Mechanism of Conversion from Heterotrophy to Autotrophy in Euglena gracilis

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Received June 4, 2007; accepted August 20, 2007

Summary Euglena grown to stationary phase in the dark without aeration accumulated lipids. When these high lipid cells are transferred to an inorganic medium and aerated, lipids were rapidly metabolized and the respiratory rate declined concomitant with the decline in cellular lipid content. Prolamellar bodies, propyrenoids and prothylakoids developed within the proplastid of dark aerated cells and the cells developed an increased capacity for chlorophyll synthesis manifested upon subsequent exposure to light. Lipid content did not decline in cells exposed to nitrogen and chlorophyll synthesis ability did not increase. The addition of an organic carbon source to cells at the start of aeration did not prevent lipid degradation. Organic carbon source addition and inhibitors of RNA and protein synthesis did however inhibit the development of an increased capacity for chlorophyll synthesis. These results suggest that oxygen triggers light independent proplastid development with the oxidative metabolism of lipids providing the carbon and energy for the synthesis of nucleic acids and proteins required for proplastid development in the dark.

Key words Euglena, Wax esters, Chlorophyll, Protochlorophyll, Metabolic inhibitors.

Growth of microorganisms requires carbon and energy for the synthesis of cellular components. Autotrophs utilize sunlight or the energy released by oxidation of inorganic compounds for growth while the breakdown of organic compounds provides the energy needed for the growth of heterotrophs. Most pathogenic bacteria, protozoa, and fungi are heterotrophs. The objective of this study was to analyze the mechanism of conversion from heterotrophy to autotrophy using the unicellular alga, Euglena gracilis.

Euglena gracilis grows heterotrophically on a variety of carbon sources in the dark showing a typical animal-type metabolism and when it is placed in the light on an inorganic medium it grows autotrophically exhibiting plant-type metabolism. Euglena is the only organism that can reversibly switch from animal-like heterotrophic metabolism to plant-like autotrophic metabolism making its taxonomic classification as a plant or animal problematic (Johnson 1968). Schiff’s group (Osafune et al. 1990, Schiff and Schwartzbach 1982) has studied the transformation from a heterotroph to a phototroph, the greening of Euglena gracilis var. bacillaris, by transferring dark grown non-dividing cells, resting cells, into the light to induce chloroplast development. They found that the breakdown of the storage carbohydrate paramylum provided carbon and energy for the light induced synthesis of proteins, nucleic acids and lipids needed for chloroplast development (Schwartzbach et al. 1975, Rosenberg et al. 1964) found that lipids, mainly wax esters, accumulated in the cytoplasm of dark grown stationary Euglena and their breakdown was induced by light exposure providing an additional source of carbon and energy for light induced chloroplast development.

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To better understand the role of lipid metabolism in the conversion from heterotrophy to autotrophy, we studied light independent chloroplast development triggered by transferring cells grown heterotrophically in the dark without aeration to an inorganic medium with aeration. *Euglena* grown to stationary phase on Hutner’s medium (Hutner et al. 1956) containing 1.5% sucrose and maintained in the dark without aeration accumulated lipids. Cellular lipid levels declined, proplastids containing prolamellar bodies, propyrenoids and prothylakoids were formed and the capacity for light dependent chlorophyll synthesis increased when these high lipid cells were transferred to an inorganic medium and aerated in the dark with 1.5% CO₂. Respiration measured with an oxygen electrode was high during the time of lipid degradation and decreased as lipid degradation ceased (Sumida et al. 1982). Addition of an organic carbon source at the start of aeration did not prevent lipid degradation but it inhibited the development of the capacity for chlorophyll synthesis. Studies with inhibitors demonstrated a clear role for nuclear gene activity in the development of the chlorophyll biosynthetic capacity. Taken together, these results suggest that lipid degradation triggered by oxygen availability provides the carbon and energy for light independent chloroplast development facilitating the cell’s transition from heterotrophic to phototrophic growth upon light exposure. The availability of an external carbon source which will allow heterotrophic growth does not appear to be inhibitory to lipid degradation but it does inhibit light independent chloroplast development ensuring that all of the carbon and energy provided by lipid breakdown is used to support heterotrophic growth.

**Materials and methods**

1. **Cells:** The test strain was *Euglena gracilis* Klebs var. *bacillaris* Cori cultured in the dark. This strain has been cultured continuously in the dark since 1964, and was kindly provided by Professor Jerome Schiff (Institute for Photobiology, Brandeis University, MA 02254, USA).

2. **Medium:** Organic medium: Hutner’s medium pH 3.5 (Hutner et al. 1956) (1.5% sucrose hydrolyzed into glucose and fructose upon autoclaving) supplemented with 5 µg per liter of vitamin B₁₂. Inorganic medium: A modified Cramer and Myers medium pH 6.8 (Cramer et al. 1952) containing per liter, 1 g KH₂PO₄, 1 g (NH₄)₂HPO₄, 0.2 g MgSO₄ · 7H₂O, 0.026 g CaCl₂ · 2H₂O, 3.3 mg FeSO₄ · 7H₂O, 0.1 mg vitamin B₁, 0.5 µg B₁₂ and 1 ml trace element solution.

3. **Culture conditions:** *Euglena* was grown aseptically in the dark without shaking at 28°C on organic medium for 5 d. Approximately 50 ml of a 5 d old culture containing 1–2×10⁶ cells per ml was added to 350 ml of fresh organic medium in a 500 ml Erlenmeyer flask and cells were maintained without shaking. Cell growth under these conditions ceased after 4 d and cells were harvested on the fifth day of culture when they had the highest lipid content. Harvested cells were washed twice with inorganic medium by centrifugation (500g, 5 min) and resuspended in inorganic medium at a concentration of 2–4×10⁵ cells per ml. The cell suspension was placed in a flat oblong vessel, aerated with air containing 1.5% (v/v) CO₂ and maintained in the dark at 25°C (Hase 1980). Aseptic technique was used throughout this work and all transfers were performed with the aid of green safelights.

4. **Cell counting:** After adding a few drops of formalin solution to a culture aliquot, cells were counted under a microscope at 100 times magnification using a Fuchs-Rosenthal counting chamber. The number of cells in 1 ml of the original cell suspension was calculated by multiplying the count by the constant (3.1×10² cells/ml).

5. **Chlorophyll measurements:** A culture aliquot was centrifuged at 3,000 rpm for 30 min at 4°C. The pelleted cells were resuspended in a known volume of 80% acetone and kept at 4°C for 30–60 min to extract chlorophyll. The mixture was then centrifuged at 3,000 rpm for 30 min at 4°C to pellet cells and the supernatant was scanned from 420–700 nm (Sumida et al. 1987) using a UV210A (Shimadzu Seisakusho) spectrophotometer. The cellular chlorophyll content (pg) was cal-
culated using the equation: Chlorophyll content (pg/cell) = \[\frac{\text{[\text{OD663} \times 8.05] + (\text{OD645} \times 20.3)}}{\text{total volume of acetone} \times (\text{cells/ml}) \times 10^{-6}}\].

(6) Measurement of chlorophyll fluorescence: A 50 ml culture aliquot containing approximately 1.0 \times 10^6 cells/ml was centrifuged at 3,000 rpm for 30 min at 4°C, and extracted with 5 ml of 80% acetone. The mixture was then centrifuged at 3,000 rpm for 30 min at 4°C to pellet cells and the fluorescence spectrum of the supernatant was determined from 610–730 nm using an excitation wavelength of 470 nm. Protochlorophyll [ide] was determined from the emission at 633 nm (Sumida et al. 1982).

(7) Lipid determination: Cells were harvested by centrifugation at 3,000 rpm for 30 min at 4°C, the cell pellet was washed by resuspension in cold distilled water and the cells recovered by centrifugation. Lipids were extracted by resuspending the cell pellet in 20 ml of a 2 : 1 mixture of chloroform/methyl alcohol and kept at 4°C for 1 h with intermittent stirring. The mixture was centrifuged at 3,000 rpm for 30 min at 4°C, and the supernatant was dried under N₂ gas flow in a water bath kept at 35°C. The dissolved residue was dissolved in a small volume of chloroform/methyl alcohol, 4 volumes of 0.88% KCl solution was added and the mixture was stirred. Phases were separated by centrifugation at 500 rpm for 10 min and the upper layer was discarded. The lower layer was washed by mixing with an equal volume of a 3 : 48 : 47 mixture of chloroform/methyl alcohol/1.76% KCl, phases were separated by centrifugation and the upper layer discarded. The washing procedure was repeated until a white turbid interface was no longer obtained. The lipid containing lower layer was dried at 50°C and weighed.

(8) Measurement of respiration: Cellular respiratory rate was measured using a Yanagimoto recording respiratory oxygen measurement system (PO-100A). Respiratory rate is reported as oxygen consumed per 10^6 cells/h.

(9) Effect of metabolic inhibitors: Stationary phase cells grown on organic medium in the dark for 5 d to accumulate lipid were washed twice by centrifugation with inorganic medium and resuspended in the dark in inorganic medium at a final concentration of 2–4 \times 10^5 cells/ml. Either 6-methylpurine (6MP), cycloheximide (CHI) or lincomycin (LIN) was added to the cultures at the start of aeration in the dark. After 15 h, the inhibitor was removed by centrifugation and resuspension in fresh inorganic medium and the cells were irradiated for 30 min at 3000–4000 lux provided by a bank of daylight fluorescent lamps and the amount of chlorophyll synthesized was determined. Drug concentrations are shown in Figs. 11–13.

(10) Electron microscopy: *Euglena* cells were fixed with 2.5% glutaraldehyde (TAAB) for 1 h at 4°C and post fixed with 1% osmium tetroxide (EM Science, USA) for 1 h at 4°C. The suspension was centrifuged and the cell pellet was embedded in 2% agarose (Nakarai Co). Samples were dehydrated by successive incubations in a 50–100% graded ethanol series followed by incubation in 100% acetone (Wako Pure Chem Co). Samples were embedded in Spurr’s resin (EM Science, USA) and serial sections were prepared using a Leica UCT microtome. Sections were stained with 3% uranyl acetate (Merck, Germany) at room temperature followed by lead citrate, after which they were examined with a JEOL CX-100 electron microscope in the EM facility of Nippon Sport Science Univ. (Osafune et al. 1980). Freeze-substitution method: Cells were placed in liquid nitrogen (−189°C) cooled liquid propane using a freeze-substitution apparatus (JFA 7000). The samples were transferred to −85°C acetone containing 1% glutaraldehyde for 18 h to replace ice within the cells. The temperature of the acetone/glutaraldehyde was slowly raised over a 2 h period to −20°C and then to room temperature over a 2 h period. Cells were washed with acetone and embedded in Epoxy resin as described previously (Osafune et al. 1980).

Results

Fifty ml of culture containing approximately 2.0 \times 10^6 cells/ml grown on organic medium with
shaking was transferred into a 500 ml Erlenmeyer flask containing 400 ml of organic medium and incubated without shaking in the dark at 28°C. Measurements of cellular lipid content over a 6 d period found that lipid accumulation began 24 h after transfer reaching 0.7 ng/cell on day 5 at which time cell division stopped (Fig. 1). Lipids accumulating in stationary phase Euglena have been shown to be wax esters (Rosenberg et al. 1964). Lipid content did not increase in the control culture which was incubated with shaking indicating that wax esters do not accumulate in aerated cultures.

Intact wax globules are seen in the cytoplasm of wax-rich cells prepared for electron microscopy by freeze-substitution (Fig. 2A). The wax globules do not have a limiting membrane in contrast to vacuoles which contain a limiting membrane. Accumulated wax appears as crystallized needle-like structures in the cytoplasm of cells prepared for electron microscopy by conventional fixation procedures (Fig. 2B). Proplastids of 5 d old stationary high lipid cells lack a prolamellar body and membranes are not seen in the stroma (Fig. 2). Large amounts of lipid are found in the cytoplasm of 5 d old stationary high wax cells (Fig. 2).
Ultrastructural and biochemical changes in *Euglena* during the transition from heterotrophic to autotrophic growth were studied by washing 5 d old stationary high lipid cells twice with inorganic medium to remove the organic carbon source, transferring them to inorganic medium and aerating them with 1.5% CO₂ in air for 5 d in the dark. Multiple proplastids containing prolamellar bodies, propyrenoids and prothylakoids.

**Fig. 3.** A–D: Electron microscopy identifies developing proplastids (PP) containing prolamellar bodies (PLB), propyrenoids (PY) and prothylakoids (PT) in cells transferred from organic medium to inorganic medium and maintained in the dark for 5 d with aeration. A: a section of a whole cell. B–D: serial sections through the developing proplastid. Serial sections show a proplastid showing a developing propyrenoid and prothylakoids.
propyrenoids and prothylakoids are seen in the 5 d old aerated cells (Fig. 3). Serial sections show the developing propyrenoid containing prothylakoids (Fig. 3B–D). These proplastids resemble the proplastids seen in 3 d old resting cells exposed to light for 30 min (Osafune et al. 1980).

Development of the biosynthetic capacity to synthesize chlorophyll during incubation of high lipid cells on inorganic medium in the dark with aeration was determined by measuring the amount of chlorophyll synthesized during the first 30 min after light exposure. Cell number begins to increase 6 h after transfer of cells to inorganic medium with aeration and by 18 h there is approximately a twofold increase in cell number (Fig. 4). The amount of chlorophyll synthesized per cell or per ml culture is maximal 18 h after transfer to inorganic medium with aeration and then declines. The development of proplastids in aerated cells is associated with a transient increase in the biosynthetic capacity to synthesize chlorophyll upon light exposure.

Light exposure triggers the photoconversion of protochlorophyll(ide) to chlorophyll(ide). To determine if the transient increase in chlorophyll biosynthetic activity was due to the accumulation of protochlorophyll(ide) in aerated cells rather than to an increase in the biosynthetic capacity to synthesize protochlorophyll(ide), a fluorometric assay was used to measure protochlorophyll(ide) levels in dark aerated cells and the levels of chlorophyll(ide) and protochlorophyll(ide) 5 min, 15 min and 30 min after light exposure (Fig. 5). Protochlorophyll(ide) is present in high lipid cells at the time of transfer to inorganic medium with aeration and protochlorophyll(ide) levels increased between 18–24 h after transfer. At all time points examined, light exposure decreased the amount of

Fig. 5. Increased chlorophyll synthesis rates result from an increase in protochlorophyll(ide) levels in cells transferred from organic to inorganic medium in the dark with aeration. At the indicated times after transfer, an aliquot of culture was exposed to light. Protochlorophyll(ide) and chlorophyll(ide) levels were determined at the time of light exposure (0 min) and 5, 15 and 30 min after light exposure.
protochlorophyll(ide) and increased the amount of chlorophyll(ide) (Fig. 5). The decrease in protochlorophyll(ide) levels and increase in chlorophyll(ide) levels was dependent upon the time of illumination and was consistent with a precursor product relationship. The increased biosynthetic capacity for chlorophyll synthesis is not the result of an increased rate of chlorophyll precursor synthesis but rather the result of protochlorophyll(ide) accumulation within the prolamellar bodies formed upon transfer of high lipid cells to inorganic medium with aeration.

The changes in proplastid ultrastructure and protochlorophyll(ide) levels suggests that the transfer to inorganic medium with aeration triggers a limited light independent transition from heterotroph to autotroph. What is the source of carbon and energy driving this phenomenon? Freyssinet (1976) and Schwartbach et al. (1975) reported that paramylum was not degraded in the dark in carbon starved cells and that light exposure triggered its utilization providing carbon and energy for plastid development. Cells grown without aeration accumulate lipid. Thus, we investigated changes in lipid content and respiratory activity in cells transferred in the dark from an organic to an inorganic medium with aeration.

Cellular lipid content decreased approximately 2 fold during the first 24 h after transferring cells to an inorganic medium with aeration (Fig. 6). Lipid utilization was much slower after this time. Cellular oxygen consumption was highest at the time of transfer to inorganic medium with aeration (0 h) and declined as lipid content decreased (Fig. 6). When cells were aerated with nitrogen rather than 1.5% (v/v) CO$_2$ in air, total cellular lipid did not decrease and the latent capacity for chlorophyll synthesis did not increase during incubation in the dark as is seen for cultures aerated with 1.5% (v/v) CO$_2$ in air (Fig. 7). It appears that transfer to inorganic medium and aeration with
Fig. 8. Transfer of cells from an organic medium to an organic medium with aeration did not prevent the aeration triggered degradation of lipids. High lipid cells were transferred in the dark from organic medium to inorganic medium containing 0.5% glucose, galactose, saccharose or to Hutner medium and aerated in the dark. At the indicated times after transfer, an aliquot was removed for lipid determination.

Fig. 9. The presence of an organic carbon source inhibited the development of the capacity for chlorophyll synthesis. High lipid cells were transferred in the dark from organic medium to inorganic medium containing 0.5% glucose, galactose, saccharose or to Hutner medium and aerated in the dark. At the indicated times after transfer, an aliquot was removed, exposed to light for 30 min and the amount of chlorophyll(ide) produced was determined.

Fig. 10. The presence of an organic carbon source reduced the rate of chlorophyll synthesis in cells incubated in the dark for 19 h with aeration. High lipid cells were transferred in the dark from organic medium to inorganic medium containing 0.5% glucose, galactose or to Hutner medium, aerated in the dark for 19 h and then exposed to light to induce chlorophyll synthesis. Chlorophyll(ide) levels were determined at the time of light exposure (0 min) and 5, 15 and 30 min after light exposure to determine the rate of chlorophyll(ide) synthesis.

Fig. 11. An inhibitor of nucleic acid synthesis, 6-methylpurine, reduced the rate of chlorophyll synthesis in a concentration dependent manner in cells incubated 15 h in the dark with aeration. High lipid cells were transferred in the dark from organic medium to inorganic medium containing different concentrations of 6-methylpurine, aerated in the dark for 15 h, washed to remove the antibiotic, resuspended in fresh inorganic medium and then exposed to light to induce chlorophyll synthesis. Chlorophyll(ide) levels were determined at the time of light exposure (0 min) and 5, 15 and 30 min after light exposure to determine the rate of chlorophyll(ide) synthesis.
1.5% (v/v) CO₂ in air triggered the oxidative degradation of lipids. Lipid breakdown apparently provided the carbon and energy for protochlorophyll(ide) accumulation resulting in an increase in the latent capacity for chlorophyll synthesis.

The triggering of lipid breakdown and increase in latent capacity for chlorophyll synthesis could be caused by transferring the high lipid cells to an inorganic medium or by aeration. To distinguish between these possibilities, lipid content was determined in high lipid cells after transfer to an organic medium with aeration. Lipid content decreased to the same extent in cells transferred to inorganic medium (control) to medium containing 1.5% sucrose (Hunter's medium) and to medium containing 0.5% glucose, galactose or saccharose with aeration (Fig. 8). The latent capacity for chlorophyll synthesis was however sensitive to the nature of the carbon source (Fig. 9). Maximum chlorophyll synthesis was seen 12 h after transferring cells to glucose containing medium while in cells transferred to inorganic medium, galactose or Hutner's medium, maximum chlorophyll synthesis was seen at 19 h. In all cases, cells on inorganic medium had a higher latent capacity for chlorophyll synthesis than cells on organic medium. Differences in latent capacity for chlorophyll synthesis reflected differences in the rate of chlorophyll synthesis upon illumination (Fig. 10). The similar decrease in lipid content seen after transfer to carbon free or carbon containing medium suggests it is the presence of oxygen provided by aeration that triggers lipid breakdown while the presence of a carbon source inhibits the development of the capacity to synthesize chlorophyll.

To determine the dependence of the development of the latent capacity for chlorophyll synthesis on macromolecule synthesis, high lipid cells were transferred to inorganic medium containing 6-methylpurine (6MP), an inhibitor of nucleic acid synthesis, cycloheximide (CHI), an inhibitor of cytoplasmic protein synthesis, or lincomycin (LIN), an inhibitor of chloroplast protein synthesis and aerated with 1.5% (v/v) CO₂ in air at 25°C in the dark. After 15 h, the inhibitor was removed by centrifugation, cells were resuspended in fresh inorganic medium and the cells were irradiated to

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Fig. 12. An inhibitor of cytoplasmic protein synthesis, cycloheximide, reduced the rate of chlorophyll synthesis in a concentration dependent manner in cells incubated 15 h in the dark with aeration. High lipid cells were transferred in the dark from organic medium to inorganic medium containing different concentrations of cycloheximide, aerated in the dark for 15 h, washed to remove the antibiotic, resuspended in fresh inorganic medium and then exposed to light to induce chlorophyll synthesis. Chlorophyll(ide) levels were determined at the time of light exposure (0 min) and 5, 10, 15 and 30 min after light exposure to determine the rate of chlorophyll(ide) synthesis.

Fig. 13. An inhibitor of chloroplast protein synthesis, lincomycin, reduced the rate of chlorophyll synthesis in a concentration dependent manner in cells incubated 15 h in the dark with aeration. High lipid cells were transferred in the dark from organic medium to inorganic medium containing different concentrations of lincomycin, aerated in the dark for 15 h, washed to remove the antibiotic, resuspended in fresh inorganic medium and then exposed to light to induce chlorophyll synthesis. Chlorophyll(ide) levels were determined at the time of light exposure (0 min) and 5, 15 and 30 min after light exposure to determine the rate of chlorophyll(ide) synthesis.
determine the latent capacity for chlorophyll synthesis.

Development of the capacity to synthesize chlorophyll was inhibited in a concentration dependent manner in cells treated with 6MP (Fig. 11). At the maximum concentration tested, $5 \times 10^{-4} \text{M/ml}$, chlorophyll synthesis was by 75%. CHI treatment produced an even greater concentration dependent inhibition of the development of chlorophyll synthetic capacity (Fig. 12). LIN was the least effective inhibitor producing a 50% inhibition of development of the latent capacity of chlorophyll synthesis at the highest concentrations tested (Fig. 13). Taken together, the inhibitor studies suggest that the synthesis of nuclear encoded cytoplasmically synthesized proteins is the major factor contributing to the development in dark aerated cells of the latent capacity for chlorophyll synthesis.

Discussion

When *Euglena* is cultured with organic nutrients in the dark, chloroplasts degenerate and become proplastids whose only internal structure is a single prothylakoid near the envelope (Fig. 2). When these dark cultured cells are transferred to an inorganic medium in the dark with aeration, chloroplasts undergo limited structural development and the latent chlorophyll synthesis increases. The curve of lipid reduction in the inorganic medium in the dark shown in Fig. 6 can be regarded as due to the degradation of wax ester, and the oxygen activity curve was similar to this curve, indicating that the degradation was oxidative (Fig. 6). No lipid degradation was noted in nitrogen, nor was there an increase in chlorophyll synthesis ability (Fig. 7), further supporting the oxidative degradation hypothesis. It is very interesting that proplastid development in the dark aerated cells is dependent upon oxygen triggered cytoplasmic lipid (wax ester) degradation in contrast to the light induced proplastid development which is dependent upon light triggered paramylum ($\beta$-1, 3-glucan) degradation (Sumida et al. 1987).

The addition of an organic carbon source to cells at the start of aeration did not prevent lipid degradation (Fig. 8) providing further evidence that it is the aeration triggered degradation of lipids that provides the carbon and energy for proplastid development in the dark (Figs. 3A, B). The presence of the organic carbon source did however inhibit the development of the latent capacity for chlorophyll synthesis (Fig. 9). Hase’s group was the first to report that an organic carbon source inhibited chloroplast development (Ochiai et al. 1970, Hase 1971) in a phenomenon termed the glucose effect. Glucose and other organic carbon sources that could be used as respiratory substrates were subsequently shown to inhibit light triggered chloroplast development in *Euglena* (Schiff and Schwartzbach 1982, Schuler et al. 1981). The organic carbon source inhibition of light triggered and aeration triggered chloroplast development in *Euglena* is very similar to the inhibition by glucose seen in *Chlorella protothecoides* (Aoki et al. 1964, Osafune and Hase 1975).

The requirement for nucleic acid and protein synthesis for the aeration triggered development of the latent capacity for chlorophyll synthesis was investigated using the nucleic acid synthesis inhibitor, 6MP (Watanabe et al. 1980), the inhibitor of protein synthesis on 80S cytoplasmic ribosomes, CHI, and the inhibitor of protein synthesis on 70S chloroplast ribosomes, LIN. All agents inhibited development of the latent capacity for chlorophyll synthesis (Figs. 11–13) suggesting a requirement for nucleic acid and protein synthesis during the dark incubation after transfer from organic to inorganic medium with aeration. The presence of a number of organic carbon sources prevented the development in the dark of the latent capacity for chlorophyll synthesis triggered by aeration. Different carbon sources produced different degrees of inhibition. Oxidative degradation of lipids provides carbon and energy for the aeration triggered development of the latent capacity for chlorophyll synthesis. Prevention of the development of the capacity for chlorophyll synthesis by an organic carbon source is thus not a general effect of increased carbon availability or metabolic rate but rather a specific effect dependent on the type of carbon source. Further analyses is required to
determine the molecular level at which external carbon and aeration interact during the conversion from heterotrophy-to-autotrophy in *Euglena*.

**Acknowledgment**

We thank Professor Steven D. Schwartzbach of Biology Department, University of Memphis for critically reading and rewriting this manuscript. This work was aided by grant (No. 15570054 to T. Osafune) from the Ministry of Education, Science, Sports and Culture, Japan.

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