

Optogenetics: controlling cell function with light

A brief description of the basic steps required to control cellular function with optogenetics is presented.

In optogenetics, exogenous genes coding for light-sensitive proteins are expressed in cells, and illumination is used to alter cellular behavior. Optogenetics involves the development of light-sensitive proteins, strategies for delivering their genes to specific cells, targeted illumination and finally, compatible readouts for reporting on changes in cell, tissue and animal behavior.

First step: light-activated proteins—the toolbox

Optogenetics requires, first of all, light-sensitive proteins, which can be naturally occurring or they can be chemically modified to become photosensitive.

Tools for modulating the membrane potential

One of the most common uses of optogenetics is for changing the membrane voltage potential of excitable cells. In neurons, membrane depolarization leads to the activation of transient electrical signals (spiking), which are the basis of neuronal communication. Conversely, membrane hyperpolarization leads to the inhibition of these signals. Controlling the ‘switch’ that operates these currents enables neuroscientists to study how neurons functionally relate to each other and how neuronal circuits control behavior. By exogenously expressing light-activated proteins that change the membrane potential in neurons, light can be used as the on-off switch.

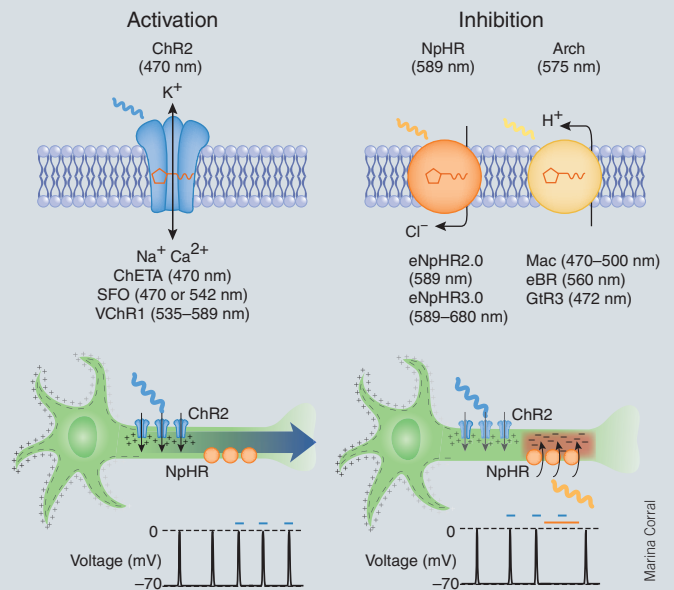
One approach is to use chemically modified so-called ‘caged ligands’ that become active upon stimulation with light and bind exogenous receptors that were genetically introduced into specific neurons. Ligands can also be tethered to the receptors themselves via a light-sensitive compound that acts as the optical switch. In both of these cases, the light-sensitive soluble or tethered ligand has to be administered to cells or tissues to render them light-sensitive.

Alternatively, naturally occurring genes encoding light-sensitive proteins, such as opsins, can be used. These light-sensitive transmembrane proteins are covalently bound to a chromophore, retinal, which upon absorption of light, isomerizes (for example, from a *trans* to a *cis* configuration), activating the protein. Notably, retinal compounds are present in most vertebrate cells in sufficient quantities, thus eliminating the need to administer an exogenous molecule.

The first genetically encoded system for optical control in mammalian neurons using opsins was established by exogenous expression of a three-gene system from *Drosophila melanogaster*. Neurons expressing these proteins responded to light with waves of depolarization and spiking over many seconds.

The recent discovery that opsins from microorganisms—which combine the light-sensitive domain with an ion channel or pump in the same protein—can also modulate neuronal signaling revolutionized the methodology by providing faster control in a single easily expressed protein.

The first of these neuronal switches used channelrhodopsin-2 (ChR2). When expressed in a neuron and exposed to blue light, this nonselective cation channel immediately depolarizes the



Optogenetic tools for modulating membrane voltage potential.

neuron and triggers a spike. Several variants of ChR2 have been developed. ChETA mutants were engineered as faster ChR2 variants, which can be used to spike neurons at frequencies greater than 40 hertz. The step function opsins, or SFO variants, in contrast, are slower versions of ChR2 that can induce prolonged stable excitable states in neurons upon exposure to blue light and then be reversed upon exposure to green light. Channelrhodopsin-1 (VChR1) acts similarly to ChR2 but is activated by red-shifted light.

Sometimes it is desirable to inhibit neuronal signaling instead of triggering it. Light stimulation of halorhodopsin (NpHR), a chloride pump, hyperpolarizes neurons and inhibits spikes in response to yellow light. Recent variants (named eNpHR2.0 and eNpHR3.0) exhibit improved membrane targeting in mammalian cells and consequently, photocurrents. Light-driven proton pumps such as archaerhodopsin-3 (Arch), Mac, bacteriorhodopsin (eBR) and rhodopsin-3 (GtR3) can also be used to hyperpolarize neurons and block signaling.

The optogenetic toolbox is quickly expanding as a result of screens that aim to identify new light-sensitive proteins in different ecological niches or by reengineering existing variants. Notably, several of these tools can be used in combination to allow multimodal control of neuronal activity. Recently, ChR2 was used to control the firing of mouse heart cells, extending the use of these tools to nonneuroscience applications.

Tools for modulating cell signaling

Optogenetic tools have also emerged that allow control of intracellular signaling cascades and molecular interactions. These tools are

generally fusions of light-absorbing domains with protein 'effector' domains. Chimeric proteins, called OptoXRs, composed of bovine rhodopsin and the intracellular components of adrenergic G protein-coupled receptors allow optical control of G protein-mediated signaling cascades.

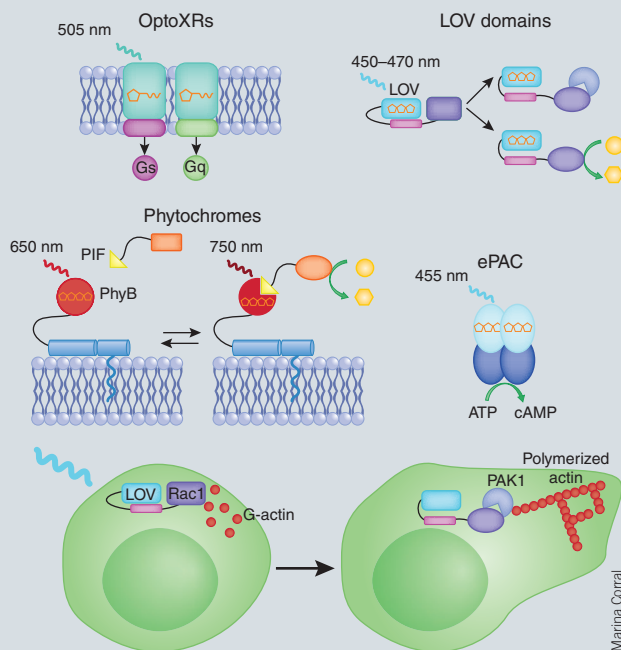
Non-membrane-associated photosensitive proteins such as light, oxygen, voltage (LOV) domains, phytochromes or cryptochromes from plants, can be fused to cellular effector proteins to create light-sensitive variants. The LOV2 domain uses naturally encoded flavin as a chromophore and is the basis of light-activated fusion proteins such as LOV2-Rac. Blue light induces a change in the conformation of the LOV2 domain, which results in the release of the allosteric block of Rac, allowing it to bind and activate downstream targets such as PAK1, leading to the polymerization of actin filaments and the generation of localized cell protrusions and cell movement. LOV domains have also been used to create, among others, light-driven DNA binding proteins, protein dimerizers and enzymes.

An alternative cytoplasmic optogenetic system consists of the photoreceptor PhyB and its protein binding partner PIF. Red light triggers the binding of PIF to PhyB, and infrared light releases PIF. Fusions of PIF with upstream activators of the Rho-family GTPases allowed light-dependent recruitment to the plasma membrane where PhyB was anchored, leading to localized activation of the actin cytoskeleton and formation of cellular extensions. Unlike the retinal and flavin-based systems described above, the PhyB chromophore phycocyanobilin has to be supplied to non-plant cells.

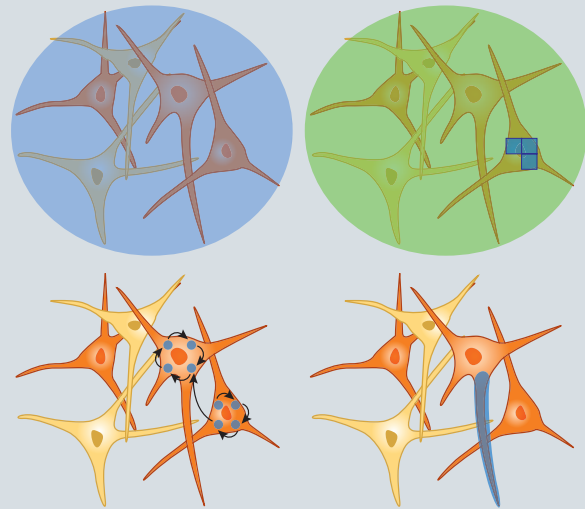
Finally, certain naturally occurring enzymes such as the light-activated adenylyl cyclase, (ePAC), can be used to modulate cell signaling events by direct production of second messenger molecules.

Second step: delivering the genes

Genes coding for these light-sensitive proteins can be delivered to the target cells by transfection, viral transduction or the creation



Optogenetic tools for modulating intracellular signaling.



Illumination of target cells using widefield, patterned and scanned light.

of transgenic animal lines. Expression can be restricted to cells of interest using specific promoters or recombinase-based conditional systems such as the Cre system. Alternatively, the use of viral vectors for gene delivery can allow targeting of specific cells without specific promoters, for example, to target neuronal populations based on their topological connections.

Third step: controlled illumination

Precise control of cellular activity via optogenetics depends on the well-defined temporal and spatial control of the illumination light. Widefield illumination can be temporally controlled using an ultra-fast shutter with a constant light source, fast switching of an LED or one-photon laser scanning microscopy. All cells expressing the light-sensitive proteins in the light path will be stimulated in concert. For *in vivo* applications, light sources coupled to optical fibers or miniaturized LEDs have been widely used.

Alternatively, light-patterning approaches can be used to selectively illuminate a subset of cells. One approach uses digital micromirror-based devices to reflect dynamic user-controlled patterns of light onto specific cells or regions of cells. Two-photon scanning microscopy can be used for targeted activation of single ChR2-expressing cells with high temporal control, and recently it was combined with temporal focusing to selectively activate small segments of a cell, an entire cell or a group of cells, individually or simultaneously.

Fourth step: reading the outcome

The effect induced by illumination of the photosensitive protein needs to be measured in cells, tissue or organisms. Electrodes can be used to monitor the effect of changes in membrane voltage. Many fluorescence-based biosensors with compatible excitation profiles, some of which are also genetically encoded, can be used to measure different cellular readouts. Finally, behavioral testing can be used to assess the effect of modulating cellular activity in whole animals.

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ACKNOWLEDGMENTS

We thank K. Deisseroth for his assistance in the preparation of this Primer.