Review

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Optogenetic control of intracellular signaling pathways

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Cells employ a plethora of signaling pathways to make their life-and-death decisions. Extensive genetic, biochemical, and physiological studies have led to the accumulation of knowledge about signaling components and their interactions within signaling networks. These conventional approaches, although useful, lack the ability to control the spatial and temporal aspects of signaling processes. The recently emerged optogenetic tools open exciting opportunities by enabling signaling regulation with superior temporal and spatial resolution, easy delivery, rapid reversibility, fewer off-target side effects, and the ability to dissect complex signaling networks. Here we review recent achievements in using light to control intracellular signaling pathways and discuss future prospects for the field, including integration of new genetic approaches into optogenetics.

Challenges in accessing the dynamic information of intracellular signal transduction

Cells are constantly sensing and responding to extracellular stimuli in their environment. A central question in cell biology is how intracellular signaling pathways respond to the external environment to make appropriate decisions and how decision-making processes go awry in disease conditions. Genomics and proteomics have been continuously expanding our knowledge base of genes and proteins that are responsible for specific cellular functions. However, much less is known about the dynamic nature of signal mechanisms, primarily due to a lack of appropriate tools to access this dynamic information.

From an engineering viewpoint, intracellular signaling pathways serve as circuits for processing extracellular inputs, computing net results, and executing outputs. For instance, multiple signaling pathways are activated by growth factors (inputs) to regulate proliferation, differentiation, migration, and apoptosis (outputs). Intriguingly, distinct cellular outputs that are elicited by different growth factors often utilize the same set of intracellular signaling pathways [1]. It has been suggested that the output specificity is achieved by regulating intracellular signaling transduction in space and time. However, a better understanding of the spatiotemporal aspect is hindered by the technical challenges inherent in controlling specific signal cascades in space and time.

Conventional methods for studying signal transduction primarily involve pharmacological and genetic approaches. These approaches characterize cellular outputs in response to changes in certain signaling components elicited by chemical (agonist or antagonist) or genetic (gain- or loss-of-function mutations) perturbations. Such approaches proved to be crucial for identifying components involved in signaling pathways. However, these approaches lack the spatial and temporal control to decode the dynamic information in intracellular signal transduction. Chemical genetic approaches have been developed to improve the flexibility of signaling control by using chemical inducers to trigger the activation of engineered proteins [2,3]. Unfortunately, the diffusive nature of chemicals still hampers their capacity for precise spatiotemporal control.

Emerging optogenetic approaches (see Glossary) have led to novel ways of studying signal transduction in live biological systems. Initial successes in optogenetics used light to regulate neuroelectric activities and have transformed experimental neurobiology [4–8]. The field of optochemical control of cell signaling, which primarily uses photo-uncaging of small molecules [9–11] or unnatural amino acids [12–14] to trigger the activation states of signaling molecules, has also seen success. However, we focus on optical control of intracellular signaling pathways based on genetically encoded photoactivatable proteins.

Glossary

Association/dissociation time: the average time it takes to induce association or dissociation of photoactivatable proteins.
Association/dissociation wavelength: the wavelength of light used to stimulate the association or dissociation of photoactivatable proteins. Some photoactivatable proteins (such as CRY2 and LOV) do not have a light-driven dissociation mechanism. Instead, the complex dissociates spontaneously in darkness.
Cofactor: photosensitive small molecules bound to photoactivatable proteins. Cofactors are required for the photoactivation of photoactivatable proteins. Common cofactors include flavin (blue-light sensitive) and bilin (red-light sensitive) and their derivatives. Some photoactivatable proteins (such as UVR8 and Dronpa) do not have cofactors and use intrinsic amino acids such as tryptophan residues to mediate their conformational changes.
Optogenetics: combines the power of light and genetics and uses light-mediated protein–protein interactions to control the open/closed state of channels or the activation/inactivation states of signaling components within live cells.
Photoactivatable proteins: also referred to as photoreceptors. These proteins undergo light-induced conformational change to initiate signal transduction.
Photoexcitation: the process of converting photon energy to conformational changes of photoreceptors.

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In this type of optogenetic control, activities of intracellular signaling components are coupled to light-induced conformational changes of photoactivatable proteins [15–19]. We summarize current achievements in optogenetic control of signaling pathways, highlight advantages of precise spatiotemporal control, and explore future prospects.

Optogenetic control of cell signaling

Photoactivatable proteins

Photoactivatable proteins are core components of optogenetic control of intracellular signal transduction. Pioneering work by several research groups has led to the discovery of several photoactivatable proteins, such as light, oxygen, and voltage (LOV) domains [20–22], phytochrome B (PhyB) [23,24], cryptochrome 2 (CRY2) [25], UV-resistance locus 8 (UVR8) [26,27], and Dronpa [28] (Table 1 and Box 1). Some photoactivatable proteins, such as split GFPs [29,30] have yet to be used in controlling live-cell signal transduction, but there has been recent success in using light-controlled protein–protein interactions to regulate intracellular signaling pathways in live cells (Table 2). The mechanisms of these photoactivatable systems are well known [19]. By absorbing energy from the photons in excitation light, photoactivatable proteins undergo conformational changes, rearrange inter- or intraprotein contacts, and modulate inter- or intraprotein interactions (Figure 1). In general, optogenetic signaling control can be achieved by two general schemes: light-induced protein translocation and light-induced protein uncaging (Figure 2). In the protein-translocation scheme, interprotein interactions change the cellular location or the oligomerization state of signaling proteins, which can lead to downstream cellular responses (Figure 2 A–D). In the protein-uncaging scheme, signaling proteins are inactive until intraprotein interactions remove the steric block (Figure 2 E and F).

Mitogen-activated protein kinase (MAPK) signaling pathway

The MAPK signaling pathway plays important roles in controlling cell proliferation, differentiation, survival, and apoptosis. Light-controlled activation of the MAPK signaling pathway was first demonstrated in yeast by membrane recruitment of the scaffold protein Ste5, which was known to activate the MAPK pathway when tethered to the plasma membrane [31]. Ste5 was fused to a PDZ domain, which bound to a membrane-anchored LOV-epitope fusion protein on blue-light stimulation and subsequently activated the MAPK pathway. In mammalian cells, a light-induced MAPK (Ras/Raf/MEK/ERK module) activation system was built based on the PhyB–phytochrome-interacting factor (PIF) 6 system [32]. PhyB was anchored to the plasma membrane and PIF6 was fused to the catalytic segment of the protein SOS (SOS_{cat}). Red light induced PhyB–PIF6 binding and membrane recruitment of SOS, which subsequently activated the Ras/Raf/MEK/ERK signaling pathway. Light-controlled activation of the Raf/MEK/ERK pathway in mammalian cells has also been achieved by the cryptochrome-interacting basic helix–loop–helix (CIB1)–CRY2 system [33]. CIB1 was anchored to the plasma membrane and CRY2 was fused to Raf1. Blue-light stimulation recruited Raf1 to the plasma membrane, where Raf1 was activated to activate its downstream kinases. This approach used Raf1 as the controlling component to avoid potential crosstalk with other signaling pathways, which may be induced by upstream factors such as SOS. Light-induced activation of the Raf/MEK/ERK pathway stimulated significant neurite outgrowth in PC12 cells in the absence of nerve growth factors. Interestingly, neurite outgrowth did not require constant ERK activation. Intermittent on/off light control revealed a 45-min threshold for the light-off interval, which still supported maximum neurite outgrowth [33].

In addition to light-induced binding between CRY2 and CIB1, CRY2 has been shown to oligomerize on blue-light illumination [34]. Such a property allows light-induced aggregation of CRY2–Raf1 in the cytoplasm [35], which was able to activate Raf1 and the downstream Raf/MEK/ERK signaling pathway. It is worth nothing that heterodimerization between Raf1–CIB1 and Raf1–CRY2 in the cytoplasm did not induce ERK activation, probably due to steric effects that block Raf–Raf interaction.

Phosphatidylinositol (PtdIns) 3-kinase (PI3K) signaling pathway

PI3Ks phosphorylate the 3-hydroxyl group of PtdIns to produce signaling lipids, such as PtdIns 3,4,5-trisphosphate

Table 1. Characterization of individual light-sensitive protein pairs in optogenetic toolboxes

Photoactivatable protein	Size (amino acids)	Cofactor	Association/dissociation wavelength (nm)	Association/dissociation time	Refs
PhyB(FL)–PIF3	1211/524	PCB	650/750	s/s	[64,65]
PhyB(NT)–PIF3	621/524	PCB	650/750	s/s	[24]
PhyB–PIF6	908/100	PCB	650/750	ms/ms	[23,66]
CRY2–CIB1	612/335	FAD	450/dark	s/6 min	[67–69]
CRY2–CIB1	498/170	FAD	450/dark	s/6 min	[25]
CRY2–CRY2	498/498	FAD	450/dark	s/6 min	[34]
CRY2olig	498	FAD	450/dark	s/23 min	[47]
EL222 (LOV fast cycler)	150	FMN	450/dark	s/s	[70,71]
FKF1–G1 (LOV fast cycler)	619/1173	FMN	450/dark	min/h	[21]
LOVpep–ePDZ	153/194	FMN	450/dark	s/s	[22,31]
VVD–VVD (LOV slow cycler)	150/150	FAD	450/dark	s/s to days	[72,73]
Dronpa–Dronpa	257/257	None	400/500	s/s	[16,28]
UVR8–COP1C340	440/340	None	Dark/290–310	1–4 h/s	[26,74]
UVR8–UVR8	440/440	None	Dark/280–315	2–24 h/s	[27,75–77]