

# Phototactic Responses of Cell Population to Repeated Pulses of Yellow Light in a Phytoflagellate *Cryptomonas* sp.<sup>1</sup>

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## ABSTRACT

Positive phototaxis in cell populations of a phytoflagellate *Cryptomonas* sp. was recorded photoelectrically when the duration and intensity of repeated pulses of monochromatic yellow light (570 nm) interspersed with darkness were varied. Irrespective of the duration of the light pulses, phototactic responses to repeated pulses were as great as those to continuous irradiation and were linearly dependent on the logarithm of total incident light energy when the dark interval was shorter than 60 milliseconds. Under these conditions, reciprocity between duration and intensity held well. In contrast, when the dark interval exceeded 250 milliseconds, the responses were remarkably reduced regardless of light duration and were not affected by increasing the intensity of actinic light pulses.

The present results clearly indicate that continuous stimulation with actinic light is not essential for the maximum effect, but that the length of dark interval is crucial in phototactic response.

early log phase of growth, containing about  $1 \times 10^5$  cells ml<sup>-1</sup>, were used throughout.

**Measurement of Phototactic Response to Repetitive Pulses of Light.** Phototactic responses of cell suspensions were measured with a photoelectric measuring apparatus as reported previously (10). Repeated pulses of light with various durations and with varying dark intervals were provided by chopping monochromatic light of 570 nm, which had been determined as the peak of the action spectrum of phototaxis in the material used (10), using a Jasco CRM-FL spectroirradiator with a rotating sector. The sector was driven by a speed-controllable induction motor (maximum output 25 w, 70-1,400 rpm; 4IK25RGK-A, Oriental Motor Co. Ltd., Tokyo) in combination with either speed-reducing gear heads 4GK-3 or 4GK-50 (Oriental Motor Co., Ltd.). The speed of revolution of the motor was monitored with an electric tachometer (PARM-2000A, Oriental Motor Co., Ltd.). The intensity of the stimulus light was adjusted with neutral density filters (Hoya Glass Co., Ltd., Tokyo) and was measured with a radiometer (YSI-Kettering, model 65).

## RESULTS

**Effects of Frequency of Repeated Light Pulses.** Actinic light of  $0.5 \text{ w m}^{-2}$  at 570 nm was chopped with a rotating sector at a frequency range between 0.125 and 32 Hz so that the duration of the light pulses was equal to that of the dark intervals. Responses of the cell population to the light pulses were compared with the response to continuous light of  $0.25 \text{ w m}^{-2}$ , a total incident energy per unit time the same as that of the repeated stimuli (Fig. 1).

The data clearly show a frequency dependence of the phototactic response: the responses at 16 and 32 Hz were as great as that to the continuous stimulus, while those at low frequencies (2 and 1 Hz) were reduced to approximately 20% of that of the continuous stimulus. In the range between 0.5 and 0.125 Hz, the lower the frequency the greater were the responses. As the incident light energy for a constant period of time was equal in all cases, the diverse responses (Fig. 1) are attributable to the different frequencies of the light-dark cycles.

**Reciprocity in Cycles with a Period of 32 msec.** The effect of the dose of actinic light on phototactic response was measured by varying the duration of light pulses (1, 2, 4, 8 and 16 msec) in fixed cycles with a period of 32 msec. The data in Figure 2 show that the phototactic response is linearly dependent on the logarithm of the duration of the light pulses at this higher frequency.

The effects of varying the intensity and duration of each light pulse were then tested at different total energies, and with various dark intervals, in fixed cycles with a period length of 32 msec (Table I). The phototactic responses correlated with the total dosages given for a unit period of time, so that the Bunsen-Roscoe reciprocity law holds in these data.

**Effects of Dark Intervals.** Phototactic responses to repeated pulses of light with various durations (16 msec to 1 sec) accompanied by dark intervals of different lengths (16 msec to 1 sec)

Phototactic responses of phytoflagellates have usually been analyzed using continuous light stimuli (2, 5, 6). It is important to study the effects of intermittent light stimuli on phototaxis, so that the over-all reaction can be separated into at least two subreactions, one requiring light and the other not requiring it.

Diehn (1) determined the rate of positive phototaxis in cell populations in *Euglena gracilis* as a function of the rate of repetition of the flashes, and explained the results as a resonance behavior depending on the fact that the cell rotates about once every second as it swims. Recently, Feinleib (4) found that both a single flash of blue light and repeated flashes at low frequency elicited net phototactic responses of a cell population of *Chlamydomonas reinhardtii*, and asserted that at least some cells became oriented in response to a short flash. However, the effects of the duration and intensity of the light pulses and of dark intervals on the phototactic responses of cell populations have not been analyzed quantitatively in any phytoflagellate.

The present study asks whether phototactic responses of cell populations of *Cryptomonas* sp. (10, 11) can be induced by repeated light pulses, and proceeds to study the effects of the photoreaction and dark interval on the phototactic response.

## MATERIALS AND METHODS

**Plant Material.** *Cryptomonas* sp., strain IAM CR-1, obtained from the Institute of Applied Microbiology, University of Tokyo, was grown as described previously (10). Cell suspensions in the

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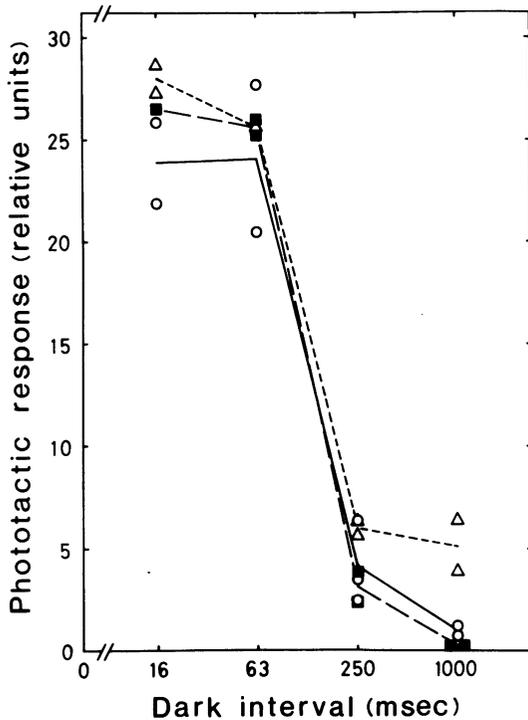


FIG. 1. Effects of the frequency of repeated light pulses on the phototactic responses of cell populations of *Cryptomonas* sp. The duration of the light pulses is equal to that of the dark intervals regardless of frequency. Light intensity was  $0.5 \text{ w m}^{-2}$ . Each point shows the value from a single measurement.

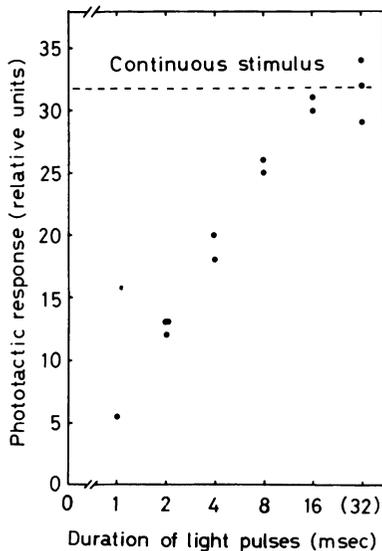


FIG. 2. Effects of durations of light pulses at about 31 Hz (cycle length = 32 msec) on phototactic responses of *Cryptomonas* sp. Light intensity was  $0.5 \text{ w m}^{-2}$ . Each point shows the value from a single measurement.

were measured to determine which of these two factors is essential in determining the level of phototactic responses (Fig. 3).

All of the responses to repeated light pulses having 16-msec, 250-msec, and 1-sec duration were fundamentally the same and were as great as that to the continuous light stimulus, provided that the dark interval was 63 msec or shorter. The responses were significantly reduced, regardless of the duration of the light pulses, if the dark interval was 250 msec or longer. It is evident that the length of the dark interval, rather than the duration of the light

Table I. Correlation of the phototactic response of *Cryptomonas* sp. to the total incident light energy.

The light was delivered per cycle at various durations of light pulses of about 31 Hz (cycle length = 32 msec).

Dose per cycle	Intensity of light	Duration		Phototactic response <sup>1</sup>
		Light	Dark	
$\text{mJ m}^{-2}$	$\text{w m}^{-2}$	msec		relative units
16.0	0.50	32	0	$31.7 \pm 2.5$
2.0	2.00	1	31	$23.5 \pm 0.5$
2.0	1.00	2	30	$17.5 \pm 1.5$
2.0	0.50	4	28	$19.0 \pm 1.0$
1.0	1.00	1	31	$14.2 \pm 2.2$
1.0	0.50	2	30	$13.4 \pm 1.8$
0.5	0.50	1	31	$5.5 \pm 0.0$
0.5	0.03	16	16	$5.5 \pm 0.6$

<sup>1</sup>Mean of two measurements  $\pm$  SE.

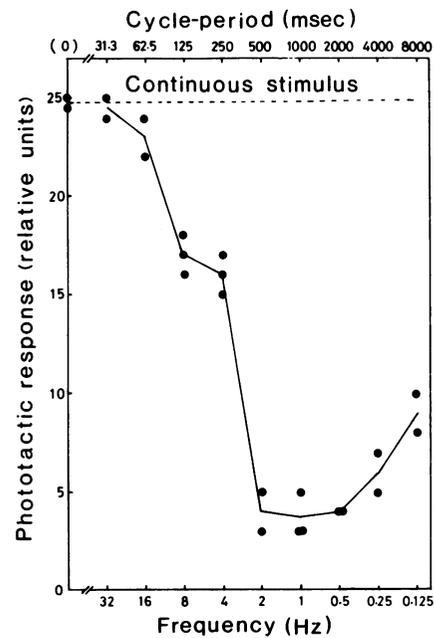


FIG. 3. Effects of dark intervals on phototactic responses of cell populations in *Cryptomonas* sp. Light pulses of monochromatic yellow light ( $0.5 \text{ w m}^{-2}$ ) were provided at durations of 16 msec (○—○), 250 msec (■—■), or 1 sec (Δ—Δ). Each point shows the value from a single measurement.

Table II. Correlation of the phototactic response of *Cryptomonas* sp. to the dark interval between light pulses at various levels of total incident light energy.

The light was delivered per cycle of about 1.85 Hz (cycle length = 540 msec).

Duration		Intensity of light	Dose per cycle	Phototactic response <sup>1</sup>
Light	Dark			
msec		$\text{w m}^{-2}$	$\text{mJ m}^{-2}$	relative units
540	0	0.50	270	$23.8 \pm 1.2$
540	0	0.17	92	$26.2 \pm 1.2$
405	135	0.50	203	$10.4 \pm 2.1$
405	135	0.17	69	$10.0 \pm 2.5$
270	270	2.00	540	$4.4 \pm 0.6$
270	270	1.00	270	$4.4 \pm 0.6$
270	270	0.50	135	$4.4 \pm 1.1$
135	405	2.00	270	$3.3 \pm 3.3$
135	405	0.50	58	$3.9 \pm 0.6$

<sup>1</sup>Mean of two measurements  $\pm$  SE.

pulses, determines the phototactic response of cell populations of *Cryptomonas* sp.

Next, an attempt was made to see whether there is any effect of intensity on the phototactic response to repeated light pulses of the lower frequency. Phototactic responses to light pulses of a constant frequency (about 1.85 Hz) (cycle length = 540 msec) with different dark intervals (0, 135, 270, and 405 msec) were measured using different intensities of actinic light. The results are summarized in Table II. Phototactic response to continuous light reached a plateau at the intensity of  $0.17 \text{ w m}^{-2}$  in this experiment. The reduction in phototactic responses with 270- and 405-msec dark intervals was not affected by increasing the intensity from  $0.5 \text{ w m}^{-2}$  to  $2.0 \text{ w m}^{-2}$ .

Thus, the responses are essentially dependent on the length of dark interval, but not on the total incident energy delivered per unit time at this lower frequency. This result is consistent with that described in the previous paragraph.

### DISCUSSION

The present study shows that repeated pulses of light can induce phototactic responses of cell populations in *Cryptomonas* sp., as in *Euglena gracilis* (1) and *Chlamydomonas reinhardtii* (4). Although the significance of the dark interval between light pulses has not been pointed out in the works cited, the data in the present work clearly demonstrate that the length of the dark intervals between light pulses is crucial in *Cryptomonas* sp.

If the dark period is shorter than 63 msec, the phototactic response in *Cryptomonas* sp. is simply dependent on the total incident energy of each light pulse and the reciprocity law holds as it does in many other photobiological phenomena (7, 9). That is, there is nothing special about the phototactic response with respect to light perception.

If the dark intervals exceed the critical length (e.g. the 250-msec interval observed in this work), the phototactic responses become weaker, being independent of total incident energy of actinic light. The findings suggest that something needed for the phototactic response is generated in the light and dissipated in the dark.

The data in Figure 1 also indicate that the longer the period of

the light-dark cycle, the greater the phototactic responses with periods longer than 2 sec, even though the duration of dark interval was much longer than the critical length of 250 msec. This can be explained as follows. Assuming that the duration of each light pulse is long enough in itself to bring about phototactic accumulation of the cell population toward actinic light, the longer dark intervals are no longer effective in canceling the light effect. Thus, stimulation with a longer duration of light pulse in such a cyclic treatment acts as it does with continuous irradiation.

In conclusion, the phototactic response in *Cryptomonas* sp. can now be separated into two elementary processes, photoreception and the following dark reaction(s), using cyclic treatments consisting of a light pulse and darkness. In this respect, it resembles many other light-dependent processes, including photosynthesis (3), vision (8), and photomorphogenesis (7, 9). Detailed microscopic observations on the behavior of individual cells may help in understanding why 250 msec is the critical length of the dark interval in cyclic treatments.

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