Ultraviolet Inactivation of Chloroplast Formation in Synchronously Dividing Euglena gracilis

Abstract. *Ultraviolet inactivation of chloroplast formation was studied in synchronously dividing cultures of* Euglena gracilis. *Sensitivity to sublethal doses given at intervals throughout the cell cycle was greater just before cell division than during division. There was approximately a twofold difference in the dose-response relationships for the periods of high and low sensitivity.*

Ultraviolet irradiation at sublethal doses bleaches *Euglena*, and its effect on chloroplast formation has been studied extensively (*1*). Target theory analysis has been applied to an investigation of this problem. These studies (*2*, *3*), which included inactivation by ultraviolet light, and fluorescence and electron microscopy, led to the formation of a general model in which self-replicating, cytoplasmic, nucleoprotein, precursor bodies are the presumed targets of the radiation. Ultraviolet radiation is believed to act by preventing the replication of these units at cell division. The number of targets involved—around 30—was compared to the "several times 10" small precursor bodies (the proplastids) which are visible in fluorescence micrographs of dark-grown cells. Observations of these proplastids at different times after dark-grown cells are returned to light showed that they lengthen, presumably by coalescence. Eventually lamellation and pigment production occur, and the mature chloroplast develops.

Certain features of the model remain unclear, for example, the correspondence between the number of precursor bodies, the number of chloroplasts in a cell, and the number of ultraviolet-sensitive targets involved in inactivation. The sequence of events during the coalescence of precursor units and the question of whether the process of chloroplast development, the chloroplast cycle, is synchronized with the cell cycle are also of interest.

The model implies that sensitivity to ultraviolet light should vary during the chloroplast cycle. First, in the proposed mechanism, the intermediary steps which include first replication and coalescence of precursor bodies, then lamellation and pigment production will probably differ in their susceptibility to ultraviolet light. Some experimental evidence for such differences has been reported. When *Euglena gracilis* is grown for a number of generations in the dark, cells contain protochlorophyll and carotenoids but lack chlorophyll completely. They are more sensitive to ultraviolet than light-grown cells, and dose-response curves indicate that the number of targets in the inactivation process is the same. What differs is the sensitivity of the individual targets. Presumably, the targets in light-grown cells are shielded from ultraviolet by chlorophyll (*3*).

Second, the model implies that during development target number will change as a consequence of the replication of precursor bodies or the coalescence of these units or both. If target theory kinetics do apply, any change in target number should be reflected by changes in the cell's sensitivity.

Euglena gracilis "Z" strain (*4*) was grown in 2-liter flasks containing 1.5 liters of inorganic medium (*5*) in constant-temperature growth chambers at 25°C. The daily light regime consisted of 12 hours of light and 12 hours of dark. The culture was sampled every 2 hours by a clock-programmed device (*6*) which dispensed 10 ml of cell suspension into vials containing formaldehyde. The samples were counted daily with a Coulter counter. Cell counts for the 24-hour experimental period, given in the figures, show that cell division was confined mainly to the dark period. During each division period an approximate doubling in cell number occurred. A 10-ml sample was also taken and placed in a sterile petri dish for irradiation. In the first experiment (Fig. 1) this was done at 2-hour intervals throughout one division cycle, and, in the second (Fig. 2), samples were taken at a point in the cell cycle just before the division period (hour 7) and half way through the division period (hour 13). Cells were irradiated (*6*) for the desired time and 0.1-ml portions were plated by a top-agar technique (*7*). These plates were incubated in the dark for 3 to 4 days before scoring to allow those cells which were not inactivated to become green. The colonies which were white were scored as bleached, and those which were green, yellow, and sectored (consisting of green cells in the center of the colony and bleached cells at the periphery) were scored as green. Controls, placed in continuous light immediately after irradiation, contained only green colonies, indicating that the doses were sublethal and completely photoreactivable.

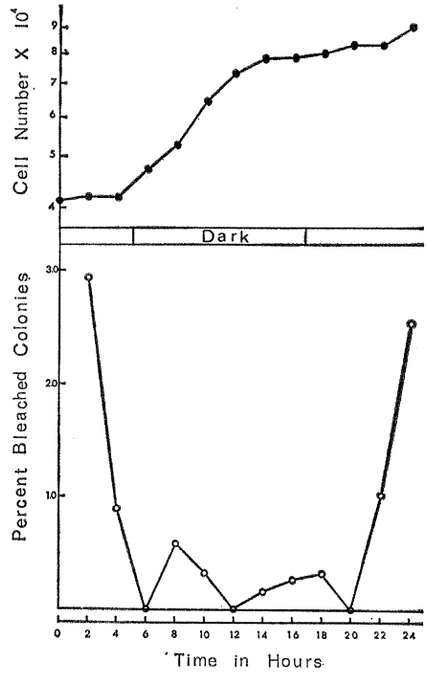

Fig. 1. In the upper portion of the figure, the number of cells per milliliter is plotted as a function of time. The light regime (12 hours light, 12 hours dark) is shown along the abscissa. Below is shown the average percentage of bleached colonies on three replicate plates from cell suspension taken at 2 hour intervals throughout the division cycle and irradiated with ultraviolet light for 0.2 minutes.

Figure 1 shows that with a low dose of ultraviolet (0.2 min) there was little or no bleaching during the division period (hours 6 through 14) or just after division (hours 14 through 20). Just prior to division (hours 2 and 4, hours 20 through 24) bleaching increased significantly.

Figure 2 shows dose-response curves for samples taken from another synchronously dividing culture at times where high sensitivity (hour 7, curve I) and low sensitivity (hour 13, curve II) were predicted from the first experiment. Compared to curve II, curve I occurs further to the left, indicating that cells irradiated just before division were more sensitive to bleaching by ultraviolet light.

The "multihit" curves in Fig. 2 show an approximate twofold difference in sensitivity in the straight-line portions of curves I and II. To evaluate how precisely the data determine the slope and intercept of these curves, values derived by a least squares analysis for goodness of fit of the experimental points were calculated for target num-

ber and slope. Just before division the target number was 67.2 [1/100 × antilog (3.870 ± 0.280)]; during division it was 78.1 [1/100 × antilog (3.8923 ± 0.374)]. The slopes for these curves were 0.4161 ± 0.0364 and 0.3664 ± 0.0204, respectively. This difference in target number indicates that the lower sensitivity to ultraviolet during division may have resulted from an increased number of entities which had to be inactivated to cause bleaching. The difference in slope indicates that the targets themselves may have been less sensitive during division. The standard deviations of these values show that target numbers and slopes are not determined precisely enough to allow a definite statement concerning the relative contribution of these factors in lowering the sensitivity of cells during division.

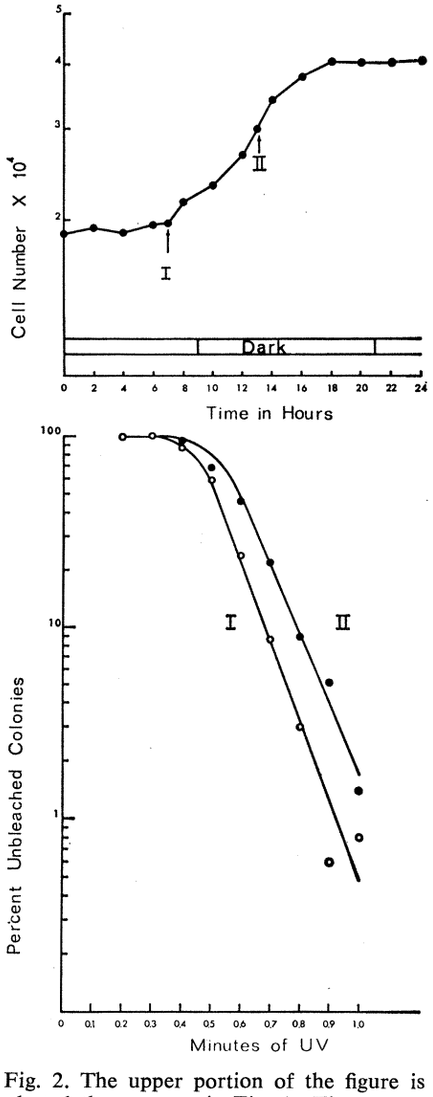

Fig. 2. The upper portion of the figure is plotted the same as in Fig. 1. The arrows mark the times at which the samples to be irradiated were removed from the culture. The lower portion represents dose-response curves for the two samples. Here, the average percentage of unbleached colonies found on four replicate plates is plotted (ordinate) as a function of UV dose (abscissa) expressed as time, in fractions of a minute, of exposure. The straight line portions of the curves are fixed by the equations derived from a least squares analysis for goodness of fit of the experimental data.

The values for target number reported here are larger than the value of 30 derived by Lyman *et al.* (*3*). Possible explanations for this discrepancy lie in the differences in culturing methods and in the strains of *Euglena gracilis* employed in the two studies. In the present work, synchronously dividing cultures of *E. gracilis* "Z" strain grown in inorganic medium were used, while exponentially dividing cultures of *E. gracilis* var. *bacillaris* grown in organic medium were used by Lyman (*3*). Although differences in ultraviolet sensitivity of the photoreactivating systems in these two strains have been reported, sensitivity to ultraviolet-induced bleaching was identical when the two strains were grown under similar conditions (*8*). Differences in culturing conditions, therefore, appears to be the more plausible explanation. The fact that values for target number obtained in studies on synchronously dividing *E. gracilis* var. *bacillaris* (*9*) are similar to the higher values obtained with *E. gracilis* "Z" strain adds support to the speculation that methods of culturing may affect target number.

Values for target number are approximations and are, therefore, subject to interpretation. Examination of the dose-response curves of Lyman *et al.* (*3*), on which the target number of 30 is based, could be interpreted as giving values of approximately 60 for dark-grown cells, and approximately 25 for light-grown cells. These figures were derived by extrapolating to the intercept of the ordinate. The value of 60 for dark-grown cells is close to the values reported here for synchronized cells. The difference in values for target numbers of light- and dark-grown cells supports the idea that culturing conditions, in this case light, can affect target number.

In relation to the model, this difference could be explained by assuming that the uncoalesced proplastids, known to be present in dark-grown cells, act as individual targets, giving higher target numbers. In light-grown cells the precursor units have coalesced and are incorporated in the mature chloroplast. To explain the high values obtained with synchronized cells a process analogous to the one occurring when cells are dark-grown might also occur during the chloroplast cycle; that is, a number of precursor units might be present and act as individual targets.

In summary, the sensitivity of synchronously dividing *Euglena gracilis* "Z" strain to ultraviolet-induced bleaching varies as a function of the time in the division cycle when the cells are irradiated. Sensitivity per cell is greater just before the onset of division in the population than during division. Two factors may contribute to the lower sensitivity at division. A large proportion of the cells in a synchronized population at this point in the division cycle have a high target number resulting from the replication of proplastids during division. In addition, the targets themselves may be less sensitive to ultraviolet action as a consequence of their physiological and morphological states at this stage. It appears that events in the chloroplast cycle are synchronized with the cell cycle and the use of synchronously dividing cultures in ultraviolet inactivation studies may serve as a tool in further elucidating the processes taking part in the formation of chloroplasts.

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References and Notes

1. M. De Deken-Grenson and A. Godts, *Exptl. Cell Res.* **19**, 376 (1960); A. Gibor and S. Granik, *J. Protozool.* **9**, 327 (1962); H. Lyman, H. Epstein, J. Schiff, *ibid.* **6**, 264 (1959); E. Pringsheim, *Rev. Algol.* (New Series) **4**, 41 (1958).
2. J. Schiff, H. Lyman, H. Epstein, *Biochim. Biophys. Acta* **50**, 310 (1961); ———, *ibid.* **51**, 340 (1961); H. Epstein and J. Schiff, *J. Protozool.* **8**, 427 (1961).
3. H. Lyman, H. Epstein, J. Schiff, *Biochim. Biophys. Acta* **50**, 301 (1961).
4. Kindly supplied by Dr. S. H. Hutner, Haskins Laboratories, New York.
5. M. Cramer and J. Myers, *Arch. Mikrobiol.* **17**, 384 (1952).
6. S. F. Petropulos, *Science* **145**, 268 (1964).
7. The model C-81 Black Light (Eastern Corp.) emits 163 $\mu w/cm^2$ at 2537 Å at 45 cm. In my experiments the distance between the lamp and the culture being irradiated was 53 cm.
8. The bottom agar consisted of inorganic salt medium (*5*) supplemented with 5 g of sodium acetate per liter and 15 g of Bacto agar per liter. The top agar was incubated in test tubes at 35°C at which temperature it was liquid. The irradiated sample (0.1 ml) was added to 5 ml of top agar, stirred, and then plated on dishes containing 15 ml of bottom agar at 25°C. At this temperature the top agar solidified.
9. J. R. Cook, *Photochem. Photobiol.* **2**, 407 (1963).
10. S. F. Petropoulos, unpublished data.
11. I thank Drs. C. S. Pittendrigh and V. G. Bruce for their interest and guidance. Supported by a predoctoral fellowship from the National Institutes of Health, and by funds from grants to C. S. Pittendrigh from the Fannie E. Ripple Foundation and the Air Force Office of Scientific Research.

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