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Optical control of cellular signaling pathways using animal opsins

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Abstract

Currently, in various biological research fields, optical control of cellular responses is widely utilized, leading to establishing a research field named "optogenetics". Successful optogenetic studies need both state-of-art optical devices illuminating target issues with a high spatiotemporal resolution and optical control tools (typically photosensitive proteins) driving cellular reactions in a light-dependent manner. Most of optogenetic analyses have used channelrhodopsins, light-sensitive ion channels, as control tools. Alternatively, animal opsins, light-sensitive G protein-coupled receptors, can be used as tools to drive a wide variety of G protein-dependent intracellular signaling pathways. Here, I characterize molecular properties of invertebrate opsins by spectroscopic and electrophysiological techniques, and introduce them as "ON-OFF" switch of GPCR signalings. Also, I discuss potentials of the animal opsins as optogenetic tools to expand the research field.

Keywords: opsin, G protein-coupled receptor, ion channel, optogenetics, membrane protein.

I Introduction

In order to understand biological phenomena, you need to characterize them, reveal detailed mechanisms, and if possible, manipulate them. Optical control is one of the most powerful techniques manipulating biological functions such as excitation of neurons, because light stimulation can be controlled with a high spatiotemporal resolution. In particular, optogenetics introducing photo-receptive proteins such as channelrhodopsin into specific neural cells has revolutionized biological research fields, in particular neuroscience[1]. In parallel, engineering has produced to powerful optical devices such as fine microscopes and devices to deliver precise light stimulus. In other words, to conduct a successful optical control research such as optogenetics, you need appropriate photo-receptive protein as optical control tool and optical devises to stimulate and/or monitor behavior of the control tool.

Current optical control/optogenetic studies utilize channelrhodopsins, light-sensitive ion channels, as control tools, and analyze neural functions as research target. Channelrhodopsin, which was originally identified from green algae[2], is an ideal tool to manipulate neural activities, since the activities are promoted or inhibited by ion conductance across the cell membranes. For example, cation-conducting and anion-conducting channelrhodopsins (and their derivatives) are used for stimulating and silencing neurons, respectively. On the other hand, in living animals, there are various "non-excitable" cells such as astrocytes. To further expand optical control techniques to a wider variety of tissues, alternative control tools are needed.

Many animals including humans possess light-sensitive G protein-coupled receptors named as opsins in their eyes or other photoreceptive tissues. Animal opsins are completely different proteins from channelrhodopsins, although both proteins have seven transmembrane helices and use retinal (aldehyde form of vitamin A) as chromophore. Animal opsins activate trimeric G proteins to drive various intracellular signaling pathways via light-dependent G protein and/or arrestin activation.

Some optogenetic studies have already utilized animal opsins as optical control tools[3, 4]. These studies use opsins that function in vertebrate visual photoreceptor cells (rods and cones). As described above, these "vertebrate visual opsins" bind retinal as chromophore, but they specifically bind "cis" isomer of retinal (Figure 1). In eyes, a specific enzyme system produces cis-retinal, but the system is absent in other tissues. The rarity of cis-retinal could be an obstacle for vertebrate visual opsins to effectively function as photoreceptive proteins in tissues outside eyes.

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In contrast to vertebrate visual opsins, invertebrate opsins can bind not only cis- but also trans-retinal (Figure. 1) that are abundant in various tissues. Furthermore, invertebrate opsins can be activated and deactivated by illumination of different wavelength light, whereas vertebrate visual ones can only be activated by light (Figure 1)[5]. That means invertebrate opsins can function as an "ON-OFF" switch of cellular activities by stimulation of different color of light. Based on these insights, I would like to insist that invertebrate opsins are better optical control tools to modulate G protein-dependent signaling pathways. In this paper, I introduce an example showing that an invertebrate opsin can effectively function as "ON-OFF" switch of a G protein-coupled cellular responses.

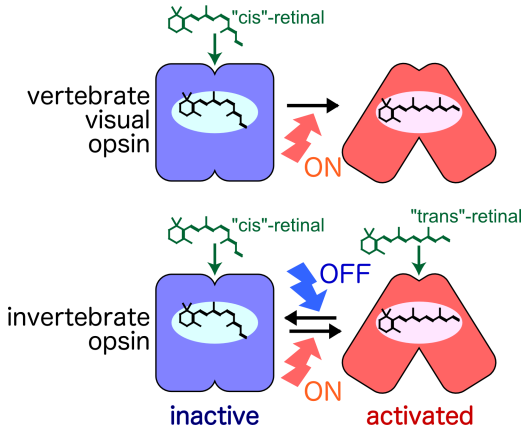

Figure 1. Retinal binding and activation schemes of animal opsins. Invertebrate opsins can bind "trans"-retinal as well as "cis"-retinal and the inactive and activated forms are interconvertible by light.

II Results and Discussion

As indicated in the "Introduction" section, invertebrate opsins can be interconvertible between inactive and activated forms. If you want to completely turn OFF an opsin, the absorption spectrum of the activated form should be largely red shifted from that of the inactive form. Spectral separation of the inactive and activated forms enables to selectively illuminate the activated form to be converted to the inactive form. However, many invertebrate opsins show the significant spectral overlaps, and cannot be fully inactivated by light. I have assessed spectroscopic properties of many invertebrate opsins, and found some opsins with sufficient spectral separation between inactive and activated forms. One of the opsins with the spectral separation is c-opsin1 from a marine ragworm *Platynereis dumerili*[6]. The inactive form of the opsin (cis-retinal bound) has an absorption maximum at 383-nm and absorb UV-light (Figure 2B). Upon UV illumination (or trans-retinal biding) convert it to the activated form that possesses a broader absorption spectrum and can absorb visible light. Yellow light (>490-nm) illumination of the activated c-opsin1 causes re-formation of the inactive form (Figure 2B)[7]. The photoreactions of c-opsin1 clearly shows that the invertebrate opsin is inter-convertible between the inactive and active form upon light absorption and visible light acts as a complete "OFF" switch of the opsin.

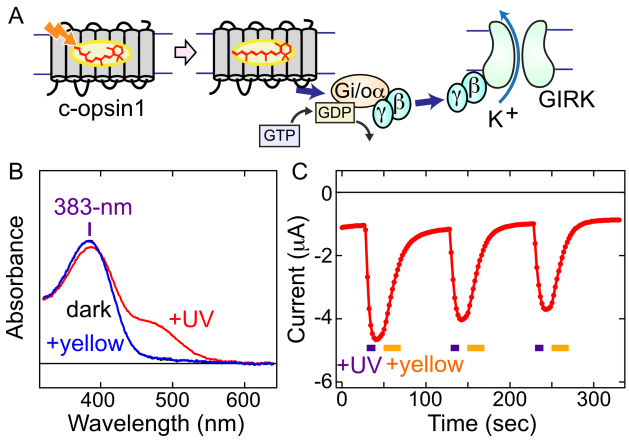

Figure 2. A, Activation pathway form c-opsin1 to GIRK channels. B, Absorption spectra of c-opsin1 before and after illumination. C, GIRK activation and deactivation by c-opsin1 in a *Xenopus* oocyte.

The *Platynereis* c-opsin1 is coupled to Gi/o-type trimeric G proteins. Activation of trimeric Gi/o leads to dissociation of α and βγ subunits, each of which drives intracellular signaling pathways. In typical neural cells, dissociated Gi/o βγ subunit directly activated a K^+ channel named GIRK leading to suppression of neural firings (Figure 2A)[4, 8]. Thus, c-opsin1 would be suitable as an optical control tool to manipulate K^+ current in neurons. The property would be useful for expanding of optogenetics, because currently, K^+ current is hard to be manipulated by channelrhodopsins.

To assess ability of c-opsin1 to modulate GIRK channel in a light-dependent manner, I introduced genes of c-opsin1 and GIRK into *Xenopus* oocytes, and analyzed light-induced changes of GIRK current in the cells using electrophysiological techniques. The obtained results clearly showed that GIRK current was increased by UV-light and decreased by yellow light (Figure 2C)[7]. Taken together, the *Platynereis* c-opsin1 possesses molecular properties suitable for optical control of K^+ currents in cells. In addition, GPCRs including the opsin can drive other intracellular signaling pathways. Based on the results and insights, at the BISC meeting, I will introduce and discuss several new optical control tools using invertebrate opsins, and how they and optical devices can contribute for progress of biological studies.

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