Complementation of a red-light-indifferent cyanobacterial mutant

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ABSTRACT   Many cyanobacteria alter their phycobili-some composition in response to changes in light wavelength in a process termed complementary chromatic adaptation. Mutant strains FdR1 and FdR2 of the filamentous cyanobacterium *Fremyella diplosiphon* are characterized by aberrant chromatic adaptation. Instead of adjusting to different wavelengths of light, FdR1 and FdR2 behave as if they are always in green light; they do not respond to red light. We have previously reported complementation of FdR1 by conjugal transfer of a wild-type genomic library. The complementing DNA has now been localized by genetic analysis to a region on the rescued genomic subclone that contains a gene designated *rcaC*. This region of DNA is also able to complement FdR2. Southern blot analysis of genomic DNA from FdR1 and FdR2 indicates that these strains harbor DNA insertions within the *rcaC* sequence that may have resulted from the activity of transposable genetic elements. The predicted amino acid sequence of RcaC shares strong identity to response regulators of bacterial two-component regulatory systems. This relationship is discussed in the context of the signal-transduction pathway mediating regulation of genes encoding phycobilisome polypeptides during chromatic adaptation.

The phycobilisomes (PBS) of prokaryotic cyanobacteria are light-harvesting complexes that absorb and transfer light energy to chlorophylls of the photosynthetic apparatus (1–3). These complexes contain two domains, each composed of chromophoric phycobiliproteins and nonchromophoric linker polypeptides (4). One domain, the core of the PBS, contains the phycobiliprotein allophycocyanin (AP; A_{max} = 650 nm) and is involved in the association of the PBS with the photosynthetic membranes. The second domain contains the phycobiliproteins phycocyanin (PC; A_{max} = 620 nm) and phycoerythrin (PE; A_{max} = 566 nm) that form rods that radiate from the core (1, 3). AP, PC, and PE are each composed of related α and β subunits that are covalently bound to linear tetrapyrrole chromophores (5). The nonchro-mophorylated linker proteins may be important in PBS assembly processes and help establish a vectorial transfer of excitation energy within the PBS and from the PBS to photosystem II that can be >95% efficient (3, 6).

Many cyanobacteria alter the phycobiliprotein composition of their PBS in response to changes in environmental light quality. This response, termed complementary chromatic adaptation, is primarily caused by altered expression of genes encoding specific PBS polypeptides and enables cells to maximize absorbance of incident light (7–9). Complementary chromatic adaptation has been most extensively characterized in the filamentous cyanobacterium *Fremyella diplosiphon* (also named *Calothrix* sp. PCC 7601) (9, 10). Green light (GL; $\lambda_{optimum}$, 540–550 nm) promotes synthesis of the red-colored PE and suppresses synthesis of a subset of the blue-colored PC (named inducible PC, PC$_i$, or PC$_2$); these cells are pigmented red in GL. Alternatively, red light (RL; $\lambda_{optimum}$, 650–660 nm) stimulates synthesis of PC$_i$ and suppresses synthesis of PE; cells grown under these conditions are pigmented blue-green. Analyses of transcript populations show that changes in the PBS pigment content reflect altered rates of transcription from *cpeBA* (encodes PE α and β subunits) and *cpcB2A2* (encodes PC$_i$ α and β subunits) (9, 11–13).

In chromatic adaptation altered gene expression is mediated by light-triggered signal transduction events. Regulation of phycobiliprotein expression in GL and RL requires either two different photoreversible photoreceptors or a common photoreceptor that initiates two significantly different signal-transduction pathways (12). Furthermore, positive regulatory elements may modulate the transcriptional activity of *cpeBA*, whereas a negative regulatory factor may control the transcriptional activity of *cpcB2A2* (12, 13).

The isolation and molecular characterization of *F. diplosiphon* mutants that exhibit aberrant chromatic adaptation have provided considerable insight into the signal-transduction mechanism involved in chromatic adaptation. In the analysis of three mutant classes (red, blue, and green denoted FdR, FdB, and FdG, respectively), altered levels of PBS polypeptides directly correlate with respective transcript abundance. The blue mutant exhibits anomalous expression of *cpcB2A2*, green mutants exhibit abnormal expression of *cpeBA* (14), and red mutants exhibit altered expression of both *cpeBA* and *cpcB2A2*. In FdR strains, there is constitutive synthesis of PE and no synthesis of PC$_i$ under any conditions of illumination. Thus, red mutants are indifferent to RL and are locked in the GL regulatory mode.

Recently, we have used (15) a *F. diplosiphon* recombinant library in the shuttle vector pPL2.7 to complement the mutant strain FdR1. The rescued complementing plasmid, designated pR1W1 (called pBR1W1 in ref. 15), was also able to complement strain FdR2. In this report, we characterize the complemented mutants and identify the gene, designated *rcaC*,† that restored normal pigmentation to the FdR mutant strains. The *rcaC* gene product shares functional domains homologous with regulatory proteins of bacterial two-component regulatory systems (16, 17). We also show that the FdR phenotype of strains FdR1 and FdR2 is likely the result of independent structural aberrations at the *rcaC* locus. This report implicates a two-component regulatory system in the control of gene expression during chromatic adaptation.

MATERIALS AND METHODS

Strains and Growth Conditions. *F. diplosiphon* Fd33 was used. This strain has a short filament phenotype and displays normal chromatic adaptation (18). Pigment mutant strains FdR1 and FdR2 were generated by electroporation and display the red mutant phenotype that has been described (14). Cells were grown in liquid or on solid BG-11 medium and

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Abbreviations: AP, allophycocyanin; GL, green light; PBS, phycobilisomes; PC, phycocyanin; PE, phycoerythrin; RL, red light.
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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M95680).

illuminated with white light at 100 μmol·m^{-2}·s^{-1} or with GL or RL at 15 μmol·m^{-2}·s^{-1} (15). Where appropriate BG-11 medium was supplemented with kanamycin at 25 μg/ml.

All *Escherichia coli* host and conjugal strains used in this work have been described (15, 19) and were propagated in liquid or on solid LB medium with antibiotics at standard concentrations (19). Plasmids harboring subcloned Fd33 genomic DNA for production of single-stranded DNA sequencing templates using helper phage M13K07 were maintained in *E. coli* XL1-Blue and propagated in liquid 2× YT medium (19). Fd33 genomic DNA subcloned into λEMBL3 was amplified in *E. coli* NM539, which was propagated on NZYM medium (19).

Gene Transfer. Transfer of DNA into *F. diplosiphon* either by conjugation or electroporation was performed as described (15).

DNA Methods. DNA restriction endonucleases and modifying enzymes were purchased from Bethesda Research Laboratories, Boehringer Mannheim, Pharmacia, and International Biotechnologies. [α-^{32}P]dCTP and deoxyadenosine 5'-[α-^{35}S]thio]triphosphate were from DuPont/NEN.

To isolate genomic DNA from *F. diplosiphon*, cells from a 50-ml culture were harvested by centrifugation at 16,000 × g for 10 min and resuspended in 3 ml of lysis buffer (50 mM glucose/25 mM Tris·HCl, pH 8.0/10 mM EDTA) supplemented with lysozyme at 2 mg/ml. The suspension was incubated at 37°C for 5 min, brought to 2% (wt/vol) SDS, and phenol-extracted, and the DNA was precipitated from 70% ethanol/0.3 M NaOAc and resuspended in TE (19).

DNA manipulations including restriction digests, agarose gel electrophoresis, ligation, and production of single-stranded DNA sequencing templates were as described by Sambrook *et al.* (19). For Southern blot hybridization analysis, DNA was transferred to a charged nylon membrane (Amersham) by the method of Reed and Mann (20). DNA probes were produced from gel-purified DNA fragments by random-primer labeling (21). Hybridizations were performed at 65°C according to Sambrook *et al.* (19). Double-stranded DNA sequencing templates were isolated and purified using a kit from Promega.

Screening the λEMBL3 Library. A λEMBL3 recombinant library of Fd33 genomic DNA was screened by plaque hybridization (19) with the genomic insert from complementing plasmid pR1W1. One identified clone, designated λGCMS5, was selected for further characterization.

DNA Sequence Analysis. DNA from λGCMS5 was digested with *Eco*RI or *Cla* I and subcloned into pUC118 or pUC119. Subclones hybridizing to the pR1W1 insert DNA were used as sequencing templates. Double- and single-stranded DNA sequencing was by the dideoxynucleotide chain-termination method (22) using Sequenase Version 2.0-modified T7 DNA polymerase (United States Biochemical). Reactions were primed with either M13 universal or reverse primers or with oligonucleotides synthesized on a Biosearch model 8600 oligonucleotide synthesizer. Sequences were analyzed using the program of International Biotechnologies and compared to sequences in the GenBank database (November 18, 1991).

Analysis of PBS Composition. Preparation of intact PBS, pigment ratio determinations, and protein separation by SDS/PAGE were as described (15).

RESULTS

PBS Composition. We have recently reported complementation of *F. diplosiphon* pigment mutants (15). One complemented exconjugant of red mutant FdR1 was designated FdR1W1 (called BR1W1 in ref. 15). To characterize chromatic adaptation in FdR1W1, axenic cells were grown in RL or GL and analyzed spectroscopically and for PBS polypeptide composition. The polypeptide composition of Fd33,

FdR1, and FdR1W1 grown in RL and GL was evaluated by SDS/PAGE (Fig. 1). Fd33 grown in RL contained low levels of PE and high levels of PC_i and its associated linker polypeptides L_R^{39} and $L_R^{37.5}$ (Fig. 1, lane 1) (8, 10). In GL, PE and the PE linker polypeptides L_R^{35} and $L_R^{35.5}$ accumulated whereas PC_i and the PC_i linker polypeptides decreased (Fig. 1, lane 2). The PBS protein composition was evaluated by examining the levels of the specific linker polypeptides in the polyacrylamide gels. In contrast to Fd33, FdR1 contained low levels of PC_i and high levels of PE in both RL and GL (Fig. 1, lanes 3 and 4) (14). Hence, the polypeptide composition of the PBS in FdR1 grown in RL was similar to that of Fd33 grown in GL (Fig. 1, compare lane 3 and lane 2). The polypeptide composition of PBS from complemented strain FdR1W1 grown in RL and GL was essentially indistinguishable from that of Fd33 (Fig. 1, compare lanes 5 and 6 with lanes 1 and 2).

Pigment ratios confirmed that FdR1W1 has normal chromatic adaptation (Table 1). In Fd33, the ratio of PC to PE in RL-grown cells was 35-fold higher than in GL-grown cells. In contrast, FdR1 and FdR2 maintained high levels of PE and low levels of PC in RL and GL. The PC/PE, PC/AP, and PE/AP ratios for strain FdR1W1 grown in RL or GL were essentially the same as those for similarly grown Fd33.

Complementing DNA. We have rescued (15) the extrachromosomal complementing plasmid, designated pR1W1, from strain FdR1W1. Reintroduction of this plasmid into FdR1 resulted in a high frequency of complementation. Plasmid pR1W1 was also able to restore wild-type pigmentation to red mutant FdR2. This restoration was apparent in the polypeptide composition of the PBS (data not shown) and the phycobiliprotein ratios (Table 1) in FdR2 harboring plasmid pR1W1 (designated strain FdR2W1). Curing both FdR1W1 and FdR2W1 of plasmid pR1W1 by removing kanamycin selection resulted in reversion to the FdR phenotype.
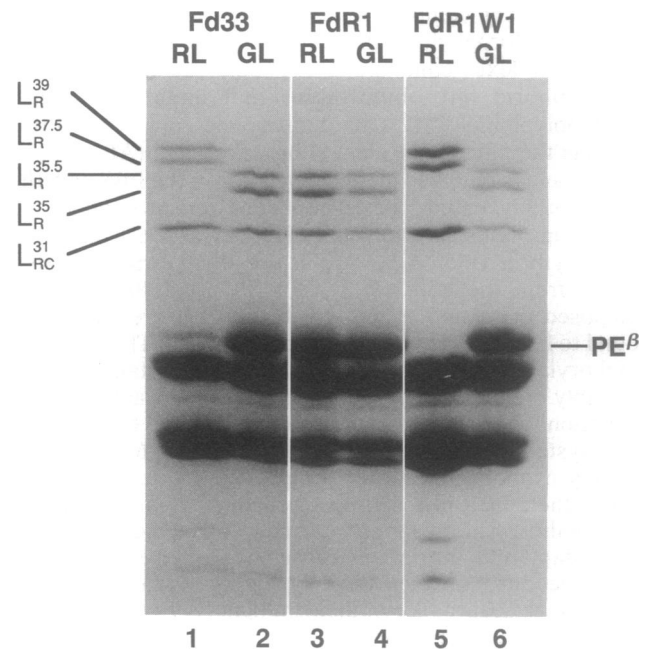
FIG. 1. Phycobiliprotein composition of PBS isolated from wild-type, the red mutant FdR1, and the complemented red mutant FdR1W1. SDS/PAGE (4–20% polyacrylamide gradient) of PBS isolated from strains Fd33 (lanes 1 and 2), FdR1 (lanes 3 and 4), and FdR1W1 (lanes 5 and 6) grown in RL (lanes 1, 3, and 5) or GL (lanes 2, 4, and 6). The linker polypeptides, designated L (3), are found in rod substructures (subscript R) or at the rod–core interface (subscript RC). Superscripts to L indicate the apparent molecular mass (kilodaltons) of each linker polypeptide. PE^{β} denotes the position of the β subunit of PE.

Genetics: Chiang *et al.*

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Table 1. Phycobiliprotein ratios in wild-type (Fd33), red mutants (FdR1 and FdR2), and complemented red mutants (FdR1W1 and FdR2W1)

Strain	Phenotype	Light	Ratio		
			PC/PE	PC/AP	PE/AP
Fd33	Wild-type	RL	7.3	1.4	0.2
		GL	0.2	0.4	1.8
FdR1	Red mutant	RL	0.2	0.3	1.7
		GL	0.2	0.3	2.0
FdR1W1	Complemented FdR1	RL	8.5	1.5	0.2
		GL	0.3	0.5	1.8
FdR2	Red mutant	RL	0.2	0.4	1.9
		GL	0.2	0.4	2.1
FdR2W1	Complemented FdR2	RL	9.0	1.5	0.2
		GL	0.2	0.4	1.9

RL, 635 nm; GL, 540 nm.

Restriction endonuclease analysis showed that plasmid pR1W1 carried a 14-kilobase-pair (kbp) genomic insert. A physical map of the region of genomic DNA that complemented the FdR phenotype is shown in Fig. 2. When a 1.6-kbp *Bst*EII fragment was excised from pR1W1, the resultant plasmid (plasmid pGCMS16; Fig. 2, line 2) was no longer able to complement the FdR phenotype. A 4.5-kbp *Bam*HI to *Xba* I fragment spanning the two *Bst*EII sites on plasmid pR1W1 was used as a probe (Fig. 2, probe 1) to screen a λEMBL3-based library of Fd33 genomic DNA. Clone λGCMS5 was isolated from the library and served as the source of DNA used for complementation analysis, generation of a physical map, and sequence determination.

Complementation of the FdR Mutants with *rcaC*. The inability of plasmid pGCMS16 to complement the FdR mutants suggested that regions proximal to the *Bst*EII sites were necessary for restoring normal chromatic adaptation. The importance of this region was also implicated in the Southern

FIG. 2. Physical map of the *F. diplosiphon* Fd33 *rcaC* region. Open box denotes 1956-bp *rcaC* open reading frame as determined by sequence analysis. Flanking and internal restriction sites are shown for enzymes used in cloning and mapping experiments. Labeled open triangles indicate mapped locations for the 4.5- and 0.4-kbp DNA insertion in mutant strains FdR1 (R1) and FdR2 (R2), respectively. Numbered horizontal bars above map identify regions that correspond to DNA probes used in screening the genomic library and Southern blot analysis of genomic DNA. Horizontal bar below map identifies the region corresponding to the 14-kbp genomic subclone harbored in λGCMS5 that extends downstream as depicted by the arrow. Numbered horizontal bars below map indicate corresponding regions harbored in the pPL2.7 shuttle vector for complementation experiments. The genomic inserts in plasmids pR1W1 (line 1), pGCMS16 (line 2), and pGCMS56 (line 3) extend 8.0 kbp upstream as depicted by the arrows. Symbols to the right of the horizontal bars denote complementation (+) or noncomplementation (−) of the FdR strains by the respective constructs. B, *Bam*HI (present only on plasmid pR1W1); Bs, *Bst*EII; C, *Cla* I; E, *Eco*RI; X, *Xba* I.

blot analysis of genomic DNA with the 4.5-kbp probe (Fig. 2, probe 1); genomic DNA isolated from the FdR mutants exhibited structural anomalies (see below). To delineate the region of DNA required for complementation, we sequenced subclones from λGCMS5 that spanned the *Bst*EII sites. Sequence analysis of this region revealed a gene designated *rcaC*[‡] (for regulator of chromatic adaptation gene C) that encodes a protein with characteristics of effector proteins of two-component bacterial regulatory systems (see below).

To confirm that *rcaC* was responsible for restoring normal chromatic adaptation, various pieces of DNA were used for complementation analysis. Fragments that were truncated in the *rcaC* gene (plasmid clones pGCMS16, pGCMS56, and pGCMS41; Fig. 2, lines 2–4, respectively) were capable of transforming the FdR strains but were unable to complement the mutant phenotype, thereby implicating lesions in *rcaC* as the cause of aberrant chromatic adaptation in FdR1 and FdR2. A 3.5-kbp *Cla* I fragment from λGCMS5 spanning *rcaC* was subcloned into the pPL2.7 shuttle vector (plasmid pGCMS37; Fig. 2, line 5) and reintroduced into the FdR strains. We were not able to readily obtain transformants using this construct. In a number of transformation attempts, we did obtain one colony that was complemented back to the wild-type phenotype. This finding suggests that multiple copies of *rcaC* are deleterious to the growth of the cells. The viable rare transformant obtained may reflect a change in either the plasmid or host that reduces expression of *rcaC*. For example, it is possible that some alteration in the sequence had occurred that prevented high-level gene expression of *rcaC* or reduced the copy number of the introduced plasmid. The fact that pR1W1, with the large genomic insert, can complement the FdR phenotype suggests that expression of *rcaC* on this plasmid is decreased. These observations require further analysis.

Characterization of *rcaC*. The gene *rcaC* is predicted to encode a 632-amino acid protein of ≈71 kDa (Fig. 3*A*). A comparison of the RcaC polypeptide sequence to proteins in the GenBank database (November 18, 1991) revealed strong homology to a number of regulatory proteins in two-component regulatory systems involved in signal transduction for cellular acclimation responses (16, 17). RcaC exhibited strong identity with PhoP, a regulatory protein involved in phosphate metabolism in *Bacillus subtilis* (23). These regulator proteins are characterized by conserved functional domains confined to ≈120 residues at the N terminus. Fig. 3*B* shows that the corresponding sequence of RcaC is 37% identical (52% conserved) to the entire sequence of PhoP.

Southern Blot Analysis. The nature of the genomic lesions in strains FdR1 and FdR2 were examined by Southern blot analysis. Total DNA from Fd33, FdR1, and FdR2 was digested with *Eco*RI, *Cla* I, or *Xba* I and hybridized to a 3.5-kbp *Cla* I probe containing the *rcaC* gene (Fig. 2, probe 2). In comparison to Fd33, the *rcaC* locus in FdR1 and FdR2 exhibited gross structural changes indicative of transposition events. The 0.85-kbp *Eco*RI fragment (see Fig. 2) from Fd33 was not present in FdR1, whereas a new band of 5.3 kbp was detected (Fig. 4*A*, compare lanes 1 and 2). A similar size increase was seen for the 3.5-kbp *Cla* I fragment in Fd33 (Fig. 4*B*, compare lanes 1 and 2). The 10-kpb *Xba* I fragment in Fd33 was detected as two *Xba* I fragments of 13.2 and 1.3 kbp in FdR1 caused by the introduction of a new *Xba* I site in the *rcaC* locus (Fig. 4*C*, compare lanes 1 and 2). This indicates that a 4.5-kbp DNA fragment has inserted into the *rcaC* gene within the 450-bp region flanked by the internal *Eco*RI and *Xba* I sites in the mutant strain (see Fig. 2). Similar analysis of genomic DNA from FdR2 indicates insertion of a 0.4-kbp
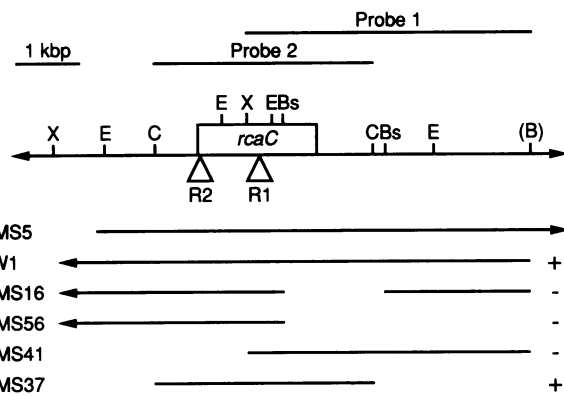
‡Designations RcaA and RcaB are used by J. Houmard (Institut Pasteur, Paris, Cedex, France; personal communication) for regulator proteins apparently involved in chromatic adaptation.
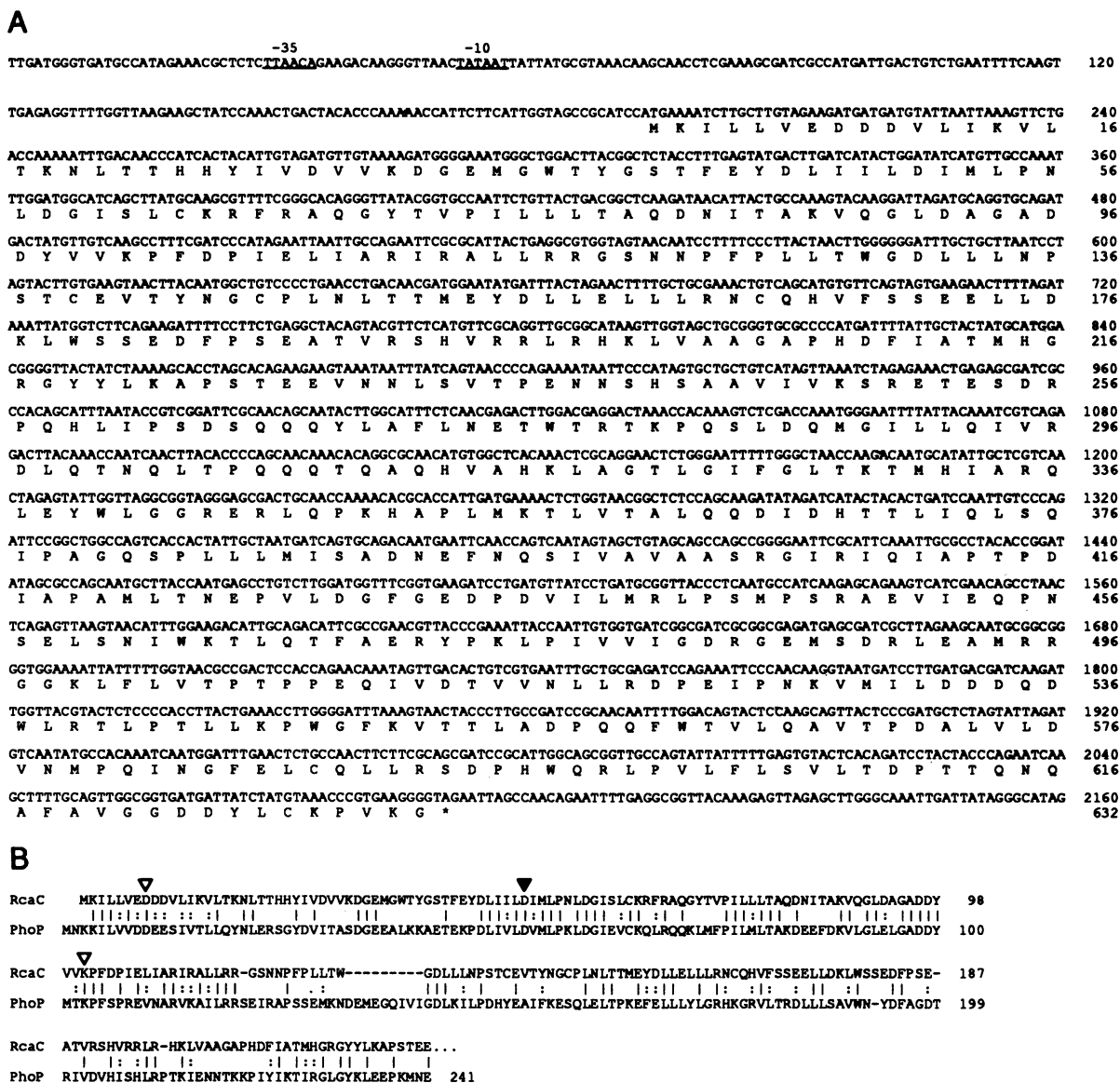
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 A  F  A  V  G  G  D  D  Y  L  C  K  P  V  K  G  *                                                                       632
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B

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        ▽                                   ▼
RcaC    MKILLVEDDDVLIKVLTKNLTTHHYIVDVVKDGEMGWTYGSTFEYDLIILDIMLPNLDGISLCKRFRAQGYTVPILLLTAQDNITAKVQGLDAGADDY    98
        |||:|:||::  ::  :|  ||    |  |    |||      |    |||:|:|:||  ||||  :||  :|  |      |||:|||   ||||  |||||
PhoP    MNKKILVVDDEESIVTLLQYNLERSGYDVITASDGEEALKKAETEKPDLIVLDVMLPKLDGIEVCKQLRQQKLMFPILMLTAKDEEFDKVLGLELGADDY   100

        ▽
RcaC    VVKPFDPIELIARIRALLRR-GSNNPFPLLTW---------GDLLLNPSTCEVTYNGCPLNLTTMEYDLLELLLRNCQHVFSSEELLDKLWSSEDFPSE-   187
        :|||  |  |:  ||::|:|||    |  .:         |||  |      | ||  |:||  |  |:  :||  ||  :|:  : ||  ||  ||  :
PhoP    MTKPFSPREVNARVKAILRRSEIRAPSSEMKNDEMEGQIVIGDLKILPDHYEAIFKESQLELTPKEFELLLYLGRHKGRVLTRDLLLSAVWN-YDFAGDT   199

RcaC    ATVRSHVRRLR-HKLVAAGAPHDFIATMHGRGYYLKAPSTEE...
        |  |:  :||  |:          :|  |::|   ||||   |     |
PhoP    RIVDVHISHLRPTKIENNTKKPIYIKTIRGLGYKLEEPKMNE   241
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FIG. 3. (*A*) Nucleotide sequence of the *rcaC* gene. The predicted amino acid sequence of RcaC is shown below the DNA sequence. Putative promoter elements are underlined. (*B*) Comparison of the amino acid sequences of the N terminus of *F. diplosiphon* RcaA and the entire *B. subtilis* PhoP protein. Identical (|) and conserved (:) amino acids between the adjacent sequences are indicated. Triangles above sequence identify conserved residues implicated in the function of regulator class proteins associated with two-component regulatory systems. Solid triangle identifies the conserved aspartate residue that is the phosphorylation substrate in these forms. Gaps in both sequences were introduced for maximum alignment.

DNA fragment into *rcaC* near the translation initiation site (see Fig. 2). In FdR2, the 0.4-kbp insert increased the size of the 3.0-kbp *Xba* I fragment seen in Fd33 (Fig. 4*C*, lane 3) and introduced a new *Cla* I site at the *rcaC* locus resulting in detection of two fragments by the probe (Fig. 4*B*). The 0.4-kbp insert in FdR2 also introduced a new *Eco*RI site at the *rcaC* locus resulting in detection of 1.7- and 0.5-kbp fragments in comparison with the 1.8-kbp fragment in the Fd33 digest (Fig. 4*A*, compare lanes 1 and 3).

DISCUSSION

Earlier, we reported isolation of plasmid pR1W1 that harbors a 14-kbp region of the Fd33 genome and restores normal complementary chromatic adaptation to red mutant strain FdR1 (15). Determination of phycobiliprotein composition and pigment content in FdR1W1 grown in RL and GL establishes that acclimation is no longer abnormal in the complemented strain (Fig. 1 and Table 1). Plasmid pR1W1 is

also able to complement another red mutant, strain FdR2 (Table 1). We have localized the gene required for complementation to a 3.5-kbp *Cla* I fragment containing a 1956-bp open reading frame (Fig. 2) designated *rcaC*.

Several observations demonstrated that the DNA fragment that encodes the regulatory protein RcaC is involved in complementing the red mutant phenotype. (*i*) Southern blot analysis of genomic DNA showed that the *rcaC* locus is structurally altered in the FdR mutants. The inserted sequences probably disrupt normal expression of *rcaC* and may represent forms of transposable genetic elements that have been reported for this strain (24). Since both FdR1 and FdR2 were generated by electroporation, it is likely that exposure of cells to a pulse of electricity induces transposition events, as suggested (14). (*ii*) Both mutants can be complemented in trans with a genomic fragment from the parental genotype Fd33 that contains the uninterrupted *rcaC* gene (Fig. 2). Any DNA fragment that does not contain the entire *rcaC* sequence is incapable of complementation.
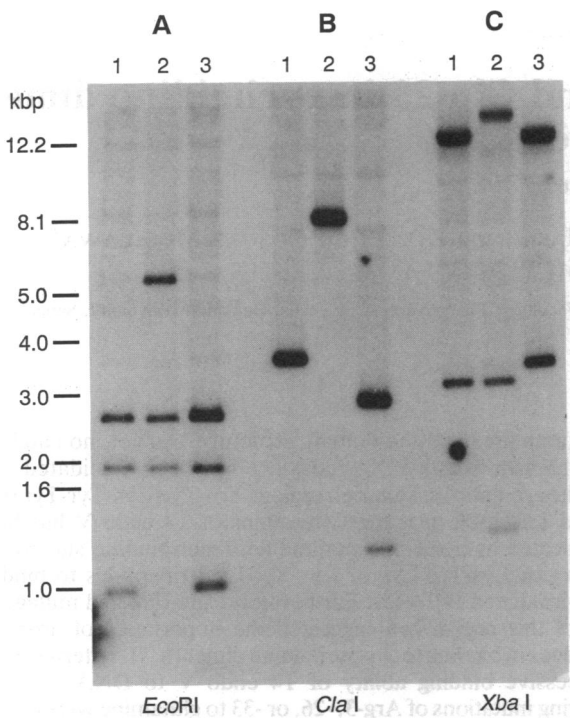
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FIG. 4.   Southern blot analysis of the rcaC genomic locus in Fd33 and FdR mutants of F. diplosiphon. Total DNA (5 μg per lane) isolated from strains Fd33 (lane 1), FdR1 (lane 2), and FdR2 (lane 3) was digested with EcoRI (A), Cla I (B), or Xba I (C) and subjected to Southern blot analysis using a radiolabeled Cla I fragment containing the rcaC gene (Fig. 2, probe 2).

The rcaC gene encodes a 71-kDa polypeptide that is homologous to a superfamily of response–regulator proteins characteristic of two-component bacterial regulatory systems (Fig. 4) (16, 17). This system involves both sensor and regulator proteins. The sensor protein detects environmental changes and communicates this information to the regulator protein by a phosphotransfer event. The regulator protein in turn causes changes in cellular function usually by altering gene transcription. The C-terminal region of the sensor activates the regulator by phosphorylation of a conserved aspartate residue located at its N terminus. RcaC is homologous to regulator proteins at the N terminus, the region that encompasses the functional domain present in all regulator proteins.

The precise role of RcaC in chromatic adaptation is not known. The red mutants exhibit constitutive synthesis of PE and no synthesis of PC_i; these strains are unable to respond to RL and appear locked in a GL regulatory mode. Complementation of the red mutants has demonstrated that RcaC is involved in suppression of PE synthesis and promotion of PC_i synthesis in RL. These two processes are predicted to be active, reflecting controlled transcription from cpeBA and cpcB2A2. Identification of a common regulator involved in expression of both cpeBA and cpcB2A2 is difficult to reconcile with the kinetic data (12, 14) and the phenotypes of pigment mutants that have been characterized. Analysis of transcript accumulation from cpeBA and cpcB2A2 suggested that an activator controls transcription from cpeBA while a repressor regulates transcription from cpcB2A2 (13). Furthermore, the kinetics of changes in the transcriptional activities of the two gene sets after transfer from RL to GL or GL to RL are markedly different, suggesting that the two gene sets are independently controlled by different regulatory elements (13). The characterization of other mutants (14) also suggests that different regulatory factors can control the activity of cpeBA and cpcB2A2. Although these results argue

against an independent regulatory role for RcaC in transcriptional control of cpeBA and cpcB2A2, they do not rule out the possibility that RcaC regulates expression of the phycobiliprotein genes in conjunction with other regulatory proteins. For example, RcaC may serve as a signal transduction chain intermediate by controlling expression of genes encoding transcriptional regulatory elements directly involved in controlling RL- and/or GL-responsive genes. Such a situation exists in the control of Ti plasmid virulence. In that system, the virA gene product contains both a sensor and a response-regulator domain and appears to control the activity of a second gene, virG, that encodes another regulator protein (25). The complementation of other mutants that display aberration in chromatic adaptation will help to elucidate the precise role of RcaC in controlling phycobiliprotein gene expression.

Note. Liang et al. (26) have reported the characterization of the patA gene, determined to be necessary for heterocyst pattern development in the cyanobacterium Anabaena 7120. Like RcaC, the PatA protein exhibits strong sequence homology to the regulatory proteins of two-component systems.

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