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Lights, cytoskeleton, action: Optogenetic control of cell dynamics

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Abstract

Cell biology is moving from observing molecules to controlling them in real time, a critical step towards a mechanistic understanding of how cells work. Initially developed from light-gated ion channels to control neuron activity, optogenetics now describes any genetically encoded protein system designed to accomplish specific light-mediated tasks. Recent photosensitive switches use many ingenious designs that bring spatial and temporal control within reach for almost any protein or pathway of interest. This next generation optogenetics includes light-controlled protein–protein interactions and shape-shifting photosensors, which in combination with live microscopy enable acute modulation and analysis of dynamic protein functions in living cells. We provide a brief overview of various types of optogenetic switches. We then discuss how diverse approaches have been used to control cytoskeleton dynamics with light through Rho GTPase signaling, microtubule and actin assembly, mitotic spindle positioning and intracellular transport and highlight advantages and limitations of different experimental strategies.

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Introduction

The 21st century has seen remarkable advances in light microscopy. Combined with improving labeling techniques, such as fluorescent proteins that just celebrated their 25th birthday, live-cell imaging has repeatedly demonstrated just how dynamic cells are. Cells continuously reorganize their shape and internal structure. Both are essential to build and operate complex multicellular organisms in which each cell type has unique functions reflected in their intracellular organization and dynamics. Forces driven by the pulling and pushing of the cell's cytoskeleton underlie cell movement, identity and many pathological processes [1,2]. Consequently, polarized and meaningful cell dynamics depend greatly on the spatial and temporal control of protein activities and interactions downstream of extracellular signaling inputs as well as intracellular control circuits. Ideally, a cell biologist would be able to specifically, locally and acutely interfere with intracellular protein activities to perturb steady-state biochemistry and interrogate cell biological consequences, and the development of technologies to achieve this goal continues to be a driving force of cell biology discovery.

Cytoskeleton filament systems assemble, disassemble and reorganize within minutes, and molecular motors transport cargo at rates of tens of micrometers per minute. Thus, compared to these time scales, experimental approaches that rely solely on removing gene expression or inhibition of protein production by RNA or CRISPR interference are quite slow and not well suited to investigate rapid intracellular dynamics at a mechanistic level. By the time one observes a phenotype, cell and cytoskeleton dynamics have reached a new steady state, which complicates the interpretation of such experiments. Cells also frequently respond to genetic alterations with compensatory mechanisms and genetic approaches do not allow subcellular spatial control.

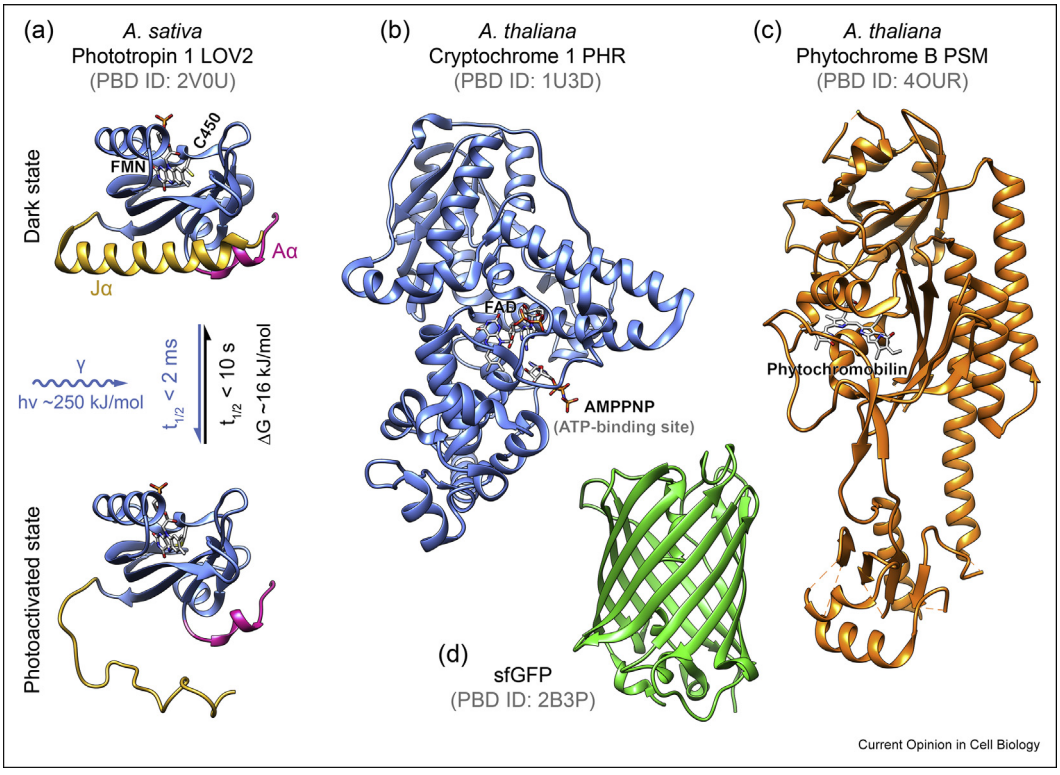
One experimental agent that, at least in non-plant cells, is orthogonal to most biological processes and that can be controlled with very high spatial and temporal accuracy is light. Although high-power lasers have been used to locally destroy and cut cellular structures, the first 'optogenetics' experiments involved expression of light-gated ion channels in neuronal cells to locally photostimulate nervous system circuits [3–5]. Nowadays, optogenetics more broadly describes any genetically encoded protein system designed to accomplish a specific light-mediated task. Many aspects of plant development and metabolism depend on the ability to sense light through photosensitive domains of

phototropins, cryptochromes and phytochromes that contain different kinds of chromophores (Figure 1) [6]. Photochemical reactions driven by photon absorption result in conformational changes that can be harnessed to accomplish optogenetic work (Figure 1a). Optogenetic experimental strategies can be roughly categorized into techniques that alter the localization of a protein of interest resulting in activation or inactivation in a specific subcellular region or organelle, approaches that uncage a constitutively active protein, and maybe most relevant to understanding physiological function, techniques that locally inactivate a specific activity of a protein or protein complex. The light-oxygen-voltage (LOV2) domain of oat phototropin 1 may be the most versatile and has been used to build protein modules that either dimerize or dissociate on blue light stimulation, or directly change protein conformation (Figure 2). Instead of once again reviewing the basic designs of optogenetic switches [7,8], we highlight examples of how optogenetic building blocks have been used to directly target cytoskeleton dynamics and function in living cells with high spatial and temporal accuracy (Table 1).

Light-controlled Rho GTPase signaling

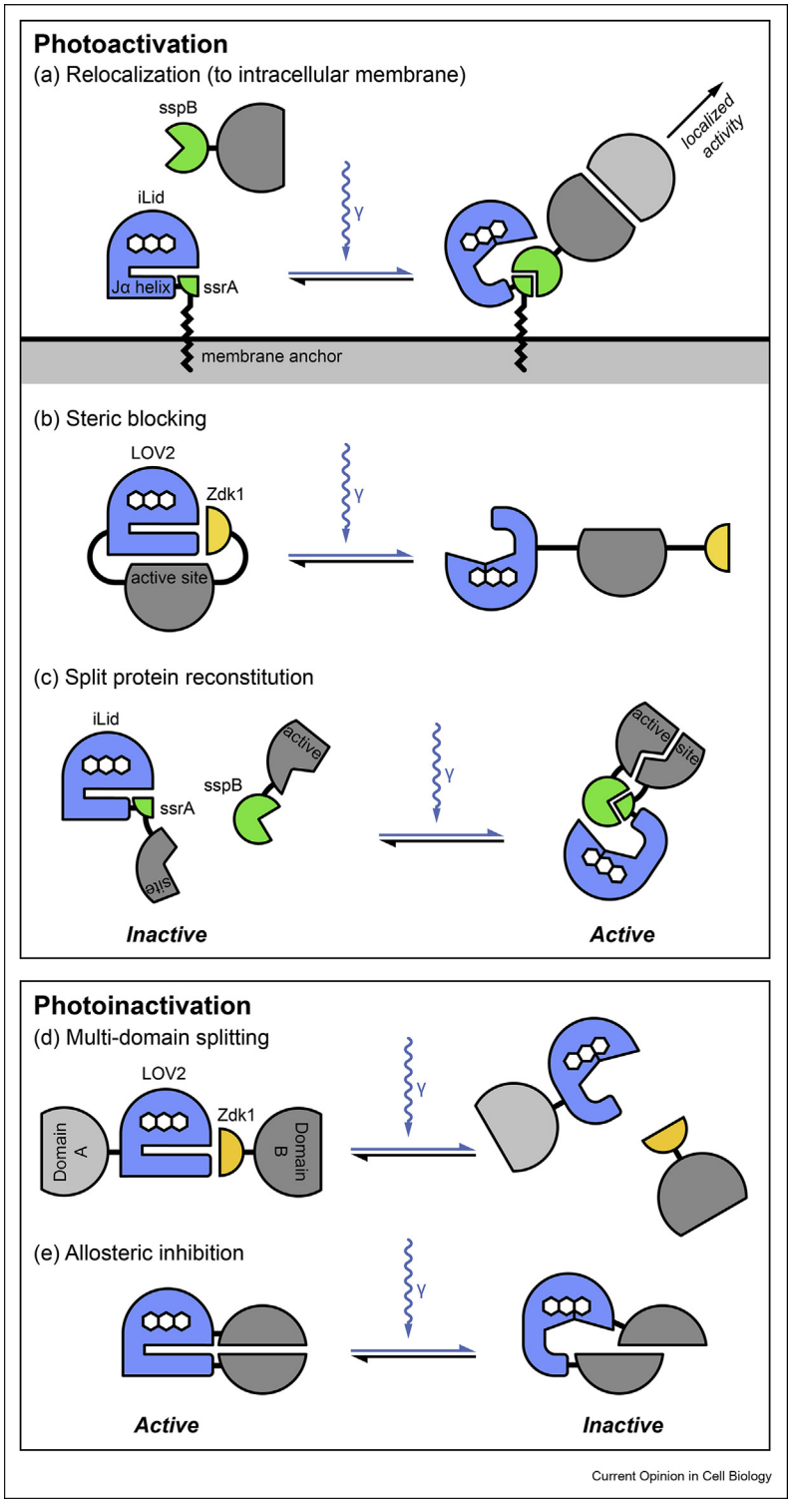
Many local cytoskeleton activities such as f-actin and microtubule dynamics during cell migration are controlled by low molecular weight Rho GTPases. It is thus no surprise that initial optogenetic approaches directed toward cytoskeleton dynamics in living cells targeted Rho GTPase activities either directly or indirectly. In one of the first demonstrations of direct optogenetic control of Rho GTPase signaling in cells, Wu et al. [9] linked a LOV2 domain to the N-terminus of a dominant active GTPase-deficient mutant of Rac1(Q61L) with the hypothesis that in the dark, LOV2

Figure 1



Comparison of plant-derived photosensitive domains. **(a)** The LOV2 domain is the smallest of the three major plant photosensors, and photochemistry and resulting structural changes have been characterized extensively [56]. Absorption of a blue light photon by the FMN co-factor drives formation of a metastable covalent adduct with a conserved cysteine, which results in the undocking of the helices flanking the LOV2 domain core and unfolding of the C-terminal Jα helix [57–59]. LOV2 photoactivation is very fast [60,61], although the light response of LOV2-based optogenetic switches will always be substantially slower. The fungal blue light sensor VVD has a very similar overall fold [62]. **(b)** The PHR of cryptochromes bind an FAD chromophore as well as ATP. Blue light induces cryptochrome dimerization although the underlying photochemistry is incompletely understood [63]. Shown is the crystal structure of cryptochrome Cry1 as a Cry2 structure is not available [64]. **(c)** The phytochrome B PSM binds a plant-specific phytochromobilin (or phycocyanobilin) chromophore that in animal cells must be added or supplied by co-expression of the biosynthetic enzyme system [65,66], although bacterial phytochromes use a BV chromophore that may be less limiting in animal cells [67]. Although not fully understood, rotation of the phytochromobilin pyrrole rings likely underlies PhyB photoconversion [68]. **(d)** sfGFP is shown for size comparison [69]. BV, biliverdin; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; PHR, photolyase homology region; PSM, photosensory module.

Figure 2



Overview of LOV2 domain–based optogenetic strategies to modulate protein activities. **(a)** Light-induced dimerization can recruit activating factors, for example, Rho GEFs, to specific intracellular sites. As indicated in the main text, this also works with other light-induced dimerizers such as Cry2/CIBN or PhyB/PIF. The same approach can also be used to sequester and thus inactivate proteins away from their normal site of action. **(b)** The light-sensitive LOV2/Zdk1 protein interaction module can sterically block access to an active or binding site in a target protein in the dark. **(c)** In principle, the activity of split proteins can be reconstituted by bringing half-domains back together by light-mediated dimerization. However, because of the difficulty in designing such proteins, this approach has not yet been used much in optogenetics. **(d)** The light-sensitive LOV2/Zdk1 module can be used to dissociate and thus disrupt the function of multidomain proteins. **(e)** Conformational changes in photosensors can propagate allosteric inhibition into split target proteins. It should be noted that swapping a light-induced dimerizer, that is, iLID/nano, for a light-sensitive interaction module, that is, LOV2/Zdk1, would be expected to relatively easily reverse the directionality of many of these designs and convert photoactivation into photoinhibition or vice versa.

Table 1

Overview of optogenetic switches to control cytoskeleton dynamics.

		Photosensor (Chromophore)	Peak excitation wavelength	Mechanism	Description
Rho-GTPase signaling	PA-Rac1	LOV2 (FMN)	On: 450 nm Off: dark	steric inhibition	Release of LO2V-mediated inhibition of Rac1(Q61L) GEF-binding [9]
	Photoactivated Cdc42 and Rac1 GEFs	PhyB/PIF (PCB)	On: 664 nm Off: 725 nm	recruitment	Activation of Rho-GTPase signaling through GEF membrane recruitment [14]
	Photoactivated Cdc42 GEF	BphP1/PpsR2 (BV)	On: 740 nm Off: 650 nm	recruitment	Activation of Rho-GTPase signaling through GEF membrane recruitment [67]
	Photoactivated Cdc42 and Rac1 GEFs	iLID/nano (FMN)	On: 450 nm Off: dark	recruitment	Activation of Rho-GTPase signaling through GEF membrane recruitment [18]
	PR_GEF	TULIPs (FMN)	On: 450 nm Off: dark	recruitment	RhoA activation through GEF membrane recruitment [20–22]
	optoGEF-RhoA	Cry2/CIBN (FAD)	On: 450 nm (dark) Off: dark (450 nm)	recruitment (sequestration)	RhoA activation (inactivation) through GEF membrane (mitochondria) recruitment [23]
	opto-PI3K	PhyB/PIF (PCB)	On: 664 nm Off: 725 nm	recruitment	Activation of Rho-GTPase signaling through PI3K membrane recruitment [15]
	Photoactivated PI3K	Magnets (FAD)	On: 450 nm Off: dark	recruitment	Membrane recruitment of PI3K activity [19]
	LOVTRAP GEF (and Rho GTPases)	LOV2/Zdks (FMN)	On: 450 nm Off: dark	sequestration	Rac1 GEF (or dominant active RhoA, Rac1) release from outer mitochondria membrane [41]
	PI-Rho GTPases (GEFs)	LOV2 (FMN)	On: dark Off: 450 nm	allosteric inhibition	LOV2 insertion in surface loops propagating allosteric conformational inactivation [47]
Cytoskeleton dynamics	SxIP-iLID	iLID/ nano (FMN)	On: 450 nm Off: dark	association	Recruitment to growing microtubule plus ends [32]
	π-EB1	LOV2/Zdk1 (FMN)	On: dark Off: 450 nm	domain splitting	Dissociation of the EB1 microtubule-binding and +TIP adaptor domains [45]
	Z-lock cofilin (αTAT)	LOV2/Zdks (FMN)	On: 450 nm Off: dark	steric inhibition	Uncaging of f-actin severing (microtubule acetylation) through LOV2/Zdk dissociation [42]
	NuMA localization	iLID/nano (FMN)TULIPs (FMN)	On: 450 nm Off: dark	recruitment	Localization of the spindle positioning factor NUMA to specific cortical sites [33,34]
	Optogenetic 'knocksideways'	iLID/nano (FMN)	On: dark Off: 450 nm	sequestration	TACC3 or KIF18A removal from mitotic spindles by outer mitochondria membrane recruitment [35]
	Clustering	Cry2olig (FAD)	On: dark (450 nm) Off: 450 nm (dark)	oligomerization	Inactivation (activation) of endocytosis (actin assembly) through crosslinking / clustering [39]
	Integrin (receptor) clustering	Cry2 CLICR (FAD)	On: 450 nm Off: dark	oligomerization	Receptor activation through cell membrane associated clustering [70]
	OptoIntegrin	PhyB/PIF (PCB)	On: 664 nm Off: 725 nm	association	Extracellular modulation of cell-matrix interaction [71]
Cargo transport	Motor recruitment	TULIPs (FMN)	On: 450 nm Off: dark	association	Recruitment of kinesin, dynein, and myosin motor domains to different organelles [26,27,29]
	Motor recruitment	PhyB/PIF (PCB)	On: 664 nm Off: 725 nm	association	Recruitment of kinesin and dynein motor domains to different organelles [30]
	Motor recruitment	CRY2/CIBN (FAD)	On: 450 nm Off: dark	association	Recruitment of kinesin and dynein motor domains to different organelles [72]
	Motor processivity	VVD (FAD)	On: 450 nm Off: dark	dimerization	Kinesin motor homodimerization [29]
	Motor gear shifting	LOV2 (FMN)	On: 450 nm Off: dark	allosteric	Control of myosin and kinesin speed/directionality through lever arm conformation changes [50]

BV, biliverdin; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; PCB, phycocyanobilin.

sterically blocks access of downstream effectors to the Rac1 binding site. Indeed, local blue light exposure of photoactivatable PA-Rac1 stimulates lamellipodia f-actin polymerization and can direct cell migration *in vitro* [9,10] and *in vivo* [11,12]. However, further analysis of the PA-Rac1 photoactivation mechanism showed that it also relies on a serendipitously introduced Ca^{2+} binding site between the LOV2 and Rac1 moieties [13], highlighting the difficulty of rational photoswitch design and explaining why this approach did not work for other Rho GTPases.

A possibly more adaptable method to locally control Rho GTPase activity is through optogenetic localization of their guanosine exchange factors (GEFs). Levskaya et al. [14] achieved this first through light-induced heterodimerization of the phytochrome PhyB photosensory domain with its downstream transcription factor effector PIF. Localized membrane recruitment of the PIF-tagged Rac1 GEF Tiam results in local lamellipodia protrusion similar to PA-Rac1 photoactivation. Moving up one step in the signaling cascade that controls Rac1 activity, PhyB/PIF-mediated phosphoinositide 3-kinase (PI3K) recruitment by localized red light exposure locally produces phosphatidylinositol (3,4,5)-trisphosphate (PIP3) [15]. In contrast to PA-Rac1, this only generates a transient increase in Rac1 activity, which is still sufficient to direct cell migration.

Because the PhyB/PIF system can be difficult to implement (Figure 1; Box 1), blue light–activated dimerization modules [16–19] have more recently been used to localize Rho GEFs (Figure 2a). For example, using a LOV2-derived dimerization system in which a PDZ domain binding peptide is inaccessible at the C-terminus of the dark-state LOV2 Jα helix, local RhoA activation through light-induced recruitment of a RhoA GEF DH domain induces f-actin contractility and cleavage furrow formation anywhere on the anaphase cell cortex [20]. The Gardel laboratory then localized RhoA GEFs in interphase cells to generate local actomyosin contractility either on stress fibers [21] or epithelial cell–cell junctions [22]. Combined with quantitative physical models of the underlying cytoskeleton, these studies highlight the strength of optogenetics to dissect cell dynamics, for example, by showing how alternating RhoA-mediated contractility and membrane internalization shortens epithelial cell–cell junctions. Similarly, Valon et al. [23] use the cryptochrome Cry2/CIB1N dimerization module to locally recruit RhoA ArhGEF11 to the membrane and induce actomyosin contractility, which induces cell migration away from the site of optogenetic stimulation by generating rear contractility and retraction [24]. Extending optogenetic RhoA activation to morphogenetic processes at the multicellular level, Cry2/CIB1N-mediated photoactivated membrane-binding of RhoGEF2 can generate surprisingly geometrically

Box 1. Limitations of optogenetic experiments

The 'dark side' of photoactivation. Diffraction sets a physical lower boundary to the spatial precision with which an area of interest can be exposed to light in a microscope, but photochemistry kinetics further limit the accuracy of intracellular photoactivation. Blue light-sensitive domains revert to the dark state through thermal decay, and the rate at which this occurs is critical for how sharp of a photoactivation boundary can be generated. Slow dark state reversal allows photoactivated molecules to diffuse further, thus broadening the boundary between activated and not activated regions. The half-life of the LOV2 photoactivated state is around 5–10 s, sufficient to generate intracellular gradients of freely diffusible molecules (Figure 3) [45]. Photoactivated Cry2 is substantially more stable, with a half-life of minutes, which can still be fast enough to generate gradients if diffusion is confined, for example, at the cell membrane or an organelle. Interestingly, a recent comparison with iLID/nano and Cry2/CIBN finds that Magnets, a dimerization system based on the fungal blue light photoreceptor VVD [19], produced the best spatial confinement, and the authors speculate that this is because both parts of the Magnet dimerizer need to be photoactivated restricting interaction to the light-exposed region [73]. In contrast to these blue light-sensitive systems, PhyB photoconverts between two relatively stable states by either red or infrared light. Thus, although longer wavelengths may be less damaging to cells and penetrate deeper into biological specimens, precise spatial control of the PhyB/PIF interaction requires complementary 650 and 750 nm illumination patterns. However, even then the PhyB/PIF dissociation rate is in the order of seconds. This is comparable to the dissociation rate of π-EB1 with a fast dark reversal LOV2(I427V) variant suggesting similar spatial accuracy [14,45]. An important trade-off for high spatial control, however, is that fast dark state reversal requires frequent (1 Hz or faster) exposure to photoactivation light, which even at low intensities can accumulate significant phototoxicity over longer periods and may also contribute to temperature increases of the specimen. Another point to consider is that dark is never truly dark as the inactive and activated states of photosensors exist in a chemical equilibrium. Even in the dark, an estimated ~1.5% of LOV2 molecules are in the activated state with the Jα helix dissociated, which increases ~60-fold in response to blue light [59]. This limits the dynamic range of individual LOV2 molecules although this can likely be increased by dimerization or other multivalent interactions.

Lights, camera and overlapping action spectra: Because of overlapping excitation spectra, it can be challenging to avoid unintended photoactivation when combining optogenetics and fluorescence microscopy. Blue light-sensitive domains such as LOV2 or Cry2 are only compatible with imaging of red or far-red fluorescent proteins. Even 515 nm, a frequently used YFP excitation line will photoactivate LOV2 [51]. Thus, combining FRET-based protein activity sensors with LOV2 optogenetics requires FRET between new near-infrared fluorescent proteins [74]. Combining fluorescence microscopy with PhyB/PIF red light-sensitive optogenetics is possibly even more problematic, because the PhyB action spectrum spans almost the entire visible light range [75]. Both blue and green light cause PhyB photoconversion, and frequent 750 nm light exposure is required to avoid accumulation of the stable PhyB photoactivated state that has a half-life of hours [30]. It is noteworthy that both PhyB isomers have absorbance minima around 515 nm, making YFP perhaps the most suitable choice for fluorescence microscopy with PhyB/PIF. Lastly, one should not forget that animal cells also express cryptochromes. Although it remains unknown if vertebrate cryptochromes are light-sensitive in a physiological context (in insects they certainly are) [76], this highlights that appropriate blue light controls in the absence of the designed photoswitch should be incorporated in any optogenetic experiment. Unfortunately, this is not always current standard practice in the field.

precise epithelial folding patterns in early *Drosophila* embryos [25].

Optogenetic relocalization to modulate cytoskeleton dynamics

Photoactivated recruitment can also localize protein activities other than Rho GTPases. For example, the Kapitein group has used TULIPs, a LOV2-based heterodimerization module, to link organelle cargoes to kinesin, dynein and myosin motor domains and demonstrated light-induced redistribution of peroxisomes, endosomes and mitochondria [26,27], and *in vitro* control of microtubule gliding [28]. However, all variations of localization-mediated photoactivation require expression of engineered constructs that become dominant active under illumination conditions, but still compete with endogenous activities and may also have unintended consequences in the dark. For example, kinesin motor activity in the absence of cargo-binding can still affect microtubule network organization. To improve dynamic range and specificity of light-activated transport systems, the Kapitein group most recently introduced a two-step system in which both cargo binding and motor processivity are blue light-activated [29]. Blue light can also be combined with red light-sensitive PhyB/PIF dimerization to independently control trafficking and distribution of different sets of organelles [30] although crosstalk challenges between channels remain (Box 1). *In vitro*, blue light-activated multimerization of kinesin motors can drive complex self-organization of dynamic microtubule arrays to analyze and direct the non-equilibrium dynamic properties of such assemblies [31]. Using a similar recruitment strategy, Adikes et al. [32] invented a system to localize proteins to growing microtubule ends through iLID/nano-mediated dimerization with the microtubule plus-end adaptor EB1 and show that light-induced microtubule plus end crosslinking with f-actin slows microtubule polymerization.

In the so far only example of spatial optogenetic control of a structural mitotic spindle protein, two independent research teams used different optimized LOV2-based dimerization modules to dissect the molecular mechanism of dynein-mediated spindle positioning in either mammalian cells or *Caenorhabditis elegans* embryos [33,34]. Both teams find that localized recruitment of NuMA to the cell cortex is sufficient to reconstitute a functional cortical dynein motor complex that can drive spindle positioning. Interestingly, in both systems, light-mediated localization of the dynein motor itself does not work possibly because the long coiled-coil NuMA molecule is required for the correct cortical dynein anchoring geometry. It should be noted that spatially precise photoactivation is difficult in mitosis because of the small size of mammalian metaphase spindles. Immobilization at the cell cortex is important here, as slow dark recovery rates present challenges in achieving sharp photoactivation boundaries of a cytoplasmic freely diffusible protein.

In a variation of the pharmacological 'knocksideways' approach, optogenetic recruitment to the cell cortex or mitochondria can also acutely remove proteins from a site of action, for example, from mitotic spindles [35,36]. However, efficient sequestration will greatly depend on relative affinities and binding kinetics of the physiological binding interaction. In addition, this or similar inactivation methods trapping proteins in the nucleus [37] or in Cry2/CIBN oligomer clusters [38,39] do not allow subcellular spatial control, and the effect of Cry2 oligomerization likely clustering many hundreds of molecules is difficult to predict. Nevertheless, replacing endogenous Delta with a Cry2-tagged variant that is inactivated by blue light-induced clustering in the cell membrane has recently been implemented to dissect temporal Notch signaling dynamics in *Drosophila* [40].

Direct photoswitching of protein activity

The above examples highlight a powerful and easily implemented optogenetic strategy that uses light-induced dimerization to recruit protein activities to ectopic sites where they were not before. Currently, only one optogenetic system exists that functions in the opposite direction. LOVTRAP relies on small engineered peptides, Zdks, that bind the LOV2 domain dark-state with high affinity [41]. Thus, in contrast to light-induced dimerization modules, blue light results in reversible dissociation of the LOV2/Zdk heterodimer. Although the LOVTRAP module was initially also used to release proteins of interest, such as the Rac1 GEF Vav2 from mitochondria [41], the Hahn laboratory recently proposed that the LOV2/Zdk interaction could directly cage protein active sites (Figure 2b) [42]. For example, blue light uncaging the f-actin binding and thus filament severing activity of Z-lock cofilin can enhance actin monomer recycling to promote leading edge polymerization. However, successful implementation of this Z-lock idea depends on fine-tuning of the relative binding affinities of the LOV2/Zdk module and the binding interaction to be disrupted, as well as on the overall active site geometry in relation to the linkers connecting LOV2 and Zdk [43]. A similar strategy uses light-sensitive oligomerization of the photochromic DRONPA fluorescent protein to locally uncage membrane-bound Cdc42 [44].

Although the vast majority of optogenetic approaches activate a protein of interest, to understand physiological intracellular protein functions in space and time, different methods are needed to acutely inactivate proteins. We recently used the LOV2/Zdk1 interaction to disrupt the function of EB1 by blue light [45]. EB1 is a dimer with an N-terminal calponin homology domain

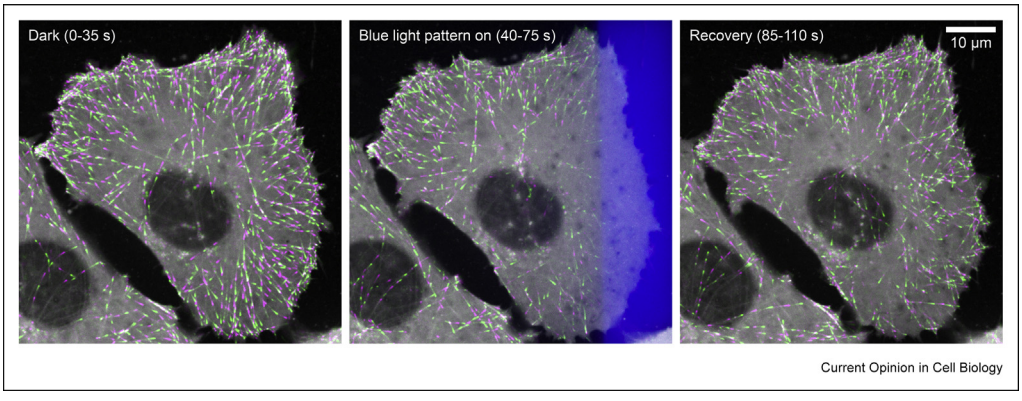
that recognizes growing microtubule plus ends and a C-terminal domain that recruits numerous other +TIP proteins to growing microtubule ends [46]. By inserting the LOV2/Zdk1 module in the disordered linker between these two domains, we demonstrated reversible light-induced dissociation of the +TIP complex and inhibition of microtubule polymerization with high spatial and temporal accuracy. In addition, migrating cells in which endogenous EB1 was replaced with this photo-inactivated π-EB1 turned away from local blue light exposure indicating that +TIP complex function is required for persistent directional cell movement. We posit that this strategy could be used to render many other multidomain proteins light sensitive (Figure 2d). To adapt this idea to more complex cell systems, we are currently developing genome editing to directly insert light-sensitive LOV2/Zdk1 modules into multidomain proteins and have been able to replace endogenous EB1 with the light-sensitive version in one step in various human cell types (Figure 3).

An even more direct way to render proteins light-sensitive is through direct insertion of a LOV2 domain into exposed surface loops predicted to propagate allosteric conformational changes (Figure 2e). Dagliyan et al. [47,48] test this idea for several different signaling molecules including the Rac1 GEF Vav2 and show that in this case, local Vav2 photoinactivation results in cell retraction from the site of blue light exposure. A related approach is to design split proteins in which inactive but properly folded subdomains are brought back together through a light-induced dimerizer to reconstitute protein activity (Figure 2c). New computational methods to predict split protein designs, which has traditionally been very difficult and may not always be possible, may open this strategy to optogenetics [49]. LOV2 conformation changes have also been used to alter lever arm geometries in both kinesin and myosin motors that can alter speed and directionality as a function of blue light exposure [50]. However, these light-controlled motors have not yet been used inside cells.

Summary and outlook

Light control of specific intracellular protein activities has only been around for a relatively short time, but optogenetic approaches are already casting new light on many dynamic cell processes. Many basic optogenetic experiments can be easily and inexpensively implemented on standard fluorescent light microscopes, but spatial control requires additional equipment to achieve localized light exposure such as laser-scanning or digital micromirror device illumination systems [51] (Box 1). Still, current photoactivation technologies are relatively static, and to gain more precise dynamic control of fast intracellular processes in cells or organisms, it will be necessary to develop feedback between microscopy and on-the-fly image analysis to direct photoactivation light patterns. It will also be interesting to see how optogenetics can break the diffraction barrier. Stimulated emission depletion (STED) reduces the point spread function diameter with a surrounding donut beam of inactivation light and recent work suggests STED nanoscale control of channelrhodopsins [52]. Similarly, STED optogenetics may be possible with phytochrome photoconversion between stable states driven by different wavelengths, but this has not yet been tested [53]. Bioluminescence resonance energy transfer between luciferase and blue light sensors [54] may provide yet another opportunity for nanoscale control by restricting activation to where the luciferase (i.e. the

Figure 3



Example of a spatiotemporal optogenetics experiment with subcellular resolution. Shown is a human tissue culture cell in which a photosensitive LOV2/Zdk1 protein–protein interaction module was inserted into the endogenous EB1 gene by CRISPR/Cas9 genome editing, which replaces the wild-type EB1 protein with the light-sensitive π-EB1 variant in one step. The images show an overlay of the mCherry-tagged π-EB1 C-terminal half on growing microtubule ends from short time-lapse sequences (eight images every 5 s) in alternating green and magenta before, during and after localized 470 nm blue light exposure. The light-exposed region is indicated by the blue overlay in the middle panel. Note the gradient of π-EB1 dissociation near the edge of the light-exposed region that results from diffusion of photoactivated molecules. Sharper boundaries can be achieved with LOV2 domain variants with faster dark recovery rates [45].

light source) and the photosensor are sufficiently close. However, spatial resolution will still be limited by the photo- and biochemical characteristics of the optogenetic switch (Box 1). In any case, it seems a safe bet that as with fluorescent proteins, there are unusual photosensors in the wild that we do not yet know about, with complementary or more desirable characteristics than what is available now, for example, narrower action spectra or faster reversion kinetics. Such properties might also be gained through further photosensor engineering or development of synthetic cofactors. The emerging field of 'photopharmacology' that uses synthetic photoswitches further broadens possible experimental approaches [55]. In summary, light-controlled proteins are an important addition to the cell biologist's toolkit. Being a relatively young field, however, many properties of optogenetic systems remain poorly characterized, and experimentalists need to be aware of the demands and limitations of their specific experiment design. Nevertheless, the future of optogenetics surely looks bright.

Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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