Laboratory of Photoenvironmental Biology †

Professor (Adjunct)
WATANABE, Masakatsu

Photosynthetic microorganisms, such as cyanobacteria and flagellate algae, respond to light in order to locate themselves in 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 1. *Euglena gracilis*, a unicellular flagellate alga. It swims forward (to the left) by shaking its flagellum, the protruding whip-like structure. 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, so-called, "eyespot" is not the "real eye" but a light shade enabling the cell to recognize the light's direction.

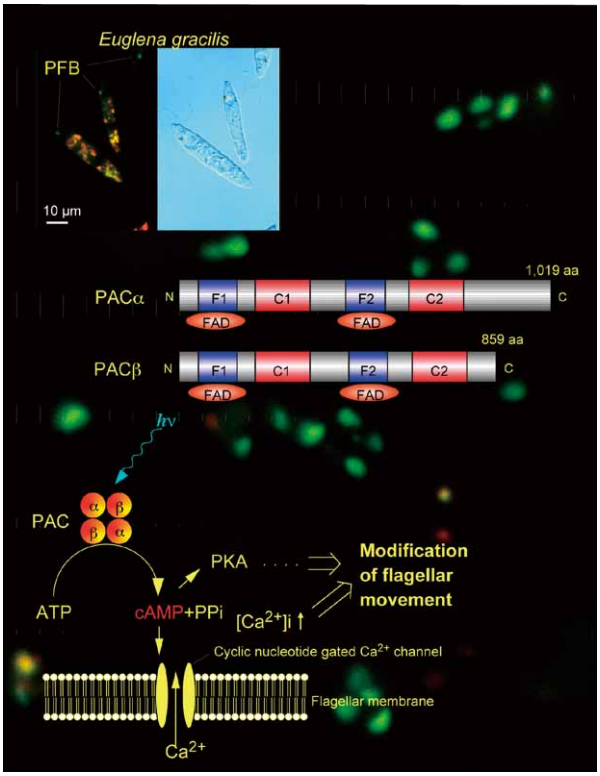

Figure 2. Domain structure of Photoactivated Adenylyl Cyclase (PAC) and its two possible action mechanisms to mediate photoavoidance behavior in *Euglena gracilis*. The green spots in the background are fluorescence microscopical images of isolated paraflagellar bodies (PFBs), the "real eyes".

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. *et al*., Nat. Meth. *4*, 39-42, 2007)

II. Differentiation of sensitivity of the photoreceptive flavin-binding domains of α- and β-subunits of PAC

In our previous report, we demonstrated that a recombinant version of the PACαF2 domain displays blue light-induced photocycle similar to those of prokaryotic BLUFs (Ito *et al*., 2005, Photochem. Photobiol. Sci., 4, 762-769). Here, we further examine the recombinant PACβF2 domain, which

†: This laboratory was closed on 31 March, 2010.

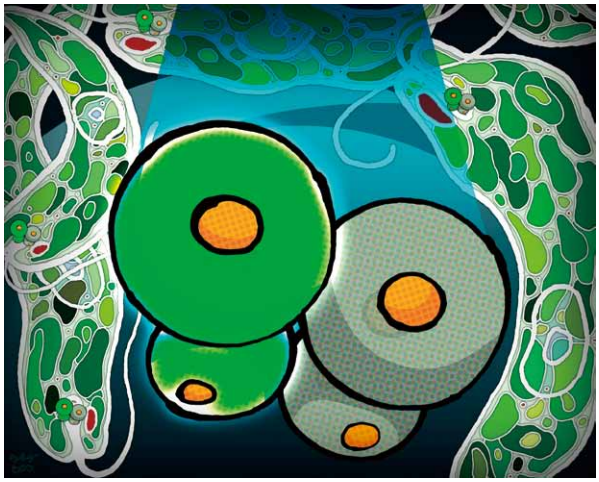

Figure 3. A schematic illustration of the putative $\alpha_2\beta_2$ heterotetrameric structure (center) of the PAC molecule of *Euglena* cells (periphery).

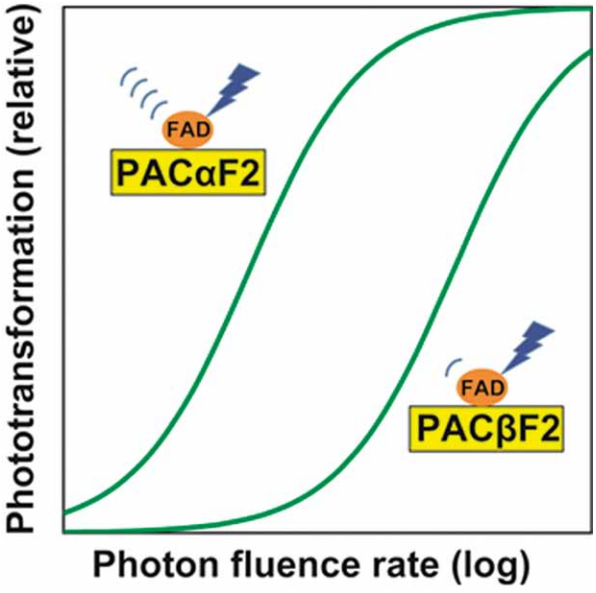

Figure 4. A schematic illustration of the differentiated light sensitivities between the homologous flavin-binding domains of the α and the β subunits of the PAC molecule. The former is estimated to be about 20 times more sensitive than the latter.

like PACαF2, exhibits blue light-induced photocycle. The estimated quantum efficiency for the phototransformation of PACβF2 was 0.06–0.08, and the half-life for dark relaxation was 3–6 s while the corresponding values for the PACαF2 were 0.28–0.32 and 34–44s. The remarkable differences between PACαF2 and PACβF2 may be related to the sensitivity of the photoactivation. In PACαF2, amino acid position 556, which is equivalent to Trp104 in the BLUF domain of the purple bacterial AppA protein, is occupied by a Leu residue, while in PACβF2, the equivalent BLUF domain site is conserved as Trp560. Amino acid substitution at this site in PACβF2-Trp560Lue markedly increased the estimated quantum efficiency (0.23) and accelerated the half-life of the dark-relaxation (2s). These results indicate that Trp560 in PACβF2 plays a main role in suppressing quantum efficiency.

Publication List

〔Original papers〕

- Fujiyoshi, S., Furuya, Y., Iseki, M., Watanabe, M., and Matsushita, M. (2010). Vibrational microspectroscopy of single proteins. J. Phys. Chem. Lett. *1,* 2541–2545.
- Ito, S., Murakami, A., Iseki, M., Takahashi, T., Higashi, S., and Watanabe, M. (2010). Differentiation of photocycle characteristics of flavin-binding BLUF domains of α- and β-subunits of photoactivated adenylyl cyclase of *Euglena gracilis*. Photochem. Photobiol. Sci. *9,* 1327-1335.
- Kim, E., Park, J.S., Simpson, A.G., Matsunaga, S., Watanabe, M., Murakami, A., Sommerfeld, K., Onodera, N.T., and Archibald, J.M. (2010). Complex array of endobionts in *Petalomonas sphagnophila*, a large heterotrophic euglenid protist from *Sphagnum*-dominated peatlands. ISME J. *4,* 1108-1120.
- Matsunaga, S., Uchida, H., Iseki, M., Watanabe, M., and Murakami, A. (2010). Flagellar motions in phototactic steering in a brown algal swarmer. Photochem. Photobiol. *86,* 374-381.