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REVIEW

Optogenetic approaches for understanding homeostatic and degenerative processes in *Drosophila*

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Abstract
Many organs and tissues have an intrinsic ability to regenerate from a dedicated, tissue-specific stem cell pool. As organisms age, the process of self-regulation or homeostasis begins to slow down with fewer stem cells available for tissue repair. Tissues become more fragile and organs less efficient. This slowdown of homeostatic processes leads to the development of cellular and neurodegenerative diseases. In this review, we highlight the recent use and future potential of optogenetic approaches to study homeostasis. Optogenetics uses photosensitive molecules and genetic engineering to modulate cellular activity in vivo, allowing precise experiments with spatiotemporal control. We look at applications of this technology for understanding the mechanisms governing homeostasis and degeneration as applied to widely used model organisms, such as *Drosophila melanogaster*, where other common tools are less effective or unavailable.

Keywords Cell signalling · Homeostasis · Tissue regeneration · Protein folding · Aging

Introduction

Adult organisms maintain their tissues and organs in a dynamic equilibrium, where cells die and are constantly replaced in a process of tissue homeostasis [1]. Homeostasis maintains the functionality of tissues and organ systems through stem cell mediated repair. This repair recruits stem cell pools which are maintained in specialized niches and regulated by extracellular signals or injury [2]. Stem cell niches have a complex, signal-rich environment which preserves tissue-specific stem cells. In aging organisms, these stem cell pools are depleted, initiating the degenerative process, where tissue homeostasis becomes less active [3, 4]. Mutations affecting maintenance signals can lead to disease, but recent studies into drug combinations show therapeutic potential, as they stimulate symmetric stem cell divisions, replenish the stem cell pool, and boost tissue regeneration [5, 6]. Given the importance of homeostasis in disease progression and aging, it is important to develop methods to study the complex interplay of these processes. Optogenetics is one such recent method being applied extensively to this field of research.

Optogenetics has been a particular success in *Drosophila* due to the many tools for gene manipulation. The most common approach has been to use the yeast-derived GAL4/UAS expression system to drive expression of optogenetic transgenes in a variety of tissues [7], especially when combined with specific integration sites to assure equal expression from different transgenes [8]. These can be combined with other binary expression systems such as lexA and QUAS to express different transgenes in different tissues [9, 10]. Temporal control of transgene expression can also be achieved using the temperature sensitive allele of the transcriptional repressor Gal80^{ts} [11], or drug inducible promoters such as RU486 inducible GeneSwitch Gal4 system [12]. The ease of fly genetics allows expression in traditional genetic mutant backgrounds as well as in combination with RNAi knockdowns available as a library of fly lines; resources which have recently been expanded to include guide RNAs for CRISPR/Cas9 and dCas9-VPR for gene knockout and overexpression [13]. To manipulate genes directly, without overexpression, many genes in the *Drosophila* genome have been modified with a landing site for recombination-mediated cassette exchange (RMCE) [14, 15]. This landing site can be used to introduce any tag as an

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additional exon allowing expression of a tagged version of the protein at endogenous levels, and has been used to introduce an optogenetic cassette directly into the genome [16].

Optogenetics encompass a toolkit of techniques to control neuronal firing, gene expression, and protein function through light-responsive proteins or protein domains [17–29]. The term "optogenetics" was used to describe approaches that combine *optical* and *genetic* manipulation of specific cell types or specific cellular processes [30]. Optogenetic tools have been applied to successfully address a number of questions in neuroscience and cell biology that require spatiotemporal and neurochemical precision in vitro or in vivo. This toolkit has expanded rapidly, generating many potential applications for research questions across disciplines which were previously difficult to address [31]. In this review, we focus on recent advances in optogenetic tools for the study of mechanisms behind homeostatic and degenerative processes. We discuss how innovative applications of optogenetics in stem cell biology, neurodegenerative diseases, and homeostasis are being used to fill existing knowledge gaps and suggest future directions for the utilization of this suite of tools.

Optogenetic approaches

A series of discoveries enabled the development of optogenetics, from basic characterisation of light-activated protein pumps to applications in neuronal firing. In 1971, well before optogenetics was established as a concept, the bacteriorhodopsin was among the first protein pumps that were characterised as being activated by visible light photons [32]. One of the earliest applications of a light-induced technique was to study gene expression through the ability of phytochrome B to bind reversibly to the transcription factor, PIF3 [33–35]. Researchers also developed other light-inducible methods which use optically gated ion channels, are non-invasive, and are able to control *Drosophila* behaviour [36] and neuronal activity with Rhodopsin [37]. These studies led to the discovery of several optically sensitive genetic proteins that can manipulate cellular physiology. In 2005, Deisseroth, Boyden, and colleagues demonstrated the ability to precisely control neural activity at a millisecond timescale by successfully introducing and activating light-sensitive, microbial opsins in mammalian neurons in vitro [38]. Deisseroth et al. coined the term "Optogenetics"[30], the short history of which was reviewed by Deisseroth [24] and by Boyden (from a first-person perspective) [39]. Optogenetics in its early stages was applied to behavioural neuroscience, but subsequently expanded to cell biology [40].

Optogenetic approaches can be separated into two broad categories. The first focuses on the use of photoactivatable protein domains to regulate protein localization or oligomerization [41]. Examples of these systems include cryptochrome 2 (CRY2) [42, 43], phytochrome B (phyB) [34, 44, 45], light, oxygen, and voltage (LOV) [46], and Dronpa [47, 48]. Another example is the functional expression of photoactivated adenylyl cyclases (PACs) for light-induced manipulation of the cAMP messenger molecule in animal cells [49]. For a review of these single and dual-protein approaches for multiscale control of protein function in signalling biology, please see [50]. The second category involves the use of antibody-like recombinant binders for a broader range of protein targets using intrabodies or nanobodies to bind to endogenous proteins, targets which were previously inaccessible by the approaches in the first category [51–53]. The light-programmable control of these recombinant binders greatly expands the list of potential protein targets that can be targeted. These protein domains can be fused to genes of interest, providing a simple modular system, as they require low levels of activation light, are reversible, and are often independent of exogenous cofactors [54]. Given the growing number of new optogenetic approaches, researchers can keep track of different systems

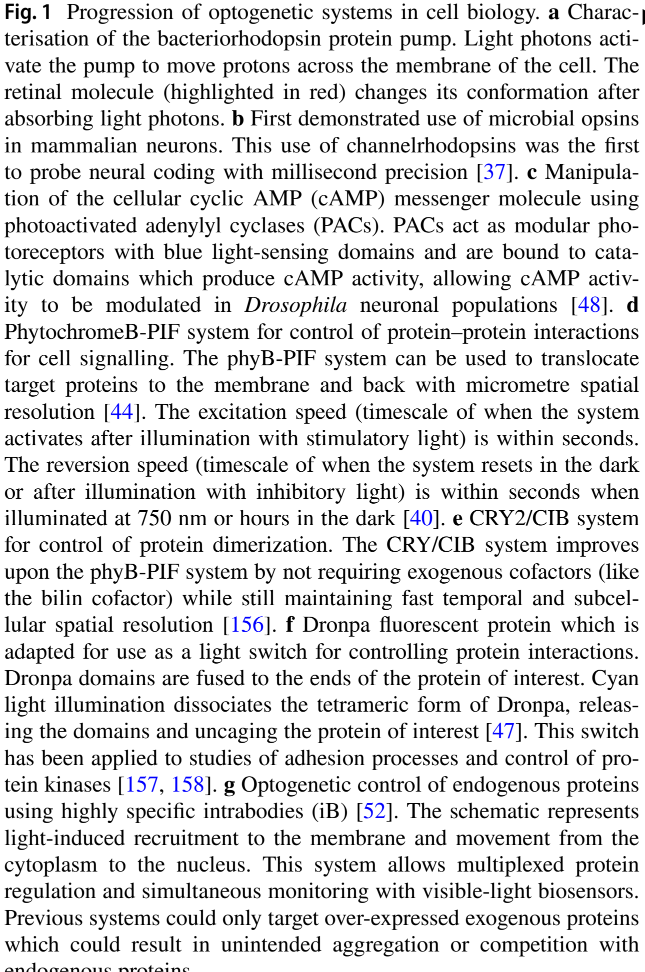


Fig. 1 Progression of optogenetic systems in cell biology. **a** Characterisation of the bacteriorhodopsin protein pump. Light photons activate the pump to move protons across the membrane of the cell. The retinal molecule (highlighted in red) changes its conformation after absorbing light photons. **b** First demonstrated use of microbial opsins in mammalian neurons. This use of channelrhodopsins was the first to probe neural coding with millisecond precision [37]. **c** Manipulation of the cellular cyclic AMP (cAMP) messenger molecule using photoactivated adenylyl cyclases (PACs). PACs act as modular photoreceptors with blue light-sensing domains and are bound to catalytic domains which produce cAMP activity, allowing cAMP activity to be modulated in *Drosophila* neuronal populations [48]. **d** PhytochromeB-PIF system for control of protein–protein interactions for cell signalling. The phyB-PIF system can be used to translocate target proteins to the membrane and back with micrometre spatial resolution [44]. The excitation speed (timescale of when the system activates after illumination with stimulatory light) is within seconds. The reversion speed (timescale of when the system resets in the dark or after illumination with inhibitory light) is within seconds when illuminated at 750 nm or hours in the dark [40]. **e** CRY2/CIB system for control of protein dimerization. The CRY/CIB system improves upon the phyB-PIF system by not requiring exogenous cofactors (like the bilin cofactor) while still maintaining fast temporal and subcellular spatial resolution [156]. **f** Dronpa fluorescent protein which is adapted for use as a light switch for controlling protein interactions. Dronpa domains are fused to the ends of the protein of interest. Cyan light illumination dissociates the tetrameric form of Dronpa, releasing the domains and uncaging the protein of interest [47]. This switch has been applied to studies of adhesion processes and control of protein kinases [157, 158]. **g** Optogenetic control of endogenous proteins using highly specific intrabodies (iB) [52]. The schematic represents light-induced recruitment to the membrane and movement from the cytoplasm to the nucleus. This system allows multiplexed protein regulation and simultaneous monitoring with visible-light biosensors. Previous systems could only target over-expressed exogenous proteins which could result in unintended aggregation or competition with endogenous proteins

System	Diagram	Innovation	Applications
(a) Characterisation of bacteriorhodopsin 1971 [32]	H+ H+ H+ 560nm Retinal	First characterisation of a proton pump which is activated by visible light photons	Channelrhodopsins [30-31, 37]
(b) Microbial opsins (ChR2-YFP) in neurons 2005 [30], [38]	ChR2-YFP construct added via lentiviral vector 450nm	First demonstrated use of microbial opsins (ChR2) in controlling neuronal activity and initial use of the term "optogenetics"	Defensive behaviour in *Drosophila* [31], odour sensing & collective behaviour [61], CsChrimson system [62]
(c) Use of modular photoreceptors to modulate cAMP messenger molecule 2007 [48]	450nm Light sensing domains Catalytic domains ATP cAMP Intracellular Signalling	First manipulation of cAMP messenger with spatiotemporal control	Olfactory stimulation & odour behaviour [63]
(d) Phytochrome-PIF system 2009 [33-35, 44-45]	PhyB Pr YFP mCh 650nm 750nm PhyB Pfr Excitation speed Seconds [40] Reversion speed Seconds (with illumination), hours (in dark) [40]	First spatiotemporal control of protein-protein interactions	optoSOS system [20]

System	Diagram	Innovation	Applications
(e) Cry2/CIB system 2010 [42-43, 156]	Gal4AD, 450nm, CIB, Cry2, Gal4BD; Excitation speed Seconds [40]; Reversion speed Minutes [40]	First system with no need for exogenous cofactors to be added. Light-induced spatiotemporal control of protein-protein dimerization	Stem cell signal transduction [16], protein aggregation [80], morphogen gradient regulation [114]
(f) Dronpa fluorescent protein 2012 [48]	500nm, 400nm; Excitation speed Seconds [40]; Reversion speed Seconds (with illumination), minutes (in dark) [40]	First use of a fluorescent protein as a light switch for controlling protein interactions	Adhesion and bio-mechanical processes [47], control of protein kinases [157-158]
(g) Intrabodies (iB) for endogenous proteins 2020 [51-53]	Recruitment to membrane: 740nm, Bacterial Phytochrome Pr, iB(GFP), QPAS1, mCh; Accumulation to nucleus: 740nm, iB(GFP), Nucleus, QPAS1, mCh	Use of intrabodies (antibody-like recombinant binders) which enables targeting of endogenous proteins	*To be determined*

Fig. 1 (continued)

using the OptoBase database [55]. The suite of optogenetic tools available has progressed significantly in the past 15 years and can be used to tackle complex questions in cell biology (Fig. 1).

Compared to classic genetic tools, optogenetic tools have the following advantages: (1) unmatched precision for spatial, temporal, and neurochemical regulation of proteins in in vitro and in vivo systems [54, 56, 57]; (2) great flexibility and reversibility, where the same approach can be used to turn pathways on or off in a variety of tissues or brain regions [56, 58]; and (3) the possibility of multiplexing [41, 59].

Applications of optogenetics

Neural signalling

Before optogenetic tools were developed in the 2000s, Francis Crick suggested in 1979 that a light-based approach would be useful to study neural signalling, so it was only natural that it was the first area of application for early optogenetics [31]. One of the first optogenetic systems used a combination of proteins including photo-reversible *Drosophila* rhodopsin NinaE, arrestin-2, and the α subunit of photoreceptor G-protein to sensitize cultured hippocampal

neurons to light stimulation [37]. Expression of channelrhodopsins in 2 distinct classes of presynaptic motoneurons, which innervate one postsynaptic cell, was used to study presynaptic homeostatic plasticity at the neuromuscular junction, demonstrating the usefulness of light-activated channels for targeted experiments [60]. Newer and improved tools, such as the red-light activated channelrhodopsin, CsChrimson, have been applied to study dopamine release in the ventral nerve cord and medial protocerebrum of the larval *Drosophila* central nervous system [61, 62], nociceptive-like escape behaviour (in response to noxious stimuli and painful experiences), and the curling and rolling escape response in the larvae [63]. Further improvements such as the addition of the Split-Gal4 system were used to map the behavioural phenotypes attributed to at least one third of all descending neurons in the fly [64]. A wide range of behaviours are being dissected using optogenetics, such as sensorimotor pathways underlying behaviours, back-walking/back-crawling [65], turning/head casting [66], escaping [67], and oviposition [68]. In vertebrates, optogenetics studies address motor control [69–72], reward system [73], learning, and memory [74, 75].

Neurodegeneration

Optogenetics can be used to study protein misfolding common in neurodegenerative diseases. For Alzheimer's disease (AD) and Parkinson's disease (PD), existing *Drosophila* models can be divided into three broad categories: (1) *Drosophila* orthologs of human disease genes; (2) transgenic constructs carrying alleles of human disease-causing genes; and (3) models used to study the effects of environmental stressors on Aβ toxicity [76]. Studies have also combined approaches, developing models that use both *Drosophila* orthologs of human disease genes and overexpression of human transgenes. Expression using the GAL4-UAS system and accumulation of Aβ-42 peptide alone is sufficient to cause neurodegeneration and behavioural defects in *Drosophila* models [77]. A novel optogenetic approach used the Cry2, blue-light sensitive oligomerizing protein to investigate the consequences of formation of Aβ oligomers in *Drosophila*, *C. elegans*, and *D. rerio* [78]. Both expression and induced oligomerization of Aβ reduced lifespan and healthspan, but the effects could be separated into metabolic problems induced by expression alone and physical damage caused by light-induced Aβ oligomerization [79, 80].

Similarly, PD is characterized by the aggregation of α-synuclein, or Lewy bodies, within cells of the central nervous system. α-synuclein is hypothesised to contribute to toxicity and subsequent death of dopaminergic neurons in the substantia nigra pars compacta which is responsible for motor control [81]. To study the fixed stages of PD, an optogenetic PD model was constructed by introduction of the halorhodopsin (NpHR) gene, a light-gated chloride channel which inhibits neurons, into the substantia nigra compacta [82]. Unlike the classical animal model of PD, this novel optogenetic approach avoids causing damage to the nigrostriatal system and can be used as a PD model for various stages of the disease by adjustment of illumination parameters [82].

Other studies looked at TDP-43, a protein excluded from the nucleus into insoluble cytoplasmic inclusions, observed in post-mortem patient tissue in neurodegenerative disorders including Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal dementia (FTD) [83, 84]. An optogenetic model of TDP-43 proteinopathy was developed by expressing Cry2 fused to human TDP-43 (optoTDP43). This in vivo model induced TDP-43 cytoplasmic aggregation and recapitulated important features of patient pathology such as progressive motor dysfunction, addressing the link between TDP-43 cytoplasmic aggregation and toxicity.

Stem cells, development, and tissue regeneration

The process of gastric epithelial homeostasis and regeneration has become an important model for understanding the regulation of stem cells [85, 86]. As these are adult tissues, genetic approaches must circumvent developmental stages using temperature-sensitive or tissue-specific overexpression, knockdown, or CRISPR/Cas9 [87–89]. One approach used signalling pathway components fused to CRY2 to regulate signalling using light in the adult fly midgut stem cell model [16]. A limitation of traditional overexpression or knockout techniques is that they affect stem cell regulation across the entire lifespan of the fly. This approach avoided the developmental effects of modulated stem cell signalling by raising flies in the dark and using light only at adult stages to activate the targeted pathway when required. Targeting intestinal stem cell signalling pathways with optogenetics allows perturbation at specific ages of *Drosophila* (e.g., young, adult, and old) to establish an age-based link to the effects of signals on gastric homeostasis.

Developmental processes such as tissue morphogenesis can be studied using optogenetic approaches. For example, morphogenesis during embryonic *Drosophila* development has been studied using optogenetics in a variety of ways [90]. A non-exhaustive list of optogenetic approaches to study *Drosophila* development includes: the structure of centrosomes [91], Erk signalling [27, 92–94], Toll signalling [95], adhesion [56, 96–98], cell shapes [99], GTPase activity [45, 100–110], anterior-to-posterior patterning through Bicoid [111], Wnt [112], and Notch signalling [113]. One approach that looked at phosphatidylinositol-4,5 biphosphate [PI(4,5)P2] during *Drosophila* ventral furrow formation showed that it was required for apical constriction, an essential tissue invagination process [114, 115]. The study

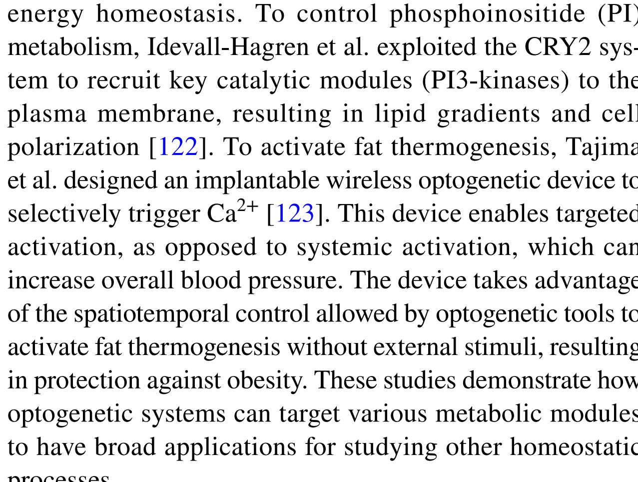
observed that optogenetics can be utilized to control cell contractility in *Drosophila* adding tissue mechanics to light-regulatable processes [116].

Optogenetic control of cell differentiation is also applicable to regenerative medicine and tissue engineering. These systems typically provide reversible changes through protein–protein interactions or temporary changes which only last for the lifetime of the modified proteins. Tissue engineering requires long-term changes in gene expression through a spatially dependent “on” switch. Polstein et al. engineered such an optogenetic switch that would permanently express a transgene upon illumination by blue light using the CRY2 system and incorporating a myogenic differentiation factor in vivo in a mouse model [117]. Their result demonstrated that sustained transgene activation of in vivo cell differentiation and tissue morphogenesis was possible after an initial dose of illumination.

Nutrient and metabolic homeostasis

To optimize their fitness levels, animals vary their food choices with their body needs. For lab-raised *Drosophila*, yeast is the primary source of protein and nutrients. When investigating the chemosensory channels mediating yeast feeding, researchers found that gustatory receptor neurons (GRNs) of the taste peg, a sensory structure, and sensillar GRNs sustain and initiate yeast feeding, respectively [118]. The response of the yeast-sensitive GRNs is intensified following deprivation from amino acids, in turn modulating nutrient homeostasis. This result was determined using the FlyPAD assay, which allows automated, high-throughput analysis of feeding behaviour with temporal resolution [119]. Incorporation of optogenetics into the FlyPAD assay, known as the optoPAD, led to a high-throughput system that allowed direct manipulation of neurons to induce appetitive or aversive effects on feeding by activating or inhibiting GRNs. [120]. The adjustable light pulse of the optoPAD system, which can be dynamically linked to observed behaviour, makes it ideal for silencing neurons or activating reinforcement circuits. OptoPAD allowed “virtual gustatory realities” and induced appetitive behaviours by activating or inhibiting sweet and bitter neurons, respectively [120]. Another system, the Sip-Triggered Optogenetic Behavior Enclosure (‘STROBE’), addressed the challenge of manipulating feeding circuits in freely moving animals when the timing of sensory inputs is affected by the animals’ behavior. STROBE was able to temporally couple neuron activation with feeding events to find that coincident activation of sweet neurons upon food contact drives appetitive behavior and activation of bitter neurons drives aversive behavior [121].

Emerging optogenetic tools focus on detection of metabolic pathway components and modulation of whole-body



energy homeostasis. To control phosphoinositide (PI) metabolism, Idevall-Hagren et al. exploited the CRY2 system to recruit key catalytic modules (PI3-kinases) to the plasma membrane, resulting in lipid gradients and cell polarization [122]. To activate fat thermogenesis, Tajima et al. designed an implantable wireless optogenetic device to selectively trigger Ca^{2+} [123]. This device enables targeted activation, as opposed to systemic activation, which can increase overall blood pressure. The device takes advantage of the spatiotemporal control allowed by optogenetic tools to activate fat thermogenesis without external stimuli, resulting in protection against obesity. These studies demonstrate how optogenetic systems can target various metabolic modules to have broad applications for studying other homeostatic processes.

Cardiac homeostasis

Optogenetic pacing of the heart allows non-invasive as well as highly precise spatiotemporal electrical stimulation. Transgenic flies expressing a light-gated cation channel, channelrhodopsin-2 (ChR2), paced contractions optogenetically at different developmental stages, including the larval, pupal, and adult stages. Cardiac function and its response to pacing stimulation were tracked using a high-speed and ultra-high-resolution optical coherence imaging system, serving as a powerful tool for basic heart research [124]. A hypersensitive variant of ChR2 (ChR2.XXL) was able to elevate the *Drosophila* larval heart rate over a range of temperatures and calcium levels, showing the potential of using light as a pacemaker in mammalian heart transplant conditions [125]. Further optogenetic studies using this variant showed that activation of cholinergic, dopaminergic, and serotonergic neurons also causes the release of cardioactive substances that increase heart rate over a range of temperatures [124–128].

Future directions in application of optogenetics to study homeostasis

Targeting regulatory signalling pathways of intestinal stem cells

Moving forward, optogenetic fusion constructs can be used with major components in signalling pathways to apply spatiotemporal control of homeostasis. For example, the Keap1–Nrf2 regulator pathway has potential for perturbation with optogenetics. Nrf2 is a master regulator of the cellular redox state. It controls the proliferative activity of ISCs and promotes intestinal homeostasis. Loss of Nrf2 in ISCs causes reactive oxygen species (ROS) accumulation and accelerates age-related degeneration of the intestinal epithelium [129]. In ISCs, loss of Keap1, a

Fig. 2 Potential optogenetic manipulation of the Keap1–Nrf2 pathway. The Keap1–Nrf2 pathway can progress to degrade Nrf2 in the cytoplasm after ubiquitination. Alternatively, if Nrf2 is activated by ROS or electrophiles, it will dissociate from the Keap1 dimer and translocate into the nucleus. Subsequently, Nrf2 will dimerize with the transcriptional activator, Maf. It will begin transcription of target genes that contain the antioxidant response element (ARE) motif. **a** Potential optogenetic perturbation of Keap1 (with a fused optogenetic construct such as CRY2/CIB) could induce clustering [16, 137] and prevent Keap1 from forming a homodimer and functioning normally. This perturbation could act as a potential gain-of-function of Nrf2 target gene transcription. **b** Potential optogenetic perturbation of Nrf2 (with a fused construct such as CRY2/CIB) could cause Nrf2 to cluster and prevent movement through the nuclear membrane. Such regulation would act as a potential loss-of-function of Nrf2 target gene transcription

negative regulator for Nrf2, decreases intracellular ROS levels and regulates redox balance to control ISC proliferation as Keap1 homodimers promote proteasomal degradation of Nrf2 in the cytoplasm [130]. Post-translational modification of Keap1 by ROS (or electrophiles) leads to the release of Nrf2, and its translocation into the nucleus [131]. Nrf2 enters the nucleus, where it dimerizes with Maf family transcription factors, inducing expression of target genes [132].

The Keap1–Nrf2 pathway could be perturbed with optogenetics at each of these three points: (1) during the homodimerization of Keap1 (as illustrated in Fig. 2a); (2) the nuclear translocation of Nrf2 (Fig. 2b); or (3) the dimerization of Nrf2 with a transcriptional activator. In this pathway, an optogenetic approach which can take advantage of

Table 1 Stem cell signalling pathways already targeted with optogenetics

Signalling pathways	Function	Protein interaction	Optogenetic system used
Wnt/Wg	ISC maintenance [159], cell fate specification, cell polarity [160]	Beta-catenin (Arm) oligomerization	CRY2 [137]
RTK	Cell proliferation, differentiation, cell cycle control, cell metabolism [161]	Membrane receptor oligomerization	CLICR [138]
Ras/ERK	Cell proliferation, cell cycle arrest, differentiation [162]	Plasma membrane recruitment	OptoSOS [22]
Toll	Immune response, cell proliferation [163]	Membrane receptor oligomerization	CRY2 [16]
EGFR	Cell division, survival, migration [164, 165]	Membrane receptor oligomerization	CRY2 [16]
BMP	Embryogenesis, adult tissue maintenance [166]	Dimerization, recruitment of SMAD proteins	optoBMP [140]
FGF	Cell proliferation, migration, patterning [167]	Membrane receptor homodimerization	OptoFGFR1/CRY2 [141]

Each pathway plays a unique but interconnected function in regulating stem cell and homeostatic activity. With an understanding of the protein interactions for each pathway, specific optogenetic systems have been applied, optimized, or developed to regulate activity

Table 2 Stem cell signalling pathways with potential for optogenetic manipulation

Signalling pathways	Function	Protein interaction
Nrf/Keap1	Redox regulation [129, 168]	Dimerization, nucleartranslocation
Notch	Neural differentiation, ISC maintenance, enterocyte differentiation [169, 170]	Nuclear translocation
Hedgehog	Embryo pattern formation, progenitor and stem cell proliferation [171]	Protein–protein interactions
Hippo/YAP	Cell proliferation, apoptosis [172]	Phosphorylation
JAK/STAT	Cell proliferation, stem cell maintenance [173]	Dimerization, nuclear translocation

This non-exhaustive list highlights signalling pathways which have not yet been targeted with optogenetics, despite their important roles in homeostasis. The specific protein interactions of these signalling pathways inform the type of optogenetic system that researchers can use for spatiotemporal control and investigation

protein dimerization would be appropriate (to prevent Keap1 dimerization, induce Nrf2 clustering to modify its size and ability to move into the nucleus, or prevent Nrf2 binding to a transcriptional activator). For example, CRY2-based tools would be useful here, because the light-sensitive CRY2 protein, fused to the protein of interest, can bind to a partner protein (such as CIB) and modulate dimerization. Alternatively, another approach to knockdown Nrf2 is to use light-controlled degradation tools which regulate protein stability by fusing the protein of interest to a degradation module and light-responsive domain like LOV [133–135]. Given the interaction of ROS with Keap1, future studies could combine this application with existing optogenetic tools of ROS production [136].

As summarized in Table 1, stem cell signalling pathways that have already been studied using optogenetics include Wnt [137], RTK (receptor tyrosine kinase) [138], Ras/ERK (extracellular signal regulated kinase) [22], Toll, EGFR (epidermal growth factor receptor) [16, 139], BMP (bone morphogenetic protein) [140], and FGF (fibroblast growth factor) [141]. Conversely, a non-exhaustive list of other potential signalling pathways which have not yet been studied with optogenetics (at the time of this review), but have significant potential include Notch, Hedgehog, Hippo/YAP (Yes-associated protein), and JAK/STAT (Janus kinase/signal transducers and activators of transcription) (Table 2). The application of optogenetics to regulate Nrf2 and other pathways involved in stem cell activity will provide researchers with more precise tools to study age-related degeneration and aging (for example, comparing median-age flies to elderly flies).

Models for protein-folding diseases

Given the recent success of optogenetics-based models, these tools have significant potential to create models of protein-folding diseases or proteopathies. One potential new model would involve optogenetically induced aggregation of amylin to study Type 2 diabetes. One of the pathological hallmarks of Type 2 diabetes is the presence of islet amyloid deposits composed of amylin (human islet amyloid polypeptide precursor or hIAPP) [142]. Amylin is a 37-amino-acid polypeptide that is toxic to pancreatic islet cells [143]. Levels of human amylin cause different extents of oligomerization, which correspond to diabetes disease stages. Diabetes phenotypes differ according to amylin overproduction levels [144], and there are similarities between Type 2 diabetes and Alzheimer's disease [145]. The toxicity of amylin fibrils is

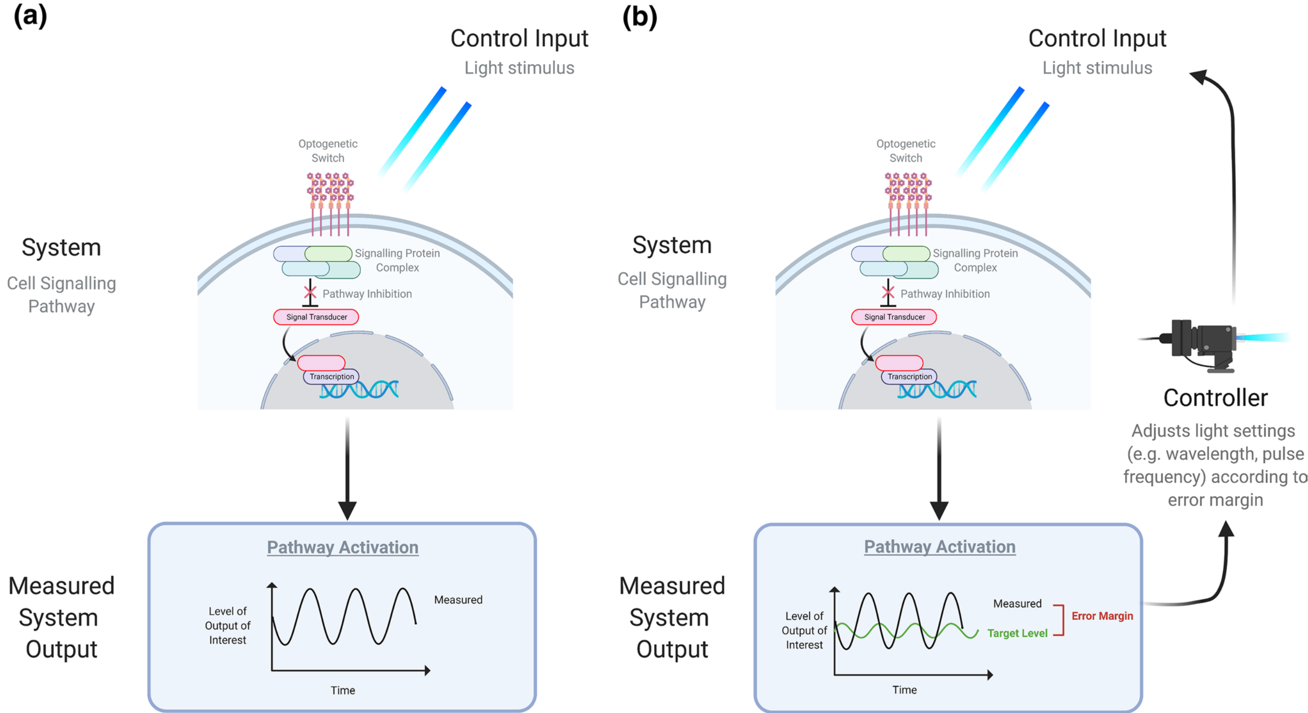


Fig. 3 Open- and closed-loop approaches for optogenetics. **a** Open-loop approach. The control input is the light stimulus and the output is a selected measure of interest for pathway activity. The measured output of interest has no impact on the control input. **b** Closed-loop approach. The target is a predetermined level of a measure of interest (e.g., pathway activation). When the measured output level differs from the expected target level, the controller considers the "error margin" between both levels, and adjusts the input accordingly. Examples of adjustments include changing the light wavelength or pulse frequency of illumination. The measured output influences the control input via a feedback loop

similar to the toxicity of Amyloid-β proteins in Alzheimer's disease [143]. Amylin oligomerization in the pancreas and amyloid formation in the brain contributes to the degeneration in AD and Type 2 diabetes [146].

Since the fat body organ in *Drosophila* plays a similar function in energy storage and metabolism to the human liver, it is a useful in vivo model to study amylin aggregation. Expression of human and mouse amylin to induce amylin aggregates in central nervous system (CNS) and fat body regions have been completed [147]. In addition, researchers have completed a 3D crystal structure analysis of hIAPP in *Drosophila* fat body tissue [148], providing useful insights for manipulation of the aggregation process. Future studies could apply optogenetic systems which regulate protein oligomerization, such as CRY2, PhyB, and LOV, to induce amylin aggregation. The optogenetic advantage of spatiotemporal control enables three variables for researchers to control when studying amylin and its effects: (1) levels of amylin aggregation; (2) age or time points at which aggregation is induced;, and (3) chosen areas of fat body tissue or central nervous system (CNS) to induce aggregation. Optogenetics would enable control of these three important dimensions for a useful Type-2 diabetes in vivo model of amylin-based pancreas degeneration in *Drosophila*.

Optogenetics can also augment models of neurodegenerative diseases, where protein misfolding plays a major role. The mechanisms by which protein aggregation relates to neurodegeneration are still subject to debate. Optogenetic control of protein aggregation can help to provide some insight into this gap. For example, in Huntington's disease patients, the Huntingtin protein mis-folds and aggregates because of an expanded polyQ sequence in the gene [149]. Studies could take advantage of the control and visible readouts of protein aggregation with models that use optogenetic fusion constructs with the Huntingtin protein or any other proteins that aggregate. These models would adopt a similar approach to previously developed Amyloid models.

Adopting a closed-loop approach

In addition to new models of disease, a closed-loop approach to designing optogenetic tools for cell signalling networks will also be an important step for the future of optogenetics in homeostasis (Fig. 3). Closed-loop control is an engineering concept that is defined as using the difference between the measured output and a desired target to guide changes in the control inputs to a system [150]. This stands in contrast to open-loop control, where the measured output of a

system has no effect on the control input. In neuroscience, this approach has been adopted and involves perturbing neural systems to have real-time control over neural dynamics and animal behaviour [120, 121, 150, 151].

Given the complexity of cell signalling networks, a similar closed-loop approach would yield important insights for the interplay between signalling pathways. A closed-loop approach requires three parts: (1) availability of a target; (2) system control inputs; and (3) measured system outputs [150]. The system control input would be light stimulus that is modulated based on measured output [150]. In cell signalling networks, the measured system output could be any of the downstream proteins or effects of the signalling pathway (such as protein activity, gene expression or membrane modification). The target would be a selected level of downstream activity of the signalling pathway. "Closing the loop" with optogenetics would help to elucidate the delicate feedback mechanisms involved in homeostatic signalling.

In cell biology, the future challenge will be to develop accurate readouts of the output of interest. For example, in the Wnt signalling pathway, a potential measured output would be the recruitment of Dishevelled (Dvl) proteins to the plasma membrane or stabilization of β-catenin when canonical Wnt signalling is activated [152]. This activity would then be used to create automatic feedback for modulating the optogenetic light stimulus. The diverse range of output types to measure and target in cell signalling will remain a challenge to be solved when applying a closed-loop control approach.

Even though optogenetics provides unparalleled spatiotemporal control, researchers should keep in mind the limitations of this tool, controls required and alternate approaches to ensure experiments are designed appropriately and data are interpreted carefully. Some examples include: (1) excitation with high frequency blue light is toxic to tissues, causes excessive heating [153], and has limited penetration to deeper tissues [154]. (2) Light can affect normal physiological processes such as brain function and temperature changes associated with light illumination during in vivo optogenetic manipulations could have both electrophysiological and behavioural consequences [155]. (3) This can be overcome by targeting optogenetic tools that are excited by near-infrared light (780–1100 nm) which causes less thermal heating and damage, in addition to displaying deep penetration [154]. (4) Careful experimental design to control the time course of light-driven responses, such as using pulses of light instead of continuous illumination which generates more heat, can help to remove confounding factors [155]. (5) The use of controls is required to substantiate that the optogenetic manipulations are physiological. (6) Different optogenetic constructs have inherent limitations such as specific on/off kinetics, excitation and reversal time (Fig. 1) that might render them unsuitable for certain applications.

Conclusion

In this review, we discussed recent advancements and applications of optogenetic approaches in homeostasis. The rapidly growing optogenetic toolkit is enabling the perturbation of a diverse range of targets and in vivo processes. Where other approaches and techniques may fail or lack in spatiotemporal control and specificity, optogenetics yields significant potential to uncover new insights for stem cells, developmental biology, protein-folding diseases, and cell signalling circuitry.

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Declarations

Conflict of interest We have no conflicts of interest to declare.

References

1. Pellettieri J, Alvarado AS (2007) Cell turnover and adult tissue homeostasis: from humans to planarians. Annu Rev Genet 41:83–105
2. Biteau B, Hochmuth CE, Jasper H (2011) Maintaining tissue homeostasis: dynamic control of somatic stem cell activity. Cell Stem Cell 9(5):402–411. https://doi.org/10.1016/j.stem.2011.10.004
3. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell 153(6):1194–1217. https://doi.org/10.1016/j.cell.2013.05.039
4. Chandel NS, Jasper H, Ho TT, Passegue E (2016) Metabolic regulation of stem cell function in tissue homeostasis and organismal ageing. Nat Cell Biol 18(8):823–832. https://doi.org/10.1038/ncb3385
5. Castillo-Quan JI, Tain LS, Kinghorn KJ, Li L, Gronke S, Hinze Y, Blackwell TK, Bjedov I, Partridge L (2019) A triple drug combination targeting components of the nutrient-sensing network maximizes longevity. Proc Natl Acad Sci USA 116(42):20817–20819. https://doi.org/10.1073/pnas.1913212116
6. Admasu TD, Chaithanya Batchu K, Barardo D, Ng LF, Lam VYM, Xiao L, Cazenave-Gassiot A, Wenk MR, Tolwinski NS, Gruber J (2018) Drug synergy slows aging and improves healthspan through IGF and SREBP lipid signaling. Dev Cell 47(1):67–79
7. Brand AH, Perrimon N (1994) Raf acts downstream of the EGF receptor to determine dorsoventral polarity during *Drosophila oogenesis*. Genes Dev 8(5):629–639

8. Groth AC, Fish M, Nusse R, Calos MP (2004) Construction of transgenic drosophila by using the site-specific integrase from phage φC31. Genetics 166(4):1775–1782. https://doi.org/10.1534/genetics.166.4.1775
9. Kockel L, Huq LM, Ayyar A, Herold E, MacAlpine E, Logan M, Savvides C, Kim GES, Chen J, Clark T, Duong T, Fazel-Rezai V, Havey D, Han S, Jagadeesan R, Kim ESJ, Lee D, Lombardo K, Piyale I, Shi H, Stahr L, Tung D, Tayvah U, Wang F, Wang J-H, Xiao S, Topper SM, Park S, Rotondo C, Rankin AE, Chisholm TW, Kim SK (2016) A *Drosophila* LexA enhancer-trap resource for developmental biology and neuroendocrine research. G3: Genes Genomes Genet 6(10):307–3026
10. Potter CJ, Tasic B, Russler EV, Liang L, Luo L (2010) The Q system: a repressible binary system for transgene expression, lineage tracing, and mosaic analysis. Cell 141(3):536–548. https://doi.org/10.1016/j.cell.2010.02.025
11. McGuire SE, Mao Z, Davis RL (2004) Spatiotemporal gene expression targeting with the TARGET and gene-switch systems in *Drosophila*. Sci STKE 2004(220):pl6
12. Osterwalder T, Yoon KS, White BH, Keshishian H (2001) A conditional tissue-specific transgene expression system using inducible GAL4. Proc Natl Acad Sci USA 98(22):12596–12601. https://doi.org/10.1073/pnas.221303298
13. Hu Y, Comjean A, Rodiger J, Liu Y, Gao Y, Chung V, Zirin J, Perrimon N, Mohr SE (2020) FlyRNAi.org—the database of the *Drosophila* RNAi screening center and transgenic RNAi project: 2021 update. Nucleic Acids Res 49(D1):D908–D915. https://doi.org/10.1093/nar/gkaa936
14. Venken KJT, Schulze KL, Haelterman NA, Pan H, He Y, Evans-Holm M, Carlson JW, Levis RW, Spradling AC, Hoskins RA, Bellen HJ (2011) MiMIC: a highly versatile transposon insertion resource for engineering Drosophila melanogaster genes. Nat Methods 8(9):737–743. https://doi.org/10.1038/nmeth.1662
15. Nagarkar-Jaiswal S, DeLuca SZ, Lee P-T, Lin W-W, Pan H, Zuo Z, Lv J, Spradling AC, Bellen HJ (2015) A genetic toolkit for tagging intronic MiMIC containing genes. Elife 4:e08469
16. Bunnag N, Tan QH, Kaur P, Ramamoorthy A, Sung ICH, Lusk J, Tolwinski NS (2020) An optogenetic method to study signal transduction in intestinal stem cell homeostasis. J Mol Biol 432(10):3159–3176. https://doi.org/10.1016/j.jmb.2020.03.019
17. Gradinaru V, Zhang F, Ramakrishnan C, Mattis J, Prakash R, Diester I, Goshen I, Thompson KR, Deisseroth K (2010) Molecular and cellular approaches for diversifying and extending optogenetics. Cell 141(1):154–165. https://doi.org/10.1016/j.cell.2010.02.037
18. Fenno L, Yizhar O, Deisseroth K (2011) The development and application of optogenetics. Annu Rev Neurosci 34:389–412. https://doi.org/10.1146/annurev-neuro-061010-113817
19. Pastrana E (2011) Optogenetics: controlling cell function with light. Nat Methods 8(1):24–25
20. Toettcher JE, Voigt CA, Weiner OD, Lim WA (2011) The promise of optogenetics in cell biology: interrogating molecular circuits in space and time. Nat Methods 8(1):35–38. https://doi.org/10.1038/nmeth.f.326
21. Yizhar O, Fenno LE, Davidson TJ, Mogri M, Deisseroth K (2011) Optogenetics in neural systems. Neuron 71(1):9–34. https://doi.org/10.1016/j.neuron.2011.06.004
22. Toettcher JE, Weiner OD, Lim WA (2013) Using optogenetics to interrogate the dynamic control of signal transmission by the Ras/Erk module. Cell 155(6):1422–1434
23. Hausser M (2014) Optogenetics: the age of light. Nat Methods 11(10):1012–1014. https://doi.org/10.1038/nmeth.3111
24. Deisseroth K (2015) Optogenetics: 10 years of microbial opsins in neuroscience. Nat Neurosci 18(9):1213–1225. https://doi.org/10.1038/nn.4091
25. Steinbeck JA, Choi SJ, Mrejeru A, Ganat Y, Deisseroth K, Sulzer D, Mosharov EV, Studer L (2015) Optogenetics enables functional analysis of human embryonic stem cell-derived grafts in a Parkinson's disease model. Nat Biotechnol 33(2):204–209. https://doi.org/10.1038/nbt.3124
26. Chen S, Weitemier AZ, Zeng X, He L, Wang X, Tao Y, Huang AJY, Hashimotodani Y, Kano M, Iwasaki H, Parajuli LK, Okabe S, Teh DBL, All AH, Tsutsui-Kimura I, Tanaka KF, Liu X, McHugh TJ (2018) Near-infrared deep brain stimulation via upconversion nanoparticle-mediated optogenetics. Science 359(6376):679–684. https://doi.org/10.1126/science.aaq1144
27. Johnson HE, Toettcher JE (2018) Illuminating developmental biology with cellular optogenetics. Curr Opin Biotechnol 52:42–48. https://doi.org/10.1016/j.copbio.2018.02.003
28. Stabel R, Stuven B, Hansen JN, Korschen HG, Wachten D, Moglich A (2019) Revisiting and redesigning light-activated cyclic-mononucleotide phosphodiesterases. J Mol Biol 431(17):3029–3045. https://doi.org/10.1016/j.jmb.2019.07.011
29. Tichy AM, Gerrard EJ, Legrand JMD, Hobbs RM, Janovjak H (2019) Engineering strategy and vector library for the rapid generation of modular light-controlled protein-protein interactions. J Mol Biol 431(17):3046–3055. https://doi.org/10.1016/j.jmb.2019.05.033
30. Deisseroth K, Feng G, Majewska AK, Miesenbock G, Ting A, Schnitzer MJ (2006) Next-generation optical technologies for illuminating genetically targeted brain circuits. J Neurosci 26(41):10380–10386. https://doi.org/10.1523/JNEUROSCI.3863-06.2006
31. Deisseroth K (2011) Optogenetics. Nat Methods 8(1):26–29. https://doi.org/10.1038/nmeth.f.324
32. Oesterhelt D, Stoeckenius W (1971) Rhodopsin-like protein from the purple membrane of *Halobacterium halobium*. Nat New Biol 233(39):149–152. https://doi.org/10.1038/newbio233149a0
33. Ni M, Tepperman JM, Quail PH (1999) Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. Nature 400(6746):781–784. https://doi.org/10.1038/23500
34. Shimizu-Sato S, Huq E, Tepperman JM, Quail PH (2002) A light-switchable gene promoter system. Nat Biotechnol 20(10):1041–1044. https://doi.org/10.1038/nbt734
35. Zhu Y, Tepperman JM, Fairchild CD, Quail PH (2000) Phytochrome B binds with greater apparent affinity than phytochrome A to the basic helix–loop–helix factor PIF3 in a reaction requiring the PAS domain of PIF3. Proc Natl Acad Sci 97(24):13419. https://doi.org/10.1073/pnas.230433797
36. Lima SQ, Miesenböck G (2005) Remote control of behavior through genetically targeted photostimulation of neurons. Cell 121(1):141–152. https://doi.org/10.1016/j.cell.2005.02.004
37. Zemelman BV, Lee GA, Ng M, Miesenbock G (2002) Selective photostimulation of genetically chARGed neurons. Neuron 33(1):15–22. https://doi.org/10.1016/s0896-6273(01)00574-8
38. Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K (2005) Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci 8(9):1263–1268
39. Boyden ES (2011) A history of optogenetics: the development of tools for controlling brain circuits with light. F1000 Biol Rep. https://doi.org/10.3410/B3-11
40. Guglielmi G, Falk HJ, De Renzis S (2016) Optogenetic control of protein function: from intracellular processes to tissue morphogenesis. Trends Cell Biol 26(11):864–874
41. Tischer D, Weiner OD (2014) Illuminating cell signalling with optogenetic tools. Nat Rev Mol Cell Biol 15(8):551–558. https://doi.org/10.1038/nrm3837
42. Yu X, Sayegh R, Maymon M, Warpeha K, Klejnot J, Yang H, Huang J, Lee J, Kaufman L, Lin C (2009) Formation of nuclear bodies of *Arabidopsis* CRY2 in response to blue light is

associated with its blue light–dependent degradation. Plant Cell 21(1):118–130
43. Park H, Kim NY, Lee S, Kim N, Kim J, Heo WD (2017) Optogenetic protein clustering through fluorescent protein tagging and extension of CRY2. Nat Commun 8(1):30. https://doi.org/10.1038/s41467-017-00060-2
44. Tyszkiewicz AB, Muir TW (2008) Activation of protein splicing with light in yeast. Nat Methods 5:303–305
45. Levskaya A, Weiner OD, Lim WA, Voigt CA (2009) Spatiotemporal control of cell signalling using a light-switchable protein interaction. Nature 461(7266):997–1001. https://doi.org/10.1038/nature08446
46. Briggs WR, Christie JM, Salomon M (2001) Phototropins: a new family of flavin-binding blue light receptors in plants. Antioxid Redox Signal 3(5):775–788. https://doi.org/10.1089/15230860152664975
47. Johr R, Bauer MS, Schendel LC, Kluger C, Gaub HE (2019) Dronpa: a light-switchable fluorescent protein for opto-biomechanics. Nano Lett 19(5):3176–3181. https://doi.org/10.1021/acs.nanolett.9b00639
48. Zhou XX, Chung HK, Lam AJ, Lin MZ (2012) Optical control of protein activity by fluorescent protein domains. Science 338(6108):810. https://doi.org/10.1126/science.1226854
49. Schroder-Lang S, Schwarzel M, Seifert R, Strunker T, Kateriya S, Looser J, Watanabe M, Kaupp UB, Hegemann P, Nagel G (2007) Fast manipulation of cellular cAMP level by light in vivo. Nat Methods 4(1):39–42. https://doi.org/10.1038/nmeth975
50. Goglia AG, Toettcher JE (2019) A bright future: optogenetics to dissect the spatiotemporal control of cell behavior. Curr Opin Chem Biol 48:106–113. https://doi.org/10.1016/j.cbpa.2018.11.010
51. Yu D, Lee H, Hong J, Jung H, Jo Y, Oh B-H, Park BO, Heo WD (2019) Optogenetic activation of intracellular antibodies for direct modulation of endogenous proteins. Nat Methods 16(11):1095–1100. https://doi.org/10.1038/s41592-019-0592-7
52. Gil AA, Carrasco-López C, Zhu L, Zhao EM, Ravindran PT, Wilson MZ, Goglia AG, Avalos JL, Toettcher JE (2020) Optogenetic control of protein binding using light-switchable nanobodies. Nat Commun 11(1):4044. https://doi.org/10.1038/s41467-020-17836-8
53. Redchuk TA, Karasev MM, Verkhusha PV, Donnelly SK, Hülsemann M, Virtanen J, Moore HM, Vartiainen MK, Hodgson L, Verkhusha VV (2020) Optogenetic regulation of endogenous proteins. Nat Commun 11(1):605. https://doi.org/10.1038/s41467-020-14460-4
54. Mansouri M, Strittmatter T, Fussenegger M (2019) Light-controlled mammalian cells and their therapeutic applications in synthetic biology. Adv Sci (Weinh) 6(1):1800952. https://doi.org/10.1002/advs.201800952
55. Kolar K, Knobloch C, Stork H, Žnidarič M, Weber W (2018) OptoBase: a web platform for molecular optogenetics. ACS Synth Biol 7(7):1825–1828
56. Krueger D, Izquierdo E, Viswanathan R, Hartmann J, Pallares Cartes C, De Renzis S (2019) Principles and applications of optogenetics in developmental biology. Development. https://doi.org/10.1242/dev.175067
57. Chan YB, Alekseyenko OV, Kravitz EA (2015) Optogenetic control of gene expression in *Drosophila*. PLoS ONE 10(9):e0138181. https://doi.org/10.1371/journal.pone.0138181
58. Wiegert JS, Mahn M, Prigge M, Printz Y, Yizhar O (2017) Silencing neurons: tools, applications, and experimental constraints. Neuron 95(3):504–529. https://doi.org/10.1016/j.neuron.2017.06.050
59. Guru A, Post RJ, Ho YY, Warden MR (2015) Making sense of optogenetics. Int J Neuropsychopharmacol. https://doi.org/10.1093/ijnp/pyv079
60. Genc O, Davis GW (2019) Target-wide induction and synapse type-specific robustness of presynaptic homeostasis. Curr Biol 29(22):3863–3873. https://doi.org/10.1016/j.cub.2019.09.036
61. Klapoetke NC, Murata Y, Kim SS, Pulver SR, Birdsey-Benson A, Cho YK, Morimoto TK, Chuong AS, Carpenter EJ, Tian Z, Wang J, Xie Y, Yan Z, Zhang Y, Chow BY, Surek B, Melkonian M, Jayaraman V, Constantine-Paton M, Wong GK, Boyden ES (2014) Independent optical excitation of distinct neural populations. Nat Methods 11(3):338–346. https://doi.org/10.1038/nmeth.2836
62. Privman E, Venton BJ (2015) Comparison of dopamine kinetics in the larval Drosophila ventral nerve cord and protocerebrum with improved optogenetic stimulation. J Neurochem 135(4):695–704. https://doi.org/10.1111/jnc.13286
63. Dason JS, Cheung A, Anreiter I, Montemurri VA, Allen AM, Sokolowski MB (2020) Drosophila melanogaster foraging regulates a nociceptive-like escape behavior through a developmentally plastic sensory circuit. Proc Natl Acad Sci USA 117(38):23286–23291. https://doi.org/10.1073/pnas.1820840116
64. Cande J, Namiki S, Qiu J, Korff W, Card GM, Shaevitz JW, Stern DL, Berman GJ (2018) Optogenetic dissection of descending behavioral control in *Drosophila*. Elife. https://doi.org/10.7554/eLife.34275
65. Sen R, Wang K, Dickson BJ (2019) TwoLumps ascending neurons mediate touch-evoked reversal of walking direction in *Drosophila*. Curr Biol 29(24):4337–4344. https://doi.org/10.1016/j.cub.2019.11.004
66. Tastekin I, Khandelwal A, Tadres D, Fessner ND, Truman JW, Zlatic M, Cardona A, Louis M (2018) Sensorimotor pathway controlling stopping behavior during chemotaxis in the *Drosophila melanogaster* larva. Elife. https://doi.org/10.7554/eLife.38740
67. de Vries SE, Clandinin T (2013) Optogenetic stimulation of escape behavior in *Drosophila melanogaster*. J Vis Exp. https://doi.org/10.3791/50192
68. Lin CC, Prokop-Prigge KA, Preti G, Potter CJ (2015) Food odors trigger *Drosophila* males to deposit a pheromone that guides aggregation and female oviposition decisions. Elife. https://doi.org/10.7554/eLife.08688
69. Aravanis AM, Wang LP, Zhang F, Meltzer LA, Mogri MZ, Schneider MB, Deisseroth K (2007) An optical neural interface: in vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology. J Neural Eng 4(3):S143-156. https://doi.org/10.1088/1741-2560/4/3/S02
70. Ljunggren EE, Haupt S, Ausborn J, Ampatzis K, El Manira A (2014) Optogenetic activation of excitatory premotor interneurons is sufficient to generate coordinated locomotor activity in larval zebrafish. J Neurosci 34(1):134–139. https://doi.org/10.1523/JNEUROSCI.4087-13.2014
71. Arrenberg AB, Del Bene F, Baier H (2009) Optical control of zebrafish behavior with halorhodopsin. Proc Natl Acad Sci USA 106(42):17968–17973. https://doi.org/10.1073/pnas.0906252106
72. Antinucci P, Dumitrescu A, Deleuze C, Morley HJ, Leung K, Hagley T, Kubo F, Baier H, Bianco IH, Wyart C (2020) A calibrated optogenetic toolbox of stable zebrafish opsin lines. Elife. https://doi.org/10.7554/eLife.54937
73. Lobo MK, Covington HE 3rd, Chaudhury D, Friedman AK, Sun H, Damez-Werno D, Dietz DM, Zaman S, Koo JW, Kennedy PJ, Mouzon E, Mogri M, Neve RL, Deisseroth K, Han MH, Nestler EJ (2010) Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. Science 330(6002):385–390. https://doi.org/10.1126/science.1188472
74. Liu X, Ramirez S, Pang PT, Puryear CB, Govindarajan A, Deisseroth K, Tonegawa S (2012) Optogenetic stimulation of a hippocampal engram activates fear memory recall. Nature 484(7394):381–385. https://doi.org/10.1038/nature11028

75. De Marco RJ, Thiemann T, Groneberg AH, Herget U, Ryu S (2016) Optogenetically enhanced pituitary corticotroph cell activity post-stress onset causes rapid organizing effects on behaviour. Nat Commun 7:12620. https://doi.org/10.1038/ncomms12620
76. Bolus H, Crocker K, Boekhoff-Falk G, Chtarbanova S (2020) Modeling neurodegenerative disorders in *Drosophila melanogaster*. Int J Mol Sci. https://doi.org/10.3390/ijms21093055
77. Iijima K, Liu H-P, Chiang A-S, Hearn SA, Konsolaki M, Zhong Y (2004) Dissecting the pathological effects of human Aβ40 and Aβ42 in *Drosophila*: a potential model for Alzheimer's disease. Proc Natl Acad Sci USA 101(17):6623–6628. https://doi.org/10.1073/pnas.0400895101
78. Lim CH, Kaur P, Teo E, Lam VYM, Zhu F, Kibat C, Gruber J, Mathuru AS, Tolwinski NS (2020) Application of optogenetic Amyloid-β distinguishes between metabolic and physical damages in neurodegeneration. Elife. https://doi.org/10.7554/eLife.52589
79. Teo E, Ravi S, Barardo D, Kim H-S, Fong S, Cazenave-Gassiot A, Tan TY, Ching J, Kovalik J-P, Wenk MR (2019) Metabolic stress is a primary pathogenic event in transgenic *Caenorhabditis elegans* expressing pan-neuronal human amyloid beta. Elife 8:e50069
80. Teo E, Ravi S, Barardo D, Kim HS, Fong S, Cazenave-Gassiot A, Tan TY, Ching J, Kovalik JP, Wenk MR, Gunawan R, Moore PK, Halliwell B, Tolwinski N, Gruber J (2019) Metabolic stress is a primary pathogenic event in transgenic *Caenorhabditis elegans* expressing pan-neuronal human amyloid beta. Elife. https://doi.org/10.7554/eLife.50069
81. Gundersen V (2010) Protein aggregation in Parkinson's disease. Acta Neurol Scand 122(s190):82–87. https://doi.org/10.1111/j.1600-0404.2010.01382.x
82. Lee EJ, Yoon HH, Park ES, Min J, Jeon SR (2018) A novel animal model of Parkinson's disease using optogenetics: representation of various disease stages by modulating the illumination parameter. Stereotact Funct Neurosurg 96:22–32. https://doi.org/10.1159/000486644
83. Otte CG, Fortuna TR, Mann JR, Gleixner AM, Ramesh N, Pyles NJ, Pandey UB, Donnelly CJ (2020) Optogenetic TDP-43 nucleation induces persistent insoluble species and progressive motor dysfunction in vivo. Neurobiol Dis. https://doi.org/10.1016/j.nbd.2020.105078
84. Mann JR, Gleixner AM, Mauna JC, Gomes E, DeChellis-Marks MR, Needham PG, Copley KE, Hurtle B, Portz B, Pyles NJ, Guo L, Calder CB, Wills ZP, Pandey UB, Kofler JK, Brodsky JL, Thathiah A, Shorter J, Donnelly CJ (2019) RNA binding antagonizes neurotoxic phase transitions of TDP-43. Neuron 102(2):321-338.e328. https://doi.org/10.1016/j.neuron.2019.01.048
85. Micchelli CA, Perrimon N (2006) Evidence that stem cells reside in the adult *Drosophila* midgut epithelium. Nature 439(7075):475–479. https://doi.org/10.1038/nature04371
86. Ohlstein B, Spradling A (2006) The adult *Drosophila* posterior midgut is maintained by pluripotent stem cells. Nature 439(7075):470–474. https://doi.org/10.1038/nature04333
87. Lin S, Ewen-Campen B, Ni X, Housden BE, Perrimon N (2015) In vivo transcriptional activation using CRISPR/Cas9 in *Drosophila*. Genetics 201(2):433–442. https://doi.org/10.1534/genetics.115.181065
88. Ni J-Q, Zhou R, Czech B, Liu L-P, Holderbaum L, Yang-Zhou D, Shim H-S, Tao R, Handler D, Karpowicz P (2011) A genome-scale shRNA resource for transgenic RNAi in *Drosophila*. Nat Methods 8(5):405–407
89. Port F, Chen H-M, Lee T, Bullock SL (2014) Optimized CRISPR/Cas tools for efficient germline and somatic genome engineering in *Drosophila*. Proc Natl Acad Sci 111(29):E2967–E2976
90. De Renzis S (2020) Morphogenesis: guiding embryonic development with light. Curr Biol 30(17):R998–R1001. https://doi.org/10.1016/j.cub.2020.07.048
91. Gallaud E, Ramdas Nair A, Horsley N, Monnard A, Singh P, Pham TT, Salvador Garcia D, Ferrand A, Cabernard C (2020) Dynamic centriolar localization of polo and centrobin in early mitosis primes centrosome asymmetry. PLoS Biol 18(8):e3000762. https://doi.org/10.1371/journal.pbio.3000762
92. Johnson HE, Goyal Y, Pannucci NL, Schüpbach T, Shvartsman SY, Toettcher JE (2017) The spatiotemporal limits of developmental Erk signaling. Dev Cell 40(2):185–192
93. Johnson HE, Djabrayan NJV, Shvartsman SY, Toettcher JE (2020) Optogenetic rescue of a patterning mutant. Curr Biol 30(17):3414-3424.e3413. https://doi.org/10.1016/j.cub.2020.06.059
94. Johnson HE, Toettcher JE (2019) Signaling dynamics control cell fate in the early *Drosophila* embryo. Dev Cell 48(3):361–370
95. Irizarry J, McGehee J, Kim G, Stein D, Stathopoulos A (2020) Twist-dependent ratchet functioning downstream from Dorsal revealed using a light-inducible degron. Genes Dev 34(13–14):965–972. https://doi.org/10.1101/gad.338194.120
96. Krueger D, Pallares Cartes C, Makaske T, De Renzis S (2020) βH-spectrin is required for ratcheting apical pulsatile constrictions during tissue invagination. EMBO Rep 21(8):e49858. https://doi.org/10.15252/embr.201949858
97. Krueger D, Quinkler T, Mortensen SA, Sachse C, De Renzis S (2019) Cross-linker–mediated regulation of actin network organization controls tissue morphogenesis. J Cell Biol 218(8):2743–2761. https://doi.org/10.1083/jcb.201811127
98. Krueger D, Tardivo P, Nguyen C, De Renzis S (2018) Downregulation of basal myosin-II is required for cell shape changes and tissue invagination. EMBO J 37(23):e100170
99. Eritano AS, Bromley CL, Bolea Albero A, Schütz L, Wen F-L, Takeda M, Fukaya T, Sami MM, Shibata T, Lemke S, Wang Y-C (2020) Tissue-scale mechanical coupling reduces morphogenetic noise to ensure precision during epithelial folding. Dev Cell 53(2):212-228.e212. https://doi.org/10.1016/j.devcel.2020.02.012
100. Bagci H, Sriskandarajah N, Robert A, Boulais J, Elkholi IE, Tran V, Lin Z-Y, Thibault M-P, Dubé N, Faubert D, Hipfner DR, Gingras A-C, Côté J-F (2020) Mapping the proximity interaction network of the Rho-family GTPases reveals signalling pathways and regulatory mechanisms. Nat Cell Biol 22(1):120–134. https://doi.org/10.1038/s41556-019-0438-7
101. Izquierdo E, Quinkler T, De Renzis S (2018) Guided morphogenesis through optogenetic activation of Rho signalling during early *Drosophila* embryogenesis. Nat Commun 9(1):1–13
102. Teets FD, Watanabe T, Hahn KM, Kuhlman B (2020) A computational protocol for regulating protein binding reactions with a light-sensitive protein dimer. J Mol Biol 432(4):805–814. https://doi.org/10.1016/j.jmb.2019.12.033
103. Wu YI, Frey D, Lungu OI, Jaehrig A, Schlichting I, Kuhlman B, Hahn KM (2009) A genetically encoded photoactivatable Rac controls the motility of living cells. Nature 461(7260):104–108. https://doi.org/10.1038/nature08241
104. Leung DW, Otomo C, Chory J, Rosen MK (2008) Genetically encoded photoswitching of actin assembly through the Cdc42-WASP-Arp2/3 complex pathway. Proc Natl Acad Sci 105(35):12797–12802. https://doi.org/10.1073/pnas.0801232105
105. Grobe H, Wüstenhagen A, Baarlink C, Grosse R, Grikscheit K (2018) A Rac1-FMNL2 signaling module affects cell-cell contact formation independent of Cdc42 and membrane protrusions. PLoS ONE 13(3):e0194716. https://doi.org/10.1371/journal.pone.0194716
106. Kim J, Lee S, Jung K, Oh WC, Kim N, Son S, Jo Y, Kwon H-B, Heo WD (2019) Intensiometric biosensors visualize the activity

of multiple small GTPases in vivo. Nat Commun 10(1):211. https://doi.org/10.1038/s41467-018-08217-3

107. Qin X, Hannezo E, Mangeat T, Liu C, Majumder P, Liu J, Choesmel-Cadamuro V, McDonald JA, Liu Y, Yi B, Wang X (2018) A biochemical network controlling basal myosin oscillation. Nat Commun 9(1):1210. https://doi.org/10.1038/s41467-018-03574-5
108. Adikes RC, Hallett RA, Saway BF, Kuhlman B, Slep KC (2017) Control of microtubule dynamics using an optogenetic microtubule plus end–F-actin cross-linker. J Cell Biol 217(2):779–793. https://doi.org/10.1083/jcb.201705190
109. Qin X, Park BO, Liu J, Chen B, Choesmel-Cadamuro V, Belguise K, Heo WD, Wang X (2017) Cell-matrix adhesion and cell-cell adhesion differentially control basal myosin oscillation and *Drosophila* egg chamber elongation. Nat Commun 8(1):14708. https://doi.org/10.1038/ncomms14708
110. Wang X, He L, Wu YI, Hahn KM, Montell DJ (2010) Light-mediated activation reveals a key role for Rac in collective guidance of cell movement in vivo. Nat Cell Biol 12(6):591–597. https://doi.org/10.1038/ncb2061
111. Huang A, Amourda C, Zhang S, Tolwinski NS, Saunders TE (2017) Decoding temporal interpretation of the morphogen bicoid in the early *Drosophila* embryo. Elife. https://doi.org/10.7554/eLife.26258
112. Kaur P, Saunders TE, Tolwinski NS (2017) Coupling optogenetics and light-sheet microscopy, a method to study Wnt signaling during embryogenesis. Sci Rep 7(1):1–11
113. Viswanathan R, Necakov A, Trylinski M, Harish RK, Krueger D, Esposito E, Schweisguth F, Neveu P, De Renzis S (2019) Optogenetic inhibition of delta reveals digital notch signalling output during tissue differentiation. EMBO Rep 20(12):e47999. https://doi.org/10.15252/embr.201947999
114. Guglielmi G, Barry JD, Huber W, De Renzis S (2015) An optogenetic method to modulate cell contractility during tissue morphogenesis. Dev Cell 35(5):646–660
115. Spagnuolo G, Genovese F, Fortunato L, Simeone M, Rengo C, Tatullo M (2020) The impact of optogenetics on regenerative medicine. Appl Sci. https://doi.org/10.3390/app10010173
116. Hu W, Li Q, Li B, Ma K, Zhang C, Fu X (2020) Optogenetics sheds new light on tissue engineering and regenerative medicine. Biomaterials 227:119546. https://doi.org/10.1016/j.biomaterials.2019.119546
117. Polstein LR, Juhas M, Hanna G, Bursac N, Gersbach CA (2017) An engineered optogenetic switch for spatiotemporal control of gene expression, cell differentiation, and tissue morphogenesis. ACS Synth Biol 6(11):2003–2013. https://doi.org/10.1021/acssynbio.7b00147
118. Steck K, Walker SJ, Itskov PM, Baltazar C, Moreira JM, Ribeiro C (2018) Internal amino acid state modulates yeast taste neurons to support protein homeostasis in *Drosophila*. Elife. https://doi.org/10.7554/eLife.31625
119. Itskov PM, Moreira J-M, Vinnik E, Lopes G, Safarik S, Dickinson MH, Ribeiro C (2014) Automated monitoring and quantitative analysis of feeding behaviour in *Drosophila*. Nat Commun 5(1):4560. https://doi.org/10.1038/ncomms5560
120. Moreira JM, Itskov PM, Goldschmidt D, Baltazar C, Steck K, Tastekin I, Walker SJ, Ribeiro C (2019) OptoPAD, a closed-loop optogenetics system to study the circuit basis of feeding behaviors. Elife. https://doi.org/10.7554/eLife.43924
121. Musso PY, Junca P, Jelen M, Feldman-Kiss D, Zhang H, Chan RC, Gordon MD (2019) Closed-loop optogenetic activation of peripheral or central neurons modulates feeding in freely moving *Drosophila*. Elife. https://doi.org/10.7554/eLife.45636
122. Idevall-Hagren O, Dickson EJ, Hille B, Toomre DK, De Camilli P (2012) Optogenetic control of phosphoinositide metabolism. Proc Natl Acad Sci 109(35):E2316. https://doi.org/10.1073/pnas.1211305109
123. Tajima K, Ikeda K, Tanabe Y, Thomson EA, Yoneshiro T, Oguri Y, Ferro MD, Poon ASY, Kajimura S (2020) Wireless optogenetics protects against obesity via stimulation of non-canonical fat thermogenesis. Nat Commun 11(1):1730. https://doi.org/10.1038/s41467-020-15589-y
124. Alex A, Li A, Tanzi RE, Zhou C (2015) Optogenetic pacing in *Drosophila melanogaster*. Sci Adv 1(9):e1500639. https://doi.org/10.1126/sciadv.1500639
125. Zhu YC, Uradu H, Majeed ZR, Cooper RL (2016) Optogenetic stimulation of *Drosophila* heart rate at different temperatures and Ca2+ concentrations. Physiol Rep. https://doi.org/10.14814/phy2.12695
126. Malloy C, Sifers J, Mikos A, Samadi A, Omar A, Hermanns C, Cooper RL (2017) Using optogenetics to assess neuroendocrine modulation of heart rate in *Drosophila melanogaster* larvae. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 203(10):791–806. https://doi.org/10.1007/s00359-017-1191-7
127. Ferenczi EA, Tan X, Huang CL (2019) Principles of optogenetic methods and their application to cardiac experimental systems. Front Physiol 10:1096. https://doi.org/10.3389/fphys.2019.01096
128. Joshi J, Rubart M, Zhu W (2019) Optogenetics: background, methodological advances and potential applications for cardiovascular research and medicine. Front Bioeng Biotechnol 7:466. https://doi.org/10.3389/fbioe.2019.00466
129. Hochmuth CE, Biteau B, Bohmann D, Jasper H (2011) Redox regulation by Keap1 and Nrf2 controls intestinal stem cell proliferation in *Drosophila*. Cell Stem Cell 8(2):188–199. https://doi.org/10.1016/j.stem.2010.12.006
130. Wu WL, Papagiannakopoulos T (2020) The pleiotropic role of the KEAP1/NRF2 pathway in cancer. Annu Rev Cancer Biol 4(1):413–435. https://doi.org/10.1146/annurev-cancerbio-030518-055627
131. Pitoniak A, Bohmann D (2015) Mechanisms and functions of Nrf2 signaling in *Drosophila*. Free Radic Biol Med 88(Pt B):302–313. https://doi.org/10.1016/j.freeradbiomed.2015.06.020
132. Blank V (2008) Small Maf proteins in mammalian gene control: mere dimerization partners or dynamic transcriptional regulators? J Mol Biol 376(4):913–925
133. Mondal P, Krishnamurthy VV, Sharum SR, Haack N, Zhou H, Cheng J, Yang J, Zhang K (2019) Repurposing protein degradation for optogenetic modulation of protein activities. ACS Synth Biol 8(11):2585–2592. https://doi.org/10.1021/acssynbio.9b00285
134. Renicke C, Schuster D, Usherenko S, Essen L-O, Taxis C (2013) A LOV2 domain-based optogenetic tool to control protein degradation and cellular function. Chem Biol 20(4):619–626. https://doi.org/10.1016/j.chembiol.2013.03.005
135. Baaske J, Gonschorek P, Engesser R, Dominguez-Monedero A, Raute K, Fischbach P, Müller K, Cachat E, Schamel WWA, Minguet S, Davies JA, Timmer J, Weber W, Zurbriggen MD (2018) Dual-controlled optogenetic system for the rapid down-regulation of protein levels in mammalian cells. Sci Rep 8(1):15024. https://doi.org/10.1038/s41598-018-32929-7
136. Wojtovich AP, Foster TH (2014) Optogenetic control of ROS production. Redox Biol 2:368–376. https://doi.org/10.1016/j.redox.2014.01.019
137. Kaur P, Saunders TE, Tolwinski NS (2017) Coupling optogenetics and light-sheet microscopy, a method to study Wnt signaling during embryogenesis. Sci Rep 7(1):16636. https://doi.org/10.1038/s41598-017-16879-0
138. Bugaj LJ, Spelke DP, Mesuda CK, Varedi M, Kane RS, Schaffer DV (2015) Regulation of endogenous transmembrane receptors

through optogenetic Cry2 clustering. Nat Commun 6(1):6898. https://doi.org/10.1038/ncomms7898
139. Lusk JB, Lam VY, Tolwinski NS (2017) Epidermal growth factor pathway signaling in *Drosophila* embryogenesis: tools for understanding cancer. Cancers (Basel). https://doi.org/10.3390/cancers9020016
140. Humphreys PA, Woods S, Smith CA, Bates N, Cain SA, Lucas R, Kimber SJ (2020) Optogenetic control of the BMP signaling pathway. ACS Synth Biol. https://doi.org/10.1021/acssynbio.0c00315
141. Kim N, Kim Jin M, Lee M, Kim Cha Y, Chang K-Y, Heo Won D (2014) Spatiotemporal control of fibroblast growth factor receptor signals by blue light. Chem Biol 21(7):903–912. https://doi.org/10.1016/j.chembiol.2014.05.013
142. Cooper GJ, Willis AC, Clark A, Turner RC, Sim RB, Reid KB (1987) Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. Proc Natl Acad Sci 84(23):8628. https://doi.org/10.1073/pnas.84.23.8628
143. Lorenzo A, Razzaboni B, Weir GC, Yankner BA (1994) Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. Nature 368(6473):756–760. https://doi.org/10.1038/368756a0
144. Zhang S, Liu H, Chuang CL, Li X, Au M, Zhang L, Phillips ARJ, Scott DW, Cooper GJS (2014) The pathogenic mechanism of diabetes varies with the degree of overexpression and oligomerization of human amylin in the pancreatic islet β cells. FASEB J 28(12):5083–5096. https://doi.org/10.1096/fj.14-251744
145. Suresh J, Khor IW, Kaur P, Heng HL, Torta F, Dawe GS, Tai ES, Tolwinski NS (2020) Shared signaling pathways in Alzheimer's and metabolic disease may point to new treatment approaches. FEBS J. https://doi.org/10.1111/febs.15540
146. Jackson K, Barisone GA, Diaz E, Jin L-w, DeCarli C, Despa F (2013) Amylin deposition in the brain: a second amyloid in Alzheimer disease? Ann Neurol 74(4):517–526. https://doi.org/10.1002/ana.23956
147. Schultz SW, Nilsson KPR, Westermark GT (2011) *Drosophila melanogaster* as a model system for studies of islet amyloid polypeptide aggregation. PLoS ONE 6(6):e20221. https://doi.org/10.1371/journal.pone.0020221
148. Xie L, Gu X, Okamoto K, Westermark GT, Leifer K (2019) 3D analysis of human islet amyloid polypeptide crystalline structures in *Drosophila melanogaster*. PLoS ONE 14(10):e0223456. https://doi.org/10.1371/journal.pone.0223456
149. Hatters DM (2008) Protein misfolding inside cells: the case of huntingtin and Huntington's disease. IUBMB Life 60(11):724–728. https://doi.org/10.1002/iub.111
150. Grosenick L, Marshel JH, Deisseroth K (2015) Closed-loop and activity-guided optogenetic control. Neuron 86(1):106–139. https://doi.org/10.1016/j.neuron.2015.03.034
151. Zhang Z, Russell LE, Packer AM, Gauld OM, Häusser M (2018) Closed-loop all-optical interrogation of neural circuits in vivo. Nat Methods 15(12):1037–1040. https://doi.org/10.1038/s41592-018-0183-z
152. Ng LF, Kaur P, Bunnag N, Suresh J, Sung ICH, Tan QH, Gruber J, Tolwinski NS (2019) WNT signaling in disease. Cells 8(8):826
153. Polesskaya O, Baranova A, Bui S, Kondratev N, Kananykhina E, Nazarenko O, Shapiro T, Nardia FB, Kornienko V, Chandhoke V, Stadler I, Lanzafame R, Myakishev-Rempel M (2018) Optogenetic regulation of transcription. BMC Neurosci 19(Suppl 1):12. https://doi.org/10.1186/s12868-018-0411-6
154. Rao P, Wang L, Cheng Y, Wang X, Li H, Zheng G, Li Z, Jiang C, Zhou Q, Huang C (2020) Near-infrared light driven tissue-penetrating cardiac optogenetics via upconversion nanoparticles in vivo. Biomed Opt Express 11(3):1401–1416. https://doi.org/10.1364/BOE.381480
155. Owen SF, Liu MH, Kreitzer AC (2019) Thermal constraints on in vivo optogenetic manipulations. Nat Neurosci 22(7):1061–1065. https://doi.org/10.1038/s41593-019-0422-3
156. Kennedy MJ, Hughes RM, Peteya LA, Schwartz JW, Ehlers MD, Tucker CL (2010) Rapid blue-light-mediated induction of protein interactions in living cells. Nat Methods 7(12):973–975. https://doi.org/10.1038/nmeth.1524
157. Zhou XX, Fan LZ, Li P, Shen K, Lin MZ (2017) Optical control of cell signaling by single-chain photoswitchable kinases. Science 355(6327):836. https://doi.org/10.1126/science.aah3605
158. Patel AL, Yeung E, McGuire SE, Wu AY, Toettcher JE, Burdine RD, Shvartsman SY (2019) Optimizing photoswitchable MEK. Proc Natl Acad Sci 116(51):25756. https://doi.org/10.1073/pnas.1912320116
159. Vanuytsel T, Senger S, Fasano A, Shea-Donohue T (2013) Major signaling pathways in intestinal stem cells. Biochim Biophys Acta 1830(2):2410–2426. https://doi.org/10.1016/j.bbagen.2012.08.006
160. Logan CY, Nusse R (2004) The WNT signaling pathway in development and disease. Annu Rev Cell Dev Biol 20(1):781–810. https://doi.org/10.1146/annurev.cellbio.20.010403.113126
161. Lemmon MA, Schlessinger J (2010) Cell signaling by receptor tyrosine kinases. Cell 141(7):1117–1134. https://doi.org/10.1016/j.cell.2010.06.011
162. Meloche S, Pouysségur J (2007) The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. Oncogene 26(22):3227–3239. https://doi.org/10.1038/sj.onc.1210414
163. Kawasaki T, Kawai T (2014) Toll-like receptor signaling pathways. Front Immunol 5:461
164. Shilo B-Z (2003) Signaling by the *Drosophila* epidermal growth factor receptor pathway during development. Exp Cell Res 284(1):140–149. https://doi.org/10.1016/S0014-4827(02)00094-0
165. Lusk JB, Lam VYM, Tolwinski NS (2017) Epidermal growth factor pathway signaling in *Drosophila* embryogenesis: tools for understanding cancer. Cancers (Basel) 9(2):16. https://doi.org/10.3390/cancers9020016
166. Wang RN, Green J, Wang Z, Deng Y, Qiao M, Peabody M, Zhang Q, Ye J, Yan Z, Denduluri S, Idowu O, Li M, Shen C, Hu A, Haydon RC, Kang R, Mok J, Lee MJ, Luu HL, Shi LL (2014) Bone morphogenetic protein (BMP) signaling in development and human diseases. Genes Dis 1(1):87–105. https://doi.org/10.1016/j.gendis.2014.07.005
167. Ornitz DM, Itoh N (2015) The fibroblast growth factor signaling pathway. Wiley Interdiscip Rev Dev Biol 4(3):215–266. https://doi.org/10.1002/wdev.176
168. Jaramillo MC, Zhang DD (2013) The emerging role of the Nrf2-Keap1 signaling pathway in cancer. Genes Dev 27(20):2179–2191. https://doi.org/10.1101/gad.225680.113
169. Bray SJ (2006) Notch signalling: a simple pathway becomes complex. Nat Rev Mol Cell Biol 7:678
170. VanDussen KL, Carulli AJ, Keeley TM, Patel SR, Puthoff BJ, Magness ST, Tran IT, Maillard I, Siebel C, Kolterud Å, Grosse AS, Gumucio DL, Ernst SA, Tsai Y-H, Dempsey PJ, Samuelson LC (2012) Notch signaling modulates proliferation and differentiation of intestinal crypt base columnar stem cells. Development 139(3):488. https://doi.org/10.1242/dev.070763

171. Briscoe J, Therond PP (2013) The mechanisms of hedgehog signalling and its roles in development and disease. Nat Rev Mol Cell Biol 14:416
172. Saucedo LJ, Edgar BA (2007) Filling out the hippo pathway. Nat Rev Mol Cell Biol 8(8):613–621. https://doi.org/10.1038/nrm2221
173. Arbouzova NI, Zeidler MP (2006) JAK/STAT signalling in *Drosophila*: insights into conserved regulatory and cellular functions. Development 133(14):2605. https://doi.org/10.1242/dev.02411

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