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Dominance between the Two Flagella during Phototactic Turning in *Chlamydomonas*

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ABSTRACT—For phototactic steering, *Chlamydomonas* detects environmental light conditions with a photoreceptor (eyespot) while rotating the cell body around its body axis. Because of the bodily rotation and the directionality of the eyespot sensitivity, the light signal perceived by the eyespot must alternate between a light period and a dark period. It is an interesting question how cells can correctly change its swimming direction while the light signals change periodically. In this study, we examined the timing of the change in cells' swimming direction with respect to the timing of the light/dark cycle occurring at the photoreceptor. Most of the cells that displayed positive phototaxis had the eyespot facing the outside of the helical swimming track. We found that when phototactic light was applied from the direction perpendicular to the swimming direction of a cell, a phototactic response was initiated when the eyespot faced the light source. This was constantly observed irrespective of the phase at which the phototactic light was turned on. The initial change observed after the light stimulation was a decrease in the pitch angle of the helical swimming path, which caused the cell to swim less farther away from the light source than when unstimulated. This change was followed by a large turn toward the light source, which occurred when the eyespot faced away from the light. These observations indicate that the dominance of the *cis*-flagellum (the flagellum nearest to the eyespot) over the *trans*-flagellum (the flagellum farthest from the eyespot) decreases during the light phase and increases during the dark phase. Thus, both light reception (on response) and the cessation of light perception (off response) by the eyespot are important for producing phototactic turns.

INTRODUCTION

Chlamydomonas, a bi-flagellate photosynthetic protist, displays phototaxis toward or away from the light source (for review, see Witman, 1993; Kreimer, 1994; Hegemann, 1997; Sineshchekov and Govorunova, 1999). It detects the environmental light with rhodopsin-like photoreceptor molecules (Foster *et al*., 1984; Deininger *et al*., 1995) contained in the eyespot, a distinct membranous structure located at a unique site on the equator of the cell body. Foster and Smyth (1980) have proposed that the *Chlamydomonas* eyespot is sensitive only to the light incident at the right angle to the surface, because pigmented particles that line the eyespot membrane function as an interference reflector. In fact, studies that measured the photoreceptor current in single cells have confirmed that the photoreceptor is about eight times more sensitive to the light incident from the external side of a cell than to the light coming from behind (Harz *et al*., 1992; Schaller and Uhl, 1997). Because a *Chlamydomonas* cell rotates around the body axis at a frequency of about 2 Hz while swimming, it is likely that a swimming cell, when illuminated sideways, must experience alternating light and dark periods. In other words, *Chlamydomonas* scans the light environment by rotating itself (Foster and Smyth, 1980).

The light intensity detected by the eyespot, modulated by the bodily rotation, should be used by the cell as the signal for controlling the flagellar beating and thereby changing the swimming direction. Previous studies have shown that a change in light intensity causes an imbalance of the beating of the two flagella. For example, an increase in light intensity has been shown to increase the beat frequency of the *trans*-flagellum (the flagellum farthest from the eyespot) and decreases that of the *cis*-flagellum (the flagellum nearest to the eyespot) (Smyth and Berg, 1982; Sineshchekov *et al*., 1991a, b). The beat amplitudes of the two flagella also change differentially following the dark/light cycle (Rüffer and Nultch, 1991). These changes may be brought about through a change in the cytoplasmic Ca^{2+} concentration (Kamiya and Witman, 1984).

The directional sensitivity of the photoreceptor and the differential regulation of flagellar beating by light signals support the hypothesis that a *Chlamydomonas* cell turns toward the light source (when displaying positive phototaxis) or away from it (when displaying negative phototaxis) at the moment when the eyespot faces the light source. However, there has

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been no report that correlates the phototactic turn with the eyespot direction. Furthermore, the position of the eyespot relative to the axis of a cell's helical swimming path has not been established. It has often been assumed that the eyespot faces outward but there is also an indirect observation that it faces inward during negative phototaxis (Schaller *et al.*, 1997). In this study, we analyzed the position of the eyespot in swimming cells and found that the direction relative to the swimming path changes with the sign of the phototaxis. More importantly, through analysis on how cells change the swimming direction during phototaxis, we found that a phototactic turn consists of two steps, each of which occurs during the light phase and the dark phase, respectively.

MATERIALS AND METHODS

Culture and solution

A wild-type strain, 137C mt+, of *Chlamydomonas reinhardtii* was used in all experiments. Cells were cultured for 3 to 4 days in the TAP medium (Gorman and Levine, 1964) under a 12 hr light/12 hr dark cycle. Before experiments, the cells were washed and suspended in solution containing 1 mM KCl, 0.3 mM $CaCl_2$, 0.2 mM EGTA, and 5 mM Hepes (pH 7.2).

Microscope observation

The cell suspension was placed in a chamber (1×1×0.3 cm) covered with a cover slip so that the solution did not come into contact with air. The movement of the cells was observed with an inverted microscope (IX-70, Olympus, Tokyo) and recorded on video tapes. For the observation of the eyespot, a polarizing filter was placed before the objective and condenser lenses. The relative direction of the polarizing plane of the two filters was slightly apart from 90 degree so that the cell body could be visualized faintly. The eyespot can be seen as a bright spot with a polarizing optics (Kamiya and Witman, 1984). A 100-W halogen lamp fitted with a heat absorbing filter and a red (>600 nm) filter were used for illumination.

Phototaxis was induced by illuminating cells with an LED emitting 500 nm light (half band width = 35 nm; NSPE510S, Nichia Chemical Inc., Anan, Japan). The fluence rate was measured with a calibrated photodiode (S874-5K, Hamamatsu Photonics, Hamamatsu, Japan) and was set at 2×10^{19} photons/m^2/s. This fluence rate was chosen because a lower fluence rate did not permit us to clearly define the onset of the phototactic turning, and a higher fluence rate often induced photoshock responses instead of phototactic turning.

For analysis of phototactic turn, cells were stimulated with two LEDs. One LED was used to preorient the cells by phototaxis. The other was placed perpendicularly to the first one and was used to phototactically change the swimming direction in the right angle. The two LEDs were switched alternately with a relay. The timing of the control signal was also recorded on the video tape.

RESULTS

Direction of the eyespot

A *Chlamydomonas* cell swims in a helical path because it rotates around the long axis of the cell and also around an axis perpendicular to the body axis. The rotation around the cell axis results from a three-dimensional component in the beating of the two flagella, whereas the rotation around the perpendicular axis results from the imbalance in strength between the beating of the two flagella. Therefore, if the *cis*-flagellum (the flagellum nearest to the eyespot) beats more strongly than the *trans*-flagellum (the flagellum farthest from the eyespot), the *cis*-flagellum as well as the eyespot must be placed on the outer side of the helix. If, on the other hand, the *trans*-flagellum beats more strongly than the *cis*, the eyespot will be positioned facing the inside of the helix (Fig. 1).

We recorded the swimming track with a polarizing microscope, which can visualize the eyespot as a bright spot when it is positioned on the edge of the cell (Kamiya and Witman, 1984). Fig. 2A shows an example of a swimming track in which the eyespot was facing the outer side of the helical path. *Chlamydomonas* displays both positive and negative phototaxis depending on the culture conditions and the daily rhythm, in a manner that cannot be controlled strictly. We found that the position of the eyespot differs in cells showing positive taxis and those showing negative taxis. In preparations of cells of which >90% showed positive phototaxis, more than 80% of the cells swam with the eyespot facing outer side of the helix (Fig. 2B). In preparations of cells of which >90% showed negative phototaxis, ~50% of the cells swam with the eyespot facing inside of the helix, and >30% cells had the eyespot facing outside of the helix. In negatively phototactic cells, the ratio of the numbers of cells with the eyespot facing inside or outside of the helical swimming path varied significantly from one

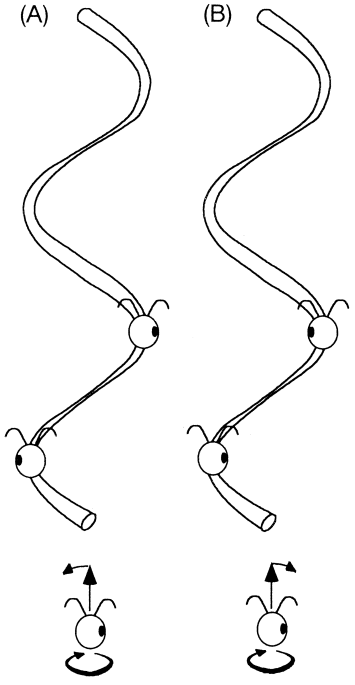

Fig. 1. Relationship between the direction of the eyespot relative to the helical swimming track and the force generated by the flagella. (A) When the *cis*-flagellum (the flagellum nearest to the eyespot) generates stronger force than the *trans*-flagellum (the flagellum farthest from the eyespot), the eyespot points toward the outside of the helix. (B) When the *trans*-flagellum generates stronger force than the *cis*-flagellum, the eyespot points toward the inside of the helix. These illustrations are based on a report by Crenshaw *et al.* (2000) that the cell rotates counterclockwise when viewed from the posterior end, i.e., the helical track is left-handed.

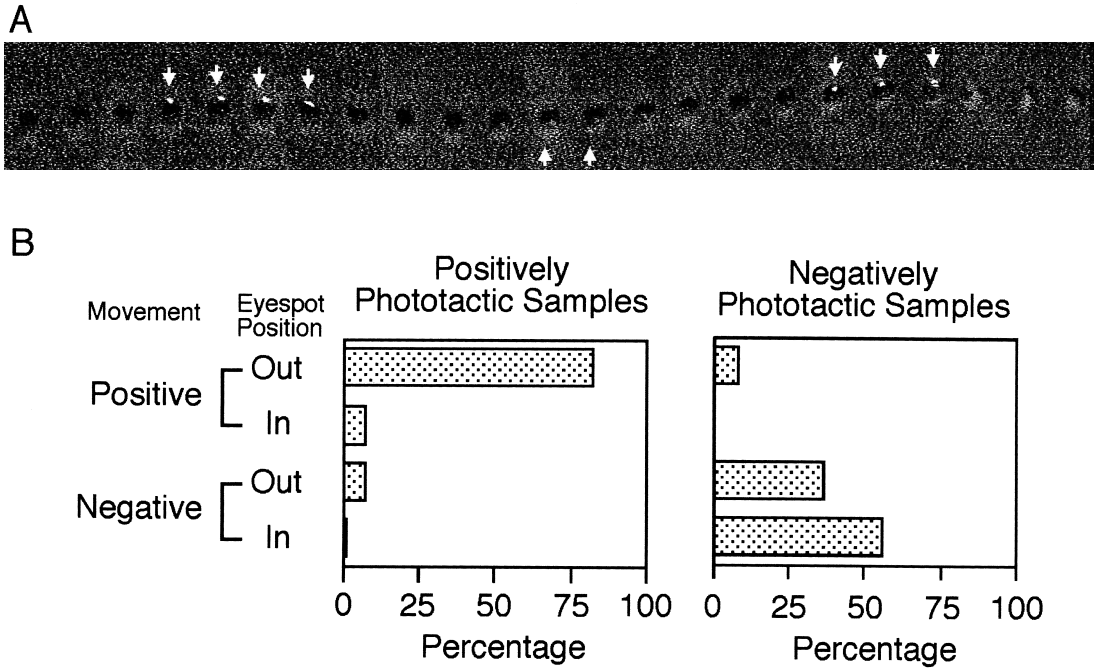

Fig. 2. Direction of the eyespot relative to the helical swimming track of *Chlamydomonas*. (A) Video record showing the position of the eyespot (arrows) of a swimming *Chlamydomonas* cell. This cell was displaying positive phototaxis toward the light source, which was located on the right side of the images. Video images taken at 1/30 sec intervals. (B) Proportion of cells that show positive or negative phototaxis (i.e., those that swam toward the light source or those that swam away from the light source) and the direction of the eyespot. Data obtained from independent samples in which most of the cells were showing positive phototaxis (left panel) or negative phototaxis (right panel).

preparation to another. These observations indicate that the position of the eyespot relative to the helical swimming path is partially correlated with the sign of phototaxis.

Change in swimming direction during phototaxis

For studying how a cell changes its swimming direction upon photo-stimulation, we examined only those cells that showed positive phototaxis. This is because the eyespot in those cells is almost always facing outside of the helix (Fig. 2B), and therefore we did not need to observe its position every time we recorded the cells' behavior.

To facilitate analysis, cells were first made to swim toward a fixed direction by their phototactic activity, by applying preorientation light for about 10 seconds (light 1 in Fig. 3A). Then light 1 was switched off and a second light directed at the right angle (light 2) was switched on simultaneously to induce phototactic turn. Since the eyespot should be positioned on the outer side of the helical swimming track, we can tell its position with respect to light 2 from the phase of the helix. For example, if light 2 is applied between point a and point b in Fig. 3, the eyespot should be facing toward the light source; conversely, if the light is applied between point b and c, the eyespot must be facing away from the light source.

When the stimulating light was suddenly changed from light 1 to light 2 (arrow 2 in Fig. 3A), cells changed their swimming directions toward the new light source (filled symbols in Fig. 3A). In the particular case shown in Fig. 3, the eyespot should be facing toward the light source 2 when the light was switched from 1 to 2 (point d). The rightward turn (c to d) was decreased in amplitude and a leftward turn followed it (d to e). The change can be more clearly seen when the swimming direction is plotted against time. The direction was calculated from the coordinates of the cell position at two successive time points. When the eyespot was on the side of the light source 2 (a to b in Fig. 3a), the swimming direction continuously changes from leftward to rightward; in the plot of the angle in Fig. 3b, this corresponds to the portion a to b, with a negative slope. When the eyespot faced against the light source 2 (b–c), the angle change corresponds to the portion with a positive slope in Fig. 3B. In Fig. 3 it can be seen that the negative slope after c was interrupted at the point of d, when the light was changed from 1 to 2. Thus the change in the direction between c and d was smaller than that between a and b. On the other hand, the change in the direction occurring after point d became greater than from b and c. Between d and e, the eyespot was expected to face against the light source 2 under the assumption that the bodily rotation occurred at a constant frequency.

Fourty different cells were examined for their phototactic turns in this manner. Almost all the cells displayed similar responses. Importantly, the manner and timing of the turn did not change greatly even if they were stimulated by the second light at different phases of the helical swimming path. Fig. 3C shows the swimming direction from six traces in which the light direction was changed at various timings (arrows in Fig. 3C). The swimming tracks of the six cells were aligned so that

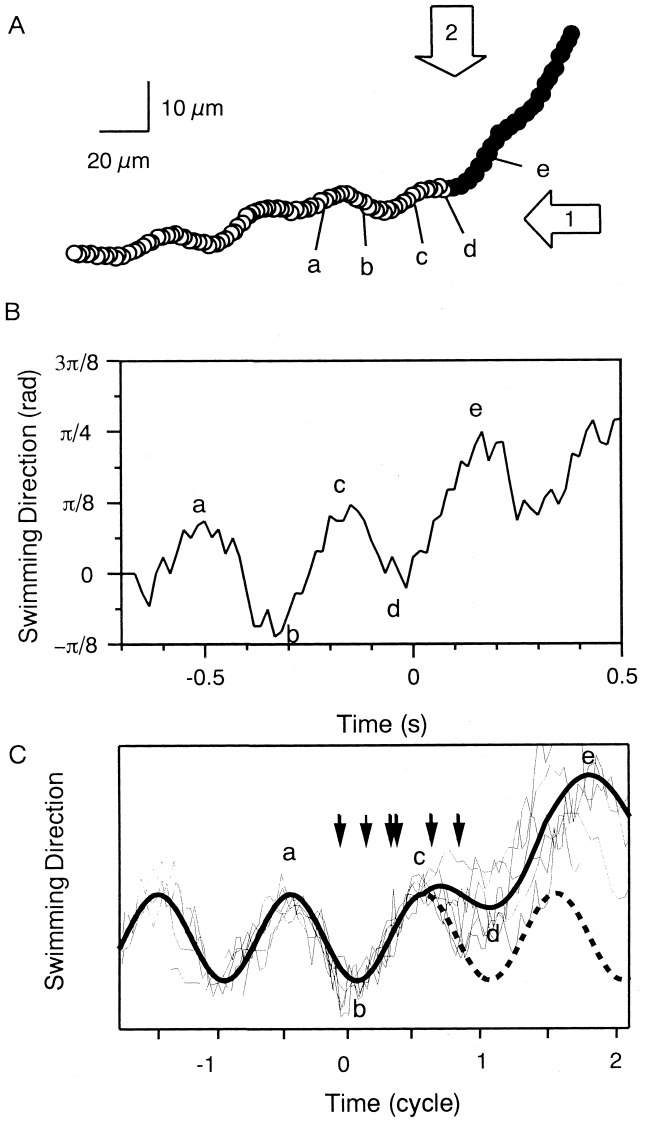

Fig. 3. Phototactic turn in a cell showing positive phototaxis. (A) Record of the cell positions taken at 1/60 sec intervals. This cell was first illuminated by light 1 during a period represented by open circles and then by light 2 during a period represented by closed circles. For ease of evaluating the change in the helical path, this image is enlarged in vertical direction. (B) The swimming direction of the cell shown in (A). The ordinate is the swimming direction relative to the direction of the light 1: angle zero is the direction toward the light source 1. The angle increases with the leftward turn. Positions a–e correspond to the positions marked with the same characters in A. (C) Superposition of records from six different cells. The time and angle are scaled by eye so that the curves in the steady state (time point –2 - 0) maximally overlap. The thick line is an approximate average of the six lines, showing the general tendency. Arrows indicate the time when the light direction was changed from 1 to 2. Note that the angular change showed a similar tendency irrespective of when the light direction was switched. The broken line shows the angular change that would be expected under constant light conditions.

the phase of their helical paths before the stimulus (all look like almost sinusoidal) match with each other. The arrows indicate the time point when the light 1 was switched to light 2. It must be noted that no change occurred right after the light direction was changed before point c; in that case, the eyespot faced away from light source 2 between b and c and a change occurred only after point c when the eyespot faced the light source. To quantify the change in the swimming direction, we measured the change between c and d (θ_{cd}). It was found to be about half the angle between a and b ($\theta_{cd}/\theta_{ab}=0.49\pm0.18$, n=11). In other words, when a cell was stimulated, it moved away from the light source less farther than it would in the same phase of helical path during steady state swimming. In contrast, in phase d–e, i.e. when the eyspot was shaded, the cell displayed about 30% larger change in the swimming direction than in the corresponding phase of unstimulated swimming (between b and c) ($\theta_{de}/\theta_{bc}=1.28\pm0.31$).

In the steady state, the rightward and leftward directional changes of the cell were identical; i.e., both θ_{ab} and θ_{bc} were 0.99 rad. On the other hand, when the cell displayed a phototactic turn, the two angles greatly differed; θ_{cd}=0.49 rad, and θ_{de}=1.27 rad. It means that a cell can change its swimming direction, in the first cycle of rotation after photostimulation, as much as 0.77 rad (44 degrees), i.e., about half of the angle needed for full orientation (90 degrees).

DISCUSSION

In this study we have examined the behavior of *Chlamydomonas* cells after a sudden directional change of stimulating light. As expected from the hypothesis that the photoreceptor in this organism is sensitive only to the light coming from the outside of the cell, we observed that the cells produced no change in swimming when illuminated from behind the eyespot (from b to c in Fig. 4). A change of the swimming path was produced only when the eyespot was directed toward the light source (from c to d in Fig. 4), and that a similar change was produced irrespective of the phase at which the light direction was changed. These observations clearly indicate that the phototactic turning is initiated only when the eyespot faces the light source.

Although a phototactic turn started to take place only when the eyespot faces the light source, the total change in the swimming direction occurred in both light and dark phases. After the photostimulation, the angular change was about 50% smaller during the light phase (c–d in Fig. 4) and about 30% larger during the dark phase (d–e) than in steady state swimming. This indicates that both the reception of the light signal (on-response) and the cessation of light reception (off-response) should be important for the phototactic turning.

In the positively phototactic cells that we used in this study, almost all cells swam with the eyespot positioned on the outer side of the helical swimming path. This should mean that the *cis*-flagellum must generate greater force than the *trans*-flagellum in the steady state swimming (Fig. 1). The reduction in amplitude of this helical path observed just after the phototactic turn started (c–d) should indicate that the dominance of the *cis*-flagellum over the *trans*-flagellum decreases when the eyespot perceives the light signal. In contrast, the increase in amplitude observed when the eyespot faces the other side

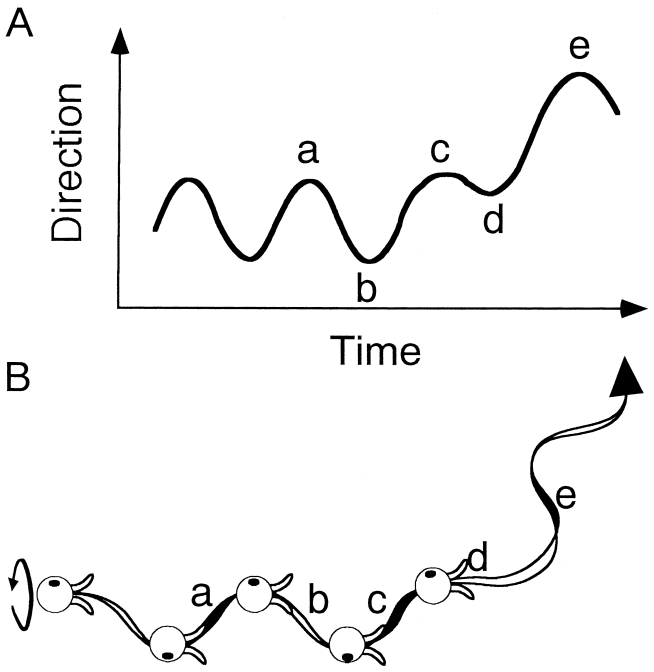

Fig. 4. Schematic diagram of the turning in positive phototaxis. The points a–e in A and B corresponds to each other. The direction of phototactic light is switched form 1 to 2 at some point between b and d. (A) Swimming direction plotted against time as in Figs. 3B and C. (B) Swimming track (ribbon) and the direction of the eyespot. The surface of the ribbon facing the inside and outside of the helix are expressed in white and black, respectively. The initial change occurs as the decrease in the angular change when the eyespot faces the light source (from c to d). In contrast, the following turn in the opposite direction (from d to e) increases.

(d–e) indicates that the dominance of the *cis*-flagellum increases under these conditions. In short, the light phase decreases the dominance of the *cis*-flagellum whereas the dark phase increases it (Table 1). If we assume that the dominance is solely due to the flagellar beat frequency, the beat frequency of the *cis*-flagellum should decrease during the light phase and increase during the dark phase. The beat frequency of the *trans*-flagellum may change inversely.

Cells showing negative phototaxis should show an opposite change in the flagellar dominance. Since the cell is expected to turn away from light when the eyespot faces the light, the dominance of the *cis*-flagellum should increase during the light phase (Table 1). Rüffer and Nultsch (1991), although not examining the relationship with the phototaxis, reported that a light-dependent change in flagellar dominance can occur in two opposite manners. Thus we speculate that the light-induced change in the flagellar dominance may be opposite in the positively and negatively phototactic cells (Table 1). Previous studies on the flagellar beat frequency during dark-light cycle have shown that the beat frequency of the *cis*-flagellum increases and that of the *trans*-flagellum decreases during the light phase (Smyth and Berg, 1982; Sineshchekov, 1991a, b). During the dark phase, the beat frequencies of the two flagella change in the opposite directions; the light phase increases the dominance of the *cis*-flagellum whereas the dark phase does the opposite. This light-induced dominance of the *cis* flagellum would produce a negatively-phototactic turn in a cell, i.e. a turn by which cells turn away from the light source (Table 1). The cell used in those previous studies may have been negatively phototactic, although no description about the sign of phototaxis can be found in the literature.

The photoreception by the eyespot has been shown to result in an opening of a Ca^{2+} channel. Also, studies on the behavior of demembranated and reactivated cell models at various Ca^{2+} concentrations indicated that the *cis*-flagellum is dominant at $<=10^{-8}$ M [Ca^{2+}] whereas trans-flagellum is dominant at higher concentrations (Kamiya and Witman, 1984). These observations lead us to a hypothesis that the intraflagellar [Ca^{2+}] increases during the light phase, which would then result in dominance of the *trans*-flagellum. This would explain the phototactic turns toward the light source such as the case shown in Fig. 3. Negative phototaxis, however, cannot be explained by this mechanism. It may be that some intra-cellular process that follows the Ca^{2+} influx is reversed in positively and negatively phototactic cells. Alternatively, flagellar dominance may be controlled by two or more independent mechanisms involving control of flagellar beat frequency and beat pattern (Omoto and Brokaw, 1985). We speculate that these different mechanisms may vary in effect depending on the cellular internal conditions such as photosynthetic activity (Takahashi and Watanabe, 1993), and that such a control may be performed through some protein phosphorylation. It is possible that the flagellar dominance is regulated by the phosphorylaiton of some dynein components as suggested by recent experiments (King and Dutcher, 1997; Habermacher and Sale, 1997). Clearly, the basis for the occurrence of the positive and negative phototaxis thus remains to be studied. However, from the results of the present study, we can at least say that the control of the flagellar dominance in both light and dark phases is most important for the

Table 1. Change in flagellar dominance during light-dark cycle at the eyespot.

	flagellum	dark	→	light	→	dark
Positive phototaxis	*cis*		↓*		↑*	
	trans		↑		↓	
Negative phototaxis	*cis*		↑		↓	
	trans		↓		↑	

* Upward and downward arrows indicate the increase and decrease in the flagellar dominance, respectively, that occur during dark-light or light-dark transition.

determination of the sign of phototactic turns.

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