ULTRAVIOLET SENSITIVE FACTORS IN THE CYTOPLASM THAT AFFECT THE DIFFERENTIATION OF *EUGLENA* PLASTIDS

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The bleaching phenomenon in *Euglena gracilis* strain z offers a unique possibility for investigating the hereditary nature of plastids. Treatment of *Euglena* cultures with streptomycin, elevated temperature, or irradiation with a mild dose of UV light causes the permanent loss of the capacity to develop chloroplasts in the growing cultures. Early explanations of this phenomenon were that the bleaching treatment differentially inhibited the growth of the plastids or killed them, thus resulting in cells which lacked plastids. In effect the bleaching treatment presumably "cured" the *Euglena* cells of their plastids.

Recently we reported (1) that the plastids of the bleached *Euglena* cells did not disappear. The presence of proplastids within cells of bleached *Euglena* was demonstrated by phase and fluorescence microscopy as well as by tests of the biochemical properties which are usually associated with plastids.[1] It was concluded that the changes induced in *Euglena* in bleaching are of the nature of mutations affecting the plastids rather than the entire loss of these structures. The bleached plastids are to be regarded as proplastids that can no longer differentiate to chloroplasts.

In a quantitative study of the action of UV light

[1] We have more recently confirmed the presence of the proplastids in bleached strains by electron microscopy.

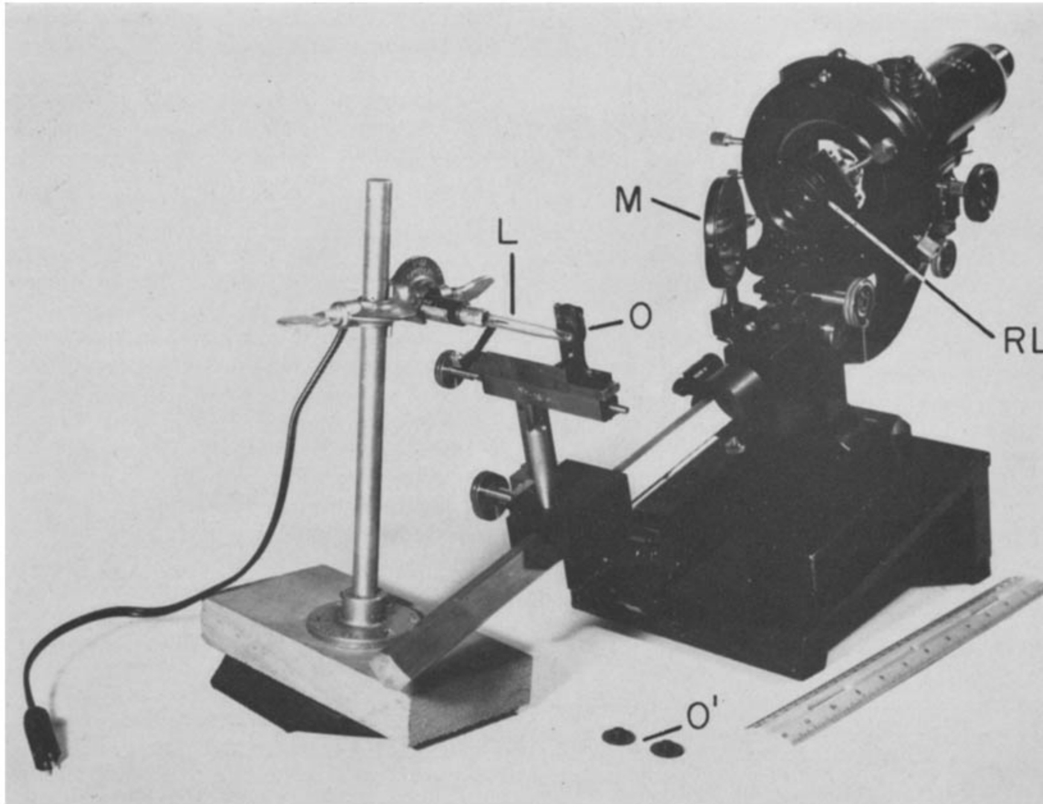

FIGURE 1

UV microbeam apparatus. *L*, UV pencil lamp (Black Light Eastern Co., Port Washington, New York); *M*, mirror; *O*, small orifice in holder; *O'*, orifices of different sizes; *RL*, reflecting lens ×50 (American Optical Co. #1200) mounted on substage.

on the bleaching of *Euglena*, Lyman, Epstein, and Schiff (2) concluded from the action spectrum for the process that nucleoprotein particles are the primary targets of the quanta of UV light. From the kinetics of the bleaching phenomenon they estimated that the cells contain about 30 such particles, all of which had to be inactivated to cause bleaching. Presumably these nucleoprotein particles are the ones concerned with the differentiation of the proplastids to chloroplasts.

To determine whether the nucleoprotein particles are located in the nucleus or in the cytoplasm we have utilized a UV microbeam apparatus. With this apparatus the entire cell, the nucleus alone, or only the cytoplasm may be irradiated.

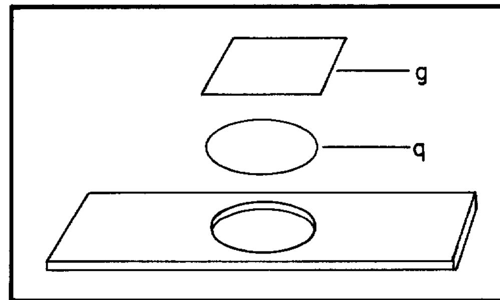

FIGURE 2

Culture chamber assembly; (× 0.7). A hole 18 mm in diameter was drilled in a microscope slide. The cells were embedded between a quartz (*q*) 22 mm coverglass and a glass (*g*) coverglass. The coverglass sandwich was placed with the quartz side down over the hole in the slide and sealed in position with paraffin-vaseline.

METHODS AND RESULTS

1) *The microbeam apparatus* (Fig. 1) was based on the principle described by Tschakholin (3). A reflecting × 50 lens was used as a condenser in a microscope which had a mechanical substage (*RL*, Fig. 1). This lens focused the image of a small orifice (*O*, Fig. 1) onto the plane of the microscope stage. The UV source was a low pressure mercury pencil lamp (*L*, Fig. 1). The lamp emits primarily at 2537 A with an intensity of 42 μw/cm^2 at a distance of 30 cm. Sufficient visible light is emitted to enable the localization of the UV beam. A shield (not shown in the picture) was installed at the plane of the orifice so that no stray UV light reached the lens. Interchangeable orifices (*O'* Fig. 1) of different diameters were used. The smallest orifice was of about 0.2 mm diameter and its focused image on the microscope stage measured about 4.5 μ diameter. A doughnut-

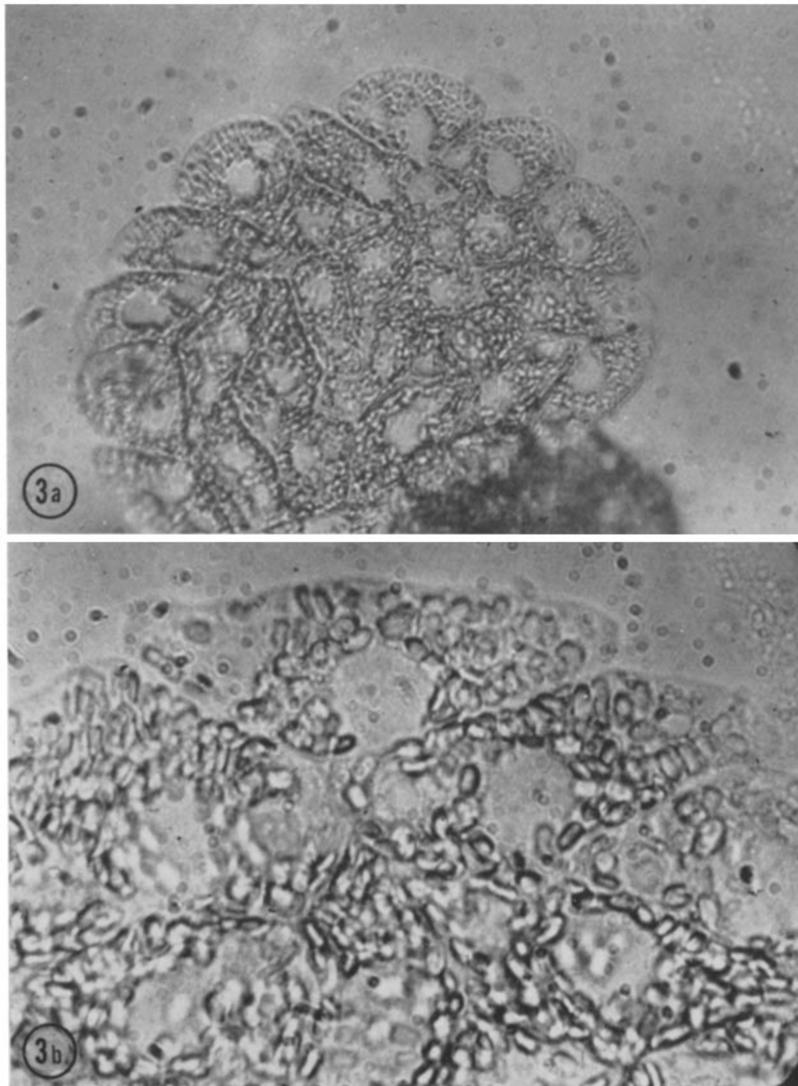

FIGURE 3

Euglena colony growing in the culture chamber. 3 *a*, colony magnified 600 times; 3 *b*, edge of colony magnified 1200 times.

shaped image which was used for irradiating the cytoplasm while shielding the nucleus was obtained by placing a quartz coverglass over the large orifice and masking the center of the orifice with a droplet of India ink.

The position of the focused UV beam in the microscope field was determined with the aid of an eye piece reticule. With the aid of the mirror (*M*, Fig. 1) the UV beam was deflected and the field illuminated with red light. Red light was used since it was established by Schiff *et al.* (4) that this part of the light spectrum does not photoreactivate the UV-inactivated plastids. The *Euglena* cell was placed in the appropriate position in the microscope field so that a selected area of it would be irradiated when the UV beam was deflected back to the stage. A shutter placed between the orifice and the mirror permitted the cell to be irradiated for a preset length of time.

2) *The culture chamber* used in this study is depicted in Fig. 2. These chambers permitted the immobilization of the *Euglena* cells and also observations on the individual irradiated cell and its progeny. The culturing procedure was as follows: To a standard culture medium (1) was added an equal volume of water and agar to a final concentration of 1.5 per cent. The solution was autoclaved and cooled to 40°C. A predetermined number of log phase, dark-grown *Euglena gracilis* strain z cells were added to provide a final concentration of about 2000 cells per ml. Small drops, about 0.01 ml of the suspension, were placed on the round 22-mm quartz coverslips (A. D. Jones Co., Cambridge, Massachusetts) (*q*, Fig. 2) and immediately covered with a square glass coverslip (*g*, Fig. 2). The optical density of the thin layer of culture medium was found to be about 0.3 in the 250 to 280 mμ region. This thin culture "sandwich" was placed with the quartz side down over an 18 mm hole which was drilled in a standard lucite microscope slide. The rim around the hole of the slide was greased before placing the culture chamber over it and the chamber itself was ringed with a paraffin-vaseline mixture. In this culture chamber the cells grew into colonies of about one mm in diameter. Cells which grew in contact with the glass surfaces were flattened and offered excellent opportunities for observation of the development of cells and colonies under high magnification. Fig. 3 *a* is a photograph of such a colony magnified 600 times and Fig. 3 *b* is the edge of the same colony magnified 1200 times. Note the nuclei of the cells as clear areas in the paramylum-filled cytoplasm. After irradiating the cells the slides were kept at 18°C under continuous red light of 50 to 100 foot-

TABLE I

Effect of Irradiating the Euglena Cell, Nucleus or Cytoplasm, with UV Light

Relative UV dose	Colony type	Target irradiated and size of UV beam (in μ^2) 16 Nucleus	32 Nucleus	380 Total cell	350* Cytoplasm
sec					
1/10	Normal	12	—	8	4
	Bleached	0	—	10	1
	Mixed	0	—	4	2
	Killed	0	—	4	0
1/5	Normal	34	13	5	9
	Bleached	0	0	14	4
	Mixed	0	0	1	2
	Killed	1	1	8	1
1/2	Normal	40	11	0	3
	Bleached	0	0	9	9
	Mixed	0	0	1	8
	Killed	7	6	10	5
1	Normal	24	15	0	3
	Bleached	0	0	1	12
	Mixed	0	0	0	4
	Killed	5	8	10	3
2	Normal	11	8	0	0
	Bleached	0	0	0	4
	Mixed	0	0	0	1
	Killed	2	7	5	9
5	Normal	7	1	0	0
	Bleached	0	0	0	1
	Mixed	0	0	0	0
	Killed	6	10	6	7
10	Normal	5	0	0	—
	Bleached	0	0	0	—
	Mixed	0	0	0	—
	Killed	9	4	2	—
Total number of cells irradiated		163	84	98	92
Total bleached or mixed colonies		0	0	40	48

* Doughnut-shaped beam, dark center shielding the nucleus.

candles (determined with a General Electric selenium light meter). The developing colonies were examined on the 3rd day after irradiation and again on the 6th day. Colonies were scored as either normal green, bleached, or mixed, the latter when they contained both types of cells. Cells which failed to divide or divided only once or twice were classified as killed cells. The results of the irradiation of more than 400 cells are summarized in Table I. Irradiation of the nucleus, only, never resulted in the appearance of bleached cells. High doses of UV light given to the nucleus inhibited further cell division, but even in these "biologically killed" cells the proplastids could enlarge and green.

Schiff, Lyman, and Epstein (4) ingeniously demonstrated the separation of the phenomenon of plastid greening from the inherited ability of the cells to form green colonies. They observed that dark-grown cells which were irradiated with UV light were capable of greening when kept under red light. After greening, these cells when grown on agar gave only bleached colonies. Our observations indicate that the proplastids of the UV-treated cells are capable of developing to chloroplasts even when the nucleus of the cell is damaged and made incapable of further divisions. Perhaps in these cells the greening in red light is due to a store of enzymes sufficient for the differentiation of one generation of plastids.

Irradiation of the entire cell or the cytoplasm while shielding the nucleus (Table I) resulted in a considerable number of bleached colonies. In several cases the irradiation of isolated regions of the cytoplasm with the 32 μ^2 beam was attempted, while avoiding the nucleus. At no time were bleached cells observed to arise from a cell that was irradiated at one or two spots in the cytoplasm. However, when five or more such areas of the cytoplasm were irradiated, mixed colonies containing green cells and bleached cells, but not totally bleached colonies, were occasionally observed. Apparently a portion of unirradiated cytoplasm is sufficient to restore the capacity of the cell to produce green progeny.

It is concluded from these experiments that the location of the presumed nucleoprotein particles which affect the differentiation of the proplastids is in the cytoplasm and not in the nucleus. Recent findings of the presence of RNA in the plastids, and possibly also DNA (5–7), lend support to the hypothesis that the plastids themselves may be the bearers of these genetic factors. Proof that the UV-sensitive particles are localized specifically within the plastids is still lacking.

SUMMARY

When *Euglena* cells were irradiated only in the nuclear region with a UV microbeam apparatus, no bleached progeny were observed. Irradiation of the entire cell or only the cytoplasm caused bleaching of the progeny of the irradiated cells. It is concluded that the UV-sensitive factors which are concerned with the differentiation of the proplastids to chloroplasts are localized in the cytoplasm and not in the nucleus.

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