

Regulation of the Synthesis of Ribulose-1,5-bisphosphate Carboxylase and Its Subunits in the Flagellate *Chlorogonium elongatum*.

II. Coordinated Synthesis of the Large and Small Subunits

Peter Westhoff, Kurt Zimmermann, Frank Boege, and Klaus Zetsche

Institut für Allgemeine Botanik und Pflanzenphysiologie der Justus-Liebig-Universität Giessen, D-6300 Giessen, Bundesrepublik Deutschland

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Transfer of heterotrophically grown cells of the unicellular green alga *Chlorogonium elongatum* to autotrophic growth conditions causes a 10–15 fold increase in the amount of the chloroplastic enzyme ribulose-1,5-bisphosphate carboxylase.

This increase was found to be due to *de novo* synthesis. The relative proportions of large and small subunits of the enzyme do not change. Their ratio is close to 3.4, the proportions in weight of the two subunits in the holoenzyme. Continuous labelling with [³⁵S]sulfate reveals that the ratios of incorporation into large and small subunits are essentially the same in autotrophic and heterotrophic cells.

Pulse-chase experiments show that the subunits are degraded synchronously.

The coordinated subunit synthesis cannot be uncoupled using inhibitors of protein and RNA synthesis or high temperature of cultivation of the alga.

The results suggest a very tightly coordinated synthesis of the large and small subunits of ribulosebisphosphate carboxylase.

Introduction

Ribulose-1,5-bisphosphate carboxylase (RuBPCase) is a chloroplastic protein which is composed of eight large and eight small subunits [1], which are encoded by two different genomes [2, 3]. Therefore this protein is a useful model for studying the molecular events by which nucleocytoplasmic and chloroplastic systems interact.

Concerning the question how the synthesis of large and small subunits is regulated, conflicting results are presented in the literature. In the unicellular green alga *Chlamydomonas* [4], in spinach leaf protoplasts [5], and in greening pea shoots [6] synthesis of large and small subunits is tightly coordinated. But several reports indicate, that in other plants the synthesis of both subunits might be not strictly coupled [7–10]; these investigators de-

tected pools of free subunits not assembled to the holoenzyme.

In the preceding paper [11] we have shown that in the phytoflagellate *Chlorogonium elongatum* the level of RuBPCase protein increases considerably when the alga is shifted from heterotrophic to autotrophic culture conditions. This rise in enzyme amount is caused by increased *de novo* synthesis of RuBPCase protein under autotrophic culture conditions and not by a reduced rate of degradation [11]. In this paper we demonstrate that the synthesis of the large and small subunits of RuBPCase is strictly coordinated and cannot be uncoupled by specific inhibitors of protein and RNA synthesis.

Materials and Methods

Materials

[³⁵S]sulfate (carrier-free) was purchased from Amersham Buchler (Braunschweig, Germany). *p*-aminobenzyl-cellulose (PAB-cellulose) was from Serva (Heidelberg, Germany). All other reagents were of analytical grade and were obtained from various commercial sources.

Abbreviations: RuBPCase, ribulose-1,5-bisphosphate carboxylase/oxygenase (EC.4.1.1.39); LS, large subunit of RuBPCase; SS, small subunit of RuBPCase; TCA, trichloroacetic acid; SDS, sodium dodecyl sulfate; PAB cellulose, *p*-aminobenzyl cellulose.

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Purification of the large and small subunits of RuBPCase and preparation of monospecific antibodies to LS and SS

RuBPCase of *Chlorogonium* was isolated by affinity chromatography of crude extracts on Sepharose-bound antibodies to tobacco RuBPCase [11]. In addition to the published procedure [11] 0.5% (v/v) Nonidet P-40 was included into the wash buffer.

The subunits were purified by SDS-gel electrophoresis on 12.5% slab gels (300 × 270 × 6 mm) [12]. Protein bands were localized by staining small longitudinal gel strips with Coomassie brilliant blue or by the technique of Higgins and Dahmus [13]. The protein was diffusion-eluted, and SDS removed according to Weber and Kuter [14]. The subunits were precipitated by dialyzing the Dowex-eluate exhaustively against water. The subunits were solubilized in 1% (w/v) SDS, 0.15 M NaCl and 5% (v/v) 2-mercaptoethanol and stored at -20 °C.

Antibodies to LS and SS were raised in rabbits using standard procedures. The final concentration of SDS in the antigen preparation was 0.5%. IgG fractions from the rabbit sera were prepared by 18% Na₂SO₄ precipitation.

Ouchterlony assays

Double immunodiffusion assays of the SDS-solubilized subunits were performed in an agarose mixture containing 0.1% (w/v) SDS and 1% (v/v) Triton X-100 [15]. Thus precipitation artifacts caused by SDS could be avoided.

Quantitative determination of RuBPCase holoenzyme and its subunits in crude extracts

The concentrations of RuBPCase holoenzyme and its subunits in crude extracts were estimated by single radial immunodiffusion [16].

Immunoprecipitation of labelled RuBPCase by PAB-cellulose-linked antibodies

Antibodies to RuBPCase holoenzyme (IgG fraction by 18% (w/v) Na₂SO₄ precipitation) were linked to PAB cellulose as described by Schütz *et al.* [17].

Crude extracts were treated with sufficient amounts of anti-RuBPCase-PAB cellulose. After 1 h of incubation the anti-RuBPCase-PAB cellulose was collected by centrifugation (7 min, 10 000 × g),

washed five times with 15 ml of 50 mM Tris-HCl, pH 7.8, 1 M NaCl, 1% (v/v) Nonidet P-40 and once with 125 mM Tris-HCl, pH 6.8. Specifically bound RuBPCase protein was eluted with 200 µl of 125 mM Tris-HCl, pH 6.8, 3% (w/v) SDS. As judged from the Coomassie brilliant blue staining and fluorography [18] this immunoprecipitation method gave a homogenous RuBPCase preparation (Fig. 1).

Anti-RuBPCase-PAB cellulose could be regenerated by washing three times with 125 mM Tris-HCl, pH 6.8, 3% (w/v) SDS, twice with 50 mM Tris-HCl, pH 7.8, 1 M NaCl, 1% (v/v) Nonidet P-40 and four times with 50 mM Tris-HCl, pH 7.8, 200 mM NaCl. In the last buffer (+ 0.02% (w/v) NaN₃) the anti-RuBPCase-PAB cellulose was stored at 4 °C. The SDS treatment however led to a loss of binding capacity. Therefore the completeness of immunoadsorption was routinely checked by testing the supernatant of the anti-RuBPCase-PAB cellulose treated crude extract with single radial immunodiffusion assay.

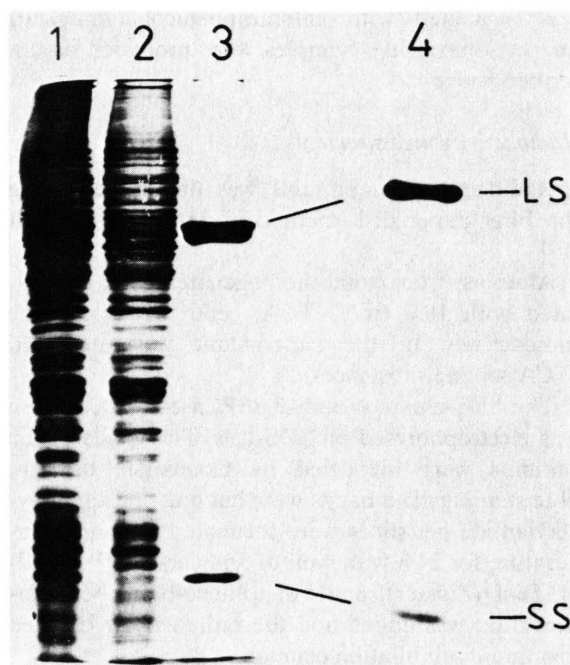


Fig. 1. Immunoprecipitation of RuBPCase with anti-RuBPCase PAB-cellulose. Samples were analysed on 12.5% SDS-slab gels [12]. 1. crude extract of *Chlorogonium* cells, 2. crude extract after immunoprecipitation, 3. polypeptides released from anti-RuBPCase PAB-cellulose by SDS treatment, 4. fluorograph of ³⁵S-labelled RuBPCase, immunoprecipitated with anti-RuBPCase PAB-cellulose.

SDS-Gel electrophoresis

Polyacrylamide gel electrophoresis in the presence of SDS was performed according to Laemmli [12].

Labelling of cells with [35 S]sulfate

In both kinds of experiments – continuous labelling and pulse-chase experiments – cells were preincubated in sulfate-free culture medium for 45 min.

Continuous labelling was performed with 2–10 μ Ci [35 S]sulfate per ml of culture. At each time 25 ml of culture were withdrawn and the incorporation was stopped by adding ice to the samples. The cells were spun down, washed once with sulfate-enriched (2.225 mM) culture medium and immediately stored at -20°C .

In the pulse-chase experiments cells were pulse-labelled for 15 min with 1.25 μ Ci [35 S]sulfate per ml of culture. Then the cells were harvested, washed once with sulfate-enriched (2.225 mM) culture medium and resuspended in the same medium. One part of the autotrophic and heterotrophic culture was incubated with chloramphenicol (70 $\mu\text{g}/\text{ml}$) and cycloheximide. Samples were processed as described above.

Radioactivity measurements

Radioactivity in proteins was determined using the filter paper disk method of Mans and Novelli [19].

Aliquots from total homogenate were precipitated with 10% (w/v) TCA, centrifuged, and the radioactivity in the supernatant was measured (TCA soluble substances).

The SDS-eluate of anti-RuBPCase-PAB cellulose was electrophoresed on SDS-gels. The bands of the subunits were identified by Coomassie brilliant blue staining. The bands were cut out, and the polyacrylamide gel slices were incubated at room temperature for 24 h with 1 ml of Soluene-350 (Packard) or TS-1 (Zinsser). 8 mls of toluene-based scintillation fluid was added and the radioactivity counted in a liquid scintillation counter.

Determination of protein

Protein in solutes containing detergents or urea was determined by the procedure of Schaffner and Weissmann [20]. In all other cases Esen's method

[21] was used. Handling of cells and the preparation of crude extracts were as described in the preceding paper [11].

Results

Characterization of the antisera

The antisera to LS and SS gave single precipitin lines when tested with their respective isolated antigen as well as with SDS-containing crude extracts of *Chlorogonium* cells (Fig. 2). The two precipitin lines were confluent indicating immunological identity of the antigens. 2-Mercaptoethanol had to be included into the subunit preparation; otherwise a doublet of precipitin lines was observed (Fig. 2). The faint of the two bands has to be considered as a result of aggregating subunits. No cross-reaction of the antisera with the other antigen was observed. It should be pointed out, that preimmune IgG fractions had no reactions with the antigens. No precipitin lines were detected between the antibodies and SDS alone (results not shown).

Taken together the antibodies to LS and SS formed precipitin lines with the SDS-denatured subunits (Fig. 2), with native subunits present in the holoenzyme (data not shown) and with the *in vitro* synthesized subunit polypeptides Westhoff *et al.* [32]. The same held true for antibodies to RuBPCase holoenzyme, except that immunoprecipitation of *in vitro* synthesized LS- and SS-polypeptides with antiserum to RuBPCase holoenzyme was not tested.

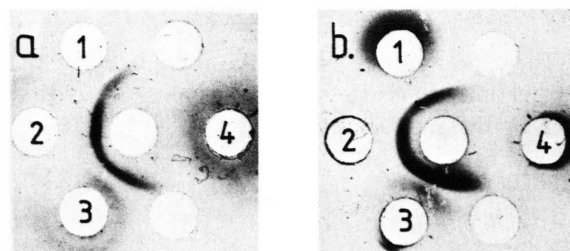


Fig. 2. Ouchterlony assays for specificity of the antisera to large and small subunits of RuBPCase. a. Antiserum to SS in the central well. 1. crude extract of *Chlorogonium* cells (0.1% SDS, 5% 2-mercaptoethanol), 2. 1.8 μg SS in 0.1% SDS and 5% 2-mercaptoethanol, 3. 3.5 μg SS in 0.1% SDS, 4. 7.5 μg LS in 0.1% SDS. b. Antiserum to LS in the central well. 1. 3.8 μg LS in 0.1% SDS, 2. 3.8 μg LS in 0.1% SDS and 5% 2-mercaptoethanol, 3. crude extract of *Chlorogonium* cells (0.1% SDS and 5% 2-mercaptoethanol), 4. 1.8 μg SS in 0.1% SDS.

Coordinated synthesis of large and small subunits in autotrophic as well as in heterotrophic cells

Using monospecific antibodies to LS and SS the pools of the subunit could be measured quantitatively by single radial immunodiffusion [16]. The characterization of the antisera had shown that they react well with all kinds of subunits. This ensured that free occurring subunits, that are subunits not assembled to the holoenzyme were recognized too.

When heterotrophically grown cells were shifted to autotrophic growth conditions the amount of RuBPCase increased due to de novo synthesis [11]. If the synthesis of both subunits is synchronous under both culture conditions, the ratio of the levels of LS and SS should not change. The relative proportions of the subunits in the holoenzyme are close to the value of 3.4.

Transfer of the cells from heterotrophic to autotrophic growth did not lead to alterations in the relative proportions of both subunits (Fig. 3b). In this experiment cells were cultured heterotrophically

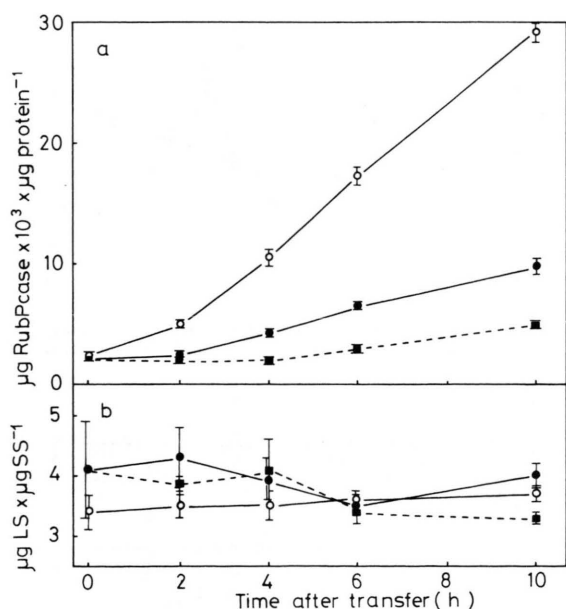


Fig. 3. Amounts of large and small subunits in cells growing under different conditions. Cells were precultured heterotrophically at 37 °C and then further cultured (start of experiment) autotrophically at 30 °C ○—○, in the dark with acetate at 30 °C ■—■ and in the dark without acetate at 30 °C ●—●. The amounts of RuBPCase holoenzyme and the subunits were estimated by single radial immunodiffusion [16]. a. Amount of RuBPCase holoenzyme, b. Ratio of large to small subunits.

at 37 °C instead of 30 °C. This higher growth temperature was used, since the differences in the levels of RuBPCase in autotrophic and heterotrophic cells could be enlarged from 5–7 to 10–15 fold.

When acetate was withdrawn in the dark synthesis of RuBPCase started immediately (Fig. 3a). Within the limitations of error – as outlined by the standard deviations – the ratio of the levels of LS and SS did not change (Fig. 3b). Taken together the relative proportions of both subunits were the same under all growth conditions tested, and the measured value was close to the expected one of 3.4. This suggested that synthesis of both subunits proceeds synchronously. Pools of free subunits were not detected. This finding was confirmed by labelling experiments. Autotrophic and heterotrophic cells were labelled continuously with [³⁵S]sulfate. Both cell types incorporated nearly the same amount of radioactive label into total soluble protein, but eight times more radioactivity could be detected in the RuBPCase of autotrophic cells [11]. The ratio of radioactivities in LS and SS, isolated from autotrophic and heterotrophic cells, is in the range of 3–4 (Table I). This value corresponds to the relative proportions of both subunits as measured by single radial immunodiffusion. It should be stressed that the large and small subunits of the RuBPCase of *Chlamydomonas*, a near relative of *Chlorogonium*, show a similar distribution of sulfur-containing amino acids [22]. The data suggested that the synthesis of large and small subunits is coupled under both growth conditions.

Pulse-chase experiments were next conducted to investigate directly the degradation rates of the

Table I. Synchronized synthesis of large and small subunits of RuBPCase in autotrophic and heterotrophic cells. Labelling of cells (10 µCi [³⁵S]sulfate/ml of culture) preparation of crude extracts, immunoprecipitation of RuBPCase protein and measurement of radioactivity in LS and SS was performed as described in Materials and Methods.

Time after addition of [³⁵ S]sulfate [min]	Ratio of radioactivity in LS and SS	
	autotrophic cells	heterotrophic cells
15	2.8	3.5
45	2.8	4.2
90	3.0	3.7
135	3.1	3.7
180	2.6	3.3

Table II. Synchronized degradation of large and small subunits of RuBPCase in autotrophic and heterotrophic cells. Labelling of cells ($1.25 \mu\text{Ci } [^{35}\text{S}]\text{sulfate/ml}$ of culture), preparation of crude extracts, immunoprecipitation of RuBPCase protein and measurement of radioactivity in LS and SS were performed as described in Materials and Methods.

Time after start of the chase [h]	Ratio of radioactivity in LS and SS			
	autotrophic cells		heterotrophic cells	
	– anti-biotics	+ CAP, + CHI	– anti-biotics	+ CAP, + CHI
0	0.6	0.6	1.3	1.3
2	0.6	0.7	1.6	1.4
4	0.6	0.8	1.7	1.3
6	0.6	0.7	1.5	1.1
8	0.7	0.7	1.5	1.6

subunits. If the subunits are not degraded synchronously, the ratio of their radioactivities should alter during the chase period. Since we did not succeed in preventing further incorporation of radioactivity out of the TCA-soluble pool into protein during the chase, one part of the autotrophic and heterotrophic cultures was incubated with cycloheximide and chloramphenicol. Thus further protein synthesis was stopped. The degradation rates of RuBPCase holoenzyme were found to be equal in both growth conditions, RuBPCase protein was degraded only very slowly [11]. The ratio of radioactivities in LS and SS in autotrophic as well as in heterotrophic cells remained nearly constant (Table II), *i.e.* the degradation of the subunits is coordinated too. It should be mentioned that the ratio of the radioactivities in LS and SS both in autotrophic and in heterotrophic cells did not reach the expected value of 3–4 (see Table I). Because of the low concentrations of $[^{35}\text{S}]\text{sulfate}$ used in this experiment the cytoplasmic compartment seems to be supplied with more $[^{35}\text{S}]\text{sulfate}$ than the chloroplast. The low concentration of $[^{35}\text{S}]\text{sulfate}$ was used in order to prevent the accumulation of too much radioactivity in the TCA soluble pool, which would interfere with the chase.

Control mechanisms in the biosynthesis of RuBPCase

Which of the two genetic systems – nucleus/cytoplasm and chloroplast – does control the synthesis of the large and small subunits? To get some informations about these regulatory processes we chose

the application of antibiotics and of elevated culture temperatures. Uncoupling of the synthesis of the subunits would indicate which of the two systems has the leadership in the biosynthesis of RuBPCase. As an experimental system the transition from heterotrophic to autotrophic growth was taken.

Effects of chloramphenicol and cycloheximide on the synthesis of large and small subunits

When the heterotrophically precultured cells were grown autotrophically in the presence of chloramphenicol or cycloheximide, inhibitors of chloroplastic and cytoplasmic protein synthesis respectively, no increase in the amount of RuBPCase could be detected (Fig. 4a). In the control culture the RuBPCase level rose more than tenfold. This inhibition of RuBPCase synthesis could not be attributed to nonspecific, toxic effects of the antibiotics because protein synthesis and cell division continued, although with obvious retardation (Table III). The relative proportions of large and small subunits were not affected by the two antibiotics,

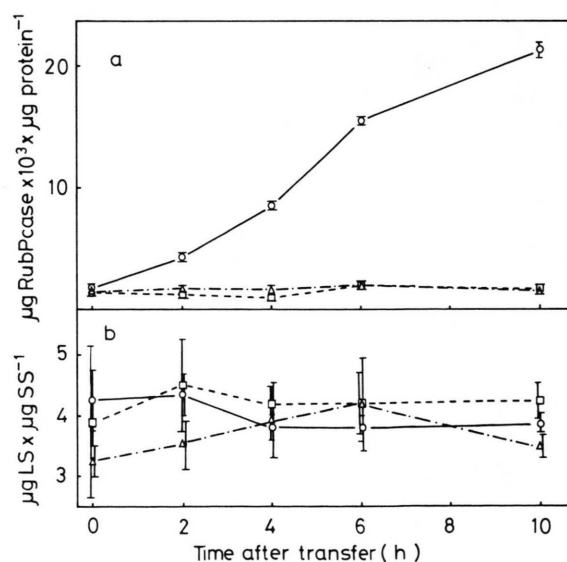


Fig. 4. Effects of chloramphenicol or cycloheximide on the amounts of large and small subunits. Cells were precultured heterotrophically at 37°C and then transferred to autotrophic conditions at 30°C . The amounts of RuBPCase holoenzyme and its subunits were estimated by single radial immunodiffusion [16]. Control $\bigcirc$ — $\bigcirc$, + chloramphenicol ($70 \mu\text{g/ml}$) $\triangle$ — $\triangle$, + cycloheximide ($30 \mu\text{g/ml}$) $\square$ — $\square$. a. Amount of RuBPCase holoenzyme, b. Ratio of large to small subunits.

Table III. Increase of protein and cell number after a 10 h-incubation with chloramphenicol (70 µg/ml) and cycloheximide (30 µg/ml) during photoautotrophic growth.

	Increase of total protein in µg per ml of culture	Increase of cell numbers cells per ml culture
control	32.5	0.72×10^6
+ chloramphenicol	21.0	0.52×10^6
control	36.0	1.15×10^6
+ cycloheximide	18.8	0.38×10^6

they did not differ significantly from the ratio measured in the control culture (Fig. 4b).

Effect of rifampicin on the synthesis of large and small subunits

Rifampicin has been found to be an efficient inhibitor of chloroplastic rRNA synthesis in *Chlorogonium* as in other algae [23]. This antibiotic led to a considerable reduction in the increase of RuBPCase amount, when heterotrophically grown cells were transferred to autotrophic growth conditions (Fig.

5a). The coupling of subunit synthesis was not affected (Fig. 5b).

Effect of high growth temperatures on the synthesis of large and small subunits

High temperatures had been shown to impair relatively specifically chloroplast formation in several higher plants and *Euglena*. Especially the synthesis of the large subunit of RuBPCase is prevented [8]. In *Chlorogonium* temperatures of about 37 °C led to a heavily decreased synthesis of chlorophyll in the light, when heterotrophically cultured cells were grown further on autotrophically at 37 °C instead of 30 °C – the usual growth temperature – (data not shown). Heterotrophic growth was not concerned negatively at 37 °C, and even more it was promoted. Synthesis of RuBPCase slowed down, but was not completely inhibited, when cells grown heterotrophically at 37 °C are transferred to autotrophic growth at the same temperature (Fig. 6a). The relative proportions of the subunits in the 37 °C-autotrophic culture did not differ from those in the control.

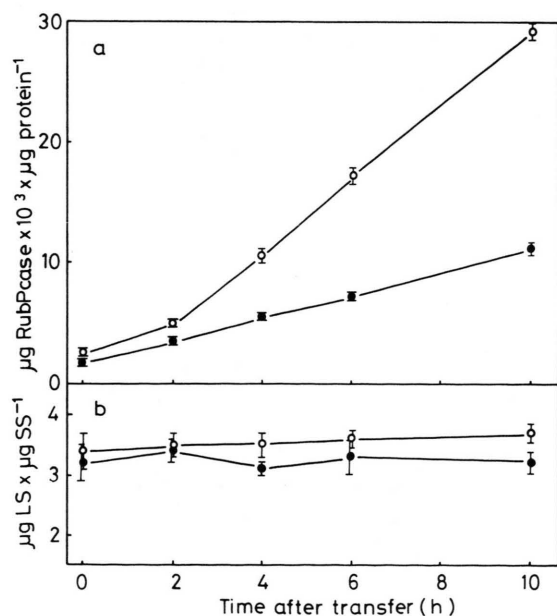


Fig. 5. Effect of rifampicin on the amounts of large and small subunits. Experimental details as described in legend to Fig. 4. Control ○—○, + rifampicin (125 µg/ml) ●—●. a. Amount of RuBPCase holoenzyme, b. Ratio of large to small subunits.

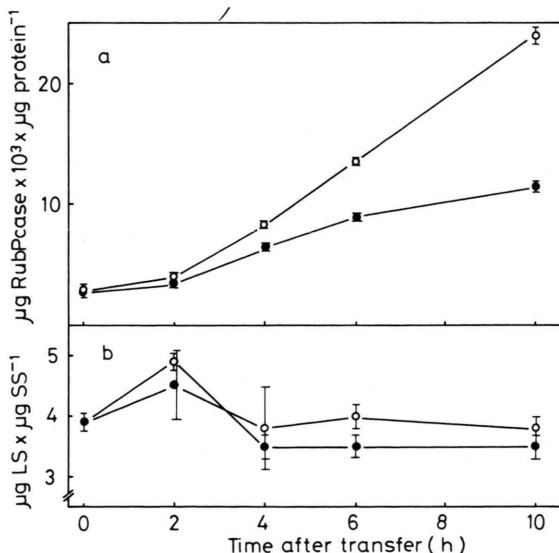


Fig. 6. Effect of high growth temperature on the amount of large and small subunits. Cells were precultured heterotrophically at 37 °C and then transferred to autotrophic growth conditions at 30 °C ○—○ or 37 °C ●—●. The amounts of RuBPCase holoenzyme and its subunits were estimated by single radial immunodiffusion [16]. a. Amount of RuBPCase holoenzyme, b. Ratio of large to small subunits.

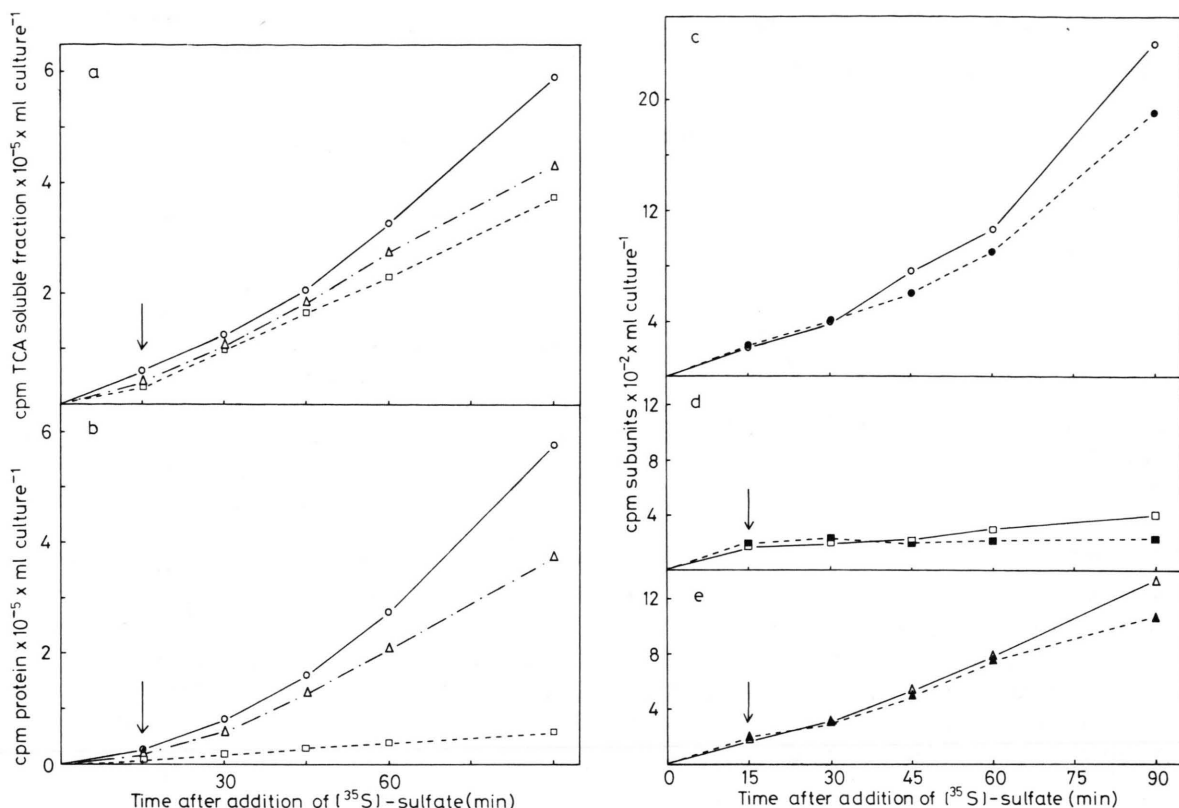


Fig. 7. Effects of cycloheximide and chloramphenicol on the incorporation of ^{35}S -radioactivity into the large and small subunits. Autotrophically grown cells were cultured further on in sulfate-free culture medium supplemented with ^{35}S -sulfate ($2 \mu\text{Ci/ml}$) and the antibiotics as indicated. For measurements of radioactivity and immunoprecipitation of RuBPCase protein see Materials and Methods. Control $\bigcirc$ — $\bigcirc$, + cycloheximide ($30 \mu\text{g/ml}$) $\square$ — $\square$, + chloramphenicol ($70 \mu\text{g/ml}$) $\triangle$ — $\triangle$. In Fig. 7. c-e: radioactivity in LS: open symbols, radioactivity in SS: closed symbols. The arrow indicates the addition of antibiotics. a. Radioactivity in the TCA-soluble pool. b. Radioactivity in total protein. c. Radioactivity in LS and SS, control. d. Radioactivity in LS and SS, + cycloheximide. e. Radioactivity in LS and SS, + chloramphenicol.

The effect of chloramphenicol and cycloheximide on incorporation of radioactivity into large and small subunits

In the case of the two inhibitors of protein synthesis the findings presented above were confirmed using an another approach. Cells were labelled with ^{35}S -sulfate in the presence of cycloheximide or chloramphenicol. Cycloheximide and chloramphenicol reduced the incorporation into total soluble protein to approximately 66% and 10% respectively as compared with the control (Fig. 7b). In the presence of antibiotics less radioactivity was found in the TCA-soluble pool (Fig. 7a). This seemed not to be significant, since in other experiments these differences in the radioactivities of the TCA-soluble

pools were not noticed. Addition of cycloheximide completely and immediately abolished incorporation into SS, but further incorporation into LS was stopped almost to the same extent too (Fig. 7d). Chloramphenicol in the concentration employed did not prevent further labelling of LS and SS totally, but considering the rate of the reduction in incorporation both subunits were affected in the same manner. Extending the labelling period to more than three hours led to the same results.

Discussion

There are five lines of evidence which suggest a stringent coordinated control of the synthesis of RuBPCase subunits in *Chlorogonium*:

(a) The relative proportions of LS and SS did not alter in autotrophic and heterotrophic growth conditions. The value of about 3–4 is in the range of the proportions in weight of LS and SS in the holoenzyme.

(b) The ratios of incorporation into the subunits were nearly the same for both growth conditions.

(c) The degradation rates were coordinated for both subunits.

(d) Inhibitors of protein and RNA synthesis and high growth temperature failed to uncouple subunit synthesis.

(e) No evidence was obtained for the existence of pools of free subunits.

The results fit well the findings obtained in another unicellular green alga, *Chlamydomonas* [4]. But the situation in higher plants is not homogeneous. In rye leaves deficiency of plastid ribosomes can be induced by high temperature [24]. Under these conditions large subunits of RuBPCase are not synthesized, but small subunits are formed and accumulated [8]. In isolated soybean leaf cells were found, that synthesis of large subunits continued, although small subunit synthesis had stopped [7]. Evidence for a pool of free small subunits was obtained also for tobacco protoplasts [9]. In contrast subunit synthesis in greening pea shoots [6] and spinach protoplasts [5] seems to be strictly coupled.

To value these findings, first the notions of “coupled and uncoupled subunit synthesis” are to be clarified. Coupled synthesis does not mean, that the amounts of both subunits match exactly, uncoupled synthesis does not imply a totally uncoordinated synthesis. Therefore it has to be investigated to which extent subunit synthesis is matched.

Unicellular green algae like *Chlorogonium* and *Chlamydomonas* have a closer coupling of subunit synthesis than higher plants. Two differences between higher plants and these two unicellular green algae are to be discussed. First these algae possess only one chloroplast, whereas higher plants are provided with several of these organelles per cell. Assuming the same copy number of DNA per plastid, the chloroplastic gene for the large subunit is much more amplified in cells of higher plants than in unicellular green algae. In contrast the nuclear gene for the small subunit exists only as single or close to single copy per genome [25]. Hence

it follows, that the dosage of large to small subunit genes differs between the two groups of plants. Second RuBPCase is the most abundant protein in higher plants comprising up to 50–60% of the total soluble protein. In *Chlorogonium* and *Chlamydomonas* 7 or 10% respectively of the total soluble protein consists of RuBPCase protein [22]. Therefore these differences make reasonable to assume that synthesis of small subunits in higher plants does not seem to be coupled so strictly as in unicellular green algae. A stock of free small subunits perhaps ensures, that these lots of plastids in cells of higher plants are provided with sufficient amounts of small subunits to build up RuBPCase holoenzyme.

To explain RuBPCase synthesis in *Chlorogonium* the model used has to bear in mind, that subunit synthesis in this alga is stringently coordinated and especially cannot be uncoupled by inhibitors of cytoplasmic and chloroplastic protein synthesis. Therefore Elli's proposal [27] of the cytoplasmic control principle does not fit our data. Our findings suggest that the two genetic compartments control one another. This requires a bilateral flow of information. Proteins or RNA are imaginable as transmitters of information. Transport of proteins from the cytoplasm into the chloroplast is well documented [28, 29], nothing is known about the reciprocal way. Transport of RNA from the chloroplast into the cytoplasm is a point of issue [30, 31], transport in the opposite direction has not been reported. It remains to examine whether one of the suggested possibilities is true.

At which level of gene expression synthesis of large and small subunits is controlled in *Chlorogonium*? Levels of translatable mRNAs for LS and SS under autotrophic and heterotrophic growth conditions have been estimated. The results indicate that the amounts of mRNAs for LS and SS are matched too (Westhoff and Zetsche, [32]). So further studies are necessary to elaborate a more sophisticated model of how synthesis of RuBPCase is controlled.

Acknowledgements

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