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Editorial

Editorial on Special Issue "The Advances and Applications of Optogenetics"

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Abstract: This Special Issue provides an update for the rapidly developing technology known as "optogenetics" that is the use of genetically encoded light-sensitive molecular elements (usually derived from lower organisms) to control or report various physiological and biochemical processes within the cell. Two ongoing clinical trials use optogenetic tools for vision restoration, and optogenetic strategies have been suggested as novel therapies for several neurological, psychiatric and cardiac disorders. The Special Issue comprises two reviews and seven experimental papers on different types of light-sensitive modules widely used in optogenetic studies. These papers demonstrate the efficiency and versatility of optogenetics and are expected to be equally relevant for advanced users and beginners who only consider using optogenetic tools in their research.

Keywords: optogenetics; photoswitching; photocontrol; all-optical electrophysiology; microbial rhodopsins; ion channels; LOV domains; membrane potential; intracellular trafficking; protein–protein interaction; signaling

1. Introduction

Broadly defined, optogenetic technology "combines genetic targeting of specific neurons or proteins with optical technology for imaging or control of the targets within intact, living neural circuits" [1]. This umbrella term encompasses both genetically encoded light-sensitive actuators and reporters of cellular activity. Historically, the reporters have been introduced first: targeting specific cell populations by heterologous expression of the gene encoding green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* predated the term "optogenetics" by >10 years [2]. Structure-directed combinatorial mutagenesis of GFP has converted this protein into a fluorescent pH indicator to monitor synaptic transmission [3].

These early developments led Francis Crick to predict the possibility also to activate neurons with light [4]. Indeed, this has soon been achieved by co-expression of several essential elements of the enzymatic cascade of animal vision in non-visual cells [5]. However, the real coming of age optogenetics experienced after the emergence of a cornucopia of photosensitive molecules from photosynthetic microbes and plants. Furthermore, synthetic chromophores—referred to as "photoswitches"—have been designed to interact with specific target proteins and confer photosensitivity to them. Currently, many different natural and synthetic photosensitive moieties are being used in optogenetic experiments in many different cellular and organismal contexts, and the field is still rapidly expanding.

2. In This Special Issue

Piatkevich et al. review recent efforts to engineer genetically-encoded fluorescence indicators to monitor the membrane voltage and the concentrations of Ca^{2+} and K^+, as well as key neurotransmitters,

changes in which accompany neuronal activity. This work serves as an excellent guide for selection of the most appropriate optogenetic reporters for a particular experiment.

Optogenetic actuators are even more diverse than sensors, in both their nature and intended uses. In some cases, such as microbial rhodopsins, the functions of the photosensor and effector are executed by the same protein domain, whereas in other proteins a photosensory domain is followed by distinct effector domains. Examples of photosensory domains found in native multidomain proteins are small flavoprotein modules known as Light, Oxygen, or Voltage sensing (LOV) and Blue-Light-Utilizing Flavin-binding (BLUF) domains that respond to UV-A/blue light (320–500 nm) [6,7]. Both these domains are widely used in optogenetic studies.

In plant phototropins that contain LOV domains, photoexcitation of the chromophore flavin mononucleotide (FMN) leads to unfolding of the C-terminal Jα helix, to which various peptides of interest, such as nuclear localization and export signals, can be attached. Wehler and di Ventura use a LOV domain-based light-inducible nuclear export system (LEXY) to manipulate cellular levels of the transcription factor p53 with blue light. In certain human cancers, excessive inactivation of p53 results from overexpression of its negative regulator, murine double minute 2 (Mdm2). The 12-amino-acid peptide, p53–Mdm2/MdmX inhibitor (PMI), binds to Mdm2 and suppresses its function. The authors show that in the dark, the PMI-LEXY fusion remains in the nucleus and prevents Mdm2 from degrading p53. Illumination caused export of the PMI-LEXY fusion to the cytosol, which released Mdm2. According to the authors, this optogenetic tool can be used to study the effects of local p53 activation within a tissue or organ.

BLUF domains are mostly found in prokaryotes and usually bind flavin adenine dinucleotide (FAD) as a chromophore. They exhibit different photochemical reactions, as compared to LOV domains. Kaushik et al. have analyzed 34 native BLUF domains from publicly accessible sequence databases. They have found functional association of these domains with several previously unknown effector domains, such as guanine nucleotide exchange factor for Rho/Rac/Cdc42-like GTPases (RhoGEF), phosphatidyl-ethanolamine binding protein (PBP), ankyrin and leucine-rich repeats. This remarkable modular diversity of BLUF domain-containing proteins expands the repertoire of potential chimeric assemblies that can be created by a combination of BLUF domains with appropriate cellular effectors.

Microbial rhodopsins, being electrogenic, are used to control the membrane voltage with light [8]. Channelrhodopsins mediate passive transport of ions along the electrochemical gradient and are therefore intrinsically more potent than rhodopsin ion pumps that translocate across the membrane only one ion per captured photon. Both cation- and anion-selective channelrhodopsins are known, abbreviated as CCRs and ACRs, respectively [9]. CCRs appear to emerge by convergent evolution by at least two independent routes. One CCR family was found in green (chlorophyte) flagellate algae, in which they act as photoreceptors for phototaxis [10]. Another CCR family that shows closer primary sequence homology to haloarchaeal rhodopsin pumps than to other known CCRs, was found in phylogenetically distant cryptophyte algae [11]. Shigemura et al. characterize channel properties of CCR4 from the cryptophyte alga *Guillardia theta* (GtCCR4). The advantages of this protein as an optogenetic tool comprise the red-shifted absorption maximum (530 nm), small desensitization during continuous illumination and the relatively high Na^+/H^+ permeability ratio, as compared to ChR2 from the chlorophyte alga *Chlamydomonas reinhardtii* (*Cr*ChR2).

H^+ permeability of CCRs is a serious problem in some optogenetic experiments, as it may lead to a decrease in the cytoplasmic pH [12]. Duan et al. show that the D156H mutation of *Cr*ChR2, and the corresponding mutations of the fast CCR variant Chronos [13] and blue-shifted ChR from *Platymonas subcordiformis* (*Ps*ChR) [14] enhanced relative permeabilities for Na^+ and Ca^{2+}, as compared to that for H^+. Moreover, in *Ps*ChR this mutation additionally increased the current amplitude, which made it the best currently available tool for optogenetic manipulation of the intracellular Ca^{2+} level.

Despite >50 native and the innumerable number of engineered CCR variants currently known, *Cr*ChR2 and its gain-of-function H134R mutant so far have remained the most frequently used optogenetic excitatory tools [15]. Two articles in this Special Issue report mechanistic studies on *Cr*ChR2,

the results of which might contribute to further improvement of this tool for optogenetic needs. Richards et al. probe the role of residual hydrophobic mismatch (RHM) by a combination of computational and functional approaches. The authors identified several residues at the intracellular/lipid interface, mutations of which were predicted to significantly reduce the RHM energy penalty. They also showed, by electrophysiological analysis of these mutants, that the reduction of the RHM penalty in the closed state compromised *Cr*ChR2 conductance, selectivity and open state stability. These results show that protein–lipid interactions have to be taken into account when engineering optogenetic tools for specific cell types.

Ehrenberg et al. examine the functional role of Thr127 located near the retinylidene Schiff base in *Cr*ChR2. Replacement of this residue with alanine or serine did not change the position of the spectral maximum, which ruled out its contribution to the counterion complex. However, the T127A mutation, unlike the conservative T127S mutation, accelerated deprotonation of the Schiff base and strongly delayed its reprotonation. These results place Thr127 in the hydrogen-bonded network connecting the Schiff base with Asp156, which the authors identified earlier as the proton donor to the Schiff base [16]. This conclusion was further corroborated by the observation of extended lifetime of the channel open state observed in both T127A and T127S mutants, as compared to the wild type.

Erofeev et al. systematically analyzed the influence of frequency, duration and intensity of optical stimulation on performance of *Cr*ChR2 in cultured mouse hippocampal neurons. Using optimal photostimulation protocols is very important in optogenetic experiments, because e.g., insufficient illumination results in poor fidelity, whereas excessive light might lead to overheating of the tissue. The authors show that at the optimal stimulation frequency 1–5 Hz the dependence of photocurrent on the light pulse duration is described by a right-skewed bell-shaped curve, whereas the dependence on the stimulus intensity is close to linear. These results complement previously published work (e.g., [17]) and provide useful guidelines for optogenetic experimentation.

Finally, the review by Kellner and Berlin summarize recent progress in the development of synthetic azobenzene switches and their optimization for two-photon excitation (2PE). Azobenzene is the most popular chromophore used in synthetic optogenetics, owing to its high quantum yield, solubility in water and minimal photobleaching. Most importantly, under photoexcitation azobenzene undergoes a rapid, robust isomerization from the *trans* to *cis* conformation that can be harnessed to drive biologically relevant conformational changes in target proteins. 2PE allows using near-infrared (NIR) light that better penetrates biological tissue to activate optogenetic molecules and provides three-dimensional single cell-level spatial resolution. However, typical azobenzene-based switches exhibit poor absorption of NIR. The authors describe several strategies that have been used to increase the 2P-absorption cross section of azobenzene-based photoswitches without compromising the rate of their response or other useful properties.

Taken together, the papers in this Special Issue are a valuable contribution towards a better understanding of photochemistry and biophysics of optogenetic tools, which provides the guidelines for further engineering to improve their performance.

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