LABORATORY OF PHOTOENVIRONMENTAL BIOLOGY

Professor (Adjunct)
WATANABE, Masakatsu

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 intrinsic effector role in *Euglena gracilis* (Figure 1), a unicellular flagellate alga, which shows blue-light type photomovements (Iseki *et al.*, Nature *415*, 1047-1051, 2002). 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, has been considered a photosensing organelle responsible for the 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 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.

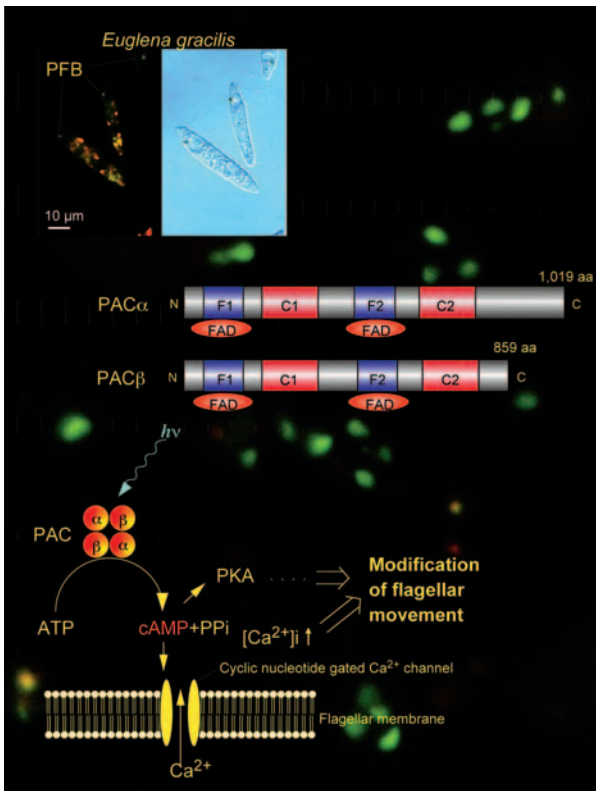

Figure 2. Domain structure of Photoactivated Adenylyl Cyclase (PAC) and its two possible action mechanisms to mediate photoavoidance behavior in *Euglena gracilis*. The green spots in the background are fluorescence microscopical images of isolated paraflagellar bodies (PFBs), the "real eyes".

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)

Ⅱ. Structures and features of the photoreceptive domains (F1 and F2) of PAC

To biophysically understand intramolecular photosignal processing, knowledge of the three dimensional structure of

the protein as well as spectroscopical analyses are essential. Due to the present difficulty of preparative heterologous expression of functional PAC, however, neither crystallography nor spectroscopical analyses of the whole PAC protein have been realized yet. To partially compensate for this situation we tried homology modeling and quantum chemical calculation of its photoreceptive domains (F1 and F2) of the α- subunit using their prokaryotic counterparts, BLUFs, as the templates (Figure 3). It was of interest that, from the viewpoint of binding energies thus calculated, F1 appeared to bind FAD less strongly than F2 does. This difference might indicate different structural and functional roles between these very similar domains.

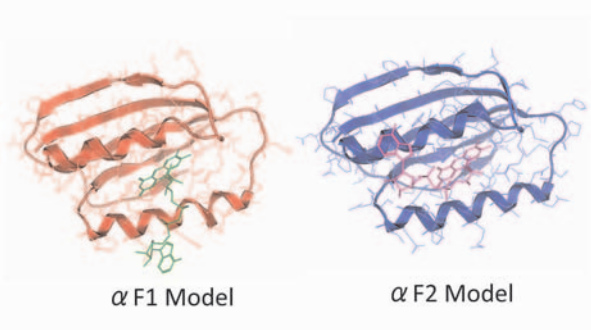

Figure 3. Three-dimentional structure model of the photoreceptor domains (F1 and F2) of PACa subunit. The template for the homology modeling is AppA, a bacterial blue-light sensor.

Publication List

〔Original papers〕

- Maruyama, S., Misawa, K., Iseki, M., Watanabe, M., and Nozaki, H. (2008). Origins of a cyanobacterial 6-phosphogluconate dehydrogenase in plastid-lacking eukaryotes. BMC Evol. Biol. *8,* 151 doi:10.1186/1471-2148-8-151.
- Ioki, M., Takahashi, S., Nakajima, N., Fujikura, K., Tamaoki, M., Saji, H., Kubo, A., Aono, M., Kanna, M., Ogawa, D., Fukazawa, J., Oda, Y., Yoshida, S., Watanabe, M., Hasezawa, S., and Kondo, N. (2008). An unidentified ultraviolet-B-specific photoreceptor mediates transcriptional activation of the cyclobutane pyrimidine dimer photolyase gene in plants. Planta *229*, 25-36.
- Mori, E., Takahashi, A., Kitagawa, K., Kakei, S., Tsujinaka, D., Unno, M., Nishikawa, S., Ohnishi, K., Hatoko, M., Murata, N., Watanabe, M., Furusawa, Y., and Ohnishi, T. (2008). Time course and spatial distribution of UV effects on human skin in organ culture. J. Radiat. Res. (Tokyo) *49*, 269-277.