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Behavior of *Euglena gracilis* under simultaneous competing optical and chemical stimuli

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ABSTRACT

In this study, we developed a microfluidic system to elucidate the behavioral response of *Euglena gracilis* cells to simultaneous competing optical and chemical stimuli. The system illuminated a nonuniform blue light of 0.5–18 mW/cm^2 on cells confined in a 2D microchamber to induce a photophobic response (step-up photo-shock). After cells accumulated in areas of weak blue light, a CO_2 gradient (0%–100%) was generated in the microchamber to induce counter chemotaxis (aerotaxis). *E. gracilis* cells showed negative chemotaxis for areas of higher CO_2 concentrations rather than strong blue light, suggesting that CO_2 chemotaxis is dominant over blue light photophobicity. We also examined phototaxis instead of photo-shock responses, using in-plane uniform blue light illumination in a counter direction to the CO_2 gradient. The cells exhibited both CO_2 chemotaxis and blue light phototaxis, and swam back and forth randomly. In both cases, some sensitive cells accumulated in niche areas, primarily with CO_2 concentration $< 25\%$ and secondarily with a weaker blue light intensity (further away from the blue light source). The results indicate that competing chemotactic and photophobic or phototactic stimuli induced superimposed responses, although *E. gracilis* was more strongly affected by CO_2 chemotaxis than by blue light. We propose that the two independent chemotaxis and photoresponse signal-transduction pathways are merged into one flagellar control mechanism. Moreover, a small proportion of cells were resistant to CO_2 or blue light, showing the diversity in cell metabolic status.

1. Introduction

Response to imposed stimuli is an essential behavior in all living organisms. Motile microalgae exhibit taxis, which involves a change in the direction of movement in response to stimuli [1–4]. Taxis or a phobic response in some cases [5–7] is a simple but effective behavior for movement to areas that are more conducive for survival. For instance, flagellar microalgae, such as *Euglena gracilis* or *Chlamydomonas reinhardtii*, can sense environmental chemical substances or strong light and change their swimming direction [8–11]. The mechanisms involved must be simple and primitive, at least superficially, as these cells have no neural systems. They have various sensing proteins [12–14] that detect environmental stimuli and produce chemical signaling substances. For instance, *E. gracilis* shows a clear phobic response to strong blue light, but not much to green or red light [15]. Iseki et al. identified the sensing protein for strong blue light as photoactivated adenylyl cyclase (PAC) [16,17], which produces cAMP upon detecting blue light. The receptor for CO_2, including those for many other chemicals, has not yet been identified. After unidentified sequential/parallel chemical reaction cascades, the signal reaches the flagellum, and its motion is modified to change the swimming direction. The details of chemical sensing proteins, signaling cascades, and flagellum motion have not yet been completely elucidated, even with modern molecular biological technologies. One of the difficulties arises from complex and interfering signaling cascades. Another difficulty is that the cells do not show identical behavior due to individual characteristics or diverse responses.

Among many studies on chemotaxis or phototaxis in motile microalgae, the study by Wakabayashi et al. reported the interference effects of redox reagents on phototaxis in *C. reinhardtii* [18]. In their study, the direction of phototaxis in *C. reinhardtii* switched when the cells were treated with redox reagents to alter the cytoplasmic redox status. This observation suggests that the signal transduction pathway from channel-rhodopsin (photoreceptor) to flagellum motion in *C. reinhardtii* is a redox-sensitive chemical reaction cascade. We previously examined the effects of redox reagents on the photophobic responses of *E. gracilis*

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and found that none of the redox reagents tested significantly affected the photophobic behavior in the cells, unlike phototaxis by *C. reinhardtii* [18]. This result showed that the mechanism of the photoresponse of *E. gracilis* markedly differs from that of phototaxis in *C. reinhardtii*.

A promising approach for investigating the characteristics, mechanisms, and signal transduction pathways involved in taxis or phobic responses of microalgae is to simultaneously impose two types of external stimuli to the cells in a manner such that each stimulus induces a response competing with the other (counter) stimulus. When one stimulus–response reaction overcomes the other, it implies that the sensitivity to the dominant stimulus is substantially larger than that to the other stimulus, suggesting that a specific signal transduction pathway exists for the dominant stimulus or that the flagellar control mechanism differs for the dominant stimulus. From the responses of microalgae, we can estimate the priority of taxis, among the other taxes and responses, and examine the signal transduction pathway for flagellar control.

The multi-modal responses of microalgae are also important to understand a sudden explosive increase in the growth of green algae in nature [19]. In many cases, this explosive increase in algae growth occurs when two counter-balanced stimuli become one-sided or when the microalgae acquire the resistance to one of the stimuli. The explosive increase in growth is beneficial when the microalgae produces massive lipids/fats for biofuel use, but sometimes results in significant environmental damage particularly when the microalgae produce toxic substances or cause oxygen depletion. At the same time, competing simultaneous stimuli play important roles in algal growth to trigger cell evolution and enhance diversity in cell characteristics. The mechanisms involved in the multi-modal behaviors of microalgae under various stimuli are, thus, key issues for understanding microalgae/algal/plant metabolism.

There are some previous studies that investigated on the two overlapping stimuli in *Euglena* as well as other microswimmers, particularly for gravitaxis versus phototaxis.

Wager noted that light and gravity are competing stimuli as strong light weaken gravitaxis [20]. Hader and Liu described that high-energetic UV light caused the swimming activity for some flagellates to stop and sink passively [21]. Hader et al. also pointed out the influence of radical oxygen species (ROS) produced by UV light on the gravitaxis [22]. As well, the effect of light illumination on gravitaxis in *Chlamydomonas* and its spectral dependence was studied [23,24]. As such, the studies on the multi-modal responses of microalgae or microswimmers are suggestive to figure out the signal transduction pathway of flagellates.

The present study aims to investigate the behavioral response of *E. gracilis* cells to competing optical and chemical stimuli using a blue light gradient and a CO_2 gradient in the counter direction. The two stimuli are the major factors dominating microalgae/algal/plant growth. We developed a microfluidic system in which *E. gracilis* cells were confined in a 2D microchamber, and a nonuniform blue light was illuminated from beneath the microchamber to induce a photophobic response (step-up photo-shock). The system also applied in-plane CO_2 gas diffusion into the 2D microchamber from one side to induce CO_2 chemotaxis (aerotaxis) in the counter direction to the photo-shock. In a separate experiment with the same system, in-plane uniform blue light was illuminated on the cells to induce negative phototaxis counter to CO_2 chemotaxis. We showed the competing effects of the two stimuli and the priority of the photophobic or phototaxis response and chemotaxis by analyzing cell behaviors and quantitatively evaluating cell distribution. Diversity in taxis or phobic responses of *E. gracilis* was also demonstrated.

2. Methods and materials

2.1. Chemotaxis in the microfluidic device

We used a polydimethylsiloxane (PDMS) microfluidic device with a

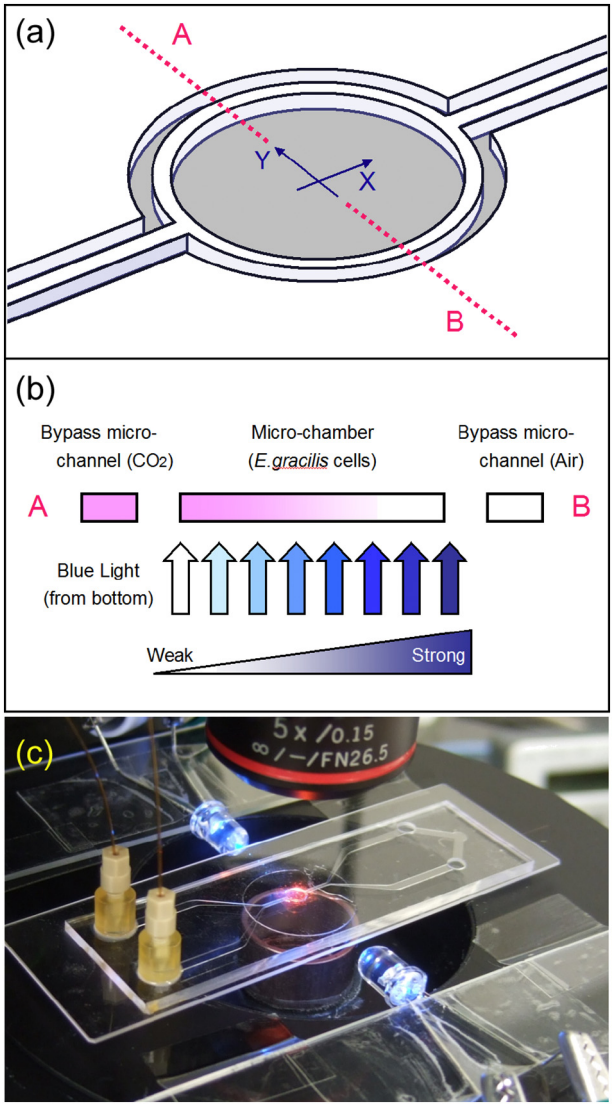

Fig. 1. (a) Schematic drawing of the microfluidic structure employed in this study. The device had a closed microchamber with two bypass microchannels running beside the chamber. The diameter of the microchamber was 2.5 mm. The cells were confined in the microchamber by a covering glass plate. The coordinate system X, Y was set as indicated with origin at the center of the microchamber, with a unit of 205 pixel/mm that was derived from the resolution of the captured images. The unit of (X, Y) coordinates values was pixel, and omitted for simplicity. The dotted line indicates the direction of the chemical gradient and the blue light gradient. (b) Cross-sectional diagram of the microchip for the chemotaxis versus step-up photo-shock experiment described in Section 2.2, with nonuniform blue light irradiation from underneath the device. The blue light gradient pattern was produced by a personal computer (PC) and pattern was sent to a liquid crystal (LC) projector in the system [26,27]. The gradient pattern from the projector was irradiated from underneath. No light-emitting diode (LED) was used in this experiment. CO_2 gas flowed in the A-side microchannel, whereas air flowed in the B-side microchannel. CO_2 diffused from A-side microchannel into the microchamber, and a chemical gradient was generated in the microchamber. (c) Photograph of the experimental setup for phototaxis versus chemotaxis experiment described in Section 2.3. Uniform in-plane blue light irradiation from an LED at the B-side was used to induce phototaxis in the cells. In this experiment, the LC projector emitted no blue light but only red light. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)