Suppression of Mutants Aberrant in Light Intensity Responses of Complementary Chromatic Adaptation†

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Complementary chromatic adaptation is a process in which cyanobacteria alter the pigment protein (phycocyanin and phycoerythrin) composition of their light-harvesting complexes, the phycobilisomes, to help optimize the absorbance of prevalent wavelengths of light in the environment. Several classes of mutants that display aberrant complementary chromatic adaptation have been isolated. One of the mutant classes, designated “blue” or FdB, accumulates high levels of the blue chromoprotein phycocyanin in low-intensity green light, a condition that normally suppresses phycocyanin synthesis. We demonstrate here that the synthesis of the phycocyanin protein and mRNA in the FdB mutants can be suppressed by increasing the intensity of green light. Hence, these mutants have a decreased sensitivity to green light with respect to suppression of phycocyanin synthesis. Although we were unable to complement the blue mutants, we did isolate genes that could suppress the mutant phenotype. These genes, which have been identified previously, encode a histidine kinase sensor and response regulator protein that play key roles in controlling complementary chromatic adaptation. These findings are discussed with respect to the mechanism by which light quality and quantity control the biosynthesis of the phycobilisome.

Some cyanobacteria grow in environments in which the fluctuation of light is extreme and frequent. They depend on light to meet their energy requirements and must effectively compete with other photosynthetic organisms for this valuable resource. To utilize light energy optimally, many cyanobacteria adjust the number and/or the pigment composition of their light-harvesting complexes, the PBS. *Fremyella diplosiphon* is a filamentous cyanobacterium that responds to changes in light intensity by varying the PBS number and rod length and to changes in wavelength by undergoing CCA. CCA is a reversible process in which the composition of the PBS rods changes (24) such that the change in protein composition is controlled at the level of transcription (7, 31). This change in protein composition is controlled at the level of transcription such that the *cpcB2A2* operon, which encodes the inducible PE polypeptides (designated PC) and associated linker polypeptides, is expressed only in RL (10, 17), and the *cpeBA* operon, which encodes the PE polypeptides, is expressed only in GL (18).

Recently, progress has been made in the elucidation of the CCA signal transduction pathway. Molecular and biochemical approaches have been used to isolate phycobiliprotein genes (11, 31), to characterize their expression patterns (18–21), and to identify promoter elements and protein factors important for their expression (4, 5, 13, 26, 28, 29). The isolation and characterization of mutants in conjunction with genetic analysis can help identify genes encoding elements that govern CCA. Several CCA regulatory mutants have been isolated and classified according to their PBS compositions and the expression of phycobiliprotein genes in RL and GL (3, 5, 7, 14, 30). The mutants that we have isolated have been placed into four classes: (i) black (designated FdBk), (ii) red (FdR), (iii) green (FdG), and (iv) blue (FdB). The FdBk and FdR mutants exhibit no CCA and exhibit aberrant expression of both the *cpcB2A2* and *cpeBA* operons. The FdBk mutants exhibit intermediate levels of expression from the *cpcB2A2* and *cpeBA* operons in both RL and GL, while the FdR mutants constitutively express *cpeBA* and never express *cpcB2A2*. In contrast, FdG mutants show normal expression of *cpcB2A2* but never express *cpeBA*, while FdB strains show relatively normal expression of *cpeBA* but constitutively express *cpcB2A2*. Since the FdBk and FdR mutants exhibit altered expression of both *cpcB2A2* and *cpeBA*, while the FdB and FdG mutants are only aberrant for the expression of a single phycobiliprotein gene set (*cpcB2A2* and *cpeBA*, respectively), the lesions in the former mutants are likely to be upstream in the signal transduction chain relative to those of the latter. Recently, a number of mutants in CCA have been found to be complemented by genes encoding components of two component regulatory systems (5, 13, 14). In this paper, we present the characterization of FdB mutants and demonstrate that the suppression of *cpcB2A2* activity is less sensitive to GL in these mutants than it is in WT cells. Two genes, *rcaE* and *rcaF*, which encode a sensor histidine kinase and a response regulator (13, 14), respectively, were shown to each suppress the mutant phenotype; however, neither of these genes is altered in the FdR strains. The mode by which RcaE and RcaF may suppress the FdB phenotype is discussed.

**MATERIALS AND METHODS**

**Abbreviations.** The following abbreviations are used in this paper: PC, phycocyanin; PE, phycoerythrin; CCA, complementary chromatic adaptation; PBS, phycobilisome; RL, red light; GL, green light; WT, wild-type cells; k**, μ**g of kanamycin/ml; GUS, β-glucuronidase.

**Strains and growth conditions.** A shortened filament strain of *F. diplosiphon*, FD33, initially designated SF33 (8), was used as our WT (25). The blue mutants, FdB1 and FdB2, were generated by electroporation (3) and nitrosoguanidine mutagenesis (10a), respectively, and display the phenotype previously described.
(3). Cells were grown in liquid or on solid BG-11 medium (23) buffered to pH 8.0 with 10 mM HEPES and illuminated with WL, RL, and GL of various intensities. Light intensities from 10 to 35 μmol of photons m⁻² s⁻¹ were attained by using a combination of white (Westinghouse 20 W cool white fluorescent tube, F20T12/R) and red (Westinghouse 20 W green fluorescent tube, F20T12/G) lamps filtered through clear plexiglass. Electroporants were grown in BG-11 liquid medium containing k₂, except when grown for plasmid rescue, in which case the growth medium was supplemented with 50 to 75 μg of kanamycin/mL.

GUS constructs and assays were previously described (5, 6). PCR amplification (4) was performed with either specific plasmids (noted in the text) or with a population of plasmids from F. diplosiphon recombinant library (14); both were purified in a CsCl gradient. After electroporation, the cells were inoculated into 10 ml of BG-11 medium and grown for 16 to 24 h in 30 μmol of photons m⁻² s⁻¹ of WL. The cells were then pelleted by centrifugation at 5000 × g for 5 min, resuspended in BG-11, and spread on solid BG-11 medium containing k₂. Kanamycin-resistant colonies and transfectants were transformed as described elsewhere (14). The junctions of all of the constructs were sequenced.

The region of the FdB genome containing the rcaE gene was used in all experiments. Fragment 1 of the pHR recombinant library (14) and fragment 2 of pEC99, designated pHK9-3 (described in DNA sequence analysis section of Materials and Methods), was digested with NotI and SmaI and cloned into the pEC100 insert. Large preparations of plasmid DNA were obtained in a CsCl density gradient. RNA was isolated from the pellet by using Tri Reagent-LS (Molecular Research Center, Inc., Cincinnati, Ohio). RNA was fractionated by electrophoresis in a formaldehyde-agarose gel, transferred to nitrocellulose membranes, and hybridized (9) to one of four 32P-labeled DNA fragments. The fragments were leader sequence [pEC100, fragment pEC(298) (4)]; (ii) a 293-bp XhoI fragment that extends from base pair 17 to 310 of the cepBA coding sequence (18); (iii) a leader sequence from cpeBA1, the constitutively expressed PC operon which encodes constitutive PC (PCr) [HindIII-XhoI fragment from pEC(A12) (4), or (iv) a 1.9-kbp XhoI fragment containing a portion of the 16S genes coding for RNA from pAN4 (32). Quantitation of RNA abundance from Northern blot hybridizations was conducted by using a PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.).

DNA sequence analysis. To generate a series of nested deletions, pEC99 was digested with Smal and Xhol. The 3.0-kbp F. diplosiphon genomic DNA fragment that was generated was cloned into pBluescript SK-, removed from the plasmid by digestion with SpeI/KpnI, and cloned into pGEM-TIZ (Promega, Madison, Wis.). The resulting plasmid, pTIG, was linearized with SacI/NorI or KpnI/HindIII, and the fragment was used to processively digest the DNA (12). pHK9-3 was digested from the pTIG plasmid linearized with KpnI/HindIII; it contains the coding region starting 55 bp downstream and ending 657 bp upstream of ORFII. The genomic DNA fragments containing the rcaE gene were isolated using approximately 500 bp upstream of rcaE from FdB1 and FdB2 were amplified from genomic DNA. The amplified primers were: (i) a 1587-bp upstream primer pair with a 10-bp nucleotide tail containing a 3′ EcoRI site that was generated was cloned and sequenced with synthetic oligonucleotide primers. The amplified DNA fragment was generated using the ABI Prism 310 Genetic Analyzer (Perkin-Elmer, Foster City, Calif.). The sequences were compiled in Sequence Navigator (Applied Biosystems–Perkin-Elmer), analyzed with the University of Wisconsin Genetic Computer Group software package, and compared to sequences in the GenBank database (1).

RESULTS

Two blue mutants, FdB1 and FdB2, were identified by their abnormally blue color when grown in GL or WL. Under these conditions, the WT strain, FdB3, is reddish in color because PE is highly expressed in GL or WL (which has a large GL component). Characterization of phycobiliprotein levels and cpeBA1 (constitutively expressed PC gene set), cpeB242 (RL-inducible PC gene set), and cpeB4 transcript levels in FdB1 at 15 μmol of photons m⁻² s⁻¹ of RL or GL was done previously (3). The FdB1 phenotype resulted from elevated levels of cpeB242 mRNA in WL and GL relative to that of WT.

Whole-cell absorbance spectra. Liquid cultures of WT, FdB1, and FdB2 cells were grown in 10 μmol of photons m⁻² s⁻¹ of RL or GL to an A₅₇₀ of between 0.3 and 0.7, and the whole-cell absorbance spectra (350 to 750 nm) were measured (Fig. 1A). The cells were grown to a relatively low density to minimize self-shading. The absorbance spectra of F. diplosiphon.
from 350 to 750 nm and the y axis is relative absorbance. The peaks of light absorbance by PC (arrow), PE, and chlorophyll a (chl), are indicated. (B) Table with a qualitative representation of PC, PE, and PC levels in WT, FdB1, and FdB2 cells grown in 10 μmol of photons m⁻² s⁻¹ of RL or GL. High levels are indicated by a plus sign and low levels are indicated by a minus sign. (C) Autoradiogram of RNA gel blot hybridization to cpcB2A2, cpeBA, cpeB1A1, and 16S rRNA-specific probes. Arrows mark the positions of the principal hybridizing transcripts.

photo grown in WL contains four major peaks which represent light absorption by (i) chlorophyll a at 680 and 450 nm, (ii) PC at 620 nm, and (iii) PE at 560 nm. The spectra for RL- and GL-grown cells demonstrated that FdB1 and FdB2 exhibited abnormal CCA. Both strains had high levels of PE in GL and low levels in RL. In FdB2 the PE levels in GL appeared to be somewhat reduced relative to that of WT. However, the difference in PC levels between that in FdB1 and FdB2 and that in WT was dramatic and accounted for the characteristic blue color of the mutants; the PC was elevated in the mutant strains in both RL and GL. FdB2 accumulated more PC and less PE than FdB1. A qualitative summary of the analysis of PE and PC accumulation in FdB1 and FdB2 grown in RL and GL is given in Fig. 1B.

Northern analysis of phycobiliprotein transcripts. Northern analyses were used to determine if the high level of PC absorption of FdB1 and FdB2 in GL reflected differences in the levels of PC mRNA. RNA was isolated from WT, FdB1, and FdB2 after growth at 10 μmol of photons m⁻² s⁻¹ of RL or GL and hybridized to DNA probes specific for cpcB1A1, cpcB2A2, and cpeBA (Fig. 1C). In the two blue mutants, the cpcB1A1 transcript levels were similar to that of WT; transcripts were present in both RL and GL. In GL, the levels of cpeBA transcripts in FdB1 and FdB2 were approximately the same as that in WT, while in RL, the levels of cpeBA transcripts in FdB1 and FdB2 were slightly greater than that in WT. This slight difference, as well as any other minor variations in PC in PE mRNA levels, was not consistently observed among repetitions of this experiment and was therefore not quantified. In contrast, the cpcB2A2 transcript levels in the FdB mutants were very different than those in WT. In WT, cpcB2A2 (1.6 kb) and cpcB2A2H2I2D2 (3.8 kb) transcripts (cpcH2I2D2 encode the nonchromophorlated PBS PC linker polypeptides) (17) were present at high levels in RL and were not detectable in GL, while in FdB1 and FdB2 they were present at high levels in both RL and GL. The absorption spectra and Northern blot hybridizations clearly illustrate that the blue phenotype is due to high levels of PC that reflect high levels of the cpcB2A2 transcript. Since the phenotypes of the FdB1 and the FdB2 mutants were similar, we focused most of our attention on FdB1.

GUS expression from the cpcB2A2 promoter. CCA is primarily controlled at the level of transcription in F. diplosiphon; the half-lives of cpcB2A2 and cpeB4 transcripts are not affected by light quality (20). To test whether the FdB1 mutant phenotype was a result of aberrant transcriptional regulation of the cpcB2A2 promoter, a WT cpcB2A2 promoter fragment was linked to the GUS gene in a translational fusion that was inserted into an autonomously replicating plasmid. This plasmid, pPCi(298)G, was electroporated into WT and FdB1, and the electroporants were assayed for GUS activity in RL and GL. In WT, this promoter caused high levels of GUS expression in RL (10 μmol of photons m⁻² s⁻¹) and background levels in GL (10 μmol of photons m⁻² s⁻¹) (Fig. 2A) (4). In contrast, when this construct was introduced into FdB1, the GUS activity was high in both RL and GL, with the activity in RL being less than twofold higher than the activity in GL (Fig. 2A). In WT cells, deletion of the cpcB2A2 promoter to 76 bp upstream of the transcription start site had no effect on RL- or GL-regulated transcription. However, when this promoter fragment was shortened to −37 bp, there was no expression in RL or GL (4). To determine whether the same cpcB2A2 promoter elements required for CCA-regulated transcription in WT cells were required for constitutive expression in FdB1, pPCi(76)G and pPCi(37)G were electroporated into FdB1, and the resulting strains were assayed for GUS activity (Fig. 2). Background levels of GUS activity were observed in FdB1 strains harboring pPCi(37)G (similar to expression in WT cells), while the GUS activity in cells transformed with pPCi(76)G in RL and GL was similar to that observed for pPCi(298)G; deletion of the promoter to −76 bp had little effect on either overall or CCA-regulated expression. These results demonstrate that the lesion in FdB1 leads to a change in the level of transcription of the cpcB2A2 operon but is not the consequence of a change in the coding or regulatory regions of that operon.
tifying GUS activity relative to protein concentration after growth of the cells at and the expression of the chimeric parentheses. The GUS plasmids were electroporated into WT and FdB1 cells, positions of the upstream endpoints relative to the transcription start site in as previously described (4). The plasmid designations are on the left with the change until the GL intensity was increased to 75 in the FdB2 strain was altered in a similar way but did not absorption spectra were similar to those of WT; they synthe-

Whole-cell absorption spectra of FdB1 and FdB2 after growth in high-intensity light. We noted that light intensity affected pigmentation in the FdB1 and FdB2 strains. To investigate this phenomenon, FdB1 was grown in RL and GL at 10, 30, 50, and 75 μmol of photons m⁻² s⁻¹. At all intensities of RL the absorption spectra of FdB1 were similar to those of WT (data not shown); the PC levels remained high, decreasing slightly at 50 and 75 μmol of photons m⁻² s⁻¹. In contrast, with increasing intensities of GL, FdB1 exhibited a dramatic change in the PC levels with little change in the PE levels. When FdB1 cells were maintained at 30 μmol of photons m⁻² s⁻¹ (Fig. 3A) or 75 μmol of photons m⁻² s⁻¹ (data not shown) of GL, their absorption spectra were similar to those of WT; they synthesized high levels of PE and low levels of PC. The pigmentation in the FdB2 strain was altered in a similar way but did not change until the GL intensity was increased to 75 μmol of photons m⁻² s⁻¹ (data not shown). This demonstrated that the FdB strains were still capable of normal CCA but required more GL than WT to suppress PC synthesis. Furthermore, the suppression of PC synthesis in the FdB2 strain was less sensitive to GL than was FdB1. A qualitative presentation of this data for FdB1 is given in Fig. 3B.

Northern blot hybridizations with RNA from FdB1 grown in high-intensity light. Northern blot hybridizations were used to determine if the decrease in PC levels in FdB1 in response to high-intensity GL was due to a change in the levels of cpcB2A2 transcripts. RNA isolated from WT and FdB1 cells grown in 10 and 30 μmol of photons m⁻² s⁻¹ of GL and RL was hybridized to cpcB2A2-, cpeBA-, and cpeB1A1-specific probes (Fig. 3C). The levels of cpeB1A1 transcript were not altered by light intensity in any of the strains. Furthermore, there was little change in the cpcB2A2 and cpeBA transcript levels in WT when the light intensity was increased from 10 to 30 μmol of photons m⁻² s⁻¹ of GL. In contrast, the cpcB2A2 as well as the longer cpcB2A2H2I2D2 transcript levels in FdB1 decreased dramatically during growth at the higher intensity GL, and the ratio of the PC transcripts in RL and GL approached that of WT (data not shown). Therefore, the low PC levels in FdB1 cells grown at higher intensity GL reflect a decrease in the quantity of cpcB2A2 mRNA.

In FdB1 electroporants containing pPC(298)G, the level of GUS activity was much higher than in WT cells grown at 10 μmol of photons m⁻² s⁻¹ of GL and dropped by 85% when the cells were grown in 30 μmol of photons m⁻² s⁻¹ of GL (twice that of WT, Fig. 2B). This reflects the drop in cpcB2A2 mRNA (Fig. 3C). In summary, the blue phenotype of FdB1 (and FdB2) is the result of high levels of PC in GL which result from overexpression of cpcB2A2, while the expression of the cpeBA operon in FdB1 (and FdB2) is similar to that of WT. With increasing intensities of GL, the FdB strains become phycocyanin-like similar to WT. While FdB2 requires 50 to 75 μmol of photons m⁻² s⁻¹ of GL to reduce the blue phenotype, FdB1 exhibits the WT phenotype at 30 μmol of photons m⁻² s⁻¹. The changes observed in the pigmentation of the FdB mutants as the intensity of GL is increased reflect changes in the transcriptional activity of the cpcB2A2 operon.

FIG. 2. Expression of GUS activity from the cpcB2A2 promoter in WT and FdB1 cells. cpcB2A2 promoter fragments were fused to the GUS gene in p2.7G1 as previously described (4). The plasmid designations are on the left with the positions of the upstream endpoints relative to the transcription start site in parentheses. The GUS plasmids were electroporated into WT and FdB1 cells, and the expression of the chimeric cpcB2A2-GUS genes was analyzed by quantifying GUS activity relative to protein concentration after growth of the cells at 10 μmol of photons m⁻² s⁻¹ of RL and GL (A) and 30 μmol of photons m⁻² s⁻¹ of GL (B). Measurements of GUS activity are means of three separate experiments with the standard errors in parentheses.

FIG. 3. WT and FdB1 cells grown at 10 or 30 μmol of photons m⁻² s⁻¹ of GL. (A) Absorption spectra of whole cells grown in 10 or 30 μmol of photons m⁻² s⁻¹ of GL. The x axis is the wavelength of light in nm, the y axis is the relative absorbance, and the numbers in parentheses above the spectra are light intensities during growth. The peaks representing light absorbed by PC, PE, and chlorophyll a (chl) are labeled accordingly, and an arrow marks the PC absorbance peak. (B) Table with a qualitative representation of PC and PE levels in WT and FdB1 cells grown in 10 or 30 μmol of photons m⁻² s⁻¹ of GL. High phycobiliprotein levels are indicated by a plus sign, and low levels are indicated by a minus sign. (C) Autoradiogram of RNA gel blot hybridization to cpcB2A2-, cpeBA-, cpeB1A1-, and 16S rRNA-specific probes. RNA was isolated from cells grown in either 10 or 30 μmol of photons m⁻² s⁻¹ of GL (designated 10 and 30, respectively, at the top of the panel). Arrows mark the positions of the principal hybridizing transcripts.
in phycobiliprotein transcript levels (Fig. 4B). Both the PC and PE levels observed in the absorption spectra of FdB1(s) are reflected in the levels of the cpcB2A2 and cpeBA mRNAs in this strain. The transcript levels for cpcB2A2 were intermediate with respect to WT and FdB1. Specifically, FdB1(s) had high levels of cpcB2A2 in RL and much lower levels in GL than FdB1. The RL to GL ratio of cpcB2A2 transcripts in WT is 18:1; in FdB1, it is 1:1; and in FdB1(s), it is 5:1. Furthermore, FdB1(s) cells grown in RL had a twofold higher level of cpeBA transcripts than WT.

**Plasmid rescue.** To ensure that the altered phenotypes of the suppressed mutant strains were dependent upon the introduced plasmid, two tests were conducted: (i) the strains were plated on solid BG-11 medium lacking kanamycin, which facilitates curing of the pPL2.7-derived plasmids, and (ii) the plasmids were rescued and reintroduced into FdB1 and FdB2. Curing the strains of this plasmid should result in the original mutant phenotype, and reintroduction of the plasmid into the original mutant should suppress the FdB phenotype. When FdB1(s) and FdB2(s) were cured of their pPL2.7 plasmids, they became kanamycin sensitive and reverted to the original blue phenotype (data not shown). FdB1(s) and FdB2(s) both contained a single, identical plasmid, designated pEC99, that when reintroduced into either of the original mutant strains restored the FdB(s) phenotype.

**DNA sequence of F. diplosiphon genomic fragment.** The plasmid pEC99, diagrammed in Fig. 5, contained three open reading frames (ORFs). ORFI or the rcaE gene is incomplete; the protein encoded by it is 351 amino acids long and extends to the end of the genomic insert. ORFII or the rcaF gene encodes a protein of 124 amino acids, begins 12 bp downstream of the end of rcaE, and is likely to be in the same operon. A third ORF is separated from rcaF by 15 bp and is transcribed from the opposite strand. Another plasmid, pDK1 (13, 14), contains rcaE (encoding 655 amino acids and with an additional 2.6 kbp upstream of the translation start site), rcaF, and a portion of ORFIII. This plasmid (Fig. 5) was also able to suppress the FdB phenotype. RcaE and RcaF are a sensor histidine kinase and a response regulator of two-component regulatory systems, respectively. The rcaE gene was recently found to complement mutants in CCA that exhibit a black phenotype, and the amino acid sequence of the protein was previously reported (14). In pEC99, rcaE is truncated and lacks the region encoding the sensor domain at the N terminus of the molecule. The rcaF gene, which complements a CCA red mutant, encodes a CheY- and Spo0F-like response regulator (15). This response regulator contains the conserved lysine and aspartate residues (including the putative phosphorylation site) that are found in this class of proteins.

To determine which of the open reading frames suppressed the FdB phenotype, the plasmids pEC100 and pEC200 were generated (Fig. 5). pEC100 contains a truncated rcaE and the entire rcaF, and pEC200 contains only ORFII. pEC100 rescued the blue phenotype while pEC200 did not. Hence, introduction of the truncated form of rcaE plus full-length rcaF shifted the mutant phenotype toward that of WT, even though the endogenous promoter responsible for the expression of rcaE (and perhaps rcaF) was absent. Expression of these two genes in pEC99 and pEC100 may be from the kanamycin gene promoter. To further define the sequences that suppress the FdB phenotype, four additional constructs (Fig. 5) were made and electroporated into FdB1. pHr (for histidine kinase and response regulator) contains 358 bp of upstream sequence, the complete rcaE and rcaF genes, and 184 bp downstream of rcaF. Two other constructs were made with the same upstream and downstream endpoints as pHr but with deletions within rcaE.
nucleotides were in frame. To generate the construct pDK4, conserved aspartate of RcaF was deleted. The remaining 145 D
R, the carboxy-terminal half of the molecule and a portion of the included the five motifs required for histidine kinase activity in encoding 526 amino acids of RcaE was removed; the region introduced into
 cleotide of the coding region) (14). In summary, if pHR is mol of photons m
rants were selected and grown in liquid medium in WL (50
FdB1 by electroporation, and kanamycin-resistant electropo-
PpHR, p
D
R, pDK1, and pDK4 were introduced into
FdB1(pHR-B1), and FdB1(pHR-B2), respectively. In all of
one might expect that FdB1(pDK4) would respond to RL and
the FdB phenotype in the FdB1(pDK4) strain was suppressed and was intermediate between those of FdB1 and FdB1(pHR). The FdB1(pDK4) electroporants had decreased levels of PC in both GL and WL relative to FdB1, but the reduction was not to the same extent as that seen in WT. In RL, they all had elevated PE (the PE absorbance peaks are marked with asterisks in Fig. 6) relative to WT. Also, in GL they had slightly greater absorption at 650 nm than WT; this elevated absorbance may be the consequence of increased levels of allophycocyanin.

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(pΔHR) or rcaF (pΔFR). In the former plasmid a region encoding 526 amino acids of RcaE was removed; the region included the five motifs required for histidine kinase activity in the carboxy-terminal half of the molecule and a portion of the N terminus with similarity to phytochrome and ethylene receptors of vascular plants (14). The remaining open reading frame (534 bp) encodes a polypeptide of 176 amino acids. In pΔHR, 225 bp encoding both the N-terminal acidic domain and the conserved aspartate of RcaF was deleted. The remaining 145 nucleotides were in frame. To generate the construct pDK4, rcaF was truncated at nucleotide 148 (relative to the first nucleotide of the coding region) (14). In summary, if pHR is introduced into F. diplosiphon cells both RcaE and RcaF should be synthesized from the plasmid. Only RcaF should be produced from pΔHR, and only RcaE should be produced from pΔFR or pDK4.

Absorption spectra of FdB1 strains with deletion constructs. pHr, pΔHR, pΔFR, pDK1, and pDK4 were introduced into FdB1 by electroporation, and kanamycin-resistant electroporants were selected and grown in liquid medium in WL (50 μmol of photons m⁻² s⁻¹), RL (10 μmol of photons m⁻² s⁻¹), and GL (10 μmol of photons m⁻² s⁻¹). The absorption spectra of the electroporants, shown in Fig. 6, demonstrate that the introduction of pHr, pΔHR, pΔFR, and pDK1 into FdB1 (data not shown for the last of these) substantially reduced the FdB phenotype; the absorption spectra of FdB1 strains harboring these plasmids (Fig. 6B) were more similar to those of WT cells (Fig. 6A). This similarity was especially apparent in

FIG. 6. Absorption spectra of WT, FdB1, and FdB1(s) strains harboring various plasmids grown in 50 μmol of photons m⁻² s⁻¹ of WL and 10 μmol of photons m⁻² s⁻¹ of RL and GL. The light conditions are indicated at the top, the strain designations are on the left, the x axis is wavelength from 350 to 800 nm, and the height of the peaks depict relative absorbances. The peaks of PC and PE absorbance are marked by arrows and asterisks, respectively. (A) As a point of reference, absorption spectra for WT and FdB1 cells in WL, RL, and GL are shown. (B) Absorption spectra of electroporants containing the plasmids pHr, pΔHR, pΔFR, and pDK4; these strains have phenotypes which are most similar to that of WT. (C) Absorption spectrum of a electroporant containing pDK4; this strain has a less suppressed phenotype.
containing rcaE and rcaF from both FdB1 and FdB2 was amplified by PCR and sequenced. Both strains had rcaE and rcaF sequences identical to those in WT. Hence, these genes were not altered in the FdB mutants, which leads to the conclusion that overexpression of either rcaE or rcaF suppresses the FdB phenotype.

Characterization of rcaC from FdB1 and FdB2. It was previously shown that the red mutant FdB1 contains a large insertion in and appears to be null for rcaC (5). This mutant constitutively expresses cpeB4 and never expresses cpeB2A2 (3). Introduction of rcaC (this gene encodes a bacterial response regulatory protein with a putative DNA binding domain) complements this phenotype (5). Furthermore, proteins specific to RL-grown cells bind to the promoter of cpeB2A2 (4). This DNA-protein interaction does not occur with protein extracts from RL-grown FdR1 (data not shown). Therefore, it is possible that in RL, RcaC binds to and activates expression of cpeB2A2 and that the FdB phenotype is a consequence of a mutation in RcaC that alters the function of RcaC such that only the expression of cpeB2A2 is affected (causing constitutive activation of cpeB2A2). To test this possibility, the rcaC gene was PCR amplified from FdB1 and FdB2 and cloned into pPL2.7, and each was introduced into FdR1 in three independent transformations. Both of these plasmids (designated pB1-RCAC and pB2-RCAC) complemented the FdR1 mutant. Each of the three independent transformants, grown in RL and GL (10 and 20 μmol of photons m⁻² s⁻¹), exhibited the WT and not the FdB phenotype (data not shown). FdR1 cells transformed with pPL2.7 alone retained the FdR phenotype (data not shown). Hence, the lesion in the FdB strains is probably not in rcaC.

DISCUSSION

In this report, we have identified a class of CCA mutants, designated FdB, in which the severity of the mutant phenotype varies with the intensity and wavelength of light. The mutants accumulate an abnormally high level of PC in GL and WL, causing the cells to appear blue. We have demonstrated, using promoter-reporter gene fusions, that the phenotype of the FdB mutants reflects constitutive expression of cpeB2A2 and that the mutation is not within the cpeB2A2 operon itself. We have also demonstrated that the FdB mutants are not altered in the known regulatory genes involved in CCA, rcaE and rcaF. Furthermore, the mutant strain probably also has a normal rcaC gene, since this gene from both the FdB1 and FdB2 mutants can complement a strain null for rcaC. Interestingly, the FdB phenotype is suppressed by the introduction of additional copies of rcaE and/or rcaF, suggesting that the lesion in each of the FdB strains is altering the effectiveness of a trans-acting factor that controls expression from the cpeB2A2 promoter but is not completely eliminating the function of that factor.

It is possible that the FdB phenotype is due to the presence of more than one mutation. However, it is more likely that this phenotype is the consequence of a single lesion. First, we have yet to detect a class of mutants that undergoes a second mutation resulting in a blue phenotype. Red mutants and black mutants undergo second mutations that result in green mutant phenotypes. Second, the mutation can be suppressed by increasing the intensity of GL as well as by introducing additional copies of either rcaE or rcaF. This suggests that the regulatory element that is altered is less efficient in performing its function or is present at a decreased level in the cell; it does not appear to be absent. If the phenotype was the result of two mutations at distinct loci, we would have to assume that the two altered loci do not include rcaE, rcaF, or rcaC and that they are both present either at lower levels or as structurally altered forms.

To understand the findings presented in this paper with respect to CCA, we must first consider the functions of the rcaC, rcaE, and rcaF gene products. RcaE and RcaF represent a sensor-response regulator pair characteristic of certain two-component regulatory systems (2, 13, 14, 22). These elements appear to act upstream of a second response regulatory protein, RcaC, in a phosphoryl signal transduction chain that controls CCA (13). Similar to a mutation in rcaC, lesions in rcaE and rcaF affect both cpeB4 and cpeB2A2 expression. Since the FdB mutation affects only cpeB2A2 expression, the mutation is likely to be either (i) downstream of rcaE-rcaF and rcaC or (ii) in a second regulatory pathway that feeds into the CCA signal transduction pathway.

Proteins that function downstream of rcaE-rcaF and rcaC may include a cpeB2A2 activator and a protein that inhibits the function of this activator. Our results are consistent with the idea that the FdB mutants have lesions in a gene encoding such an activator such that the cpeB2A2 operon becomes constitutively expressed. Activator control of cpeB2A2 is supported by the observation that an RL-specific protein binds to a region of the cpeB2A2 promoter that is necessary and sufficient for RL induction (4) and that this binding interaction does not occur when the cpeB2A2 promoter elements are incubated with proteins from mutants that are unable to express cpeB2A2. A mutation in such an activator may cause increased expression from cpeB2A2, which would explain why several attempts to complement the FdB phenotype have been unsuccessful, even though the library has been used to complement several other CCA mutant classes. Even if a wild-type copy of the altered gene were introduced into the mutant, the aberrant gene copy would always be present and may have a dominant or semidominant effect. Mutations that block the function or expression of this activator would be more prevalent than those that cause constitutive activity. However, our screen for mutants, performed in RL, did not allow for the isolation of mutants deficient for only cpeB2A2 expression. In RL, neither PC nor PE would be expressed in such mutants, resulting in extremely slow growth and pale green pigmentation; such strains would look unhealthy and would not have been picked. In GL or WL, the cells deficient in cpeB2A2 expression would only express PE and would look like WT. Hence, we would need to develop a different screen to identify mutants that are null for activator function.

A mutation that increased the activity of a cpeB2A2 activator could also explain the finding that the FdB phenotype is suppressed in high-intensity WL and GL. When WT cells are exposed to 10 μmol of photons m⁻² s⁻¹ of GL, the CCA photoreceptor receives and transmits the information necessary to increase cpeBA transcription and inhibits that of cpeB2A2. Decreased expression of cpeB2A2 and increased expression of cpeBA appear to require the activation of molecules that are specific for each of the phycobiliprotein operons. In one model, a protein (X) that inhibits the cpeB2A2 activator (A) by some form of modification (or protein-protein interaction) is synthesized or activated exclusively in GL. In FdB strains, the activator is in a mutant form (Am) that readily binds to and activates the cpeB2A2 promoter but is inefficiently modified by X in GL. Hence, in low-intensity GL, there is only a small quantity of active X, and since Am is not readily modified by X, most of Am remains active, promoting transcription of the cpeB2A2 operon. At a higher intensity of GL the amount of active X increases, and even though modification of Am is inefficient, elevated levels of active X cause the equilibrium to shift towards modified, inactive Am.
An alternative model could place the FdB mutation in a pathway separate from CCA. In this case, the lesion may be in a GL-specific photoreceptor that may integrate light intensity into the CCA response. Since twice as much light is required to activate or repress cpeBA expression than to regulate cpeBA2 expression (20), it is possible that there are two different photoreceptors or two distinct domains of a single photoreceptor that each respond to GL and send separate signals to alter the levels of cpeBA2 or cpeBA expression in an intensity-dependent manner; the altered response of FdB to light intensity is a common feature of photoreceptor mutants (27). The FdB phenotype can simply be explained by a lesion in a GL photoreceptor, or a reduction in its expression, such that the minimal fluence for activation has increased; thus, more light would be required to repress transcription of cpeBA2.

The final question to be addressed is how increased production of RcaE and RcaF in the FdB strains can suppress the mutant phenotype. RcaE, the putative CCA photoreceptor (14), is thought to interact directly with the response regulator RcaF (13). Overproduction of either of these proteins might favor the interaction of RcaE and RcaF, thereby facilitating the transmission of a signal through the phosphorelay system and altering the minimal fluence required for generating a specific effect; less light would be required to suppress cpeBA2 transcription in an FdB strain overproducing RcaE or RcaF. With respect to the model presented above, elevated levels of RcaE or RcaF could facilitate signal transmission in low-intensity GL, which would lead to repression of cpeBA by stimulating the modification of Alm by X. Alternatively, overexpression of a histidine kinase or response regulator may result in cross talk; RcaE and RcaF may partially substitute for the components of a second signaling pathway in suppressing the FdB phenotype. Further studies of the FdB strains and identification of the components altered in these strains may provide some key insights into intensity control in photosynthetic organisms.

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