

**LABORATORY OF PHOTOENVIRONMENTAL  
BIOLOGY**

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

### I. Photoactivated Adenylyl Cyclase (PAC)

In 2002, we found a novel blue-light receptor with an effector role from *Euglena* (Iseki *et al.*, *Nature* 415, 1047-1051, 2002): *Euglena gracilis*, a unicellular flagellate, shows blue-light type photomovements. The action spectra indicate the involvement of flavoproteins as the photoreceptors mediating them. The paraflagellar body (PFB), a swelling near the base of the flagellum, has been considered as a photosensing organelle 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  $\alpha$ - and  $\beta$ -subunits. Predicted amino acid sequences of each of the subunits were similar to each other and contained two FAD-binding domains (BLUF: sensor of blue light using FAD) each followed by an adenylyl cyclase catalytic domain. The flavoprotein showed an 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 cyclic AMP level without any other signal transduction proteins.

#### 1-1 Kinetic properties of PAC photoactivation

Although PAC appeared to be a photoreceptor for the step-up photophobic response (Figure 1), physiological evidence demonstrating that the photoactivation of PAC actually causes the step-up photophobic response is lacking. Recently, we reported the kinetic properties of in vitro activation of PAC by light, comparing them with those of the step-up photophobic response (Yoshikawa *et al.* 2005). We showed that activation of PAC is dependent both on photon fluence rate and the duration of irradiation and that reciprocity held well in the range of 2-50  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (total fluence of 1,200  $\mu\text{mol m}^{-2}$ ), suggesting that activation of PAC is entirely dependent on total photon fluence. We also examined the effects of intermittent

irradiation on PAC activation and showed that intermittent irradiation using pulses of light and dark of equal length (0.1-180 s) caused activation of PAC to almost the same extent irrespective of the cycle periods. This means that elevation of PAC activity occurs only during the light period and that elevated PAC activity falls off within 0.1 s after the termination of irradiation. Such responsiveness is fast enough for PAC to mediate the step-up photophobic response that occurs with several subseconds' latency.

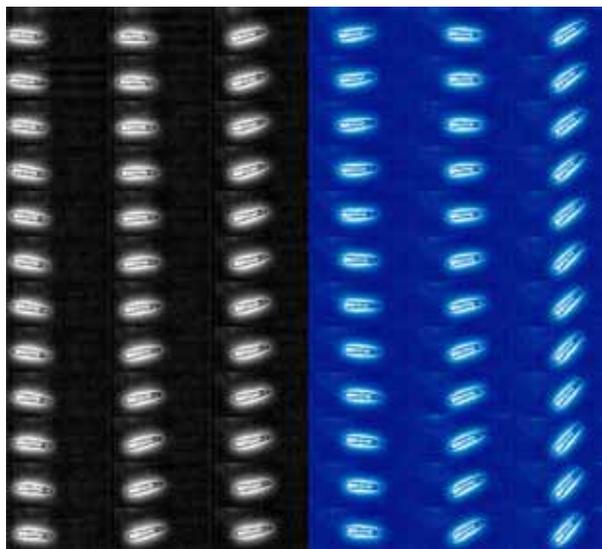


Figure 1. A serial recording of the step-up photophobic response of a *Euglena* cell by a high-speed video camera under infrared observation light (from top of left column to bottom of right column, 20 ms intervals).

We also reported wavelength dependency of PAC activation between 260-650 nm at equal quanta. The curve of wavelength dependency showed prominent peaks at 290 nm, 390 nm and 450 nm, which agrees well both with the absorption spectrum of FAD and the action spectrum for the step-up photophobic response of *Euglena*. All the above properties of PAC activation confirm that PAC actually acts as a photoreceptor for the step-up photophobic response.

#### 1-2 The change in intracellular cAMP levels upon blue light irradiation

Since in vitro activity of PAC was elevated by blue light irradiation, it is easy to think that an increase in intracellular cAMP level evoked by photoactivation of PAC is a major cause of the step-up photophobic response. So far, however, there has been no experimental evidence that blue light irradiation induces an increase in intracellular cAMP level of *Euglena*. We measured intracellular cAMP before and after onset of blue light irradiation, which showed that the intracellular cAMP level remarkably increased within 1 s of the onset of irradiation and the increased cAMP level decreased within 10 s and gradually returned to the initial level even if irradiation continued. The time course of intracellular

cAMP coincided well with the process of the step-up photophobic response. This strongly suggests that the increase in intracellular cAMP evoked by photoactivation of PAC is a key event in the step-up photophobic response.

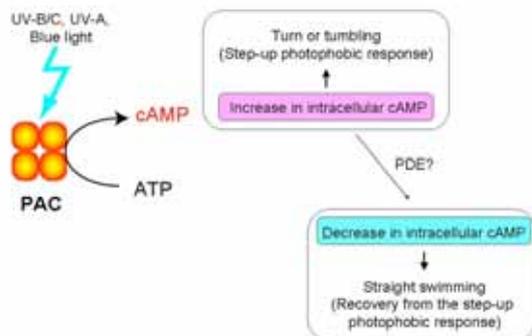


Figure 2. Schematic representation of PAC involvement in the step-up photophobic response of *Euglena*. PAC is activated by UV-B/C, UV-A and blue lights in a photon fluence-dependent manner to produce cAMP, which increases intracellular cAMP. The increase in intracellular cAMP triggers the step-up photophobic response, being followed by a decrease in intracellular cAMP, probably mediated by PDE, which brings about recovery from the step-up photophobic response.

### 1-3 Photoinduced spectral shift of BLUF domains

The mechanism of photoactivation of PAC is a subject of great interest. However, it is difficult to obtain enough PAC from *Euglena* cells for photochemical and structural analyses because the efficiency of PAC purification is low; only several micrograms of PAC can be obtained from 30 litres of *Euglena* culture. It is also difficult to obtain heterologously expressed PAC while keeping its activity intact because most of the expressed protein goes into the insoluble fraction, so-called inclusion bodies. Recently, we succeeded in obtaining the soluble recombinant flavin binding domain (F2) of PAC $\alpha$  by heterologous expression in *E. coli* by fusing the domain to glutathion-S-transferase (GST). The recombinant F2 sample contained both FAD and FMN with trace amounts of riboflavin and showed a spectral red shift upon blue-light irradiation followed by recovery in darkness. Such photoinduced spectral shifts were first reported and well characterized for the BLUF domain of AppA, a regulator of photosynthesis gene expression in *Rhodobacter sphaeroides*. The spectral shift is considered to be caused by alterations in  $\pi$ - $\pi$  stacking and hydrogen bonding between FAD and the tyrosine residue (Tyr21). Recent reports on the crystal structures of BLUF domains of AppA and a cyanobacterial protein TII0078 indicate that the glutamine residue (Gln63 in AppA and Gln50 in TII0078) play a crucial role in rearrangement of the

hydrogen bond network to the flavin. We reported that when recombinant F2 proteins were mutated at the tyrosine (Tyr472) or glutamine (Gln514) residues, corresponding to Tyr21 and Gln63 in AppA, no photoinduced spectral shift was observed. Thus, the mechanism of photoactivation of the recombinant F2 in PAC $\alpha$  seems essentially the same as that of the prokaryotic BLUF proteins, though the kinetic properties are slightly different from each other: e. g., the half-life was 34-44 s at 25 °C for the recombinant PAC $\alpha$  F2, whereas that reported for prokaryotic BLUF domains varies from ca. 3.5 s (TII0078) to ca. 900 s (AppA).

### Publication List:

#### Original papers

- Fujita, S., Iseki, M., Yoshikawa, S., Makino, Y., Watanabe, M., Motomura, T., Kawai, H., and Murakami, A. (2005). Identification and characterization of a flagellar fluorescent protein of brown alga *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae): a flavoprotein homologous to Old Yellow Enzyme. *Eur. J. Phycol.* **40**, 159-167.
- Ito, S., Murakami, A., Sato, K., Nishina, Y., Shiga, K., Takahashi, T., Higashi, S., Iseki, M., and Watanabe, M. (2005). Photocycle features of heterologously expressed and assembled eukaryotic flavin-binding BLUF domains of photoactivated adenylyl cyclase (PAC), a blue-light receptor in *Euglena gracilis*. *Photochem. Photobiol. Sci.* **4**, 762-769.
- Ohnishi, N., Allakhverdiev, S.I., Takahashi, S., Higashi, S.-I., Watanabe, M., Nishiyama, Y., and Murata, N. (2005). Two-step mechanism of photodamage to photosystem II: step 1 occurs at the oxygen-evolving complex and step 2 occurs at the photochemical reaction center. *Biochemistry* **44**, 8494-8499.
- Okajima, K., Yoshihara, S., Fukushima, Y., Geng, X., Katayama, M., Higashi, S.-I., Watanabe, M., Sato, S., Tabata, S., Shibata, Y., Itoh, S., and Ikeuchi, M. (2005). Biochemical and functional characterization of BLUF-type flavin-binding proteins of two species of cyanobacteria. *J. Biochem.* **137**, 741-750.
- Yoshikawa, S., Suzuki, T., Watanabe, M., and Iseki M. (2005). Kinetic analysis of the activation of photoactivated adenylyl cyclase (PAC), a blue-light receptor for photomovements of *Euglena*. *Photochem. Photobiol. Sci.* **4**, 727-731.

#### Review article

- Watanabe, M., and Iseki, M. (2005). Discovery and characterization of photoactivated adenylyl cyclase (PAC), a novel blue-light receptor flavoprotein, from *Euglena gracilis*. In *Handbook of Photosensory Receptors*, W.R. Briggs, and J.L. Spudich, eds. (Weinheim, Wiley-VCH Verlag), pp. 247-260.