Published in final edited form as:

Biochem Soc Trans. 2013 October ; 41(5): 1183–1188. doi:10.1042/BST20130150.

Optobiology: Optical control of biological processes via protein engineering

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Abstract

Enabling optical control over biological processes is a defining goal of the new field of optogenetics. Control of membrane voltage by natural rhodopsin-family ion channels has found widespread acceptance in neuroscience, due to the fact that these natural proteins control membrane voltage without further engineering. In contrast, optical control of intracellular biological processes has been a fragmented effort, with various laboratories engineering light-responsive properties into proteins in different manners. Here, we review the various systems that have been developed for controlling protein functions with light based on vertebrate rhodopsins, plant photoregulatory proteins, and, most recently, the photoswitchable fluorescent protein Dronpa. By allowing biology to be controlled with spatiotemporal specificity and tunable dynamics, light-controllable proteins will find applications in the understanding of cellular and organismal biology and in synthetic biology.

Keywords

optogenetics; LOV domain; cryptochrome; phytochrome; UVR8; fluorescent protein

Introduction

Light-controllable proteins have been of immense interest due to their beneficial properties of studying cellular processes and biological events with precise spatial and temporal specificity [1, 2]. Previously, small molecule agonists or antagonists of signaling proteins have been used, but low spatial and temporal resolution, unexpected endogenous reactions, and off-target effects remain as inherent disadvantages when studying real-time events in living cells or organisms [3]. Thus, developing tools that evade these shortcomings could greatly advance our understanding of biology.

Recently, many genetically encoded systems have been engineered to modulate protein activities in response to light. In general, in these systems, a light-induced conformational change in a chromophore-containing protein domain changes its interactions with other protein domains or influences the functional output of a fused protein domain. We present a short summary of recent developments of engineered light controllable proteins with a goal

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of pointing out the unique aspects each system and providing an overview of current research in the field.

Vertebrate rhodopsins: light-induced G protein activation

Rhodopsins are seven-pass transmembrane receptors that bind retinaldehyde as a cofactor. In bacteria and protists, many function as light-dependent ion channels or pumps. These have been enormously useful in mediating optical control of firing of vertebrate neurons with no or minimal modification, and the advantageous combination of controlling neuronal activity by light in genetically defined subpopulations of neurons gave rise to the term optogenetics [4]. In vertebrates, rhodopsins are G-protein coupled receptors that initiate light-induced intracellular signaling in photoreceptor cells by activating the specialized heterotrimeric G protein transducin expressed only in photoreceptor cells.

Airan et al. modified vertebrate rhodopsins to activate the ubiquitous heterotrimeric G proteins G_q and G_s to allow control of G protein-mediated processes in non-photoreceptor cells [5]. G_q and G_s activate adenylate cyclase and phospholipase C, respectively, inducing the second messengers cyclic adenosine monophosphate (cAMP) and inositol triphosphate (IP_3). Airan et al. replaced the intracellular loops of green-absorbing rhodopsin with those from the G_q -coupled α_{1a} -adrenergic receptor (α_1 -AR) or the G_s -coupled β_2 -adrenergic receptor (β_2 -AR) (Figure 1). Upon green light stimulation, these chimeras, Opto- α_1 -AR and Opto- β_2 -AR, significantly upregulated levels of cAMP and IP_3 respectively, as expected. Thus engineered rhodopsins are able to mediate optical activation of G protein signaling in non-neuronal cells.

LOV domains: light induced unfolding and heterodimerization

Plants and bacteria contain a variety of photoreceptors that activate intracellular signaling in response to light [6–8]. Among these, the most extensively studied are light-oxygen-voltage (LOV) domains, cryptochromes, and phytochromes, which utilize the small chemical compounds flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and phytychromobilin as cofactors, respectively.

Upon blue light (peak 450 nm) absorption, the FMN cofactor in the LOV domain forms a covalent bond with a cysteine residue [9]. This process reverts in the dark over the course of seconds to minutes via hydrolysis of the FMN-cysteine bond. In plant flavin-binding/kelch-repeat/F-box 1 (FKF1), the FMN-cysteine bond formation leads to interaction of the FKF1 N-terminus with GIGANTEA (GI) [10]. Yazawa et al. took advantage of this feature to relocalize protein domains of interest to specific locations within mammalian cells in response to light [10]. They used light to recruit a FKF1 fusion with Rac1 to a GI fusion with a membrane anchor, enabling Rac1 activation by endogenous membranebound activators (Figure 2a). They similarly recruited a FKF1 fusion to a transcriptional activation domain to a GI fusion to a DNA-binding domain to activate transcription (Figure 2a). This transcriptional regulation scheme using FKF1 and GI has been recently reproduced and extended by another group [11].

In the second LOV domain (LOV2) of plant phototropin, the FMN-cysteine bond formation leads to partial unfolding of the C-terminal α -helix (J α) from the rest of the LOV2 domain. This was initially used to confer light responsiveness in an allosteric manner, specifically to the enzyme dihydrofolate reductase by linkage to a surface site and to a transcription factor by mutually exclusive folding of J α and the N-terminal α -helix of the transcription factor [12, 13]. Subsequently, phototropin LOV2 has been fused to the N-terminus of target domains or peptides to create steric blocks that can be relieved by light-induced J α unfolding (Figure 2a). In the first example of this approach, Wu et. al. tested different linkages of the LOV2 domain to the N-terminus of the guanine nucleotide triphosphatase (GTPase) Rac1 and selected for those that showed Rac1 activation by blue light [14]. The resulting construct, photoactivable Rac1 (PA-Rac1), enabled optical control of membrane ruffling in mammalian cells [14] and optical guidance of cell migration in zebrafish embryos [15]. Crystallography revealed unexpected surface contacts between the LOV2 and Rac1 domains and enabled the rational development of a photoactivatable Cdc42 through targeted mutation of residues on LOV2 [14]. Recently, a lightresponsive caspase-7 has been engineered by fusion to the C-terminus of the LOV2 domain [16]. Strickland created LOV2 fusions capable of light-mediated heterodimerization by fusing the LOV2 domain to peptides that bind to postsynaptic density protein-95/discs large/zona occludens-1 (PDZ) domains. Light induced peptide exposure and PDZ binding in yeast and mammalian cells, as demonstrated by recruitment of PDZ fusion proteins to LOV2-peptide fusions located at the plasma membrane [17].

Cryptochrome domains: light-induced heterodimerization and aggregation

Cryptochromes are FAD-binding proteins that participate in growth processes in plants and regulate circadian clocks in animals [18]. In plant cryptochromes, blue light induces reduction of FAD and a conformational change in the protein, with the process reversing spontaneously in seconds in the dark. In *Arabidopsis* Cryptochrome 2 (CRY2), the light-induced conformational change allows binding of calcium and integrin-binding protein 1 CIB1 [19]. Similarly to Yazawa et al., Kennedy et al. used the light-inducible interaction of CRY2 and CIB1 to relocate a protein to the cell membrane and a transcriptional activation domain to a DNA-binding domain [20] (Figure 2b). They also used CRY2 and CIB1 to induce reconstitution of the enzyme Cre recombinase from two fragments in response to light, enabling optical control of recombination at loxP sites (Figure 2b). CRY2-CIB1 heterodimerization is robust and rapid, as demonstrated by optical control of phosphoinositide distributions via light-induced recruitment of a inositol phosphatase to the membrane [21] and by its applicable to control gene expression in zebrafish [22].

Bugaj et al. discovered that CRY2 undergoes aggregation in response to light [23] (Figure 2b). They found that light-induced aggregation of CRY2 fusions to the Wnt receptor low density lipoprotein receptor 6 (LRP6) or to the GTPase RhoA was sufficient to activate downstream effectors. This method may enable optical control of certain cellular pathways with the expression of a single fusion protein whose construction may be fairly simple.

Phytochrome domains: light-induced heterodimerization

Phytochromes are light-responsive signaling proteins in plants and cyanobacteria that contain a covalently linked tetrapyrrole as the chromophore. Plant phytochromes utilize phytochromobilin while cyanobacteria use the structurally similar phycocyanobilin [6]. Red light induces isomerization of the tetrapyrrole cofactor, inducing a conformational change in the protein from a red-absorbing (Pr) to a far-red absorbing (Pfr) state. The Pfr state then relaxes slowly back to the Pr state, or the conversion can be induced quickly by far-red light. In plant phytochromes, the Pfr state selectively binds to phytochrome-interacting factors (PIFs).

Shimizu-Sato et al. used the light-dependent interaction of PhyA with PIF3 to control transcription in yeast cells fed with phytochromobilin [24]. Light-induced recruitment of a transcriptional activation domain fused with PIF3 to a DNA-binding domain fused with PhyA induced gene transcription (Figure 2c). Levskaya et al. used the PhyB-PIF6 interaction to control recruitment of the Rac activator Tiam to the membrane in mammalian cells fed with phytochromobilin [25] (Figure 2c). Local illumination resulted in local lamellipodia formation, as expected for Rac activation.

The absorption of red light by phytochromes contrasts with the blue light absorption by flavin-binding proteins and would be desirable for the lower phototoxicity and enhanced tissue penetration of red light. However, this system has seen slower adoption by cell biologists than the LOV domain or cryptochrome-based systems. The only other report of Phy-PIF interactions being regulatable in animal cells comes from a recent study reproducing the approach of Shimizu-Sato et al. in mammalian cells [26]. One reason for the slow adoption of Phy-PIF could be sensitivity of Phy domains to fusion and a requirement for high Phy expression levels [27].

UVR8 domains: light-induced dissociation and heterodimerization

Recently, a plant protein that demonstrates light-regulated binding and does not utilize any cofactors has been characterized. ULTRAVIOLET RESPONSE 8 (UVR8) forms homodimers that dissociate upon UV illumination, after which the monomers are able to bind CONSTITUTIVELY MORPHOGENIC 1 (COP1) [28, 29]. The responsible chromophores in UVR8 are actually a pair of tryptophan residues that are engaged in cation- π interactions with arginine residues at the dimeric interface. It is believed that photon absorption induces breakage of the cation- π interactions, leading to subtle conformational changes that in turn break hydrogen bonding interactions at the dimeric interface [28, 29]. The conformational change is remarkably long-lived, lasting many hours.

Two groups used UVR8 and COP1 to control protein heterodimerization in mammalian cells with UV light. Müller et al. fused UVR8 to a DNA-binding domain and COP1 to a transcriptional activation domain [30, 31], while Crefcoeur et al. reversed the positions of UVR8 and COP1 [31] (Figure 3a). Going beyond transcriptional activation, Crefcoeur et al. also tagged COP1 with a nuclear localization signal to enable UV-induced localization of UVR8 fusions, and created a UVR8 tandem dimer fused to histone to enable UV-induced chromatin localization of COP1 fusions [31] (Figure 3a). However, the UVR8-COP1 system

has the disadvantage that prolonged exposure to UV light is mutagenic and phototoxic, although acute phototoxicity can be minimized by applying UV light intermittently [30, 31].

Fluorescent protein domains: light-induced dissociation

Photoswitchable fluorescent proteins are fluorescent proteins that alter their optical properties in a reversible manner upon illumination [32]. Like other fluorescent proteins, the chromophore in photoswitchable fluorescent proteins is created autocatalytically from three adjacent amino acids. In Dronpa, an extensively studied photoswitchable green fluorescent protein, fluorescence is switched off upon illumination with cyan light and switched on again upon illumination with violet light. The off-switching is associated with a cis-to-trans isomerization of the chromophore and an unfolding of the portion of the beta-barrel wall of the protein adjacent to the chromophore [33, 34]. Thus, Dronpa has the essential characteristic of natural photoregulatory proteins, light-induced conformational change.

In dimeric and tetrameric coral fluorescent proteins such as 22G, the natural precursor to Dronpa, the region of the beta barrel adjacent to the chromophore forms a dimerization interface [34]. We hypothesized that mutants of Dronpa that formed weak tetramers at baseline would undergo dissociation upon blue light illumination and reassociation upon violet light illumination. We indeed found this to be the case with purified protein in vitro [35]. When we expressed in mammalian cells a fusion of the weakly tetramerizing Dronpa mutant with a plasma membrane anchor, and another fusion of Dronpa to a fluorescent protein, we found that the fluorescent protein was membrane-localized at baseline. Cyan light induced immediate release from the membrane and violet light induced re-recruitment to the membrane, demonstrating that Dronpa interactions could be regulated by light in cells (Figure 3c). Interestingly, as the photoswitching behavior of Dronpa was evolved in the laboratory rather than in nature, the light-regulated tetramerization of Dronpa may be the first example of an artificial light-regulated protein-protein interaction.

We used this property of light-induced dissociation as the basis for creating single-chain light-regulatable proteins. Specifically, we fused one copy of weakly tetramerizing Dronpa via flexible linkers to each termini of domains whose activity we desired to regulate. We hypothesized that, if the N- and C-termini of a domain flanked a functional interface, then assembly of the Dronpa tetramer in the dark would occur in front of the interface and inhibit protein function via steric blockade. The light-induced disassembly of the Dronpa tetramer could then remove the steric block and allow protein activity (Figure 3c). We tested this idea on the catalytic domain of the Cdc42 activator intersectin and on the hepatitis C virus (HCV) protease domain, as these domains have N- and C-termini flanking their active sites. Using a cellular morphology assay for intersectin and a substrate membrane release assay for HCV protease, we found that both proteins were inactivated by the fusion of weakly tetramerizing Dronpa to both termini, and reactivated by illumination with cyan light [35]. In each case, only a few constructs with linkers of different lengths needed to be constructed and tested, and several variants in each case showed light-dependent activity. The creation of single-chain light-controllable proteins by fusion at each end to Dronpa thus appears to be a generalizable strategy.

Besides its relatively easy generalizability, two additional advantages of this approach may arise from the use of a fluorescent protein as the light-responsive domain. First, the chromophore absorbs visible rather than UV light, yet is completely autocatalytically generated without the need for any endogenous metabolites such as flavins or exogenous chemicals such as phytochromobilin or phycocyanobilin. The lack of involvement of flavins not only makes the maturation of the light-controllable protein independent of cellular metabolism but also assures that wavelengths used for optical control do not excite flavins in endogenous flavoproteins, which is the major cause of phototoxicity in mammalian cells [36, 37]. The second potential advantage is that it may be possible to evolve photoswitchable fluorescent proteins that absorb yellow light to also undergo light-induced dissociation, providing the possibility for multiple channels for optical control and further reducing phototoxicity versus cyan light.

Conclusion

The goal of this review was to provide an overview of the various approaches for implementing optical control over intracellular signals in animal cells, with an emphasis on recent research. Efforts in optical control have been focused on adapting natural light-responsive proteins from plants and microorganisms, but recent discoveries suggest that light-dependent interactions can be evolved in fluorescent proteins artificially. For a more comprehensive description of natural light-responsive systems, recent detailed reviews are available [38, 39].

Light-controllable proteins offer the potential for novel insights into biology, due to the ability to control protein activities with high spatiotemporal resolution and tunable dynamics. For optical control of proteins to become a widespread approach, however, it will be critical for to move beyond light-regulated transcription and light-regulated subcellular localization to achieve light-regulated control of a larger gamut of protein functions. The ability to easily create light-responsive versions of proteins of interest is important. Protein relocalization by CRY2-CIB1 or FKF1-GI and protein aggregation by CRY2 appear to be generally reliable approaches, when the pathway being investigated can be activated by relocalization or aggregation. Light-induced protein fragment complementation, as demonstrated with CRY2-CIB1, is in principle also possible for any light-dependent heterodimerization system, and may be another general approach to create light-activatable proteins, although with protein fragment complementation, activation is typically permanent. When regulation of protein activity needs to be transient and cannot be achieved through relocalization or aggregation, creating light-controllable single-chain proteins through fusion of the phototropin LOV2 domain or photoswitchable fluorescent protein domains may be necessary. How generalizable these two approaches are remains to be determined.

As optical approaches to biology are becoming more common, it may be appropriate at this time to introduce a new term. While the term optogenetics is used by some scientists to include a wide variety of efforts to confer light responsiveness on proteins [39–41], in most usages, optogenetics refers to its original meaning of functional dissection of neuronal circuits using expression of microbial rhodopsins in genetically defined populations of

neurons [4, 42, 43]. To avoid confusion, we propose using the term “optobiology” to refer to efforts to investigate and control biology using light-responsive proteins. The term optobiology has the further benefit of improved accuracy. It reflects the idea that the field is defined by the interaction of light with biological processes — similar to how the term optoacoustics refers to the interaction of light with sound and optoelectronics to the interaction of light with electrons — something that optogenetics, as mentioned by others, does not [44].

In summary, recent years have witnessed the creation of a wide variety of tools for manipulating protein activity with light. Similarly to how fluorescent proteins transformed the study of biology by making optical sensing of protein localization and activity routine, the tools described here are poised to enable another light-based revolution where optical control of protein localization and activity enables the investigation and control of biology at very fine time and length scales. Another analogy can be made to molecular biology. Just as technical breakthroughs in DNA engineering enabled molecular biology to become a routine method, bringing control of protein expression and structure to biological experimentation, likewise advances in protein engineering may enable optobiology to become a standard method, bringing control of protein activity in space and time to the understanding of biological processes.

Acknowledgments

MZL is supported by NIH grant 1R01NS076860-01, the Rita Allen Foundation, and the Burroughs Wellcome Foundation. MZL is an author on a patent application describing the construction of light-controllable proteins by fusion to fluorescent protein domains.

Abbreviations used

α_1-AR	α_1 -adrenergic receptor
β_2-AR	β_2 -adrenergic receptor
cAMP	cyclic adenosine monophosphate
CIB1	Calcium and integrin-binding protein 1
COP1	CONSTITUTIVELY MORPHOGENIC 1
CRY	Cryptochrome
DHFR	Dihydrofolate reductase
FAD	flavin adenine dinucleotide
FKF1	Flavin-binding, Kelch Repeat, F-Box 1
FLIP	fluorescent light-inducible proteins
FMN	flavin mononucleotide
GI	GIGANTEA
GTPase	guanine nucleotide triphosphatase

IP₃	inositol triphosphate
LOV	Light-oxygen-voltage
PA-Rac1	photoactivable Rac1
Phy	phytochrome
Pr	phytochrome in red-absorbing state
PIF	phytochrome-interacting protein
Pfr	phytochrome in far red-absorbing state
UVR8	ULTRAVIOLET RESPONSE 8

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Opto- α_1 -AR **Opto- β_2 -AR** **Figure 1.**

Optical control of G protein signaling by engineered vertebrate rhodopsins. Opto- α_1 -AR (chimera of green-absorbing rhodopsin and α_1 adrenergic receptor) activates adenylyl cyclase via G_q, while Opto- β_2 -AR (chimera of green-absorbing rhodopsin and β_2 adrenergic receptor) activates phospholipase C via G_s. The rhodopsin chromophore is the cofactor retinaldehyde. AC, adenylyl cyclase; PLC, phospholipase C.

Figure 2.

Systems for optical control of proteins based on plant photosensory proteins using chemical cofactors. (a) Light-induced heterodimerization by the LOV domain of FKF1 and light-induced conformational changes in the LOV domain of phototropin has been used to control protein localization, transcription, and the activity of a fused protein domain. The chromophore of LOV domains is FMN. (b) Light-induced heterodimerization or aggregation by cryptochromes has been used to control protein localization, transcription, protein fragment complementation, and signaling protein activity. The chromophore of cryptochromes is FAD. (c) Light-induced heterodimerization by phytochromes has been used to control protein localization and transcription. The chromophore of phytochromes is phytochromobilin. POI, protein of interest; DNABD, DNA-binding domain; TAD, transcriptional activation domain; NT frag, N-terminal protein fragment; CT frag, C-terminal protein fragment. The right-angle blue arrow indicates transcription, while the diagonal blue arrow indicates downstream protein activities.

Figure 3.

Systems for optical control of proteins that do not require chemical cofactors. (a) Light-induced heterodimerization by UVR8 has been used to control transcription and protein localization. (b) Bidirectional light-controlled switching between tetrameric and monomeric states in Dronpa mutants, or between dimeric and monomeric states, has been used to control protein localization and the activity of single-chain fusion proteins. NLS, nuclear localization sequence.