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.

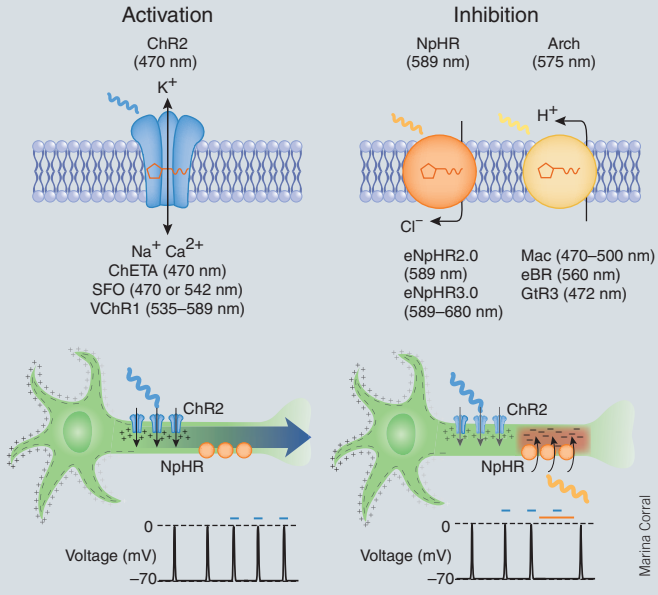
One approach is to use chemically modified so-called 'caged ligands' that become active upon stimulation with light and bind exogenous receptors that were genetically introduced into specific neurons. Ligands can also be tethered to the receptors themselves via a light-sensitive compound that acts as 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 quantitities, 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 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.



Optogenetic tools for modulating membrane volage potential.

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

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