ournal of Photoscience (2002), Vol. 9(2), pp. 98–101

# Photactivated adenylyl cyclase, a novel blue-light receptor flavoprotein, mediates photoavoidance in the unicellular flagellate *Euglena gracilis*

Mineo Iseki[1,2,3], Shigeru Matsunaga[4], Akio Murakami[5], Kaoru Ohno[1], Kiyoshi Shiga[6], Kazuichi Yoshida[1], Michizo Sugai[7], Tetsuo Takahashi[8], Terumitsu Hori[4] and Masakatsu Watanabe[1,9*]

[1]National Institute for Basic Biology, Okazaki, 444-8585 Japan
[2]Bio-oriented Technology Research Advancement Institution, 3-18-19, Toranomon, Minato-ku, Tokyo, 105-0001 Japan
[3]Center for Gene Research, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, 464-8602 Japan
[4]Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305-8572 Japan
[5]Kobe University Research Center for Inland Seas, Iwaya, Awaji, Hyogo, 656-2401 Japan
[6]Department of Physiology, Kumamoto University School of Medicine, Honjo, Kumamoto, 860-8556 Japan
[7]Department of Biology, Faculty of Science, Toyama University, Toyama, 930-8555 Japan
[8]School of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, 274-8510 Japan
[9]Department of Photoscience, School of Advanced Sciences, Graduate University for Advanced Studies, Shonan Village, Hayama, Kanagawa, 240-0193 Japan.

*Euglena gracilis* abruptly changes its swimming direction after a sudden increase or decrease in incident light intensity, that is, step-up or step-down photophobic responses, resulting in photoavoidance or photoaccumulation, respectively. To identify the photoreceptor molecules for these UV-A/blue-light type photobehaviors, we purified a flavoprotein from isolated putative photosencory organelles (PFBs) of *Euglena*. The purified flavoprotein , which noncovalently bound flavin adenine dinucleotide (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 each followed by an adenylyl cyclase catalytic domain. The purified flavoprotein actually showed adenylyl cyclase activity, being drastically elevated by blue-light irradiation. Suppression of gene expression of the flavoprotein (Photoactivated Adenylyl Cyclase, PAC) by RNA interference (RNAi) caused loss of the step-up photophobic response, demonstrating that PAC actually mediates photoavoidance of *Euglena*.

**Key words:** blue-light, photoreceptor, flavoprotein, adenylyl cyclase, photomovement, *Euglena gracilis*

## INTRODUCTION

Blue-light signal crucially regulates various biological processes such as plant morphogenesis, fungal developments, and microbial behaviors. Two different types of photoreceptor molecules for the blue-light responses have been identified in plants, i.e. cryptochromes [1, 2] and phototropins [3, 4]; the former have also been found in animals with evidence that they have a role in circadian rhythms [2]. Recently, we found a novel type of blue-light receptor flavoprotein in the photosensory organelle of *Euglena gracilis* [5]. Here we summarize the story and discuss future prospects of studies on this unique molecule.

Photobehavior of *Euglena gracilis*, a unicellular flagellate, has been the subject of extensive study for more

*To whom correspondence should be addressed.
E-mail: machakou@nibb.ac.jp

than a century [6]. The *Euglena* cell rapidly changes its swimming direction upon sudden increase or decrease in incident light intensity, thus exhibiting step-up or step-down photophobic responses, which are considered to be elemental processes for photoavoidance and photoaccumulation, respectively. Action spectroscopy has suggested the involvement of flavin(s) as the chromophore(s) of the photoreceptor molecules for the responses [7]. The *Euglena* cell has a paraflagellar body (PFB), a small ellipsoidal structure near the base of its flagellum, which is considered as a photosensory organelle for photomovements [8]. The PFB exhibits a bright green autofluorescence, consistent with the hypothesis that a flavoprotein localized in the PFB acts as the photoreceptor molecule. To examine this hypothesis, we isolated and characterized the flavoproteins from PFBs, leading to the discovery of a novel blue-light receptor having a unique feature for photosignal transduction.

## RESULTS AND DISCUSSION

*The 400-kDa flavoprotein purified from photosensory organelle of* Euglena

PFBs were isolated by cell-disruption and subsequent sucrose density gradient centrifugation. The isolated PFBs were lysed in an appropriate buffer and a 400-kDa flavoprotein was purified chromatographycally. The fluorescence excitation- and emission spectra of the boiled 400-kDa fraction matched well with that of flavins. The fluorescence intensity showed obvious pH dependency that is characteristic to flavin adenine dinucleotide (FAD), suggesting that the chromophore of the protein is most probably FAD.

From the results of SDS-polyacrylamide gel electrophoresis, the 400-kDa flavoprotein seemed to be a heterotetramer composed of 105-kDa- and 90-kDa subunits. We found that the amino acid sequences of both subunits are similar to each other and that they have four characteristic domains (F1, F2, C1, C2 in Fig. 1) in each sequence. F1 and F2 showed similarity to the N-terminal region of AppA, a redox regulator of photosystem formation in *Rhodobacter sphaeroides*. Since it is reported that AppA noncovalently bind FAD at the N-terminal region with an apparent 1:1 molar ratio [9], we assume that the 105-kDa and 90-kDa subunits each bind two FAD molecules per polypeptide, at F1 and F2.

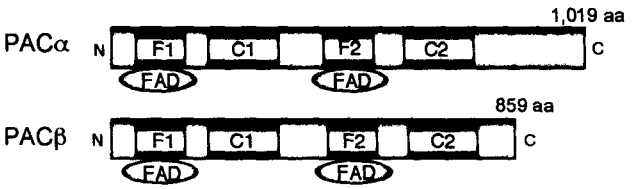


Figure 1. Diagrammatic representations of the 105-kDa subunit (PACα) and the 90-kDa subunit (PACβ).

*Photoactivated adenylyl cyclase, a sensor for photoavoidance*

The other characteristic regions, C1 and C2, quite unexpectedly but significantly, show homology with class III adenylyl cyclase catalytic domains, especially with bacterial ones; the class III adenylyl cyclase is a large family of adenylyl cyclases widely distributed in prokaryotes and eukaryotes [10]. The 400-kDa flavoprotein actually showed slight but significant adenylyl cyclase activity in darkness. Amazingly, the adenylyl cyclase activity under blue light was drastically higher, up to 80 times, than that in darkness. Thus the 400-kDa flavoprotein appeared to be a peculiar adenylyl cyclase whose activity is regulated by blue light. Accordingly, we named the flavoprotein PAC (photoactivated adenylyl cyclase) and its subunits, 105-kDa and 90-kDa polypeptide, PACα and PACβ, respectively.

To examine whether PAC actually acts as a photosensory receptor in *Euglena*, we tried to suppress gene expression of PAC by RNA interference (RNAi). Double stranded RNAs of PAC subunits were synthesized and electroporated into *Euglena* cells, which made

endogenous PAC mRNAs undetectable and caused loss of PFB in the cells. The step-up photophobic response disappeared in the RNAi-treated cells even at high intensity of light, while the step-down photophobic response remained normal. From these results, we conclude that PAC is the major constituent of the PFB and it acts as the photoreceptor for step-up photophobic response (photoavoidance) in *Euglena*, whereas it is not involved in step-down photophbic response (photoaccumulation).

*Possible mechanisms of the step-up photophobic response*

In visual and olfactory sensing systems of animals, signals are sensed by receptor molecules and activate heterotrimeric guanine nucleotide-binding proteins (G-proteins) which in turn stimulate cGMP-phosphodiesterases or adenylyl cyclases to change intracellular cyclic nucleotide concentrations, leading to closure or opening of the cyclic nucleotide-gated channels respectively. In contrast, PAC by itself can act both as a photoreceptor and an effector to catalyze cAMP formation without any other intervening signal transducers. This mechanism is extremely simple and advantageous for eliciting a quick response to light like photomovements of *Euglena*. In this connection, it is noteworthy that cyclic nucleotide-gated channels that control $Ca^{2+}$ entry and may be related to flagellar motility have been identified in mammalian sperm [11]. Considering also the fact that $Ca^{2+}$ affects flagellar movements of *Euglena* [12], the process of step-up photophobic response may well be simply explained as follows (Fig. 2): blue light activates PAC to induce a local increase of cAMP concentration around PFB, then the elevated cAMP level induces $Ca^{2+}$ entry into the flagellar apparatus and the $Ca^{2+}$ modifies the direction of flagellar beating. Another possibility is that the elevated cAMP level causes phosphorylation of flagellar proteins by activating a cAMP-dependent protein kinese (PKA), which modifies the flagellar motility as reported in *Chlamydomonas* [13] and *Paramecium* [14].

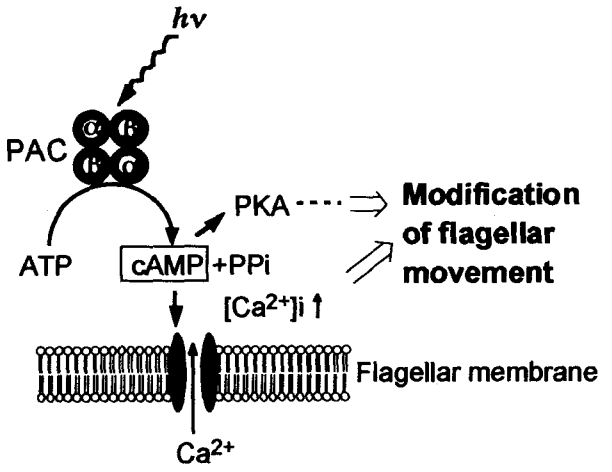


Figure 2. A working hypothesis on the mechanism of the step-up photophobic response in *Euglena*.

*What is the sensor for photoacummulation?*

It was really a surprise for us that the RNAi treatment abolished only step-up, without affecting step-down, photophobic response, because it has long been believed that PFB is the photosensory organelle for the step-down photophobic response [6, 8]. In this study, it became clear that the PFB itself is not essential for the step-down photophobic response. Since the action spectrum for the step-down photopobic response is slightly different from that of the step-up photophobic response, a similar but distinct photoreceptor molecule may be located near the PFB. A search for PAC-like sequences in *Euglena* may provide a clue to the question.

*PAC as a tool for cell biological study*

Since cAMP mediates various biological functions, we can imagine that PAC may be used as a cell biological tool for controlling cellular cAMP level by light: i.e., targeted heterologous expression of PAC by gene manupilation in nerve and other cells would enable us to pin-point control cellular processes such as axon guidance [15], synaptic plasticity (including long-term depression and long-term potentiation) [16], and cell differenciation [17]. These would include reforming PAC into PGC (photoactivated

guanylyl cyclase which produce cGMP) by point-mutations and replacing the flavin chromophore with some analogs with different absorption peaks, thus enabling us to use these combinations as wavelength-sensitve switches of biological activities. We hope these ideas will be realized in near future.

## REFERENCES


1. Ahmad, M. and Cashmore, A.R. (1993) *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. *Nature* 366, 162-166.
2. Cashmore, A. R., Jarillo, J. A., Wu, Y.-J. & Liu, D. (1999) Cryptochromes: blue light receptors for plants and animals. *Science* 284, 760-765.
3. Huala, E., Oeller, P. W., Liscum, E., Han, I.-S., Larsen, E. and Briggs, W. R. (1997) *Arabidopsis* NPH1: a protein kinase with a putative redox-sensing domain. *Science* 278, 2120-2123.
4. Briggs, W. R. and Christie, J. M. (2002) Phototropins 1 and 2: versatile plant blue-light receptors. *Trends Plant Sci.* 7, 204-210.
5. Iseki, M., Matsunaga, S., Murakami, A., Ohno, K., Shiga, K., Yoshida, K., Sugai, M., Takahashi, T., Hori, T. and Watanabe, M. (2002) A blue-light-activated adenylyl cyclase mediates photoavoidance in *Euglena gracilis*. *Nature* 415, 1047-1051.
6. Lebert, M. (2001) Phototaxis of Euglena gracilis – flavins and pterins. *In: Comprehensive Series in Photosciences 1: Photomovement.* Ed. By Häder, D. -P. & Jori, G. Elsevier, Amsterdam pp.297-341.
7. Matsunaga, S., Hori, T., Takahashi, T., Kubota, M., Watanabe, M., Okamoto, K., Masuda, K. and Sugai, M. (1998) Discovery of signaling effect of UV-B/C light in the extended UV-A/blue-type action spectra for step-down and step-up phototropic responses in the unicellular flagellate alga *Euglena gracillis*. *Protoplasma* 201, 45-52.
8. Colombetti, G., Lenci, F. and Diehn, B. (1982) Responses to photic, chemical, and mechanical stimuli. *In: The Biology of* Euglena *III.* Ed. by Buetow, D. E. Academic Press, New York pp.169-195.
9. Gomelsky, M. and Kaplan, S. (1998) AppA, a redox regulator of photosystem formation in *Rhodobacter sphaeroides* 2.4.1, is a flavoprotein. Identification of a novel FAD binding domain. *J. Biol. Chem.* 52, 35319-35325.
10. Danchin, A. (1993) Phylogeny of adenylyl cyclases. *Adv. Sec. Mess. Phosphoprot. Res.* 27, 109-162.
11. Wiesner, B., Weiner, J., Middendorff, R., Hagen, V., Kaupp, U. B. and Weyand, I. (1998) Cyclic nucleotide-gated channels on the flagellum control $Ca^{2+}$ entry into sperm. *J. Cell Biol.* 142, 473-484.
12. Doughty, M. J. and Diehn, B. (1979) Photosensory transduction in the flagellated alga, *Euglena gracilis.* I. Action of divalent cations, $Ca^{2+}$ antagonists and $Ca^{2+}$ ionophore on motility and photobehavior. *Biochim. Biophys. Acta* 588, 148-168.
13. Hasegawa, E., Hayashi, H., Asakura, S. and Kamiya, R. (1987) Stimulation of in vitro motility of *Chlamydomonas* axonemes by inhibition of cAMP-dependent phosphorylation. *Cell Motil. Cytoskeleton* 8, 302-311.
14. Noguchi, M., Ogawa, T. and Taneyama, T. (2000) Control of cililary orientation through cAMP-dependent phosphorylation of axonemal proteins in *Paramecium caudatum*. *Cell Motil. Cytoskeleton* 45, 263-271.
15. Song, H.-j. and Poo, M.-m. (1999) Signal transduction underlying growth cone guidance by diffusible factors. *Curr. Opin. Neurobiol.* 9, 355-363.
16. O'Dell, T. (1999) Long-term potentiation. *In: Molecular and Cellular Physiology of Neurons.* Fain, G. L. Harvard University Press, Cambridge, USA, London pp.476-507.
17. Aubry, L. and Firtel, R. (1999) Integration of signaling networks that regulate *Dictyostelium* differentiation. *Annu. Rev. Cell Dev. Biol.* 15, 469-517.