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Molecular Characterization of Phycobilisome Regulatory Mutants of *Fremyella diplosiphon*†

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Three classes of pigment mutants were generated in *Fremyella diplosiphon* in the course of electroporation experiments. The red mutant class had high levels of phycoerythrin in both red and green light and no inducible phycocyanin in red light. Thus, this mutant behaved as if it were always in green light, regardless of light conditions. Blue mutants exhibited normal phycoerythrin photoregulation, whereas the inducible phycocyanin was present at high levels in both red- and green-light-grown cells. Furthermore, the absolute amount of allophycocyanin was increased threefold in comparison with our wild-type strain. Green mutants lost the capacity to accumulate phycoerythrin in green light but showed normal photoregulation of phycocyanin. Analyses of transcript abundance in these mutants demonstrated that changes in the levels of the different phycobilisome components correlated with changes in the levels of mRNAs encoding those components. The characterization of these mutants supports hypotheses previously discussed concerning molecular mechanisms involved in the regulation of the phycobiliprotein gene sets during chromatic adaptation.

The light-harvesting complexes of cyanobacteria are macromolecular structures called phycobilisomes (PBS). PBS are attached to the photosynthetic membranes and absorb light in the range of 500 to 650 nm, transferring energy with high efficiency to chlorophyll molecules of photosystem II (37, 38). They consist of two structural domains: the core, which is in direct contact with the thylakoid membranes, and the rods that radiate from the core. Both domains are composed of chromophoric biliproteins and nonpigmented linker polypeptides (40). In PBS from cyanobacteria, there are often three major classes of pigmented polypeptides, phycocyanin (PC; absorbance maximum at 615 nm), phycoerythrin (PE; absorbance maximum at 562 nm), and allophycocyanin (AP; absorbance maximum at 652 nm), each consisting of an α and a β subunit. The core of the PBS is composed mainly of AP, which forms trimeric disks; the rods contain PC and PE in the form of hexameric double disks. Linker polypeptides are present in both the rods and the core and function in maintaining the structure and efficient energy transfer within the light-harvesting complex (20, 21).

In *Fremyella diplosiphon* and many other cyanobacteria, the composition of the PBS varies with the spectrum of light in the environment. This acclimation, termed complementary chromatic adaptation, allows the organism to adjust its pigment composition so as to maximize absorption of the prevalent wavelengths of available light (8, 22, 39). Changes in PBS composition during complementary chromatic adaptation have been examined after shifting cells from red illumination (R) to green illumination (G) or from G to R. The levels of AP in the core and the PC hexamers proximal to the core (the constitutive PC, or PC_1) remain unchanged during such shifts. However, exposing the cells to R causes the accumulation of a second PC species (the inducible PC, or PC_2). PE also differentially accumulates; low levels are present in PBS from R-grown cells, and high levels are present in PBS from G-grown cells. The linker polypeptides associated with PC_2 and PE are also induced in R and G, respectively (9), whereas the linker polypeptide which attaches the first PC hexamer to the core of the PBS is constitutively synthesized.

Recently, genes encoding both phycobiliproteins and linker polypeptides were isolated and characterized (2, 6, 10, 11, 13–17, 23, 28, 29, 33, 34, 36; D. A. Bryant, R. D. Porter, R. de Lorimier, G. Guglielmi, V. L. Stirewalt, J. M. Dubbs, B. Illman, and S. E. Stevens, Jr., V Int. Symp. Photosynth. Procaryotes, abstr. no. 103, 1985). In *F. diplosiphon* there are three sets of genes, designated *cpcB1A1*, *cpcB2A2*, and *cpcB3A3* (13–15), which encode PC subunits. *cpcB1A1* and *cpcB2A2* encode the constitutive and inducible PC subunits, respectively, whereas *cpcB3A3* is expressed at very low levels under normal culture conditions. Analyses of transcript accumulation and determination of transcript half-lives (R. Oelmüller, P. B. Conley, N. Federspiel, W. R. Briggs, and A. R. Grossman, Plant Physiol., in press) indicate that *cpcB1A1* is transcribed at a similar rate in both R and G as a single mRNA of 1,500 bases and that *cpcB2A2* is transcribed only during growth in R as two mRNAs, one of 1,600 bases and the other of 3,800 bases. The latter transcript also encodes the linker polypeptides associated with PC_2 (30). The role of the PC subunits encoded by *cpcB3A3* in PBS biosynthesis is unclear, although recent work demonstrated that transcription from this gene set occurred at high rates during sulfur deprivation (V. Capuano, D. Hazel, G. Guglielmi, T. Coursin, J. Houmard, and N. Tandeau de Marsac, VI Int. Symp. Photosynth. Procaryotes, abstr. no. G9, 1988). PE and AP gene sets (*cpeBA* and *apcA1B1*, respectively) have also been isolated and characterized. The former is transcribed at high rates in G (22, 33; Oelmüller, et al., in press), whereas the latter is constitutively transcribed (15).

Although many of the genes encoding structural proteins of the PBS have been isolated and their transcripts have been defined, the mechanisms for regulated expression from these genes are still poorly understood. One possible means of exploring regulated expression is through the use of mutants. In the past 10 years, mutant analyses have helped to establish a detailed structural picture of the PBS (1, 19, 31, 32, 42). Recently, mutants aberrant in the regulation of PBS

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components have been isolated in cyanobacteria (3, 5, 12, 39). Some of these arose spontaneously (35), whereas others were generated by UV light (3, 12) or nitrosoguanidine (5) treatment. We describe here three classes of pigment mutants of *F. diplosiphon*. They display abnormal regulation of phycobiliprotein synthesis and may be useful in defining the transduction chain from photoperception to altered PBS composition during complementary chromatic adaptation.

MATERIALS AND METHODS

Growth conditions. (i) Liquid culture. *F. diplosiphon* was grown to mid-exponential growth phase at 32°C in BG-11 medium (24) in an atmosphere of 3% CO_2 and 97% air. This *F. diplosiphon* strain (denoted Fd33; 12) grows as short filaments, which makes the isolation of individual colonies possible. Cultures were continuously illuminated with cool white fluorescent light (F20T12.CW; General Electric Co., Schenectady, N.Y.) filtered through either green (Plexiglas 2092, 6.6-mm thick, maximal transmission at 535 nm, no transmission below 470 or above 600 nm; Rohm & Haas Co., Philadelphia, Pa.) or red (Acrylite 210-0, 6.6-mm thick, maximal transmission at 635 nm, no transmission below 585 nm; TAP Plastics, Mountain View, Calif.) Plexiglas. Light intensities were 15 μmol/m^2 per s.

(ii) Solid medium. *F. diplosiphon* cells were maintained on plates of BG-11 medium solidified with 1.5% agar (Difco Laboratories, Detroit, Mich.) in continuous, red-enriched (Gro-Lux, F72T12; Sylvania) or green-enriched (cool white; General Electric) light at approximately 19 μmol/m^2 per s at 32 and 25°C, respectively.

Electroporation. Cells were grown in liquid cultures to mid-exponential growth phase, washed three times with distilled water, and concentrated 40- to 50-fold. A 40-μl portion of this suspension was exposed to high-voltage pulses (1,100 V, time constant for discharge of 4.9 ms; Gene Pulser; Bio-Rad Laboratories, Richmond, Calif.) and placed in 10 ml of BG-11 for 12 h. After this treatment, cells were plated on solid BG-11 medium without antibiotics and grown under Gro-Lux or cool white illumination for 5 to 10 days. Since the purpose of these experiments was to optimize electroporation conditions, cells were electroporated in the presence of plasmids containing the origin of replication from a *Fremyella* plasmid, the basis of mobility and origin of replication of pBR322, and a kanamycin resistance gene. To determine the number of filaments surviving the treatment, cells were plated on solid medium without antibiotics. In the course of these experiments, colonies with altered pigmentation were isolated.

Absorption spectra. A 2-ml suspension of exponentially growing cells was rapidly frozen in liquid nitrogen and stored at −20°C. Samples were thawed at 37°C and split into two 1-ml portions. One sample was treated with methanol to extract chlorophyll, and the other was treated with lysozyme (final concentration, 5 μg/ml) for 30 min at room temperature with occasional shaking. Cell debris was removed by centrifugation at 14,000 × *g* for 20 min. The supernatants were adjusted to equal chlorophyll (3.3 μg/ml), and absorption spectra were measured from 450 to 700 nm with a Cary 17 spectrophotometer. Phycobiliprotein concentrations were calculated according to the method of Bennett and Bogorad (7).

Fluorescence emission spectra. Exponentially growing cultures were diluted with BG-11 to an optical density of 0.1 at 750 nm. Emission spectra, after excitation with either 500- or 600-nm light, were obtained at room temperature with an MPF/3 L fluorescence spectrophotometer (The Perkin-Elmer Corp., Norwalk, Conn.). Slit widths of the excitation and emission beams were 8 and 10 nm, respectively. All curves were corrected for differential output of the monochromator and the sensitivity of the photomultiplier.

PBS isolation and analysis by electrophoresis. PBS were isolated by using a four-step gradient of 0.65, 1.0, 1.5, and 2.0 M sucrose (4). The region of the gradient containing intact PBS was collected, diluted fivefold with sodium phosphate buffer (0.65 M, pH 7.5), and pelleted by centrifugation at 100,000 × *g* for 2 h. Pelleted PBS were suspended in 0.1 M $NaCO_3$–0.1 M dithiothreitol and made to 1.7% sodium dodecyl sulfate and 10% sucrose. Polypeptides from the isolated PBS were separated by polyacrylamide gel electrophoresis, using the Laemmli buffer system (27), in a gradient of 7 to 20% polyacrylamide. Protein bands were stained with Coomassie brilliant blue R-250 and destained in 7% acetic acid–10% methanol.

Isolation and characterization of RNA. Cells were filtered onto GF/A filters (Whatman, Inc., Clifton, N.J.), suspended in 6 M guanidine hydrochloride and 1% lauroylsarcosine, and passed through a French pressure cell before RNA isolation (15, 26). For Northern (RNA) analyses, 5 μg of total RNA was loaded in each lane of a denaturing agarose gel (1.3% agarose in 20 mM morpholinepropanesulfonic acid [MOPS], 5 mM sodium acetate, 1 mM EDTA, 6% formaldehyde) (13). After electrophoresis in 20 mM MOPS–5 mM sodium acetate–1 mM EDTA at 150 V, the RNA was transferred to nitrocellulose membranes (Schleicher & Schuell, Inc., Keene, N.H.) (41). For slot blot hybridizations, an aqueous solution with 5 μg of denatured total RNA was placed in each slot and slowly passed through the nitrocellulose membrane (25). The membranes were baked in vacuo for 2 h, and hybridizations were performed with DNA fragments, labeled by random priming (18), specific for the different phycobiliprotein genes. A 287-base-pair *Hin*dIII fragment (14), a 3,800-base-pair *Hin*dIII fragment (13, 15), a 282-base-pair *Xba*I fragment (Oelmüller et al., in press; A. R. Grossman, unpublished data), and a 693-base-pair *Pst*I/*Hae*III fragment (Oelmüller et al., in press; P. G. Lemaux and A. R. Grossman, unpublished data) were used to quantitate the levels of transcripts encoding PC_1, PC_2, PE, and AP, respectively. Data obtained by slot blot hybridizations may vary between 15 and 20% (Oelmüller et al., in press).

RESULTS

Mutants. On solid BG-11 medium, Fd33 grows as green colonies when maintained in R and as red colonies when maintained in G. After exposure of R- or G-grown cells to high-voltage pulses, colonies with abnormal pigmentation were observed at a frequency of 10^{-3} to 10^{-4} relative to colonies surviving this treatment. Spectral and molecular analyses were performed for a variety of pigment mutants to classify mutants with similar phenotypes. The mutants isolated were placed into three different classes. A mutant with red coloration in both R and G (red mutant class) was called *F. diplosiphon* R1 (FdR1), a mutant which was blue-green in G but appeared to have normal pigmentation in R (blue mutant class) was called *F. diplosiphon* B1 (FdB1), and a mutant which remained green in both R and G (green mutant class) was called *F. diplosiphon* G7 (FdG7). We observed only a single blue mutant, but several with phenotypes similar to the red and green mutant classes were isolated.

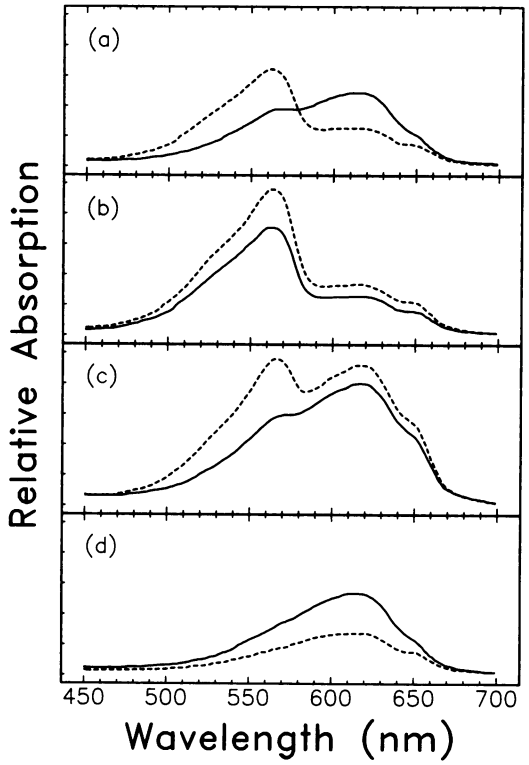

FIG. 1. Absorption spectra of cell extracts from Fd33 (a), FdR1 (b), FdB1 (c), and FdG7 (d) grown in R (——) or G (- - -). Cell extracts were adjusted to the same amount of chlorophyll (3.3 μg/ml) before measurement.

Three members of the red and two of the green mutant classes were analyzed in detail and showed, within their classes, the same phenotype. One member from each mutant class is described in detail below.

Absorption properties. To investigate the capacity of the mutants for chromatic adaptation, they were grown in liquid medium in R or G. Figure 1a shows absorption spectra of extracts of Fd33. To compare spectra, cell extracts were adjusted to equal chlorophyll (3.3 μg/ml) before the measurements were made. Extracts from R-grown cells showed maximal absorption at approximately 620 nm, reflecting high levels of PC subunits. A shoulder at 560 nm indicates the presence of some PE, and a second shoulder near 650 nm is characteristic of AP. In contrast, G-grown cells showed maximal absorption at 560 nm, reflecting high PE levels. PC levels were reduced, as indicated by low absorption at 620 nm, and AP levels were approximately the same in R- and G-grown cells. Under the conditions of illumination used here, there was between 5- and 10-fold more PE in G- than in R-grown cells (Table 1). The ratio of PC to PE changed by a factor of approximately 20 when R- and G-grown Fd33 were compared (9.4 in R-grown and 0.5 in G-grown cells; Table 1).

Figure 1b through d shows spectra of extracts from the three mutant classes. Extracts of FdR1 maintained in either R or G were spectrally similar to those of Fd33 grown in G (compare Fig. 1a and b), although absorption due to PE was slightly higher than that of Fd33 in the spectrum shown here. However, the PC-to-PE ratio and the absolute levels of all the phycobiliproteins, in an average from three experiments, were essentially the same in FdR1 maintained in R or G and similar to values for G-grown Fd33 (Table 1).

TABLE 1. Phycobiliprotein levels and ratios in Fd33 and pigment mutants

Strain	Type of light	Phycobiloprotein level (μg/3.3 μg of chlorophyll)			Ratio		
		PC	PE	AP	PC/PE	PC/AP	PE/AP
Fd33	R	0.028	0.003	0.006	9.4	4.7	0.5
	G	0.011	0.022	0.006	0.5	1.8	3.7
FdR1	R	0.011	0.024	0.007	0.5	1.8	4.0
	G	0.012	0.027	0.007	0.44	1.7	3.9
FdB1	R	0.042	0.006	0.019	7.0	2.5	0.4
	G	0.036	0.019	0.017	1.9	2.1	1.1
FdG7	R	0.029	0.000	0.006		5.8	0.1
	G	0.010	0.002	0.006	5.0	2.0	0.4

Spectra of FdB1 cell extracts (Fig. 1c) revealed high levels of PC and AP in both R- and G-grown cells. The levels of PC and AP in extracts of G-grown FdB1 were approximately threefold higher, when normalized to values for chlorophyll, than in G-grown Fd33 (Table 1). Extracts from R-grown FdB1 had spectral features similar to those of R-grown Fd33. However, the absolute levels of PC and AP were 50 and 200% higher, respectively, than in Fd33 grown in R. Elevated levels of AP in FdB1 resulted in a low PC-to-AP ratio in R and a low PE-to-AP ratio in G (Table 1).

Spectra of cell extracts from both R- and G-grown FdG7 (Fig. 1d) showed extremely low PE levels. In this mutant, the PC content was regulated normally and the absolute levels of PC and AP, after growth in both R and G, were comparable to those of Fd33 (Table 1).

Fluorescence emission spectra. Energy transfer properties of Fd33 and the mutants were assayed by fluorescence emission measurements of intact cells adjusted to the same cell suspension density (based on optical densities measured at 750 nm). Figure 2 shows fluorescence emission spectra of Fd33 and mutant cells after growth in either R or G. Cells were irradiated with either 500-nm light to excite PE or 600-nm light to excite PC.

Excitation of R-grown Fd33 with 500-nm light resulted in a peak of fluorescence emission from AP at approximately 660 nm, a weaker PC emission peak at 640 nm, and a shoulder at 680 nm due to chlorophyll fluorescence. There was also low-level fluorescence at 580 nm, since R-grown cells do contain a small amount of PE. G-grown Fd33 showed higher fluorescence emission from PE and peaks of AP and chlorophyll fluorescence emission at 660 and 680 nm, respectively (Fig. 2a). Excitation of R-grown Fd33 with 600-nm light resulted in elevated levels of both AP and chlorophyll fluorescence emission (note the difference in scales for Fig. 2a and b). G-grown cells, in contrast, exhibited much less fluorescence emission at 660 nm with 600-nm illumination. These findings reflect the fact that PBS in G-grown cells contain high levels of PE and low levels of PC, whereas PBS in R-grown cells contain high levels of PC and low levels of PE.

Fluorescence emission spectra of R- and G-grown FdR1 measured after excitation with 500- and 600-nm light are shown in Fig. 2c and d, respectively. Cells grown in either light quality exhibited fluorescence emission characteristics essentially identical to those of G-grown Fd33. In contrast, excitation of R-grown FdB1 with 500-nm light (Fig. 2e) yielded a fluorescence emission spectrum similar to that of R-grown Fd33, whereas the spectrum of G-grown FdB1 exhibited approximately threefold more fluorescence at 660-nm than did the spectrum of G-grown Fd33. Fluorescence

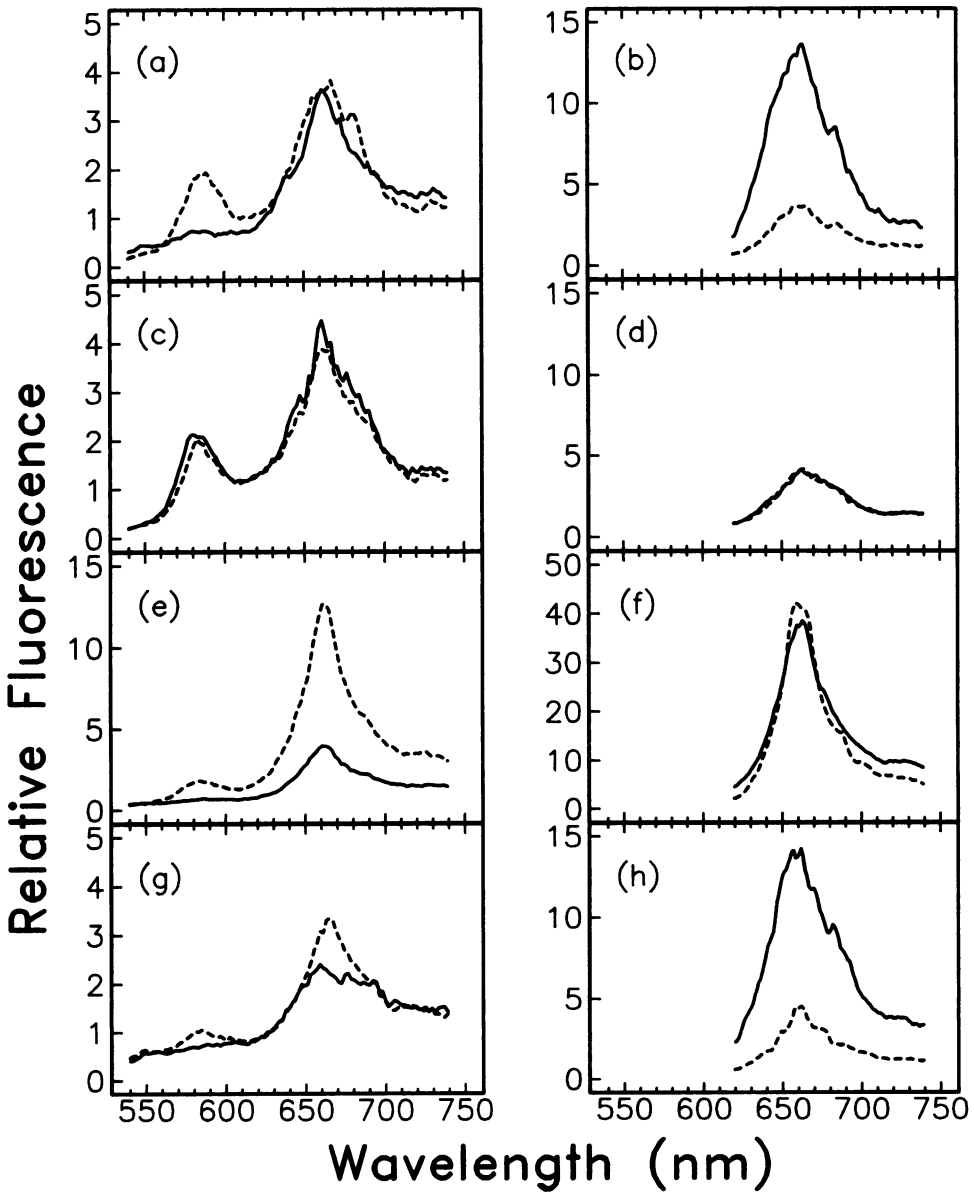

FIG. 2. Fluorescence emission spectra of R (——)- or G (- - -)-grown Fd33 (a and b), FdR1 (c and d), FdB1 (e and f), and FdG7 (g and h) after excitation with light of 500 (a, c, e, and g) or 600 (b, d, f, and h) nm. Cell suspensions were diluted to an optical density of 0.1 at 750 nm before measurement.

from PE was similar to that of Fd33, whereas chlorophyll fluorescence appeared somewhat higher. This indicated that PE was efficiently transferring energy to AP and chlorophyll and corroborated the finding that this mutant contained threefold more AP than did Fd33. After excitation with 600-nm light, fluorescence emission at 660 nm of R-grown FdB1 was approximately threefold higher than that of Fd33. G-grown FdB1 had 8- to 10-fold more fluorescence emission at 660 nm (which reflects both the high PC and AP levels in these cells) than did G-grown Fd33.

When R-grown FdG7 was excited with 500-nm light (Fig. 2g), there was approximately 50% less fluorescence emission at 660 nm than was observed for R-grown Fd33. G-grown FdG7 showed considerably lower fluorescence emission at 580 nm and slightly lower fluorescence emission at 660 nm than did G-grown Fd33. Excitation of either R- or G-grown FdG7 with 600-nm light (Fig. 2h) revealed fluorescence emission profiles similar to those observed for R- or G-grown Fd33.

PBS proteins. Figure 3 shows the polypeptide composition, as resolved by polyacrylamide gel electrophoresis, of PBS isolated from both Fd33 and the mutant strains grown in either R or G. In PBS isolated from R-grown Fd33 (Fig. 3, lane 1) both the PC_2 and PC_1 subunits (not well resolved in this gel system) and their associated linker polypeptides were present. Linker polypeptides with apparent molecular masses of 39 and 37.5 kilodaltons (L_R^{39} and $L_R^{37.5}$, respectively; see legend to Fig. 3 for explanation of designations) were coordinately regulated with the PC_2 subunits (Fig. 3, compare lanes 1 and 2). PBS from G-grown cells contained PE subunits (only PE^{β} [the β subunit of PE] was clearly resolved) and PE-associated linker polypeptides of 35.5 and

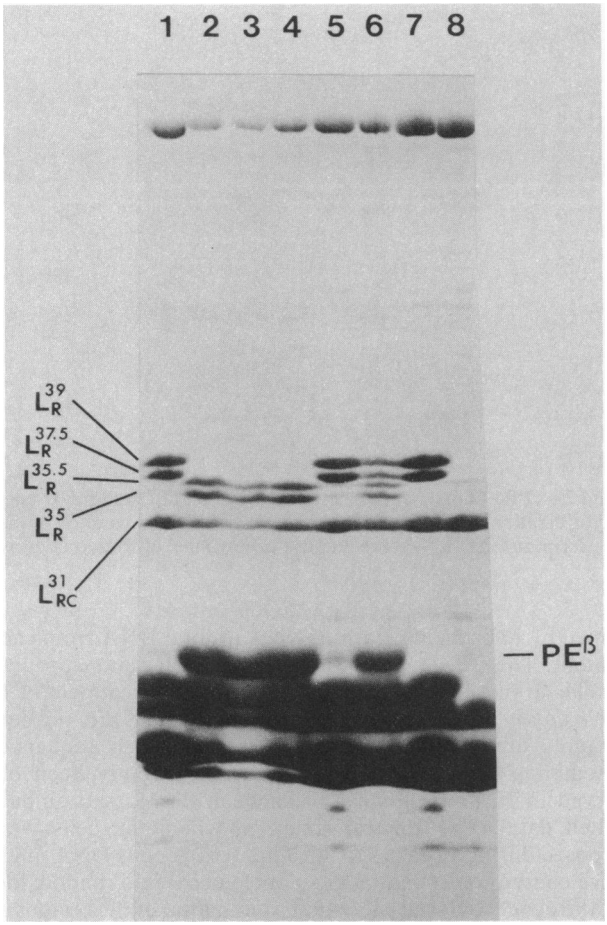

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7.5 to 20% polyacrylamide gradient) of PBS isolated from Fd33 (lanes 1 and 2), FdR1 (lanes 3 and 4), FdB1 (lanes 5 and 6), and FdG7 (lanes 7 and 8) grown in R (lanes 1, 3, 5, and 7) or G (lanes 2, 4, 6, and 8). The linker polypeptides, designated L, are in the rod substructure (indicated by the subscript R) or positioned at the rod-core interface (indicated by the subscript RC). The apparent molecular masses (in kilodaltons) of these polypeptides are indicated as superscripts to L.

35 kilodaltons ($L_R^{35.5}$ and L_R^{35}, respectively) (Fig. 3, compare lanes 1 and 2). The linker polypeptide of 31 kilodaltons (L_{RC}^{31}) was present under both light conditions.

In PBS isolated from FdR1 grown in either R or G, both the chromophoric and the linker polypeptides present were the same as those in PBS of G-grown Fd33 (Fig. 3, compare lanes 2, 3, and 4). These included $PE^{\alpha,\beta}$, $PC_1^{\alpha,\beta}$, and the linker polypeptides L_R^{35}, $L_R^{35.5}$, and L_{RC}^{31}. No change in PBS composition occurred upon transfer of cultures from G to R.

PBS from R-grown FdB1 had a polypeptide composition similar to that of PBS isolated from R-grown Fd33 (Fig. 3, compare lanes 5 and 1). High levels of AP as revealed in the spectral analyses were difficult to discern in the gel profiles presented here, since the different phycobiliprotein species are not well resolved. The PBS from G-grown FdB1, in addition to containing PE and its associated linker polypeptides, contained high levels of PC and the linker polypeptides $L_R^{37.5}$ and L_R^{39}, which are coordinately regulated with PC_2 (Fig. 3, compare lanes 1, 2, and 6).

The PBS composition of R-grown FdG7 (Fig. 3, lane 7) was similar to that of R-grown Fd33. The PBS from G-grown FdG7 had very low levels of PE and undetectable levels of the linker polypeptides L_R^{35} and $L_R^{35.5}$, which are associated with PE.

Transcript analysis. To compare the levels of transcripts encoding PBS polypeptides in the mutants with those in Fd33, RNA was isolated from cells grown in both R and G. Figure 4 shows the results of Northern analyses. The different mRNA preparations were hybridized to DNA fragments specific for PC_1, PC_2, PE, and AP (gene designations *cpcB1A1*, *cpcB2A2*, *cpeBA*, and *apcA1B1*, respectively). The *cpcB2A2*-specific DNA fragment hybridized to two transcripts, one of 1,600 bases, encoding $PC^{\alpha,\beta}$, and the other of 3,800 bases, encoding $PC^{\alpha,\beta}$, plus the three rod linker polypeptides L_R^{39}, $L_R^{37.5}$, and L_R^{9} (30). These transcripts were present only in RNA isolated from R-grown cells (13, 30). A single RNA species of 1,500 bases encodes PE and was abundant only in RNA from G-grown cells (22, 33), whereas a transcript encoding $PC_1^{\alpha,\beta}$ (1,500 bases) and two transcripts encoding $AP^{\alpha,\beta}$ (1,400 and 1,800 bases) were present at approximately equal levels in RNA preparations from R- and G-grown cells (15, 22). RNA isolated from FdR1 grown in either R or G (Fig. 4) did not contain detectable levels of the mRNAs which encode PC_2, whereas the transcript encoding PE was present at high levels under both

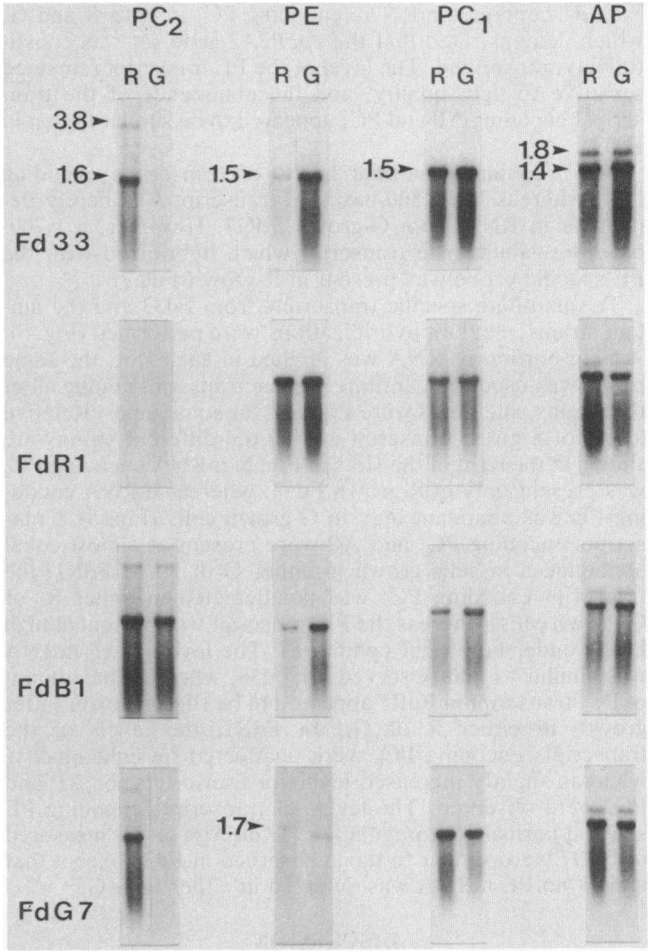

FIG. 4. Hybridization of gene-specific DNA fragments to total RNA from Fd33 and the pigment mutants grown in either R or G. The strain from which the RNA was isolated is indicated at the right; the gene-specific probe (see Materials and Methods) used in each hybridization is indicated at the top.

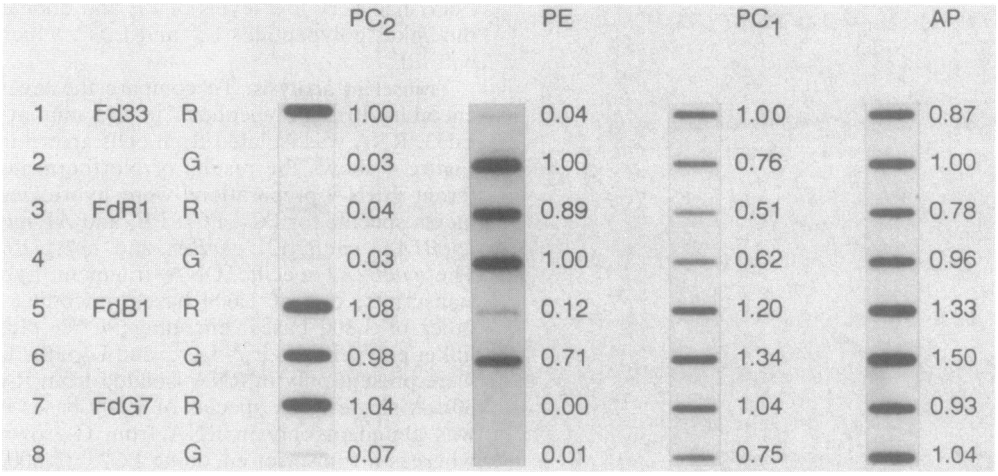

FIG. 5. Quantitation of transcripts from R- and G-grown Fd33 (slots 1 and 2), FdR1 (slots 3 and 4), FdB1 (slots 5 and 6), and FdG7 (slots 7 and 8) by slot blot hybridizations. The gene-specific probes used for the hybridizations are indicated at the top. The signals were quantified by densitometric scanning and normalized to the signal obtained for that transcript in Fd33 RNA (the higher of the values of R- and G-grown Fd33 was made 1.0).

light conditions. Levels of mRNAs encoding PC_1 and AP were similar to those determined for Fd33.

FdB1 contained mRNAs encoding PC_2 in both R and G, which demonstrated that the *cpcB2A2* gene set was constitutively transcribed. The level of the PE transcript remained sensitive to light quality, and the abundance of the transcripts encoding AP and PC_1 appeared to be similar to that in Fd33.

FdG7 contained normal levels of transcripts encoding PC_2, whereas the 1,500-base PE transcript was barely detectable in RNA from G-grown FdG7. However, a 1,700-base, low-abundance transcript which hybridized with the PE-specific probe was present in R-grown FdG7.

To quantitate specific transcripts from Fd33 and the mutant strains, slot blot hybridizations were performed (Fig. 5). A 5-μg portion of RNA was applied in each slot, the same probe was used to quantitate a given transcript among all of the strains, and filters were exposed for equal times. Relative levels of a given transcript among the different strains are shown at the right of the slots in Fig. 5. mRNA encoding PC_2 was present only in R-grown Fd33, whereas mRNA encoding PE was abundant only in G-grown cells (Fig. 5). Transcripts encoding PC_1 and AP were present at almost equal abundances in cells grown in either G or R. In FdR1, the transcript encoding PC_2 was not detected in either R- or G-grown cells, whereas the PE transcript was present at high levels under both light conditions. The level of AP mRNA was similar to that observed for Fd33, whereas the amount of PC_1 transcript in FdR1 appeared to be slightly lower (after growth in either R or G). In FdB1, the levels of the transcripts encoding PC_2 were unaffected by light quality, whereas slightly increased levels of transcripts for AP and PC_1 were observed. The levels of transcripts encoding PE showed normal photoregulation. Transcript levels measured in FdG7 were similar to those observed in Fd33 except that almost no PE mRNA was detected in either R or G.

DISCUSSION

The pigment mutants characterized here were isolated from control plates after electroporation of various plasmids into Fd33. These mutants, which appear at a frequency of 10^{-3} to 10^{-4}, do not carry the plasmid constructs as determined by hybridizing plasmid DNA to total DNA from each mutant. The frequency at which pigment variants appear is similar to that reported for nitrosoguanidine treatment of *F. diplosiphon* (39). The mechanism by which the pigment mutants are generated is not clear, although sequences resembling transposable elements have recently been observed in *F. diplosiphon* (N. Tandeau de Marsac, unpublished data). The electric shock to which the cells were exposed during attempts to introduce exogenous DNA might have caused transposition of such elements into random loci on the cyanobacterial genome. Examination of the regions of the genome encoding PC_1, PC_2, and PE indicate no gross changes at the DNA level (A. R. Grossman and B. U. Bruns, unpublished data). Electroporation might also generate mutations by causing the formation of free radicals. Further analysis is required to clarify the mechanism of mutant generation.

Whereas characterizations of cyanobacterial pigment mutants have been used to define the structure of the PBS (3, 19, 20, 32, 42), few studies have been aimed at exploiting mutants for examining the regulatory processes involved in complementary chromatic adaptation. Tandeau de Marsac (39), Cobley and Miranda (12), and Beguin et al. (5) have reported the isolation of mutants exhibiting aberrant regulation of PBS synthesis, although essentially no data at the molecular level were presented. In the three classes of mutants that we have characterized, the PBS contain an abnormal complement of phycobiliproteins in R, in G, or in both R and G, although these biliproteins do serve in energy transfer to chlorophyll, as demonstrated by fluorescence emission. All of the mutants are likely to have defects in regulated transcription of biliprotein genes. Red mutants, as exemplified by FdR1, exhibit high levels of PE and its coordinately regulated linker polypeptides and low levels of PC and its coordinately regulated linker polypeptides in both R and G. The abundance of the different biliproteins in this mutant is reflected in the abundance of the mRNAs which encode these components; hence, FdR1 grown in either R or G has no mRNA encoding PC_2 but high levels of mRNA encoding PE. The red mutants have lost the ability to acclimate to R and are fixed in the G regulatory mode. This phenotype may result from a lesion in the gene encoding the

photoreceptor or genes encoding proteins required for signal transduction (at a step close to photoperception).

FdB1 exhibits a more complex phenotype than does FdR1. In this mutant, the PE levels are regulated normally, whereas PC_2 and its associated linker polypeptides are constitutively synthesized. Transcripts encoding PC_2 are also present at high levels in both R and G. In addition, on the basis of both absorption and fluorescence emission measurements, there appears to be a threefold increase in the amount of AP in this mutant. Chlorophyll levels in FdB1 are probably not changing, since the accumulation of PE per microgram of chlorophyll is similar to that of Fd33 and the ratios of PC and PE to AP are dramatically different. The threefold increase in the level of AP is not paralleled by a threefold increase in the level of AP mRNA (although there is a small increase relative to Fd33), which indicates that posttranscription processes (e.g., elevated translation of AP mRNA) may be partly responsible for the phenotype observed in the mutant.

If the level of AP in FdB1 does reflect an increase on a per-cell basis and the structure of the PBS core is not altered, then there would be approximately three times as many PBS cores per cell and either fewer or shorter rod substructures associated with these cores. The arrangement of the inducible PC and PE hexamers, both synthesized at high levels in FdB1 in G, in the rod substructure also needs to be examined.

The most dramatic change in FdB1 at the level of transcript abundance is the constitutive accumulation of the mRNA encoding PC_2 and its coordinately regulated linker polypeptides. Previous work has suggested that transcription of the *cpcB2A2* gene set might be controlled by a repressor element (Oelmüller et al., in press). A lesion in a gene encoding this hypothetical repressor would cause constitutive synthesis of PC_2. The reason for the change in the level of core components in FdB1 is not easily explained, although altered synthesis and accumulation of one PBS component may affect the synthesis, assembly, and stability of others.

In the green mutants such as FdG7, PC levels are regulated normally, but the cells contain almost no PE (even after growth in G), as revealed by measurements of absorption and fluorescence emission as well as by analyses of the levels of PE subunits and PE mRNA. This reduced capacity to harvest light energy in the 560-nm region of the spectrum causes these mutants to grow slowly in G. Whereas only extremely low levels of PE mRNA in R- or G-grown FdG7 can be detected, a minor mRNA species from R-grown cells which hybridizes to a PE-specific probe is approximately 200 bases larger than the prevalent PE transcript. We have previously observed a similar, low-abundance transcript which hybridizes to the PE-specific probe in R-grown Fd33 (A. R. Grossman, S. Robbins, P. G. Lemaux, and P. B. Conley, unpublished data). This larger species may have a transcription initiation site different from that of the smaller mRNA or may represent a species with some homology to PE which becomes apparent on the autoradiogram only after long exposures. Kinetic analyses of changes in the synthesis of PE transcripts during acclimation to R and G have suggested that a positive regulatory element functions in transcriptional control (Oelmüller et al., in press). Inactivation of this positive regulatory element as a consequence of the electroporation might result in the phenotype observed in FdG7.

From the data presented above, we suggest that all three of the mutants characterized are aberrant in the regulation of phycobiliprotein genes during complementary chromatic adaptation. For FdR1, neither of the inducible biliprotein genes (*cpcB2A2* and *cpeBA*) is properly regulated, which suggests a common link in their regulation, perhaps at the step of photoreception or soon after that in the signal transduction chain. The R-induced PC gene set in FdB1 has become constitutive and the G-induced PE gene set in FdG7 is not transcribed, which suggests that protein factors involved in the regulation of these genes may have been altered. This conclusion is preliminary, and especially in the case of FdG7, any change in the nature of the PE transcript may alter its stability and result in the phenotype observed. Further analyses at the molecular level need to be performed.

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