DEGENERATION AND REGENERATION OF
CHLOROPLASTS IN EUGLENA GRACILIS GROWN IN
THE PRESENCE OF ACETATE: ULTRASTRUCTURAL
EVIDENCE

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SUMMARY

When green cells of Euglena gracilis, strain Z, were light-grown for several months on a solid
medium containing an excess of sodium acetate (1.0% instead of the normal 0.1%), some 30% of
the cells were colourless. The 'acetate-bleached' organisms, isolated by plating methods and
subsequently incubated in the light in a liquid medium, regained the capacity to form chlorophyll
in a few days in the absence of any organic carbon source, and within 1-2 weeks in the presence of
0.1% acetate. A number of bleached colonies, however, gave rise to populations in which the delay
in pigment synthesis initiation was at least 2 months.

Besides numerous paramylum granules and lipid inclusions, the acetate-bleached cells exhibited
variably shaped and sized plastids, apparently lacking in ribosomes and showing a deeply disor-
ganized membrane system. In the alga greened in the presence of 0.1% acetate, the pattern of
plastidome reorganization was altered; the thylakoids were often unpaired and vesiculated in dif-
ferent degrees, owing primarily to the swelling of the lumen. A complete recovery of normal
chloroplast structure occurred only after several weeks of exponential growth. The entire population
greened in the absence of acetate constantly showed normal chloroplasts with perfectly reassociated
thylakoids and clear partitions.

INTRODUCTION

Because of its plasticity in nutritional requirements, the protist Euglena gracilis
can be placed between the animal and plant kingdoms. For example, when light and
reduced organic compounds are supplied together, photosynthesis and heterotrophic
nutrition cooperate in cell growth and multiplication of the alga (Cook, 1968; Nigon
& Heizmann, 1978). The two metabolisms, however, interfere at some points. When
Euglena is grown in the presence of acetate, which is its preferred substratum (Bates
& Hurlbert, 1970; Droop, 1974), the acetate brings about a reduction in the
chlorophyll content and rate of photosynthesis activity (App & Jagendorf, 1963;
Buétow, 1967), and it enters lipids and paramylon only. On the other hand, acetate
does not enter proteins, which are formed almost exclusively by carbon fixed through
photosynthesis (Cook, 1965).

Increasing amounts of acetate, as well of other nutrients, increasingly inhibit
chlorophyll formation (Buétow, 1967). However, the total repression of the latter
process by an organic source has never been observed. Thus the view that no
nutritional condition can induce the total disorganization of the pre-existing
chloroplasts in *Euglena* has obtained widespread credibility (Mego, 1968; Nigon & Heizmann, 1978; Schiff, 1980).

In this paper we provide evidence that, in a defined condition, an excess of acetate (1% instead of the normally supplied 0.1%) produces bleached cells in which chloroplast structures are almost completely degraded. In addition, the process of regreening of the white organisms is disturbed by acetate at the relatively low concentration of 0.1%.

**MATERIALS AND METHODS**

*E. gracilis* Klebs, strain Z, was cultivated on agar slants prepared with Starr's medium (Starr, 1960) containing 0.1% or, alternatively, 1% acetate. The cultures were maintained in laboratory conditions like those described by Starr (1973).

After 3 months, cells from each culture type were plated in Mego's agar medium (Mego, 1964), and the dishes were kept in continuous fluorescent light (130–50 ft-candles; 1 footcandle = 10.764 lx) at 24–26°C. After 1 week, the ratio of green to white colonies was calculated for each dish.

Colourless organisms, which developed only from cells previously grown in the presence of 1% acetate, were taken for fluorescence and electron microscopic examinations and to inoculate Mego’s liquid medium with or without 0.1% acetate. The cultures so obtained were constantly stirred and illuminated at 24–26°C and kept in the logarithmic phase of growth by frequent subculturing. In addition, some cultures were allowed to reach the stationary phase of growth or were prepared in the resting medium of Stern, Epstein & Schiff (1964).

The regreening process in all cultures was followed by determining chlorophyll content in acetone 80 extracts (MacKinney, 1941), and by fluorescence and electron microscopic observations. The examination under ultraviolet light was made on living cells using a Zeiss Photomicroscope II equipped with an epi-fluorescence condenser and a high-pressure mercury lamp HBO W/4 (Vannini, Bonora & Dall'Olio, 1981).

The specimens for the ultrastructural investigation consisted of organisms harvested once a day from the regreening cultures and then routinely fixed, dehydrated and embedded in an Araldite/Epon mixture (Vannini, Fasulo, Bruni & Dall'Olio, 1978). The ultrathin sections, doubly stained with uranyl acetate and lead citrate, were observed and photographed with a Siemens Elmiskop 101 (Electron Microscopy Center of Ferrara University).

**RESULTS**

Once plated, the cells harvested from the tubes containing 0.1% acetate raised 98–100% green colonies. On the other hand, the algae sampled from the medium supplemented with 1% of the organic source produced about 30% of white colonies. The latter consisted of organisms, that can be named 'acetate-bleached' cells, in which chlorophyll was undetectable by both fluorescence examination *in vivo* and acetone extraction.

When examined at the ultrastructural level, the bleached microorganisms exhibited cytoplasm filled with numerous paramylon grains (Fig. 1) and lipid inclusions consisting either of fat globules (Fig. 2) or membrane whorls with varying degrees of organization. These membranous components often appeared in close connection with

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Figs 1, 2. Sections through acetate-bleached cells of *E. gracilis* showing abundant paramylon granules (pa) and lipid inclusions (l). Both ×18,000.
Acetate and chloroplast regression in Euglena

Figs 1 and 2
Acetate and chloroplast regression in Euglena bodies of uncertain nature, possibly lipid drops or organelles resembling the microbodies described by Osafune, Klein & Schiff (1980) in etiolated Euglena cells (Fig. 3).

Especially noteworthy was the constant presence of variably shaped plastids, which apparently lacked ribosomes and possessed a deeply disorganized thylakoid system (Fig. 4). These organelles, which often contained a great number of plastoglobules (Fig. 5), were larger than the proplastids of the dark-grown cells.

The acetate-bleached algae kept in a liquid medium depleted of acetate regained the ability to form chlorophyll within 2—3 days. After this lag period, the kinetics of pigment synthesis, as well as that of the structural development of the chloroplasts, were virtually the same as have been described repeatedly for cells first cultivated in the dark and then exposed to the light (see Nigon & Heizmann, 1978). In particular, the thylakoids appeared perfectly associated in all phases of the developmental process (Fig. 6).

When 0.1 % acetate was present in the medium, the acetate-bleached cells underwent a 1 to 2-week lag period in chlorophyll formation and in plastid structural development. Subsequently, the pattern of plastidome organization went through an abnormal phase characterized by the presence of organelles, often possessing a well-structured pyrenoid (Fig. 7). The plastids were unusual in that the thylakoids were vesiculated to varying degrees, due primarily to the swelling of the lumen (Figs 8, 9). This stage of vesiculation lasted approximately 1—2 weeks when the cultures were maintained in the exponential phase of growth, and longer for organisms in a resting medium or in the stationary phase of growth. In either case, the algae characterized by vesiculated plastids contained an amount of chlorophyll virtually the same as that in the control group, which was formed by etiolated cells exposed for several days to continuous light in a medium containing 0.1 % acetate.

Finally, a complete recovery of chloroplast structure occurred, with perfectly reassociated thylakoids and clear partitions (Fig. 10). All the stages in plastid regeneration were accompanied by a decrease in paramylon and lipid inclusions.

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Fig. 3. Section through a cell as in Fig. 1, showing relationship of lipid bodies (l) and membrane whorls (mvw). X55 000.

Fig. 4. Degraded plastids in an acetate-bleached cell. X40 000.

Fig. 5. Plastid of a bleached cell displaying a stroma with several plastoglobuli, few thylakoids and apparently no ribosome. X48 000.

Fig. 6. Section through a bleached cell cultivated in the light in a liquid medium deprived of acetate. The plastids show a normally developed and arranged thylakoid system. X30 000.

Figs 7—9. Sections through bleached cells cultivated for 2 weeks in the light in a liquid medium containing 0.1 % acetate.

Fig. 7. A vesiculated chloroplast with a well-developed pyrenoid (p) surrounded by paramylon granules (pa). X28 000.

Fig. 8. An abnormally shaped chloroplast with numerous and large vesicles. X23 000.

Fig. 9. Highly malformed chloroplast, partly occupied by large vesicles and partly showing an almost normal structure. X23 000.
Figs 5 and 6. For legend see p. 417.
Acetate and chloroplast regression in Euglena

Figs 7–9. For legend see p. 417.
DISCUSSION

Contrary to published studies (Cook, 1968; Nigon & Heizmann, 1978), we have provided evidence of nutrition-induced chloroplast degeneration in *Euglena* grown in the light.

On the whole, 1% acetate in defined conditions apparently acts on this alga like excess glucose on *Chlorella protothecoides*. Excess glucose is commonly used to investigate the conversion of green cells into white organisms and *vice versa* under controlled nutritional conditions (Hase, 1971; Osafune & Hase, 1975).

Caution, however, is required in comparing experiments in which different nutrients are employed. In addition, *Euglena* and *Chlorella* are not equivalent systems, because the latter alga forms chloroplasts both in the light and in the dark. Despite this, it is reasonable to assume that some common event occurs in the nutrition-induced regression of chloroplasts into plastid remnants in facultative photo-organotrophs. More precisely, in accordance with most studies of the influence of carbon sources on both the above-mentioned algae (see Harris & Kirk, 1969; Hase, 1971; Schuler, Brandt & Wiessner, 1981), we believe that an unknown repressor
Acetate and chloroplast regression in Euglena

Substance(s) forms during acetate utilization. In particular, over prolonged exposure to the carbon source, the slowly dividing cells of Euglena could accumulate numerous paramylon grains and lipid inclusions (according to the biochemical data of Cook, 1965) as well as repressor intermediate(s). The latter may form in such large amounts that the internal structures of the pre-existing chloroplasts cannot be maintained. When the acetate-bleached cells are transferred into an acetate-free medium, the toxic substance(s) may be readily degraded and/or diluted among the generations. On the contrary, when 0.1% acetate is present, the amount of bleach-inducing intermediate(s) decreases more slowly, so that chloroplast morphogenesis starts after several days and follows an abnormal pattern. This observation seems to show that one or more regulatory interactions are disturbed, presumably until the level of toxic substance(s) exceeds a critical value.

The morphological data presented here do not furnish indications as to which cell compartment concerned with plastid development is more affected by the repressor compound(s) (see Herrmann, Borner & Hagemann, 1980; Schiff, 1980). However, an initial study with etiolated Euglena cells, first grown in the dark and then illuminated in a resting medium supplemented with 1% acetate, shows that chlorophyll a to b ratio during both the lag period and the major period of chloroplast development (see Nigon & Heizmann, 1978; Schiff, 1980) is constantly less than 2.5.

Therefore, the repressive effects seem to interact primarily with the synthesizing system for the thylakoid particles known to have a high chlorophyll a to b ratio (PS I complex), whereas the formation of particles in which this ratio is low (PS II complex) (Brandt, 1980, 1981) is conceivably less affected by acetate. If so, differences in the mechanism of action of acetate and glucose exist, because the latter primarily affects PS II when applied in excess to regreening Euglena (Schwelitz, Cisneros & Jagielo, 1978; Schuler et al., 1981). However, it should be remembered that glucose, under the conditions used, does not act as a bleach-inducing agent for Euglena.

Although considerable work remains to be done to determine the mode in which acetate acts on chloroplasts, this carbon source appears to be a unique tool in helping to explain the nutritional control of plastid development in Euglena.

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REFERENCES


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