STUDIES OF CHLOROPLAST DEVELOPMENT IN *EUGLENA*

XIII. VARIATION OF ULTRAVIOLET SENSITIVITY WITH EXTENT OF CHLOROPLAST DEVELOPMENT

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ABSTRACT Ultraviolet (UV) inactivation of green colony-forming ability of several different types of *Euglena gracilis* var. *bacillaris* was studied. The observed target numbers are not widely different, while the doses required to produce a single inactivation event (D_o) vary with the type of cell used. In dark-grown cells adapting to the light in resting medium and in an X-ray-induced mutant, D_o is proportional to the chlorophyll content of the cells. However, in hyperdeveloped cells which contain abnormally high amounts of chlorophyll, the correlation does not hold, suggesting that it is not chlorophyll per se which is responsible for the differences observed. D_o's of colony-forming ability (viability) of light-grown and dark-grown cells are found to differ by the same factor as those of green colony-forming ability. Stationary phase and exponential phase cells show a small difference in D_o with no obvious difference in target multiplicity. The multiplicity of the various target curves has been re-evaluated by computer and found to be between 30 and 40.

INTRODUCTION

Previous work (1) indicated that the two effects of ultraviolet (UV) light could be distinguished in *Euglena gracilis* var. *bacillaris*. Low doses of UV impaired the cell's ability to transfer chloroplasts and proplastids to its progeny, resulting in colorless clones. Higher doses produced cell death.

UV-inactivation curves of green colony-forming ability in light-grown and dark-grown cells were found to have multiplicities of about 30 in agreement with the observed numbers of proplastids in the dark-grown cells (1). In both cases, the UV inactivation was completely reversible by photoreactivation (2), but the UV doses required to produce a single inactivation event were found to differ by a factor of about 1.7 for the light-grown compared with the dark-grown cells (1).

In this paper, we detail further experiments which bear on the differences in UV sensitivity between light-grown and dark-grown cells.

METHODS

The parent strain was *Euglena gracilis* var. *bacillaris*. This strain was originally obtained from Dr. Seymour Hutner of the Haskins Laboratories, New York City (1, 3). Dark-grown cells (1) contain proplastids and protochlorophyll, but lack chlorophyll (4); light-grown cells contain chlorophyll.

P_1BXL is a mutant obtained by X-irradiation of the light-grown parent strain. It produces less chlorophyll per cell when grown in the light (5) and has abnormal chloroplasts as judged by fluorescence and electron microscopic observations.

Hyperdeveloped cells (HDC) were obtained by the method of Stern (5). They contain abnormally large amounts of chlorophyll and display hyperdevelopment of photosynthetic 0_2 evolution and CO_2 fixation.

All cells [except HDC which are in resting medium (5)] were maintained on Hutner's medium and were plated as previously described (1).

Pigments were extracted and determined as described previously (4).

UV-irradiation experiments were conducted in approximately the same manner as previously described (1) except that suspensions of cells were irradiated in 3.4 ml of resting medium, and in most experiments, a 4 w germicidal lamp (General Electric G4T4) with a rated output of 8 mw/ft^2 at a distance of 3 ft was used.

In survival experiments, plates were incubated under nonphotoreactivating conditions after UV.

The computer program used (6, 7) was designed to fit a variety of models and functions. The data could be compared with the desired model (in this case, a multitarget equation) by an iterative process, and the best least squares fit could be obtained. The UV-inactivation data were compared with the equation

$$G = 1 - (1 - e^{-D/D_0})^n \quad (1)$$

Where G is the expected fraction of green colonies, D_0 is a parameter which varies with the cells used, D is the UV dose in ergs/mm^2, and n is the multiplicity of the inactivation curves. The machine used was an IBM 7090 digital computer located at the National Bureau of Standards, Washington, D. C.

RESULTS AND DISCUSSION

Variation of Sensitivity with Chlorophyll Content. Fig. 1 shows the curves for UV inactivation of green colony–forming ability or initially dark-grown cells at various stages of chloroplast formation. For these experiments, dark-grown cells were incubated in resting medium for 3 days before use (under these conditions, there is no further growth). At zero time and after various times of light exposure, these cells were exposed to a range of UV doses and were plated under nonphotoreactivating conditions. After 9 days, the colonies were scored as green or white. The dose required for a single inactivation event (D_0) changes progressively as chloroplast development proceeds, spanning the extremes of light-grown and dark-grown cells. An obvious parameter to be correlated with this change is the amount

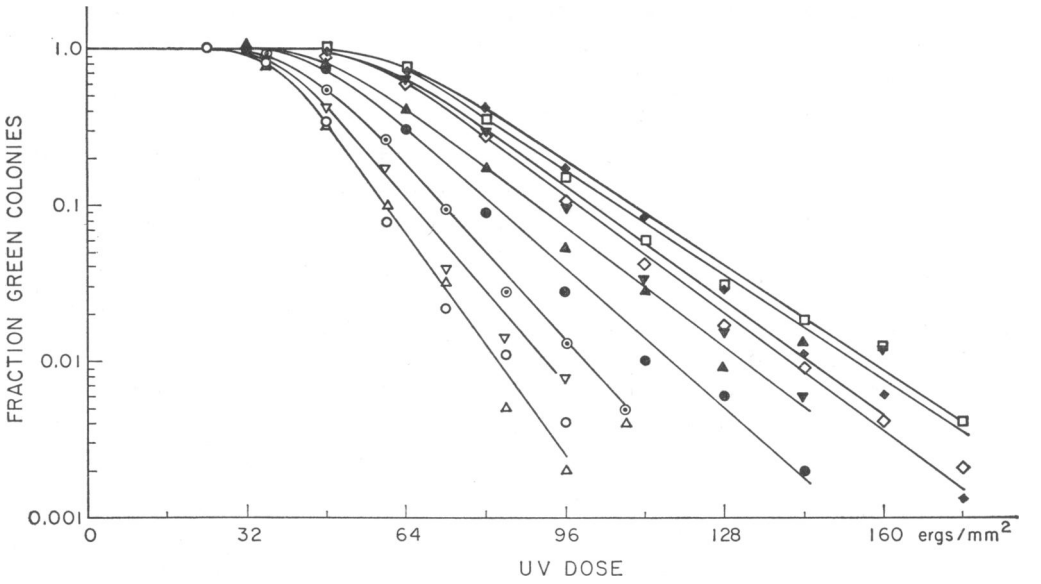

FIGURE 1 The change in sensitivity to UV of green colony–forming ability when dark-grown cells are placed in the light. Dark-grown cells were incubated in resting medium in the dark for 3 days. At 0 time, they were placed in light of about 150 ft-C. Aliquots were irradiated at the following times: ○ 0 time (before exposure to light), △ 8 hr, ▽ 16 hr, ⊙ 24 hr, ● 36 hr, ▲ 48 hr, ▼ 60 hr, □72 hr, ◆ 84 hr, ◇ 108 hr.

of chlorophyll which is increasing progressively during this time. Fig. 2 shows the similarity between the kinetics of the two processes while Fig. 3 shows a linear correlation between amount of chlorophyll synthesized and the variation in D_0 for inactivation of green colony formation.

This correlation also holds for a mutant of *Euglena,* P_1BXL, with impaired chlorophyll-synthesizing ability, as shown in Fig. 4 and Table I. Amounts of chlorophyll per cell can be changed by lighting conditions (5) and HDC which contain more chlorophyll than normal can thereby be prepared. Fig. 5, which compares chlorophyll and D_0 for HDC and normal light-grown cells, shows that the correlation found before does not hold in this instance, since the D_0's are essentially the same while the chlorophyll is nearly 70% higher in the HDC.

It appears, then, that chlorophyll per se is not causally related to the differences in D_0 between light-grown and dark-grown cells. This is further borne out by the finding (Fig. 6) that UV inactivation of cell viability at higher UV doses shows that same difference in sensitivity as does green colony–forming ability. Since the effect of UV on the green colony–forming system is thought to be cytoplasmic and is probably localized in the plastids (1, 8) while loss of viability is doubtless due to nuclear events, we must visualize a more generalized mechanism for the differences in sensitivity. If the differences are only apparent (i.e. if the actual target

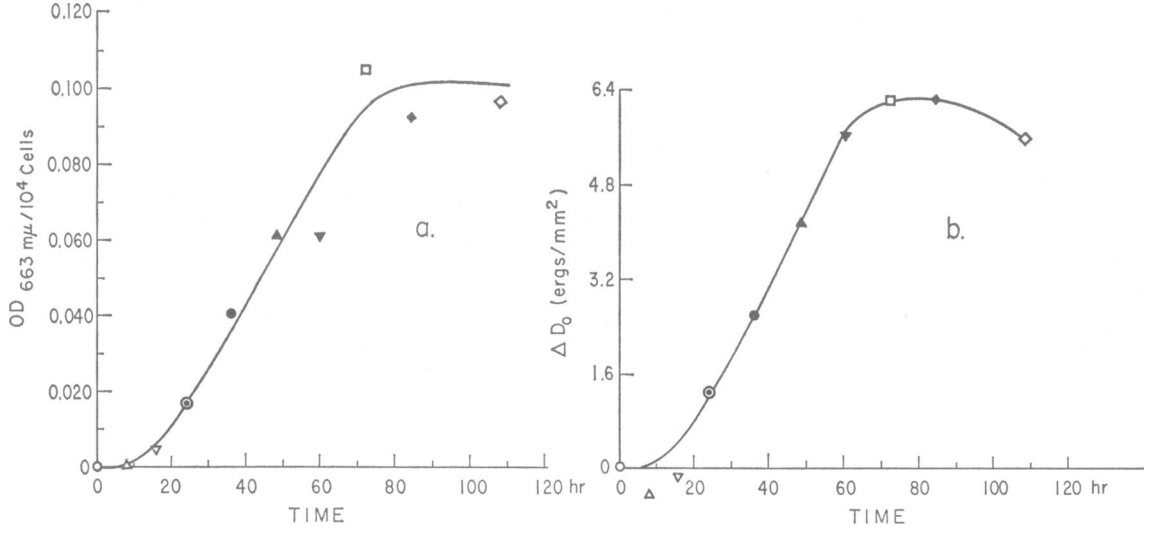

FIGURE 2(*a*) Chlorophyll synthesis in dark-grown cells placed in the light. Aliquots of the suspension used in the experiment in Fig. 1 were analyzed for chlorophyll at the times indicated. (*b*) The change in $D_o(\triangle D_o)$ of dark-grown cells placed in the light. D_o's were estimated from the straight line portions of the curves in Fig. 1. $\triangle D_o$ is obtained by subtracting the dark-grown D_o from those of the light-adapting cells.

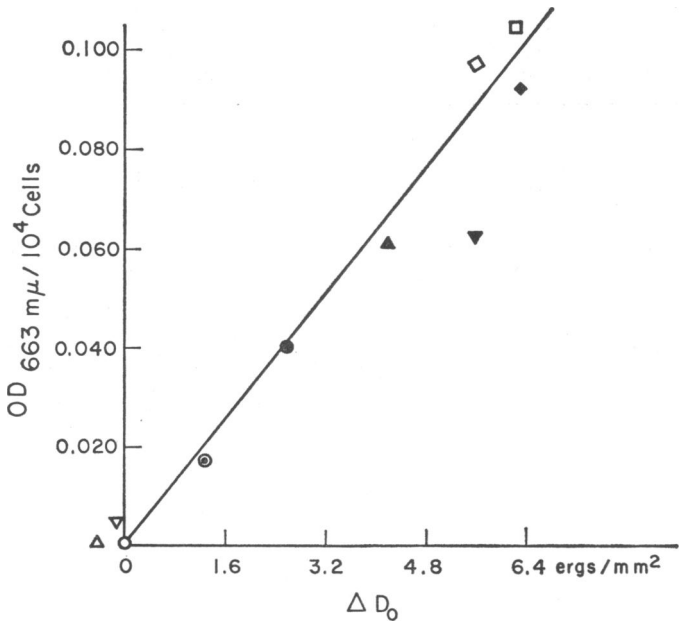

FIGURE 3 The relationship of chlorophyll per cell to $\triangle D_o$.

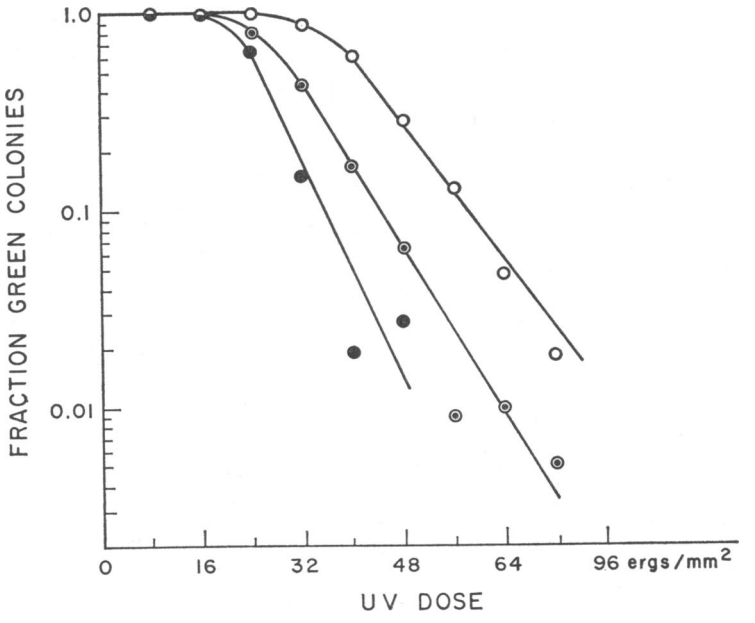

FIGURE 4 The relative sensitivities to UV inactivation of green colony-forming ability in the parent strain (*E. gracilis, b.*) and the mutant P_1BXL. Dark-grown parent strain ●, light-grown parent strain ○, P_1BXL ⊙.

TABLE I

THE RELATIONSHIP OF THE UV SENSITIVITIES OF LIGHT-GROWN *EUGLENA* AND THE MUTANT P_1BXL TO THEIR CHLOROPHYLL CONTENT

	Dark-grown parent strain	Light-grown		
		P_1BXL	Parent strain	Δ Parent strain/ΔP_1
D_0 *ergs/mm²*	5.6	8.8	9.2	
ΔD_0	—	2.2	3.6	1.6
Chlorophyll $OD_{663}/5 \times 10^5$ cells	—	0.215	0.310	1.4

D_0's were estimated from Fig. 4. The ratios in column 4 are obtained by dividing the values in column 3 by the values in column 2.

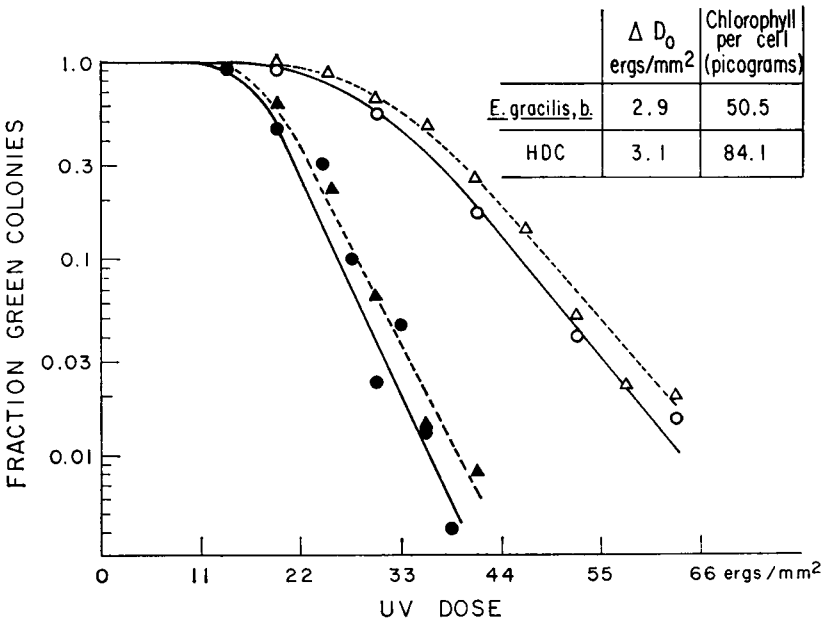

FIGURE 5 The relative sensitivities to UV inactivation of green colony-forming ability in the parent strain and HDC. Dark-grown (-●—●-) and light-grown (-○—○-) parent strain; dark-grown (-▲—▲-) and light-grown (-△—△-) HDC.

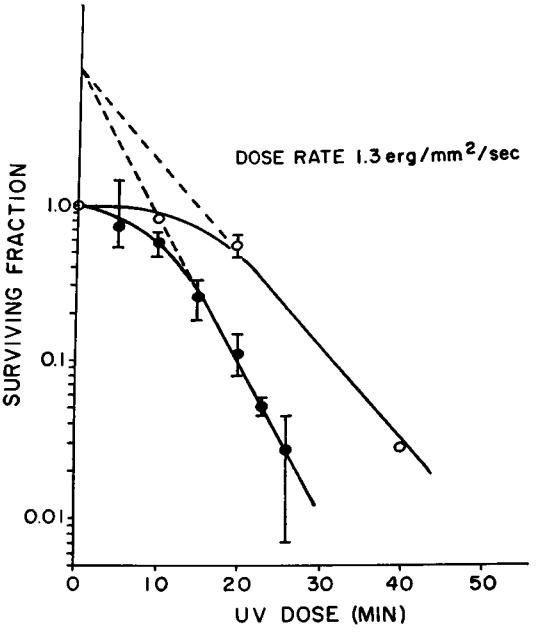

FIGURE 6 Colony-forming ability of dark-grown (●) and light-grown (○) cells.

has not changed in sensitivity but there has been the interposition of absorption or scattering material between the source and the target) this must reside at or near the membrane of the cell to affect plastids and nucleus in the same way. While the absorbing or scattering material is apparently not chlorophyll, it is probably some other substance which is related to the photosynthetic apparatus but which is not present in abnormal amounts in hyperdeveloped cells. Brawerman et al. (9) have shown that RNA and protein increase about 40% over the dark controls in non-growing cells induced to form chloroplasts. Most of this new material appears in the plastid fraction, the rest in the supernatant and microsome fractions. On the other hand, ergosterol, which has a large UV absorption cross-section is not associated with the chloroplasts of *Euglena* (10) but is present in larger amounts in light-grown than in dark-grown cells, and could also serve as a UV filter. The killing curves presented in Fig. 6 are typical of several such experiments and indicate multiplicities of about 8 suggesting that this strain of *Euglena* is polyploid and most probably octaploid.

Differences in Sensitivity During Growth of Cells. Fig. 7 compares the UV sensitivities of dark-grown cells in the exponential and stationary phases of growth. There is a small difference (about 1.2) in the D_0 values for the two conditions but no obvious change in multiplicity.

Re-evaluation of Multiplicities for Inactivation of Green Colony-Forming

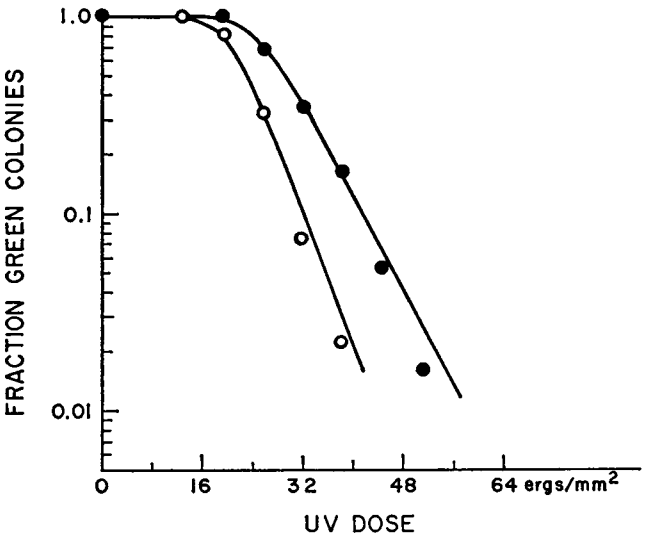

FIGURE 7 The sensitivity to UV inactivation of green colony-forming ability in exponential (●) phase and stationary (○) phase cells.

Ability. With the accumulation of further data it has been possible to undertake a statistical analysis of the value of n, the multiplicity, in the multitarget equation as applied to inactivation of green colony-forming ability and to thus obtain a better estimate than was available from our earlier data (1). A total of 20 (and in one case, 28) curves were analyzed by computer including the curves shown in the preceding portions of this paper, as well as others. The method of weighting the data points can have considerable influence on the results, and, in the program used, several methods were available: (*a*) the standard deviation of each point could be used to calculate a weight based on the reciprocal of the variance; (*b*) equal per cent variation could be allowed for each point, i.e. a point with a value of 1.0 might have a variation of 0.1, while a point with a value of 0.1 would have a variation of 0.01; (*c*) equal weight could be put on each point (all standard deviations were thereby set at 0.01). The second method of weighting effectively puts larger weight on points with smaller values as compared with the other methods. It was considered that the first method, using the standard deviations was the one of choice, although, in practice, the third did not differ greatly from the first.

In the first computation, using method (*a*) for weighting, each of 20 curves was fitted to equation (1) and D_0 and n were calculated for each. The computer gave a standard deviation along with each result, and this was used to obtain a weighted mean and standard error. The average value of n thus calculated was 40.3 ± 3.7. Using the second method of weighting the data, but everything else the same, the average value for n was computed at 29.8 ± 4.1. In another computation, 28

curves (8 additional ones) were analyzed with equal weight on each point. The weighted average value for n and its standard error were found to be 43.5 ± 10.5. Since it is very difficult to count the proplastids in dark-grown cells, the microscopical estimate of 30 is in reasonable agreement with the computer values for the number of UV sensitive entities.

Petropulos (11) estimated target numbers of about 60 for synchronous cells of the *Z* strain of *Euglena gracilis* and quotes the results of Cook (12) on synchronous cells of the *bacillaris* strain to support this high number. The values presented above, calculated from many experiments, indicate that the variation in the data is great enough to make the interpretation of individual experiments ambiguous. In addition, Petropulos has apparently not considered the evidence (13) that in some strains of *Euglena,* chloroplast division is not synchronous with cell division, and may occur before or after cytokinesis. Since Petropulos used synchronized cells in his studies, it is possible that his target curves represent multiplicities for cells which have a double chloroplast compliment as a transient stage in the division process.

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