Interrogating cellular perception and decision making with optogenetic tools

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Optogenetics promises to deepen our understanding of how cells perceive and respond to complex and dynamic signals and how this perception regulates normal and abnormal function. In this study, we present our vision for how these nascent tools may transform our view of fundamental cell biological processes.

Recent years have seen a maturation in the application of optogenetics in cell biology. There has been acceleration in the development and accessibility of diverse optogenetic modules that allow the manipulation of cells using light-sensitive protein actuators. Many flavors of modular "plug-and-play" optogenetic proteins have now been reported, and several groups have undertaken the necessary work of benchmarking these tools against each other in various experimental contexts (Pathak et al., 2014; Hallett et al., 2016). Furthermore, there is increasing development of illumination hardware, providing solutions to programmatically administer light onto cells in high throughput (Gerhardt et al., 2016; Hannanta-anan and Chow, 2016).

As the technical barriers to implementation of optogenetic tools are continually lowered, we anticipate that these tools will become instrumental in our understanding of cell biology, particularly in understanding cell signaling and signal perception: how a cell senses, filters, and ultimately decides how to respond to its environment. Here, we do not focus on specific technological advances (reviewed elsewhere; Beyer et al., 2015), but rather we outline a vision for general strategies by which optogenetics can be used to uncover important insight into cell signaling networks, cellular regulation, and cell dysfunction in disease.

Cellular virtual reality through optogenetics

The notion that the dynamics of a stimulus can encode information should be intuitive from our daily experience: without this, we could not track down a moving tennis ball or avoid an approaching car. On a microscopic level, cells in our body are also faced with dynamic challenges: neighboring cells secrete quantized amounts of mitogens and morphogens that constantly fluctuate, nutrients are delivered during discrete feeding times, and circadian cycles regulate natural oscillations of gene expression and protein activity.

It has become clear that cellular regulatory networks process information that the cell receives over time and that the cell integrates this dynamic information to make complex decisions. However, standard cell biological tools are poorly suited to recapitulate the input variations that cells may naturally experience. Classical tools (e.g., genetic knockouts/overexpression and small-molecule inhibition) can merely present cells with static stimuli that represent only a single point on a multidimensional stimulus landscape. One of the most powerful aspects of optogenetic tools is that they can be used to surgically probe how living cells respond to dynamic signals. Although optogenetic tools can be used to control particular molecular activities in both space and time, we believe that dynamic controllability is one of the most important and unique advantages of light-based control.

Optogenetics is analogous to a "virtual reality" system for cells (Fig. 1 A), through which we can now stimulate cells with user-defined programs that systematically apply a range of stimuli. This provides a powerful tool to uncover the principles by which the dynamics and variation of cell signals can regulate cellular behavior. In addition to having high spatiotemporal precision, optogenetic methods are programmable, scalable, and genetically encodable. The programmability of illumination and the scalability of microwell experiments will enable us to rapidly scan stimulus landscapes with an expansive set of input profiles. This will provide a systematic understanding of cellular dynamic response. Their genetic encodability makes these methods fully compatible with standard cell culture processes, conventional microscopy, and in vivo application. Importantly, because light is a largely orthogonal cue, we can be confident that the inputs we project on the cell will specifically manipulate the node and pathway of interest. Together, these properties enable us to build quantitative input–output maps of signaling networks and cell fate responses, permitting a high-resolution functional understanding of the interconnected cellular machinery.

Dissecting complex signaling networks with precise molecular knobs

Optogenetics can be used to understand dynamic signal perception on multiple scales. On a molecular level, optogenetics can be used to perform essentially any biochemical experiment in the biochemical context of a living cell. We are learning to optogenetically intervene at diverse network nodes (especially when using universal light-controlled currencies like induced recruitment). For those nodes that we can control, intensity titration can be used to populate dose–response and

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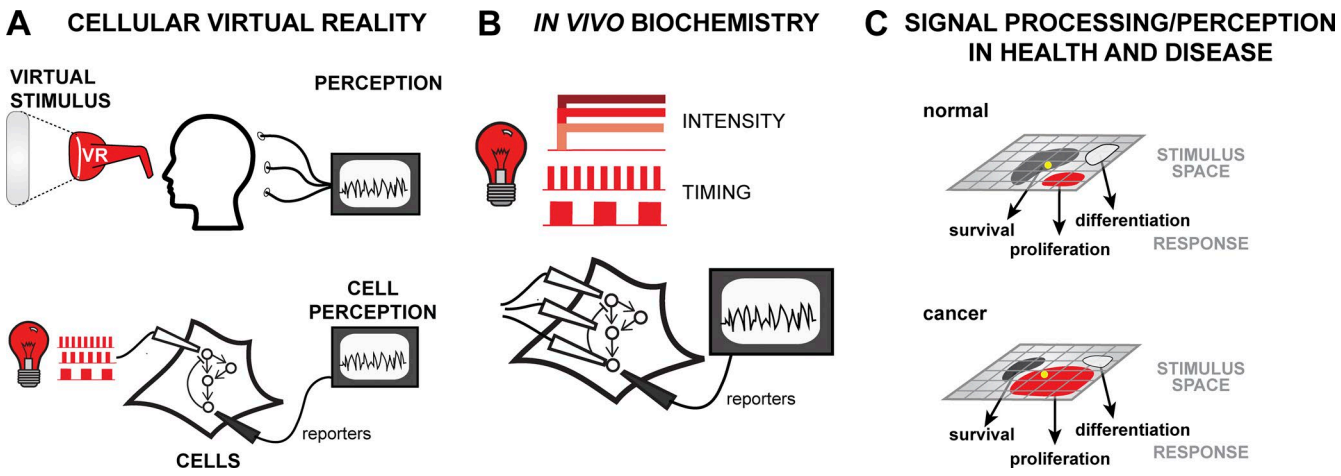

Figure 1. **Understanding cellular input responses through optogenetics.** (A) Optogenetics provides a virtual reality platform for cells, capable of probing cells with user-programmable dynamic inputs. This enables a systematic examination of how cells perceive complex and time-varying input signals. (B) Optogenetic tools enable dissection of molecular networks in the native cellular context. Signal intensities and timings can be tuned at arbitrary nodes within a signaling network, permitting a quantitative understanding of information flow and a functional mapping of network interactions. (C) Mapping how signaling inputs drive cell fate outputs may reveal fundamental rewiring of input responses within cancer or other diseases. In the depicted conceptual example, a given stimulus (yellow dot) promotes survival in a normal cell but induces proliferation in a tumor cell as a result of cancer-induced input response rewiring. This would result in hyperproliferation.

Michaelis-Menten–like curves, all with the reaction conditions and output parameters set by the native cellular biochemistry (Fig. 1 B). Reaction kinetics can be measured at different points in the cascade (using different reporters), allowing us to track how quickly information flows from one point to the next. In the case of the phytochrome B/phytochrome interaction factor (PhyB/PIF) optogenetic system (Levskaya et al., 2009), the ratio of on (650 nm) and off (750 nm) light can also be varied such that the overall PhyB/PIF K_d remains constant while the underlying rate constants, k_{on} and k_{off}, vary. In this way, the relative contributions to signaling outputs of biochemical kinetics versus thermodynamics may be determined. We can also use control hardware to stimulate the cells with precise, time-variant patterns of stimulation (input over time) to systematically map dynamic stimulus response relationships. Importantly, conducting these molecular biochemistry experiments in a live cell can link specific biochemical nodes to transcriptional and cell fate outcomes—the ultimate downstream outputs for these integrated dynamic decisions. To the extent that it is possible, these optogenetic experiments should complement more traditional in vitro biochemical experiments using purified components. The most interesting questions may exist at the intersection between live cell, in vivo, and in vitro biochemistry: which biochemical and biophysical features exist in vitro but not in vivo, and vice versa.

Deciphering information flow through intact cell signaling networks further relies on dissecting the branched and nonlinear structure of these pathways. Resultant feedback and feed-forward linkages can yield sophisticated signaling behaviors such as adaptation, fold change detection, and hysteresis. Optogenetic tools, which are often reversible on the second scale, allow us to analyze these linkages by comparing the forward and backward input–output responses (Alon, 2007). As we continue to endow new signaling proteins with optogenetic control, we will be able to orthogonally "walk down" a pathway and observe system response to activation of its component nodes (Fig. 1 B). We can thus map the sources and strengths of network connectivity by observing how the system responds when sequential nodes of the pathway are activated. As an early example, Xu et al. (2016) recently dissected the roles of PI3K and Akt in the insulin response of glucose transporter 4 (GLUT4) translocation. Using independent optogenetic control over PI3K and Akt signaling, the authors showed that Akt activation only partially recapitulates the PI3K- and insulin-induced GLUT4 response, implicating Akt-independent processes mediated through PI3K. Such analysis will be especially powerful for characterizing signaling nodes that are not targetable with drugs (e.g., Ras). Mapping network function in this manner will enable us to design or predict the effect of small-molecule or biological drugs or to redesign signaling networks for therapeutic or other purposes.

In addition to uncovering network responses to individual signals, optogenetics allows us to probe signal integration from multiple pathway inputs simultaneously. Orthogonal optogenetic tools, for instance blue light– and red light–inducible systems, could be multiplexed to perform two or even three "color" stimulation experiments to mimic and disentangle the effects of multiple native signal inputs. In this manner, optogenetic tools are complementary to chemical-induced dimerizing tools (Spencer et al., 1993), which can provide additional orthogonal control channels.

Functionally interrogating cellular perception

On the cellular scale, optogenetics allows us to characterize the cell as a black box: we can systematically probe the cell with variable inputs (naturally occurring or not), and we can measure the resultant cellular output (e.g., transcription and cell fate). Optogenetics provides causal understanding of how complex signals can regulate cell function.

Our group recently applied optogenetic control of Ras signaling to investigate how cells interpret dynamic Ras inputs through the Ras–Erk pathway (Toettcher et al., 2013). Using sophisticated microscopy and computer-controlled light-emitting diode illumination, we showed that the Ras–Erk signaling module behaves like a high-bandwidth low-pass filter, able to transmit all signals lasting >4 min. At the transcriptional level, however, we found that transcriptional circuits can be

highly sensitive to signal dynamics. Whereas some genes respond to a transient, 30-min Ras–Erk signal, others required a sustained 2-h stimulus.

In conceptually related work, Hannanta-anan and Chow (2016) used optogenetics to dissect how transcription factors interpret information in Ca^{2+} dynamics. Here, the authors generated optically defined Ca^{2+} oscillations to determine that—contrary to long-standing belief—the Ca^{2+}-sensitive nuclear factor of activated T cells (NFAT) promoter responds primarily to the integrated Ca^{2+} signal and not to its frequency.

There is also great potential in probing how cells respond to complex signals beyond those that a cell has evolved to experience. Recent work from our group revealed a weakness within the yeast osmoresponse by pulsing it with a non-natural square-wave osmotic stimulus pattern (Mitchell et al., 2015). The adaptive nature of the osmoresponse network caused the cell to interpret these pulses as an increasing input ramp, hyperactivating the stress response and inhibiting growth. Because wild-type yeast had likely never experienced such an oscillatory input pattern in the wild, the network's weakness was not evolutionarily selected against. With our ability to define arbitrary cell inputs, we could now reveal and exploit this feature. Uncovering analogous circuits within mammalian cells using the more generalizable tools of optogenetics for variable input control may bring about future dynamic interventions to leverage such network weaknesses for therapeutic purposes.

Probing disease-specific changes in cellular perception

By understanding cellular input response, we may also glean unique insights into cellular dysfunction in disease. In cancer, many of the signaling nodes defining the cell's input response are mutated. A common view is that these mutations drive high levels of cell-autonomous signaling to promote tumor growth. However, overexpression of such proteins does not necessarily yield proliferation, but instead—paradoxically—can promote alternative fates like senescence (Serrano et al., 1997). This indicates that (a) fully mapping input–output relationships in oncogenic pathways will be important to understanding oncogenic transformation (what is the role of signal intensities and signal timings?) and that (b) the mechanisms by which oncogenes induce cancer can be complicated and varied.

An untested, yet optogenetically tractable, hypothesis is that cancer processes may fundamentally alter the cellular input–output response map (Fig. 1 C). Subtle alterations in abundance, affinities, or kinetics of any network node could change the cell's response properties such that input signals driving one phenotype in normal cells (e.g., survival) may now drive a different phenotype in diseased cells (e.g., proliferation). Although we currently classify cancer largely through its mutated components, optogenetics offers a complementary approach to understand how those mutated components impact cellular function. Ultimately, an enhanced functional understanding of diseases like cancer may offer new metrics for disease stratification and drug selection. In addition, functional profiling of cancer cells could provide treatment insights for the large number of tumors whose mutations are not known or understood.

Future outlook and concluding remarks

Optogenetic tools are well equipped to study principles of signal perception and collective cell function in vivo, and initial studies have already exploited spatiotemporally precise control to probe the principles of embryo development in model organisms (Guglielmi et al., 2015; Buckley et al., 2016). In moving to larger animals, challenges emerge regarding sufficient light delivery for optogenetic actuation. These challenges are continually being overcome with reports of new red-shifted optogenetic proteins (Kaberniuk et al., 2016; Reichhart et al., 2016) and other technologies such as up-converting nanoparticles (He et al., 2015) and wirelessly powered light-emitting diode implants (Montgomery et al., 2015). Future advances may further permit us to leverage optogenetic precision directly in vivo for therapeutic purposes. Although significant challenges remain, it would in principle be powerful to tune the strength and location of a cellular therapy (e.g., adoptive immunotherapy) with optical precision.

In the nearer future, we anticipate that optogenetics will provide an indispensable framework to understand how interacting cellular components coordinate cell sensing and response through space and time in health and disease. Although our current wealth of "omics" tools can give us a comprehensive molecular description of cells, they cannot provide information on how these molecular networks dynamically interact to respond to inputs and regulate cell behaviors. Optogenetics promises powerful mechanistic tools to fill this gap, which will ultimately deepen our understanding of cell biology.

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References

Alon, U. 2007. Network motifs: theory and experimental approaches. *Nat. Rev. Genet.* 8:450–461. http://dx.doi.org/10.1038/nrg2102

Beyer, H.M., S. Naumann, W. Weber, and G. Radziwill. 2015. Optogenetic control of signaling in mammalian cells. *Biotechnol. J.* 10:273–283. http://dx.doi.org/10.1002/biot.201400077

Buckley, C.E., R.E. Moore, A. Reade, A.R. Goldberg, O.D. Weiner, and J.D. Clarke. 2016. Reversible optogenetic control of subcellular protein localization in a live vertebrate embryo. *Dev. Cell.* 36:117–126. http://dx.doi.org/10.1016/j.devcel.2015.12.011

Gerhardt, K.P., E.J. Olson, S.M. Castillo-Hair, L.A. Hartsough, B.P. Landry, F. Ekness, R. Yokoo, E.J. Gomez, P. Ramakrishnan, J. Suh, et al. 2016. An open-hardware platform for optogenetics and photobiology. *Sci. Rep.* 6:35363. http://dx.doi.org/10.1038/srep35363

Guglielmi, G., J.D. Barry, W. Huber, and S. De Renzis. 2015. An optogenetic method to modulate cell contractility during tissue morphogenesis. *Dev. Cell.* 35:646–660. http://dx.doi.org/10.1016/j.devcel.2015.10.020

Hallett, R.A., S.P. Zimmerman, H. Yumerefendi, J.E. Bear, and B. Kuhlman. 2016. Correlating in vitro and in vivo activities of light-inducible dimers: a cellular optogenetics guide. *ACS Synth. Biol.* 5:53–64. http://dx.doi.org/10.1021/acssynbio.5b00119

Hannanta-anan, P., and B.Y. Chow. 2016. Optogenetic control of calcium oscillation waveform defines NFAT as an integrator of calcium load. *Cell Syst.* 2:283–288. http://dx.doi.org/10.1016/j.cels.2016.03.010

He, L., Y. Zhang, G. Ma, P. Tan, Z. Li, S. Zang, X. Wu, J. Jing, S. Fang, L. Zhou, et al. 2015. Near-infrared photoactivatable control of Ca^{2+} signaling and optogenetic immunomodulation. *eLife.* 4:e10024. http://dx.doi.org/10.7554/eLife.10024

Kaberniuk, A.A., A.A. Shemetov, and V.V. Verkhusha. 2016. A bacterial phytochrome-based optogenetic system controllable with near-infrared light. *Nat. Methods.* 13:591–597. http://dx.doi.org/10.1038/nmeth.3864

Levskaya, A., O.D. Weiner, W.A. Lim, and C.A. Voigt. 2009. Spatiotemporal control of cell signalling using a light-switchable protein interaction. *Nature.* 461:997–1001. http://dx.doi.org/10.1038/nature08446

Mitchell, A., P. Wei, and W.A. Lim. 2015. Oscillatory stress stimulation uncovers an Achilles' heel of the yeast MAPK signaling network. *Science.* 350:1379–1383. http://dx.doi.org/10.1126/science.aab0892

Montgomery, K.L., A.J. Yeh, J.S. Ho, V. Tsao, S. Mohan Iyer, L. Grosenick, E.A. Ferenczi, Y. Tanabe, K. Deisseroth, S.L. Delp, and A.S.Y. Poon. 2015. Wirelessly powered, fully internal optogenetics for brain, spinal and peripheral circuits in mice. *Nat. Methods.* 12:969–974. http://dx.doi.org/10.1038/nmeth.3536

Pathak, G.P., D. Strickland, J.D. Vrana, and C.L. Tucker. 2014. Benchmarking of optical dimerizer systems. *ACS Synth. Biol.* 3:832–838. http://dx.doi.org/10.1021/sb500291r

Reichhart, E., A. Ingles-prieto, a.-m. tichy, c. mckenzie, and h. janovjak. 2016. a phytochrome sensory domain permits receptor activation by red light. *Angew. Chem. Int. Ed. Engl.* 55:6339–6342. http://dx.doi.org/10.1002/anie.201601736

Serrano, M., A.W. Lin, M.E. McCurrach, D. Beach, and S.W. Lowe. 1997. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell.* 88:593–602. http://dx.doi.org/10.1016/S0092-8674(00)81902-9

Spencer, D.M., T.J. Wandless, S.L. Schreiber, and G.R. Crabtree. 1993. Controlling signal transduction with synthetic ligands. *Science.* 262:1019–1024. http://dx.doi.org/10.1126/science.7694365

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

Xu, Y., D. Nan, J. Fan, J.S. Bogan, and D. Toomre. 2016. Optogenetic activation reveals distinct roles of PIP3 and Akt in adipocyte insulin action. *J. Cell Sci.* 129:2085–2095. http://dx.doi.org/10.1242/jcs.174805