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CpcF-dependent regulation of pigmentation and development in *Fremyella diplosiphon*

Melissa J. Whitaker [a], Juliana R. Bordowitz [a,b], Beronda L. Montgomery [a,b,c,*]

[a] Department of Energy—Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA
[b] Cell and Molecular Biology Graduate Program, Michigan State University, East Lansing, MI 48824, USA
[c] Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA

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ABSTRACT

Cyanobacteria harvest light for photosynthesis using photosynthetic light-harvesting complexes called phycobilisomes (PBSs). Lyases are enzymes responsible for covalent attachment of light-absorbing chromophores to the phycobiliproteins (PBPs) contained in PBSs. We isolated a pigmentation mutant in the filamentous cyanobacterium *Fremyella diplosiphon* and determined that it possesses an insertional mutation in *cpcF*, which encodes one component of a heterodimeric phycocyanin lyase. Here, we discuss the implications of the mutation in *cpcF* on light-dependent pigmentation and morphology responses characteristic of complementary chromatic adaptation in *F. diplosiphon*. Although *cpcF* encodes a phycocyanin lyase, significant decreases in the levels of all classes of PBPs are associated with CpcF deficiency in *F. diplosiphon*. Notably, CpcF deficiency has a limited effect on the shape of *F. diplosiphon* cells, but significantly impacts filament length. Possible mechanisms for the broad impact of CpcF deficiency on pigmentation and filament morphology are discussed.

Introduction

Photosynthetic organisms must be able to sense and respond to changes in the ambient light environment for long-term survival. A number of light-dependent physiological responses have been identified including photoperiodism, phototaxis, and photoregulation of the light-harvesting complexes to maximize photosynthesis. A long-recognized light-dependent process that occurs in some cyanobacteria is called complementary chromatic adaptation (CCA), which results in changes in the protein composition of the photosynthetic light-harvesting phycobilisome (PBS) complexes of cyanobacterial cells in response to changes in the ratio of red-to-green wavelengths in the ambient light environment [5,7]. In cyanobacteria that exhibit CCA, the PBS of wild-type (WT) cells grown in red light (RL) accumulate high levels of the RL-absorbing phycocyanin (PC) pigment, resulting in cells that have a blue-green color. Conversely, high levels of the green-light (GL)-absorbing phycoerythrin (PE) pigment accumulate under green illumination, leading to cells that are red to brick red in color.

Exploiting the contrasting color of cells that is dependent upon the prevalent light environment under which the strains are grown, the isolation and complementation of pigmentation mutants has led to significant advances in our understanding of the signal transduction pathway controlling CCA and the structural components of the PBS in the filamentous cyanobacterium *Fremyella diplosiphon* (recently reviewed in [19,22]). Pigmentation mutants isolated in *F. diplosiphon* (also known as *Calothrix* sp. strain PCC 7601) have resulted largely from gene inactivation as a result of the introduction of an endogenous insertion element [2,4,11,12,17,18,28]. Numerous screens for pigmentation mutants have led to the isolation of structural and regulatory genes involved in the CCA response. For example, the isolation of black (FdBk) mutants, which accumulate intermediate levels of PE and inducible PC (PCi) independent of growth conditions, led to the identification of a sensor-kinase-class biliprotein, RcaE [18]. Red (FdR) mutants, which accumulate high levels of PE and low levels of PCi under both RL and GL conditions, resulted from insertional mutations in *rcaE* or genes encoding two response regulators, i.e., *rcaF* or *rcaC* [11,17]. Genetic analysis led to the suggestion that RcaE, RcaF, and RcaC are sequential members of a photosensory phosphorelay signaling system [17].

Here, we report the isolation of a mutant in *F. diplosiphon* that is chartreuse (FdCh) in color. This mutant contains a disrupted *cpcF*

Abbreviations: CCA, complementary chromatic adaptation; GL, green-light; PBP, phycobiliprotein; PBS, phycobilisome; PC, phycocyanin; PE, phycoerythrin; RL, red light; WT, wild-type

* Corresponding author. Address: MSU-DOE Plant Research Lab, Michigan State University, 106 Plant Biology Building, East Lansing, MI 48824-1312, USA. Fax: +1 517 353 9168.
E-mail address: montg133@msu.edu (B.L. Montgomery).

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gene and provides a genetic tool for investigating the role of CpcF in *F. diplosiphon* in greater detail than previously reported. *cpcE* and *cpcF* genes encode enzymes central to PC biosynthesis in cyanobacteria [13]. It was determined previously that *cpcE* is co-transcribed with PC apoprotein-encoding genes *cpcB1* and *cpcA1* in *F. diplosiphon*; however, *cpcF* reportedly is transcribed as a monocistronic message [32]. Initial reports suggested that a *cpcF* mutant is defective in PCi accumulation in RL specifically due to a defect in chromophore attachment to the α subunit of PC [33]. A *cpcF* mutant also was described as defective in accumulating PE in GL conditions [31,32] and as lacking constitutive PC (PCc), which is present in both RL and GL conditions, as well [32]. The PBSs isolated from this *cpcF* mutant were reported to be smaller in size than those isolated from WT cells [32]. The pleiotropic phenotype of the *cpcF* mutant was hypothesized to reflect a regulatory role for chromophore molecules in the accumulation of PBPs and/or PBS synthesis [31,33]. In other cyanobacteria, including *Synechococcus* sp. PCC 7002 and *Mastigocladus laminosus* PCC 7603, CpcE and CpcF have been shown to operate as a heterodimeric lyase enzyme that catalyzes the attachment of the PCB chromophore to Cys-84 of the α subunit of PC, as well as catalyzing chromophore detachment and transfer [13,14,29,36]. In these non-PE-producing organisms, disruption of *cpcE* or *cpcF* resulted in a specific defect in PC accumulation [6,29,37]. In *Anaebaena* sp. PCC 7120, which produces phycoerythrocyanin (PEC) in addition to PC, insertional mutagenesis of *cpcE* or *cpcF* resulted in a lack of PC accumulation, but notably an overproduction of PEC, particularly under low light conditions [10].

Similar to earlier reports, the FdCh1 mutant that we isolated is severely deficient in the accumulation of PE and PC in both GL and RL. In addition to studying the effect of CpcF deficiency on these two PBPs, we also assessed the impact of CpcF deficiency on the levels of allophycocyanin (AP) and the cellular morphology of the *cpcF* insertional mutant. We provide evidence that CpcF contributes to the regulation of pigmentation and filament morphology during CCA in *F. diplosiphon*.

Materials and methods

Strains and growth conditions. We used strain SF33, the *F. diplosiphon* strain that forms discrete colonies on plates, as the WT pigmentation strain (hereafter WT). The FdCh1 strain was isolated from mutagenized SF33 cells that were heat-shocked and screened for pigmentation mutants as previously described [2]. *F. diplosiphon* cultures were routinely grown in BG-11 [1] containing 20 mM Hepes at pH 8.0 (hereafter BG-11/Hepes) at 28 °C. Liquid cultures were grown under GL or RL illumination as previously described [8]. Light fluence rates were measured using a Li-Cor light meter (model LI-250, Li-Cor, Lincoln, NE) with a connected Li-Cor quantum sensor (model LI-190SA).

Cell density and absorption spectrum measurements. The densities of cyanobacterial cell cultures were determined by measuring absorbance at 750 nm (A750) using the cuvette port of a SpectraMax M2 microplate reader (Molecular Devices, Sunnyvale, CA). Whole-cell spectral scans were obtained from 350 to 800 nm for cells that were adjusted to an A_{800} of ~0.1.

Isolation of F. diplosiphon genomic DNA. We isolated genomic DNA from *F. diplosiphon* cell cultures using a phenol–chloroform-based method. Briefly, cells were broken open in TE buffer (pH 8) in the presence of glass beads using vortexing. Genomic DNA was extracted with phenol/chloroform (1:1 vol/vol), precipitated with ethanol in the presence of NaOAc (pH 5), and resolubilized in TE buffer (pH 8). Isolated genomic DNA was treated with RNaseA to digest RNA.

PCR amplification. PCR analysis of *cpcF* was accomplished using standard PCR procedures and genomic DNA as a template [27]. Primers used for PCR amplification were 5′ cpcF (GTTAA GATGTTGTTGCCC) and 3′ cpcF (TTATTCATCCGAATGTTG).

Pigment extraction and quantification. Chlorophyll *a* (chl*a*) and PBPs were extracted and quantified as previously described [8].

Microscopy. Slides of *F. diplosiphon* cells were prepared and analyzed with an inverted Axiovert 200 Zeiss LSM 510 Meta confocal laser scanning microscope (CLSM: Carl Zeiss MicroImaging, Thornwood, NY) using differential interference contrast (DIC) optics and fluorescence excitation/emission filters as previously detailed [8].

Results

A F. diplosiphon chartreuse mutant contains an insertional mutation in cpcF

We isolated a chartreuse mutant (FdCh1) in an SF33 WT pigmentation background after heat-shock treatment. Based on whole-cell spectral analyses, we observed that the FdCh1 mutant exhibits a near complete reduction in the levels of PE and PC under GL-growth conditions, as we were not able to observe any peaks corresponding to these PBPs in whole-cell spectral scans (Fig. 1)—conditions which lead to the accumulation of high levels of PE in SF33. We were also unable to detect quantitatively these PBPs in extracts from the FdCh1 mutant (Table 1). Under RL growth conditions, FdCh1 contained severely reduced levels of PC relative to SF33 (Fig. 1). PC was reduced to ~3.2% of SF33 PC levels for the FdCh1 strain grown in RL (Table 1). The reduction in PE levels in the FdCh1 mutant in RL also was acute, as these cells accumulated on average 5% of SF33 PE levels. Markedly, the PE/PC ratios for SF33 and FdCh1 under RL were not significantly different (Table 1).

Levels of allophycocyanin (AP) were lower under GL in the mutant, with the FdCh1 mutant accumulating only ~19% of the SF33 AP levels (Table 1). Similar to GL conditions, AP levels of the FdCh1 mutant were reduced significantly relative to SF33 under RL, with the mutant accumulating ~52% of SF33 AP levels (Table 1). Nota-

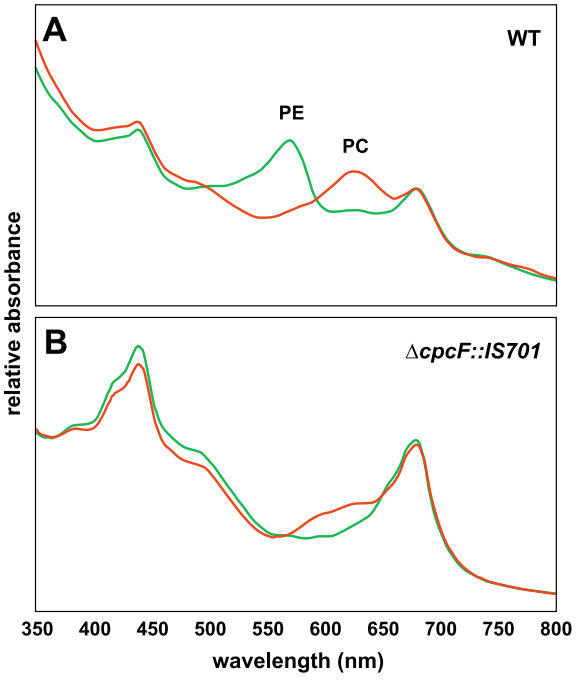

Fig. 1. Whole-cell absorbance spectral scans of wild-type and Δ*cpcF* mutant strains. Representative whole-cell spectral scans of (A) SF33 wild-type pigmentation (WT) and (B) FdCh1 (Δ*cpcF::IS701*) mutant strains. Cells were grown in GL (green line) or RL (red line).