Plant Physiol. (1990) 92, 802–808
0032-0889/90/92/0802/07/$01.00/0

Received for publication August 29, 1989
and in revised form October 19, 1989

Immunogold Localization of Ribulose-1,5-Bisphosphate Carboxylase with Reference to Pyrenoid Morphology in Chloroplasts of Synchronized *Euglena gracilis* Cells[1]

Tetsuaki Osafune*, Akiho Yokota, Shuji Sumida, and Eiji Hase

Department of Microbiology, Tokyo Medical College, 6-1-1 Shinjuku, Tokyo 160 (T.O., S.S.); *Department of Agricultural Chemistry, University of Osaka Prefecture, Sakai, Osaka 591* (A.Y.); *and Laboratory of Chemistry, Faculty of Medicine, Teikyo University, Hachioji, Tokyo 192-03, Japan* (E.H.)

ABSTRACT

Euglena gracilis **strain (Z) cells were synchronized under photoautotrophic conditions using a 14 hour light:10 hour dark regimen. The cells grew during the light period (growth phase) and divided during the following 10 hour period either in the dark or in the light (division phase). Changes in morphology of the pyrenoid and in the distribution of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) within the chloroplasts were followed by immunoelectron microscopy during the growth and division phases of *Euglena* cells. Epon-embedded sections were labeled with an antibody to the holoenzyme followed by protein A-gold. The immunoreactive proteins were concentrated in the pyrenoid, and less densely distributed in the stroma during the growth phase. During the division phase, the pyrenoid could not be detected and the gold particles were dispersed throughout the stroma. Toward the end of the division phase, the pyrenoid began to form in the center of a chloroplast, and the immunoreactive proteins started to concentrate over that rudimentary pyrenoid. During the growth phase, small areas rich in gold particles, called 'satellite pyrenoid,' were observed, in addition to the main pyrenoid. From a comparison of photosynthetic CO_2-fixation with the total carboxylase activity of Rubisco extracted from *Euglena* cells in the growth phase, it is suggested that the carboxylase in the pyrenoid functions in CO_2-fixation in photosynthesis.**

The pyrenoid is a specialized region of the chloroplast of many algae, taking the form of a proteinaceous mass of material (5, 7, 8). Holdsworth (9) showed that pyrenoids isolated from the green alga *Eremosphaera viridis* had high specific activity of ribulose-1,5-bisphosphate carboxylase, and that two polypeptides of the same size as the two subunits of the carboxylase made up 90% of the total pyrenoid protein. Similar observations were made for pyrenoids isolated from the prasinophycean alga *Micromonas squamata* (26) and the brown alga (*Pilayella littoralis*) (11, 12). Satoh *et al.* (27) suggested that much of the Rubisco[2] of the green alga *Bryopsis maxima* is localized in the pyrenoid, and that 10 minor polypeptides comprised about 15% of the total pyrenoid protein. Recently, nitrate reductase has been found in the pyrenoids of several green algae (16).

Vladimirova *et al.* (28) studied the distribution of Rubisco in the chloroplasts of two green algae *Chlamydomonas reinhardtii* and *Dunaliella salina* by the immunofluorescence method, and observed an intense fluorescence over the pyrenoid. The distribution of Rubisco in the pyrenoid and thylakoid region of the chloroplast of *C. reinhardtii* was also studied by Lacoste-Royal and Gibbs (15) by immunoelectron microscopy. They reported that about 40% of the total small subunit in the plastid and 30% of the large subunit are localized in the thylakoid region, presumably in the stroma, the remaining portions existing in the pyrenoid. In a recent immunoelectron microscope study of *Chlorella pyrenoidosa* by McKay and Gibbs (21), it was shown that in either light-limited or light-saturated cells, the pyrenoid was heavily labeled by antibody to each subunit of Rubisco, whereas chloroplast stromal labeling was not above background levels, suggesting that the pyrenoid Rubisco is functional *in vivo*. Kuchitsu *et al.* (14) characterized polypeptides of the pyrenoid isolated from *C. reinhardtii*, and found that substantial amount of Rubisco protein is present as a particulate form in the pyrenoid.

Recent studies in our laboratory (25) showed that the pyrenoid is absent in proplastids of dark-grown cells of *Euglena gracilis*, and when these cells are transferred to an inorganic medium, rudimentary pyrenoids are formed in the dark in developing plastids, with fully developed pyrenoids only appearing after exposure of these cells to light. Cook *et al.* (4) reported that the pyrenoid is recognized in the chloroplasts of synchronized cells of *E. gracilis* only in the first half of the light period. Recently, Kiss *et al.* (13) showed by immunofluorescence method that the pyrenoid of *E. gracilis* is labeled by a Rubisco antibody, and that the presence and disappearance of the pyrenoid occurring under different nutritional conditions are related to the distribution of Rubisco in the chloroplast.

In the present study, we followed changes in morphology of the pyrenoid and distribution of Rubisco in the chloroplasts of synchronized cells of *E. gracilis* during the cell cycle by immunoelectron microscopy, and found that the appearance and disappearance of the pyrenoid is accompanied by concentration of Rubisco in the pyrenoid and its dispersion through-

[1] This work was aided by grants (Nos. 01540582 and 01304004, to T. O.) from the Ministry of Education, Science and Culture, Japan.

[2] Abbreviations: Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase.

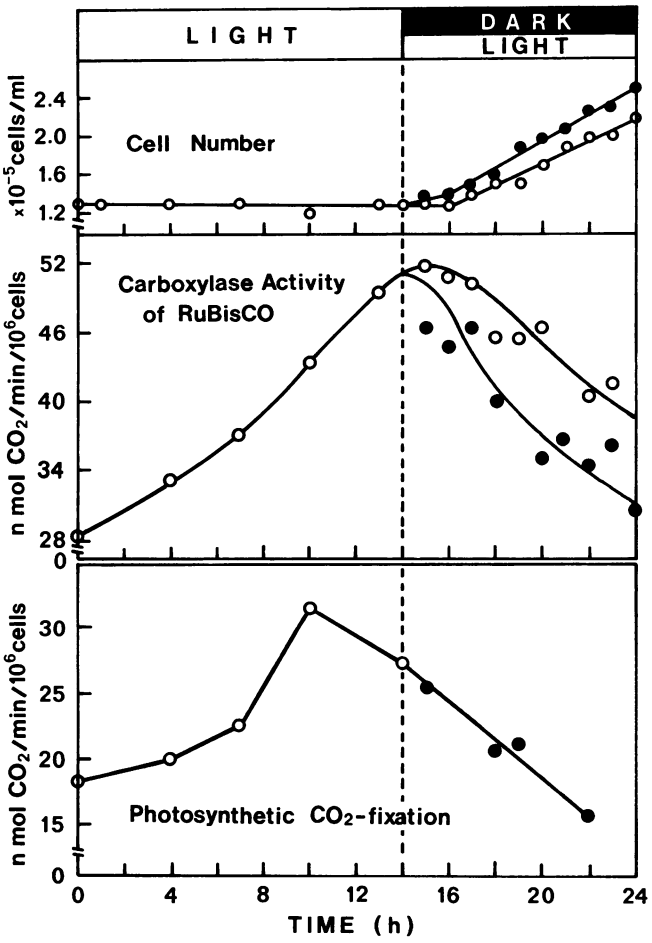


Figure 1. Time course of changes in photosynthetic CO_2-fixation and the carboxylase activity of Rubisco of *Euglena* cells during the cell cycle in synchronized cultures. Starting cells had been synchronized under the 14 h light:10 h dark regimen, and were placed under the same light:dark cycle (solid circles) as well as in continuous light (open circles) at 25°C. The same light intensity (6000 lux) was used in synchronized cultures and that in measurements of photosynthetic CO_2 fixation. The enzyme activity was measured at 30°C. See "Discussion" for the activity at 25°C. The experiment was repeated several times, and similar results were obtained.

out the stroma. Measurements of photosynthetic CO_2-fixation of cells and total activity of Rubisco extracted from those cells strongly suggest that the carboxylase in the pyrenoid functions in photosynthetic CO_2-fixation.

MATERIALS AND METHODS

Synchronized Culture

Euglena gracilis strain Z was obtained from the Algal Culture Collection of the Institute of Applied Microbiology, University of Tokyo (IAM Z-6), and has been maintained in Department of Microbiology, Tokyo Medical College. Cells were synchronized under a 14 h-light:10 h dark regimen at 25°C under photoautotrophic conditions. The cell suspension was aerated with 1.5% CO_2 in air. Details of the synchronized culture have been described previously (24). The light intensity was 6000 lux at the surface of the culture container in this work. In the experiments, the synchronized cells were placed under a 14 h-light:10 h dark cycle or in continuous light.

Measurements of Photosynthetic CO_2-Fixation

Two mL of cell culture taken at appropriate times were mixed with 2 mL of 100 mM (Hepes)-KOH buffer (pH 6.8) in a 5 mL vial and then aerated for 10 min. Photosynthetic CO_2 fixation was assayed in the presence of added 300 mM $NaH^{14}CO_3$ (520 dpm/nmol) under the same light and temperature conditions as in the cultivation of the cells. Part of the reaction mixture (1 mL) was withdrawn at appropriate times, and the reaction was stopped by adding 0.5 mL of 4 M formic acid. The acidified mixture was dried in an oven at 70°C. Acid, heat-stable ^{14}C was counted with a scintillation counter.

Extraction and Assay of Rubisco

Euglena cells were suspended in 25 mM Tris-HCl buffer (pH 7.8) containing 1 mM EDTA and 10 mM mercaptoethanol, and disintegrated by sonication at 0°C for 2 min. The homogenate was centrifuged at 10,000*g* for 10 min at 0°C. The carboxylase activity of Rubisco in the extract was measured as described in the previous paper (29).

Preparation of Antibody Against Rubisco

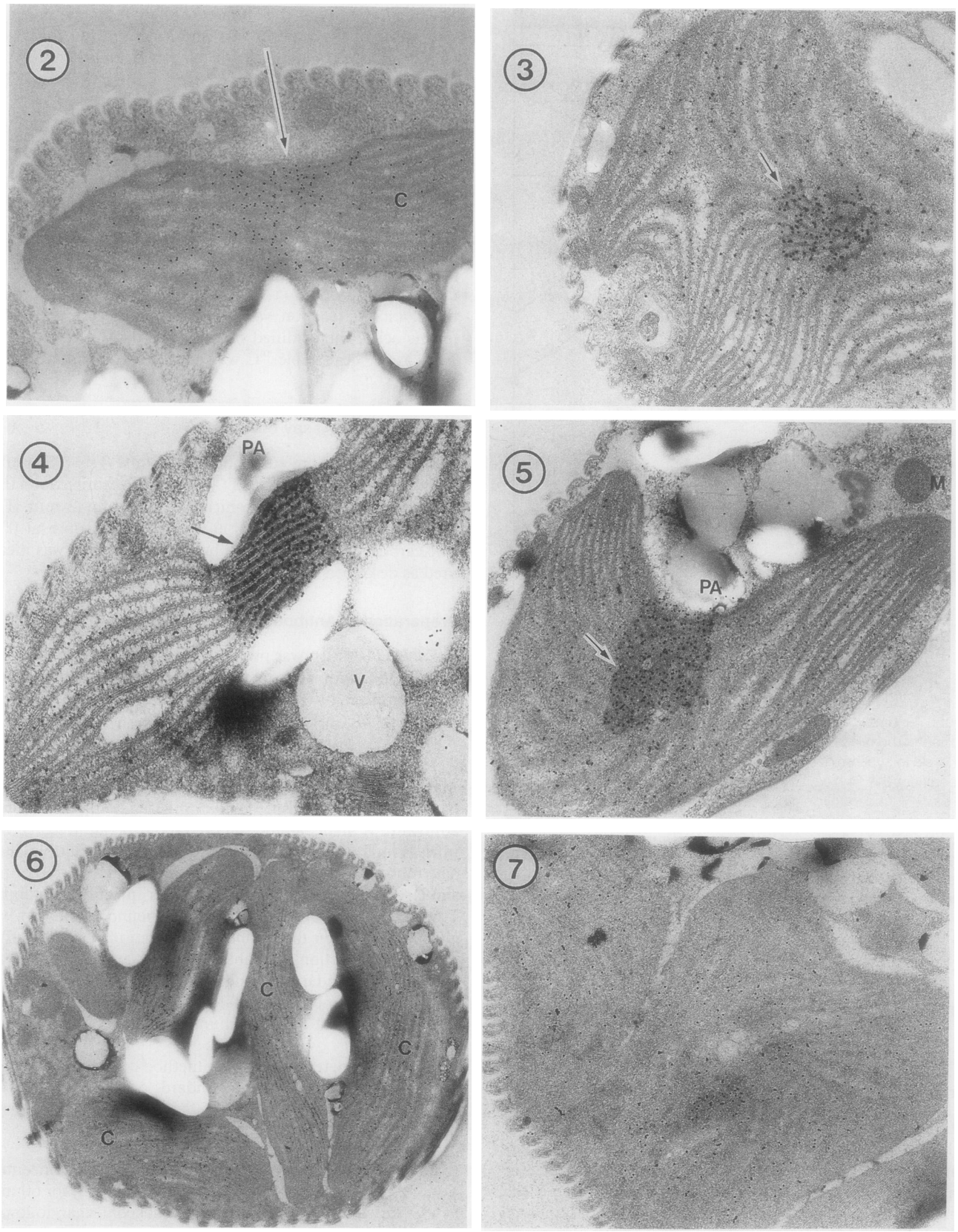
Euglena Rubisco was purified as described previously (30). Possible contaminants in the purified Rubisco preparation were removed by DEAE-cellulose chromatography by the method of McCurry *et al.* (20), and stored as ice beads of the polyethylene glycol-undissolved enzyme (30).

Three mg of purified Rubisco was dissolved in 1 mL of 10 mM potassium phosphate buffer (pH 7.4) containing 0.9% (w/v) NaCl, and emulsified with 1 mL of a complete Freunds adjuvant. A male albino rabbit (about 2 kg weight) was intradermally injected with the emulsion at multiple sites. The rabbit was again immunized with 3 mg of Rubisco in a similar way 3 weeks later, and a booster injection containing 1 mg of Rubisco and an incomplete Freund's adjuvant was given 3 weeks after the second immunization. Two days after the booster injection, the rabbit was bled, and the serum was prepared by standard methods. The γ-globulin fraction was isolated by ammonium sulfate fractionation, and dialyzed thoroughly against 10 mM sodium phosphate buffer (pH 7.4) containing 0.9% (w/v) NaCl.

Protein was determined by the method of Bradford (1) with bovine serum albumin as the standard.

Immunogold Electron Microscopy

Euglena cells were fixed in glutaraldehyde (final concentration 2%; w/v) in 0.01 M phosphate-buffer (pH 7.2) for 60 min at 4°C, rinsed in the same buffer and blocked in agar, followed by dehydration in ethanol series and then acetone. Samples were embedded in Epon-812 resin (Tabb Lab.). Thin sections were placed on nickel slit grids. These were floated section-



Figures 2–7. **Figure 2.** A cell at the beginning of the light period (0-h cell) labeled with anti-Rubisco followed by protein A-gold (×20,000). The arrow indicates the pyrenoid region, where gold particles are denser than in the rest of the chloroplast. C: chloroplast. In control cells treated with preimmune serum, the labeling with gold was insignificant in all immunogold experiments.

side down on drops of 0.01 M PBS with 0.5% BSA for 30 min at room temperature, and then incubated in PBS with 0.5% BSA containing antibody to Rubisco or in preimmune rabbit serum at 37°C for 10 min. The sections were washed twice in PBS, and incubated in PBS containing protein A-gold for 20 min. Sections were subsequently stained with uranyl acetate and viewed in a JEOL 100B electron microscope at 80 kV. In control experiments, cells were treated with preimmune serum prior to protein A-gold labeling.

RESULTS

Synchronized *Euglena* cells grew during the 14 h light period ('growth phase') and divided during the following 10 h period either in the dark or in the light ('division phase'), as seen from Figure 1 (upper panel). The cell division in the light was delayed by a few hours. The carboxylase activity of Rubisco extracted from these synchronized cells increased nearly twofold during the growth phase and decreased, in terms of the activity per cells, during the division phase (Fig. 1, middle panel). As seen in Figure 1 (lower panel), the rate of photosynthetic CO_2-fixation by the cells increased during the initial several hours in the light, attaining the maximum level around 10 h and gradually decreased thereafter even in the light.

As seen from Figures 2 and 3, the chloroplasts of the cells at the beginning of the light period (0-h cells) contained rudimentary pyrenoids, and when sections were labeled with anti-Rubisco followed by protein A-gold (see "Materials and Methods"), gold particles were more concentrated in the pyrenoid region than in other areas of the chloroplasts. These rudimentary pyrenoids rapidly changed into typical pyrenoids with adjacent paramylum granules, and the gold particles also became markedly concentrated over the pyrenoid, as seen from Figures 4 and 5. Chloroplast division began soon after the beginning of the cell division (6). Shortly before the start of the chloroplast division, the pyrenoid began to disappear concurrently with the dispersion of gold particles from the pyrenoid region into the stroma, and became totally undetectable in the chloroplasts during the division phase of the cell cycle either in the dark (Figs. 6 and 7) or in the light (Figs. 10 and 11). Gold particles in these chloroplasts were completely dispersed throughout the stroma. Toward the end of the division phase, gold particles in the chloroplasts appeared to be arranged in short lines in the narrow area of stroma between thylakoids, either in the dark (Fig. 8) or in the light (Fig. 12). Subsequently, the gold particles were again seen to be clustered in the center of chloroplast (Fig. 9), concurrently with the reappearance of the rudimentary pyrenoid (Fig. 13).

When immunoelectron micrographs of serial sections through the chloroplasts in actively growing cells were examined in further details, some small areas distinctly rich in gold particles were observed, besides the main pyrenoid region. These areas, called 'satellite pyrenoids,' could not be detected in the cells of the division phase. As seen from Figure 14, some larger satellite pyrenoids were in association with the paramylum granule as was the main pyrenoid. Table I shows the relative distribution of gold particles among the main and satellite pyrenoids and the stroma in four chloroplasts of a serially sectioned 10-h cell.

DISCUSSION

The pyrenoid may be regarded as a device which has become established in the algae for the storage of Rubisco, the vital enzyme of the photosynthetic carbon-fixation cycle (8). It is not known, however, whether Rubisco in the pyrenoid is functional or is simply stored there. Recent studies (14, 21) suggested that pyrenoid Rubisco can be functional *in vivo*. The data in Figure 1 show that the maximum CO_2-fixation observed in cells (10-h cell) grown under 6000 lux was nearly the same as the total activity of Rubisco extracted from the cells at the same stage. The enzyme activity was measured at 30°C, and the activity at 25°C, the temperature at which the CO_2-fixation of cells was determined, can be estimated as 75% of the measured value according to the result obtained by Yokota *et al.* (30). When photosynthetic CO_2-fixation was measured at higher light intensities (10,000–20,000 lux), the maximum fixation reached more than 100% of the total enzyme activity of cells (our unpublished data). It was confirmed that at these higher light intensities, the pyrenoid was present in the chloroplasts of growing cells. This might imply that Rubisco in the pyrenoid can function in photosynthetic CO_2-fixation, although kinetic characteristics of the enzyme existing in the pyrenoid remain to be worked out.

Considering a possible relationship between the algal pyrenoid and certain inclusions of higher plant chloroplasts, Griffiths (8) described that a gradual progression can be traced through the plant kingdom, starting with the massive, single pyrenoid, then to smaller multiple pyrenoids, and finally to very small proteinaceous areas distributed throughout the chloroplast. He pointed out that the pyrenoid of the liverwort *Anthoceros* has particular significance in this connection. The liverwort pyrenoid is reported to consist of small units (25 to many hundreds) which may form an aggregated central mass within the chloroplast, or may be distributed throughout the chloroplast as small fragments (17–19). Kaja (10) and Burr (2) described the possible evolutionary steps in pyrenoid disintegration in a comparison of different species of *Anthoceros*, which show a range of forms from a homogenous pyrenoid to complete dispersal throughout the chloroplast. Different morphologies of the pyrenoid, including satellite pyrenoids, in *Euglena* chloroplasts observed during the cell cycle appears

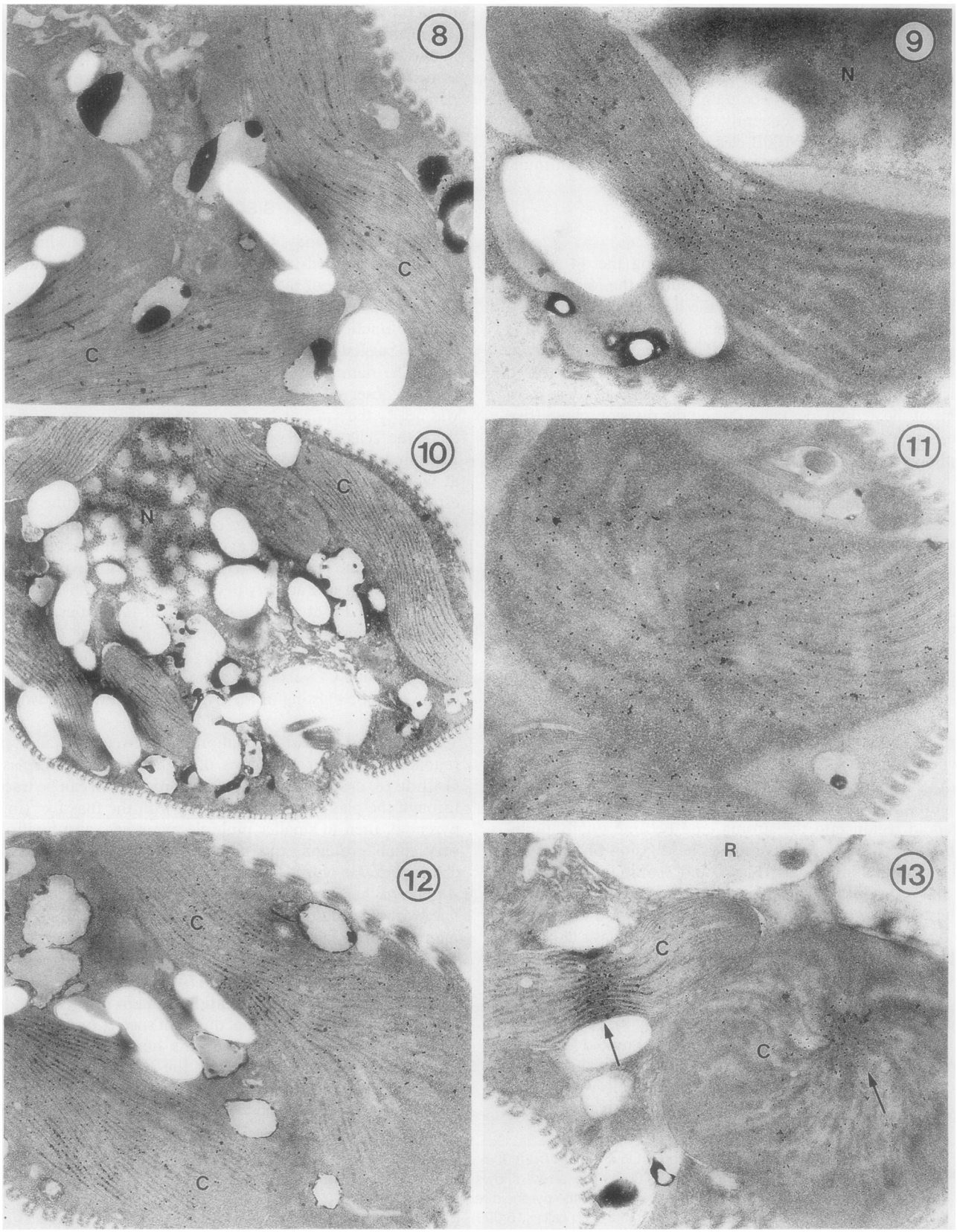
Figure 3. A transverse section of a chloroplast in a 0-h cell (×28,000). The arrow indicates the pyrenoid region.

Figure 4. A chloroplast in a 13-h cell (×23,000). The arrow shows the pyrenoid, where gold particles are densely localized. Note that paramylum (PA) is present surrounding the chloroplast at the pyrenoid region. V: vacuole.

Figure 5. A transverse section of a chloroplast in a 13-h cell (×15,000). The arrow indicates the pyrenoid.

Figure 6. A section through chloroplasts in a 18-h cell (4 h in the dark) (×11,500). Serial sections showed that no pyrenoid was detectable in these chloroplasts. Note that gold particles are dispersed throughout the chloroplast.

Figure 7. A transverse section of a chloroplast in an 18-h cell (4 h in the dark) (×19,000). No pyrenoid is seen.



Figures 8–13. Figure 8. A section through chloroplasts in a 20-h cell (6 h in the dark) (×17,000). Gold particles are localized in rows in the spaces between the thylakoids.

Table I. *Relative Distribution of Rubisco-Gold Particles Among the Stroma and Pyrenoid Regions in Chloroplasts of a Cell from the Growth Phase Culture*

The cell sample was taken from a synchronized culture at the 10th h from the beginning of the light period (10-h cell). Gold particles were counted in each of 41 serial sections of the cell and were summed.

Chloroplast	No. of Gold Particles in the Stroma	No. of Gold Particles in Pyrenoids								Sum Total
		Main	Satellite						Total	
			1	2	3	4	5	6		
1	339	1451	300	23	18				1792	2131
2	307	1780	37	18	13				1848	2155
3	187	969							969	1156
4	316	1005	551	46	22	18	16	12	1670	1986

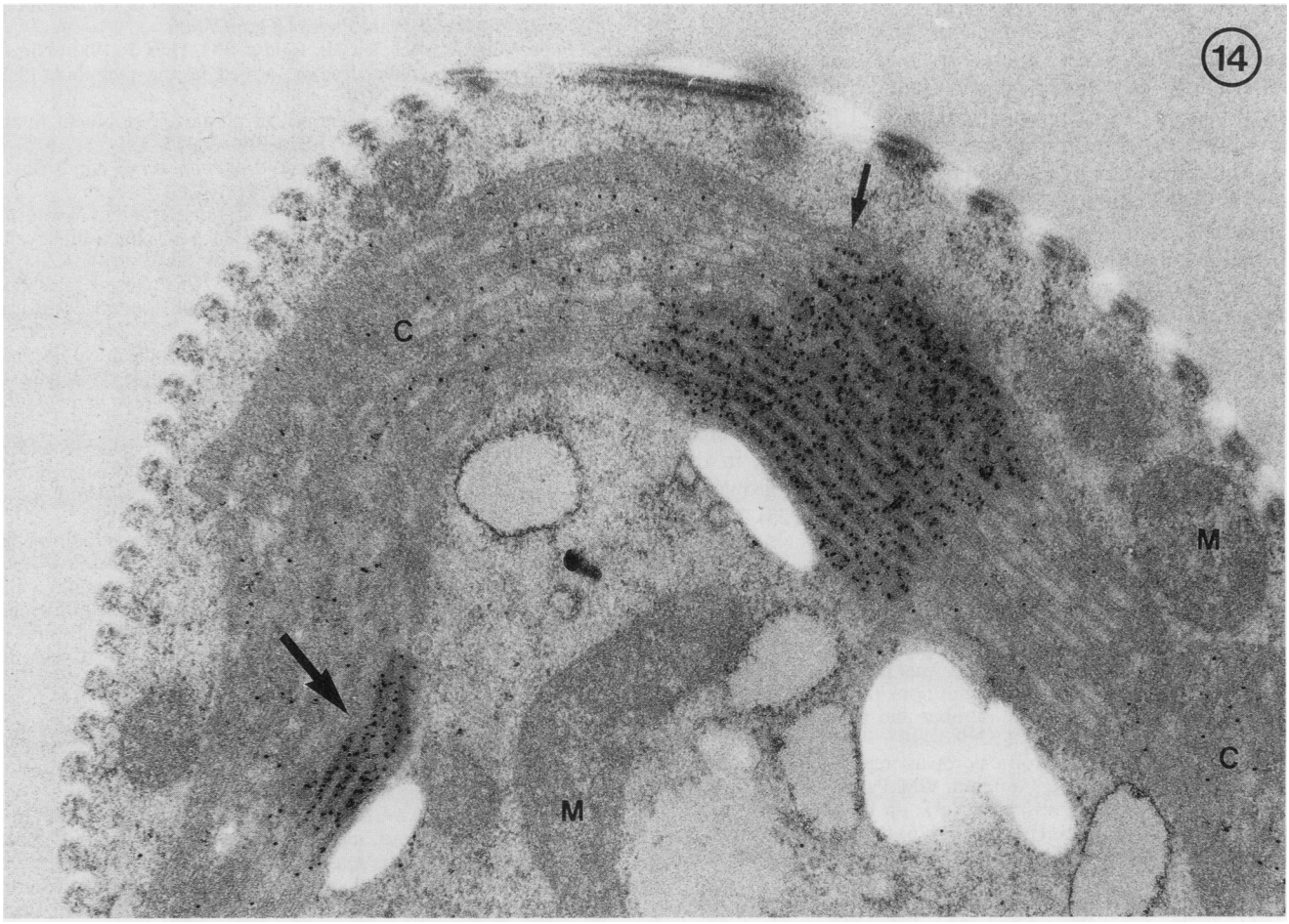


Figure 14. A section through a chloroplast in a 10-h cell (×22,400). The upper arrow indicates the main pyrenoid and the lower one a satellite pyrenoid. Note that both pyrenoids have a paramylum granule associated with them.

Figure 9. A section of a chloroplast in a 23-h cell (9 h in the dark) (×22,000). Gold particles are again concentrated in the center of the chloroplast (the pyrenoid region).

Figure 10. A section of a chloroplast in an 18-h cell (18 h in continuous light) (× 8,800). *Euglena* cells synchronized under the 14 h light: 10 h dark regimen were placed in continuous light. No pyrenoid was detected in the chloroplasts, and gold particles were dispersed throughout the chloroplast, as in the cases in Figures 6 and 7.

Figure 11. A transverse section through a chloroplast in an 18-h cell (18 h in continuous light) (× 22,000). No pyrenoid is seen.

Figure 12. A section through chloroplasts in a 21-h cell (21 h in light) (×18,000). Gold particles appear to be gathering in a row, as in Figure 8.

Figure 13. A section through chloroplasts in a 24-h cell (24 h in light) (×13,000). The arrows indicate pyrenoids. Gold particles are concentrated over the pyrenoid. Note that the paramylum granule is present near the pyrenoid.

to be analogous to the different morphologies of the pyrenoid in the liverwort that might represent evolutionary steps.

The dispersion of pyrenoid in *Euglena* chloroplasts was observed to begin shortly before the start of the chloroplast division in the *Euglena* cell cycle. Chloroplast division begins shortly after cell division (6). The dispersion of the pyrenoid might facilitate distribution of Rubisco between daughter chloroplasts during chloroplast division. According to Cook *et al.* (4), the pyrenoid was present only in the first half of the light period in the light:dark synchronized *Euglena* cells. Cook (3) reported that chloroplast division took place slightly before cell division. Presumably, the pyrenoid had dispersed and become undetectable long before the chloroplast division under the conditions used by Cook.

Kiss *et al.* (13) showed by transmission electron microscopy and immunofluorescence microscopy that the appearance and disappearance of pyrenoids concomitant with concentration and dispersion of Rubisco in chloroplasts of *Euglena gracilis* are controlled by nutritional conditions. Low nutrient levels are related to the presence of pyrenoid, while high nutrient levels with the absence of the pyrenoid. Orcival-Lafont and Calvayrac (23) also found a similar correlation between nutrient levels and pyrenoid presence. Miyachi *et al.* (22) reported that the pyrenoid is affected by environmental CO_2 concentration. The pyrenoid and the starch sheaths around the pyrenoids develop much more in low-CO_2 cells than in high CO_2-cells of three unicellular algae, *Chlorella* and *Scenedesmus*.

We have observed that the pyrenoid is newly formed, concurrently with accumulation of Rubisco over it, in the dark in the proplastids of dark-organotrophically grown *E. gracilis* cells transferred to an inorganic medium (25).

LITERATURE CITED

1. **Bradford MM** (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72:** 248–254
2. **Burr FA** (1970) Phylogenetic transitions in the chloroplasts of the Anthocerotales I. The number and ultrastructure of the mature plastids. Am J Bot **57:** 97–110
3. **Cook JR** (1966) Studies on chloroplast replication in synchronized *Euglena*. *In* IL Cameron, GM Padilla, eds, Cell Synchrony. Academic Press, New York, pp 153–168
4. **Cook JR, Haggard SS, Harris P** (1976) Cyclic changes in chloroplast structure in synchronized *Euglena gracilis*. J Protozool **23:** 368–373
5. **Dodge JD** (1973) The pyrenoid. *In* The Fine Structure of Algal Cells. Academic Press, London, pp 105–124
6. **Ehara T, Hase E** (1987) Interactions between the nucleus and cytoplasmic organelles during the cell cycle of *Euglena gracilis* in synchronized cultures: nucleus-chloroplasts association. (abstract No. 4-106-5) XIV Intal Botanical Cong. Berlin (West), Germany
7. **Griffiths DJ** (1970) The pyrenoid. Bot Rev **36:** 29–58
8. **Griffiths DJ** (1980) The pyrenoid and its role in algal metabolism. Sci Prog, Ocf (1980) **66:** 537–553
9. **Holdsworth RH** (1971) The isolation and partial characterization of the pyrenoid protein of *Eremosphaera viridis*. J Cell Biol **51:** 499–513
10. **Kaja H** (1954) Untersuchungen über die Chromatophoren und Pyrenoid der Anthocerotales. Protoplasma **44:** 136–153
11. **Kerby NW, Evans LV** (1978) Isolation and partial characterization of pyrenoids from the brown alga *Pilayella littoralis* (L.) Kjellm. Planta **142:** 91–95
12. **Kerby NW, Evans LV** (1981) Pyrenoid protein from the brown alga *Pilayella littoralis*. Planta **151:** 469–475
13. **Kiss JZ, Vasconcelos AC, Triemer RE** (1986) Paramylon synthesis and chloroplast structure associated with nutrient levels in *Euglena* (Euglenophyceae). J Phycol **22:** 327–333
14. **Kuchitsu K, Tsuzuki M, Miyachi S** (1988) Characterization of the pyrenoid isolated from unicellular green alga *Chlamydomonas reinhardtii*: particulate form of Rubisco protein. Protoplasma **144:** 17–24
15. **Lacoste-Royal G, Gibbs SP** (1987) Immunocytochemical localization of ribulose-1,5-bisphosphate carboxylase in the pyrenoid and thylakoid region of the chloroplast of *Chlamydomonas reinhardtii*. Plant Physiol **83:** 602–606
16. **Lopez-Ruiz A, Verbelen JP, Roldan JM, Diez J** (1985) Nitrate reductase of green algae is located in the pyrenoid. Plant Physiol **79:** 1006–1010
17. **Manton I** (1962) Observations on plastid development in the meristem of *Anthoceros*. J Exp Bot **13:** 325–333
18. **McAllister F** (1914) The pyrenoid of *Anthoceros*. Am J Bot **1:** 79–95
19. **McAllister F** (1927) The pyrenoids of *Anthoceros* and *Notothylas* with especial reference to their presence in spore mother cells. Am J Bot **14:** 246–257
20. **McCurry SD, Gee R, Tolbert NE** (1982) Ribulose-1,5-bisphosphate carboxylase/oxygenase from spinach, tomato, or tobacco leaves. Methods Enzymol **90:** 515–521
21. **Mckay RML, Gibbs SP** (1989) Immunocytochemical localization of ribulose-1,5-bisphosphate carboxylase/oxygenase in light-limited and light-saturated cells of *Chlorella pyrenoidosa*. Protoplasma **149:** 31–37
22. **Miyachi S, Tsuzuki M, Maruyama I, Gantar M, Miyachi S, Matsushima H** (1986) Effects of CO_2 concentration during growth on the intracellular structure of *Chlorella* and *Scenedesmus* (Chlorophyta). J Phycol **22:** 313–319
23. **Orcival-Lafont AM, Calvayrac R** (1974) Le plastidome chez *Euglena gracilis* Z. J Phycol **10:** 300–307
24. **Osafune T, Mihara S, Hase E, Ohkuro I** (1975) Formation and division of giant mitochondria during the cell cycle of *Euglena gracilis* Z in synchronous culture I. Some characteristics of changes in the morphology of mitochondria and oxygen-uptake activity of cells. Plant Cell Physiol **16:** 313–326
25. **Osafune T, Ehara T, Sumida S, Hase E** (1988) Light-independent and dependent processes of the formation of pyrenoid and thylakoids in dark-grown cells of *Euglena gracilis* Z. *In* Proceedings of the IVth Asia-Pacific Conference and Workshop on Electron Microscopy. Bangkok, pp 519–520
26. **Salisbury JL, Floyd GL** (1978) Molecular, enzymatic and ultrastructure characterization of the pyrenoid of the scaly green monad *Micromonas squamata*. J Phycol **14:** 362–368
27. **Satoh H, Okada M, Nakayama K, Miyaji K** (1984) Purification and further characterization of pyrenoid proteins and ribulose-1,5-bisphosphate carboxylase-oxygenase from the green alga *Bryopsis maxima*. Plant Cell Physiol **25:** 1205–1214
28. **Vladimirova MG, Markelova AG, Semenenko VE** (1983) Use of the cytomimmunofluorescent method to clarify localization of ribulose bisphosphate carboxylase in pyrenoids of unicellular algae (in Russian). Fiziologia Rastenii **29:** 941–950
29. **Yokota A, Kitaoka S** (1987) Rates of glycolate synthesis and metabolism during photosynthesis of *Euglena* and microalgae grown on low CO_2. Planta **170:** 181–189
30. **Yokota A, Harada A, Kitaoka S** (1989) Characterization of ribulose-1,5-bisphosphate carboxylase/oxygenase from *Euglena gracilis* Z. J Biochem **105:** 400–405