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Photo- and Metabolite Regulation of the Synthesis of Ribulose Bisphosphate Carboxylase/Oxygenase and the Phycobiliproteins in the Alga *Cyanidium caldarium*[1]

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ABSTRACT

In the eukaryotic and unicellular alga *Cyanidium caldarium* the synthesis of the plastid enzyme ribulose bisphosphate carboxylase/oxygenase (RuBPCase) and the light gathering proteins phycocyanin (PC) and allophycocyanin (APC) is under the control of light and glucose, which is a metabolizable carbon source for this organism. Light promotes the synthesis of these proteins while glucose has a strong inhibitory effect on this process. All subunits of the proteins mentioned above are *in vitro* translation products of poly (A)$^-$-RNA (Steinmüller, Kaling, Zetsche 1983 Planta 159: 308–313). Both factors—light and glucose—exert their effects mainly by modulation of the level of translatable messenger RNA for these proteins. Under autotrophic growth conditions the level of translatable RuBPCase-, PC-, and APC-messenger RNA is high, whereas in the presence of glucose the level of these mRNAs is low or not detectable at all.

Hitherto, the regulation of the synthesis of plastid proteins has mainly been studied in green algae and higher plants. For these plants it is now well established that the synthesis of plastid proteins is under the control of light and phytohormones and in the case of several algae under the control of a metabolizable carbon source in the culture medium (5, 8, 20, 27). The enzyme RuBPCase[3] is one of the most thoroughly studied plastid proteins in green algae and higher plants.

It is composed of eight large and eight small subunits. In green algae and higher plants the LSU is encoded by plastid DNA and synthesized on 70 S ribosomes, while the SSU is encoded in nuclear genes and is synthesized on the 80 S ribosomes of the cytoplasm as a precursor polypeptide (11, 13, 17, 22).

In a previous paper (25) we have demonstrated that in the unicellular eukaryotic alga *Cyanidium caldarium*, the SSU of RuBPCase is synthesized together with the LSU of the enzyme by poly(A)$^-$-RNA. Both subunits are synthesized in the size of the mature subunits. These findings suggest that the gene for the SSU of RuBPCase may be encoded in the plastid DNA of this alga.

The systematic position of *C. caldarium* is still uncertain. *Cyanidium* shares several properties with the unicellular red algae of the genus *Porphyridium* (10, 23, 26). But in contrast to red algae, *Cyanidium* can exist in a hot and acidic environment and furthermore, it is able to grow heterotrophically in the dark with glucose as carbon source. Under heterotrophic culture conditions the plastid is strongly reduced in size and thylakoid make up. Moreover, it completely loses its photosynthetic pigments Chl, PC, and APC (6, 12, 24). The subject of this paper is to investigate how light and glucose control the concentration of RuBPCase and the phycobiliproteins and their corresponding mRNAs.

MATERIALS AND METHODS

Culture of Algae. *Cyanidium caldarium* Geitler (Sammlung von Algenkulturen, Göttingen, FRG, strain 107.79, originally Allen's strain 14.1.1) was cultured as described previously (25) with the modification that the medium was supplemented with the micronutrients used by Ascione *et al.* (3) and with 1% soil extract. In the [^{35}S]sulfate labeling experiments, $(NH_4)_2SO_4$ was replaced by the same amount of NH_4Cl.

Isolation and Purification of RuBPCase and Phycobiliproteins. The subunits of RuBPCase, PC, and APC were purified as described by Steinmüller *et al.* (25). Separation of the proteins of the $(NH_4)_2SO_4$ precipitation (33–55% saturation) on DEAE-sephacel yields three protein peaks (Fig. 1). identified as Ru-

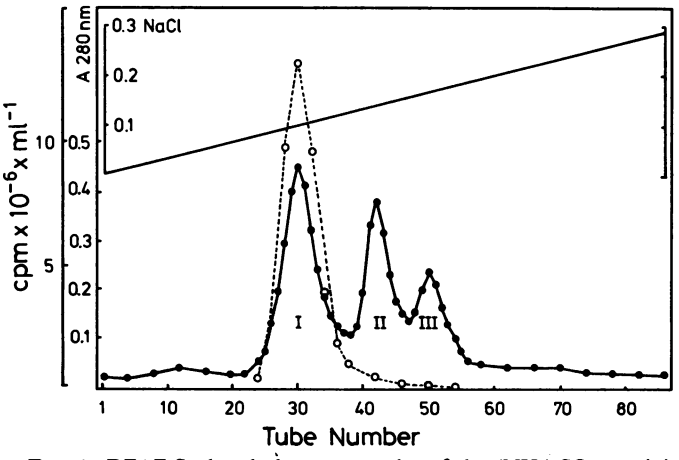

FIG. 1. DEAE-Sephacel chromatography of the $(NH_4)_2SO_4$ precipitate (33–55% saturation) of the homogenate supernatant. (●——●), *A* at 280 nm, (O --- O), activity of RuBPCase. Peak I, RuBPCase; peak II, PC; peak III, APC.

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[3] Abbreviations: RuBPCase, ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39); SSU, small subunit; LSU, large subunit; PC, phycocyanin; APC, allophycocyanin; RuBP, ribulose-1,5-bisphosphate.

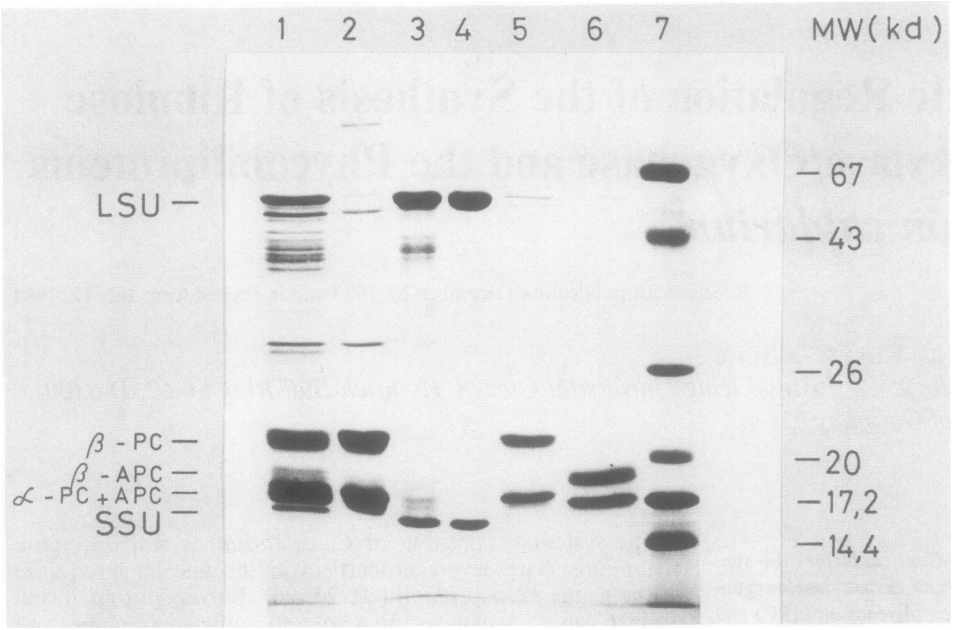

FIG. 2. Purification of RuBPCase, PC, and APC from autotrophically grown cells. SDS-polyacrylamide electrophoresis of various fractions of the proteins. 1, Proteins of the supernatant of the cell homogenate; 2, proteins precipitated between 0 and 33% $(NH_4)_2SO_4$; 3, proteins precipitated between 33 and 55% $(NH_4)_2SO_4$; 4, peak I fraction of the DEAE-sephacel chromatography; 5, peak II fraction of the DEAE-sephacel chromatography (PC); 6, peak III fraction of the DEAE-sephacel chromatography (APC); 7, standard proteins.

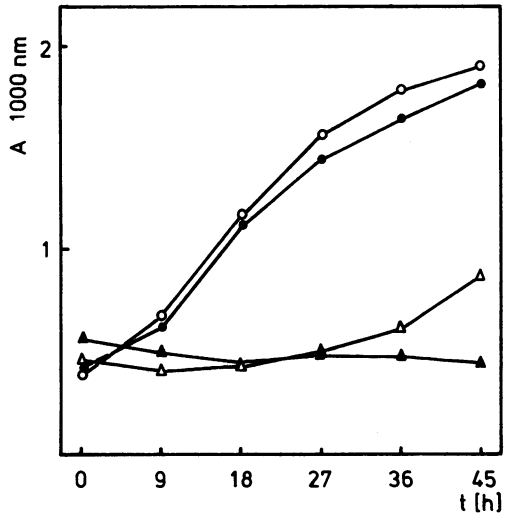

FIG. 3. Growth of *C. caldarium* cells under different culture conditions. Cells were heterotrophically pregrown and then transferred to the following conditions: (△), autotrophic; (●), mixotrophic; (○), heterotrophic; (▲), dark without glucose.

BPCase by measurement of the enzyme activity. The blue colored peaks II and III correspond to PC and APC, respectively, as determined from their absorption spectra (23). Yet the bulk of phycobiliproteins has already been removed with the first $(NH_4)_2SO_4$ precipitation (0–33% saturation) as is shown in Figure 2. The purity of the isolated proteins was examined by SDS-electrophoresis on 12.5% polyacrylamide slab gels (Fig. 2). Mol wt determinations reveal 55 kD for the LSU and 15.5 kD for the SSU of RuBPCase. These values are in good agreement with the results of Ford (14). PC and APC were also separated into two subunits with the following mol wt: α-PC, 16.6 kD; β-PC, 20.2 kD; α-APC, 16 kD; and β-APC, 17.8 kD. Antibodies against each subunit were raised in rabbits using standard procedures. Antisera were used without any further purification. Identification of the subunits was performed with the corresponding antibodies (25, 27).

Determination of RuBPCase Activity and Concentration. Activity of the enzyme was measured by incorporation of $^{14}CO_2$ in acid stable products. The test system consisted of 200 μl 50 mM

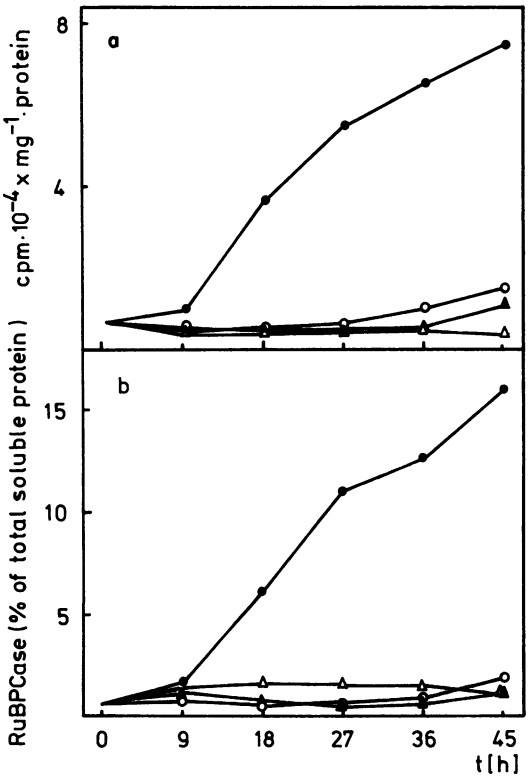

FIG. 4. RuBPCase activity (A) and concentration (B) of cells grown under different conditions: (●), autotrophic; (○), mixotrophic; (▲), heterotrophic; (△), dark without glucose.

Tris-HCl-buffer (pH 7.8), 15 mM $MgCl_2$, 2 mM DTE, 5 μl $NaH^{14}CO_3$, and 50 μl enzyme extract. The reaction was started by addition of 10 μl 5 mM RuBP. The reaction mixture was incubated at 35°C for 10 min. The reaction was stopped by the addition of 200 μl CH_3COOH. Aliquots of 100 μl were removed and pipetted on paper disks. The disks were dried and counted in a Beckman L 7500 scintillation counter using 3 ml Quickszint L 501 (Zinsser, Frankfurt, FRG) as scintillator. RuBPCase protein was determined by the single radial immunodiffusion method of Mancini *et al.* (19). Other protein determinations were performed according to Bradford (9).

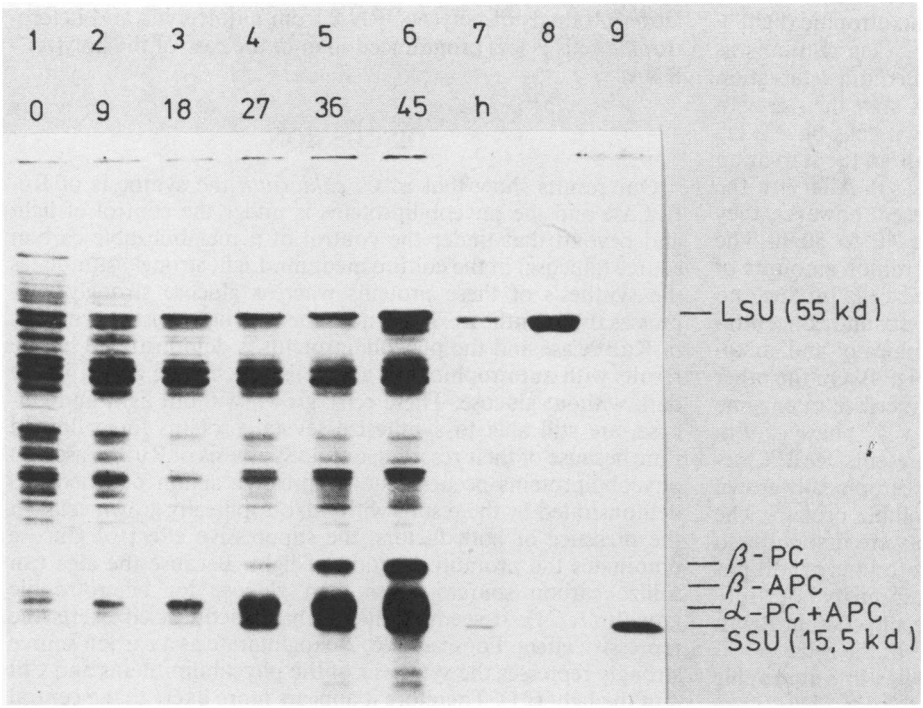

FIG. 5. Changes in protein pattern after the transfer of cells from heterotrophic to autotrophic culture conditions. The soluble proteins were separated by SDS-polyacrylamide electrophoresis. Lane 1–6 from left to right 0, 9, 18, 27, 36, 45 h after transfer of the cells; 7 purified RuBPCase, 8, 9 purified large and small subunits of RuBPCase. The same amount of proteins (60 μg) was applied to each slot (1–6). Staining was with Coomassie blue.

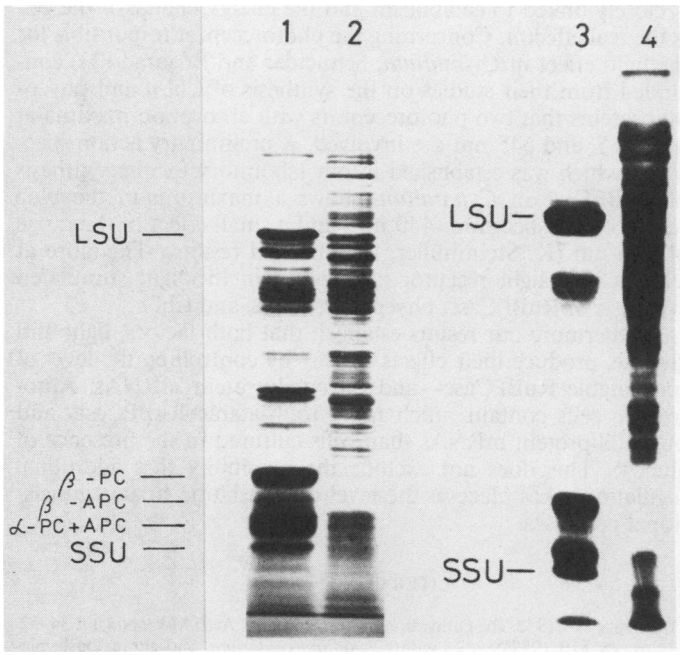

FIG. 6. ^{35}S-Labeling of proteins in autotrophic and heterotrophic cells. Cells were harvested in the logarithmic growth phase, and then suspended in a sulfate-poor medium to which [^{35}S]sulfate (4 μCi/ml) was added. The cells were labeled for 2 h. The soluble proteins were separated by SDS-PAGE and stained with Coomassie blue or fluorographed. Lanes 1 and 2 proteins of autotrophic and heterotrophic cells, respectively, stained with Coomassie blue; lanes 3 and 4 fluorographs of the proteins of autotrophic and heterotrophic cells, respectively.

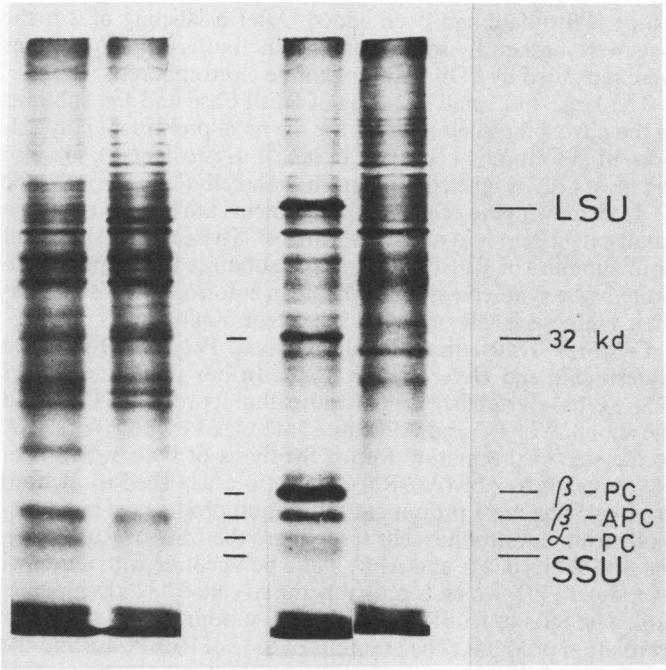

FIG. 7. Translation products (TP) of poly(A)$^+$- and poly(A)$^-$-RNA of autotrophic and heterotrophic cells. 1, TP of poly(A)$^+$-RNA from autotrophic cells; 2, TP of poly(A)$^+$-RNA from heterotrophic cells; 3, TP of poly(A)$^-$-RNA from autotrophic cells; 4, TP of poly(A)$^-$-RNA from heterotrophic cells.

SDS-PAGE. Electrophoretic separation of polypeptides was carried out as described by Laemmli (18). Labeled proteins were localized by fluorography according to Bonner and Laskey (7).

RNA Isolation and Translation. RNA was isolated by the phenol/chloroform/isoamyl alcohol method. The isolated RNA was separated into poly(A)$^+$- and poly(A)$^-$-RNA by chromatography on oligo (dT)-cellulose. The RNA-fractions were translated in a nuclease-treated rabbit reticulocyte-lysate system. Details of the procedures are as reported by Steinmüller *et al.* (25).

Chemicals. $NaH^{14}CO_3$ (2.19 GBq/mmol), [^{35}S]sulfate, carrier free (>185 GBq/mg S), and [^{35}S]methionine (>22 TBq/mmol) were purchased from Amersham Buchler, Braunschweig, FRG. RuBP came from Sigma.

RESULTS

Activity and Concentration of RuBPCase and Phycobiliproteins under Different Growth Conditions. Heterotrophically pre-

grown cells were placed under autotrophic, mixotrophic (light + glucose), or heterotrophic growth conditions. One culture was placed in the dark without glucose in the medium (starvation culture). Mixotrophic and heterotrophic cells start their growth without any delay while autotrophically grown cells have a lag period of 10 to 15 h before they resume growth. In the starvation culture the cell number decreases slowly (Fig. 3). After 9 h the autotrophically grown cells look markedly green; however, they obtain their full blue-green color only after 40 to 50 h. The mixotrophically grown cells synthesize only minor amounts of pigments. Heterotrophically grown or starved cells produce no detectable photosynthetic pigments. The activity and concentration of RuBPCase is very low in heterotrophically and mixotrophically grown cells and in starved cells (Fig. 4). On the other hand, in autotrophically grown cells a large increase in enzyme activity and concentration takes place after a lag phase of 9 h. After 45 h, 16% of the soluble protein represents RuBPCase-protein, whereas in heterotrophically and mixotrophically grown cells the enzymes achieve only 1% of the soluble protein. The changes in the protein pattern of cells which were first cultured heterotrophically and then transferred to autotrophic conditions are shown in Figure 5. Some polypeptides, especially both subunits of RuBPCase and the subunits of the phycobiliproteins, increase markedly during autotrophic growth of the cells.

Synthesis of RuBPCase and Phycobiliproteins in Autotrophic and Heterotrophic Cells. Cells growing in the log-phase were harvested and then resuspended in a sulfate-poor medium to which [^{35}S]sulfate had been added. After a labeling of 2 h the cells were broken by sonication and the buffer-soluble proteins were separated by SDS-polyacrylamide electrophoresis.

The large and small subunits of RuBPCase and the subunits of the phycobiliproteins, which are the most prominent polypeptides in autotrophic cells (Fig. 6, lane 1 *versus* lane 2), are also the most heavily labeled proteins in these cells (lane 3 *versus* lane 4). In heterotrophic cells the same proteins are labeled only to a small extent or not at all (lanes 3 and 4). These results show that both subunits of RuBPCase and the subunits of the phycobiliproteins are synthesized at a high rate in autotrophic cells whereas their synthesis is low or absent in heterotrophic cells.

Cell-Free Translation of Poly(A)$^+$- and Poly(A)$^-$-RNA from Autotrophic and Heterotrophic Cells. In our preceding studies (25) we have established that both subunits of RuBPCase and the subunits of PC and APC are synthesized by poly(A)$^-$-RNA in the size of the mature forms. Synthesis of both subunits of phycocyanin by poly(A)$^-$-RNA was also established by Belford *et al.* (4). The most prominent translation products of poly(A)$^-$-RNA from autotrophic cells (Fig. 7) are the LSU of RuBPCase, the β-subunits of PC and APC, and a polypeptide with a mol wt of about 32 kD, which is probably the so called "32 kD protein" (16). The SSU of RuBPCase and the α-subunit of PC are minor translation products. The amounts of SSU of RuBPCase and the α-subunit of PC change with different RNA preparations. This may be the consequence of the several washings of the isolated RNA which are necessary in order to obtain translatable RNA preparations (25). The subunits of RuBPCase, PC, and APC were identified with the help of specific antibodies as described (25). We were not successful in precipitating the α-subunit of APC, probably because the amount of this subunit is too small for detection. No immunoprecipitation of translation products of poly(A)$^+$-RNA with antibodies against the subunits of RuBPCase, PC, and APC were detectable. Translation products of poly(A)$^+$-RNA in the region of the subunits of RuBPCase, PC, and APC are therefore other polypeptides of unknown function.

A comparison of the translation products of poly(A)$^-$-RNA from autotrophic and heterotrophic cells demonstrates the absence of the subunits of RuBPCase, PC, and APC among the products of heterotrophic cells. The difference between the translation products of poly(A)$^+$-RNA from autotrophic and heterotrophic cells is less pronounced than in the case of the poly(A)$^-$-RNA.

DISCUSSION

Our results show that in *C. caldarium* the synthesis of RuBPCase and the phycobiliproteins is under the control of light and beyond that under the control of a metabolizable carbon source (glucose) in the culture medium. Light strongly stimulates the synthesis of these proteins whereas glucose strongly suppresses their synthesis. The requirement of light for the synthesis of RuBPCase and the phycobiliproteins is demonstrated by the results with autotrophic cells and cells which were grown in the dark without glucose. These cells, grown without light and glucose, are still able to synthesize several proteins for a limited time because of their reserves, but no synthesis of RuBPCase and phycobiliproteins occurs. The suppressive action of glucose is demonstrated by the results with mixotrophically grown cells. In the presence of both factors, the suppressive effect of glucose dominates the promotive action of light. Because the alga can utilize carbon sources other than glucose for heterotrophic growth (1, 21), it seems unlikely that glucose itself exerts the repressive effect. For example, 2-oxoglutarate as a carbon source strongly represses the synthesis of the phycobiliproteins and Chl *a* in the light (21). Therefore it appears more likely that a central metabolite of catabolic metabolism or a signal substance which is closely linked to catabolism and the energy charge of the cell is the real effector. Concerning the photoreceptor responsible for the light effect in *Cyanidium*, Schneider and Bogorad (23) concluded from their studies on the synthesis of Chl *a* and phycobiliproteins that two photoreceptors with absorption maxima at 440, 575, and 645 nm are involved. A preliminary action spectrum, which was established in our laboratory for the synthesis of RuBPCase in *Cyanidium*, shows a maximum in the blue region of the spectrum (440 nm) and a small effect in the range of 580 nm (K. Steinmüller, unpublished results). Therefore at least, a blue light receptor is involved in the light stimulated synthesis of RuBPCase, phycobiliproteins, and Chl *a*.

Furthermore our results establish that both factors, light and glucose, produce their effects mainly by controlling the level of translatable RuBPCase—and phycobiliprotein mRNAs. Autotrophic cells contain much more translatable RuBPCase- and phycobiliprotein mRNAs than cells cultured in the presence of glucose. This does not exclude the possibility that additional regulation takes place at the level of translation or posttranslational processes.

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