PRELIMINARY NOTES

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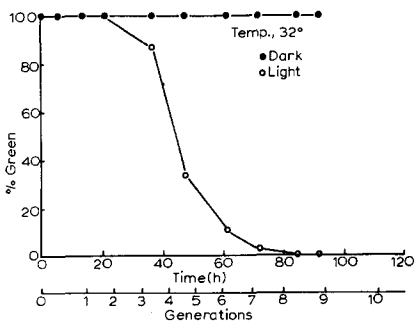
Light dependence of temperature-induced bleaching in *Euglena gracilis*

Euglena gracilis has been found to be sensitive to a wide range of exogenous physical and chemical agents that induce specific and permanent bleaching. We define bleaching to mean the permanent loss of the ability to form green colonies upon plating. This is taken to mean the loss of the ability of the chloroplast to be replicated. Growth at elevated temperatures[1] and streptomycin[2] were among the earliest agents observed to cause this loss. BRAWERMAN AND CHARGAFF[3] proposed that chloroplast synthesis and function was unimpaired at elevated temperatures but chloroplast replication was inhibited. They also suggested that chloroplast replication was autonomous and controlled by a factor that was affected by elevated temperatures. These deductions were based on the kinetics of chloroplast formation during growth at elevated temperatures and recovery upon return to room temperature. With the availability of quantitative plating methods for Euglena[4] it has become possible to



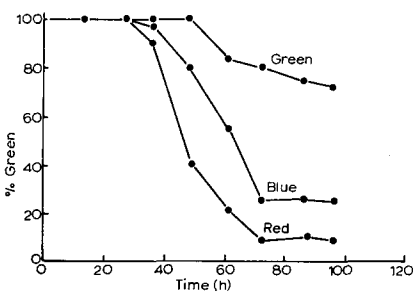


Fig. 1. Kinetics of light-dependent temperature-induced loss of the ability to form green colonies at 32°. *E. gracilis*, var. *bacillaris*, Strain Z (Pringsheim) was grown on a defined organic medium[9] at 32° in a rotary water-bath shaker at 120 rev./min, illuminated with cool white fluorescent lamps at about $5 \cdot 10^3$ ergs/cm^2/sec or in darkness. Aliquots were taken at intervals for cell counting and plating. Generation times shown were the same for both light- and dark-grown cultures. Plating was done by the method of LYMAN *et al.*[4]. Plates were incubated in the light at 25° and scored by counting green and white colonies. Plates preincubated in the dark for several days prior to incubation in the light showed similar results. Control cultures grown at 26° and treated similarly yielded all green colonies. There was no loss of viability in the cultures at 32°.

Fig. 2. Kinetics of light-dependent temperature-induced loss of ability to form green colonies at 32° in red, green and blue light. The filters were made from 0.5-cm-thick plexiglass sheet (Rohm and Haas No. 2444, 2092 and 2424 for red, green and blue, respectively). Illumination was provided by a series of 150-W cool-beam flood lamps with the light intensity adjusted to about $5 \cdot 10^3$ ergs/cm^2/sec for each color. Adjustments were made in the final calculations for approximately equal numbers of quanta at the peak wavelength for each filter. The red filter gave essentially no transmission below 650 nm; the green and blue filters had maximum transmissions at 525 and 470 nm, respectively. Cells were grown and treated as in Fig. 1. Growth rates for all cultures were essentially the same.

examine more closely the progress of temperature-induced bleaching on a dynamic basis. We report here that temperature-induced bleaching at 32° requires light.

Fig. 1 shows the kinetics of loss of the ability to form green colonies at 32°. Loss in the light begins at about the end of the third cell generation, progresses almost linearly to about the sixth generation, and is essentially complete by the end of the eighth generation. No bleaching was seen in cells grown in the dark at this temperature. There was no difference in growth rates of light- and dark-grown cultures at 32°, and the growth rates were similar at 26° where there was no bleaching. Similar results were obtained at 34° in the light. Dark-grown cells at 34° showed some bleaching, but this was delayed and occurred more slowly than in the light. There was no growth and no bleaching at 37°.

It is well known that light mediates the formation of chloroplasts from proplastids and chlorophyll synthesis may be a necessary part of this development. The light-induced synthesis of chloroplasts from their precursors, the proplastids, was measured at 32° using nondividing cells with chlorophyll synthesis as an index of chloroplast synthesis. Chlorophyll and carotenoid synthesis by dark-grown cells[5] placed in the light at 32° was found to be essentially the same as at 26° (Table I), and plating showed that these resting cells were not bleached. This experiment and others on resting cells indicate that chloroplast synthesis is not affected by elevated temperatures but that growth is necessary to obtain bleaching.

The light requirement for temperature-induced bleaching at 32° implies the existence of a chromophore that promotes the effect of temperature. A preliminary survey of the effectiveness of broad areas in the red, green and blue regions of the spectrum is shown in Fig. 2. Red or blue light appear to be almost as effective as white light, while green light is relatively ineffective. The partial bleaching in green light may be the result of overlap between the transmissions of the green and blue filters.

TABLE I

LIGHT-INDUCED PIGMENT FORMATION BY *E. gracilis* AT 32°

Flasks containing defined organic medium were inoculated with dark-grown *E. gracilis* and allowed to grow to near stationary phase in the dark at 26° and then transferred into the light at 26° and 32° under the conditions given for Fig. 1. Little cell division was observed. Samples were taken at intervals for cell number, total chlorophyll and carotenoid determinations and plating. Plating procedures were as for Fig. 1. Chlorophyll was determined by the method of ARNON[10] and carotenoids by the method of KIRK AND ALLEN[11].

Time (*h*)	*Temp.*	*Chlorophyll* (*pg/cell*)	*Carotenoid* (*pg/cell*)	*Green colonies* (%)
0	26°	0	0.31	100
	32°	0	0.30	100
20	26°	0.7	0.5	100
	32°	1.8	0.7	100
45	26°	2.5	1.7	100
	32°	3.5	1.1	100
73	26°	5.8	2.0	100
	32°	5.8	1.5	100
95	26°	7.3	2.0	100
	32°	6.8	1.6	100
141	26°	8.9	2.5	100
	32°	7.9	2.4	100

Kinetics similar to those for temperature-induced bleaching were observed by SCHIFF *et al.*[6] for the loss of photoreactivability of ultraviolet-inhibited chloroplast replication in Euglena. Their calculations indicate the loss by dilution of 10–12 particles which happens to coincide with the number of chloroplasts. We have noted a precipitous drop in the number of targets responsible for ultraviolet-induced bleaching during growth in the light at 32° as well as a proportional drop in photoreactivability. We are investigating the possible relation between the loss of ultraviolet-sensitive sites and the sites of light-dependent temperature-induced bleaching. Cells derived from heat-bleached cultures have been shown to lack the characteristic satellite DNA that has been attributed to the chloroplast[7]. On the other hand, it has been shown[8] that heat-bleached cells can incorporate δ-aminolevulinic acid and form fluorescent sites. Whether these sites represent proplastid-like structures remains to be shown.

We are continuing to examine the effects of elevated temperature on the ability of Euglena to form green colonies and the role of light in these effects. A detailed action spectrum of the light effect is in progress coupled with biochemical analyses to identify the cellular sites involved in temperature-induced bleaching. An electron and fluorescence microscopic examination of cells during temperature-induced bleaching is also in progress.

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The identity of X in $F_1 \cdot X$ with F_c

Recently VALLEJOS *et al.*[1] described the isolation and the properties of a coupling factor which they called $F_1 \cdot X^*$. This soluble factor, which contains a Mg^{2+}-dependent, oligomycin-insensitive and cold-labile ATPase, resembles very much the soluble ATPase F_1, described by PULLMAN *et al.*[2]. The major difference between the two

* VALLEJOS *et al.*[1] called the factor F_1–X. Since, however, this might imply a covalent bond between F_1 and X, we prefer to refer to it in this paper as $F_1 \cdot X$, the central dot implying an association between F_1 and X.