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Photoreversibility of the Effect of Red and Green Light Pulses on the Accumulation in Darkness of mRNAs Coding for Phycocyanin and Phycoerythrin in *Fremyella diplosiphon*[1]

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ABSTRACT

DNA fragments encoding a red light-inducible phycocyanin gene and a green light-inducible phycoerythrin gene have been used to investigate the effect of red and green pulses on the accumulation of phycocyanin and phycoerythrin mRNA in subsequent darkness. A red pulse promotes phycocyanin and suppresses phycoerythrin mRNA accumulation while a green pulse has an opposite effect on both transcript levels. The effect of a saturating light pulse is canceled by a subsequently given pulse of the other light quality. For a given mRNA, the positive and negative effects require the same fluence for saturation, whereas to saturate the phycoerythrin mRNA response requires at least twice as much light as to saturate the phycocyanin mRNA response. Calculations of the apparent extinction coefficients for the pigments mediating the light-regulated mRNA increase and decrease are of the order of 2×10^4 for phycocyanin mRNA and less than 10^4 for phycoerythrin mRNA. The data are consistent with the hypothesis that the light-induced increase and decrease of a particular phycobiliprotein mRNA is controlled by a single red/green photoreversible photosystem, but that phycoerythrin and phycocyanin mRNA levels are either controlled by two distinct photoreversible systems or that marked differences occur in the chain of events leading from photoperception to gene activation. These system(s) differ from most phytochrome systems in several ways: First, they remain fully on or off depending upon the light quality of the terminal irradiation. Second, they can be completely reversed by light of the appropriate wavelength after several hours of darkness without diminution of the effectiveness of the reversing light pulse. These two features argue against the existence of dark reversion or dark destruction of the biologically active moiety. Third, signal transduction is rapid—measurable mRNA changes occur even during a 10 minute irradiation.

Many cyanobacteria, such as *Fremyella diplosiphon*, alter the composition of their phycobilisomes in response to the environmental light quality, a phenomenon first described by Engelmann (15) who related the water depth at which blue-green algae grew with their ability to synthesize pigments complementary to the color of the incident light. This phenomenon, termed complementary chromatic adaptation (4, 38), is the consequence of alterations in the relative concentrations of the red-colored phycobiliprotein, phycoerythrin (PE[2]) and blue-colored phycobiliprotein, phycocyanin (PC) (3, 5, 8); red light (R) promotes PC and suppresses PE and green light (G) promotes PE and suppresses PC accumulation (37, 40). The action maximum for PE synthesis is between 540 and 550 nm (22, 40), while the highest rate of PC synthesis occurs at wavelengths between 650 and 660 nm. Since R and G pulses potentiate synthesis of PC and PE in subsequent darkness (D) and since the effects of the light pulses are photoreversible (16, 17, 21), it has been postulated that chromatic adaptation is controlled by a photoreversible photoreceptor with action maxima in the R and G region of the visible spectrum (4, 40).

Each phycobiliprotein consists of an α and β subunit and recently many genes encoding these phycobiliproteins and their associated linker polypeptides have been isolated from cyanobacteria (2, 9–13, 25, 32–35) and eukaryotic algae (9, 30, 31). Using a DNA fragment which encodes the end of the PE^{β} and the first 161 nucleotides of the PE^{α} from *F. diplosiphon* Grossman *et al.* (20, and unpublished data) have shown that both subunit genes are transcribed as a single mRNA. Similar results have been obtained by Mazel *et al.* (34). Moreover, transcripts are detectable at high levels in cells maintained in G, and only at low levels in R-acclimated cells. This finding is consistent with previous results, obtained with inhibitors of transcription, suggesting that PE accumulation in G is regulated at the level of transcription (18).

However, PC is present both in R- and G-acclimated cells (7). Recently Conley *et al.* (10, 12) have shown that in *F. diplosiphon* at least 2 PC gene sets are located on the cyanobacterial genome: while transcripts from one (inducible) gene set are only detectable in cells grown in R (10) transcription from a second (constitutive) gene set occurs in both R- and G-acclimated algae (12). A third 'PC-like' gene set has also been isolated and partially sequenced (20), although expression from the gene set could not be detected. These observations are consistent with the findings of Bryant (7) and Bryant and Cohen-Bazire (8) that at least two distinct sets of PC subunits are present in cyanobacteria which exhibit complementary chromatic adaptation, one present in cells grown either in R or G and the other only in cells maintained in R. As for the PE genes, the α- and β-subunit genes for PC are transcribed as a dicistronic mRNA (10, 12, 31) which might insure that both subunits are produced in a 1:1 ratio, *i.e.* the ratio observed in the phycobilisomes.

The aim of the present study is to investigate PC and PE mRNA regulation by a postulated R/G-photoreversible photoreceptor (4). Using DNA fragments specific for the light-inducible PC and PE genes we have measured changes of the transcript

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[2] Abbreviations: PE, phycoerythrin; AP, allophycocyanin; D, darkness; G, green light; PC, phycocyanin; R, red light.

levels after transfer of R- and G-acclimated cells to D. The light treatments were terminated with R and G pulses in order to study the effect of these light pulses on the accumulation of PC- and PE-mRNA levels during subsequent D. It was found that the mRNA levels of both phycobiliproteins respond rapidly to light pulses. Moreover, the data indicate that both the light-stimulated increase and decrease of PC and PE mRNA levels in *F. diplosiphon* are controlled by R/G reversible photosystem(s) with some characteristics similar to those of phytochrome, but with some important differences.

MATERIALS AND METHODS

Growth Conditions. *Fremyella diplosiphon* (*Calothrix* sp., PCC 7601), a subculture of UTEX #481, was grown at 32°C in 40 mL of Gorham's medium (26) in an atmosphere of 3% CO_2/97% air. Cultures were illuminated with 15 μmol m^{-2} s^{-1} of red (R) and green (G) light. White light was either filtered through a R plexiglass filter (Acrylide 210-0, 6.7 mm thick, maximal transmission at 635 nm, no transmission below 585 nm) or a G plexiglass filter (Rohm & Haas Plexiglas 2092, 6.7 mm thick, maximal transmission at 540 nm, no transmission below 480 or above 605 nm). After 5 d of growth the temperature was reduced to 25°C and the cells were allowed to adapt to this temperature for 6 h before the onset of the light-pulse treatments, because changes in the mRNA abundance were too fast for reasonable kinetic studies at 32°C.

Light Pulses. Saturating light pulses of R and G light were given for 10 min at a fluence rate of 30 μmol m^{-2} s^{-1}. For illumination with lower fluences, irradiation times were varied between 2 and 1000 s with a constant fluence rate of 15 μmol m^{-2} s^{-1}.

RNA Extraction. For total RNA extraction 40 mL of cultures in the early log growth phase were collected by vacuum filtration on a Whatman filter disc (GF/C-filter) and the filter was immediately transferred to liquid nitrogen. RNA extraction was performed by immersing the discs in 7 mL of 0.1 M Tris-HCl (pH 6.5) containing 6 M guanidine-HCl, 0.01 M dithiothreitol, and 1% *N*-laurylsarcosine. Further RNA extraction was performed as described by Kranetz and Anwar (29).

Quantitation of mRNA. The method used for determination of relative amounts of mRNA levels by slot blot hybridization has been described in detail (27, 39). For hybridization a 3.7 kb *Hin*dIII DNA fragment was used which contains the R-inducible *cpc*A_2B_2 (genes encoding inducible $PC^{\alpha,\beta}$) (10). A 294 bp *Xba*I fragment encoding amino acids 6–104 of PE^{β} (20) (AR Grossman, personal communication) was used for quantitations of the PE-mRNA levels. The DNA fragment encoding the β-subunit of the AP gene set (*apc*AB) is a 693 bp *Pst*I/*Hae*III fragment which begins 37 nucleotides from the site of translation initiation and ends 240 nucleotides downstream from the termination codon (PG Lemaux, AR Grossman, manuscript in preparation). Hybridization was performed at 67°C (15–18 h, 10^4 Cerenkov counts/slot). The 3.7 kb *Hin*dIII fragment encoding the PC genes was hydrolyzed to 400 to 800 bp by boiling in 0.5 M NaOH for 4 to 5 min prior to hybridization (31). The filters were washed 3 × 10 min with 0.01 M PO_4^--buffer (pH 7.0), 0.02 M EDTA, 0.1% SDS at room temperature, 2 min with 0.05 M PO_4^--buffer pH 7.0, 0.02 M EDTA, 0.1% SDS at 67°C and 3 × 10 min at room temperature and dried before exposure to a Kodak XAR-5 film (−80°C for 1–8 h). Under these conditions the PE-containing DNA fragment hybridizes only to a 1500 base transcript and the PC DNA fragment to a major 1600 base transcript and a minor 3800 base transcript as previously shown by Northern hybridization (10, 19, 20).

All values are based on three to four independent experiments. Representative slot blots are shown in the accompanying paper. Those mRNA levels which were compared to each other were bound to the same nitrocellulose sheet and hybridized against the above-mentioned DNA fragments. The autoradiogram was scanned and the mRNA levels (operationally the signals obtained by scanning the autoradiogram) determined relative to one mRNA level (the reference signal) which was taken as 100%. This procedure was repeated at least three times with RNAs from different extractions. The variation in the data is of the order of 10% as compared to the reference signal.

RESULTS

Figure 1, top, shows that the PC mRNA level is high in R-acclimated cultures and hardly detectable in cultures kept in G. On the other hand (Fig. 1, bottom), the PE mRNA level is high under continuous G and is low in R. Under the growth conditions used for these studies we always observed approximately 10% of the PE mRNA level in R-grown cultures compared to G-grown cultures. The high PC and PE mRNA levels present under light conditions favorable for their accumulation decrease after transfer of cultures of *F. diplosiphon* to darkness (D) and the extent of the decrease is modulated by R and G pulses. In the case of PC mRNA the decrease is fast if R-acclimated cultures receive a terminating G pulse before the onset of D and significantly slower following a R pulse (Fig. 1, top), while the opposite

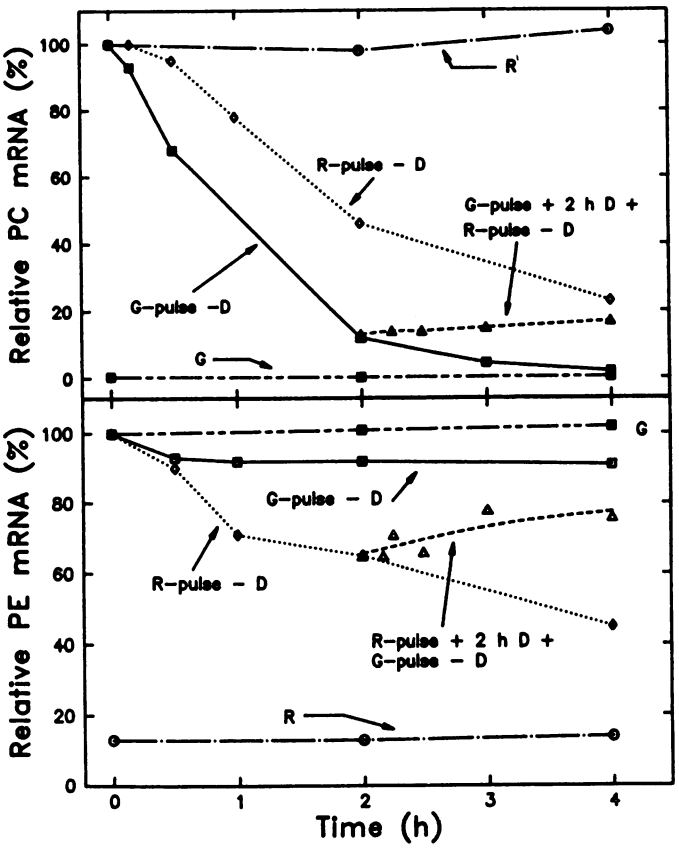

FIG. 1. The effect of saturating R and G pulses on the relative amounts of PC and PE mRNA in total RNA isolated from cultures of *F. diplosiphon*. Cells were either grown in R (○) or in G (■). For the determination of the effect of light pulses on the PC (PE) mRNA level, R- (G-) acclimated cells received a R (◇) or G (□) pulse before transfer to D. △, relative amount of PC mRNA in D after the following treatment: R-acclimation + G pulse + 2 h D + R pulse (for PE mRNA: G-acclimation + R pulse + 2 h D + G pulse). The mRNA level of R-acclimated cultures (in case of PC mRNA) and G-acclimated cultures (for PE mRNA) at time point 0 was taken as 100% and all other mRNA levels normalized to these levels.

effect of the light pulse treatment is observed for the PE mRNA level (Fig. 1, bottom). However, in general the decrease of the mRNA level in D is far slower for PE than for PC.

In cells adapted to G and therefore low in PC mRNA (Fig. 1, top) an increase in PC mRNA can be induced by a single R pulse (Fig. 2, top). Indeed, the slower PC mRNA decrease in D after a R pulse (Fig. 1, top) may be a consequence of the persistence of the inductive effect of R. In comparable fashion, in cells maintained in R and low in PE mRNA (Fig. 1, bottom), an increase in PE mRNA can be induced by a single G pulse (Fig. 2, bottom). Again, the slower PE mRNA decrease in D after a G pulse than after a R pulse (Fig. 1, bottom) may be a consequence of the persistence of the inductive effect of G (Fig. 2, bottom). The low PC mRNA level in G-acclimated algae (approximately 2% of that in R-acclimated cells) is increased fivefold by an inductive R pulse followed by 4 h of D (Fig. 2, top) while the PC mRNA level in D drops even below the level detectable in cultures kept in continuous G if the light treatment is terminated with a saturating G pulse (Fig. 2, top). The same phenomena were observed for the PE mRNA levels except that a G pulse was inductive and a R pulse repressive (Fig. 2, bottom).

The effect of an inductive light pulse on PC and PE mRNA accumulation in D can be canceled by a second light pulse of the other light quality (Figs. 1 and 2). A R pulse given after 2 h of D prevents further PC mRNA decrease (Fig. 1, top) and causes a decrease in PE mRNA levels (Fig. 2, bottom) almost immediately while a G pulse after 2 h D increases the PE mRNA level again (Fig. 1, bottom) and decreases the PC mRNA level in subsequent D (Fig. 2, top). These results explain previous observations that PE and PC protein syntheses in D are controlled by R and G pulses. The type of phycobiliprotein synthesized depends on the quality of the last light treatment alone (14, 16, 40). Moreover, the data show that under our experimental conditions the effect of light pulses on PE and PC mRNA accumulation in subsequent D occurs almost immediately without a detectable lag phase, and a pulse of the opposing light quality can reverse the system in all four possible cases even after 2 h of D (Figs. 1 and 2). The initial light-induced mRNA changes shown in Figures 1 and 2 occur essentially without a detectable lag period.

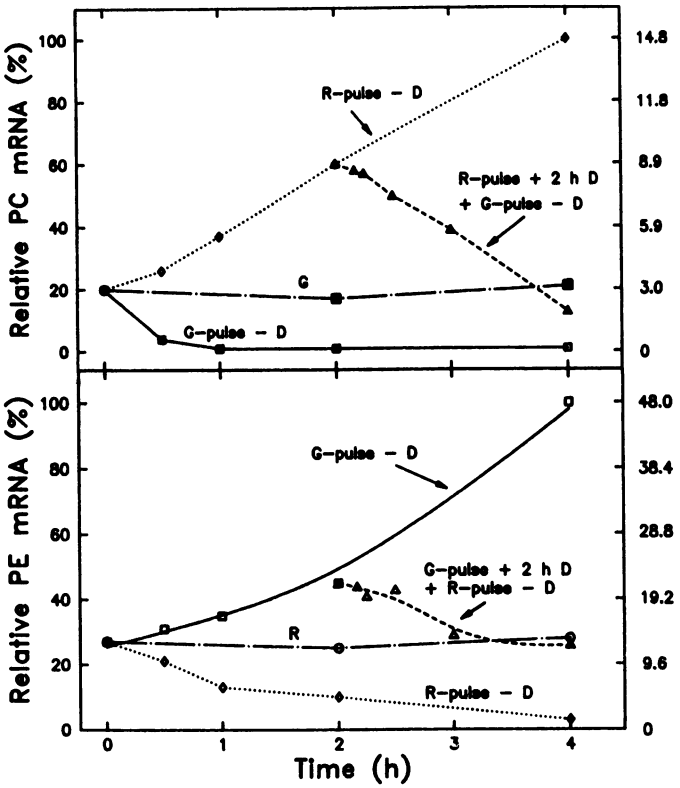

FIG. 2. The effect of saturating R and G pulses on the relative amount of PC and PE mRNA in total RNA isolated from cultures of *F. diplosiphon* which were either grown in R (○) or in G (■). Light treatment as described in the legend to Fig. 1, except that the pulses were given after G- acclimation in case of PC mRNA and R-acclimation for PE mRNA. R- (G-) acclimated cells received a R (◇) or G (□) pulse before transfer to D. △, relative amount of PC (PE) mRNA in D after the following treatment: G- acclimation + R pulse + 2 h D + G pulse (for PE mRNA: R-acclimation + G pulse + 2 h D + R pulse). The highest value of each graph was taken as 100% and all other mRNA levels referred to these levels. In order to be consistent with the data shown in Figure 1 the numbers on the right side of the graph indicate the amount of mRNA (in %) compared to the PC (PE) mRNA levels in R- (G-) acclimated cultures.

Figure 3 shows that the effect of a 10 min saturating inductive light pulse (R in case of PC mRNA and G for PE mRNA) cannot be canceled entirely by a subsequently given saturating pulse of the other light quality even if given immediately, indicating that the signal transduction from the perception of light to the alteration of PC and PE mRNA levels is fast. Thus as a longer D period is interposed between the initial pulse and that of the other light quality, progressively more of the change in mRNA levels has already taken place at the onset of the second light pulse. However, Figures 1 to 3 show that the inductive effect of a light pulse on PE and PC mRNA accumulation can be canceled by a counteractive light pulse at any time tested. Thus the response which can be measured following 90 min of D after the first (promotive) pulse depends on the length of time the initial promotive light pulse is permitted to act (Fig. 3).

The amount of mRNA coding for the allophycocyanin protein (AP) is not affected by R and G (Fig. 4). With the same RNA samples probed for PC and PE mRNA it was found that the level of transcript encoding AP is almost the same in R- and G-acclimated cells and that the decrease of this mRNA level upon transfer of G-acclimated cells to D is not significantly affected by a R and G pulse given at the end of the light treatment (Fig. 4). Thus the level of AP mRNA is not differentially controlled by R and G even though light is required for its maintenance. Two other mRNA species, that for the core linker protein of the phycobilisomes (20) (PG Lemaux, AR Grossman, manuscript in preparation), and that thought to encode the anchor protein (PG Lemaux, AR Grossman, manuscript in preparation) behaved in a similar fashion in that they required light for maintenance, but decayed equally fast after R or G treatment (data not shown).

Figures 5 and 6 show the dependency of the PC and PE mRNA levels, respectively on the fluence of the R and G pulses given prior to a 2 h D incubation period. Cultures were either grown in G and received pulses of different fluences of R or in R followed by pulses of different fluences of G before transfer to D. Under our growing conditions fluences up to 100 (log 2.00) μmol m^{-2} are ineffective in causing significant changes in the amount of PC and PE mRNA. For PC mRNA, light pulses at a fluence of 3×10^2 (log 2.48) μmol m^{-2} give a threshold response, 10^3 (log 3.00) μmol m^{-2} gives half saturation of the response, and 3×10^3 (log 3.48) μmol m^{-2} is saturating (Fig. 5). However, in the case of PE mRNA, both the inductive effect of a G pulse as well as the reduction of the high PE mRNA level in G-acclimated cells by a R pulse requires at least 1.5×10^3 (log 3.18) μmol m^{-2} to be half saturated (Fig. 6).

In the above experiments, a change in total fluence was obtained by varying irradiation time (between 2 and 100 s) at a constant fluence rate. Table I shows that at least over the fluence range used for our experiments the induction of PC and PE mRNA depends only on the total fluence of the light treatment

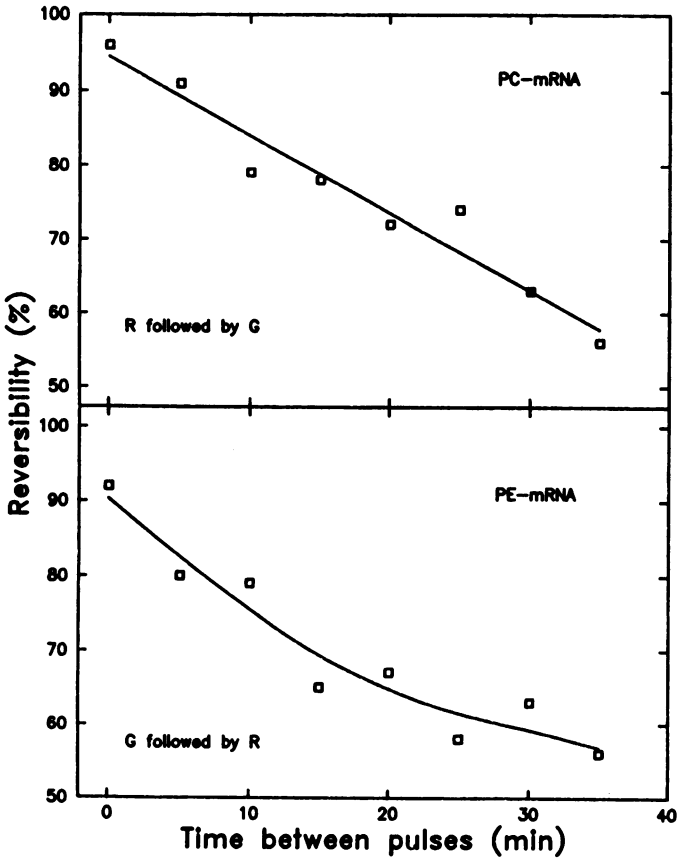

FIG. 3. The extent of reversibility of the effect of an inductive light pulse by a reversible light pulse on the amount of PC and PE mRNA in the total RNA isolated from *F. diplosiphon*. G-acclimated cells received a saturating R pulse (for PC mRNA determination) and R-acclimated cells a saturating G pulse (for PE mRNA determination) before transfer to D for 90 min. The D period was interrupted by a reversible saturating light pulse (G for PC mRNA and R for PE mRNA) the onset of which is indicated on the abscissa. Reversibility (%) means:

$$\frac{[\text{mRNA}]_{\text{inductive pulse}} - [\text{mRNA}]_{\text{inductive pulse+D+reversible pulse}}}{[\text{mRNA}]_{\text{inductive pulse}} - [\text{mRNA}]_{\text{reversible pulse}}}.$$

The amount of mRNA detectable after the inductive light pulse (R for PC mRNA and G for PE mRNA) was taken as 100% and the other mRNA levels referred to these values. The data are based on 3 different RNA extractions, and the variation of the original data is of the order of 6% (for PC mRNA) and 12% (for PE mRNA).

and is independent of fluence rate or duration of irradiation. Within the errors of experimentation the reciprocity law is valid. Longer exposures at lower fluence rates were not included as with irradiation times longer than 500 s measurable mRNA changes are already under way.

Figures 7 and 8 show the fluences required in a second light pulse to cancel the effect of an initial pulse of the opposite light quality. Cultures were either grown in R and received a saturating G pulse followed immediately by a variable R pulse or were grown in G and received a saturating R pulse followed immediately by a variable G pulse before transfer to D for 4 h. The data are in agreement with the results shown in Figures 5 and 6: (a) A final R pulse induces PC mRNA (Fig. 7, top) and reduces the PE mRNA level (Fig. 8, bottom), while a final G pulse reduces the abundance of PC mRNA (Fig. 7, bottom) and promotes PE mRNA accumulation (Fig. 8, top). (b) For a given mRNA the final inductive and repressive light pulses require almost the same fluence to be saturating. (c) Canceling of the effect of the

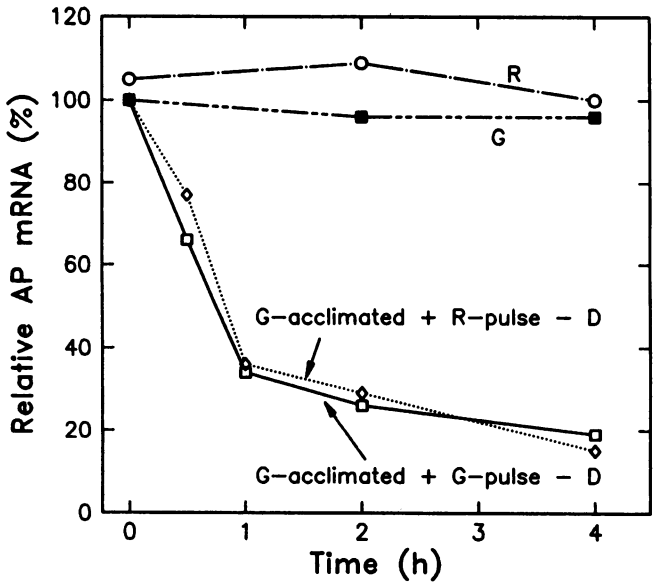

FIG. 4. Relative amount of AP mRNA in total RNA isolated from R- (○) or G- (■) acclimated cultures of *F. diplosiphon* as well as the effect of a saturating R (◇) and G (□) pulse given after G-acclimation on the level of AP mRNA in subsequent D. The amount of AP mRNA in G-acclimated cultures at time point 0 was taken as 100% and all other mRNA levels referred to this amount.

first light pulse by a second light pulse of the other light quality requires more light in the case of PE than for PC mRNA (about 2.5×10^3 (log 3.40) μmol m^{-2} for PC mRNA and about 7.5×10^3 (log 3.88) μmol m^{-2} for PE mRNA).

From the data shown in Figures 5 and 6 the apparent extinction coefficients for the induction and reversion of PC and PE mRNA were calculated (Table II) by the classical approach of Hendricks *et al.* (23) for phytochrome action. These calculations are made under two general assumptions: first, that production of active photoproduct, P, is a consequence of first order photochemistry, and, second, that mRNA abundance is linearly related to photoproduct concentration. If these assumptions are correct, then $P(t) = P(0)e^{-I\phi\epsilon t}$, where I is the fluence rate in moles of quanta m^{-2}, ϕ is the quantum efficiency of the photoprocess, e is the molar extinction coefficient, and t is the time in s. Under these assumptions, and an added assumption of a quantum efficiency of 0.1 for each photoreaction, the extinction coefficients are of the order of 2×10^4 based on PC-mRNA measurements whereas the coefficients for both the induction and reversion are below 10^4 when the PE mRNA data are used for the calculation.

DISCUSSION

The data show that light pulses affect the accumulation of the R-inducible PC mRNA and the G-inducible PE mRNA in subsequent D. R causes an increase in PC and a decrease in PE mRNA levels and G has exactly the opposite effect (Figs. 1–3). The fact that for a particular mRNA the apparent extinction coefficient is almost the same for the increase and suppression is compatible with the hypothesis that light control of this mRNA accumulation is regulated by a single photoreversible photoreceptor (4, 40).

Calculations of the apparent extinction coefficient based on PC and PE mRNA measurements suggest that at least twice as much light is required to affect the level of PE mRNA as is required for the PC mRNA response (Table II, Figs. 5–8). This difference cannot be caused by differences in screening of the effective wavelengths by the different photosynthetic pigmenta-

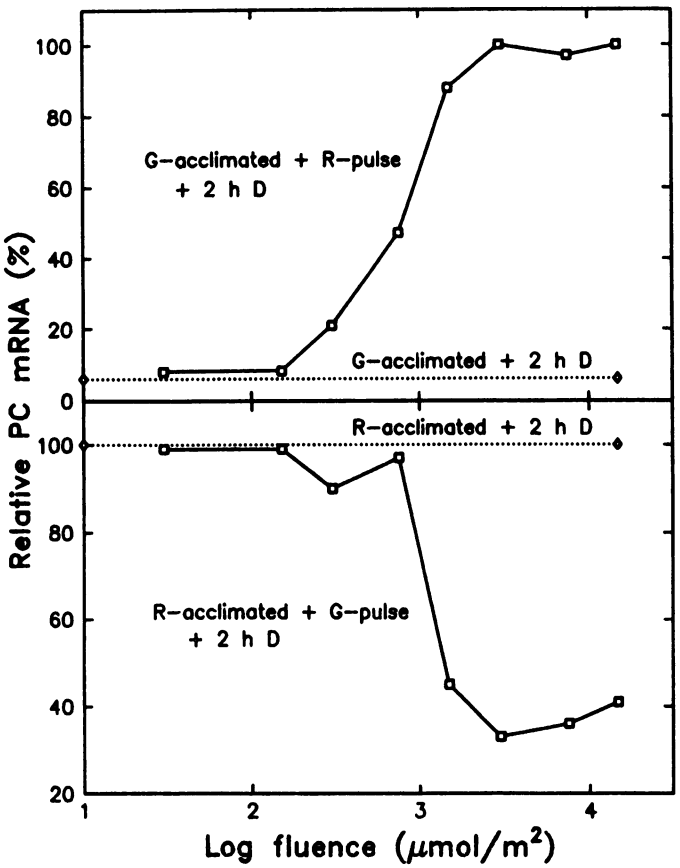

FIG. 5. The effect of various fluences of a R and a G pulse on the relative amount of PC mRNA in total RNA isolated from cultures of *F. diplosiphon*. G-acclimated cultures received a R and R-acclimated cultures a G pulse before transfer to D for 2 h. The logarithm of the fluences of the light pulses is indicated on the abscissa. (·····), controls, *i.e.* cultures, which were transferred to D without light pulse. The highest value (including controls) of each curve was taken as 100% and all other mRNA levels referred to this amount.

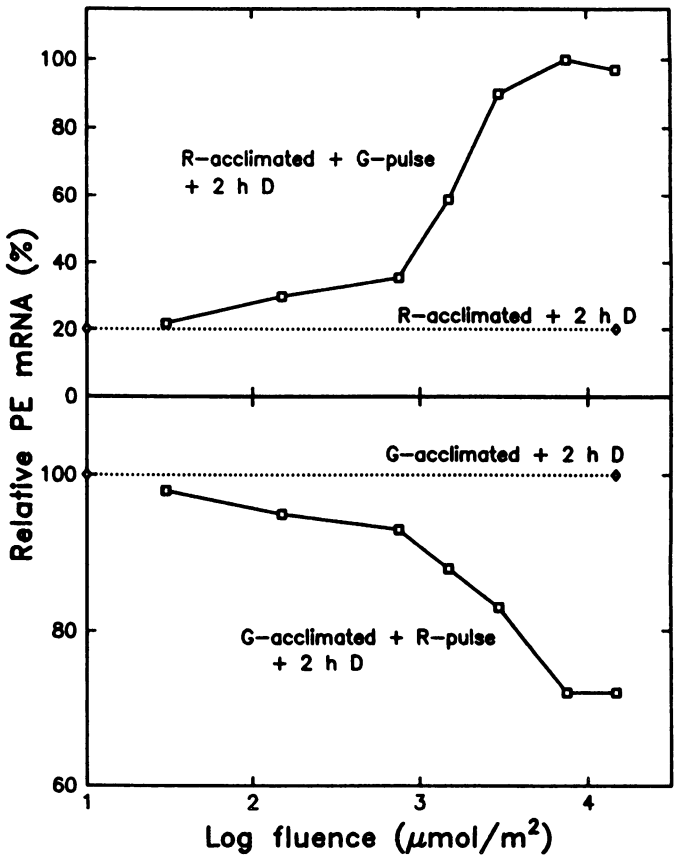

FIG. 6. The effect of various fluences of a G and of a R pulse on the relative amount of PE mRNA in total RNA isolated from cultures of *F. diplosiphon*. R-acclimated cultures received a G and G-acclimated cultures a R pulse before transfer to D for 2 h. The logarithm of the fluences of the light pulses is indicated on the abscissa. (·····), controls, *i.e.* cultures, which were transferred to D without a light pulse. The highest value (including controls) of each curve was taken as 100% and all other mRNA levels referred to this amount.

tion of R- and G-acclimated cells. For example, it requires less R to induce PC mRNA than to cause a decrease in PE mRNA in the identical G-acclimated cells; conversely, it requires less G to cause a decrease in PC than to induce PE mRNA in the identical R-acclimated cells. The lower sensitivity of the PE mRNA responses persists in the absence of any difference in the screening pigments. If we assume that R/G reversible changes in the state of a photoreceptor causes a proportional change in the amount of mRNA, light regulation of PE and PC mRNA would likely occur via two different photoreceptors, both of which are photoconvertible. However, it is yet to be determined whether fluence-response relations measured at the level of steady-state mRNA reflect differences in the fluence-response relationships of the underlying primary photoreactions. Differential fluence requirements of the promoting and suppressing light pulses for PC and PE mRNA accumulation might also be explained on the basis of a single photoreversible photoreceptor with PC and PE mRNA-specific differences in the signal transduction chain(s).

The fluences required for the reversal of the positive and negative light effects (Figs. 7 and 8) are significantly higher than those required for the positive or negative responses themselves (Figs. 5 and 6). This difference is probably because the wavelengths of the reversing light pulses shown in Figures 7 and 8 are in spectral regions where there is strong absorption by the existing photosynthetic pigmentation of the algae (R pulse applied to R-acclimated cells and vice versa), whereas in the experiments shown in Figures 5 and 6 the wavelength of the light pulses are in spectral regions where such absorption is much weaker (R pulse applied to G-acclimated algae and vice versa). This inter-

Table I. *Test for Validity of the Reciprocity Law*

Test fluence 1500 μmol m^{-2}. Results are the averages of three separate experiments.

Light Treatment		Relative Amount of mRNA	
		PC mRNA	PE mRNA
		%	
G-acclimated cells: saturating R pulse, 2 h D		100	
G-acclimated cells: 2 h D		8	
R-acclimated cells: saturating G pulse, 2 h D			100
R-acclimated cells: 2 h D			21
Fluence Rate	Time		
μmol m^{-2} s^{-1}	s		
30	50	71[a]	59[b]
15	100	67	64
3	500	69	53

[a] G-acclimated cells: variable R pulse, 2 h D. [b] R-acclimated cells: variable G pulse, 2 h D.

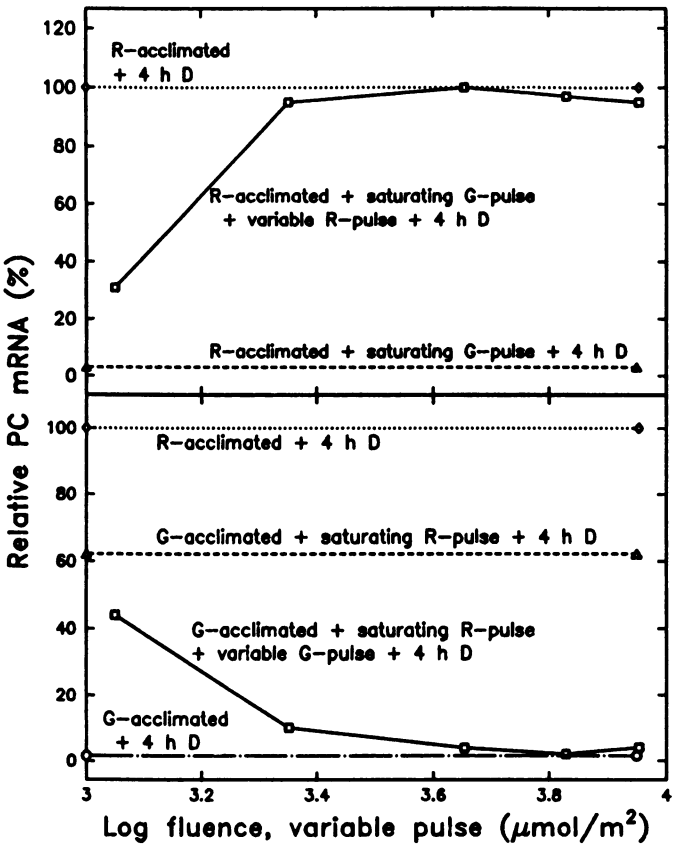

FIG. 7. Reversibility of the effect of a saturating G pulse by R pulses of different fluences and of a saturating R pulse by G pulses of different fluences on the relative amount of PC mRNA. Cultures were acclimated to R before application of the saturating G pulse and to G before the saturating R pulse, respectively. After the light treatments cells were kept in D for 4 h until harvested for total RNA extraction. The fluence of the variable light pulse is indicated on the abscissa. The amount of PC mRNA of R-acclimated cultures transferred to D for 4 h was taken as 100% and all other mRNA levels referred to this level.

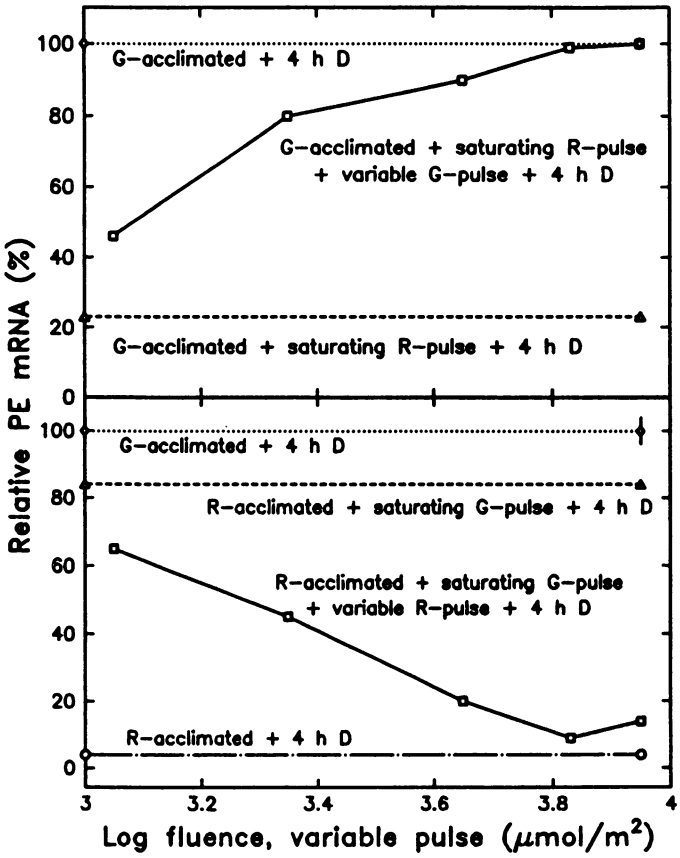

FIG. 8. Reversibility of the effect of a saturating R pulse by G pulses of different fluences and of a saturating G pulse by R pulses of different fluences on the relative amount of PE mRNA. Cultures were acclimated to G before application of the saturating R pulse and to R before the saturating G pulse, respectively. After the light treatments cells were kept in D for 4 h until harvest for total RNA extraction. The fluence of the variable light pulse is indicated on the abscissa. The amount of PE mRNA of G-acclimated cultures transferred to D for 4 h was taken as 100% and all other values referred to this level.

pretation has been confirmed by transmission measurements of R and G through R- and G-acclimated algal cultures (data not shown).

In previous studies on chromatic adaptation in *Tolypothrix tenuis*, the effect of R and G pulses on the accumulation of the PC and PE chromoproteins in subsequent D has also been investigated (17). Cells were kept in nitrate-free medium to prevent phycobilisome formation. When nitrate was added, phycobiliprotein synthesis occurred even in D and predominantly PC was formed if the last light treatment was R while PE was synthesized if the light period was terminated with G. In this case as well, the effect of R illumination could be reversed by subsequently given G and the effect of G by a subsequent R treatment. An induction of PE synthesis by a G pulse and its reversibility by R has also been demonstrated in *F. diplosiphon* (40). However, since PC synthesis occurs in both R- and G-acclimated cyanobacteria (7, 22) the effect of light pulses on PC accumulation in D has not previously been investigated in detail. The use of a probe specific for the light-inducible PC gene (10), *i.e.* one which hybridizes only to R-induced PC transcripts and not to transcripts of the constitutively expressed PC gene set, allowed us to compare the effect of R and G pulses on both PC and PE mRNA levels.

Even though nothing is known about the steps occurring between the perception of light and the observed changes in the mRNA abundance, Figures 1, 2, and 3 show that the events are

Table II. *Calculated Extinction Coefficients for the Effect of R or G on the Abundance of PC and PE mRNA (for further details, see Ref. 23)*

Direction of Effect	Estimated Extinction Coefficients[a]	
	Phycocyanin	Phycoerythrin
Positive	18,800	7,500
Negative	19,800	6,500

[a] Calculated on the basis of an assumed quantum efficiency of 0.1 for all photoreactions.

rapid under our experimental conditions and without detectable lag phases. Even a 10 min inductive light pulse brings about a change in mRNA abundance that cannot be fully canceled by a subsequently given light pulse of the other light quality (Fig. 3). Moreover, a 10 min inductive light pulse suffices to cause an increase in the mRNA accumulation over at least 4 h in D and a cancelling light pulse, given 2 h after the inductive pulse prevents further mRNA accumulation almost immediately (Fig. 2). Thus the effect of the light is already complete within the 10 min illumination period before the cultures are transferred to D.

The kinetics of mRNA accumulation in D following R and G pulse treatments are different for PC and PE mRNA. As an example: Figure 1 shows that the decline of PC mRNA in D is much faster than the decline of the PE mRNA level irrespective

of whether the light treatment was terminated by an inductive or repressive light pulse. Experiments indicate that the half-lives of both mRNAs are of the order of 10 to 30 min (34a). Thus we conclude that unlike PC mRNA a substantial PE mRNA synthesis still occurs in D even after a R pulse. Another difference noted above is that while hardly any PC-mRNA is detectable in cultures kept in continuous G, *i.e.* under noninductive light conditions (Fig. 1, top), the PE-mRNA level in R is about 10% of the level detectable in G (Fig. 1, bottom). This difference may simply be a function of the spectral quality of the light sources and the absorption properties of the pigments. For example, the G source might cease transmitting at wavelengths sufficiently short that it would not excite the photoreceptor that induces an increase in PC mRNA, while the red source might emit light at sufficiently short wavelengths to turn on at least partially the photoreceptor system inducing PC mRNA.

The action of the photoreceptor(s) on PE and PC mRNA accumulation is in some way comparable with phytochrome-induced changes of gene expression in higher plants. However, the photobiological properties of the cyanobacterial system indicate that the underlying mechanisms between perception of light and alteration of gene expression might be different from phytochrome-controlled gene expression (36). First, in *F. diplosiphon*, the response to light pulses occurs immediately and persists in subsequent D as though a single switch were turned on or off and remained in that configuration, dependent entirely on the light quality of the last irradiation. Second, the position of the switch as set by one pulse can be reversed by the appropriate light treatment after several hours of D without any diminution in the effectiveness of the second pulse. Taken together, these two properties of the cyanobacterial system indicate that the kinds of dark reactions well known for phytochrome systems (dark reversion, destruction) (24) are lacking in *F. diplosiphon*. Third, even immediate irradiation with a second light pulse of the opposite quality fails to reverse the effect of the first light pulse completely (Fig. 3). Thus a fraction of the final response, in this case a change in mRNA abundance, has already taken place by the end of a 10 min irradiation and the passage of signal through the transduction chain has been rapid. Escape kinetics are too rapid to be measured with the present techniques, though the time course for escape might be measurable by use of shorter light pulses of a fluence higher than available in the present study or at lower temperatures. Changes in mRNA abundance as rapid as those reported here are not usual in systems in which mRNA abundance is regulated by phytochrome (*e.g.* 1; see 36) though some rapid responses at the mRNA level, mediated by phytochrome, have been reported (28). Finally, both R and G induce both positive and negative responses. If single photoreversible pigment systems are involved, one cannot state with confidence which form is biologically active—indeed both forms might be (leading to repression in one configuration and activation in the other). This situation is unlike that for phytochrome where there is abundant evidence that Pfr is the biologically active form (6).

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