Optogenetics comes of age: novel inhibitory light-gated anionic channels allow efficient silencing of neural function

Alex Peralvarez-Marin¹ and Pere Garriga²

¹Unitat de Biofísica, Departament de Bioquímica i de Biologia Molecular, Facultat de Medicina, and Centre d'Estudis en Biofísica, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.

²Grup de Biotecnologia Molecular i Industrial, Centre de Biotecnologia Molecular, Departament d'Enginyeria Química, Universitat Politècnica de Catalunya, Edifici Gaia, Rambla de Sant Nebridi 22, 08222 Terrassa, Spain.

***Corresponding author:** Professor Pere Garriga, Centre de Biotecnologia Molecular, Departament d'Enginyeria Química, Universitat Politècnica de Catalunya, Rambla de Sant Nebridi 22, 08222 Terrassa, Spain. Tel: +34 937398568; FAX: +34 937398225; E-mail: pere.garriga@upc.edu

Summary

Optogenetics, the pushing field of research that uses light-switchable biochemical tools in a sophisticated technological approach for monitoring or controlling neural function, is rapidly evolving with the discovery and development of novel microbial rhodopsins. Light absorbing membrane proteins, as tools for brain research, are promoting new applications within the novel discipline of optogenetics. Finding light-gated rhodopsin ion channels with increased intrinsic better light sensitivity and improved resolution features is needed to overcome some of the current limitations of existing molecules. The recent discovery of light-gated inhibitory anion channels opens new opportunities for studying physiological neural processes and represent, at the same time, a powerful approach to elucidate the mechanisms of neurological and mental disorders that could benefit from this approach.

Keywords: rhodopsins; light-activated ion channels; signal transduction; brain function.

Microbial rhodopsins

The discovery of bacteriorhodopsin in the 1970s [1] opened new research perspectives in the use of bacterial photoreceptor proteins for a wide range of potential technological applications. Until then, only visual receptors in vertebrates and invertebrates were known to use retinal as the light-absorbing molecule that mediated their specific function [2]. Rhodopsin had been known almost one century ago as visual purple due to its pinkish color. This visual photoreceptor has been thoroughly studied for its main role in vision and as a prototypical member of the G-protein coupled receptor superfamily. Visual rhodopsin can be found in the retina that was termed the approachable part of the brain because it could be studied by means of non-invasive techniques. Brain function, however, could only be monitored by imaging technologies. The prospects for other more focalized and versatile approaches that could provide higher resolution, and higher manipulation ability, has been met by the development of novel uses of microbial opsins whose efficient activation can be light-controlled and targeted to specifically-located cell types.

Microbial rhodopsins have had a preeminent site in science books for several decades. Initially, proteins such as bacteriorhodopsin were thought to provide new clues for the treatment of visual impairment derived from genetic defects in human rhodopsin such as those associated with the retinal degenerative disease retinitis pigmentosa [3]. The notable thermal and conformational stability of the robust bacteriorhodopsin contrasted with the delicate and labile conformation of visual rhodopsin. Soon it was realized that, in spite of sharing a photoexcitable mechanism mediated by a retinal chromophore, the two proteins have a completely distinct physiological function. Visual rhodopsin is the light-absorbing pigment that initiates the visual phototransduction process in the retinal leading to a visual stimulus in the brain through a cascade of biochemical reactions and secondary messengers. In contrast, bacteriorhodopsin is a direct transducer of light into proton transport that eventually generates an electric

current by means of an electrochemical gradient across the bacterial membrane. Therefore, the completely different function of the two retinal proteins precluded any use of bacteriorhodopsin in visual research. However, the discovery of novel microbial rhodopsins and their fine-tuned engineered versions has recently provided new hope to restoring visual impairment in humans [4].

After this initial attempt to use these receptors as models in vision research, novel applications were foreseen for microbial rhodopsins into the technology field towards optical-based or photocurrent-based applications. Molecular biology techniques were employed to produce engineered bacteriorhodopsin metastable mutants that yielded two-state photocycles for long-lasting optical biomemories and photocurrent-based devices. The initial prospects have not been fulfilled as expected, but research on microbial rhodopsin by means of a plethora of biophysical methods has allowed gathering important experimental information for the structural elucidation of such light-gated molecular mechanisms. In the last 15 years, the intensive use of metagenomics boosted the identification of novel naturally-occurring microbial rhodopsins in diverse ecosystems, such as proteorhodopsin, a eubacteria microbial rhodopsin found in the ocean [5]. The scientific explosion took place when the field of microbial rhodopsins met neuroscience, a field that had been looking for tools to efficiently control specific brain cell types (Fig. 1) without altering other surrounding, or distant, cell types. Optogenetics was about to emerge.

Optogenetics

The quest for photoreceptors capable of generating light-switched tools, which can serve to bypass the initial steps of the cellular machinery, is paving the way for the new optogenetics field with breakthrough applications in neurobiology research. Initial attempts of controlling cell function by using microelectrodes were put aside because of the additional advantages that triggered optical stimulation of excitable cells provides: lower tissue invasion and damage and higher spatiotemporal resolution. Chemical

genetics approaches were designed to trigger specific ion channels. The aim was to engineer chemical photo-switches to target ion channels that could trigger action potentials. This strategy has been proven particularly useful in model systems such as zebrafish, *Drosophila*, and *Caenorhabditis elegans* providing superior temporal and spatial resolution in light-driven neuron control. However, chemical genetics was soon outrun by optogenetics with the discoveries of the first naturally occurring light-gated ion channel, Channelrhodopsin-1, and the first light-gated cation channel Channelrhodopsin-2 (ChR2) from the eukaryotic unicellular microorganism *Chlamydomonas reinhardtii* [6,7].

Microbial rhodopsins and optogenetics

Microbial rhodopsins, and especially channelrhodopsins (ChR), have been the starting point for the field of optogenetics as a way to monitor and/or control cellular and physiological processes. The ideal optogenetics toolbox should contain depolarization as well hyperpolarization channels, to alternatively activate and inhibit excitatory processes with high temporal and spatial resolution. So far, ChR2 and related ChRs were identified as light-gated cation channel rhodopsins (CCRs), thus capable of activating excitable cells by promoting membrane depolarization. Bacteriorhodopsins and halorhodopsins have been, until now, the only naturally occurring microbial rhodopsins capable of promoting membrane hyperpolarization. These kind of microbial rhodopsins act as light-gated ion pumps with limited temporal resolution (one ion per photon) as compared to a light-gated ion channel moving thousand of ions per photon, with the obvious difference on current levels between ion pumps and ion channels. Thus, initial steps to generate inhibitory ChRs started with the high-resolution structure of ChR2 towards directed mutagenesis to design ChR-based anion light-gated channels and chimeras. The judicious combination of state-of-the art sequencing methods and structural biology techniques for membrane proteins deserve special attention to fully

elucidate the molecular mechanism of microbial rhodopsins, in an effort to use protein engineering for improved optogenetics applications.

New tools for optogenetics

In a recent report, Govorunova *et al.* [8] provide a potential solution for hyperpolarizing optogenetics. The Spudich lab takes advantage of nuclear metagenomics studies of the cryptophyte *Guillardia theta* to identify three genes sharing sequence similarity, and key functional residues, with type I microbial rhodopsins and ChRs. These newly discovered genes were well expressed in human derived cell lines, and the two out of three exhibiting photocurrents were named *GtACR1* and *GtACR2* (*Guillardia theta* anion channel rhodopsins 1 and 2). *GtACR1* and *GtACR2* showed the highest sensitivity at specific wavelengths, 515 nm for *GtACR1*, and 470 nm for *GtACR2*, which showed additional absorbance bands at 445 nm and 415 nm. The current level of these light-gated anion channel rhodopsins (ACRs) is 6 to 8 times higher than ChR2, showing also lower inactivation. *GtACR1* and *GtACR2* show no pH-dependence, i.e. no proton transport, and none of the tested cations was transported. ACRs strictly transported anions, in comparison to Cl-conducting ChR mutants [9], which showed residual cation permeability. The photocurrent intensity for both ACRs is similar, but the transport kinetics for *GtACR2* is faster than *GtACR1*. The naturally occurring ACRs newly discovered possess photocurrent amplitudes and kinetics orders of magnitude higher than hyperpolarizing systems based on ion transporters, such as archaerhodopsin-3 proton pump or the ChR-based light-gated chloride channel ChloC mutant [10].

The identification of naturally-occurring ACRs finally addresses the light-dependent membrane hyperpolarization issue, i.e. excitable cells silencing with rapid kinetics, high-sensitivity and strict anion selectivity. ACRs together with CCRs provide a complete toolbox (Fig. 1) for high spatiotemporal neuronal control to understand physiological and pathophysiological neurobiology processes towards defining new therapies for neuronal diseases. However, the promising outreach of these *in vitro* tools

needs to be translated into successful applications in cellular and *in vivo* systems to become not just a promise but also a reality.

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Acknowledgements

This work has been supported by grant CIVP16A1861 from Fundación Ramón Areces (to PG).

Figure 1. The new type of light-gated inhibitory anion channels recently discovered [8] expands, and completes, the molecular repertoire of biochemical tools for monitoring and controlling neuronal function.

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Naturally-occurring rhodopsins recently discovered, that function as light-gated inhibitory anion channels, expand the current optogenetics toolbox and represent new opportunities for studying physiological neural processes and the mechanisms of neurological and mental disorders.