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Regulation of Phycobilisome Structure and Gene Expression by Light Intensity[1]

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ABSTRACT

The cyanobacterium *Agmenellum quadruplicatum* PR-6 (*Synechococcus* sp PCC 7002) was grown turbidostatically in white light at three levels of irradiance: 20, 200, and 1260 microeinsteins per square meter per second. Phycobilisomes were isolated from each culture and analyzed by absorbance, gel electrophoresis, and electron microscopy. The ratio of phycocyanin to allophycocyanin decreased 1.8-fold from the lowest to highest irradiance. This change was due entirely to an approximately 2.5-fold decrease in one structural unit of rod domains, the complex of phycocyanin, and a 33-kilodalton linker polypeptide (LR33). For a given irradiance, phycobilisomes from cells grown on ammonium as the nitrogen source had 10 to 20% more phycocyanin than those from nitrate cultures. Total RNA was isolated from all cultures and probed with gene fragments specific to phycocyanin and allophycocyanin subunits and LR33. The relative level of RNAs encoding phycocyanin and allophycocyanin was found to vary with light intensity in parallel with the phycobiliprotein ratio. Hence, the light-harvesting capacity of phycobilisomes is directly regulated by relative levels of phycobiliprotein mRNA. The LR33 transcript occurs as a 3′ extension on about 10% of phycocyanin transcripts. The ratio of RNA encoding LR33 to that encoding phycocyanin did not vary with irradiance, although the protein ratio changed 1.7- to twofold between extremes. Based on these and other observations, we propose that the LR33 protein is constitutively synthesized at a rate higher than that required to complex with available phycocyanin.

PAR varies in its spectrum and flux. In response to such changes, photosynthetic organisms alter the composition and organization of their light-harvesting pigments. For example, cyanobacteria change the composition of phycobilisomes, a major antenna complex, in response to spectral shifts (18, 23). We sought to determine whether alterations in total irradiance, in the absence of spectral shifts, could elicit changes in phycobilisome structure and composition. A second goal was to determine whether the expression of antenna genes was also affected by such shifts.

The cyanobacterium *Agmenellum quadruplicatum* PR-6 (*Synechococcus* sp PCC 7002) was chosen for this study. The phycobilisomes of this organism are well described in terms of overall structure and the function of individual polypeptide components (reviewed in refs. 2 and 6). The major components of this complex are the pigmented proteins, PC[5] and AP. The former is localized in rod substructures, where it is bound to linker polypeptides called LRC29, LR33, and LR9. Rods are linked to a central core domain by LRC29. The core contains AP and its associated linker polypeptides.

The genes encoding the α and β subunits of PC (*cpcA* and *cpcB*, respectively) are linked in tandem with those encoding LR33 (*cpcC*) and LR9 (*cpcD*) (7–9, 20) (Fig. 4). Genes encoding AP α and β subunits (*apcA* and *apcB*, respectively) and one linker polypeptide (LC8.5) are similarly clustered (4). Transcripts encoding these *cpc* and *apc* loci have been mapped (12). The major transcript of each locus encodes only the α and β subunits of the respective phycobiliproteins. Minor transcripts extend further in the 3′ direction to encode linker polypeptides. Having this knowledge of phycobilisome structure and gene organization in *A. quadruplicatum*, we could readily investigate responses to changes in light intensity.

In addition to irradiance, we simultaneously examined the effect of nitrogen source on phycobilisome structure. This is of interest because phycobiliproteins are strongly regulated by the concentration of assimilable nitrogen in the medium. Hence, the particular nitrogen compound supplied could also influence phycobilisome structure.

MATERIALS AND METHODS

Culture Conditions

The marine cyanobacterium *Agmenellum quadruplicatum* PR-6 (*Synechococcus* sp PCC 7002) was cultured in medium A (22) containing as the sole nitrogen source either sodium nitrate at 1 g/L (11.8 mM) or ammonium chloride at 0.5 g/L (9.3 mM). The culture vessel, described by Myers and Clark (19), consists of three concentric glass cylinders that define two annular spaces. The outer annulus contains circulating water at a constant temperature of 39°C. The inner annulus, 0.9 cm wide, holds the culture. The length of the vessel is 110 cm and has a working volume of 2 L of culture. The culture was constantly aerated with CO_2 in air (1%, v/v) to provide the carbon source and ensure thorough mixing. Illumination was provided by cool-white fluorescent bulbs (Sylvania

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[5] Abbreviations: PC, phycocyanin; AP, allophycocyanin; kb, kilobase.

F40TR/CW) which were parallel to the vessel and extended its full length. Irradiance was adjusted by varying the number of lamps and/or their distance from the vessel. PAR was measured from the hollow central core of the vessel with a spherical sensor (Biospherical Instruments, model QSL-100P) connected to a digital scalar irradiance meter (model QSP170A). The A_{550} of the culture was kept between 0.4 and 0.8 (1-cm light path) by periodically draining a portion and replacing with fresh medium. This relatively low and constant cell density, combined with the short path length of the culture, minimized *in situ* variation of incident irradiance resulting from self-shading by the cells. Transmitted PAR fluctuated <10% in the dilution cycle and was within 20% of the incident value (*i.e.* in medium lacking cells). Irradiance values cited in the text are the mean of PAR in medium alone and with cells at an $A_{550} \simeq 0.5$.

Phycobilisomes

Phycobilisomes were purified and fractionated by gel electrophoresis as previously described (3). Within 1 h of isolation, the absorption spectrum was measured with a Cary 14 spectrophotometer modified for computer-controlled data acquisition. The molar ratio of PC/AP in phycobilisomes was determined by dissociating the purified particles (by dialysis in 10 mM $KHPO_4$ pH 7), measuring the resulting absorption spectrum, and applying the absorption coefficients of Bryant *et al.* (5). Mounting for EM was performed as previously described (8). Gels were stained with Coomassie Brilliant Blue R250 and scanned with an LKB Ultroscan laser densitometer, with the results recorded on chart paper. Peaks of interest were excised and weighed to assess relative staining intensity.

RNA

Total RNA was isolated as described before (14), but the CsCl centrifugation step was replaced with precipitation in 4 M LiCl. RNA was fractionated in agarose gels (1.5%, w/v) containing 6% (w/v) formaldehyde, 50 mM Hepes (pH 7.8), and 1 mM EDTA (12). Northern blots were performed and probed according to the method of Gasparich (12). All samples contained the same mass of total RNA (±10%). Lengths of RNA species were determined by comparing to the migration of endogenous rRNAs, assuming that they match the lengths of rRNAs from *Anacystis nidulans* (11, 17). Some gels also contained RNA molecular weight standards (Bethesda Research Laboratories). Slot blots were made to quantitate the relative amounts of specific RNAs. Total RNA in gel-loading buffer (minus dye) was heated to 68°C for 3 min and brought to 1.5 M NaCl/0.15 M sodium citrate, pH 7.0. To each aperture of a slot blot manifold (Schleicher and Schuell, model Minifold II), 40 to 120 ng of RNA in a volume of 20 to 60 μL was applied and then drawn to a nitrocellulose membrane by vacuum. The membrane was treated and probed exactly as for Northern blots. Exposures of Kodak X-AR film were made without intensifying screens. Autoradiographic signals were measured by scanning densitometry with a Helena Laboratories Quick-Scan Jr., using the internal integrator to evaluate the areas of peaks. Different concentrations of each RNA sample were applied in triplicate, both as a single twofold dilution and as two serial threefold dilutions. The mean area of triplicate peaks was taken as the signal intensity. Autoradiograms were used for analyses only if the signal intensity of a sample was linearly correlated with the RNA mass applied to the filter.

Probes

Probes consisted of gel-purified restriction fragments labeled with [α-^{32}P]dATP by the random primer method (Boehringer Mannheim, catalog No. 1004 760). Restriction fragments used to probe the *cpc* operon (Fig. 4) were the 1.7-kb *Bgl*II/*Xho*I fragment of pAQPR1 (*cpcBA*) (7), the 0.9-kb *Xho*I/*Hin*dIII fragment of pAQPR1 (*cpcC*) (9), and the 0.36-kb *Hin*dIII fragment of pAQPR50 (*cpcD*) (8). RNA encoding α-AP and β-AP was probed with a 1.1-kb *Hin*dIII fragment containing *apcAB* and a portion of *apcC* (4).

RESULTS

A. quadruplicatum was grown turbidostatically at three levels of irradiance. At each level the fluctuation in total irradiance was <8% as measured at the center of the culture vessel. Furthermore, because cell concentration varied at most twofold throughout the experiment, the spectrum of light transmitted by the culture also deviated minimally. Finally, the cultures were grown for at least three doublings under a given irradiance before harvesting. Thus, the cells were considered to be free of ongoing physiological changes that might attend adaptation to varying light intensity. Under these conditions, the only significant variable was the flux of light.

We also examined the effect of nitrogen source. Nitrogen was provided as nitrate or ammonium. The concentration of these ions in fresh medium was close to 10 mM. The actual concentrations in culture were not determined, but they did not limit the growth rate.

Phycobilisome Composition and Structure

Absorption spectra of phycobilisomes are shown in Figure 1, A and B. They are normalized at A_{660}, a wavelength at which only core components absorb, and thus the spectra directly reflect differences in PC content. Molar ratios of PC/AP determined from absorbance data are shown in Table I. The ratio is seen to decrease 1.6- to 1.7-fold in the range from 20 to 1260 $\mu E \cdot m^{-2} \cdot s^{-1}$. Difference spectra of phycobilisomes from NH_4^+ cultures are shown in Figure 1C. Both difference spectra are superimposable when normalized at a maximum A_{629}. Thus, phycobilisomes from different light intensities differ by a constant type of light-absorbing unit. The absorbance of this unit corresponds to that of the PC-LR33 complex (9). This is also true for phycobilisomes from NO_3^- cultures (data not shown).

At each irradiance, the ratio PC/AP is higher in NH_4^+ than NO_3^- cultures, as exemplified by the absorbance of phycobilisomes from high-light (1260 $\mu E \cdot m^{-2} \cdot s^{-1}$) cultures shown in Figure 1D. There is 10 to 20% more PC in phycobilisomes from NH_4^+ cultures (Table I). The absolute difference in the molar ratio of PC/AP averages 0.25, corresponding to 1.5 PC hexamers more per phycobilisome in NH_4^+ (because a molar ratio of 1.0 is equivalent to six PC hexamers per core) (3, 13).

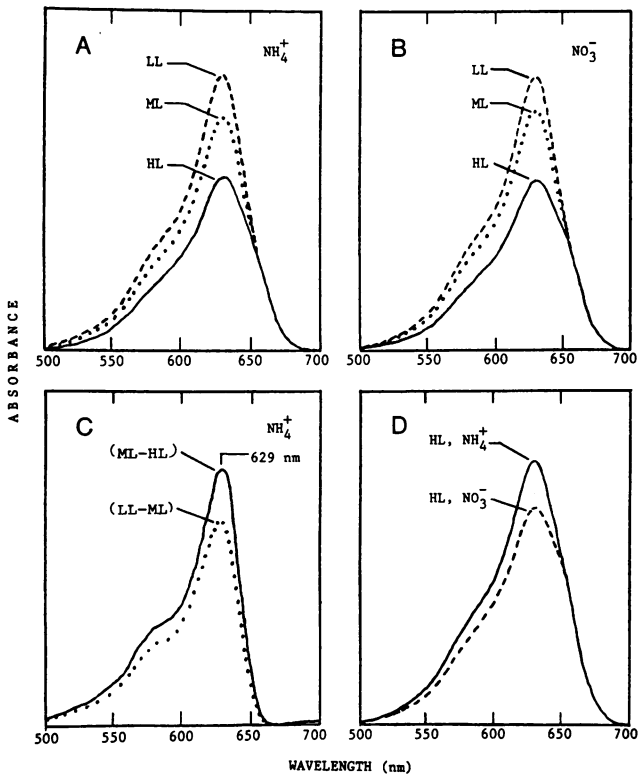

Figure 1. Absorbance and absorption-difference spectra of phycobilisomes from cultures differing in light intensity and nitrogen source. A, From cultures grown in NH_4^+ under 20 (LL), 200 (ML) or 1260 (HL) $\mu E \cdot m^{-2} \cdot s^{-1}$. B, From cultures grown in NO_3^- under LL, ML and HL. C, Absorption-difference spectra of phycobilisomes from NH_4^+ cultures. LL-ML, Low light minus medium light; ML-HL, medium light minus high light. D, Phycobilisomes grown under HL, in NH_4^+ or NO_3^-. Spectra in A, B, and D are normalized at A_{660}. Spectra in C are not normalized, reflecting the relative difference between the respective pairs of samples.

Values of PC/AP were also determined from electron micrographs of isolated phycobilisomes. It was assumed that each disc of a rod substructure is composed of six α-PC-β-PC monomers and that each core substructure contains 36 α-AP-β-AP monomers (3, 13). For the purpose of both absorbance and electron microscopic analyses, the β-18 and the α-AP-B subunits were considered as AP subunits. Primary data are displayed in Table II. The mean number of discs per phycobilisome was converted directly to the molar ratio PC/AP (Table I). The values thus calculated correspond closely to those obtained from absorbance measurements but are consistently lower by about 9%. One explanation is that the extinction coefficients used to calculate PC and AP give overestimates of PC/AP for this organism. Another is that systematic artifacts in the electron microscopic determination of this quantity cause underestimates. In any case, the parameter of greatest interest is the change in the ratio of PC/AP. Absorbance and electron microscopic data both give similar values for this change, as seen in Table I, where PC/AP at each intensity is shown relative to the value in low light (20 $\mu E \cdot m^{-2} \cdot s^{-1}$).

Linker polypeptides in phycobilisomes were quantitated by scanning densitometry of electrophoretically resolved polypeptides. A stained SDS gel of phycobilisomes is shown in Figure 2. All samples have the same components; however, phycobilisomes from high-light cultures show contaminants due to their low sedimentation coefficient. Visual inspection reveals a decrease in the ratio of LR33/LRC29 with increasing light intensity. In Table I are listed the values for LR33/LRC29 as ratios of stain absorbance. More relevant are the normalized values, as seen in the adjacent column, which reflect the change in the amounts of these linkers. Quantitation of LR9 in polyacrylamide gels was not attempted because it was not resolved from LC8.5.

We found that the ratio of LRC29 to the core linker LCM94 was constant among all samples (data not shown), regardless of irradiance or nitrogen source. Because LRC29 joins a rod to the core (9), the constancy of LRC29/LCM94 reflects an invariant number of rods per phycobilisome. Hence, variation in the amount of PC is due solely to changes in the average number of PC-LR33 complexes per rod and not to differences in the number of rods.

Table I. *Molar Ratios of Phycobilisome Components*

Nitrogen Source	Irradiance	PC/AP				LR33/LRC29			
		A[a]	Rel[b]	EM[c]	Rel	Gel[d]	Rel	EM	Rel
	$\mu E \cdot m^{-2} \cdot s^{-1}$								
	20	2.37	1.00	2.11	1.00	1.62	1.00	1.28	1.00
NO_3^-	200	2.03	0.86	1.83	0.87	1.32	0.82	0.99	0.77
	1260	1.37	0.58	1.22	0.58	0.78	0.48	0.40	0.31
	20	2.63	1.00	2.57	1.00	2.22	1.00	1.68	1.00
NH_4^+	200	2.21	0.84	2.06	0.80	1.55	0.70	1.18	0.70
	1260	1.69	0.64	1.46	0.57	0.97	0.44	0.63	0.38

[a] Determined by absorbance. [b] Relative (Rel) to the value for 20 $\mu E \cdot m^{-2} \cdot s^{-1}$. [c] Determined by EM. [d] Determined by scanning densitometry of stained gels. These are not molar ratios but ratios of stain absorbance.

Table II. *Evaluation of Phycobilisome Rod Structure by EM*

Nitrogen Source	Irradiance	Phycobilisomes Counted	No. of Rods with *n* Discs						Total Discs	Mean Discs/ Phycobilisomes
			n = 1	*n* = 2	*n* = 3	*n* = 4	*n* = 5	*n* > 6		
	$\mu E \cdot m^{-2} \cdot s^{-1}$									
NO_3^-	20	100	34	368	128	22	4	1[a]	1268	12.68
	200	99	70	422	50	5	1	0	1089	11.00
	1260	113	359	227	5	0	0	0	828	7.33
NH_4^+	20	100	8	323	149	57	26	12[b]	1540	15.40
	200	100	37	422	79	24	3	1[c]	1236	12.36
	1260	115	233	380	6	0	0	0	1011	8.79

[a] Six discs. [b] Seven with six discs, one with seven discs, four with eight discs. [c] Seven discs.

The ratio of linker polypeptides was also determined by EM. We (9) previously demonstrated that LRC29 is associated with the core-proximal disc of rods and that discs beyond this contain only LR33. Thus, the number of LRC29 linkers in a sample is equal to the number of rods, and the number of LR33 linkers is the difference between the total number of discs and the number of rods. Calculated molar ratios of LR33/LRC29 are listed in Table I along with normalized values. The latter closely match those obtained by scanning densitometry. An exception is found in phycobilisomes from high-light cultures in nitrate. The discrepancy may be due to underestimation of LR33/LRC29 by EM because of the small size of these phycobilisomes. As with gel scanning data, we found that the number of rods per phycobilisome varied little with irradiance or nitrogen source (5.5 ± 0.2 [mean ± SD] for all six samples).

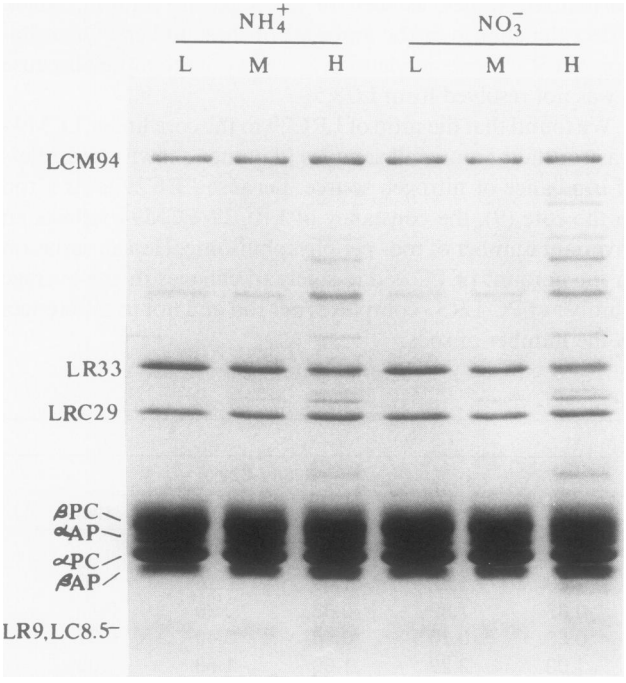

Figure 2. Gel electrophoresis of phycobilisome polypeptides. Phycobilisomes were purified from cultures grown in NH_4^+ or NO_3^- under irradiance of 20 (L), 200 (M), or 1260 (H) $\mu E \cdot m^{-2} \cdot s^{-1}$. The identities of resolved components are indicated on the left.

The magnitude of LR33/LRC29 ratios deduced from electron micrographs match closely those expected from the observed PC/AP ratios, assuming the mode of phycobilisome structure mentioned above (3, 9). For example, phycobilisomes from nitrate cultures grown under 200 $\mu E \cdot m^{-2} \cdot s^{-1}$ have a PC/AP ratio of 2.03 (by absorbance), equivalent to an average of (6)(2.03) = 12.18 PC discs per phycobilisome. Of these discs, six are hypothesized to be complexed with LRC29, the remaining 6.18 with LR33. Thus, the molar ratio of LR33/LRC29 should be 1.03, which is close to the value of 0.99 calculated from electron micrographs.

In summary, we found that phycobilisome structure is modified solely by changes in irradiance under a constant spectrum of illumination. The source of nitrogen in the medium also influences phycobilisome structure, with NH_4^+ yielding more PC than NO_3^-. Finally, the observed changes in phycobilisome structure are solely due to variation in the number of PC-LR33 complexes.

In addition to altering phycobilisome structure, *A. quadruplicatum* also varies the number of phycobilisomes, *i.e.* the ratio of phycobiliprotein to Chl varies inversely with irradiance (data not shown). This has been described in other cyanobacteria grown under a constant (15, 24) or varying (18) spectrum of illumination. Interestingly, in *A. quadruplicatum*, the number and structure of phycobilisomes can be regulated independently (9). For example, cultures shifted directly from 1260 to 20 $\mu E \cdot m^{-2} \cdot s^{-1}$ exhibited the low phycobiliprotein to Chl ratio characteristic of high-light cultures, even after many cell divisions (data not shown). However, their phycobilisome structure and composition did respond to the shift, as demonstrated here. A more gradual shift from high to low irradiance leads to the same changes in phycobilisome structure but also to an increase in phycobiliprotein to Chl.

RNAs for Phycobilisome Components

From each culture, total RNA was purified and analyzed by Northern blotting, using as probes cloned DNA specific to *apcAB*, *cpcBA*, *cpcC*, or *cpcD* ("Materials and Methods"). Resulting autoradiograms are shown in Figure 3.

RNA homologous to *apcAB* consists of three species with lengths of 1.3, 1.45, and 1.75 kb, as has been described by

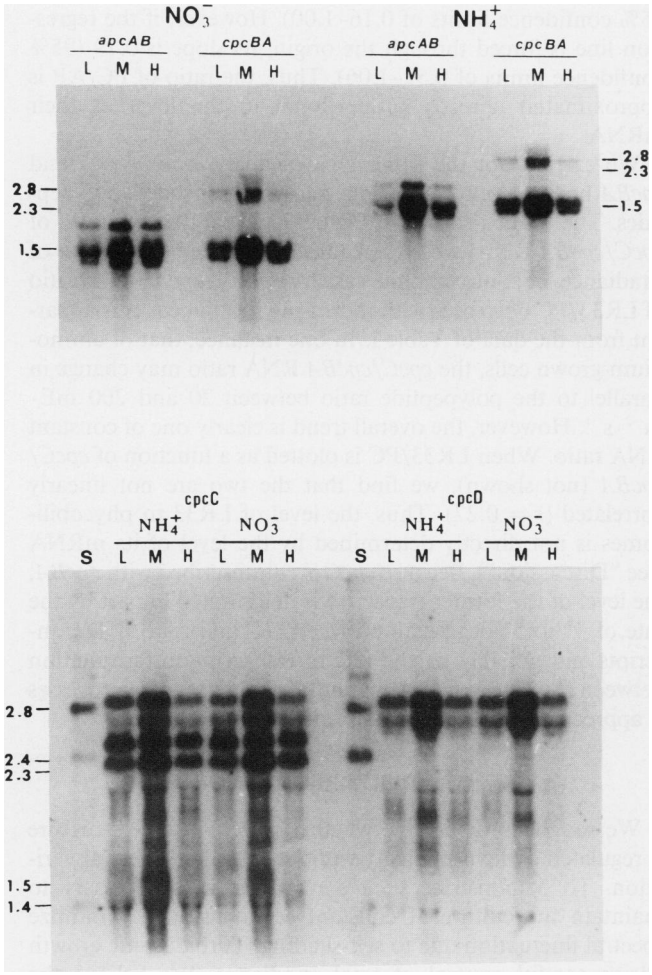

Figure 3. Northern blots of RNA isolated from cultures grown in varying irradiance and nitrogen sources, probed with phycobiliprotein and linker genes. All samples contained the same mass of total RNA (±10%). Top, RNA probed with *apcAB* or *cpcBA*. Positions corresponding to the major rRNA species (2.3 and 2.8 kb = 26S rRNA, 1.5 kb = 16S rRNA) are indicated. Bottom, RNA probed with *cpcC* or *cpcD*. Positions corresponding to rRNA species (as above) and a commercially supplied RNA standard (1.4 and 2.4 kb in lane S) are indicated. Irradiances are 20 (L), 200 (M), or 1260 (H) $\mu E \cdot m^{-2} \cdot s^{-1}$.

Gasparich (12) (Fig. 3). The two species of lowest mass contain only *apcAB* but differ by 165 base pairs in their 5′-end point. The 1.75-kb species contains *apcABC*. The same overall pattern of fragment lengths and hybridization intensity is seen in RNA from all cultures, regardless of irradiance or nitrogen source (Fig. 3).

In the case of *cpcBA*-homologous transcripts, two major species, 1.4 and 1.55 kb, are present in all cultures (Fig. 3). These both contain only *cpcBA* and differ in the location of their 5′-end points (12). The more weakly hybridizing transcript of approximately 2.5 kb consists of four resolvable species, as seen in Figure 3, bottom. All four, 2.35, 2.5, 2.7, and 2.85 kb, are homologous to *cpcC*. The 2.7- and 2.85-kb species contain *cpcD* as well. The difference in length within each pair of transcripts (*cpcBAC* or *cpcBACD*) is 0.15 kb, which is equal to the difference found between the 5′ ends of the two major *cpcBA* transcripts (12). Based on the size and location of the *cpcD* gene, the difference in length between *cpcBAC* and *cpcBACD* transcripts is expected to be approximately 300 base pairs (8). This matches the difference found in the Northern blots of Figure 3. Therefore, each of the four transcripts apparently possesses one of two possible 3′-end points and one of two possible 5′-end points. From the relative intensities of the bands seen by hybridization to *cpcC*, we estimate that 20% of the transcripts that contain *cpcC* also contain *cpcD*. This is illustrated in Figure 4 (which does not show very minor transcripts extending beyond *cpcD* [12]). The overall pattern of *cpcC*- and *cpcD*-homologous RNAs does not vary with irradiance or nitrogen source.

It is apparent that the fraction of total RNA as phycobiliprotein gene transcripts varies between samples, as reflected by the different band intensities in lanes of the gels shown in Figure 3. This correlates with changes in total phycobiliprotein per unit of Chl, as mentioned above. For example, medium-light RNA shows stronger hybridization to *apcAB* and *cpcBA* probes than high-light RNA. Low-light RNA in these blots was prepared from cultures that had been directly shifted from high light. They retained a low phycobiliprotein to Chl ratio, which is reflected in low hybridization to phycobiliprotein gene probes.

We sought to determine the relative levels of *apcAB*, *cpcBA*, and *cpcC* transcripts in the various cultures. Attempts to quantitate these RNAs by scanning densitometry of Northern blot autoradiograms was unsuccessful because of the high variability of the results. This probably arose from uneven transfer of RNA to the nitrocellulose membrane. To overcome this problem, RNA in aqueous solution was applied directly to membranes using a slot blot apparatus and then probed with gene-specific restriction fragments. The resulting hybridization signals, quantitated by scanning densitometry, were very reproducible for any blot. Furthermore, for properly exposed autoradiograms, the hybridization signal was proportional to the mass of RNA applied. Under these conditions, the relative densitometric signal of two RNAs, *e.g. cpcBA/*

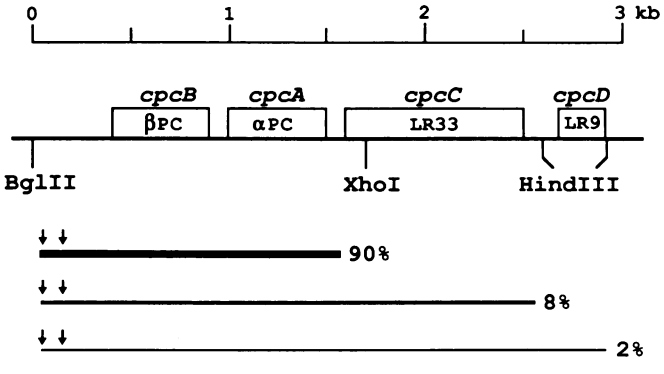

Figure 4. Map and transcripts of the *cpcBACD* locus. Restriction sites used to generate probes for specific segments are indicated. Lines below the map show the approximate bounds of three transcripts, each having one of two possible 5′ ends (indicated by arrows) (12). The estimated amount of each species, as a fraction of total *cpcBACD* transcripts, is given at the right.

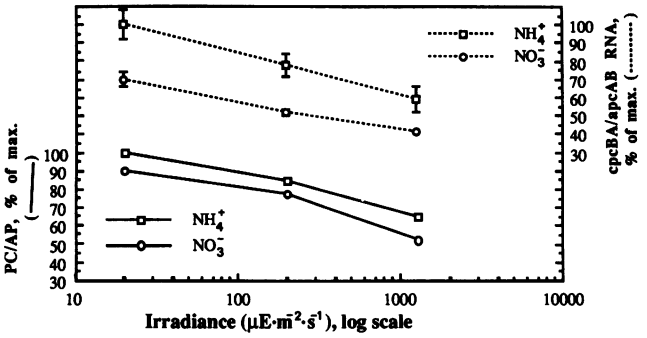

Figure 5. Dependence on irradiance of phycobiliprotein ratio (PC/AP) and of phycobiliprotein gene transcripts (*cpcBA/apcAB*). Both ratios are plotted as percentages of the culture with the maximum value. Error bars for RNA ratios are the SD of four replicates, each normalized to the high-light nitrate culture.

apcAB, for each culture was determined. Ratios obtained from four replicate measurements were compared by normalizing to the value of one culture, which we chose to be the high-light nitrate culture. This does not provide a measure of the absolute molar or mass ratio of two RNA species. However, changes in relative densitometric signals are proportional to changes in the absolute ratio of two RNAs. Results for the *cpcBA/apcAB* RNA ratio are displayed in Figure 5. Two trends are apparent. First, the ratio at each irradiance is higher for the culture containing ammonium than for that containing nitrate. Second, for each nitrogen source, the *cpcBA/apcAB* RNA ratio decreases with increasing irradiance. For comparison, the protein ratio, PC/AP, for each culture (obtained from Table I, absorbance column) is also plotted in Figure 5 as a percentage of the maximum value. The trends in RNA and polypeptide ratios are parallel. This is seen directly in Figure 6, where relative protein ratios are plotted against the corresponding RNA ratios. The drawn line is that of least squares regression and has a correlation coefficient of 0.91. Thus, the ratio of PC/AP varies linearly with the proportion of their respective mRNAs. The slope of the line is 0.58 (with 95% confidence limits of 0.16–1.00). However, if the regression line is forced through the origin, its slope is 0.79 (95% confidence limits of 0.52–1.06). Thus, the ratio of PC/AP is approximately directly proportional to the level of their mRNAs.

We carried out the same type of analysis for *cpcC*- and *cpcBA*-homologous transcripts and their encoded polypeptides. The results, shown in Figure 7, reveal that the ratio of *cpcC/cpcBA* RNA is constant throughout the tested range of irradiance, in contrast to the case for *cpcBA/apcAB*. The ratio of LR33/PC decreases with increasing irradiance, as is apparent from the data of Table I. In one instance, that of ammonium-grown cells, the *cpcC/cpcBA* RNA ratio may change in parallel to the polypeptide ratio between 20 and 200 mE·$m^{-2}·s^{-1}$. However, the overall trend is clearly one of constant RNA ratio. When LR33/PC is plotted as a function of *cpcC/cpcBA* (not shown), we find that the two are not linearly correlated ($r = 0.27$). Thus, the level of LR33 in phycobilisomes is not directly determined by the level of its mRNA (see "Discussion"). Because *cpcC* is cotranscribed with *cpcBA*, the level of the former transcript is determined in part by the rate of 3′ to 5′ degradation in *cpcBAC* and *cpcBACD* transcripts and possibly by the rate of transcription termination between *cpcA* and *cpcC*. Apparently, neither of these processes is appreciably affected by light intensity.

DISCUSSION

We sought to determine whether phycobilisome structure is regulated by light intensity without attendant spectral variation. To demonstrate such a response, it is necessary to maintain the culture at constant cell density to minimize spectral fluctuation due to self-shading. Turbidostatic growth allows careful control of total irradiance. We believe the present results demonstrate that light flux alone is a factor in determining the antenna capacity of phycobilisomes.

By analysis of ultrastructure and composition, we determined that the only irradiance-dependent variable in phycobilisomes is the number of PC-LR33 complexes. *A. nidulans* exhibits a similar response. One study showed that the ratio of total cellular PC/AP in this species varied inversely with irradiance (24). Although the spectrum of illumination was

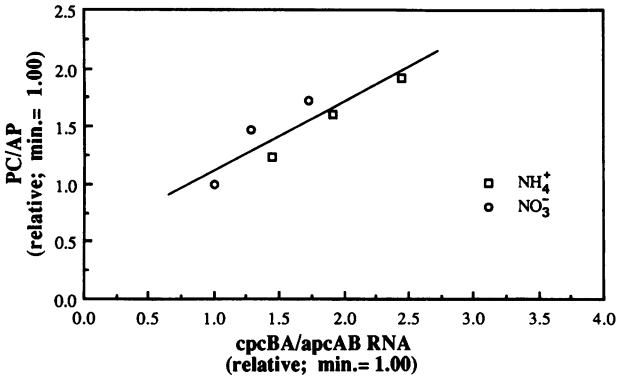

Figure 6. Dependence of phycobiliprotein ratio (PC/AP) on the ratio of the corresponding RNAs (*cpcBA/cpcAB*). Values plotted are normalized to the minimum, which is set at 1.0. The line drawn is that of least squares regression.

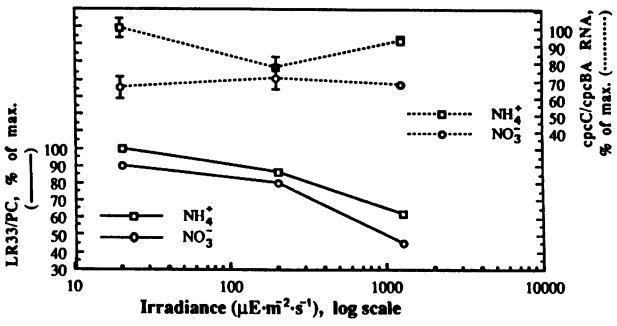

Figure 7. Dependence on irradiance of protein ratio (LR33/PC) and corresponding RNA ratio (*cpcC/cpcBA*). Both ratios are plotted as percentages of the maximum value. Error bars for RNA ratios are the SD of four replicates, each normalized to the high-light nitrate culture.

kept constant, the cultures were not grown turbidostatically and thus encountered intensity and spectral variation. Lonneborg *et al.* (18) showed that shifting *A. nidulans* cultures from white to red illumination induced changes in the level of PC relative to AP and of two PC-associated linkers, LR33 and LR30. Similar changes in these polypeptides occur when a spectral shift is accompanied by changes in temperature and CO_2 concentration (25). Our results with *A. quadruplicatum* suggest that these changes in *A. nidulans* phycobilisome composition are reflective of ultrastructural changes, *i.e.* the number of discs per rod, and are due to changes in light intensity that attend the shift in illumination from white to red light. Interestingly, in both *A. nidulans* (25) and *A. quadruplicatum* (present study), the PC/AP ratio varies by a factor of 2 between its extremes. Rod components distal to PC are more strongly regulated by light intensity. For example, phycoerythrin varies fivefold with respect to PC over a wide range of irradiance (15).

Other studies of phycobilisome composition have failed to detect an effect of incident spectrum or intensity. In one case, *A. nidulans* grown in white or red light showed no difference in the ratio of PC/AP, although the ratio of phycobiliprotein to Chl was responsive (16). It is possible that red illumination was sufficiently intense such that total PAR absorbed by cells was the same as for white illumination. Another cyanobacterium, *Microcystis aeruginosa*, showed no change in phycobilisome composition with differing irradiance (21). However, the CO_2 concentration supplied to low- and high-irradiance cultures differed, and high-irradiance cultures were allowed to attain a greater cell density. These variables may have obscured an actual dependence of phycobilisome composition on light intensity.

We also examined the relative levels of RNA encoding PC, AP, and LR33 as a function of irradiance. Changes in the PC/AP polypeptide ratio were nearly directly proportional to changes in the ratio of their RNAs. Thus, the relative amounts of these proteins are regulated by relative RNA abundance. We do not know whether RNA levels are governed by rates of transcription or degradation. The rate of *cpcBA* transcription is known to vary with light intensity (12), but *apcAB* transcription has not yet been examined.

In contrast to PC and AP, the level of LR33 in phycobilisomes is not directly related to the level of its transcript, *cpcC*. For example, the ratio of LR33/PC in nitrate-grown cultures decreases by a factor of 1.9 between 20 and 1260 $\mu E \cdot m^{-2} \cdot s^{-1}$; yet, the RNA ratio *cpcC*/*cpcBA* remains constant. Two hypotheses can account for this. The first is that translational regulation of *cpcC* RNA governs the level of LR33 to maintain the appropriate stoichiometry with PC. The second is that the level of total LR33 is indeed directly related to the level of its RNA, but the polypeptide is synthesized in excess; thus, only that portion that is incorporated in phycobilisomes is accounted for. We prefer the second hypothesis. It is supported by studies of *A. quadruplicatum* strains that contain an extra copy of the *cpcBA* locus in addition to the wild-type *cpc* operon (10). These strains synthesize twice the wild-type level of PC, some of which is incorporated into phycobilisomes. The extra phycobilisome-bound PC is accompanied by a stoichiometric increase in LR33, in spite of the fact that *cpcC* is unaltered. The simplest explanation of these results is that LR33 is normally synthesized in excess. In the presence of additional PC, as in a PC-overproducing strain, the excess is detected. If LR33 is not overproduced, but in fact translationally regulated, then these results suggest that PC itself is a regulatory factor. An inherent feature of both of these hypotheses is that PC-LRC29 complexes form preferentially to PC-LR33 complexes. Such a postulate is necessary to explain the constant number of PC-LRC29 complexes per phycobilisome. Thus, if LR33 is translationally regulated, then its synthesis must be deferred to that of LRC29. If LR33 is synthesized at a constant level, then PC must preferentially associate with LRC29. PC synthesized in excess of that required to bind available LRC29 would then be accessible to LR33.

The levels of *cpc* operon transcripts in *Anabaena* PCC 7120 have been examined as a function of light intensity (1). It was found that the *cpcC* transcript, relative to *cpcBA*, was 10-fold higher at low irradiance than high. The significance of this finding is unknown because levels of phycobilisome components were not measured. However, it seems unlikely that phycobilisomes contain 10-fold more LR33, relative to PC, in low light. We found in *A. quadruplicatum* a twofold change in LR33/PC over a 60-fold range of light intensity. Data for *A. nidulans* also suggest an approximate twofold change in this ratio over a large change in illumination (Fig. 8 of ref. 18). Thus, the level of LR33 in *Anabaena* phycobilisomes, as in *A. quadruplicatum*, may not be directly regulated by the level of its mRNA.

We found phycobilisome structure to be dependent on nitrogen source. Particles isolated from ammonium-grown cultures consistently contained more PC than those from nitrate-grown cultures. This effect is evidently regulated by relative mRNA abundance as seen in Figure 5, where ammonium-grown cultures at a given irradiance always have a higher *cpcBA*/*apcAB* RNA ratio than the corresponding nitrate-grown cultures. We speculate that this regulation is mediated by the intracellular concentration of some reductant because NH_4-N is assimilated directly, whereas NO_3-N must be reduced from the +5 to −3 oxidation state. The proportions of various transcripts within the *cpc* operon may also depend on nitrogen source. At low and high (but not medium) irradiance, the *cpcC*/*cpcBA* RNA ratio is greater in ammonium than nitrate cultures (Fig. 7). Because *cpcC* transcripts occur only as extensions of *cpcBA* transcripts, the ratio of *cpcC*/*cpcBA* RNA is a function of either the rate of transcription termination between *cpcA* and *cpcC* or the rate of degradation 3′ to *cpcA*. Conceivably, these rates could be influenced by nitrogen source and lead to altered levels of intraoperon transcripts.

Located between *cpcC* and *cpcD* are two closely spaced inverted repeats that are expected to form pronounced hairpin loop structures in RNA (8). Deletion of these inverted repeats is associated with a partial loss of LR33, providing evidence that these putative secondary structures stabilize 5′-RNA sequences (8). In the present work, we noted that RNA-encoding *cpcD* occurs as a 3′ extension of a fraction of *cpcBAC* transcripts (Fig. 3). This finding strengthens the postulated 5′ stabilization function of these hairpin loops, because they should coincide with a transcript ending between *cpcC* and *cpcD*, as is observed. Located near the 3′ end of *cpcD* is

another pronounced inverted repeat (our unpublished data) that likely defines the 3′ end of the *cpcBACD* transcript.

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