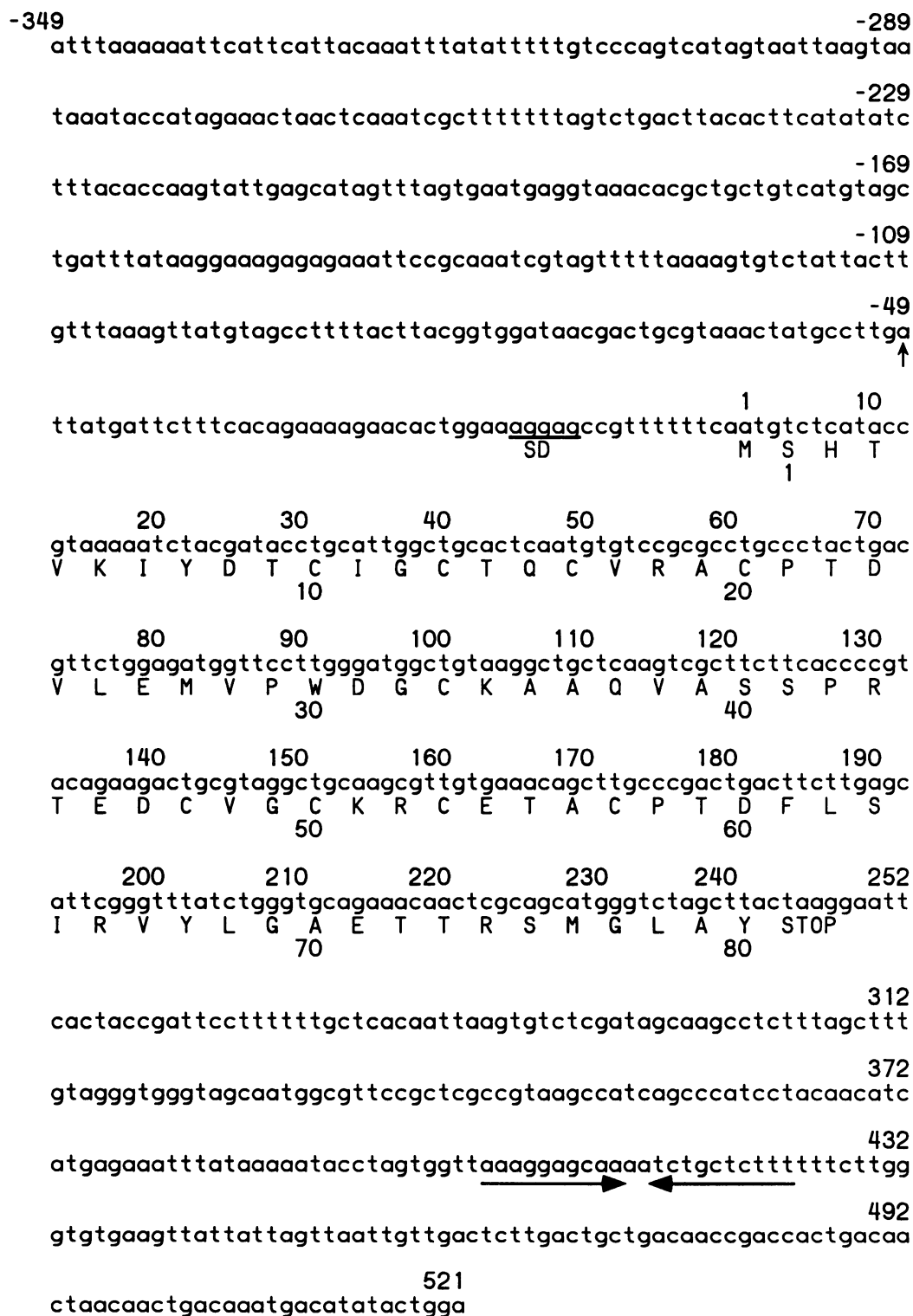
In cyanobacteria, green algae, and higher plants, the *psaC* gene encodes PsaC, the apoprotein for two iron-sulfur clusters, F<sub>A</sub> and F<sub>B</sub>, located in the PSI complex in the thylakoid membrane. F<sub>A</sub> and F<sub>B</sub> act as the terminal electron acceptors in the PSI complex (reviewed in ref. 2). Our research objective is to develop a genetic system in which various hypotheses concerning the structural and functional roles of the PsaC protein can be tested. Our experimental organism is the filamentous nitrogen-fixing cyanobacterium *Anabaena variabilis* ATCC 29413 (henceforth called *Anabaena* 29413) for the following reasons: (a) *Anabaena* 29413 is capable of dark chemotrophic growth (8), and (b) it is possible to introduce exogenous DNA into these cells *via* conjugal transfer from *Escherichia coli* cells (1). In addition, positive selection vectors have been constructed so that gene replacement mutations may be created *via* homologous double-reciprocal recombination events (4). As a first step in the molecular genetic analysis of the PsaC protein, we recently engineered and characterized a *psaC* interruption mutant of *Anabaena* 29413 (Table I) (5).

Figure 1 shows the nucleotide sequence of the *psaC* gene and its flanking regions. It also shows the derived amino acid sequence of the PsaC protein. A sequence of 37 N-terminal amino acid residues of the PsaC polypeptide from a purified PSI preparation from *Anabaena* 29413 matches completely with the amino acid sequence deduced from the nucleotide sequence except the first Met residue (K. Nyhus, M. Ikeuchi, Y. Inoue, J. Whitmarsh, and H.B. Pakrasi, manuscript in preparation). Thus, the N-terminal Met residue of this protein is posttranslationally removed in *Anabaena* 29413. The PsaC protein from *Anabaena* 29413 is highly similar to the same protein from other organisms (2). The only difference in the sequence of the PsaC protein of *Anabaena* 29413 from that of all other species is a conservative replacement of Ile at position 38 by Val (2).

**Table I. Characteristics of the *psaC* Gene from *Anabaena* 29413**

Organism:	<i>Anabaena variabilis</i> ATCC 29413, a filamentous cyanobacterium.
Location on Chromosome:	Present as a single copy and located on a 595-base pair-long <i>SspI</i> / <i>EcoRI</i> restriction fragment.
Function:	PsaC is the apoprotein for two (4Fe-4S) centers F <sub>A</sub> and F <sub>B</sub> , acting as the terminal electron acceptors in the PSI complex.
Techniques:	λEMBL 3 genomic DNA library of <i>Anabaena</i> 29413 was screened with the <i>psaC</i> gene from tobacco chloroplast genome (7). Restriction fragments of the λ clone containing <i>psaC</i> gene were subcloned in pUC 118 and pUC 119 plasmid vectors, and dideoxynucleotide sequencing was performed using universal primers (6). A 22-mer oligonucleotide corresponding to the sequence of the noncoding strand around the translation start site of the <i>psaC</i> gene was used in the primer extension analysis for the identification of the 5'-end of the <i>psaC</i> mRNA (3).
Gene Structure:	A single, monocistronic, 420-base-long <i>psaC</i> transcript was detected in <i>Anabaena</i> cells grown in light as well as in complete darkness. Transcription begins at an A residue at position –49 with respect to the translation initiation site. A Shine-Dalgarno sequence was found upstream of the initiation codon of the <i>psaC</i> open reading frame.
Protein Structure:	The mature PsaC protein is expected to be 80 amino acids long with a predicted molecular mass of 8685 D and a predicted isoelectric point of 5.74. The most notable feature is the presence of nine conserved Cys residues at positions 10, 13, 16, 20, 33, 47, 50, 54, and 57, respectively.
EMBL Accession No.:	X57153

<sup>1</sup> Supported by grants from the National Institutes of Health (GM 41841) and Lucille B. Markey Charitable Trust to H.B.P.



**Figure 1.** Nucleotide sequence of the *psaC* gene and its flanking regions in *Anabaena* 29413. The translated amino acid sequence (in one letter codes) is shown below the DNA sequence in the coding region. The numbering of the nucleotides begins with the A of the initiating Met codon. The numbering of the amino acids begin with Ser, the first residue of the mature protein. The Shine-Dalgarno (SD) ribosome-binding site is underlined. ↑, start site of the *psaC* transcript; → and ←, a potential stem-loop structure.

**ACKNOWLEDGMENTS**

We thank Dr. M. Sugiura for providing the tobacco plastid DNA library from which we obtained the *psaC* probe and Dr. T. Thiel for the construction of the  $\lambda$ EMBL3 library of *Anabaena* 29413 genomic DNA in our laboratory.

**LITERATURE CITED**

1. Elhai J, Wolk CP (1988) Conjugal transfer of DNA to cyanobacteria. *Methods Enzymol* **167**: 747-754
2. Golbeck JH, Braynt DA (1991) Photosystem I. In CP Lee, ed, *Current Topics in Bioenergetics*, Vol 16. Academic Press, New York, pp 83-177
3. Golden SS, Brusslan J, Haselkorn R (1986) Expression of a family of *psbA* gene encoding a photosystem II polypeptide in the cyanobacterium *Anacystis nidulans* R2. *EMBO J* **5**: 2789-2798
4. Maldener I, Lockau W, Cai Y, Wolk C (1991) The calcium-dependent protease of the cyanobacterium *Anabaena*: molecular cloning and expression of the gene in *Escherichia coli* and site directed mutagenesis. *Mol Gen Genet* **225**: 113-120
5. Mannan RM, Whitmarsh J, Nyman P, Pakrasi HB (1991) Directed mutagenesis of an iron sulfur-protein of the photosystem I complex in the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413. *Proc Natl Acad Sci USA* **88**: 10168-10172
6. Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
7. Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chunwangse J, Obokata J, Yamaguchi-Shinozaki K, Ohto C, Torazawa K, Meng BY, Kusuda J, Takaiwa F, Kato A, Tohdoh N, Shimada H, Sugiura M (1986) The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. *EMBO J* **5**: 2043-2049
8. Wolk CP, Shaffer PW (1978) Heterotrophic micro- and macrocultures of a nitrogen-fixing cyanobacterium. *Arch Microbiol* **110**: 145-147