Plant Physiol. (1988) 88, 1077–1083
0032-0889/88/88/1077/07/$01.00/0

Changes in Accumulation and Synthesis of Transcripts Encoding Phycobilisome Components during Acclimation of *Fremyella diplosiphon* to Different Light Qualities[1]

Received for publication April 5, 1988 and in revised form June 16, 1988

R. OELMÜLLER*, P. B. CONLEY, N. FEDERSPIEL, W. R. BRIGGS AND A. R. GROSSMAN
Department of Plant Biology, Carnegie Institution of Washington, Stanford, California 94305-1297

ABSTRACT

We have used gene-specific DNA fragments as hybridization probes to quantitate the levels of transcripts encoding several phycobilisome polypeptides in the cyanobacterium *Fremyella diplosiphon* in response to changes in the light environment. While the levels of transcripts encoding allophycocyanin, the core linker polypeptide, and the constitutive phycocyanin subunits are similar in *F. diplosiphon* grown either in red or green light, the levels of other transcripts change dramatically. Transcripts encoding the inducible phycocyanin subunits are barely detected in green light-grown cells and very abundant in red light-grown cells, while the level of phycoerythrin mRNA is approximately 10-fold more in green than red light-grown cells. Quantitation of the phycoerythrin and inducible phycocyanin transcripts after transfer of cultures from green to red light and red to green light demonstrate that both increase rapidly upon exposure of cells to inductive illumination. The decrease in the phycoerythrin mRNA level in red light is much slower than the decline in the levels of the inducible phycocyanin transcripts in green light. Since the half-lives of the inducible phycocyanin and phycoerythrin transcripts do not change when *F. diplosiphon* is exposed to red or green illumination, the steady state levels of these mRNAs are primarily controlled by the rate of transcription. Therefore, the high level of phycoerythrin mRNA maintained for several hours after cultures are transferred from green to red illumination must result from continued transcription of the phycoerythrin gene set. Differences in expression from the phycoerythrin and inducible phycocyanin gene sets in response to light quality are discussed in terms of possible mechanisms involved in their regulation.

The phycobilisome, the major light harvesting complex of eukaryotic red algae and procaryotic cyanobacteria (16, 18–21, 51, 52) is composed of two domains: the core, which is attached to the thylakoid membranes and includes the major biliprotein allophycocyanin (AP,[2] absorption maximum approximately 650 nm), and the rods which are associated with the core and composed of hexameric arrays of either phycocyanin (PC, absorption maximum approximately 620 nm) alone or PC and phycoerythrin (PE, absorption maximum approximately 560 nm). PC hexamers are proximal to the core while PE hexamers are distal. Overlapping absorption and emission spectra of the different phycobiliproteins result in unidirectional energy flow in the phycobilisome to the reaction centers in the thylakoid membranes (PE to PC to AP to Chl) that is better than 90% efficient (43). The interactions of the biliproteins with the nonpigmented linker polypeptides (47) cause minor shifts in the spectral properties of the associated chromophores and may increase the efficiency of energy transfer between hexamers (18–20). Moreover, specific phycobiliproteins in the core of the phycobilisome (35, 36, 44) serve as energy bridges between the AP and the reaction center Chl of PSII (18, 38, 39).

The influence of light on the levels of phycobiliprotein components has been investigated intensively (see Refs. 3, 22, 23, 45, and 46 for reviews of pertinent literature). Both light quality (3, 22, 23, 46) and quantity (27, 41, 50) can influence the composition of the phycobilisome. Generally, high light fluence rates result in reduced numbers of chromophoric proteins in the complex (50) and a decrease in the number of phycobilisomes per cell (27, 34). Some cyanobacteria, such as *Fremyella diplosiphon*, alter the components of their phycobilisomes in response to changes in the wavelengths of light in the environment, a phenomenon termed complementary chromatic adaptation (3). Red light (R) promotes PC synthesis while green light (G) promotes PE synthesis (2, 14, 15, 17). Differences in the complement of linker polypeptides are also observed in phycobilisomes from cells maintained in R and G (4, 5, 33).

Several genes encoding both phycobiliprotein subunits and linker polypeptides (1, 6, 8–11, 25, 30–33, 39, 42) have recently been isolated. It has been demonstrated with gene-specific DNA fragments that changes in the protein composition of the light-harvesting complex correspond, at least qualitatively, to changes in the levels of transcripts encoding these polypeptides (22, 23). In *F. diplosiphon* high levels of PE mRNA are detectable in G-acclimated cells while low levels are present in R-acclimated cells (22, 23, 39). Modulation of the level of PC subunits appears to be accomplished by differential expression from at least two PC gene sets. Transcripts from one PC gene set (*cpc*A_2B_2) are only detectable in cells grown in R (9) while transcripts from the other set (*cpc*A_1B_1) are constitutively expressed and detected in cells grown in either R or G (10). In agreement with these findings, previous reports have demonstrated that in some cyanobacteria which exhibit complementary chromatic adaptation a set of constitutively expressed PC subunits is present in R- and G-acclimated cells while an additional set of PC subunits appears in R-acclimated cells (4, 5).

In the present study we have acclimated *F. diplosiphon* to a constant fluence rate of R or G and measured changes in the

[1] CIW-DPB publication no. 1008. This work was supported by National Science Foundation (NSF) Grant DCB 8615606 awarded to A. R. G. and W. R. B. N. F. was supported by NSF grant DCB-8615606 while R. O. was supported by the Alexander von Humboldt-Stiftung (Feodor Lynen-Research Fellowship).

[2] Abbreviations: AP, allophycocyanin; $AP^{\alpha,\beta}$, α and β subunits of allophycocyanin; G, green light; PC, phycocyanin; $PC^{\beta,\alpha}$, β and α subunits of phycocyanin; PE, phycoerythrin; $PE^{\beta,\alpha}$, β and α subunits of phycoerythrin; R, red light.

levels of mRNAs encoding the different phycobilisome components after transfer of R-acclimated cells to G and G-acclimated cells to R. The changes observed in the levels of PE and inducible PC transcripts during acclimation to the different light qualities are mostly an effect on mRNA synthesis since the rate of mRNA degradation under the different conditions of illumination appears to remain constant.

MATERIALS AND METHODS

Growth Conditions. *Fremyella diplosiphon* (*Calothrix* sp., PCC 7601), a subculture of UTEX#481, was grown for 5 d at 32°C in 40 mL of Gorham's medium (26) in an atmosphere of 3% CO_2 in air. Cultures were illuminated with 15 μmol $m^{-2}s^{-1}$ of R or G. Light from cool white fluorescent lamps (General Electric F20T12.CW) was either filtered through a R plexiglass filter (Acrylide 210-0, 6.6 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 and above 600 nm). The spectral distributions of the light incident on the samples for each source, measured with a Li-1800 Spectra-Radiometer (LiCor, Lincoln, NE), are shown in Figure 1.

Gene-Specific DNA Fragments Used for Quantitation of Transcripts. For quantitation of the inducible PC mRNA a 3.7 kb *Hin*dIII fragment containing *cpc*A_2B_2 and part of *cpc*C (which encodes a linker polypeptide associated with the rod substructure) (33) was used. This fragment was previously shown to hybridize specifically to the inducible PC transcripts (a 1600- and 3800-base transcript; the former encodes $PC^{\beta,\alpha}$ and the latter $PC^{\beta,\alpha}$ plus three linker polypeptides) (8, 9, 33). The level of PE mRNA was determined with a 294 bp *Xba*I fragment from the *cpe*BA gene set, which encodes amino acids 6–104 of PE^{β}. The level of the constitutive PC mRNA was determined with a 287 bp *Hin*dIII fragment internal to *cpc*A_1 (8). The species used to quantitate the level of AP mRNAs was a 693 bp *Pst*I/*Hae*III fragment from the *apc*A_1B_1 gene set which begins 54 nucleotides downstream from the first bp of the coding region of AP^{β}, while a 250 bp *Hae*III fragment starting 6 nucleotides downstream from the beginning of the coding region of *apc*C and ending 55 nucleotides downstream of the termination codon was used to quantitate transcripts encoding the core linker protein (PG Lemaux, AR Grossman, unpublished data). The *cpc*B_2A_2 and *cpe*BA fragments were purified from an agarose gel after digestion of pUC8 (containing appropriate inserts) with *Hin*dIII and *Xba*I, respectively. The other specific fragments were in M13, and single stranded templates were labeled using random primers (13).

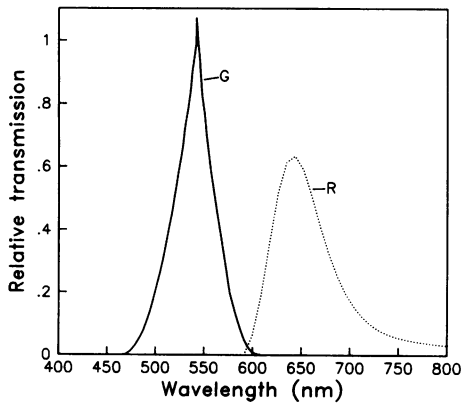

FIG. 1. Spectral distribution of energy from G- and R-light source. The sharp peak at 546 nm is the mercury emission line from the fluorescent lamp.

RNA Isolation and Quantitation. RNA was isolated as described previously (10). An equal amount of RNA was either resolved by electrophoresis on a 1.5% agarose gel under denaturating conditions (9) and transferred directly to nitrocellulose paper (48), or directly bound to nitrocellulose paper for slot blot hybridizations as described by Kaufman *et al.* (28), prior to probing.

Hybridizations were performed at 67°C (15–18 h, 10^4 Cerenkov counts/slot) in 0.5 M NaCl, 0.1 M NaH_2PO_4, 0.1 M Tris, 2 mM EDTA, 0.1% SDS. After hybridization, the filters were washed three times (10 min each time in 0.01 M phosphate buffer [pH 7.0], 0.02 M EDTA, 0.1% SDS) at room temperature and once at 67°C for 2 min with the same buffer except that the phosphate concentration was 0.05 M, and exposed to a Kodak XAR-5 film (−80°C for 1–8 h).

Rifampicin Treatment. Cells were grown to mid-logarithmic phase in either R or G. Rifampicin was dissolved in methanol at a concentration of 100 mg/ml and added to the cultures to a final concentration of 50 μg/ml. R- and G-acclimated cultures were each placed in either the same light in which they were grown, or the complementary light, immediately following the addition of the transcription inhibitor. Aliquots of cells were removed from the cultures at regular intervals and RNA isolated.

The average of three to seven independent experiments was used to obtain the data presented in the figures. The variation in the data is of the order of 10% or less.

RESULTS

Characterization of Light Conditions. The G filter used in these experiments (Fig. 1) was chosen because its transmission spectrum closely matches the action spectrum for PE synthesis in another cyanobacterium, *Tolypothrix tenuis* (12). Vogelmann and Scheibe (49) also observed maximal PE synthesis at 540 nm, the peak transmission through the G filter, with essentially no synthesis below 440 nm and above 590 nm for *F. diplosiphon* (see also 24). After growth of *F. diplosiphon* in G, essentially no transcript for inducible PC subunits is observed (see below). The red filter used (Fig. 1) eliminates all wavelengths below 585 nm, even though 585 nm still allows low levels of PE synthesis (12, 49). Using this filter we observe 5 to 10% of the level of PE subunits measured in G-acclimated cells (BU Bruns, AR Grossman, WR Briggs, unpublished data), and approximately 10% of the level of PE transcript (see below).

Effect of R and G on the levels of phycobiliprotein transcripts. RNA was isolated from R- and G-acclimated *F. diplosiphon* cells and from cells transferred from R to 6 h of G and from G to 6 h of R. The DNA fragment encoding the inducible PC subunits (see "Materials and Methods") hybridized to a 1600- and 3800-base transcript present in cells maintained in R but not detected in cells maintained in G (Fig. 2A, lanes 1 and 2). These transcripts have been previously characterized (8, 9, 33). Moreover, in G-acclimated cells transferred to R for 6 h (Fig. 2A, lane 4), the inducible PC transcripts accumulate to levels observed in cells grown continuously in R. Essentially no inducible PC mRNAs are detectable 6 h after transfer of R-acclimated cells to G (Fig. 2A, lane 3).

The PE-specific probe (see "Materials and Methods") hybridizes to an mRNA species of approximately 1500 bases which is detectable at low levels in R- and high levels in G-acclimated cells (Fig. 2B, lanes 1 and 2). In contrast to the results for the inducible PC transcripts, only a small reduction in the level of PE mRNA is observed when *F. diplosiphon* cultures are transferred from inductive to 6 h of noninductive light (Fig. 2B, lane 4). However, 6 h of G suffices to raise the level of PE mRNA to that present in RNA preparations from cultures maintained continuously in G (Fig. 2B, compare lanes 2 and 3).

The levels of other transcripts encoding phycobilisome com-

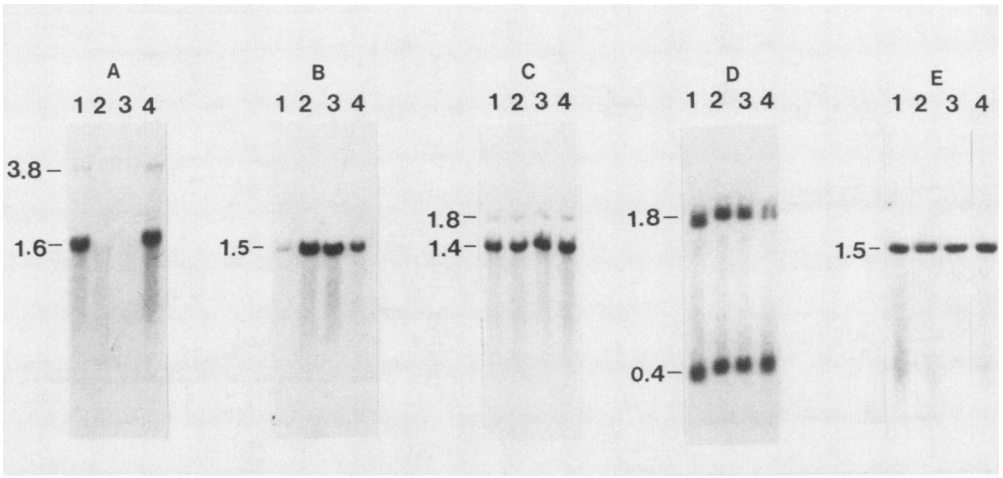

FIG. 2. Hybridization of DNA fragments encoding phycobiliproteins to RNA from *F. diplosiphon* cells grown in R (lanes 1), G (lanes 2), transferred from R to G for 6 h (lanes 3), transferred from G to R for 6 h (lanes 4). Equal amounts of RNA were separated on denaturing agarose gels and hybridized to DNA fragments encoding the inducible PC (A), PE (B), AP (C), the core linker protein (D), and the constitutive PC (E). Sizes of the hybridizing RNA species, in kilobases, are given to the left of each panel.

ponents are not significantly affected by light quality. Similar levels of hybridization to the AP fragment are observed with RNA from cells grown in R, G, G to R (6 h) and R to G (6 h), as shown in Figure 2C. The predominant transcript which encodes AP is 1400 bases and is present at approximately equivalent levels in all four of the RNA preparations. Similar levels of a 400-base transcript encoding a linker polypeptide associated with the core of the phycobilisome (33) are also observed among the four RNA preparations (Fig. 2D). The gene encoding the core linker polypeptide is contiguous to the gene encoding AP^{α}. In addition to the 400-base transcript, an 1800-base transcript (23) (apparent in Fig. 2, C and D) which is a polycistronic mRNA, encodes the core linker polypeptide plus $AP^{\alpha,\beta}$ (23). The abundance of both the 400- and 1800-base transcripts are similar in R and G. Recently, low abundance mRNAs of 5200 and 5900 bases (not detectable in these autoradiograms) have also been shown to encode the AP subunits and core linker polypeptide (PG Lemaux, AR Grossman, unpublished data). The level of the constitutive PC transcript, under the conditions of illumination used here, is not detectably different in R and G. Under different conditions of illumination we have observed approximately two-fold more constitutive PC mRNA in R-acclimated cells (10).

Figure 3 shows typical slot blot hybridizations which were used for quantitating the levels of inducible PC and PE mRNAs upon transfer of cultures from R to G and G to R. Quantitation of these mRNA species is presented in the curves of Figure 4. These results demonstrate that the inducible PC mRNA decreases or increases rapidly upon transferring cells from R to G or G to R, respectively. Changes in PC mRNA levels are essentially complete within 2 h of the transfer to either inductive or noninductive illumination (Fig. 3, panels 1 and 2; Fig. 4, top). In contrast, the level of PE mRNA increases to a maximum approximately 4 h following the transfer of cells from R to G, while only a small decrease in the PE mRNA is observed 4 h after the transfer of G-acclimated cells to R (Fig. 3, panels 3 and 4; Fig. 4, bottom). Even 15 h after transferring G-acclimated cells to R, the level of PE mRNA is significantly higher than in cells maintained continuously in R (Fig. 4, bottom). Thus, changes in the level of the PE transcript during acclimation to different light conditions are slower than the analogous changes for the inducible PC transcripts.

Effect of Rifampicin on the Steady State Levels of mRNAs Encoding PE and Inducible PC Subunits. Rifampicin has been used to block transcription in several prokaryotic organisms, including cyanobacteria (29). Over the incubation times used in these experiments, we have found that rifampicin at 50 μg/ml prevents the incorporation of radioactive uridine into trichloroacetic acid precipitable material, indicating the cessation of RNA synthesis (data not shown). Moreover, accumulation of the inducible PC transcripts after transfer of G-acclimated cells to R, and of the PE transcript after the transfer of R-acclimated cells to G is totally prevented if rifampicin is included in the medium at the time of transfer (data not shown). The effect of rifampicin on the levels of the mRNAs encoding the inducible PC, the PE, and the AP transcripts (quantitated by slot blot hybridizations) in G and R, or upon transfer of cultures from G to R or R to G, is presented in Figure 5. Within 60 min of the addition of rifampicin to R-acclimated cells, almost no inducible PC transcripts can be detected (Fig. 5, top). Similar results are presented for the PE transcripts in both R- and G-acclimated cultures (Fig. 5, middle) and for the AP transcripts in cultures grown in R and transferred from R to G at the time of rifampicin addition (Fig. 5, bottom). Similar kinetics of the decrease in AP mRNA are observed if the cultures are maintained in G or transferred from G to R (at the time of rifampicin application) (data not shown). Furthermore, the kinetics of decay of the inducible PC and PE mRNAs do not change if R-acclimated cells are transferred to G and G-acclimated cells are transferred to R at the same time as the addition of rifampicin to the cultures (Fig. 5, top and middle). From these experiments we estimate the half-lives ($t_{1/2}$) of the transcripts encoding the inducible PC, the PE, and the AP subunits to be approximately 13, 25, and 20 min, respectively. These results strongly indicate that the maintenance of high levels of PE and inducible PC mRNAs under inductive light conditions requires continuous and high level mRNA synthesis. These data also demonstrate that light quality does not affect the rate of degradation of the inducible PC, PE, or AP transcripts. Hence, the effect of light quality on the relative abundance of inducible PC and PE mRNAs must occur at the level of transcription. Since the abundance of inducible PC and PE mRNAs decreases upon transfer to noninductive illumination much faster when rifampicin is included in the culture medium (compare the data in Figs. 4 and 5), the synthesis of both of these transcripts must continue following the transfer to noninductive illumination, with the level of PE mRNA synthesis being much higher (for a longer time following the transfer) than the level of inducible PC mRNA synthesis.

Levels of mRNA Synthesis during Inductive and Noninductive

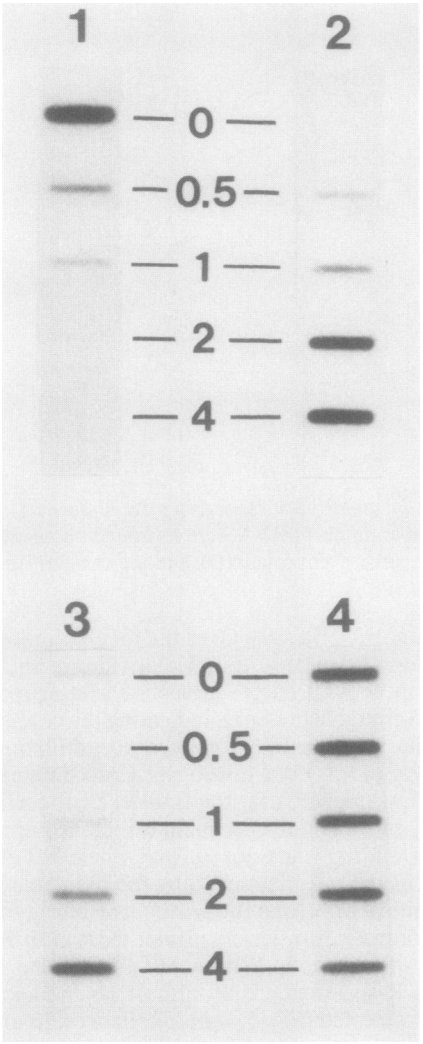

FIG. 3. Slot blot hybridizations of RNA (2.5 μg in each slot) to a 3.8 kb *Hin*dIII fragment encoding the inducible PC (panels 1 and 2) and a 294 bp *Xba*I fragment encoding PE (panels 3 and 4). R-acclimated cells were transferred to G (panels 1 and 3) and G-acclimated cells to R (panels 2 and 4), for various times (given as hours between panels 1 and 2 and panels 3 and 4) prior to RNA isolation.

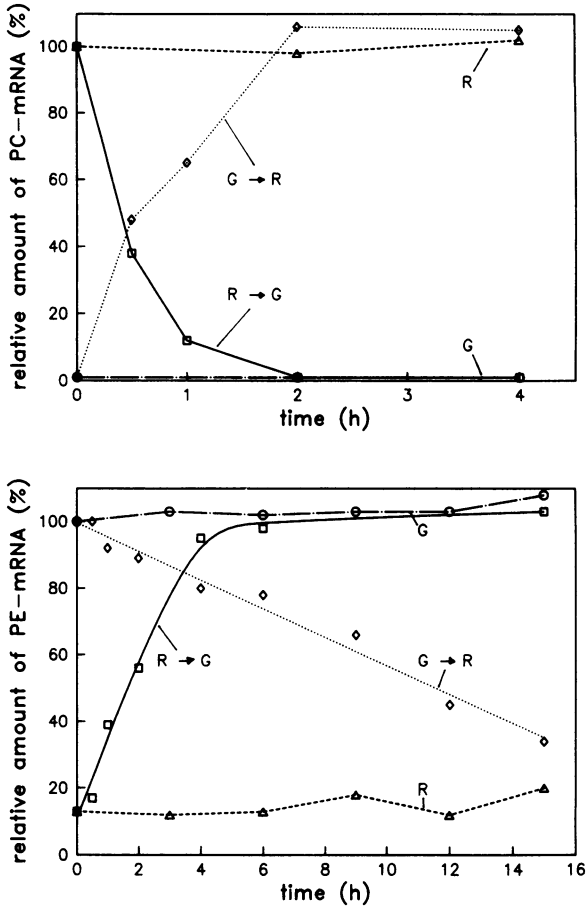

FIG. 4. Relative amount of inducible PC and PE mRNA in *F. diplosiphon* grown in R (△), G (○), R-acclimated cells transferred to G (□) and G-acclimated cells transferred to R (◇). Equal amounts of RNA (2.5 μg in each slot) were used for slot blot hybridizations and the radioactive signals were quantitated with a scanning densitometer (Hoefer Scientific Instruments, USA). The level of inducible PC mRNA in R-acclimated cells, and the level of PE mRNA in G-acclimated cells (time 0 for R- and G-grown cells, respectively) was taken as 100%. The data presented are from six independent RNA extractions.

Illumination. If steady-state transcript levels reflect both light-dependent synthesis and light-independent degradation, mRNA synthesis can be described as

$$^0k_s(t) = d[\text{mRNA}](t)/dt + {}^1k_d[\text{mRNA}](t),$$

where $^0k_s(t)$ is the zero-order rate constant of synthesis at time t, and $^1k_d(t)$ the first order rate constant of degradation at time t. From the data in Figures 4 and 5, ^0k_s was calculated for the inducible PC and PE transcripts from R- and G-acclimated cells and from cells transferred from R to G and G to R. The rate constant for the synthesis of the inducible PC mRNA increases to a maximum value 90 min after transferring G-acclimated cells to R and to a minimum 180 min after transferring R-acclimated cells to G (Fig. 6, top). The rate of PE mRNA synthesis reaches a maximum 3 h following the transfer of cultures from R to G, while the rate of synthesis after the transfer of cultures from G to R decreases very slowly, and even after 13 h in R, 50% of the maximum rate is observed (Fig. 6, bottom) (note different time scales). The striking difference in the rate of synthesis of the PE and inducible PC transcripts after switching from inducing to noninducing light conditions suggests that different mechanisms may be involved in regulating the expression from these gene sets.

DISCUSSION

The data presented above result from an initial examination of the effect of light quality on the accumulation and synthesis of transcripts encoding components of the phycobilisome. The levels of transcripts encoding the AP subunits, the core linker polypeptide, and the constitutive PC subunits are similar in the R and G used in these experiments. In contrast, very large increases in the levels of PE and inducible PC mRNAs occur rapidly after transferring *F. diplosiphon* from R to G and G to R, respectively. The G used in these experiments was effective in eliminating essentially all inducible PC mRNA synthesis while the R did not eliminate all PE mRNA synthesis. Under our condition of R the cells contain between 5 and 10% of the PE chromoprotein present in cells maintained in G (BU Bruns, AR Grossman, WR Briggs, unpublished data) and the level of PE mRNA is approximately 10% of that observed in G-acclimated cells. PE mRNA reaches a maximum level of accumulation 4 h after transferring R-acclimated cells to G, while the inducible PC mRNA reaches a maximum 2 h after the transfer of G-acclimated cells to R. The decline in PE and inducible PC transcripts upon

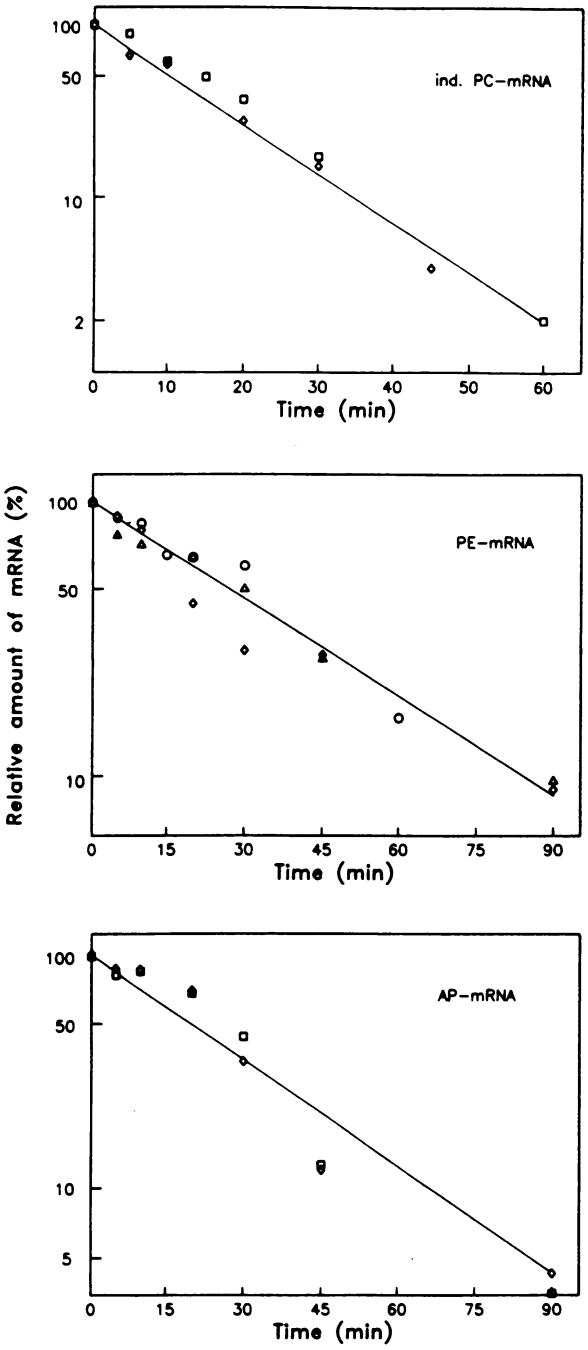

FIG. 5. The effect of rifampicin (50 μg/ml) on inducible PC (upper panel), PE (middle panel) and AP (lower panel) mRNA levels in *F. diplosiphon* cultures grown in R (□), G (○), and transferred from R to G (◇) or G to R (△) at the time of rifampicin application. The mRNA levels measured were normalized to mRNA levels determined at $t = 0$ (taken as 100%).

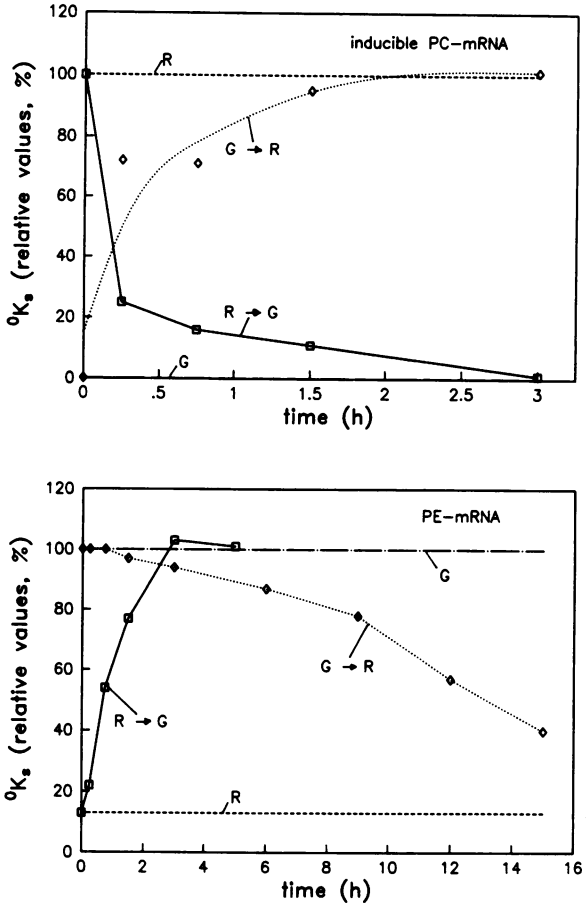

FIG. 6. Estimations of the rate constants of synthesis (^0k_s) for inducible PC and PE mRNA at various times after the transfer of R-acclimated cells to G (□) and G-acclimated cells to R (◇) using the data in Figs. 4 and 5 and the equation shown in "Results." (-----) ^0k_s in R-acclimated cells; (-·-·-·-) ^0k_s in G-acclimated cells. For the inducible PC mRNA (PE mRNA) the ^0k_s value in R (G) was taken as 100% (to which all other values were normalized).

transfer of cells to noninductive conditions of illumination proceeds much more slowly for the former than for the latter. Indeed, for the PE transcript only a 20 to 30% decrease is observed 8 h following the transfer of G-acclimated cells to R. In contrast, 2 h after the transfer of R-acclimated cells to G, the inducible PC mRNA is barely detectable.

In the experiments presented above, we used a mutant strain of *F. diplosiphon*, Fd33 (7) which has reduced filament length and therefore grows as individual colonies on agar plates. This is of considerable benefit in genetic studies and mutant analyses where it is essential that the organism grow as discrete colonies. This mutant exhibits complementary chromatic adaptation qualitatively similar to that of the wild type strain (7) (BU Bruns, AR Grossman, WR Briggs, unpublished data). Furthermore, the kinetics of accumulation of the PE and inducible PC transcripts in wild-type cells during shifts from one light quality to the other are similar to those observed in Fd33 (R Oelmüller, WR Briggs, AR Grossman, unpublished data).

Both synthesis and degradation are involved in determining the absolute level of a transcript. To determine the contribution of these processes in the differential accumulation of the biliprotein transcripts during complementary chromatic adaptation, we measured the $t_{1/2}$ of the specific biliprotein mRNAs, using rifampicin to block *de novo* RNA synthesis, under the different conditions of illumination. The $t_{1/2}$ of the inducible PC, PE and AP transcripts are approximately 13, 25, and 20 min, respectively. These values do not appear to be significantly affected by the conditions of illumination. Therefore, the slow decline in PE mRNA upon transfer of cultures from G to R relative to the decline in inducible PC mRNA when cultures are transferred from R to G, is not a consequence of a differential light effect on transcript stability, but must be due to sustained synthesis of PE mRNA even after cultures are placed in noninductive light. Moreover, the slower decrease in the rate of PE-mRNA synthesis—compared to the inducible PC-mRNA synthesis—after transfer of the cultures from inductive to non-inductive light conditions cannot be caused just by the leakiness of our G light

source which still allows 10% PE-mRNA synthesis (Fig. 6), but must be the result of differences in the mechanism of their light regulation. In the case of inducible PC mRNA, acclimation of the rate of mRNA synthesis to new light conditions occurs within 3 h while even after 15 h in R, PE mRNA synthesis is not yet acclimated (Fig. 6). These results are in agreement with the finding that there is an increase in the levels of the PE subunits (normalized to AP) for approximately 24 h after transferring cultures from G to R (BU Bruns, AR Grossman, WR Briggs, unpublished data). They also strongly suggest that light regulation of PE and inducible PC mRNA abundance occurs via transcriptional control.

Although it is unknown whether the PE gene set is controlled by a negative or positive regulatory element, the gradual decline in the level of PE mRNA after switching cultures from G to R, in our opinion, favors the latter, and may result from dilution of a cellular factor important in promoting expression from the PE gene set. The more rapid decline in inducible PC mRNA synthesis (synthesis declines by 50% approximately 10 min after transferring cultures from R to G, see Fig. 6) suggests either that a negative regulatory element is synthesized rapidly after the cells are transferred from R to G, or, that if a positive regulatory protein is responsible for the activation of the PC gene set, it is unstable in G. These ideas may be testable using inhibitors of protein synthesis. Such experiments, combined with other biochemical and genetic analysis, will help define the molecular species responsible for altered PE and PC synthesis, as well as the synthesis of other light-regulated gene products during complementary chromatic adaptation. Furthermore, since the light effect observed appears to be primarily on transcription, this system lends itself to a more quantitative photobiological analysis to examine some properties of the photoreceptor(s) involved. Such studies are presented in the accompanying article (40).

Acknowledgments—The authors would like to thank P. G. Lemaux who first isolated the AP and core linker polypeptide-specific DNA fragments which were used in experiments presented here. We also thank B. U. Bruns for helpful discussions.

LITERATURE CITED

1. Belknap WR, R Haselkorn 1987 Cloning and light regulation of expression of the phycocyanin operon of the cyanobacterium *Anabaena*. EMBO J 6: 871–884
2. Bennett A, L Bogorad 1973 Complementary chromatic adaptation in a filamentous blue-green alga. J Cell Biol 58: 419–435
3. Bogorad L 1975 Phycobiliproteins and complementary chromatic adaptation. Annu Rev Plant Physiol 26: 265–272
4. Bryant DA 1981 The photoregulated expression of multiple phycocyanin species. General mechanism for control of phycocyanin synthesis in chromatically adapting cyanobacteria. Eur J Biochem 119: 425–429
5. Bryant DA, G Cohen-Bazire 1981 Effects of chromatic illumination on cyanobacterial phycobilisomes. Evidence for the specific induction of a second pair of phycocyanin subunits in *Pseudanabaena* 7409 grown in red light. Eur J Biochem 119: 415–424
6. Bryant DA, R de Lorimier, DH Lambert, JM Dubbs, VL Stirewalt, SE Stevens Jr, RD Porter, J Tam, E Jay 1985 Molecular cloning and nucleotide sequence of the α and β subunits of allophycocyanin from the cyanelle genome of *Cyanophora paradoxa*. Proc Natl Acad Sci USA 82: 3242–3246
7. Cobley JG, RD Miranda 1983 Mutations affecting chromatic adaptation in the cyanobacterium *Fremyella diplosiphon*. J Bacteriol 153: 1486–1492
8. Conley PB, PG Lemaux, AR Grossman 1988 Molecular characterization and evolution of sequences encoding light harvesting components in the chromatically adapting cyanobacterium *Fremyella diplosiphon*. J Mol Biol 199: 447–465
9. Conley PB, PG Lemaux, AR Grossman 1985 Cyanobacterial light harvesting complex subunits encoded in two red light-induced transcripts. Science 230: 550–553
10. Conley PB, PG Lemaux, TL Lomax, AR Grossman 1986 Genes encoding major light-harvesting polypeptides are clustered on the genome of the cyanobacterium *Fremyella diplosiphon*. Proc Natl Acad Sci USA 83: 3924–3928
11. de Lorimier R, DA Bryant, RD Porter, W-Y Liu, E Jay, SE Stevens Jr 1984 Genes for α and β phycocyanin. Proc Natl Acad Sci USA 81: 7946–7950
12. Diakoff S, J Scheibe 1973 Action spectrum for chromatic adaptation in *Tolypothrix tenuis*. Plant Physiol 51: 382–385
13. Feinberg AP, B Vogelstein 1983 A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132: 6–13
14. Fujita Y, G Hattori 1960a Formation of phycoerythrin in preilluminated cells of *Tolypothrix tenuis* with special reference to nitrogen metabolism. Plant Cell Physiol 1: 281–292
15. Fujita Y, G Hattori 1960b Effect of chromatic lights on phycobilin formation in the blue-green alga *Tolypothrix tenuis*. Plant Cell Physiol 1: 293–303
16. Gantt E 1981 Phycobilisomes. Annu Rev Plant Physiol 32: 327–347
17. Gendel S, I Ohad, L Bogorad 1979 Control of phycoerythrin synthesis during chromatic adaptation. Plant Physiol 64: 786–790
18. Glazer AN 1985 Light harvesting by phycobilisomes. Annu Rev Biochem 14: 47–77
19. Glazer AN 1984 A macromolecular complex optimized for light energy transfer. Biochim Biophys Acta 768: 29–51
20. Glazer AN 1982 Phycobilisomes: structure and dynamics. Annu Rev Microbiol 36: 173–198
21. Glazer AN, DJ Lundell, G Yamanaka, RC Williams 1983 The structure of a simple phycobilisome. Ann Microbiol 134B: 159–180
22. Grossman AR, PG Lemaux, PB Conley 1986 Regulated synthesis of phycobilisome components. Photochem Photobiol 44: 827–837
23. Grossman AR, PG Lemaux, PB Conley, BU Bruns, LK Anderson 1988 Characterization of phycobiliprotein and linker polypeptide genes in *Fremyella diplosiphon* and their regulated expression during complementary chromatic adaptation. Photosynth Res 17: 23–56
24. Haury JF, L Bogorad 1977 Action spectra for phycobiliprotein synthesis in a chromatically adapting cyanophyte. Plant Physiol 60: 835–839
25. Houmard J, D Mazel, C Moguet, DA Bryant, N Tandeau de Marsac 1986 Organization and nucleotide sequence of genes encoding core components of the phycobilisomes from *Synechococcus* 6301. Mol Gen Genet 205: 404–410
26. Hughes EO, PR Gorham, A Zehnder 1958 Toxicity of a unialgal culture of *Microcystis aeruginosus*. Can J Microbiol 4: 225–236
27. Jahn W, J Steinbiss, K Zetsche 1984 Light intensity adaptation of phycobiliprotein content of the red alga *Porphyridium*. Planta 161: 536–539
28. Kaufman LS, WF Thompson, WR Briggs 1984 Differential red light requirements for phytochrome induced accumulation of cab RNA and rbs RNA. Science 226: 1447–1449
29. Leach CK, NG Carr 1974 In vitro protein synthesis and measurement of the stability of messenger RNA in the blue-green alga *Anabaena variabilis*. J Gen Microbiol 81: 47–58
30. Lemaux PG, AR Grossman 1985 Major light-harvesting polypeptides encoded in polycistronic transcript in eukaryotic algae. EMBO J 4: 1911–1919
31. Lemaux PG, AR Grossman 1984 Isolation and characterization of a gene for a major light-harvesting polypeptide from *Cyanophora paradoxa*. Proc Natl Acad Sci USA 81: 4100–4104
32. Lind LK, SR Kalla, A Lönneborg, G Öquist, P Gustafsson 1985 Cloning of the β phycocyanin gene from *Anacystis nidulans*. FEBS Lett 188: 27–32
33. Lomax TL, PB Conley, J Schilling, AR Grossman 1987 Isolation and characterization of light-regulated phycobilisome linker polypeptide genes and their transcription as a polycistronic mRNA. J Bacteriol 169: 2675–2684
34. Lönneborg A, LK Lind, SR Kalla, P Gustafsson, G Öquist 1985 Acclimation processes in the light-harvesting system of the cyanobacterium *Anacystis nidulans* following a light shift from white to red. Plant Physiol 78: 110–114
35. Lundell DJ, AN Glazer 1983 Molecular architecture of light-harvesting antenna. Core substructure in *Synechococcus* 6301 phycobilisomes: two new allophycocyanin and allophycocyanin B complexes. J Biol Chem 258: 902–908
36. Lundell DJ, G Yamanaka, AN Glazer 1981 A terminal energy acceptor of the phycobilisome: the 75,000 dalton polypeptide of *Synechococcus* 6301 phycobilisomes, a new biliprotein. J Cell Biol 91: 315–319
37. Manadori A, M Alhadeff, AN Glazer, A Melis 1984 Photochemical apparatus organization in *Synechococcus* 6301 (*Anacystis nidulans*). Effect of phycobilisome mutation. Arch Microbiol 139: 117–123
38. Manadori A, A Melis 1985 Phycobilisome PSII association in *Synechococcus* 6301 (Cyanophyceae). FEBS Lett 181: 79–82
39. Mazel D, G Guglielmi, J Houmard, W Sidler, DA Bryant, N Tandeau de Marsac 1986 Green light induces transcription of phycoerythrin operon in the cyanobacterium *Calothrix* 7601. Nucleic Acids Res 14: 8279–8290
40. Oelmüller R, AR Grossman, WR Briggs 1988 Photoreversibility of the effect of red and green light pulses on the accumulation of mRNAs coding for phycocyanin and phycoerythrin in *Fremyella diplosiphon*. Plant Physiol 88: 1084–1091
41. Öquist G 1974 Light induced changes in pigment composition of photosynthetic lamellae and cell-free extracts obtained from the blue-green alga *Anacystis nidulans*. Physiol Plant 30: 45–48
42. Pilot TJ, JL Fox 1984 Cloning and sequencing of the genes encoding α and β subunits from the cyanobacterium *Agmenellum quadruplicatum*. Proc Natl Acad Sci USA 81: 6983–6987
43. Porter G, CJ Tredwell, GFW Searle, J Barber 1978 Picosecond time-

resolved energy transfer in *Porphyridium cruentum*. Part I. In the intact chloroplast. Biochim Biophys Acta 501: 532–545

44. REDLINGER T, E GANTT 1982 An M, 95,000 polypeptide in *Porphyridium cruentum* phycobilisomes and thylakoids. Possible function in linkage of phycobilisomes to thylakoids and in energy transfer. Proc Natl Acad Sci USA 79: 5542–5546
45. TANDEAU DE MARSAC N 1977 Occurrence and nature of chromatic adaptation in cyanobacteria. J Bacteriol 130: 82–91
46. TANDEAU DE MARSAC N 1983 Phycobilisomes and complementary chromatic adaptation in cyanobacteria. Bull Inst Pasteur 81: 204–254
47. TANDEAU DE MARSAC N, G COHEN-BAZIRE 1977 Molecular composition of cyanobacterial phycobilisomes. Proc Natl Acad Sci USA 74: 1635–1639
48. THOMAS P 1980 Hybridization of denatured RNA and DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 77: 5201–5203
49. VOGELMANN TC, J SCHEIBE 1978 Action spectrum for chromatic adaptation in the blue-green alga *Fremyella diplosiphon*. Planta 143: 233–239
50. YAMANAKA G, AN GLAZER 1981 Dynamic aspects of phycobilisome structure: modulation of phycocyanin content of *Synechococcus* phycobilisomes. Arch Microbiol 130: 23–30
51. ZUBER H 1983 Structure and function of the light-harvesting phycobiliproteins from the cyanobacterium *Mastogocladus laminosus*. *In* G Papageorgiou, L Packer, eds, Photosynthetic Prokaryotes: Cell Differentiation and Function. Elsevier, Amsterdam, pp 23–41
52. ZUBER H 1986 Structure of light harvesting antenna complexes of photosynthetic bacteria, cyanobacteria and red algae. Trends Biochem Sci 11: 414–419