Optogenetic Application to Investigating Cell Behavior and Neurological Disease

Danqing Zhu¹, Hunter J. Johnson²,³,⁴, Jun Chen¹ and David V. Schaffer¹,²,⁵,⁶,⁷*

¹California Institute for Quantitative Biosciences, University of California, Berkeley, Berkeley, CA, United States, ²Department of Bioengineering, University of California, Berkeley, Berkeley, CA, United States, ³Graduate Program in Bioengineering, University of California, San Francisco, San Francisco, CA, United States, ⁴Graduate Program in Bioengineering, University of California, Berkeley, Berkeley, CA, United States, ⁵Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, United States, ⁶Department of Chemical and Biomolecular Engineering, University of California, Berkeley, Berkeley, CA, United States, ⁷Helen Wills Neuroscience Institute, University of California, Berkeley, Berkeley, CA, United States

Cells reside in a dynamic microenvironment that presents them with regulatory signals that vary in time, space, and amplitude. The cell, in turn, interprets these signals and accordingly initiates downstream processes including cell proliferation, differentiation, migration, and self-organization. Conventional approaches to perturb and investigate signaling pathways (e.g., agonist/antagonist addition, overexpression, silencing, knockouts) are often binary perturbations that do not offer precise control over signaling levels, and/or provide limited spatial or temporal control. In contrast, optogenetics leverages light-sensitive proteins to control cellular signaling dynamics and target gene expression and, by virtue of precise hardware control over illumination, offers the capacity to interrogate how spatiotemporally varying signals modulate gene regulatory networks and cellular behaviors. Recent studies have employed various optogenetic systems in stem cell, embryonic, and somatic cell patterning studies, which have addressed fundamental questions of how cell-cell communication, subcellular protein localization, and signal integration affect cell fate. Other efforts have explored how alteration of signaling dynamics may contribute to neurological diseases and have in the process created physiologically relevant models that could inform new therapeutic strategies. In this review, we focus on emerging applications within the expanding field of optogenetics to study gene regulation, cell signaling, neurodevelopment, and neurological disorders, and we comment on current limitations and future directions for the growth of the field.

Keywords: optogenetics, cell signaling, spatiotemporal, gene regulation, neuroscience

INTRODUCTION

Cellular signaling is mediated by highly dynamic and intertwined pathways that control crucial cell behaviors. During organismal development, these signaling processes are spatially and dynamically regulated to orchestrate cell expansion, cell differentiation, and tissue morphogenesis. Likewise, complex signaling pathways enable and protect adult organismal function. Investigating these complex mechanisms has required the development of tools to perturb their activity.
TABLE 1 | Summary of common optogenetic systems, mechanisms, and applications.

| Systems       | Origin         | Mechanism                                  | Applications                                      | References         |
|---------------|----------------|---------------------------------------------|---------------------------------------------------|--------------------|
| Cry2/CIB1     | A. thaliana    | Dimerization                               | protein proximity, activation, membrane targeting | Kennedy et al. (2010) |
|               |                | Blue Light (420–490 nm)                     |                                                   |                    |
|               |                | Binding of the PHR of photoexcited Cry2 to |                                                   |                    |
|               |                | the CIB1 or CIBN                            |                                                   |                    |
| Cry2 PHR      | A. thaliana    | Oligomerization/clustering                 | receptor clustering, protein interactions/signaling, subcellular localization | Bugaj et al. (2013) |
|               |                | Blue Light (420–490 nm)                     |                                                   |                    |
| AsLOV2        | A. sativa Phot1| Caging/conformation change                 | Protein activation, deactivation, subcellular localization | Wu et al. (2009)   |
|               |                | Blue Light (< 500 nm)                      |                                                   |                    |
| Magnets       | Neurospora crassa | Heterodimerization (nMag, pMag)          | Protein interactions, subcellular localizations, genome editing | Kawano et al. (2015) |
| Phy/PIF       | Arabidopsis    | Dimerization                               | Deep tissue penetration for protein signaling and interactions control | Levskaya et al. (2009) |
|               | thaliana       | Red light (650 nm) activation              |                                                   |                    |
|               |                | Reversed by far-red (750 nm) light         |                                                   |                    |
|               |                | Exogenous chromophore                      |                                                   |                    |
| BphP1/PpsR2   | Rhodopseudomonas palustris | Dimerization                        | Deep tissue penetration for protein signaling and interactions control | Kaberniuk et al. (2016) |
|               |                | Near-infrared light (740–780 nm) activation|                                                   |                    |
|               |                | Chromophore is endogenously present in mammalian cells |                                                   |                    |
| UVR8/COP1     | Plants         | Dimerization                               | Inversible protein signaling and interactions   | Favory et al. (2009) |
|               |                | UV Light (290–315 nm)                      |                                                   |                    |

Conventional chemical perturbations can offer some levels of control but have limited capability in their spatial and temporal resolution (Webster et al., 1988; Gossen et al., 1995; Metzger et al., 1995; Rivera et al., 1996; Stanton et al., 2018). As an alternative approach, optogenetics offers the potential for unprecedented precise temporal, spatial, and dosage control of signaling. Specifically, the spatial precision can be achieved on the order of ~micrometers (µm), and the temporal precision can be achieved at ~ms (<1 ms; Shemesh et al., 2017). Optogenetics, which combines genetic and optical tools for the precise perturbation of target cellular processes, initially applied naturally derived photosensitive opsins to study and control the activity of individual neurons and by extension investigate brain circuitry. In particular, opsins, derived from various microbial species, were re-purposed for mammalian cell expression and upon illumination at specific wavelengths unleashed ions flow across cell membranes to activate or inhibit target neurons (Zhang et al., 2006; Deisseroth, 2015), as extensively reviewed in recent literature (Looger, 2011; Zhang et al., 2011). This review will focus instead on newly developed light-responsive systems and optics hardware that have enabled applications in new areas such as control of protein activity, signaling dynamics, up- or down- regulation of gene expression, subcellular localization, and other applications. We will discuss how the high spatiotemporal precision of optogenetics has led to novel insights into cellular signaling networks and future trends in the field of optogenetics for a variety of applications in basic science and therapeutics.

STATE-OF-THE-ART OPTOGENETIC TOOLS AND OPTICS TECHNOLOGY

In recent years, optogenetic tools have extended to many classes of proteins containing light-responsive domains (summarized in Table 1) discovered in plants, bacteria, invertebrates, etc. Their photoreceptor domains exhibit light-inducible properties such as reversible protein-protein interactions, oligomerization, and conformational changes, which can be harnessed to engineer photoswitches for regulating gene activation, repression, protein localization, and other functions (Figure 1).

One important system involves light, oxygen, and voltage domains (LOV), isolated from plant photosensor phototropin 1 (phot1). Phot1 molecule has two LOV domains, with the second domain (LOV2) primarily involved in kinase activation (Christie et al., 1999, 2012; Wu et al., 2009). In particular, Avena sativa phot1 LOV2 (AsLOV2) has a tightly associated C-terminal Jα helix in a folded state, but upon exposure to blue light, the bound flavin mononucleotide (FMN) chromophore undergoes a conformational change to displace the Jα helix from the protein core, leading to uncaging of an effector kinase domain (Harper et al., 2003). By engineering fusions between other signaling effectors to LOV domains, this light-induced conformational change can be used to unleash signaling activity.

In another system, cryptochrome 2 (Cry2), derived from the plant A. thaliana, has a photolyase homology region (PHR) that interacts with a partner CIB1 in a blue light responsive manner, due to a flavin adenine dinucleotide (FAD) bound to Cry2 as a chromophore cofactor (Kennedy et al., 2010). In one optogenetic design, the Cry2PHR domain can dimerize with CIB1 lacking the basic helix-loop-helix domain (CIBN) within seconds of exposure. In addition to dimerization, Cry2PHR can also be harnessed for self-oligomerization with blue light activation (Bugaj et al., 2013, 2015). Specifically, the detection of protein clusters can be achieved with ~10 s, and dissociation occurs with a half-life ~5.5 min.

In a common system for red light activation, phytochrome (Phy), derived from Arabidopsis thaliana, contains two structural
domains (Quail, 2010). The N-terminal photosensory domain binds to a tetapyrrole chromophore, such as phycocyanobilin (PCB), and can be triggered by red light (650 nm) to photoisomerize and bind to phytochrome-interacting factors (PIFs) to modulate downstream pathways (Quail, 2002). This Phy/PIF dimerization has been harnessed to stimulate protein-protein interactions in mammalian cells (Levskaya et al., 2009). More recently, a near-infrared-light (∼750 nm) responsive dimerization of bacterial phytochrome (BphP1) with its partner PpsR2 system has reportedly been harnessed for optogenetics (Kaberniuk et al., 2016). In addition to the deep tissue penetration of red- and far-red light, an advantage of Phy/PIF and Bphp1/PpsR2 systems is their reversibility and speed. Specifically, the dissociation of these protein pairs can be induced with lights of different wavelengths, enabling studies to perturb dynamics of signaling networks with a fast on/off rate.

Magnets, an improved system that incorporates positive and negative amino acids into the Vivid (VVD) N-terminal helix, also enables heterodimerization with electrostatic attraction upon uncaging of the protein (Kawano et al., 2015). VVD contains LOV domain that can be excited with blue light illumination to uncage the effector domain (Zoltowski et al., 2007).

FIGURE 1  |  Representative schematics of four major optogenetic-based gene regulations using different systems (created with Biorender). (A,B) Light-induced uncaging of affinity domains (positive pMag) and (negative nMag) results in dimerization of attached domains and reassembly of functional Cas9 for indel mutation and split Cre recombinase for DNA recombination. (C) Upon light-induction, the Ja helix unfolds from LOV2 core to uncage a fused protein (PAH1, RILPN313) that inhibits the binding and activity of targeted transcription factor (REST). (D) Cry2 clusters an attached protein (LRP6c) in response to light that activates the downstream target genes, e.g., Wnt signaling.
Optogenetics has been extensively applied for the spatiotemporal modulation of gene expression, including genome editing, gene activation, and repression.

(i) Genome Editing: CRISPR/Cas9 (clustered, regularly interspaced, short palindromic repeats, CRISPR-associated protein 9) can be engineered for targeted genome modification in mammalian cells at specific DNA loci, which enables the causal dissection of gene functions (Cong et al., 2013; Jinek et al., 2013; Mali et al., 2013). Optogenetic control of targeted genome editing can facilitate our improved understanding of gene networks. The first reported photoactivatable Cas9 (paCas9) was based on the dimerization system called Magnets (Kawano et al., 2015). In this work, the Cas9 nuclease was split into two parts fused to photoinducible dimerizing domains (positive Magnet and negative Magnet), which assemble and reconstitute Cas9 activity upon blue light illumination (Nihongaki et al., 2015a). This photoactivatable Cas9 cleaved a targeted endogenous genomic locus and induced indel mutation by nonhomologous end joining (NHEJ) or homology-directed repair (HDR) in a light-dependent fashion (Figure 1A). Using multiple sgRNAs targeting human genes EMX1 and VEGFA, the light-induced Cas9 indel mutations in both genes simultaneously. Though the reported study focused on HEK293T cells, EMX1 is ubiquitously expressed in neural stem cells and guides differentiation of layer-specific neuronal phenotypes (Schuurmans and Guillemot, 2002), such that this system could be further harnessed for investigating corticogenesis or other neurodevelopmental processes.

Another study adapted a photoactivatable Cre (PA-Cre) system for site-specific genome recombination, where low intensity or short pulsed illumination enabled efficient activation with high spatial and temporal precision in vivo (Kawano et al.,

Table 2: Existing optic hardware used in optogenetic illumination and their characterizations.

| Device Type                | Applications                  | Specifications                                           | References        |
|----------------------------|--------------------------------|----------------------------------------------------------|-------------------|
| **In vitro**               |                                |                                                          |                   |
| Cell culture plate         | Whole plate illumination       | • Uniform illumination                                   | Tucker et al. (2014) |
| Digital micromirror device (DMD) | Microscope illumination | • High temporal resolution (4,000 Hz)                     | Allen (2017)      |
| LAVA board                 | 24-well/96-well plate illumination | • Single color illumination                      | Repina et al. (2020) |
| Light Plate Apparatus (LPA) | 24-well plate illumination     | • Two color illumination                               | Gerhardt et al. (2016) |
| optoPlate-96               | 96-well plate illumination     | • Three color illumination                              | Bugaj and Lim (2019) |
| **In vivo**                |                                |                                                          |                   |
| Optic fiber                | Laser-coupled optical fiber mounted and passed through the skull | ~ hundreds of micrometers ~ milliseconds | Aravanis et al. (2007) |
| Wireless optoelectronics   | Injectable multimodal sensors with an array microLEDs | ~ 50 µm ~ radio frequency scavenging | Kim et al. (2013) |
In particular, Cre recombinase was split (CreN59/CreC60) into two fragments and fused to components of the Magnet system for heterodimerization under blue light (Figure 1B). This design yielded a substantial increase in DNA recombination compared to a prior design split system (Kennedy et al., 2010). Recently, an improved version of this PA-Cre system was reported to enhance efficiency and reduce dark background recombination when targeting active neurons in the murine dorsal raphe nucleus (Morikawa et al., 2020). Successful recombination was observed under blue light condition for mouse brains in vivo and in neural cells in vitro, demonstrating the promise of this system for future applications in studying neural pathways in mouse models. Other groups have applied analogous systems with different recombinases to modulate excitatory neurons and parvalbumin interneurons in the mouse brain, which can potentially be combined with PA-Cre for dual locus genome editing (Li et al., 2020).

(ii) Gene Activation: Optogenetic control of gene expression has been achieved with several different approaches, and a typical strategy combines a photosensory module with a split transcription factor that can be reassembled upon light illumination. The first reported optogenetic system for regulating gene expression, published in 2002 (Shimizu-Sato et al., 2002), was based on the fusion of the Phy-Gal4-DNA binding domain and a PIF3-Gal4-activation domain to induce downstream gene activation.

Engineering DNA-binding became far easier with the advent CRISPR/Cas9 genome editing, and this capability can be harnessed for other applications. For example, Zhang and colleagues (Konermann et al., 2013) developed light-inducible transcriptional effectors (LITEs) that enable direct optical control of mammalian cell gene expression. Cry2 systems rely upon photoactivated dimerization with its interacting partner CIB1 or the self-oligomerization of the Cry2-PHR. In this study, a customizable DNA-binding domain was fused to the light-sensitive module of Cry2, and upon blue light illumination, its dimerization partner CIB1, fused to a desired effector domain, became recruited to a target locus to modulate gene expression. This approach enabled independent, multiplexed genomic anchors to target a range of genomic loci. More recently, to improve the targeting of multiple sequences in the same gene simultaneously for robust activation, Cry2 was fused to the VP64 transactivation domain while its interacting partner CIB1 was fused to dCas9 (Jinek et al., 2012), the catalytically inactive form of Cas9. With multiple gRNAs co-transfected into mammalian cells to direct the binding of dCas9 to the targeted promoter, transcription could be induced robustly in the presence of blue light. The advantage of such a system compared to the conventional chemical-based strategy in gene activation is reversibility by the removal of light illumination, enabling dynamic patterns of gene modulation (Polstein and Gersbach, 2015). While transcriptional upregulation has been achieved in multiple studies, the magnitude of upregulation can be too low to induce an effective biological response (Nihongaki et al., 2015a,b). To address this shortcoming, a modified system incorporating synergistic activation mediators has been developed (Nihongaki et al., 2017). In this system, a single gRNA is attached to MS2 binding sequences to recruit the MS2 coat protein fused with two activators (p65 and heat shock factor 1) onto the same dCas9 protein, resulting in a 50–100-fold higher activation (ASCL1) than the previous constructs in HEK293T cells.

Researchers have applied this system to target NEUROD1 expression to induce neuronal differentiation in human iPSCs. Using the modified gene activation system, the upregulation was improved by 860-fold after 1 d of blue light illumination, and activated cells stained positively for the neuronal marker β-III tubulin (Tuj1) after 4 d of illumination. Another research has induced functional neuronal differentiation using a far-red light-mediated system, which offers low phototoxicity and deep tissue penetration (Shao et al., 2018).

(iii) Gene Repression: In addition to activating target gene expression, multiple systems have been developed to repress transcription. For example, repressive histone modifiers have been fused to light-sensitive proteins to suppress Grm2 and Neurog2 gene expression in primary neurons and Neuro2a cells with light illumination (Konermann et al., 2013). Another example harnessed a small peptide degron. Specifically, a small peptide degron RRRGN was fused to the C-terminus of the photosresponsive Ja helix of LOV2 domain. The LOV2 domain contains a flavin-binding core domain, and blue light illumination induces a side-chain rotation of the conserved flavin-interacting residue followed by dissociation and unfolding of the C-terminal Ja helix (Shcherbakova et al., 2015). This dissociation from the LOV core domain under blue light induction exposed the degron, which subsequently stimulated protein degradation (Bonger et al., 2014). Another study further engineered a system to include an additional Cry2-based system together with the LOV-domain, such that the blue light can simultaneously block new mRNA transcription while inducing the degradation of existing proteins (Pathak et al., 2017), showed a further reduction in targeting protein level compared to the single LOV2-system reported previously.

Researchers have also leveraged this LOV2 conformation change to engineer chimeric proteins to inhibit the repressor element (RE) 1-silencing transcription factor (REST) in primary neurons (Paonessa et al., 2016). REST is a transcriptional repressor that is normally expressed at low levels by mature neurons, but in various brain pathologies becomes up regulated (Mandel et al., 2011; Pozzi et al., 2013). A minimal interacting REST sequence was fused to the LOV2 C-terminus. On blue light illumination, LOV2 unfolded and freed the C-terminal domain to interact with and inhibit the binding of REST (Figure 1C). With effective light-mediated REST inhibition, neurons showed increased brain-derived neurotrophic factor transcription and boosted Na+ currents and neuronal firing (Paonessa et al., 2016). Future investigation can apply these modulating systems in vivo for brain pathologies associated with REST overexpression or hyperactivity (Zuccato et al., 2003; Lu et al., 2014).

Optogenetic tools generally allow modulation of the single gene expression using a single light color system; however, recent studies have highlighted the possibility for multiplexed control of several genes to regulate biological processes.
neurodevelopment and adult neurogenesis. Optogenetic control of cellular signaling is an exciting approach in signal presentation and temporal activation. In contrast, signal activation has poor spatial and temporal control, limited function and malfunction. Differentiation, migration, and survival. Protein ligands or peptide; Guinn and Balázsi, 2019). Such synthetic circuits can also be multiplexed orthogonally to probe multigene expressions in contribution of cell phenotypes (Szenk et al., 2020) and buffering gene dosage variation across cell populations for a more homogeneous gene expression control (Yang et al., 2021).

**OPTOGENETICS IN ORGANISMAL DEVELOPMENT**

While optogenetics has not yet been broadly applied to study development, there is considerable potential. For example, the nervous system is a highly ordered, complex, and tightly regulated system central to organismal survival and function. This system develops through the expansion and differentiation of various neural stem and progenitor cells, processes that are tightly orchestrated by extracellular ligand signals. These ligands bind to receptor tyrosine kinases (RTKs; Schlessinger, 2014), adhesion, G-protein-coupled (Rosenbaum et al., 2009), and other receptors to initiate signal transduction cascades and trigger downstream cellular responses such as growth, differentiation, migration, and survival. Protein ligands or synthetic agonists have been broadly useful for probing cellular function and malfunction in vitro and in vivo. However, such signal activation has poor spatial and temporal control, limited by slow diffusion and convection as well as technical limitations in signal presentation and temporal activation. In contrast, optogenetic control of cellular signaling is an exciting approach for spatiotemporal control of signaling (Repina et al., 2017). These approaches thereby offer a valuable toolbox to investigate the complex spatiotemporal role of major signaling pathways in neurodevelopment and adult neurogenesis.

Following gastrulation, precise spatiotemporal presentation of morphogen signals regionally patterns the neural plate, neural groove, and neural tube and thereby the full central nervous system (Nikolopoulou et al., 2017). More specifically, the induction of the neuroectoderm and patterning of both the dorsal-ventral and anterior-posterior axis of the central nervous systems rely on spatiotemporal activation of Wnt, BMP, SHH, FGF, and retinoic acid (RA; Kiecker and Lumsden, 2012). However, challenges with ectopically modulating morphogen signals with temporal and spatial control have classically limited the generation of accurate in vitro models of neurodevelopment (Kelava and Lancaster, 2016).

Optogenetic activation of signaling cascades typically relies on a genetically encoded light responsive dimerizing/oligomerizing protein fused to a signaling effector to mimic the effect of upstream ligand binding (Kramer et al., 2021), and it can do so with spatiotemporal control. In particular, several optogenetic systems controlling morphogen signals relevant to development have recently been reported, leveraging Cry2 (Kennedy et al., 2010; Bugaj et al., 2013). One of the first examples of leveraging Cry2 oligomerization to control cellular signaling was optogenetic control of canonical Wnt activation. Our optoWnt system involves fusion of the intracellular portion of the canonical Wnt co-receptor LRP6 to the PHR domain of Cry2 (Figure 1D), and we have reported optogenetic activation of Wnt signaling in both adult neural stem cells (NSCs; Bugaj et al., 2013) and human embryonic stem cells (hESCs; Repina et al., 2020). Other emerging and relevant morphogen signals to neurodevelopment include optoFGFR1(Kim et al., 2014), optoTGF (Li et al., 2018), optoBMP (Humphreys et al., 2020), and optoBrn2 (Sokolik et al., 2015), which have clear applications in modeling neurodevelopment and neurogenesis if translated to either pluripotent stem cells or adult neural stem cells.

OptoWnt has been recently deployed to investigate the temporal role of β-catenin activity dynamics on adult neurogenesis (Rosenbloom et al., 2020). It was determined that sustained optoWnt activation promoted proliferation and differentiation, while transient or disrupted activation induced cellular apoptosis. This discrepancy may explain the cell death observed during adult neurogenesis, indicating a potential inherent mechanism that corrects for deviations in differentiation cues.

In studies outside the nervous system, optogenetic control over Ras/ERK signaling in Drosophila, relying on the association of Phy-PIF for the membrane localization of SOS proteins (Son of Sevenless), revealed the role of this pathway in promoting endodermal differentiation at the cost of mesodermal fate during a critical time window (McFann et al., 2021). Specifically, Ras signaling was controlled using a specific region of SOS with catalytic activity (SOScat), which activates Ras signal when recruited to the plasma membrane (optoSOS). This was accomplished via PIF-SOScat fusion and a membrane PhyB (Toettcher et al., 2013). In hESCs, mixed optoWnt activation in a subpopulation of cells has been linked to mesendoderm specification and migration from a non-Wnt stimulated population, providing insight into gastrulation and early ectoderm specification (Repina et al., 2020). In an alternative application, investigating the temporal dynamics of optoBrn2 in hESC reveals a positive-feedback mechanism, where
pluripotency networks do not respond to optoBrn2 signals below a certain magnitude or duration threshold (Sokolik et al., 2015). This result may indicate how hESCs are able to distinguish signal vs. stochastic fluctuations during development. Finally, optogenetics can function as a precision perturbation tool in developmental biology (Krueger et al., 2019). In vivo application include decoding signaling dynamics during Xenopus development, where light-mediated Raf/MEK/ERK activation, accomplished by membrane localization of Raf1 via CRY2-CIBN association, was demonstrated to induce a tail-like structure after germ layer formation (Krishnamurthy et al., 2016, 2017), and optoWnt activation results in axis duplication (Krishnamurthy et al., 2021).

Overall, optogenetic toolkits represent a powerful and exciting approach to understand and probe the mechanisms underlying neurodevelopment and neurogenesis. In the future, optogenetic control of morphogen signals may better inform the spatiotemporal signal dynamics governing neurulation, neural tube patterning, and neural differentiation and enable more physiologically relevant in vitro models of these stages.

**OPTOGENETICS IN CELLULAR FUNCTION AND SYNAPTIC COMMUNICATION**

Beyond early-stage work in neurodevelopment, recent optogenetic systems provide novel approaches to investigate the mechanisms behind cellular survival, function, and synaptic communication in the adult nervous system. One important class of cellular signaling pathways in the nervous system is the tropomyosin-related kinase (Trk) family of RTKs, as they are known to be important for neuronal survival, neurite outgrowth, synaptic function, and synaptic plasticity (Chang et al., 2014). Additionally, Trk signaling cascades are implicated in neurodegenerative disorders, including Alzheimer’s, chronic pain, inflammation, and cancer (Amatu et al., 2019). Upon binding to the respective neurotrophins, members of the Trk family, namely A, B, and C, activate several major signal transduction pathways, including the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated protein kinase (ERK) kinase (MEK), phosphatidylinositol 3-kinase (PI3K)/Akt, and phospholipase Cγ1 (PLCγ1)/Ca2+ pathways.

Recently, several such pathways have been placed under light control using Cry2, enabling investigations into mechanisms underlying cellular behavior in the nervous system. For example, an optogenetic TrkA (optoTrkA) system (Duan et al., 2018; Khamo et al., 2019), involving the fusion of the intracellular domain of TrkA to the Cry2-CIB1 dimerizing system, successfully stimulated nerve growth factor (NGF)/TrkA signaling in PC12 cells. Under light stimulation, the group observed both neurite outgrowth and survival of dorsal root ganglion nerves, similar to the effects of exogenous NGF. Another group recently reported that a fusion of the PHR region of Cry2 to the TrkB receptor (optoTrkB) induced robust activation of canonical TrkB signaling (Chang et al., 2014). They demonstrated that sustained light illumination resulted in upregulated extracellular signal related kinase (ERK) activity, neurite outgrowth, and filopodia formation, which mimicked brain-derived neurotrophic factor (BDNF) ligand signaling.

Following similar approaches, Cry2-based optogenetic toolkits for additional signaling cascades have also been recently reported, including optoRaf (Su et al., 2020), optoRhoA (Bugaj et al., 2013), optoCdc42 (Bugaj et al., 2013), optoEphB2 (Locke et al., 2017), and light sensitive myosin motors (Zhang et al., 2021). Overall, these newly developed optogenetic toolkits enable the spatiotemporal profiling of signal activation in vitro and enable applications in understanding the signaling dynamics underlying key neurotrophin cascades and cellular function in the nervous system.

In addition to Cry2-based optogenetic systems, the LOV domain from phototropin (Christie et al., 2012) has been harnessed in neurons. For example, in photoactivatable Rac1 (Wu et al., 2009) the LOV domain sterically occluded the binding of Rac1 to its effector. Upon light illumination, a conformational change induced unwinding of the Jα helix from the LOV domain and thereby released the inhibition of Rac1 signaling. The researchers thereby demonstrated that selectively activating Rac1 signaling could induce cell motility and control the direction of cell movement, enabling analysis of Rac1 regulation in RhoA in cell mobility. Rac1 is a key GTPase that regulates actin cytoskeletal dynamics (Rafitopoulos and Hall, 2004), and in dendritic spines, Rac1 specifically regulates the spine size via rearrangement of actin cytoskeleton (Hayashi-Takagi et al., 2010; Yuste, 2011). Previous work has implied the important role of structural plasticity of spines in synaptic transmission for learning-related activities (Hayashi-Takagi et al., 2015). Given the role of Rac1 in neurites and neurons, modulating Rac1 activity using precise and reversible optogenetic tool may offer novel insights into neuronal function and health (Penzes et al., 2011; Hayashi-Takagi, 2017).

Finally, optogenetic control of cellular signaling pathways not only offers exciting new tools to probe biological questions in vitro, but it also presents a potential novel perturbation in vivo to study mechanisms of neuroregeneration and disease in model organisms. Optogenetic domains have been harnessed for the recruitment of ligands to a specified localization to induce Ca2+ signaling in vivo. In particular, the PHR domain of Cry2 was fused to STIM1, and light inducible oligomerized STIM1 translocated to the plasma membrane and bound endogenous calcium release-activated calcium (CRAC) channels to trigger Ca2+ influx into the cell (Kyung et al., 2015). Using the OptoSTIM1 system, expressed in the hippocampus of living mice, researchers observed an increase of contextual fear memory in the light-illuminated group, suggesting potential mechanisms of Ca2+ dependent memory formation. Finally, a recent study demonstrated optoAKT and optoRAF (for the control of ERK signaling) in Drosophila enhanced axon regeneration and proper axon guidance in the peripheral nervous system (PNS) as well as axon regrowth and functional recovery in the CNS (Wang et al., 2020). In this example, optogenetics may provide a potential strategy in the intervention of neural degeneration.

Overall, optogenetic control of protein activity is a powerful toolkit to probe the spatiotemporal role of signaling dynamics.
with high precision and throughput. As new optogenetic systems emerge, future applications in understanding neurodevelopment and function may benefit from in vitro and in vivo optogenetic approaches.

**OPTOGENETICS IN NEUROLOGICAL DISORDERS AND FUTURE THERAPEUTIC DIRECTIONS**

Beyond studying signaling pathways and advancing understanding of complex cellular networks in neurodevelopment and physiological brain functions, optogenetics has also been extended to medical therapeutics and for modulation of cell behaviors in the context of understanding of disease pathologies. The need to develop efficient therapies for neurodegenerative diseases is urgent, given the increasing percentages of the population living longer. One of the reasons for the healthcare burden of neurological disorders is the lack of effective targeted treatments. The simultaneous development of genome engineering and optogenetics has the potential for interrogating mechanistically the pathology involved in genetically related brain disorders and possibly offering more targeted therapies for clinical applications.

For example, stable, optogenetically-modified mammalian cells could be implanted for relevant clinical conditions (Oggu et al., 2017). Given the deeper tissue penetration and low toxicity of red or NIR, recently developed red light systems developed for high tissue penetration, transgene, or signal induction with controlled localization, timing, and dosage is increasingly possible in the adult brain. For example, the implantation of wireless, minimally invasive illumination devices can enable optogenetic modulation of neural systems in large organisms (Montgomery et al., 2015; Park et al., 2015). Some previous studies have also shown that light can be efficiently delivered by a helmet-type configuration applied to the brain (Lanzafame et al., 2014). Using engineered stem cells, one can also introduce a therapeutic target gene with a light inducible expression system, which then can be spatiotemporally controlled for producing or inhibiting specific responses (Pomeroy et al., 2017). For example, human embryonic stem cell–mesencephalic dopaminergic neurons have been optogenetically controlled in a model of Parkinson’s disease using a fiber-optic cannula, which exploited optogenetics as an off-on switch for neuronal activity, allowing this function of the cells to be tested independently of other possible functions (Steinbeck et al., 2015). While the study demonstrated the utility of optogenetics to dissect the mechanism underlying graft function, the proof-of-concept of using engrafted, engineered stem cells with optogenetic control is also promising for potential clinical applications of optogenetic cell-based therapies in neurological disorders.

However, there are also some potential limitations in the translation of optogenetic systems to clinical usages, including: (1) the gene cassette size that can be packaged in vectors for transfection; (2) safe and efficient delivery vehicles that can target the cell or tissue of interest (though engineering of viral delivery systems such as engineered adeno-associated viruses can help accelerate progress); (3) potential inflammation and immunogenicity caused by optogenetic tools; (4) limited red-light or NIR light sensitive proteins available with high tissue penetration and low phototoxicity, especially in wavelengths ranges not currently well-explored; (5) potential long-term effects of permanent introduction of non-human proteins with optical properties; and (6) a lack of appropriate illumination devices that present narrower-wavelengths for mitigating surrounding tissue phototoxicity (Wojtovich and Foster, 2014), or high spatial illumination resolution that can circumvent subcellular protein diffusion problems.

To overcome some of these limitations and further improve the light-responsive protein properties, systematic protein engineering processes such as directed evolution (Packer and Liu, 2015; Wu et al., 2019) can be applied to engineer the next generation of optogenetic systems with enhanced efficiency/specificity (Herwig et al., 2017; Bedbrook et al., 2019). Some characteristics to consider can include: (a) specificity in response to an activating wavelength, with low or no background activity under the dark condition, (b) rapid and greater sensitivity to low levels of activation light to allow for longer exposures at low illumination intensity, (c) reduced size of protein components to facilitate delivery.

**CONCLUSIONS**

In summary, state-of-the-art optogenetic systems are enabling numerous discoveries and insights into fundamental functions of cellular signaling networks. With the substantial acceleration in the range of applications, the role of optogenetics will broadly enable the examination of how genes, proteins, cells, and cellular connections modulate local and global network activity to develop complex tissue structures and encode behaviors. Such fundamental knowledge gained from optogenetic research will also potentially enable clinical therapeutics.

**AUTHOR CONTRIBUTIONS**

DZ and DS substantially contributed to the conception and design of the article and interpreting the relevant literature. DZ wrote the first draft of the manuscript. HJ and JC wrote sections of the manuscript. DS revised the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

**FUNDING**

This work was supported by NIH R01NS074831 (to DS), a Chan-Zuckerberg Biohub award (DS, DZ), the Tang Scholars program (JC), and the National Science Foundation Graduate Research Fellowship Program (HJ).

**ACKNOWLEDGMENTS**

Cartoon graphics created with BioRender.com.
REFERENCES

Allen, J. (2017). Application of patterned illumination using a DMD for optogenetic control of signaling. Nat. Methods 14:1114. doi: 10.1038/nmeth.f402

Amatu, A., Sartore-Bianchi, A., Bencardino, K., Pizzutilo, E. G., Tosi, F., and Siena, S. (2019). Tropomyosin receptor kinase (TRK) biology and the role of NTRK gene fusions in cancer. Ann. Oncol. 30, viii15–viii15. doi: 10.1093/annonc/mdy383

Aravind, A. M., Wang, L. P., Zhang, F., Meltzer, L. A., Mogri, M. Z., Schneider, M. B., et al. (2007). An optical neural interface: in vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology. J. Neural Eng. 4, S143–S156. doi: 10.1088/1741-2560/4/3/S02

Bedbrook, C. N., Yang, K. K., Robinson, J. E., Mackey, E. D., Gradinaru, V., and Arnold, F. H. (2019). Machine learning-guided channelrhodopsin engineering enables minimally invasive optogenetics. Nat. Methods 16, 1176–1184. doi: 10.1038/s41592-019-0588-3

Benzing, D., and Khammash, M. (2018). Pulsatile inputs achieve tunable attenuation of gene expression variability and graded multi-gene regulation. Nat. Commun. 9:3521. doi: 10.1038/s41467-018-05882-2

Bugaj, L. J., Rakshit, R., Payumo, A. Y., Chen, J. K., and Wandless, T. J. (2014). High-throughput multicolor optogenetics in microwell plates. Nat. Protoc. 14, 2205–2228. doi: 10.1038/nprot.2014.196

Bugaj, L. J., Spelke, D. P., Mesuda, C. K., Varedi, M., Kane, R. S., and Schaffer, D. V. (2013). Optogenetic protein clustering and signaling activation in mammalian cells. Nat. Methods 10, 249–252. doi: 10.1038/nmeth.2360

Bonger, K. M., Rakhit, R., Choksi, A. T., Mesuda, C. K., Kane, R. S., and Schaffer, D. V. (2013). C-terminal region of the UV-B photoreceptor UVR8 initiates signaling through interaction with the COP1 protein. Proc. Natl. Acad. Sci. U S A 110, 16366–16370. doi: 10.1073/pnas.1208981109

Chang, K. Y., Woo, D., Jung, H., Lee, S., Kim, S., Won, J., et al. (2014). Light-inducible receptor tyrosine kinases that regulate neurotrophin signalling. Nature 525, 333–338. doi: 10.1038/nature15257

Chen, X., Yu, X. R., Guo, S., Ong, Q., François, A., Kaplan, L., et al. (2019). Noise-reducing optogenetic negative-feedback gene circuits in human cells. Nucleic Acids Res. 47, 7703–7714. doi: 10.1093/nar/gkz562

Duan, L., Guo, S., Ong, Q., François, A., Kaplan, L., et al. (2015). Optical activation of TrkB signaling. ACS Synth. Biol. 4, 1633–1639. doi: 10.1021/acssynbio.5b00126

Ewen-Campen, B., Yang-Zhou, D., Fernandes, V. R., González, D. P., Liu, L. P., Tao, R., et al. (2017). Optimized strategy for in vivo Cas9-activation in Drosophila. Proc. Natl. Acad. Sci. U S A 114, 9409–9414. doi: 10.1073/pnas.1706635114

Evans, P. N., Chen, X., Mitra, R. K., and Balázs, G. (2019). Noise-reducing optogenetic negative-feedback gene circuits in human cells. Nucleic Acids Res. 47, 7703–7714. doi: 10.1093/nar/gkz562

Harper, S. M., Neil, I. C., and Gardiner, K. H. (2003). Structural basis of a phototropin light switch. Science 301, 1541–1544. doi: 10.1126/science.1086810

Hayashi-Takagi, A. (2017). Synapse pathology and translational applications for schizophrenia. Neurosci. Res. 114, 3–8. doi: 10.1016/j.neuresci.2016.09.001

Hayashi-Takagi, A., Takaki, M., Graziante, N., Seshadri, S., Murdock, H., Dunlop, A. J., et al. (2010). Disrupted-in-Schizophrenia 1 (DISC1) regulates spines of the glutamate synapse via Rac1. Nat. Neurosci. 13, 327–332. doi: 10.1038/nn.2487

Herwig, L., Rice, A. J., Bedbrook, C. N., Zhang, R. K., Lignall, A., Cahn, J. K. B., et al. (2017). Directed evolution of a bright near-infrared fluorescent rhodopsin using a synthetic chromophore. Cell Chem. Biol. 24, 415–425. doi: 10.1016/j.chembiol.2017.02.008

Humphreys, P. A., Woods, S., Smith, C. A., Bates, N., Cain, S. A., Lucas, R., et al. (2020). Optogenetic control of the BMP signaling pathway. ACS Synth. Biol. 9, 3067–3078. doi: 10.1021/acssynbio.0c00315

Jia, Y., Xu, R. G., Ren, X., Ewen-Campen, B., Rajakumar, R., Zirin, J., et al. (2018). Next-generation CRISPR/Cas9 transcriptional activation in Drosophila using fysAM. Proc. Natl. Acad. Sci. U S A 115, 4719–4724. doi: 10.1073/pnas.1806077115

Jin, M., Chelysheva, K., Konopka, I., Hauer, M., Doudna, J. A., and Charpentier, E. (2012). A programmable dual-RNA-Guided DNA endonuclease in adaptive bacterial immunity. Science 337, 816–821. doi: 10.1126/science.1225829

Jin, M., East, A., Cheng, A., Lin, M., Sa, E., and Doudna, J. (2013). RNA-programmed genome editing in human cells. elife 2:e00471. doi: 10.7554/elife.00471

Kabernik, N. A., Shemesh, A. A., and Verkhusha, V. V. (2016). A bacterial phytochrome-based optogenetic system controllable with near-infrared light. Nat. Methods 13, 591–597. doi: 10.1038/nmeth.3866

Kim, N., Kim, J. M., Lee, M., Kim, C. Y., Chang, K. Y., and Heo, W. D. (2014). Injectable, cellular-scale optoelectronics with applications for wireless nervous system. Annu. Rev. Neurosci. 37, 347–367. doi: 10.1146/annurev-neuro-052314-125115

Kim, N., Kim, J. M., Lee, M., Kim, C. Y., Chang, K. Y., and Heo, W. D. (2014). Spatialtemporal control of fibroblast growth factor receptor signals by blue light. Chem. Biol. 21, 903–912. doi: 10.1016/j.chembiol.2014.05.013

Kornermann, S., Brigham, M. D., Trevino, A. E., Hsu, P. D., Heidenreich, M., and Weiss, R. (2015). Optogenetic control of mammalian endogenous transcription and epigenetic states. Nature 500, 472–476. doi: 10.1038/nature14266

Kramer, M. M., Lataster, L., Weber, W., and Radziwill, G. (2021). Optogenetic approaches for the spatialtemporal control of signal transduction pathways. Int. J. Mol. Sci. 22, 5300. doi: 10.3390/ijms22105300
Optogenetic control of the canonical Wnt signaling pathway during embryonic development. *J. Mol. Biol.* 433, 167050. doi: 10.1016/j.jmb.2021.167050

Krishnamurthy, V. V., Hwang, H., Fu, J., Yang, J., and Zhang, K. (2021). Optogenetic control of the canonical Wnt signaling pathway during *Xenopus laevis* embryonic development. *J. Mol. Biol.* 433, 167050. doi: 10.1016/j.jmb.2021.167050

Krishnamurthy, V. V., Khamo, J. S., Mei, W., Turgeon, A. J., Ashraf, H. M., Mondal, P., et al. (2016). Reversible optogenetic control of kinase activity during differentiation and embryonic development. *Development* 143, 4085–4094. doi: 10.1242/dev.140889

Krishnamurthy, V. V., Turgeon, A. J., Khamo, J. S., Mondal, P., Sharan, S. R., Mei, W., et al. (2017). Light-mediated reversible modulation of the mitogen-activated protein kinase pathway during cell differentiation and *Xenopus* embryonic development. *J. Vis. Exp.* doi: 10.3791/55823

Krueger, D., Izquierdo, E., Viswanathan, R., Hartmann, J., Pallares Cartes, C., and De Renzis, S. (2019). Principles and applications of optogenetics in developmental biology. *Development* 146, 175067. doi: 10.1242/development.175067

Kyung, T., Lee, S., Kim, J. E., Cho, T., Park, H., Jeong, Y.-M., et al. (2015). Neural tube closure: cellular, molecular and biomechanical mechanisms. *Development* 142, 552–566. doi: 10.1242/dev.145904

Kogo, G., Sasaki, S., Reddy, N., Ella, K. K. R., Rao, C. M., Bokara, K. K., et al. (2017). Gene delivery approaches for mesenchymal stem cell therapy: strategies to increase efficiency and specificity. *Stem Cell Rev. Rep.* 13, 723–740. doi: 10.1007/s12015-017-9760-2

Packer, M. S., and Liu, D. R. (2015). Methods for the directed evolution of proteins. *Nat. Rev. Genet.* 16, 379–394. doi: 10.1038/nrg3927

Paonessa, F., Criscuolo, S., Sacchetti, S., Amoroso, D., Scarongella, H., Pecoraro Bisogni, F., et al. (2016). Regulation of neural gene transcription by optogenetic inhibition of the REI-silencing transcription factor. *Proc. Natl. Acad. Sci. U S A* 113, E91–E100. doi: 10.1073/pnas.1507355112

Park, S. I., Brenner, D. S., Shin, G., Morgan, C. D., Copis, B. A., Chung, H. U., et al. (2015). Soft, stretchable, fully implantable miniaturized optoelectronic systems for wireless optogenetics. *Nat. Biotechnol.* 33, 1280–1286. doi: 10.1038/nbt.3415

Pathak, G. P., Spillot, J. L., Höglund, C., Polstein, L. R., Heine-Koskinen, S., Gersbach, C. A., et al. (2017). Bidirectional approaches for optogenetic regulation of gene expression in mammalian cells using *Arabidopsis* cryptochrome 2. *Nucleic Acids Res.* 45:e167. doi: 10.1093/nar/gkx260

Penzes, P., Cahill, M. E., Jones, K. A., VanLeeuwen, J. E., and Woolfrey, K. M. (2011). Dendritic spine pathology in neuropsychiatric disorders. *Nat. Neurosci.* 14, 285–293. doi: 10.1038/nn.2741

Polstein, L. R., and Gersbach, C. A. (2015). A light-inducible CRISPR-Cas9 system for control of endogenous gene activation. *Nat. Chem. Biol.* 11, 198–200. doi: 10.1038/nchembio.1753

Pomeroy, L. E., Nguyen, H. X., Hoffman, B. D., and Bursac, N. (2017). Genetically encoded photoactivators and photosensors for characterization and manipulation of pluripotent stem cells. *Theranostics* 7, 3539–3558. doi: 10.7150/thno.20593

Pozzi, D., Lignani, G., Ferrea, E., Contestabile, A., Paonessa, F., D’Alessandro, R., et al. (2013). REST/NRSF-mediated intrinsic homeostasis protects neuronal networks from hyperexcitability. *EMBO J.* 32, 2994–3007. doi: 10.1038/embr.2013.231

Quill, P. H. (2002). Phytochrome photosensory signalling networks. *Nat. Rev. Mol. Cell Biol.* 3, 85–93. doi: 10.1038/nrm7278

Quill, P. H. (2010). Phytochromes. *Curr. Biol.* 20, R504–R507. doi: 10.1016/j.cub.2010.04.014

Rafipoupolou, M., and Hall, A. (2004). Cell migration: Rho GTPases lead the way. *Dev. Biol.* 265, 23–32. doi: 10.1016/j.ydbio.2003.06.003

Redchuk, T. A., Kaberniuk, A. A., and Verkhusha, V. V. (2018). Near-infrared light-controlled systems for gene transcription regulation, protein targeting and spectral multiplexing. *Nat. Protoc.* 13, 1121–1136. doi: 10.1038/nprot.2018.022

Repina, N. A., McClave, T., Johnson, H. J., Bao, X., Kane, R. S., and Saffer, D. V. (2020). Engineered illumination devices for optogenetic control of cellular signaling dynamics. *Cell Rep.* 31:107737. doi: 10.1016/j.celrep.2020.107737

Repina, N. A., Rosenbloom, A., Mukherjee, A., Saffer, D. V., and Kane, R. S. (2017). At light speed: advances in optogenetic systems for regulating cell signaling and behavior. *Annu. Rev. Chem. Biomol. Eng.* 8, 13–39. doi: 10.1146/annurev-chembioeng-060816-101254

Rivera, V. M., Clackson, T., Natesan, S., Pollock, R., Amara, J. F., Keenan, T., et al. (1996). A humanized system for pharmacologic control of gene expression. *Nat. Med.* 2, 1028–1032. doi: 10.1038/nm9996-1028

Rosenbaum, D. M., Rasmussen, S. G. F., and Kobilka, B. K. (2009). The growth of human scalp hair in females using visible red light laser and LED sources. *Sklar, J. A.* (2014). The growth of human scalp hair in females using visible red light laser and LED sources. *Sklar, J. A.* (2014). The growth of human scalp hair in females using visible red light laser and LED sources.
Shimizu-Sato, S., Huq, E., Tepperman, J. M., and Quail, P. H. (2002). A light-switchable gene promoter system. *Proc. Natl. Acad. Sci. U S A* 115:E6722. doi: 10.1073/pnas.1802448115

Shcherbakova, D. M., Shemovet, A. A., Kaberniuk, A. A., and Verkhusha, V. V. (2015). Natural photoconverters as a source of fluorescent proteins, biosensors and optogenetic tools. *Annu. Rev. Biochem.* 84, 519–550. doi: 10.1146/annurev-biochem-060614-034411

Shemesh, O. A., Tanese, D., Zampini, V., Linghu, C., Piatkevich, K., Ronzitti, E., et al. (2017). Temporally precise single-cell-resolution optogenetics. *Nat. Neurosci.* 20, 1796–1806. doi: 10.1038/s41593-017-0018-8

Shimizu-Sato, S., Huq, E., Pefferman, J. M., and Quail, P. H. (2002). A light-switchable gene promoter system. *Nat. Biotechnol.* 20, 1041–1044. doi: 10.1038/nbt734

Sokolik, C., Liu, Y., Bauer, D., McPherson, J., Brooker, M., Heimberg, G., et al. (2015). Transcription factor competition allows embryonic stem cells to distinguish authentic signals from noise. *Cell Syst.* 1, 117–129. doi: 10.1016/j.cels.2015.08.001

Stanton, B. Z., Chory, E. J., and Crabtree, G. R. (2018). Chemically induced proximity in biology and medicine. *Science* 359:eaao5902. doi: 10.1126/science.aao5902

Steinbeck, J. A., Choi, S. J., Mrejeru, A., Ganat, Y., Deisseroth, K., Sulzer, D., et al. (2015). Optogenetics enables functional analysis of human embryonic stem cell-derived grafts in a Parkinson’s disease model. *Nat. Biotechnol.* 33, 204–209. doi: 10.1038/nbt.3124

Su, Y., Huang, X., Huang, Z., Huang, T., Li, T., Fan, H., et al. (2020). Early but not delayed optogenetic RAF activation promotes astrocytogenesis in mouse neural progenitors. *J. Mol. Biol.* 432, 4358–4368. doi: 10.1016/j.jmb.2020.06.020

Szenk, M., Yim, T., and Balázsi, G. (2020). Multiplexed gene expression tuning with orthogonal synthetic gene circuits. *ACS Synth. Biol.* 9, 930–939. doi: 10.1021/acssynbio.9b00534

Toettcher, J. E., Weiner, O. D., and Lim, W. A. (2013). Using optogenetics to interrogate the dynamic control of signal transmission by the Ras/Erk module. *Cell* 155, 1422–1434. doi: 10.1016/j.cell.2013.11.004

Tucker, C. L., Vrana, J. D., and Kennedy, M. J. (2014). Tools for controlling protein interactions using light. *Curr. Protoc. Cell Biol.* 64:17.6.120.

Wang, X., Chen, X., and Yang, Y. (2012). Spatiotemporal control of gene expression by a light-switchable transgene system. *Nat. Methods* 9, 266–269. doi: 10.1038/nmeth.1892

Wang, Q., Fan, H., Li, F., Skeeters, S. S., Krishnamurthy, V. V., Song, Y., et al. (2020). Optical control of ERK and AKT signaling promotes axon regeneration and functional recovery of PNS and CNS in *Drosophila*. eLife 9:e57395. doi: 10.7554/eLife.57395

Webster, N. J., Green, S., Jin, J. R., and Chambon, P. (1988). The hormone-binding domains of the estrogen and glucocorticoid receptors contain an inducible transcription activation function. *Cell* 54, 199–207. doi: 10.1016/0092-8674(88)90552-1

Wojtovich, A. P., and Foster, T. H. (2014). Optogenetic control of ROS production. *Redox Biol.* 2, 368–376. doi: 10.1016/j.redox.2014.01.019

Wu, Y. I., Frey, D., Lungo, O. I., Jaehng, A., Schlichting, I., Kuhlman, B., et al. (2009). A genetically encoded photoactivatable Rac controls the motility of living cells. *Nature* 461, 104–108. doi: 10.1038/nature08241

Wu, D., Hu, Q., Yan, Z., Chen, W., Yan, C., Huang, X., et al. (2012). Structural basis of ultraviolet-B perception by UVR8. *Nature* 484, 214–219. doi: 10.1038/nature10931

Wu, Z., Kan, S. B. J., Lewis, R. D., Wittmann, B. J., and Arnold, F. H. (2019). Machine learning-assisted directed protein evolution with combinatorial libraries. *Proc. Natl. Acad. Sci. U S A* 116:8852. doi: 10.1073/pnas.1901979116

Yang, J., Lee, J., Land, M. A., Lai, S., Igoshin, O. A., and St-Pierre, F. (2021). A synthetic circuit for buffering gene dosage variation between individual mammalian cells. *Nat. Commun.* 12:4132. doi: 10.1038/s41467-021-23889-0

Yuste, R. (2011). Dendritic spines and distributed circuits. *Neuron* 71, 772–781.

Zhang, Z., Denans, N., Liu, Y., Zhulyn, O., Rosenblatt, H. D., Wernig, M., et al. (2021). Optogenetic manipulation of cellular communication using engineered myosin motors. *Nat. Cell Biol.* 23, 198–208. doi: 10.1038/s41555-020-00625-2

Zhang, F., Vierock, J., Yizhar, O., Fenno, L. E., Tsunoda, S., Kianianmomeni, A., et al. (2011). The microbial opsin family of optogenetic tools. *Cell* 147, 1446–1457. doi: 10.1016/j.cell.2011.12.004

Zhang, F., Wang, L. P., Boyden, E. S., and Deisseroth, K. (2006). Channelrhodopsin-2 and optical control of excitable cells. *Nat. Methods* 3, 785–792. doi: 10.1038/nmeth936

Zolowska, B. D., Schwedtfeger, C., Widom, J., Loros, J. J., Bilwes, A. M., Dunlap, J. C., et al. (2007). Conformational switching in the fungal light sensor vivid. *Science* 316, 1054–1057. doi: 10.1126/science.1137128

Zuccato, C., Tartari, M., Crotti, A., Goffredo, D., Valenza, M., Conti, L., et al. (2003). Huntingtin interacts with REST/NRSE to modulate the transcription of NRSE-controlled neuronal genes. *Nat. Genet.* 35, 76–83. doi: 10.1038/ng1219

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher’s Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

**Copyright © 2022 Zhu, Johnson, Chen and Schaffer. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.**