Cell

Resource

Molecular and Cellular Approaches for Diversifying and Extending Optogenetics

Viviana Gradinaru,[1,2,6] Feng Zhang,[1,5,6] Charu Ramakrishnan,[1] Joanna Mattis,[1,2] Rohit Prakash,[1,2] Ilka Diester,[1] Inbal Goshen,[1] Kimberly R. Thompson,[1] and Karl Deisseroth[1,3,4,*]
[1]Department of Bioengineering
[2]Program in Neuroscience
[3]Department of Psychiatry and Behavioral Sciences
[4]Howard Hughes Medical Institute
Stanford University, Stanford, CA 94305, USA
[5]Present address: Society of Fellows, Harvard University, Cambridge, MA 02138, USA
[6]These authors contributed equally to this work
*Correspondence: deissero@stanford.edu
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SUMMARY

Optogenetic technologies employ light to control biological processes within targeted cells in vivo with high temporal precision. Here, we show that application of molecular trafficking principles can expand the optogenetic repertoire along several long-sought dimensions. Subcellular and transcellular trafficking strategies now permit (1) optical regulation at the far-red/infrared border and extension of optogenetic control across the entire visible spectrum, (2) increased potency of optical inhibition without increased light power requirement (nanoampere-scale chloride-mediated photocurrents that maintain the light sensitivity and reversible, step-like kinetic stability of earlier tools), and (3) generalizable strategies for targeting cells based not only on genetic identity, but also on morphology and tissue topology, to allow versatile targeting when promoters are not known or in genetically intractable organisms. Together, these results illustrate use of cell-biological principles to enable expansion of the versatile fast optogenetic technologies suitable for intact-systems biology and behavior.

INTRODUCTION

A fundamental goal in biology is fast control of defined cells within functioning tissues. Temporal precision of control is important since cells may carry out fundamentally different computations and deliver different outputs depending on the timing and context of input signals. For example, it is almost meaningless to ask the causal role of "activation" or "inhibition" of a neuron type in the brain, since changes in context (Fleischmann et al., 2008) or millisecond-scale shifts in timing (Bi and Poo, 1998; Silberberg et al., 2004) can change the magnitude or flip the sign of neuronal action on the circuit. Similarly, pancreatic β cells execute synchronized oscillations in membrane potential resulting in pulsatile insulin secretion with precise timing across the population of β cells that may be important for the action of insulin on target tissues (Tengholm and Gylfe, 2009). Traditional genetics delivers cell type-specific control if adequate targeting strategies exist, but genetic approaches lack the temporal precision needed to control events with behaviorally or environmentally relevant triggering and timing; moreover, pharmacological control strategies lack cell type-specificity, temporal precision, or both. The general need to study molecular and cellular events not only in reduced systems, but also within intact biological systems, has driven recent awareness of the opportunities of fast control.

To enable temporally precise control of specific cell types within behaving animals, fast "optogenetic" (Deisseroth et al., 2006) technologies have been developed involving single-component light-responsive proteins that transduce brief pulses of light into well-defined action potential trains and effector functions in vivo (Boyden et al., 2005; Zhang et al., 2007a, 2007b). Through the use of optogenetics, precisely timed gain-of-function or loss-of-function of specified events can be achieved in targeted cells of freely moving mammals and other animals (Adamantidis et al., 2007). For example, we have found that direct light-triggered excitation of cellular electrical activity (depolarization and precisely timed action potentials) can be achieved via expression of the microbial opsin genes encoding *Chlamydomonas* channelrhodopsin-2 (ChR2) (Boyden et al., 2005) or *Volvox* channelrhodopsin-1 (VChR1) (Zhang et al., 2008). On the other hand, direct light-triggered inhibition of electrical activity (precisely timed hyperpolarization) can be achieved via expression of the *Natronomonas* halorhodopsin (NpHR) in vivo (Zhang et al., 2007a; Han and Boyden, 2007; Gradinaru et al., 2008); this halorhodopsin was selected for its step-like and highly stable photocurrents compared with other microbial generators of inhibitory current (Zhang et al., 2007a). The

halorhodopsin achieves inhibition by pumping into neurons an ion (chloride) that constitutes part of the natural mechanism of neuronal inhibition, and neurons are therefore well designed to process chloride influx (Zhang et al., 2007a); microbial proton pumps additionally can be used to achieve outward currents (Chow et al., 2010). Finally, more subtle (but still temporally precise) optical modulatory strategies are also possible, including changes in the input-output relationships of targeted cells via expression of engineered step function opsins, or "SFOs," that alter excitability (Berndt et al., 2009), and fast selective control of modulatory G_s or G_q signaling using synthetic rhodopsin/G protein-coupled receptor chimeras (optoXRs) (Airan et al., 2009). This collection of tools, along with the development of versatile devices to deliver light in vivo (Aravanis et al., 2007; Adamantidis et al., 2007; Gradinaru et al., 2007), has enabled widespread application of optogenetics.

To further expand the optogenetic toolbox, we have previously carried out genomic screening strategies to successfully identify and validate novel classes of opsins for optogenetic control (Zhang et al., 2007a, 2008) and carried out rationally designed mutagenesis to achieve new classes of opsin functionality (Berndt et al., 2009; Gunaydin et al., 2010). Here, we apply a third type of intervention, namely application of molecular trafficking strategies, to derive a panel of tools that both quantitatively and qualitatively enhance the power of optogenetics and open distinct avenues of investigation. In particular, tools are developed that allow targeting of cells solely by virtue of their topological relationships within tissue and that extend the reach of optical control to the infrared border, with effector function enhanced beyond the other known tools and covering the entire visible spectrum.

RESULTS

Membrane Trafficking and Microbial Opsin Genes

Deriving optogenetic tools from multiple classes of microbes promises substantial diversity of triggering and effector functions (Zhang et al., 2008), given the ecological diversity of microbial organisms occupying niches with a broad array of environmental signals of informational or energetic value (Yooseph et al., 2007). Moreover, as a necessary adaptation to small cell volume and genome size, microbes carry out sensation, transduction, and action via highly compact mechanisms, often all encompassed within a single open genetic reading frame (as with the microbial opsins, in which both photon sensation and ion flux effector function are implemented within a single compact protein) (Kalaidzidis et al., 1998; Lanyi and Oesterhelt, 1982; Lozier et al., 1975; Nagel et al., 2003). In contrast, metazoan or vertebrate cells may transduce energy or information with more complex multicomponent signaling cascades that afford greater opportunities for modulation but are much less portable (as with the vertebrate opsins). Optogenetic tools from simpler organisms therefore present clear opportunities, but may not express or be tolerated well by more complex cells. Indeed, archaeal halorhodopsin (the light-activated electrogenic chloride pump that can be used for optogenetic inhibition in metazoans) displays impaired subcellular localization when expressed at high levels in mammalian neurons (Gradinaru et al., 2008; Zhao et al., 2008). An early trafficking step, export from the endoplasmic reticulum (ER), was found to be impaired for this first-generation NpHR, leading to intracellular accumulations that colocalized with the ER marker KDEL (Figure 1A; Figure 1B, left). Fusion of the FCYENEV ER export motif from a vertebrate inward rectifier potassium channel to the NpHR C terminus prevented aggregate formation (Figure 1B, center) and greatly enhanced tolerability at high expression levels.

This second-generation enhanced tool (eNpHR, now eNpHR2.0) has been successfully employed in vivo and in intact tissue in a number of studies (Gradinaru et al., 2009; Sohal et al., 2009; Tønnesen et al., 2009; Arrenberg et al., 2009); expression was well tolerated, and additionally Thy1::eNpHR2.0 mice have been generated with well-tolerated long-term expression of eNpHR2.0 at high functional levels throughout the brain (G. Feng, G. Augustine, and K.D., unpublished data). As with many kinds of native inhibition, optogenetic inhibition could be overcome by strong excitatory activity (Sohal et al., 2009). Potential additional molecular modifications for enhancing photocurrents from known and emerging opsin gene family members would include signal peptides, additional ER export motifs, Golgi trafficking signals, transport signals, and other motifs involved in transport of membrane proteins along the secretory pathway to the cell surface (Simon and Blobel, 1993). We therefore sought to apply combinatorial membrane trafficking strategies that could be generally applicable in a systematic, principled fashion to candidate microbial membrane proteins for translation to metazoan applications.

Examination of eNpHR2.0-expressing hippocampal neurons revealed the absence of globular ER accumulations, as previously reported, but nevertheless persistent intracellular labeling and poor membrane localization (Figure 1B, center), suggesting that additional modifications subsequent to the ER export step would indeed be important. Examination of primary-sequence differences between two forms of an inward rectifier potassium channel with differential membrane localization (Kir2.1 and Kir2.4) revealed differences not only in C-terminal ER export motifs but also in N-terminal Golgi export signals and in C-terminal trafficking signals (Hofherr et al., 2005). Surprisingly, we found that provision of the Golgi export signal did not significantly affect surface expression (data not shown), but that addition of the trafficking signal from Kir2.1 either between eNpHR and the EYFP fusion, or at the C terminus of the fusion protein, dramatically reduced intracellular labeling and increased apparent surface membrane expression (Figure 1B, right) and also improved labeling of cellular processes (Figure 1B, right). Indeed, high-resolution confocal imaging (Figure 1C) revealed marked localization in processes, with identifiable labeled membranes spanning intracellular regions apparently devoid of the opsin-EYFP fusion protein, in a pattern never previously observed with NpHR or its derivatives.

If improved membrane targeting were indeed achieved with this modification, increased photocurrents would be anticipated to result. We therefore examined photocurrents, using whole-cell patch clamp recordings to quantify bona fide functional plasma membrane localization of halorhodopsin pump molecules. Photocurrents were indeed profoundly increased (to a level ~20-fold larger than the initially described NpHR currents;