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Acclimation Processes in the Light-Harvesting System of the Cyanobacterium *Anacystis nidulans* following a Light Shift from White to Red Light[1]

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ABSTRACT

Cyanobacteria acclimate to changes in light by adjusting the amounts of different cellular compounds, for example the light-harvesting macromolecular complex. Described are the acclimatization responses in the light-harvesting system of the cyanobacterium *Anacystis nidulans* following a shift from high intensity, white light to low intensity, red light.

The phycocyanin and chlorophyll content and the relative amount of the two linker peptides (33 and 30 kilodaltons) in the phycobilisome were studied. Both the phycocyanin and chlorophyll content per cell increased after the shift, although the phycocyanin increased relatively more. The increase in phycocyanin was biphasic in nature, a fast initial phase and a slower second phase, while the chlorophyll increase was completed in one phase. The phycocyanin and chlorophyll responses to red light were immediate and were completed within 30 and 80 hours for chlorophyll and phycocyanin, respectively. An immediate response was also seen for the two phycobilisome linker peptides. The amount of both of them increased after the shift, although the 33 kilodalton linker peptide increased faster than the 30 kilodalton linker peptide. The increase of the content of the two linker peptides stopped when the phycocyanin increase shifted from the first to the second phase. We believe that the first phase of phycocyanin increase was due mainly to an increase in the phycobilisome size while the second phase was caused only by an increase in the amount of phycobilisomes. The termination of chlorophyll accumulation, which indicates that no further reaction center chlorophyll antennae were formed, occurred parallel to the onset of the second phase of phycocyanin accumulation.

The light-harvesting complex of cyanobacteria is composed mainly of Chl *a* and phycobiliproteins. Chl *a* resides in the thylakoid membranes and the phycobiliproteins in the phycobilisomes, which are water-soluble complexes and attached on the thylakoids. The cyanobacterium *Anacystis nidulans*, used in this study, has phycobilisomes containing two core complexes and three rods exiting from each core (24). The core complex contains the chromoproteins allophycocyanin (λ_{max} ~ 650 nm), allophycocyanin B (λ_{max} ~ 665 nm), a 75 kD peptide (λ_{max} ~ 680 nm), and an 18.3 kD peptide, together with one peptide without any chromophores with the apparent mol wt of 10.5 kD (10, 11). Together with the chromoprotein phycocyanin (λ_{max} ~ 625 nm) the rod contains three different linker peptides, a 27 kD peptide that anchors the rod to the core, a 33 and a 30 kD peptide, linking phycocyanin hexamers with each other, outward toward the terminal end (9).

A. nidulans can respond with changes in the amount and the size of the phycobilisomes to altered growth conditions. High levels of phycocyanin in the cell is favored by high temperature, high CO_2 concentrations (4), low light intensities (13, 18), and light that mainly is absorbed by Chl *a* (3, 5). During nitrogen starvation, the total amount of phycocyanin decreases (1).

Much work has been done in comparing the light-harvesting complex of *A. nidulans* grown under different light conditions (7, 14, 18, 19, 20, 26). However, to date no extensive study has been published where the time course of acclimation to new light environment has been followed until the process is completed. Such studies are crucial for a better understanding of the physiology of photosynthetic adaptation and they are also fundamental for further studies of how photosynthetic adaptation, *e.g.* the biogenesis of the phycobilisome, is regulated at the molecular level.

In this paper we present data on the acclimative pigment changes in an *A. nidulans* culture during and after a shift from HW[2] (300 $\mu E \cdot m^{-2} \cdot s^{-1}$) to LR (15 $\mu E \cdot m^{-2} \cdot s^{-1}$). The $A^{PC}_{625}/A^{Chl}_{675}$ ratio, the relative amounts of the 33 and 30 kD linker peptides, and the amounts of phycocyanin and Chl *a* per cell were followed.

MATERIALS AND METHODS

Culture Conditions. *Anacystis nidulans* 625 (*Synechococcus* 6301) was used throughout the study. The bacteria were grown in a medium described by Siva *et al.* (20) but it contained 14.4 μM $FeCl_3 \cdot 6H_2O$ and 65 μM EDTA instead of Fe-*N*-hydroxylethyl-EDTA. The cells were grown under incandescent light (Philips PAR 38, 150 w/220 v) at 39 ± 1°C using equipment as described by Öquist (17). Low intensity red light was obtained by the use of a red plexiglass filter and a blue celluloid filter. The quantum flux densities were adjusted to 300 and 15 $\mu E \cdot m^{-2} \cdot s^{-1}$ for HW and LR, respectively. The spectral quanta distribution in the two light regimes, measured by QSM-2500, Techtum Instrument AB, Umeå, Sweden, are presented in Figure 1.

Spectroscopic measurements. Cell densities were determined from absorbance measurements at 750 nm (Zeiss Spectrophotometer M4 Q III, equipped with Multiblank 171 and Multilog

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[2] Abbreviations: HW, high intensity white light; LR, low intensity red light.

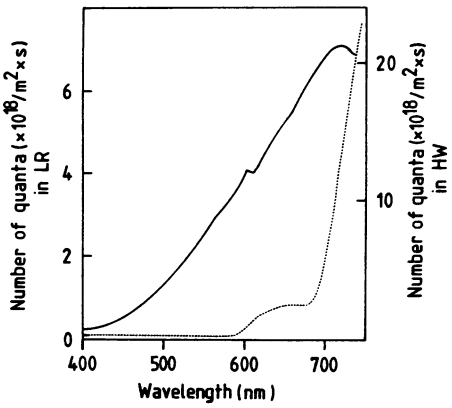

FIG. 1. The spectral quanta distribution between 400 and 745 nm in HW (——) and in LR (·····).

311 from Optilab). To correlate A_{750} to the cell number for both HW and LR conditions, the cells were counted in a hemacytometer at different densities. All measurements have been made with cultures in exponential phase of growth. A_{750} was kept <0.5 by dilution in order to minimize effects of self-shading.

Absorption spectra were measured between 600 and 750 nm on a Shimadzu MPS-50 L spectrophotometer. From these spectra, the amount of phycocyanin and Chl *a* per cell and the $A_{625}^{PC}/A_{678}^{Chl}$ ratio was estimated as described by Myers *e al.* (15).

Preparation of Phycobilisomes. Phycobilisomes were prepared according to Yamanaka *et al.* (24) with some modifications. A 0.60 M Na-phosphate buffer of pH 8.0 was used to avoid band splitting on the SDS-polyacrylamide gels. Aliquots (0.45 ml) of crude phycobilisome preparations were layered on sucrose step gradients consisting 0.40, 1.18, 1.18, 0.92, and 0.88 ml of 2.0, 1.0, 0.75, 0.50, and 0.25 M sucrose solutions, respectively, all in 0.60 M Na-phosphate buffer (pH 8.0). These gradients were centrifuged for 1 h in a VTi 65-rotor at 40,000 rpm and 18°C. If two blue bands were obtained in the gradient, the lower band was used. The quality of phycobilisomes was analyzed by recording the 77 K fluorescence emission spectra excited at 578 nm by the fluorescence spectrometer described by Ögren and Öquist (16). If the 680/650 nm emission ratio was greater than 2, the sample was considered to contain mainly intact phycobilisomes.

PAGE. After sucrose density centrifugation, the samples for PAGE were dialyzed against 6 mM Na-phosphate buffer (pH 8.0) followed by lyophilization and dilution with water to ~1 μg protein/μl. To 7.5 μl sample (~7.5 μg protein) was added an equal volume of sample buffer containing 0.5 M Tris-HCl (pH 6.8), 4% (w/v) SDS, 20% (w/v) glycerol, 1.3 mM β-mercaptoethanol, and 8% (w/v) bromophenol blue. Immediately before applying the sample on the gel, the sample was heated to 100°C for 1 min.

PAGE was performed as described by Byström and Björk (2). After destaining, the gels were dried on Whatman 3 MM paper and scanned with a densitometer (Zeiss PMQ 3 spectrophotometer equipped with a gel scanner).

RESULTS

Cell Growth. To correlate A_{750}, where neither Chl *a* nor phycobiliproteins have any significant absorbance, to cell number, a correlation curve was made for both HW and LR-grown cells (Fig. 2). It can be seen that there is a good correlation (r^2 = 0.90) between the *A* at 750 nm and the cell number. No significant difference between HW- and LR-grown cells can be seen. Since the medium has a slight *A* at 750 nm and the blank used was water, the line in Figure 2 does not cross the origo. This spectroscopic method makes it easy to follow the growth characteristics of the culture.

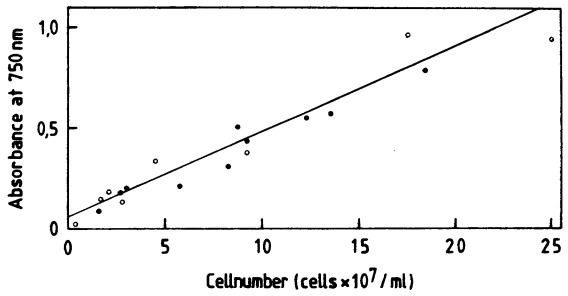

FIG. 2. Absorbance at 750 nm for HW- (○) and LR- (●) grown *A. nidulans* plotted against the cell number, counted in a hemacytometer.

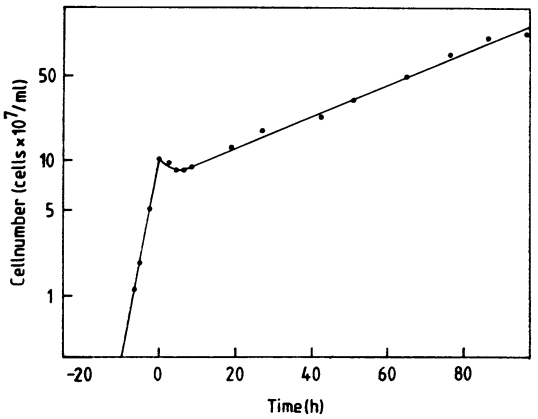

FIG. 3. Growth curve for *A. nidulans* during a light shift from HW to LR conditions. Time 0 indicates the shift from HW to LR conditions.

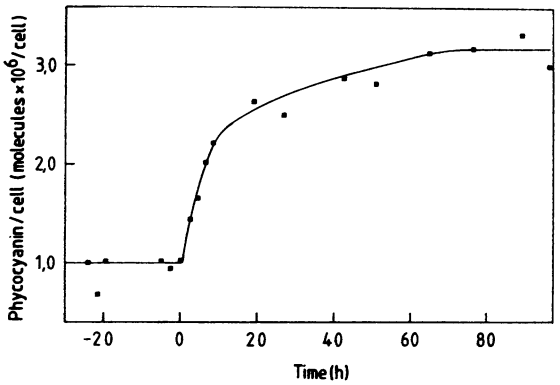

FIG. 4. The amount of phycocyanin hexamer molecules per cell during a light shift from HW to LR conditions. Time 0 indicates the shift from HW to LR conditions.

In the HW conditions, *A. nidulans* had a generation time of 4 ± 1 h (Fig. 3). When shifted to LR conditions, the increase in cell number immediately stopped and even decreased during the first 5 to 7 h. After the initial lag phase, the cells began to grow at a new steady state rate, but with a generation time of 33 ± 5 h.

Pigment Changes. After a shift from HW to LR conditions, the phycocyanin content per cell started to increase immediately and increased rapidly, about 2.4 times during the first 10 to 15 h. After the first rapid initial phase, the increase was slower reaching a steady level within 80 h (Fig. 4). The total increase of phycocyanin per cell was 3.2 times. The increase of Chl content per cell (Fig. 5) also started immediately after the shift and proceeded rapidly during an initial phase of 12 to 17 h (1.9 times). After 20 h, however, no further increase of Chl was obtained. Thus, a shift from HW to LR conditions induced

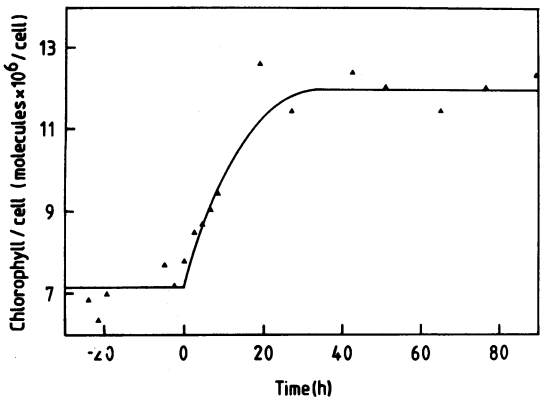

FIG. 5. The amount of Chl molecules per cell during a light shift from HW to LR conditions. Time 0 indicates the shift from HW to LR conditions.

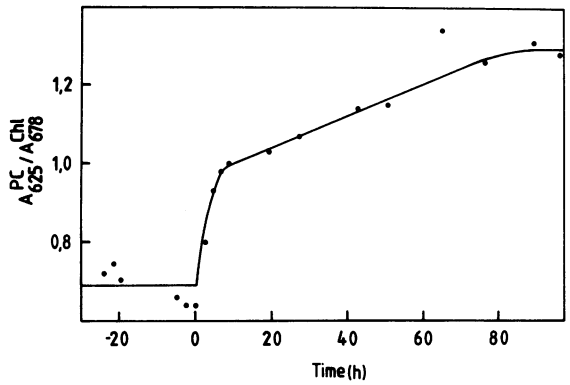

FIG. 6. The $A^{PC}_{625}/A^{Chl}_{678}$ ratio measured during a light shift from HW to LR conditions. Time 0 indicates the shift from HW to LR conditions.

pigment changes where phycocyanin increased in two steps, an initial rapid phase followed by a slow phase, whereas Chl increased in only one rapid initial phase.

The $A^{PC}_{625}/A^{Chl}_{678}$ ratio after a shift from HW to LR was calculated from the results shown in Figures 4 and 5 and presented in Figure 6. A fast initial increase (1.4 times) during the first 5 to 7 h after the shift was revealed. After that the ratio continued to increase, though at a slower rate until 70 to 90 h after the shift. The $A^{PC}_{625}/A^{Chl}_{678}$ ratio finally reached 1.3 after the shift to LR conditions; the $A^{PC}_{625}/A^{Chl}_{678}$ ratio accordingly increased 1.8 times after the shift to LR conditions.

Phycobilisome Changes. Because all phycocyanin is located within the phycobilisome (24), the increase of the phycocyanin per cell must be caused by an increase in the phycobilisome size and/or an increase in the amount of phycobilisomes. To see if there was any change in the size of the phycobilisomes during acclimation to LR, we studied the protein composition of isolated phycobilisomes by SDS-PAGE through the experiment (Figs. 7 and 8).

Proteins that belong to the core and the rods of the phycobilisomes can be seen on the polyacrylamide gel (Fig. 7). Core proteins are the 75 kD peptide and the 18.2 kD peptide which is the α-subunit of allophycocyanin, while the 33, 30, 27, and 19 kD peptides are rod peptides; the peptide band of 17.7 kD consists of the β-subunit of allophycocyanin and the α-subunit of phycocyanin.

It has been found that the size of the phycobilisome depends only on the rods; the core is not affected (26). According to Yamanaka and Glazer (26), the 27 kD linker peptide and one phycocyanin hexamer is always present in the phycobilisome, depending on the environmental conditions. To measure the changes in the rod lengths, the amounts of the 33 and 30 kD linker peptides relative the 27 kD linker peptides were calculated and presented in Figure 8. The 27 kD peptide was used in these calculations instead of the 75 kD peptide, since this peptide sometimes was partly degraded. The 33 and 30 kD linker peptides are present at HW steady state growth conditions, but at very low amounts. When shifting to the LR conditions, the ratios 33/27 kD, and 30/27 kD increased steadily, the rate of incorporation in the phycobilisome was faster for the 33 kD linker peptide compared with the 30 kD linker peptide. Both ratios reached a steady state maximum 15 to 18 h after the shift, at levels of 1.0 and 0.75 for the 33/27 kD, and 30/27kD ratios, respectively. The increase coincided in time with the first initial step of phycocyanin increase per cell (Fig. 4). The increase of the PC/APC ratio as judged from absorption spectra of isolated phycobilisomes (data not shown) also indicates that the size of the phycobilisomes increased during LR acclimation.

DISCUSSION

The lag or even decrease in cell growth after transfer of *A. nidulans* from HW to LR conditions (Fig. 4) is thought to depend on (a) terminated or strongly retarded cell growth during the initial course of acclimation and/or (b) death of a cell fraction that was unable to acclimate (this would explain the slightly decreased cell content immediately after the shift). However, the analyses of phycocyanin per cell (Fig. 4) and Chl *a* per cell (Fig. 5) revealed that the acclimating cells were very active adjusting the pigment composition directly after the change of the light environment.

The phycobilisome, which contains the phycocyanin, is known to function as a light-harvesting antenna for PSII while most of the Chl *a* deliver its light energy to PSI (23). When shifted to LR, PSI receives relatively more energy than PSII, because Chl *a* absorbs LR more effectively than does phycocyanin. The strategy to increase the phycocyanin content relatively more than Chl *a* under long wavelength red condition (14, 18) will enable the plants to reach an improved excitation balance between the two photosystems, *i.e.* relatively more excitation energy is transferred to the rate limiting PSII, thereby improving the quantum yield of photosynthesis under far red light conditions.

Myers *et al.* (15) have shown that the ratios of Chl *a* to reaction centers within each photosystem remained remarkably constant, around 118 in PSI and 52 in PSII, in *A. nidulans* whether they were grown under gold fluorescent or far red (>650 nm) light. At the same time the Chl *a* per cell ratio varied considerably. The approximate doubling of Chl *a* per cell after the LR shift (Fig. 5) is therefore thought to be paralleled by a concomitant increase of the number of reaction centers per cell, during the first 12 to 17 h after the shift. The termination of Chl *a* accumulation at about 17 h after the light shift is therefore interpreted to show that after this time, the number of reaction centers per cell, either P_{680} or P_{700} or both of them, have reached their maximum steady state levels and that no further changes in the ratios of P_{680} per cell or P_{700} per cell occur.

The two linker peptides of 33 and 30 kD in the phycobilisome, increased during the initial acclimation phase after the shift but after 15 to 18 h in LR they reached stable levels (Fig. 8). This indicates that the small phycobilisomes existing in HW did grow in size after transfer to LR conditions. This growth of the phycobilisome paralleled in time the first phase of phycocyanin accumulation per cell. The continued increase in the phycocyanin accumulation of cells during phase two must therefore be caused by an increase in the number of the phycobilisomes in the cell. If there was any increase in the number of phycobilisomes in the cells during phase one of phycocyanin, accumulation can not be evaluated from these experiments.

A model for the acclimation process from HW to LR condi-

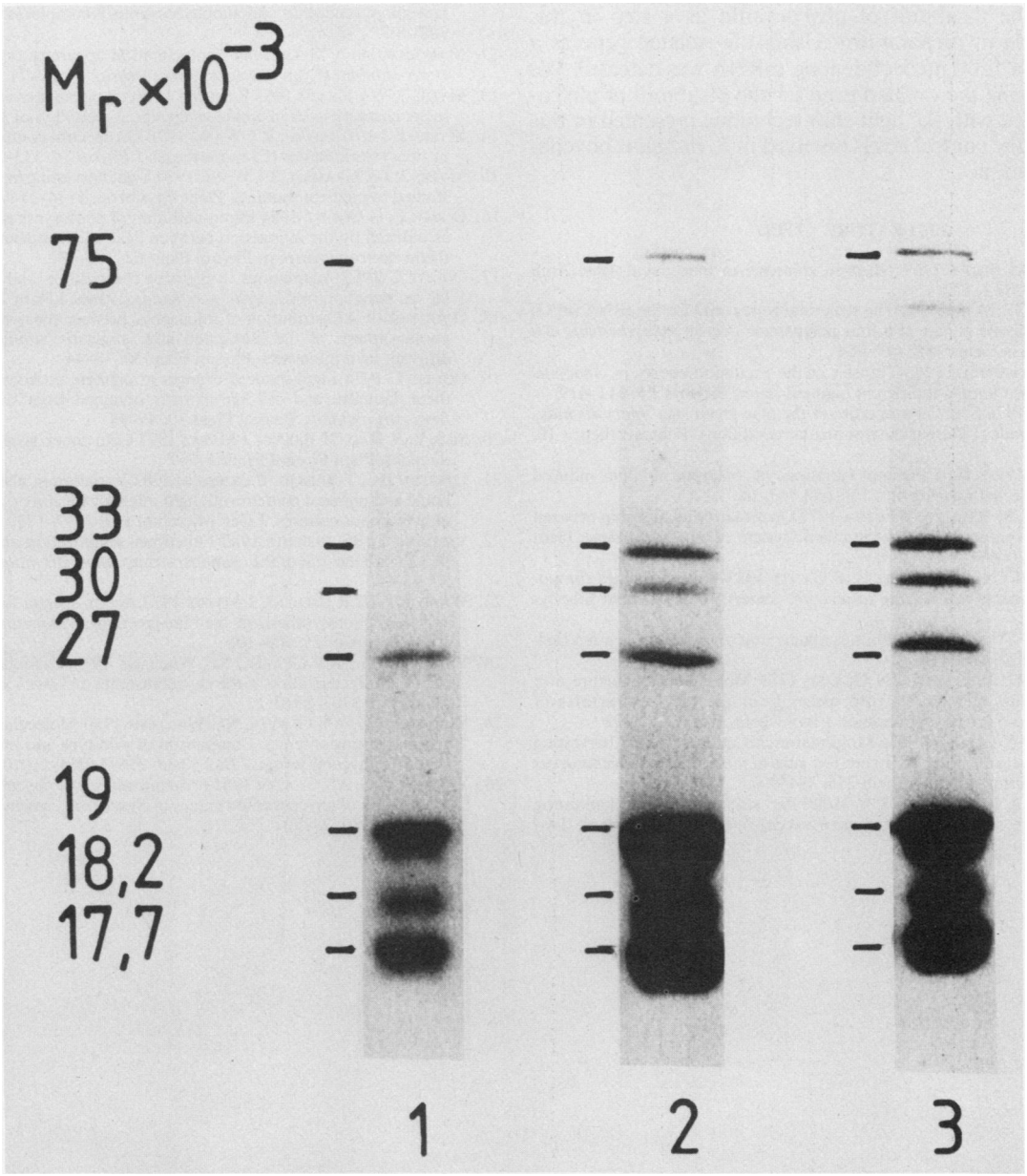

FIG. 7. Polypeptide composition of phycobilisomes from *A. nidulans*. Samples are taken just before and at different times after the shift from HW to LR conditions. The phycobilisomes in lane 1 are taken just before the shift; those in lane 2, 9 h after the shift; and those in lanes 3, 45 h after the shift. The peptides of 75 and 18.2 kD are peptides belonging to core, and the peptides of 33, 30, 27, and 19 kD are peptides belonging to the rods. The peptide band at 17.7 kD is a mixture of the α-subunit of phycocyanin and the β-subunit of allophycocyanin.

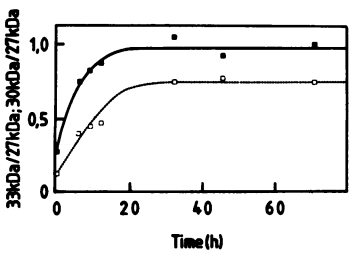

FIG. 8. Ratios between the absorbances of the Coomassie blue G-stained bands of the 33 kD and 27 kD linker peptides (■——■) and between the 30 kD and 27 kD linker peptides (□——□). The samples are taken just before and at different times after a shift from HW to LR conditions.

tions can be as follows. Immediately after the shift, the cells start producing more reaction center Chl *a* antennae of the two photosystems, as indicated by the increase in Chl *a*. At the same time, existing phycobilisomes start to extend in size by growth of the rods and new phycobilisomes are possibly synthesized. This acclimation period is completed within 12 to 17 h. After this period, synthesis of new phycobilisomes is the only acclimation process going on. These new phycobilisomes then occupy PSII reaction center antennae that previously had lacked phycobilisomes. The feasibility of such a mechanism is supported by the finding that *A. nidulans* may have PSII reaction centers that are not attached to phycobilisomes (8, 12).

This fact indicates that acclimation processes need an active control. One way to reveal the control mechanisms of light acclimation is to apply molecular genetics tools. We have isolated

the gene for the β-subunit of phycocyanin as a step in this direction (article in preparation). Using the isolated gene as a specific probe, a 1200 nucleotide long mRNA was detected. We are presently using the isolated gene for the β-subunit of phycocyanin combined with the light shift technique presented in this study to reveal the control levels involved in *A. nidulans* phycobilisome acclimation.

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