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Commentary and Perspective

A variety of photoreceptors and the frontiers of optogenetics

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Lives have acquired a variety of photoreceptive proteins which absorb light in the UV to far-red region during the evolution, such as many different types of rhodopsin, blue-light receptors including cryptochrome and phototropin, and red/far-red light photochromic phytochromes. After the long-time studies on the molecular mechanism of their action, they have been applied to various photobiological studies. Recent advancement in the research field is remarkable and brought many fruitful results especially in optogenetics. To introduce some of these results, we organized a symposium named "A variety of photoreceptors and the frontiers of optogenetics" at the 59th annual meeting of the Biological Society of Japan (BSJ) in November 2021. The symposium was co-organized by a research area of the Precursory Research for Embryonic Science and Technology Program (PRESTO) named "Optical Control", directed by Prof. Shichida (Ritsumeikan University), sponsored by Japan Science and Technology Agency (JST). We invited 4 PRESTO members and 2 other researchers to cover the light absorption region from blue to far-red (Figure 1).

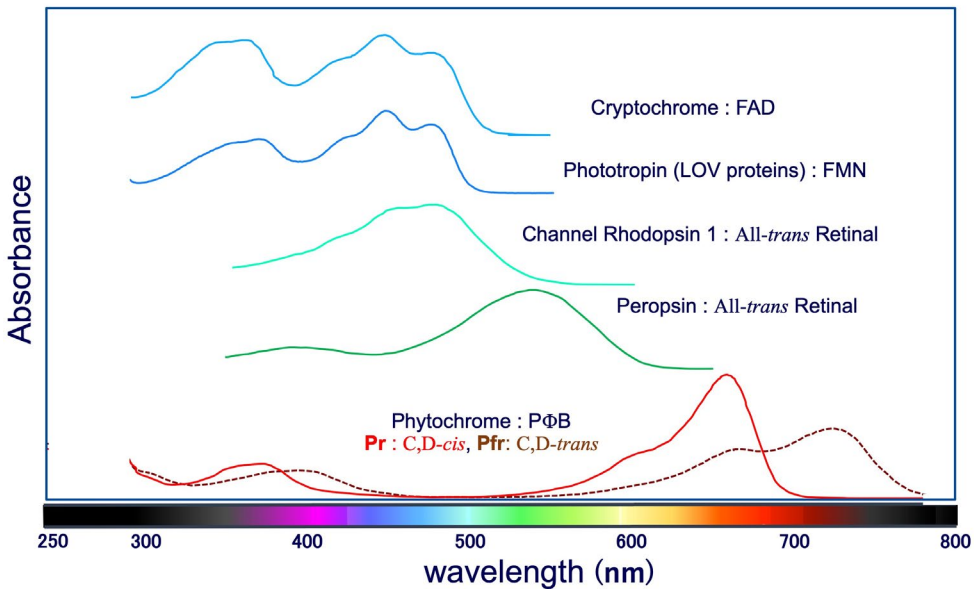

Figure 1 Absorption spectra of the photoreceptors appeared in the presentations of the Symposium. FAD; flavin adenine dinucleotide, FMN; flavin mononucleotide, PΦB; phytochromobilin.

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Optogenetics is now a common and powerful tool in the brain research field. The first speaker Dr. Takuma Kitanish (Osaka City Univ., PRESTO) presented the latest results on the conveying mechanism of a variety of spatial information through the hippocampus using optogenetic techniques [1, 2]. His group investigated the mechanism how the information is distributed to multiple downstream areas by identifying axonal projections using optogenetics during large-scale extracellular recordings from the rat subiculum, the major hippocampal output structure. They found that speed and trajectory information was selectively sent to the retrosplenial cortex and nucleus accumbens, respectively, while, place information was distributed uniformly to the retrosplenial cortex, nucleus accumbens, anteroventral thalamus, and medial mammillary body. Thus, they revealed that a development of the subiculum robustly routes diverse spatial information to downstream areas.

Cryptochrome (CRY) is a principal clock component in the molecular feedback loop of the mammalian clock system, while CRY of other species such as fly and plants acts as a photoreceptor. During the study on the function of mammalian CRY in photoreception, Dr. Arisa Hirano (Univ. Tsukuba, PRESTO) and her co-workers have found that CRY1 interacts with G protein signaling molecule and enhances the signaling mediated by OPN4 (melanopsin) [3] in cell culture. Then, she switched the research theme to the development of optogenetics tool using modified OPN4 and presented the establishment of a hyper-sensitive optogenetic method with modified human OPN4 to induce and maintain long-term hibernation-like hypothermic state [4] in mice. OPN4 is a bi-stable photopigment with a high sensitivity and was expected to be suitable for long-term optogenetics. Her group ectopically expressed C-terminally truncated OPN4 (OPN4dC) in QRFP-producing neurons in the anteroventral periventricular nucleus for inducing hypothermia. Photo-stimulation by low-power light to OPN4dC maintained hypothermia and suppressed cardiovascular function for a long period with a high temporal resolution in mice. Furthermore, they succeeded in fiber-less transcranial stimulation with a blue LED to induce a robust drop in body temperature in a non-invasive way. The OPN4dC-induced hypothermia recapitulated the kinetics of physiological changes observed in the natural hibernation and revealed that QRFP-producing neurons contributed to not only thermoregulation but regulation of cardiovascular function *in vivo*.

Optogenetics is a powerful tool in the regenerative medicine. Dr. Takashi Nagata (Univ. Tokyo, PRESTO) reported the application of optogenetics to the vision regeneration by peropsin. Peropsin is an all *trans*-retinal-binding rhodopsin, acting as a dark-active, light-inactivated GPCR upon replacement of its intracellular domain [5]. This unique property of peropsin may enable a new approach for vision restoration instead of a common use of rhodopsin [6] that brings a negative image. He presented the strategy to develop a peropsin-based optogenetic tool for vision restoration and a recent result that the tool activated the Go-type G protein. The result could be useful for restoring the light-responsiveness of retinal interneurons after photoreceptor degeneration.

Many photoreceptors used in Optogenetics come from plant. LOV domains of plant phototropin act as useful tools in optogenetics including the regulation of cell-signaling mostly in animal cells. Phototropin is a blue-light-activated protein kinase that regulates various cellular processes by phosphorylating their downstream targets in plants. Dr. Hiromasa Shikata (NIBB, PRESTO) has been studying the roles of AGC kinases on the plant development and cell growth and found that a polar localization of the kinases on the plasma membrane [7] correlates closely with re-orientation of root hair growth, suggesting that the kinases could regulate the growth direction. He presented the recent development of optogenetic tools using phototropin to directly control the kinase activity at the subcellular level to verify the assumption. Furthermore, he reported the light-regulation of the AGC kinase activity on the cell membrane by miniSOG, one of LOV domain-derived optogenetic tools [8] that controlled the hair root growth direction.

The light-induced dimerization (LID) system, in which photoresponsive proteins are rapidly and reversibly dimerized in response to light, allows controlling protein-protein interactions and cell signaling. Dr. Kazuhiro Aoki (NIBB, NINS)'s group utilize one of the red/far-red responsive LID systems, phytochrome B (PhyB)–phytochrome interacting factor (PIF), which has a unique property of controlling both association and dissociation by light on the second time scale. PhyB requires a linear tetrapyrrole chromophore such as phycocyanobilin (PCB), however, such chromophores are present only in higher plants and cyanobacteria. He presented the developments of an efficient biosynthesis system of PCB in mammalian cells to overcome this problem [9, 10] and the application to the control of cell signaling in mammalian cells, fission yeast, and *C. elegans*.

The last speaker Dr. Rei Narikawa (Tokyo Metro. Univ) has been studying cyanobacteriochrome (CBCR) photoreceptors. CBCRs are a distant relative of plant canonical photoreceptors, phytochromes, and highly diversified in their spectral properties. Among their domain structures, only the GAF domain is needed for chromophore incorporation and proper photoconversion. He focused on the species of binding chromophore and presented a few topics on the discovery and engineering of the CBCR that include the rational conversion of the CBCR GAF domains to accept the mammalian intrinsic biliverdin chromophore and its application to a near-infrared fluorescent probe for *in vivo* imaging in living mice [11]. He also developed the evolution-inspired design of multicolored photoswitches from a single CBCR GAF domain covering the wavelength blue-to-orange region [12].

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