



Professor (Adjunct)
WATANABE, Masakatsu

Photosynthetic microorganisms, such as cyanobacteria and flagellate algae, respond to light to locate themselves at appropriate photoenvironments. 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, characterization, and application 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 (Figure 1), 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 α - and β -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.



Figure 1. *Euglena gracilis*, a unicellular flagellate alga

1-1 Fast manipulation of cellular cAMP level by light *in vivo*.

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, *X. laevis* oocytes and HEK293 cells. Moreover, transgenic *D. melanogaster* flies demonstrated functional PAC expression by showing blue light-induced behavioral changes as described below.

To determine the reliance and kinetics of the light-induced change in behavior, the the grooming reflex¹⁵ was analysed: when covered with a fine powder, fruit flies instantaneously display vigorous and continuous grooming activity lasting up to 30 min. Monitoring this behavior for a total time of 5 min with irradiation alternating between dim white light and intense blue light for 1 min each, revealed high grooming activity in wild-type flies irrespective of stimulation by light (Figure 2). In contrast, neuronal expression of PAC (elav-Gal4/UAS-PAC) resulted in hyperactivity and a substantial decline in grooming activity under blue-light stimulation. When irradiation was switched back to dim white light, thus turning off blue light-induced PAC activity, flies returned to grooming behavior within several seconds. These results demonstrate that transgenic expression of PAC in fruit flies results in a functional protein that is rapidly and reversibly activated by blue light. Moreover, the fast action observed at the on- and offset of irradiation demonstrates the feasibility of rapid control of cAMP levels in a freely moving animal.

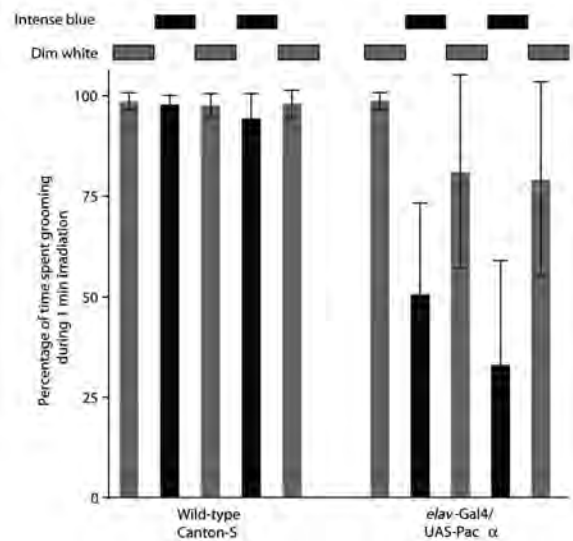
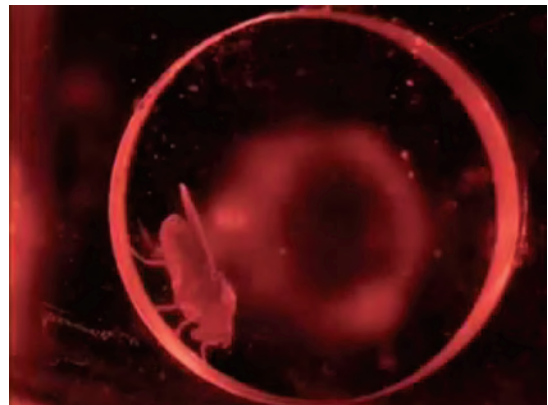


Figure 2. Photostimulation of PAC alters behavior in freely moving *D. melanogaster*.

1-2 Functional transplant of photoactivated adenylyl cyclase (PAC) into *Aplysia* sensory neurons.

In collaboration with Prof. T. Nagahama, (Faculty of Pharmaceutical Science, Toho University), functional transplant of photoactivated adenylyl cyclase (PAC) into *Aplysia* sensory neurons was performed to explore whether PAC can produce cAMP in the neurons by light stimulation. Serotonergic modulation of mechanoafferent sensory neurons in *Aplysia* pleural ganglia has been reported to increase intracellular cAMP level and promotes synaptic transmission to motor neurons by increasing spike width of sensory neurons. When cAMP was directly injected into the sensory neurons, spike amplitude temporarily decreased while spike width temporarily increased. We therefore explored these changes as indicators of appearance of the PAC function. PAC or the PAC expression vector (pNEX-PAC) was injected into cell bodies of sensory neurons. Spike amplitude decreased in both cases and spike width increased in the PAC injection when the neurons were stimulated with light (Figure 3), suggesting that the transplanted PAC works well in *Aplysia* neurons. These results indicate that we can control cAMP production in specific neurons with light by the functional transplant of PAC.

Publication List

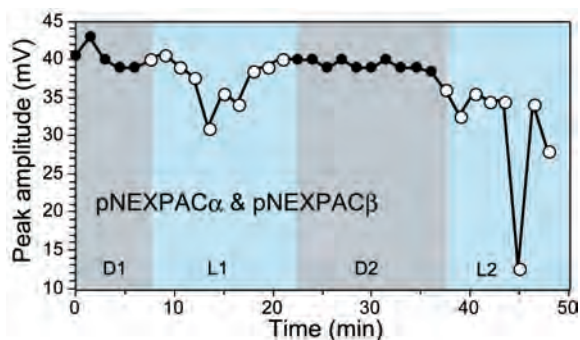
[Original papers]

- Nagahama, T., Suzuki, T., Yoshikawa, S., and Iseki, M. (2007). Functional transplant of photoactivated adenylyl cyclase (PAC) into *Aplysia* sensory neurons. *Neurosci. Res.* 59, 81-88.
- Schröder-Lang, S., Schwärzel, M., Seifert, R., Strünker, T., Kateriya, S., Looser, J., Watanabe, M., Kaupp, U.B., Hegemann, P., and Nagel, G. (2007). Fast manipulation of cellular cAMP level by light *in vivo*. *Nat. Meth.* 4, 39-42.

A



B



C

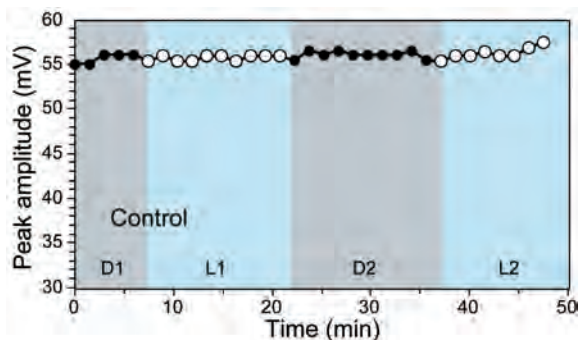


Figure 3. Effect of blue light on spike amplitude in the *Aplysia* sensory neurons into which plasmid vectors carrying PAC cDNAs were injected