LABORATORY OF PHOTOENVIRONMENTAL BIOLOGY

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Photosynthetic microorganisms, such as cyanobacteria and flagellate algae, respond to light in order to locate themselves at appropriate photoenvironments. Our research is aimed at the elucidation of the photoreceptive and signal transduction mechanisms of light responses in microorganisms. This approach has led us to the discovery, characterization, and application of a remarkably unique light sensor molecule as described below.

I. Photoactivated Adenylyl Cyclase (PAC), an algal photoreceptor protein with intrinsic effector function to produce cAMP

In 2002, we found a novel blue-light receptor with an effector role in *Euglena* (Iseki *et al*., Nature *415*, 1047-1051, 2002): *Euglena gracilis*, a unicellular flagellate, which shows blue-light type photomovements (Figure 1). The action spectra indicate the involvement of flavoproteins as the photoreceptors mediate them. The paraflagellar body (PFB), a swelling near the base of the flagellum, is thought to be a photosensing organelle responsible for photomovements. To identify the photoreceptors in the PFB, we isolated PFBs and purified the flavoproteins therein. The purified flavoprotein (ca. 400 kDa), with noncovalently bound FAD, seemed to be a heterotetramer of α- and β-subunits. Predicted amino acid sequences for each of the subunits were similar to each other and contained two FAD-binding domains (BLUF: sensor of blue light using FAD) (F1 and F2) each followed by an adenylyl cyclase catalytic domain (C1 and C2). The flavoprotein showed adenylyl cyclase activity, which was elevated by blue-light irradiation. Thus, the flavoprotein (PAC: photoactivated adenylyl cyclase) can directly transduce a light signal into a change in the intracellular cAMP level without any other signal transduction proteins (Figure 2).

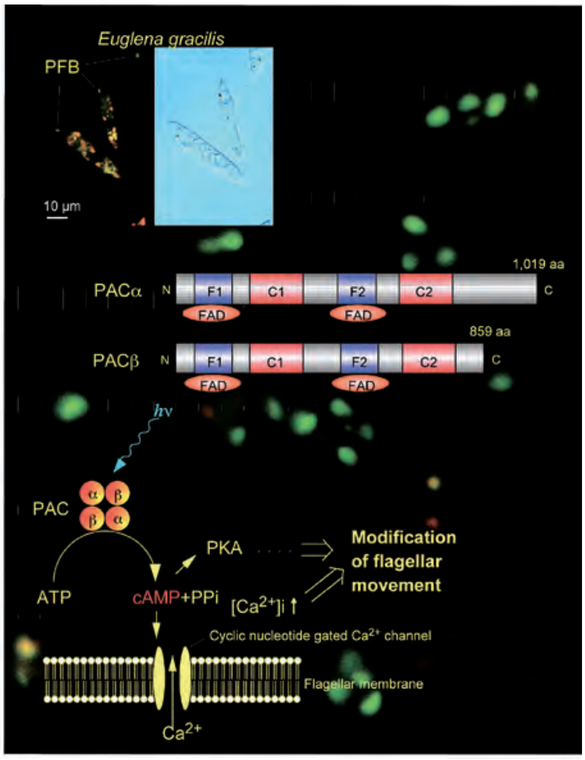

Figure 2. Domain structure of Photoactivated Adenylyl Cyclase (PAC) and its two possible action mechanisms to mediate photoavoidance behavior in *Euglena gracilis*. The green spots in the background are fluorescence microscopical images of isolated paraflagellar bodies (PFBs), the "real eyes".

A unique function such as this is best suited not only for the rapid control of the flagellar motion of the *Euglena* cell but also for a variety of biotechnological photocontrol of cAMP-controlled biological functions, including neuronal functions and developmental processes in a variety of organisms in which PAC can be heterologously expressed. For example, in collaboration with Max-Planck-Institut für Biophysik (Frankfurt) and other German groups, expression of PAC in cells was performed, which allowed the manipulation of cAMP with exquisite spatiotemporal control. We functionally expressed PACs in two popular expression systems, *Xenopus laevis* oocytes and HEK293 cells. Moreover, transgenic *Drosophila melanogaster* flies demonstrated functional PAC expression by showing blue light–induced behavioral changes (Schröder-Lang, S., Nat. Meth. *4*, 39-42, 2007)

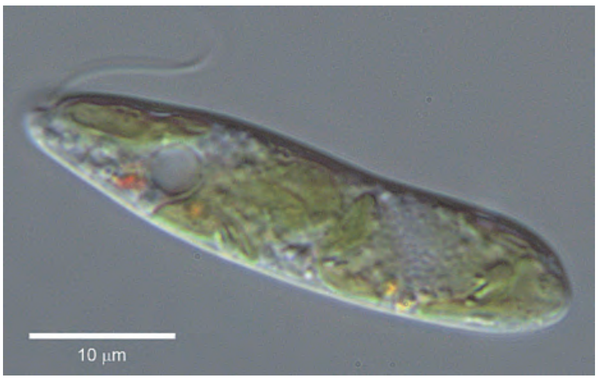

Figure 1. *Euglena gracilis*, a unicellular flagellate alga. It swims forward (to the left) by shaking the flagellum, the protruding whip-like structure. The flagellar motion is controlled by ultraviolet to blue light signals sensed by the photoreceptor molecules in the "real eye" located adjacently to the basal part of the flagellum, so that the cell can locate itself in appropriate light environments for its survival. The orange spot, so-called "eyespot" is not the "real eye" but a light shade to enable the cell to recognize the light direction.

II. Flagellar motions during algal phototactic steering

To understand the mechanism of blue-light-induced algal phototactic steering, we observed, using infrared high-speed video microscopy, light-triggered transitory flagellar motions in flagellate reproductive cells (swarmers) of a brown alga, *Scytosiphon lomentaria*, under primary helical swimming conditions before and during negative phototactic orientation to unilateral actinic light (Figure 3).

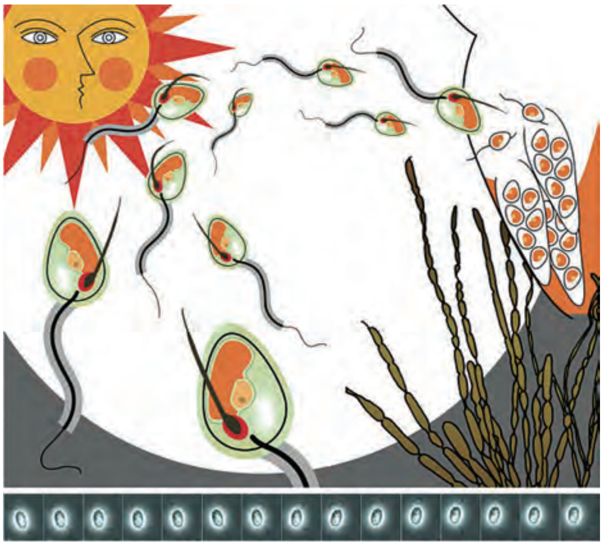

Figure 3. Photo-orientation of swarmer cells released from matured thalli of the brown alga, *Scytosiphon lomentaria*. Each swarmer has a hairy long anterior flagellum and a smooth short and autofluorescent posterior one. Cover illustration of Photochem. Photobiol. 86 (2) drawn by Hiroko Uchida

The posterior flagellum, which is autofluorescent and thought to be light-sensing, was passively dragged in the dark and exhibited one to several rapid lateral beats during orientation changes for phototactic steering (Figure 4). Notably, a brief cessation of anterior flagellar beating was occasionally observed concomitantly with rapid beats of the posterior flagellum. This behavior caused a pause in helical body rotation, which may contribute to the accuracy of phototactic steering (Figure 5). Thus, coordinated regulation of the movement of the two flagella plays a crucial role in phototactic steering.

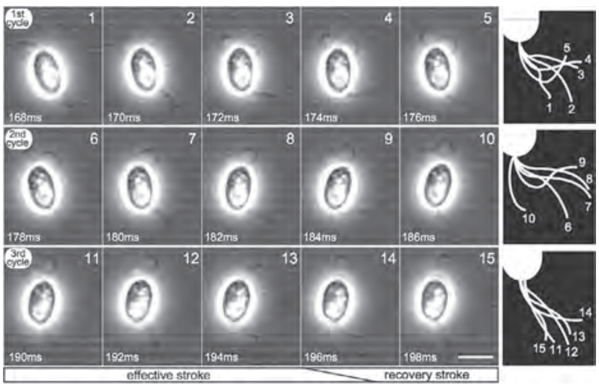

Figure 4. Rapid beats of posterior flagellum after onset of light stimulus observed at 2 ms intervals.

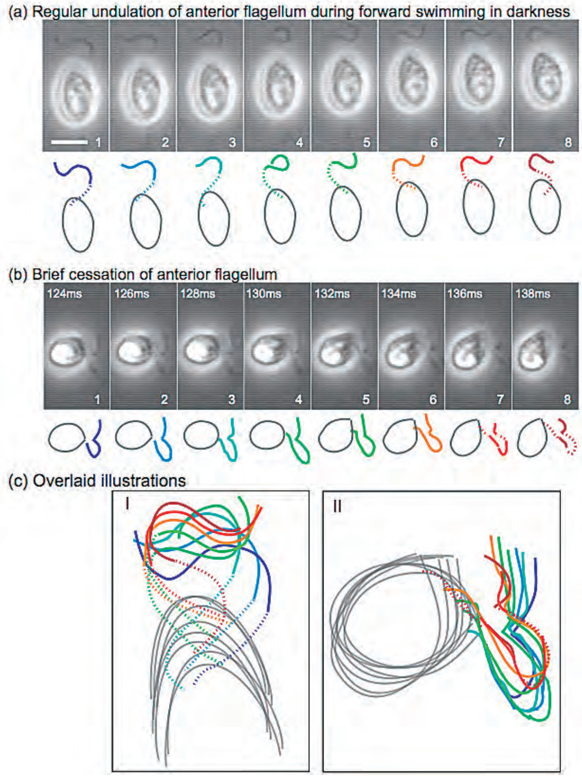

Figure 5. A brief cessation of anterior flagellum after onset of the light stimulus.

Publication List

〔Original paper〕

● Izawa, N., Suzuki, T., Watanabe, M., and Takeda, M. (2009). Characterization of arylalkylamine N-acetyltransferase (AANAT) activities and action spectrum for suppression in the band-legged cricket, *Dianemobius nigrofasciatus* (Orthoptera: Gryllidae). Comp. Biochem. Physiol. Part B *152*, 346–351.

〔Original paper (E-publication ahead of print)〕

● Matsunaga, S., Uchida, H., Iseki, M., Watanabe, M., and Murakami, A. Flagellar motions in phototactic steering in a brown algal swarmer. Photochem. Photobiol. 2009 Dec. 14.