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Optogenetic interrogation and control of cell signaling

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Signaling networks control the flow of information through biological systems and coordinate the chemical processes that constitute cellular life. Optogenetic actuators — genetically encoded proteins that undergo light-induced changes in activity or conformation — are useful tools for probing signaling networks over time and space. They have permitted detailed dissections of cellular proliferation, differentiation, motility, and death, and enabled the assembly of synthetic systems with applications in areas as diverse as photography, chemical synthesis, and medicine. In this review, we provide a brief introduction to optogenetic systems and describe their application to molecular-level analyses of cell signaling. Our discussion highlights important research achievements and speculates on future opportunities to exploit optogenetic systems in the study and assembly of complex biochemical networks.

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Introduction

Cells are dynamic, out-of-equilibrium systems in which complex networks of biomolecular interactions — or signaling networks — regulate cellular metabolism, movement, and differentiation [1–3]; coordinate the assembly of multicellular structures [4]; and permit sophisticated biological feats (i.e. cognition and memory [5]). The maintenance of these networks is centrally important to the function of living systems. Their anomalous regulation gives rise to death and disease [6]. A detailed understanding of the organization and function of signaling networks — their size, topology, and spatiotemporal evolution — remains a major focus of biological research.

A signaling network does not have a beginning or an end. It is a web of functionally interconnected biomolecules [7•]. The epidermal growth factor receptor (EGFR) pathway, which contributes to several common types of cancer [8], provides an illustrative example. This network includes mitogen-activated protein kinase (MAPK) signaling, EGFR transactivation and trafficking, phosphatidylinositol polyphosphate (PIP) signaling, and cell cycle control, among other processes. By one accounting, it comprises 211 reactions and 322 species, including 202 proteins, 73 oligomers, 22 "simple" molecules, eight degraded products, seven genes, seven RNAs, and three ions [9•]. The molecular details of this pathway took decades to work out.

Questions concerning the structure and function of signaling networks most commonly include the following: 'What molecules are involved?' 'Where do they meet?' 'How do they regulate cellular physiology?' And 'How does that regulation go awry in the context of disease?' Answers to these questions require both (i) a static map, which captures the *what* and *where* of network structure, and (ii) a set of rules that describe how that map changes in response to diverse sets of physicochemical cues that vary over time and space.

Over the last two decades, optogenetic actuators — proteins that undergo light-induced changes in activity or conformation — have emerged as powerful tools for studying the dynamics of complex signaling cascades [10••]. These proteins can control biomolecular transport, binding, and activity with impressive spatiotemporal precision [11••]. In this review, we provide a brief overview of different types of optogenetic actuators, describe their application to innovative studies of cellular biochemistry, and comment on important challenges and opportunities that lie ahead.

Methods for placing proteins under optical control

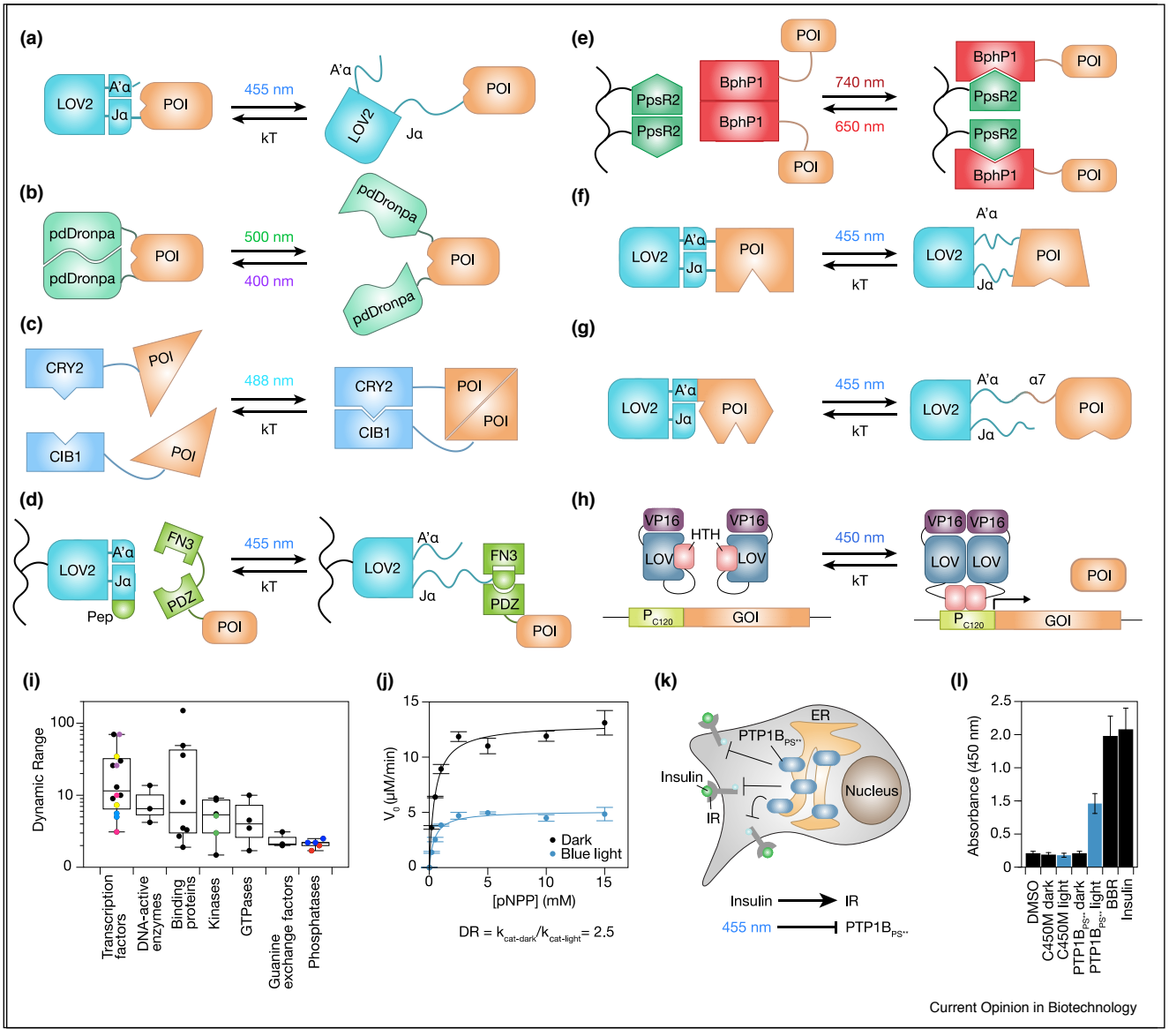
The term 'optogenetics' originated in the field of neuroscience, where light-gated ion channels found early use in experimental studies of neuronal circuits [12]. It has grown, in years since, to describe the application of genetically encoded, light-gated proteins to biochemical research, medicine, and engineering [13,14]. We will begin our discussion of signaling studies by outlining different approaches for using light-sensitive proteins to build optogenetic tools.

Cage-based systems

One version of an 'ideal' optogenetic construct is a protein that is biochemically inert, or caged, in its dark state and

active, or uncaged, in its light state. Caging typically requires the attachment of a light-sensitive protein to an essential binding site. Prototypical proteins include the light-oxygen-voltage (LOV) domain of phototropin 1 and the photodissociable mutant of Dronpa (pdDronpa; Figure 1a–b). The LOV domain derives its optical activity from a noncovalently bound flavin mononucleotide (FMN); when exposed to blue light, this cofactor forms an

Figure 1



Optical control of protein activity. Common strategies for integrating an optogenetic actuator (OA) into a protein of interest (POI) include **(a–b)** attachment near the active site, where the OA controls substrate access through (a) a conformational change [16••] or (b) photodimerization [19••], **(c)** attachment to each half of a split POI, where the OA controls POI assembly [26••], **(d–e)** attachment to the N-termini or C-termini of a POI, where the OA guides subcellular localization [27••,29••], **(f)** insertion within a catalytic domain [35••] or **(g)** attachment to an allosteric switch of a POI, where the OA triggers an activity-modulating conformational change [36••], and **(h)** attachment to an obligate homodimer, where OA controls dimerization [65••]. **(i)** The dynamic range of optogenetic constructs grouped by enzyme class [16••,19••,65••,84–90,26••,27••,28,29••,31,35••,36••,39•]. Colored points denote individual constructs examined under different experimental conditions or cellular contexts. Box plots show the median, first and third quartiles, and 1.5 times the interquartile range (whiskers). **(j)** Michaelis-Menten curves show the activity of a PTP1B-LOV2 fusion, denoted $PTP1B_{PS}$, in the presence and absence of light. **(k)** A cartoon of a mammalian cell expressing $PTP1B_{PS^{**}}$, a natively localized $PTP1B_{PS}$. Insulin stimulates phosphorylation of membrane-bound insulin receptor (IR); PTP1B dephosphorylates this receptor. **(l)** ELISA-based measurements of IR phosphorylation in HEK293 T/17 cells that are unmodified, or stably expressing either

intermolecular carbon-sulfur bond that destabilizes the N-terminal and C-terminal helices of the protein [15]. An early study used LOV to block the binding of effectors to Rac1 in the dark (and, correspondingly, to permit effector binding in the light). The resulting LOV-Rac1 fusion enabled spatial control of cellular motility, ruffling, and protrusion in live-cell imaging experiments [16••]. LOV has since emerged as an extremely versatile tool for optogenetic studies [17]. More recently, pdDronpa, which shifts between monomeric and dimeric forms under cyan and violet light, has also shown immense promise [18]. Researchers used this protein to cage several kinases in the Raf-MEK-Erk signaling pathway by attaching it to their N-termini and C-termini (i.e. its dimeric form blocked their active sites [19••]). The simplicity of cage-based systems makes them useful for controlling different classes of proteins.

Dimerizing systems

Many proteins are obligate dimers and can be rendered photoswitchable with actuators that control dimerization. Prominent examples of optical dimerizers include the bacterial phytochrome BphG1 from *Rhodobacter sphaeroides* and cryptochrome 2 (CRY2) from *Arabidopsis thaliana* [20•,21]. BphG1 is a homodimer with a photosensory core that aligns and unaligns diguanylate cyclase domains under red and far red light, respectively (its chromophore is biliverdin IXα [21]). CRY2 binds a flavin adenine dinucleotide (FAD) and associates with a cognate protein, termed CIB1, under blue light (Figure 1c, [20•]); CRY2 and its truncation variants (e.g. CRY2PHR, CRY2olig, and CRY2clust) can also oligomerize on their own [22,23]. In a proof-of-concept study, researchers used BphG1 to control the alignment of a type III adenylyl cyclase (AC), an obligate homodimer [24]. Their BphG1-AC chimera permitted optical control of cholinergic neurons — and body bending — in *Caenorhabditis elegans* (*C. elegans*). The CRY2-CIB1 system, by contrast, has been used in a much larger number of studies [25] and was recently optimized to create variants with small sizes, reduced dark-state activities, and adjustable interaction lifetimes [26••]. One particularly impressive CRY2-CIB1 pair permitted high-DR control over a split Cre recombinase (and, thus, DNA recombination) in yeast.

Cellular localization

Optical dimerizers can also guide protein localization. Three versatile examples include (i) tunable, light-controlled interacting protein tags (TULIPs), which consist of LOV2-caged peptide epitope that binds to a PDZ domain under blue light (i.e. unwinding of the Jα helix exposes an attached peptide; Figure 1d, [27••]), (ii) LOV-TRAP, which exploits a Zdk domain that binds to the dark state of LOV2 [28], and (iii) BphP1 and PpsR2, two proteins that associate and disassociate under near-infrared and red light, respectively (Figure 1e, [29••]). These proteins have allowed researchers to control MAPK activation, protrusion-retraction cycles, and transcriptional activation, among other processes [27••,28,29••]. Two architecturally similar tools worth mentioning are (i) Magnets, or pairs of electrostatically complementary Vivid (VVD) proteins that permit optical control over heterodimerization [30], and (ii) LOV2-caged sequences that direct protein binding (e.g. the SsrA peptide and the SspB adaptor protein for iLID [31]) or nuclear localization (e.g. the light-inducible nuclear localization signal, or LiNUS [32], and the light inducible nuclear export system, or LEXY [33]). Optical dimerizers, many of which rely on LOV2 [34], provide a powerful set of kinetically and spectrally distinct tools for controlling protein localization.

Allosteric modulation

The aforementioned architectures for optogenetic modulation interfere — by design — with native patterns of protein interaction and localization and could, thus, complicate detailed analyses of those patterns (which can vary by target) in signaling cascades. Allosteric photocontrol systems, which position actuators far from the active site, offer a less disruptive alternative. In one seminal study, researchers inserted LOV2 within poorly conserved loops on the surfaces of motility signaling proteins (kinases, guanosine triphosphatases, and guanine exchange factors); optically induced unwinding of LOV2 disrupted enzyme activity and permitted optical control of cellular morphodynamics (Figure 1f, [35••]). In a more recent study, we connected the N-terminal A'α helix of LOV2 to a C-terminal allosteric switch of protein tyrosine phosphatase 1B (PTP1B, Figure 1g; [36••]). This allosteric architecture, which enabled optical modulation of catalytically essential loop dynamics, preserved the native structure, activity, and subcellular localization of PTP1B. It highlights the promise of allosteric photocontrol systems as tools for probing native protein behaviors in living cells.

Design parameters

Important design parameters for optogenetic constructs include dynamic range (DR), background activity, response time, recovery time, activation wavelength, and size. These attributes, which have received comprehensive coverage in recent reviews [37,38], are summarized in Table 1. Activation wavelength, modulatory mechanism, and size tend to motivate actuator selection, while background activity and DR guide construct optimization [39•]. Figure 1i plots values of DR for a diverse set of optogenetic tools; close inspection reveals several trends: Binding partners and transcription factors (Figure 1h) tend to have the highest DRs, which extend up to ~150, while the DRs of photoswitchable enzymes rarely exceed 10. (Transcription factors, we note, control an amplification reaction, which contributes to their high DRs). Strikingly, with the exception of PTP1B, the DRs of constructs measured under different experimental

Table 1

This table details important design parameters for optogenetic actuators

System	Origin	Size (kDa)	Cofactor	Mechanism	λ_1 (λ_2)* (nm)	I_1 (I_2)* (mW cm^{-2})	t_1 (t_2)* (s)	t_r* (s)	Ref
LOV2	*Avena sativa*	16.5	FMN	When exposed to blue light, FMN forms an intermolecular carbon-sulfur bond that destabilizes the N- and C-terminal helices of LOV2.	447–488 (Dark)	2	<5	27–50	[35,90–94]
iLID nano,micro (LOV2)	*Avena sativa, Escherichia coli*	17.5, 13 (SspB)	FMN	An engineered SsrA peptide is fused to the Jα helix of LOV, which blocks the binding of SsrA to SspB in the dark. .	450–488 (Dark)	1.2–6	seconds	<30	[31]
VVD	*Neurospora crassa*	21.3	FAD	When exposed to blue light, FAD forms an intermolecular carbon-sulfur bond that permits the formation of a VVD homodimer.	450 (Dark)	N/A	N/A	10 080	[79]
Magnets (VVD)	*Neurospora crassa*-derived	17.1	FAD	VVD variants with opposite charges form heterodimers under blue light.	450–490 (Dark)	3	<30	6.8	[30]
pdDronpa	*Pectiniidae*-derived	25.5	cys-tyr-gly[a]	The pdDronpa domains dimerize under violet light and dissociatiate under cyan light (which causes a β strand at the dimer interface to become flexible).	500 (400)	20 (10)	120 (3)	N/A	[19,95,96]
CRY2PHR, CRY2clust, CRY2olig	*Arabidopsis thaliana*	57.2, 58.2, 57.1	FAD	Blue light induces CRY2 homo-oligomerization.	470–488 (Dark)	3	1 [40] $_{t1/2}$ 1 [4.6]$_{t1/2}$ 1 [110]$_{t1/2}$ [c]	267/238/408	[23]
CRY2-CIB1	*Arabidopsis thaliana*	69.5, 21.7	FAD	The N-terminal domain of CIB1 binds to the light state of CRY2 but not its dark state.	450–488 (Dark)	4.5	0.3	330	[20]
LOVTRAP (LOV2-Zdk)	*Avena sativa*, *Staphylococcus aureus* protein A-derived	16.5, 9.9 (Zdk)	FMN	The Jα of LOV2 binds to Zdk1 in the dark and dissociates under blue light.	426–466 (Dark)	100 W Hg arc lamp with 5% ND filter	<5	1.7–496 (18.5 for WT LOV2)	[28]
BphP1-PpsR2	*Rhodopseudomonas palustris*	82, 51 (PpsR2)	Biliverdin IXα	Far-red light causes a conformational change in the chromophore of BphP1 that allows it to bind to PpsR2.	740 (636)	0.2–27 (0.35–45)	300 [60]$_{t1/2}$ (300 [30]$_{t1/2}$) [c]	900	[29]
TULIPs (LOV2-PDZ)	*Avena sativa*, synthetic	17, 21 (ePDZ)	FMN	The Jα of LOV2 contains a peptide epitope that is uncaged under blue light.	468–473 (Dark)	0.005 J cm^{-2} s^{-1}	Seconds	168	[27]
BphG1	*Rhodobacter sphaeroides*	54.7	Biliverdin IXα	BphG1 homodimerizes in response to red light.	694–700 (768)[b]	0.2	N/A	46–197	[21,24]
PhyB-PIF	*Arabidopsis thaliana*	99.3, 11.3 (PIF)	Phycocyanobilin (PCB)	PhyB and PIF form a heterodimer under 650 nm light and dissociate under 750 nm light.	650 (750)	N/A	1.3 (4)	N/A	[44,97]
EL222	*Erythrobacter litoralis*	23.1	FMN	Under blue light, EL222 forms a homodimer that binds to DNA.	450–465 (Dark)	0.8	<10	<50	[64]

Table 1 (*Continued*)

System	Origin	Size (kDa)	Cofactor	Mechanism	λ_1 (λ_2)* (nm)	I_1 (I_2)* (mW cm^{-2})	t_1 (t_2)* (s)	t_r* (s)	Ref
ChR2	*Bovine rhodopsin*	39.0	Retinal	Illumination of retinal causes a conformational change in ChR2 that (i) allows ions to passively diffuse into the cell or (ii) modulates the activity of fusion partners (e.g., CXCR4, a GPCR).	488–505 (Dark)	48–367	Seconds	Seconds	[67]
CcaS-CcaR	*Synechocystis* PCC6803	86.1, 26.4 (CcaR)	PCB	Under green light, CcaS switches to the Pr state and phosphorylates CcaR. Phosphorylated CcaR binds to the cpcG2 promoter, leading to gene transcription.	520–535 (650–670)	0.008–0.403 (0.008–0.105)	N/A	N/A	[68,98]
Cph8-OmpR	*Synechocystis* PCC6803	83.1, 27.4 (OmpR)	PCB	Under red light, Cph8 switches from the Pr state to the Pfr state and dephosphorylates OmpR. Dephosphorylated OmpR unbinds from the ompC promoter which prevents transcription.	650 (740)[b]	0.008–0.105	N/A	N/A	[68,98,99]

[a] This chromophore is formed autocatalytically from residues Cys62,Tyr63 and Gly64 [95,96].
[b] These wavelengths were not explored in the papers in focus but were characterized in other studies.
[c] The half-lives required for activation/deactivation appear in brackets.
* λ_1, activation wavelength; λ_2, deactivation wavelength (reversible systems); I_1, activating intensity; I_2, deactivation intensity; t_1, illumination duration for activation in referenced experiment; t_2, illumination duration for deactivation in referenced experiment; t_r, half-life for thermal recovery. This table shows experimentally reported values (i.e. it does not necessarily include the full range of activation and deactivation wavelengths).

conditions tend to show significant context-dependence, an important consideration when using *in vitro* studies to interpret *in vivo* behaviors.

Our recent analysis of PTP1B urges some restraint in efforts to optimize DR. PTP1B undergoes several post-translational modifications outside of its active site that cause modest (i.e. approximately twofold) changes in its activity [40,41]. This effect seems small, but a photoswitchable variant that yielded similar changes in activity caused large shifts in insulin receptor phosphorylation in mammalian cells (Figure 1j–l, [36••]). This surprising finding highlights the value of constructs with DRs that match, rather than artificially exceed, physiologically relevant changes in the activities of enzymes under study.

Optogenetic analysis of signaling networks

Signaling networks often rely on nonlinear information flows and interdependent inputs that make them difficult to dissect with bulk chemical perturbations (e.g. the addition of an inhibitor [42]). Optogenetic tools enable isolated, spatiotemporally precise perturbations of signaling nodes and, thus, provide a means of dissecting them. We will outline a series of studies that have used optogenetic tools to garner useful insights into complex signaling cascades.

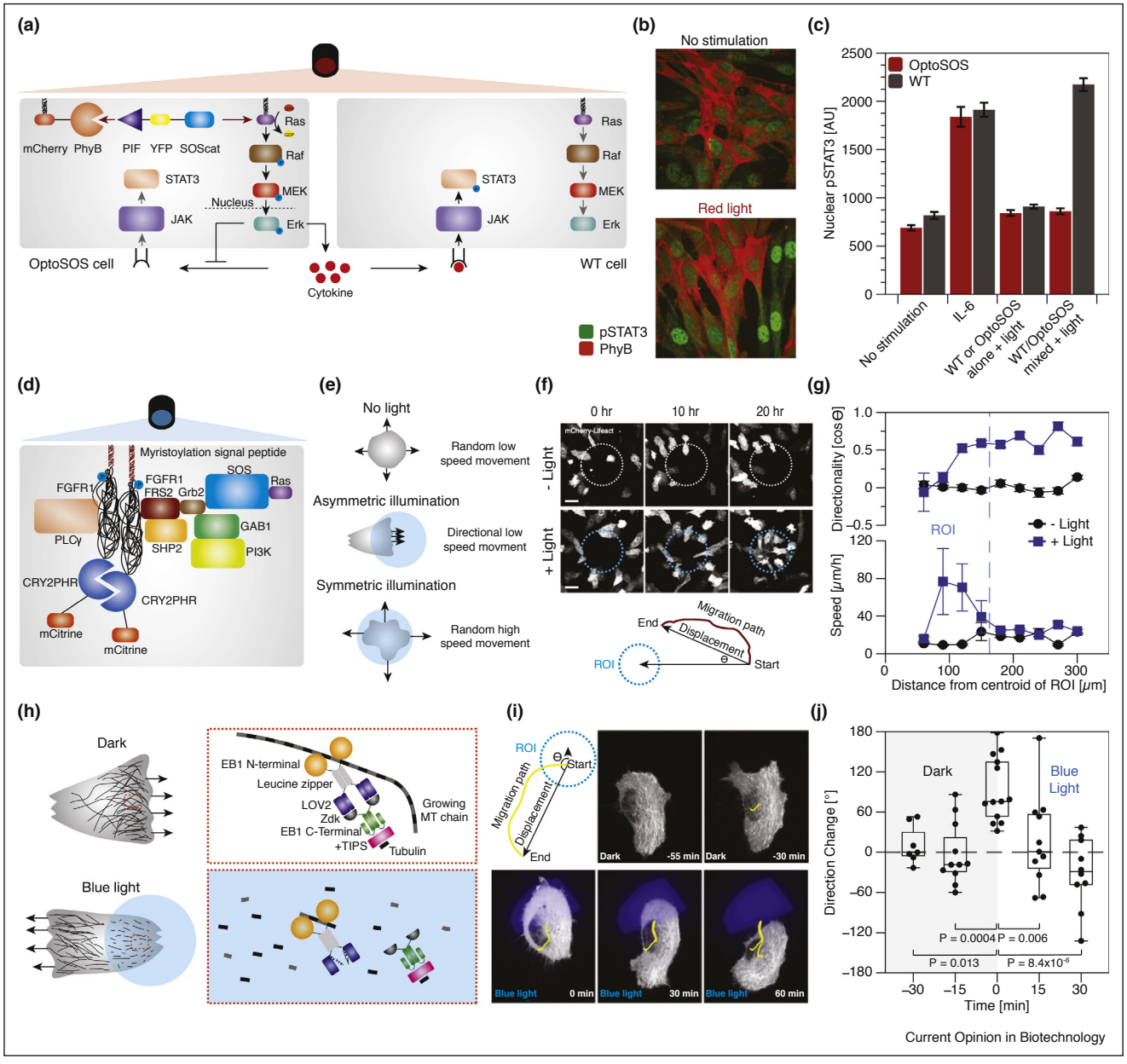
Signal transmission, integration, and filtering

Many extracellular signals activate shared signaling nodes, yet trigger distinct biological responses [43]. How? One study of the Ras/Erk pathway set out to answer this question by placing Ras under optical control [44••]. The authors fused (i) phytochrome B (PhyB) to a membrane anchor and (ii) PIF—the binding partner of PhyB—to a catalytic segment of SOS (SOS_{cat}; Figure 2a); optically induced PhyB-PIF dimerization localized SOS to the plasma membrane, activating Ras. With this construct in hand, the authors explored different signaling inputs: Short bursts of illumination (<4 min) failed to cause nuclear translocation of Erk; medium-duration illumination (>10 min) stimulated the mitogen-activated protein kinase (MAPK) cascade, causing Erk translocation; and prolonged illumination (>1 hour) stimulated cytokine release and STAT3 activation in neighboring wild-type cells (Figures 2b–c). These time-dependent outputs indicate that downstream signaling modules can filter and decode dynamic stimuli in Ras/Erk signaling. Subsequent analyses of the Ras/Erk pathway have used similar tools to probe pathway inputs [45,46].

Cellular motility

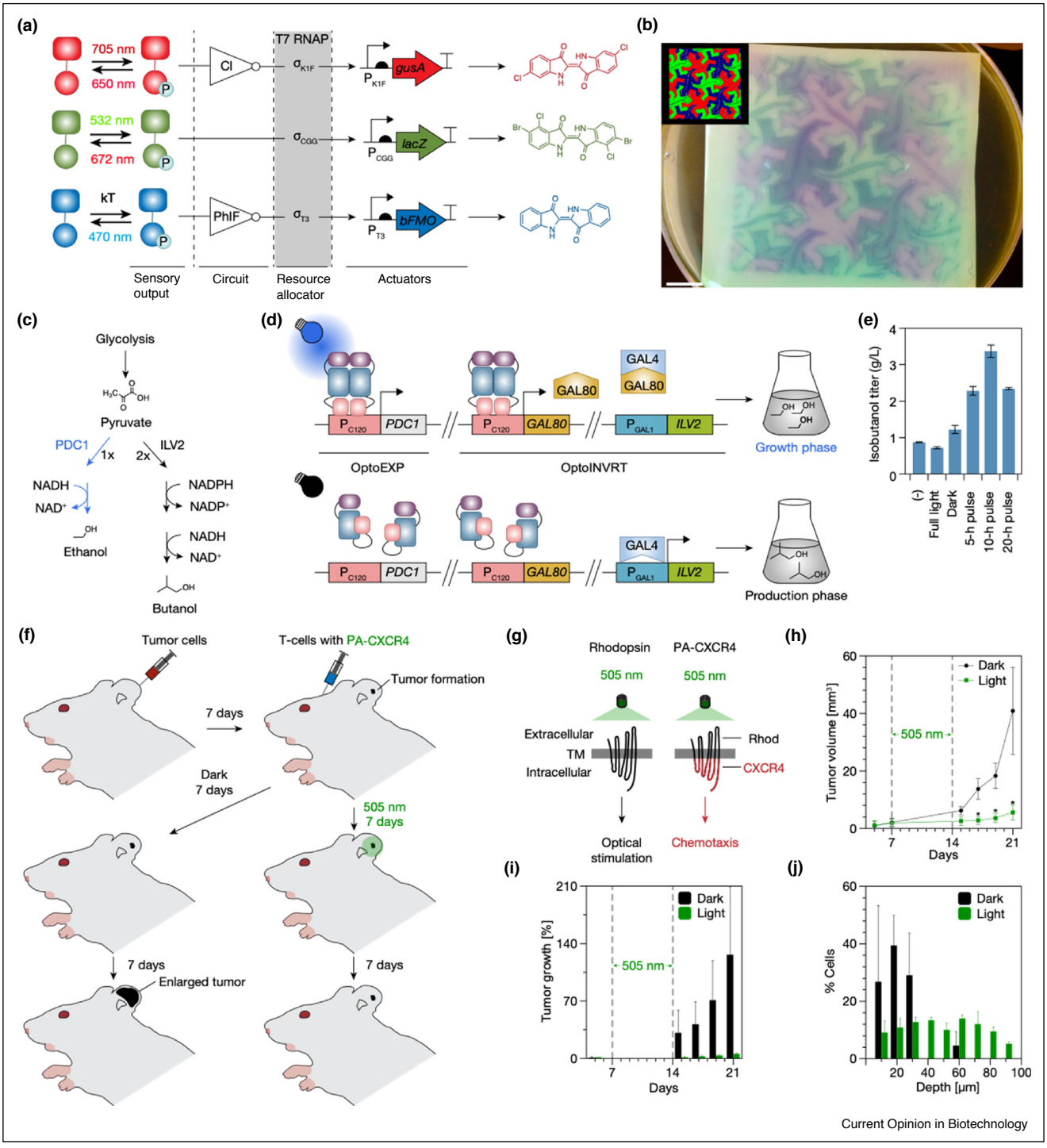
The signaling cascades that guide cellular movement and morphogenesis are spatiotemporally complex [47,48]. Several studies have used optogenetic tools to dissect them [49]. In an important proof-of-concept study, investigators fused fibroblast growth factor receptor 1 (FGFR1) to CRY2PHR [50••]. Illumination of this fusion, termed

Figure 2



Optogenetic analysis of cell signaling. **(a)** Ras/Erk signaling. Illumination of an opto-SOS cell causes heterodimerization of membrane-localized PhyB and PIF-tagged SOScat, leading to Ras activation and nuclear translocation of Erk. Prolonged illumination releases cytokines that activate JAK/STAT signaling in a neighboring WT cell, but not in the opto-SOS cell ([44••]). **(b–c)** Data to support the model in (a). (b) Immunofluorescence images of mixed WT and opto-SOS cells (NIH 3T3) with and without optical stimulation. Upon red light illumination, STAT3 activation occurs only in WT cells. (c) Quantification of nuclear pSTAT3 under various conditions. Note: IL-6 activates STAT3. Bars show the mean and SEM ($n \geq 50$ in all groups). **(d)** FGFR signaling. Illumination of an opotoFGFR1 cell causes homodimerization and autophosphorylation, creating binding sites for downstream components that mediate cytoskeletal reorganization [50••]. **(e)** A cartoon and **(f)** collection of micrographs depict optoFGFR1-expressing HUVECs migrating towards a region of illumination (ROI). Scale bar = 100 μm. Bottom: A schematic used to calculate the angle of migration. **(g)** As HUVECs enter the ROI, the directionality (cos θ) and speed of their migration decrease and increase, respectively. Data points show the mean and SEM (n unreported). **(h)** Microtubule dynamics. Illumination of a π-EB1 cell triggers LOV2-Zdk dissociation, which separates the MT-binding and +TIP adapter domains, leading to decreased MT growth rates [51••]. **(i)** Illumination of a π-EB1 cell causes it to turn away from the light. Yellow line: centroid trajectory. Left: A schematic used to calculate the change in migration direction (i.e. θ). **(j)** Illumination of π-EB1 cells causes a rapid change in the direction of migration. Box plots show the median, first and third quartiles, and 1.5 times the interquartile range (whiskers, $n = 13$). Figures in (b-c), (e-g), and (h-j) were adapted with permission from Refs. [44••,50••,51••], respectively.

Figure 3



Engineering applications. **(a)** A synthetic pathway links the wavelength-specific illumination of three phytochromes to the production of red, green, and blue pigments in *E. coli* [63••]. **(b)** A plate of 'RGB' *E. coli* illuminated with a colored image (inset) produces corresponding pigments (i.e. a photograph; scale bar = 1 cm). **(c)** A metabolic pathway with optically responsive valves produces ethanol under blue light (OptoEXP) and butanol in the dark (OptoINVRT) [65••]. Note: The numbers indicate the required number of pyruvate molecules for each molecule of ethanol or butanol. **(d)** Illumination with blue light activates transcription of PDC1 and inhibits transcription of ILV2. **(e)** Production of isobutanol by *S. cerevisiae* with the system from (c-d) with blue light pulses of 15 s on and 65 s off for 30 min every 5, 10, and 20 hour. Bars show the mean and SD ($n \geq 3$). **(f)** Light-

optoFGFR1, caused FGFR1 dimerization and *trans*-autophosphorylation, and enabled the recruitment of downstream components (Figure 2d). Local illumination of human umbilical vein endothelial cells (HUVECs) expressing the fusion caused lamellipodia formation in the region of illumination (ROI) and membrane retraction on the opposite side of the cell (Figures 2e–g). This finding indicates that asymmetric activation of FGFR1 can control cell polarity and the direction of cellular migration.

In another interesting study of cell polarization, researchers examined microtubule dynamics [51••]. This study focused on end-binding (EB) proteins, which recruit plus-end-tracking proteins (+TIPS) to the ends of growing microtubules. To study EB1, an influential EB, the authors used the LOVTRAP system to modulate the assembly of its two domains: (i) an N-terminal calponin homology (CH) domain, which recognizes growing microtubules, and (ii) a C-terminal EB-homology (EBH) domain, which recruits + TIPs (Figure 2h). Under blue light, the engineered construct, termed π-EB1, inhibited microtubule growth and caused aversive turning of migrating cancer cells (Figures 2i–j). In a particularly dramatic demonstration, migrating cells could not escape a box of blue light projected around them. Findings indicate that EB1-mediated interactions with +TIP complexes are required to maintain leading-edge identity and directionality during cell migration. This type of study — an examination of a protein's contribution to cellular motion — is well suited for optogenetic experiments and will remain common (and important) for years to come.

Calcium signaling

We would be remiss not to mention optogenetic studies of calcium signaling, which regulates synaptic transmission, muscle contraction, cellular motility, gene expression, and other processes [52••,53]. Recent work has focused on the development of photoswitchable Ca^{2+} release-activated Ca^{2+} (CRAC) channels, which are located in the plasma membrane. These channels are composed of four-pass transmembrane proteins known as ORAI, which are gated by ER-bound stromal interaction molecules (STIMs). When Ca^{2+} is depleted in the ER lumen, STIMs oligomerize on their cytosolic side, activating CRAC channels at the plasma membrane [54]. Several studies have used actuators such as CRY2 or LOV2 to control CRAC activation by caging the cytosolic domain of STIM1. These constructs, which are the subject of detailed reviews [52••,53], have enabled optical control of memory formation [55], mouse behavior (e.g. freezing [56•]), the immunoinflammatory response [57], and cell signaling and migration [58,59]. Their application to the rapidly expanding fields of neuroscience and immunology could yield an enormous number of novel insights.

Engineering applications

As Richard Feynman and others have stated, creation is the best test of understanding (here, we paraphrase) [60]. The concomitant growth of the fields of optogenetics and synthetic biology has provided numerous opportunities for using artificial signaling systems to control cellular behavior. We will highlight a few examples with diverse applications.

Biochemical printing

Signaling cascades use environmental stimuli to guide pattern formation, materials synthesis, and information storage [61•]. Two prominent studies have recreated similar control systems by using phytochromes to modulate pigment production in *Escherichia coli* [62,63••]. In the most recent of the two, researchers linked two synthetic modules in *E. coli*: (i) an array of three DR-optimized phytochromes and (ii) and a set of three phytochrome-modulated pathways for pigment production (Figure 3a). Long-term (i.e. 18 hour) illumination of bacteria harboring these modules allowed them to reproduce images projected on growth plates (Figure 3b).

Chemical biosynthesis

Signaling pathways regulate cellular metabolism and provide a valuable means of adjusting it. A recent study used EL222, a LOV protein that binds to DNA under blue light [64], to develop two new optogenetic systems: OptoEXP and OptoINVRT, which activate and repress gene expression, respectively, under blue light [65••]. The authors used these systems to control two competing pathways in yeast: (i) ethanol biosynthesis via pyruvate decarboxylation (which is essential for growth) and (ii) butanol production via acetolactate synthesis (Figure 3c–d). Pulsed illumination of an engineered strain that produced ethanol in the light and butanol in the dark maximized butanol yields (Figure 3e). This study highlights the nonlinearity of cellular metabolism and provides an experimental framework for using optogenetic systems to fine-tune metabolic flux — and optimize yield — in microbial fermentations.

Immunotherapy

T-cell therapies are most effective when directed to diseased tissues [66]. In an interesting proof-of-concept study, researchers created a photoactivatable chemokine receptor (i.e. C-X-C motif receptor 4) by fusing the parent

(Figure 3 Legend Continued) responsive T-cell immunotherapy. Mice are injected with tumor cells on day 0 and T-cells with a photoactivatable variant of CXCR4 on day 7. Illumination for 7 days arrests tumor growth [67••]. **(g)** Design of the photoactivatable CXCR4. **(h–j)** Measurements of (h) the tumor volume, (i) tumor growth (% change in volume compared with day seven), and **(j)** the penetration depth of T-cells into tumors for the experiment depicted in (f). Data points (h–j) show the mean and SEM ($n = 5$ for h and i). Figures in (a–b), (c–e), and (f–j) were adapted with permission from Refs. [63••,65••,67••], respectively.

receptor with rhodopsin (Figure 3f–g, [67••]); this engineered chimera enabled optical control of T-cell migration. The authors, in brief, injected B16/OVA tumor cells into the ears of mice, waited seven days for solid tumors to form, and injected T-cells with the engineered chimera. Under green light, solid tumors showed a reduction in tumor volume and growth rate and an increase in T-cell infiltration (Figure 3i–j). This finding suggests that local illumination could improve the quality of cell transfer therapies.

Modeling and control of optogenetic systems

As the capabilities of optogenetic systems continue to expand, tools for modeling and controlling their activities in biological systems have become increasingly sophisticated. In an early study, researchers paired (i) orthogonal two-component optogenetic systems — each based on a bifunctional kinase-phosphatase domain that activates/deactivates a cognate transcription factor under green, red, and/or far red light — with (ii) model-guided light sequences to generate user-defined protein expression dynamics in *E. coli* [68••]. These 'function generators' provide valuable tools for studying and controlling dynamically regulated biochemical processes (e.g. circadian clocks, metabolic pathways, and synthetic circuits). Kinetic models have also proven useful to guide (i) the integration of orthogonal light-inducible and ligand-inducible promoters into dynamically reconfigurable logic gates [69] and (ii) the optical stimulation of yeast pheromone response pathways [70•]. The latter study, which used a closed-loop optical system to link upstream stimulation to downstream pathway outputs, demonstrates an innovative approach for studying feedback regulation in biological circuits.

Conclusion and outlook

Many of the most impressive biological feats are multicellular. Optogenetic studies of intracellular control systems, which guide embryo development [71•], maintain heart and brain function [72–75], and control the gut microbiome [76], have emerged recently. These studies are beginning to show how differences in cell-specific network structures guide tissue development and community-level microbial processes. Optogenetic genome editing could further inform these studies by revealing the context dependence of essential gene function [77]. The advent of powerful optogenetic tools is making multicellular systems an increasingly accessible frontier for detailed, molecular-level signaling analyses.

Optogenetic systems have expanded rapidly from light-gated ion channels to include many other proteins. The current palette of optogenetic actuators and 'actuate-able' proteins; however, remains limited to specific wavelengths of light (mainly blue and green, and red for cellular localization) and a small number of protein classes. These limitations hinder multi-color actuation experiments, which could permit multiplexed analyses of complex signaling pathways. Notably, there are several approaches for using optogenetic actuators to place protein kinases under optical control [19••,35••,45], but only one for controlling protein phosphatases, which contribute to an enormous number of cellular processes [36••,78]. This discrepancy highlights the need for general platforms that permit the assembly, optimization, and evolution of different varieties of optogenetic constructs.

The concept of an 'optimum', as it applies to optogenetic tools, merits brief discussion. DR is a common figure of merit (though other photophysical attributes have certainly been adjusted [79•]). Signaling networks, however, rely on amplification and crosstalk [80,81], and optogenetic constructs that effect large — and, perhaps, physiologically irrelevant — shifts in target activity could lead to experimental artifacts. Minimally disruptive approaches for triggering shifts in protein activity that match those observed *in vivo* are more likely to resolve native — rather than artificially enhanced or expanded — signaling dynamics.

We conclude by noting that signaling networks — which distinguish cells from bags of chemicals — are centrally important to biological adaptation, resilience, information processing, and memory [82,83]. Our understanding of them guides our understanding of biological function and, as it grows, provides new opportunities for dissecting, adjusting, and exploiting the biochemical processes responsible for biology's most impressive feats.

Conflict of interest statement

A.H. and J.M.F. are inventors on a patent application that includes optogenetic systems. This patent was filed by the University of Colorado and optioned by Think Bioscience. J.M.F. is a founder and officer of Think Bioscience. E.T.L. declares no conflict of interest.

CRediT authorship contribution statement

Akarawin Hongdusit: Visualization, Writing - review & editing. **Evan T Liechty:** Visualization, Writing - review & editing. **Jerome M Fox:** Project administration, Conceptualization, Writing - review & editing.

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