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Optogenetics 3.0

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Mechanism aside, the main point to appreciate is that the association of p120 with cadherin is truly the limiting factor that determines whether a given cadherin molecule will persist on the cell surface or will be targeted for destruction. We know that cadherins are internalized by endocytosis when p120 dissociates from the juxtamembrane domain of cadherin (Figure 1) and that factors like clathrin and ubiquitin ligase Hakai are likely players in the demise of p120-deprived cadherins (Davis et al., 2003; Xiao et al., 2003). We also know that α - and β -catenins are essentially irrelevant because they stay attached to cadherins and thus are internalized simultaneously.

What we don't know is how these events unfold under normal circumstances in the cell. Presumably, the biophysical characteristics identified by Ishiyama et al. (2010) and described above do not alone drive cadherin turnover. If other mechanisms contribute, what might they look like? The simplest mechanism is shown in Figure 1 (right panel). In this model, p120 is modified, most likely by a kinase, in response to specific signals. Such a kinase could phosphorylate p120, the cadherin jux-

tamembrane domain, or both, resulting in separation of p120 from the complex and subsequent internalization of cadherin. A second model (not shown) supposes that the role of p120 is to recruit an additional factor to the cadherin complex, whose presence is required for cadherin retention and stability at the cell surface. In this scenario, the absence of p120 leads to the loss of the stability factor, and the hapless cadherin is sacrificed due to lack of support. Both of these mechanisms might be tuned up or down as needed, but the relevant players have not been clearly identified.

The first visualization of p120's molecular makeup and its interaction with the cadherin juxtamembrane domain (Ishiyama et al., 2010) likely marks the beginning of a new generation of experiments that will take advantage of these exquisite molecular insights. Minimally, the structure will lead to increasingly elegant reagents for selectively uncoupling distinct functions of p120 and improved interpretation of experimental results. However, given that the p120/cadherin interaction controls almost all of the classical cadherins and that p120 is frequently downregulated in most of the

major cancers, the results presented by Ishiyama et al. (2010) will probably have far-reaching consequences.

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Optogenetics 3.0

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Optogenetic methods use light to modulate the activities of target cells in vivo. By improving inter- and intracellular trafficking of light-sensitive switch proteins called opsins, Gradinaru et al. (2010) have developed a new generation of optogenetic tools capable of regulating the activity of targeted neurons with exquisite precision and efficiency.

Switching a well-defined cell population on and off at will is a desirable goal for systems biology research. Scientists have developed various methods across different species to target specific cells and make them controllable by diverse

external factors such as temperature and chemicals (Alexander et al., 2009; Liu and Davis, 2006). However, when it comes to temporal precision, literally nothing beats the speed of light. The successful marriage of optical technolo-

gies with molecular genetics has resulted in the new kid on the techniques block: optogenetics. Optogenetics is a rapidly developing technique that is being used by neuroscientists to manipulate the activity of selected neuronal populations

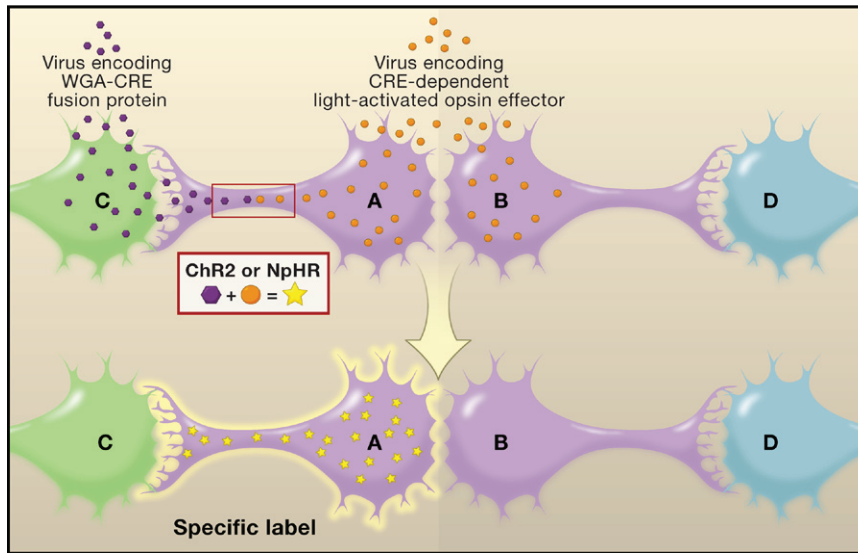


Figure 1. Targeting Specific Neurons by Optogenetics

Neurons A and B are neighboring neurons of the same type, with axonal projections ending in different brain regions containing neurons C and D, respectively. A virus encoding a WGA-CRE fusion protein is taken up by the soma of neuron C and the fusion protein product WGA-CRE *trans*-synaptically traffics into neuron A. Another virus coding for a CRE-dependent light-activated opsin effector was taken up by both neurons A and B because of their close spatial proximity. Only in neuron A, in the presence of WGA-CRE, is the construct processed and the final light-sensitive protein product (ChR2 or NpHR) made. Thus, neuron A is specifically labeled and can be distinguished from neuron B on excitation with the correct wavelength of light. Such optogenetic approaches can be used to manipulate the activity of a single neuron in a cell population in living animals with great precision.

in living animals. Optogenetics combines tissue- and cell type-specific expression of light-sensitive proteins called opsins and advanced optical methods to reach, record, and control the activity of a specific cell population. In this issue, Gradinaru et al. (2010) unveil the next-generation optogenetics toolbox.

Originally identified in microbes, the light-sensitive proteins channelrhodopsin-2 (ChR2) from the alga *Chlamydomonas reinhardtii* and halorhodopsin (NpHR) from the archaeon *Natronomonas pharaonis* are two of the most commonly used tools in optogenetics (Zhang et al., 2007). Channelrhodopsin-2 is a cation channel that allows the influx of Na^+ when illuminated by ~ 470 nm blue light (Nagel et al., 2003), resulting in activation of the neurons ectopically expressing this molecule. Halorhodopsin (Duschl et al., 1990), on the other hand, is a chloride ion pump activated by ~ 580 nm yellow light, which inhibits the neurons expressing it. The functions of ChR2 and NpHR are complementary, which enables bidirectional control of neuronal activity. ChR2 is reasonably well tolerated by cells and is readily

transported into the axons and dendrites of neurons. However, when the original version of NpHR was expressed at high levels in neurons, it formed aggregates that led to cellular toxicity manifested by intracellular blebs and the swelling of dendrites (Zhao et al., 2008). These difficulties greatly diminished the usefulness of the first generation NpHR protein as a light-sensitive switch. More recently, improvements have been made by grafting signal peptides from mammalian membrane receptors onto NpHR to enhance its membrane targeting and ER export, resulting in eNpHR2.0 (Gradinaru et al., 2008). Nevertheless, eNpHR2.0's inability to counter strong excitation of neurons still limited its application, leaving the inhibitory arm of optogenetics significantly weaker than the excitatory one.

Different groups have taken various paths to deal with this imbalance. For example, Chow et al. (2009) identified a different type of molecule, archaeorhodopsin-3 (Arch) from the archaeon *Halo-rubrum sodomense*. It is a proton pump activated by yellow-green light. Arch is capable of generating photocurrents

approaching 900 pA, which represents a huge increase compared to the original version of NpHR (typically around 100 pA), though the light power required to elicit this response (36 mW/mm^2) is at the higher end permissible for *in vivo* experiments. Instead of turning to a light-sensitive protein from a new species, Deisseroth and colleagues (Gradinaru et al., 2010) decided to tame the very beast of NpHR by disciplining it using the fundamental principles of eukaryotic protein trafficking. Their effort has paid off: the addition of the C-terminal trafficking signal from the potassium ion channel Kir2.1 gave birth to eNpHR3.0. This version shows both dramatically improved localization to the plasma membrane and, more importantly, substantially enhanced inhibitory capacity—even with relatively weak illumination of 3.5 mW/mm^2 , the resulting photocurrent exceeded 1 nA.

These quantitative changes also led to qualitative changes—the inhibition efficiency of the eNpHR3.0 is so high that it can be stimulated by suboptimal wavelengths (including far red/infrared), and this allows for full-spectrum control of neuronal activity. This is a double-edged sword, however, when eNpHR3.0 is used in concert with an excitatory switch to establish bidirectional control within a single cell. As the authors coexpressed ChR2 and eNpHR3.0 in the same neuron, they found that blue-light stimulation of ChR2 also weakly activated eNpHR3.0, and vice versa. This resulted in a 40% decrease in peak currents for both excitation and inhibition when compared to cells expressing either molecule individually. Nevertheless, eNpHR3.0 is a tremendous improvement over earlier versions, and the authors have successfully used it in combination with ChR2. It is likely that a combination of ChR2 and eNpHR3.0 (or Arch) will be sufficient in many cases where bidirectional control of cell membrane potential is desired.

A second major breakthrough for optogenetics described by Gradinaru, Deisseroth, and their colleagues concerns an important objective of current molecular genetic technology: how to precisely target the expression of effector proteins to a specific type of cell. For model organisms where

molecular genetics is underdeveloped, such as primates, this is almost out of the question. Even for organisms with a good collection of transcriptional promoters, such as mice and fruit flies, targeting a subpopulation of cells within a genetically and anatomically “homogeneous” cell population is a challenge. To address these issues, the authors resorted to *trans*-synaptic trafficking. They used two viruses: one encoding CRE recombinase fused to the transcellular tracer protein wheat germ agglutinin (WGA) and a second encoding a CRE-dependent opsin. They delivered these two viruses to a pair of remote but anatomically connected brain regions in rats or mice, one virus each to one of the two regions, and successfully labeled and optically controlled the subpopulation of neurons with projections connecting these two brain regions (Figure 1). This approach also raises an intriguing possibility that activation or inhibition may be targeted to specific axonal branches, rather than to the neuronal soma (cell body), potentially increasing the precision of optogenetic manipulation. Overall, *trans*-synaptic labeling of

anatomically connected neurons with a WGA-CRE fusion protein enabled targeting of specific neurons on the basis of their synaptic connection patterns, thus opening new doors for the precise manipulation of neural circuits.

These optogenetic techniques described by Deisseroth and his team, as well as by others, provide powerful new tools for neuroscience research. Although these methods based on light-gated ion channels are effective only in cells (neurons, muscle, endocrine cells, etc.) that can be rendered excitable by these channels, some additional recent developments promise broadening of the range of target cell types that can be manipulated by optogenetics. For example, new light-sensitive G protein-coupled receptors (dubbed optoXRs) have the potential to influence signaling cascades in cell types other than neurons (Airan et al., 2009). Theoretically, light-gated calcium ion channels could also be useful, as calcium ions are a universal secondary messenger in all known cell types. Expanding optogenetic tools so that they can be applied more broadly is the goal of optogenetics 3.0 and beyond.

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Time for Bacteria to Slow down

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The speed of the bacterial flagellar motor is thought to be regulated by structural changes in the motor. Two new studies, Boehm et al. (2010) in this issue and Paul et al. (2010) in *Molecular Cell*, now show that cyclic di-GMP also regulates flagellar motor speed through interactions between the cyclic di-GMP binding protein YcgR and the motor proteins.

Cyclic di-GMP is the molecule of the moment in bacteriology. This ubiquitous secondary messenger has been implicated in myriad processes from pathogenicity to synthesis of pili (hairlike appendages involved in bio-

film production) (reviewed in Hengge, 2009). Now two new papers, one in this issue of *Cell* (Boehm et al., 2010) and one in the upcoming issue of *Molecular Cell* (Paul et al., 2010), reveal the direct involvement of cyclic di-GMP in the

regulation of flagellar movement and bacterial swimming.

Cyclic di-GMP is synthesized from two molecules of GTP by diguanylate cyclase domains and is broken down by phosphodiesterase domains. The