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Review Article

Controlling gene expression with light: a multidisciplinary endeavour

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The expression of a gene to a protein is one of the most vital biological processes. The use of light to control biology offers unparalleled spatiotemporal resolution from an external, orthogonal signal. A variety of methods have been developed that use light to control the steps of transcription and translation of specific genes into proteins, for cell-free to *in vivo* biotechnology applications. These methods employ techniques ranging from the modification of small molecules, nucleic acids and proteins with photocages, to the engineering of proteins involved in gene expression using naturally light-sensitive proteins. Although the majority of currently available technologies employ ultraviolet light, there has been a recent increase in the use of functionalities that work at longer wavelengths of light, to minimise cellular damage and increase tissue penetration. Here, we discuss the different chemical and biological methods employed to control gene expression, while also highlighting the central themes and the most exciting applications within this diverse field.

Introduction

Light is an ideal stimulus to control biological systems. It acts orthogonally to cellular signals in a large number of organisms and allows for the tightest spatiotemporal control of any input [1]. A major area of research is the use of light to control, arguably, the most fundamental biological pathway, gene expression. This includes methods to control either the transcription (DNA to mRNA) or translation (mRNA to protein) steps (Figure 1). It is possible to either activate or silence each step, or perform both reversibly. Applications of these methods include the control of cell-free systems, gene circuits, and drug/gene delivery. Specific endogenous genes can also be controlled with light by modifying existing technologies.

Controlling gene expression with light can be approached through both chemical and biological means [2]. One approach is to use chemical photocages, light-sensitive molecules that are linked to a bioactive molecule, blocking its activity. Illumination with a specific wavelength of light causes the photocage to break the linking bond, which reforms the original molecule. Photocages have been attached to small molecule, nucleic acid, or protein regulators of expression or directly to the DNA or mRNA nucleic acid templates themselves [3] (Figure 1A–C). Alternatively, several naturally light-sensitive proteins, which function through multiple different pathways, have been engineered and fused to proteins involved in expression [4] (Figure 1D). Within this review we have covered multiple different approaches that have been used to control gene expression with wavelengths of light from ultraviolet (UV) to near-infrared (NIR) (Figure 2). It is vital to cover multidisciplinary topics to highlight the collective themes and methods used. Simple reporter proteins, such as fluorescent proteins, β-galactosidase, or luciferase, are used as a common way to measure the efficiency of each method. Current applications focus on control of cell-free to *in vivo* gene expression. Human cell lines are widely used to demonstrate application to endogenous pathways. Light-controlled systems are also widely applied *in vivo* to zebrafish, as they are transparent. A few systems have also shown application in mouse models. However, when working *in vivo* it is important proper controls are in place due to the presence of endogenous proteins that respond to light.

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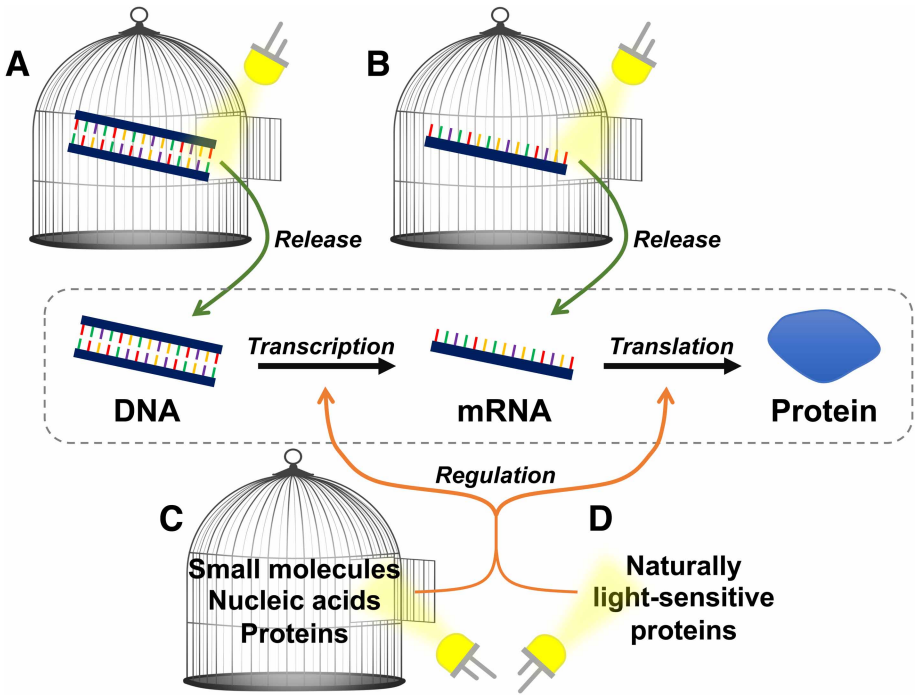

Figure 1. Methods of controlling transcription and translation with light discussed in this review.
Uncaging the (**A**) DNA or (**B**) mRNA template of a gene of interest with light allows for activation of transcription/translation. Whereas regulation of gene expression with light can be achieved by using (**C**) caged small molecules, nucleic acids, and proteins or (**D**) engineering naturally light-sensitive proteins.

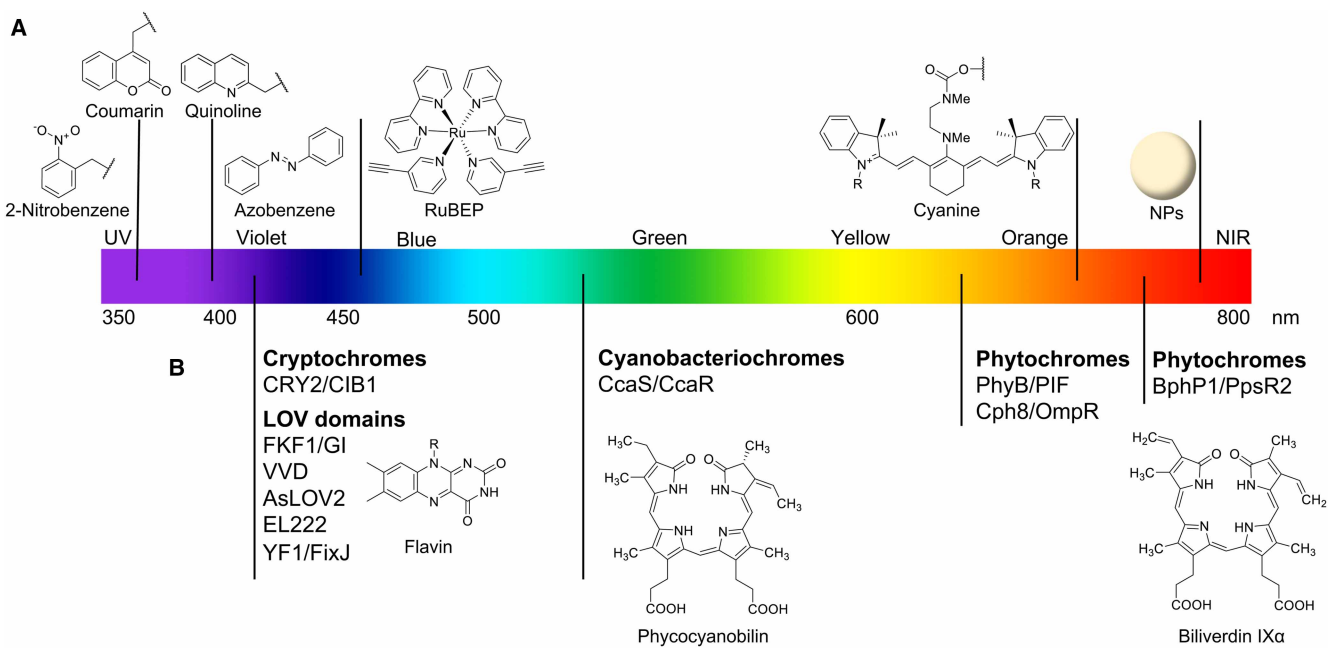

Figure 2. An illustration of the approximate wavelength of activation of a number of photocages (**A**) and engineered naturally light-sensitive proteins (**B**, with chromophores) discussed in this review. NIR = near-infrared, NP = nanoparticle.

Chemical photocages

The most commonly used photocages are 2-nitrobenzyl derivatives [5] (Figure 2A). They are small, easy to synthesise and are often available commercially; they do, however, cleave in the UV (regularly 365 nm), which can cause cellular damage. Another commonly encountered photocage are coumarin derivatives [5]. These are also irradiated with UV (365–400 nm), but can be chemically modified to allow longer wavelengths of illumination; derivatives absorbing in the green have been prepared [6]. Other photocages that have been used within this review include quinoline (UV irradiation) [7], RuBEP (blue irradiation) [8], and cyanine. Cyanines can be activated up to the NIR [9], but are not often used due to lengthy syntheses. Visible or NIR irradiation is preferable as it causes less cellular damage than UV.

Alternative photocages are azobenzenes and styryl derivatives. They are not photocleavable, but photoswitches, as they change configuration upon irradiation. *Trans-Cis* isomerisation of azobenzene and styryl derivatives occurs following UV irradiation [10,11], however modified azobenzenes can be controlled with visible or NIR irradiation [12].

Light-controlled gene expression using small molecules

Small molecules are widely used to regulate gene expression and have been extensively photocaged to control their activity with light (Figure 2A). The largest group of photocaged molecules are estradiol and tamoxifen analogues, agonists of the estrogen receptor. These have been photocaged with 2-nitrobenzyl-, coumarin- and cyanine-derivatives to control reporter gene expression in mammalian cells [13–16]. Using 2-nitrobenzyl- or thiocoumarin-caged cyclophen, reporter gene expression and phenotype, respectively, could also be controlled in zebrafish [17,18].

A widely used inducer system in mammalian cells is the Tetracycline (Tet) system, under the control of doxycycline. Nitrobenzyl-photocaged doxycyclines have controlled reporter gene expression in mammalian cells [19], tobacco leaves [19] and developing mouse embryos and *xenopus* tadpoles [20]. A number of other small molecule agonists of expression in mammalian cells have been photocaged, including 2-nitrobenzyl-caged nuclear hormones [21] and β-ecdysone [22], as well as a coumarin-caged CREB inhibitor [23].

Carbohydrates are a well-known class of bacterial gene-regulatory molecules. Bacterial gene expression is often placed under control of the lac operator, which is activated using isopropyl-β-D-thiogalactopyranoside (IPTG), a non-hydrolysable allolactose mimic. 2-Nitrobenzyl-photocaged IPTG enabled the efficient regulation of reporter genes and (+)-valencene biosynthesis [24,25]. Arabinose is also widely used to control gene expression via the arabinose operator. 2-Nitrobenzyl-photocaged arabinose was shown to efficiently control expression of Violacein biosynthesis [26].

Nucleotides are vital biological signals and building blocks for transcription. Photocaged analogues are therefore able to control these functions. *In vitro* transcription has been controlled by photocaging adenosine triphosphate (ATP) on the terminal phosphate with a coumarin [27]. This inhibited RNA synthesis until uncaging of ATP with light. In a similar manner, synthetic cells have been activated with 2-nitrobenzyl-caged ATP [28]. Alternatively, uracil and guanine triphosphates, photocaged on the nucleobase with a 2-nitrobenzyl, prevented Watson–Crick base pairing prior to uncaging [29].

Photocaging has also been demonstrated on small molecules that directly interact with DNA or RNA. Toyocamycin is an ATP analogue that controls translation by binding to a ribozyme in mRNA. Light-activated gene expression in mammalian cells was achieved through 2-nitrobenzyl-photocaging [30]. Theophylline is another small molecule used to control translation by binding to an mRNA riboswitch. 2-Nitrobenzyl-photocaged theophylline was used to control expression in bacteria [31]. G-quadruplex (G4) structures are involved in regulation of gene expression. Photocaging the G4-stabilising ligand pyridostatin with a 2-nitrobenzyl allowed the light-activated downregulation of cancer-associated genes in mammalian cells [32]. Cell-free expression can also be controlled via light-activated compaction of DNA/RNA using AzoTAB, which contains an azobenzene photoswitch and has strong nucleic acid affinity in the trans-form and weak in the cis-form [33].

Light-controlled gene expression using nucleic acids

Modification of DNA or RNA with photocages (Figure 2A) can be used to control transcription and translation with light (Figure 3). Generally, light-activated regulation of transcription and translation with nucleic acids can be broadly classified into two categories: the light-induced activation of gene expression (by using the

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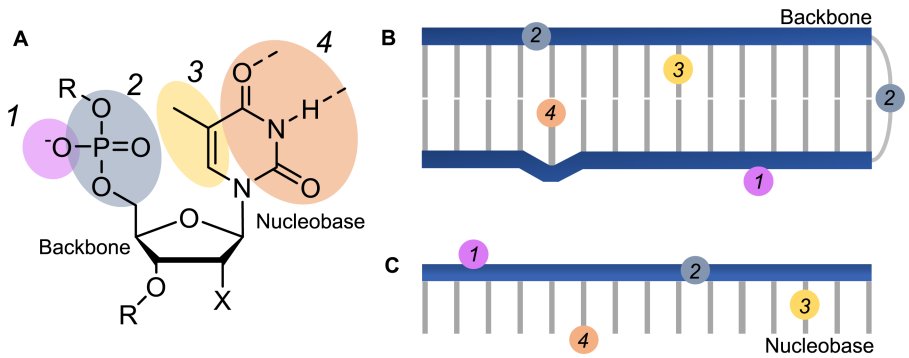

Figure 3. Attachment points of photocages onto DNA (X = H) or RNA (X = OH).

(**A**) Photocages have been attached to various positions on DNA/RNA strands to control function, including the phosphate backbone (1), in the backbone (2), on the nucleobase (3), and on the Watson–Crick face of the nucleobase (4). These photocages can be attached to double stranded (**B**) or single stranded (**C**) nucleic acids.

DNA or mRNA template) and the light-induced gene knockdown (via caged antisense oligonucleotides (ASOs) or small interfering RNAs (siRNAs)).

Gene activation

One approach of inhibiting transcription from a DNA template is by attaching a photocage onto the Watson–Crick face of the nucleobase, impeding base pairing. In this way, incorporation of 2-nitrobenzyl-caged thymidine into a DNA promoter has allowed control of cell-free transcription [34] and reporter gene expression in zebrafish [35]. Alternatively, a covalent inter-strand crosslink was made in a DNA promoter, using a psoralen modified DNA base-pair, to inhibit unwinding of the double helix and therefore transcription [36]. Irradiation initiated cell-free expression through decrosslinking via an adjacent pyrene.

Attachment of photocleavable molecules to other positions on the DNA or RNA can also disrupt recognition of the substrate. One approach is to modify the nucleobase with photocleavable groups at non-Watson–Crick base pairing sites. Amino-modified thymine bases were incorporated into a DNA promoter sequence and reacted with a photocleavable 2-nitrobenzyl biotin to generate a PCR primer, to allow amplification of a gene of interest. Subsequent binding of monovalent streptavidin allowed light-activated cell-free expression. Using this method, expression of protein pores created patterned conductive pathways through synthetic tissues [37,38]. In a similar manner, integration of 2-nitrobenzyl-caged 5-hydroxymethyl(hm)-cytosine and 5-hm-uracil into a DNA template controlled cell-free transcription with light [39]. A photocage was also installed on a nucleobase of a DNA template by using a methyltransferase with a 2-nitrobenzyl-modified S-adenosyl methionine substrate, which was used to control cell-free expression [40]. Enzymatic insertion of photocleavable biotin-coumarin-modified nucleobases into mRNA also enabled controlled expression in mammalian cells [41,42].

Photocages can also be directly attached on the oligonucleotide backbone. A plasmid with a streptavidin/biotin-coumarin backbone-modified promoter was used to control expression in mammalian cells [43]. Coumarin- [44,45] and 2-nitrobenzyl- [46,47] photocages have also been reacted with the phosphate backbone of plasmids and mRNA to allow control of reporter gene expression or phenotype in zebrafish and mammalian cells, and cell-free expression in synthetic cells. Study of head-specific overexpression in zebrafish was achieved using caged mRNA in this way [44].

Photoswitches have also been incorporated into DNA and RNA to reversibly control transcription and translation. By incorporation into a DNA promoter, azobenzenes controlled cell-free transcription [48] and gene expression [49], using UV or blue light as a stimulus. Incorporation of photoswitchable nucleobases into a G4 sequence of a promoter allowed for controlled expression of a reporter protein in zebrafish [50]. Reversible strategies for translation were also developed by capping the 5′ end of the mRNA with azobenzene or styryl photoswitches [50,51]. UV light inhibited the recruitment of a translation initiation factor, while blue light activated recruitment and expression to control differentiation in mammalian cells and zebrafish.

Gene silencing

ASOs are commonly used to knock down gene expression in cells via targeting complementary mRNA. Base pairing-inhibited 2-nitrobenzyl-caged thymidines have been incorporated into ASOs to photoactivate gene knockdown of developmental genes in zebrafish [52] and cancer-associated genes in mammalian cells [53]. By incorporating a photoreactive nucleobase into an ASO, covalent interstrand crosslinking to its complementary mRNA was achieved in mammalian cells [54].

ASOs can also be light-activated by removal of a complementary inhibitor strand. Inclusion of a 2-nitrobenzyl in the backbone of the complementary strand enabled endogenous gene knockdown and controlled development in zebrafish [55]. The complementary strand has also been attached to the ASO via a 2-nitrobenzyl-containing hairpin, which allowed for photocontrolled knockdown of a cancer-associated gene in mammalian cells [56] and developmental genes in zebrafish [57,58].

ASOs have also been circularised with a photocleavable linker, disrupting mRNA binding until photocleavage. Circularisation, achieved with 2-nitrobenzyl- [59], coumarin- [60], quinoline- [7] and Ru-BEP linkers [8] was applied to control expression with UV or blue light in zebrafish and mammalian cells.

Synthetic siRNAs are double stranded RNA molecules that are widely used to knock down expression of target genes via RNA interference (RNAi). Modification of the phosphate backbone of siRNAs with 2-nitrobenzyl allowed for light-activated knockdown in zebrafish [61]. Similarly, by attaching 2-nitrobenzyl-moieties to the 5′ [62,63] or 5′ and 3′ phosphate termini [64] cell patterning was achieved [65], and the RNAi off-state was improved by adding larger moieties [66]. Attaching an anthracene functionality along with a porphyrin photosensitiser onto the termini of siRNA controlled its function with green or red light [67]. Hybridisation of the siRNA strands was also controlled through incorporation of an azobenzene into the backbone and controlled with red light [68]. 2-Nitrobenzyl-photocaging of the Watson–Crick face of a single nucleobase in siRNA inhibited siRNA:mRNA duplex formation, and thus knocking down a reporter protein [69] and an endogenous gene [70]. An inhibitor of microRNA (miRNA), the natural substrate of RNAi knockdown, has been developed from an RNA hairpin connected through a 2-nitrobenzyl in the backbone [71]. Cleavage with UV allowed binding of the inhibitor to the miRNA, causing altered development in nematodes.

Light-responsive nanoparticles have been extensively used to allow spatiotemporal control of siRNA function. NIR light can release siRNA bound to gold nanoparticles (AuNPs). siRNA attached to AuNPs via terminal thiol-modified siRNA [72–74], DNA bridges [75], or electrostatically [76] enabled NIR-activated knockdown in mammalian cells and therapeutic effects on tumour models *in vivo*. Improved delivery was also accomplished by attaching cell-penetrating peptides to the constructs [73]. NIR-activated knockdown within mammalian cells was also achieved by attachment of siRNA, via a 2-nitrobenzyl, to upconverting nanoparticles (UCNPs), which convert NIR to UV irradiation [77]. Nanoparticles of siRNA have also been formed with positively charged block-copolymers, which aided cellular delivery, containing 2-nitrobenzyls [78].

A number of alterative methods have been used to photocontrol the silencing of gene expression. ASOs have been attached to AuNPs and activated with NIR in mice [79]. Azobenzenes have also been used to reversibly photocontrol binding of an inhibitory hairpin DNA attached to a DNAzyme to activate and deactivate cell-free expression of a reporter gene [80]. Gene knockdown has also been controlled by using a hairpin DNA decoy, modified with photocaged thymidines [81]. Implementing 2-nitrobenzyl-photocaged cytidines in a triplex-forming oligonucleotide also allowed for the light-controlled activation and silencing of gene expression in mammalian cells [82]. Modification of siRNA has also been used to control cell delivery with visible light [83,84].

Light-controlled gene expression using proteins

Proteins involved in gene expression have been extensively modified, both chemically and with naturally light-sensitive proteins, to control transcription and translation with light. Additionally, by combining these modified proteins with genome targeting technologies, it is possible to light-activate the expression of specific genes on a genome.

Chemical modification of proteins

Chemically modified proteins can be produced by engineering orthogonal ribosomes and tRNA/tRNA synthetase pairs to accept unnatural amino acids at a TGA codon. This allows precise placement of a photocage within a protein of interest. Using this method, a 2-nitrobenzyl was site-specifically incorporated into an RNA

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polymerase to demonstrate light-activated gene expression [85] and light-activated RNAi [85] in mammalian cells. Photocages have also been installed into gene editing tools, including Cre recombinases, which enable site specific recombination between DNA sequences called LoxP sites, and Zinc Finger (ZF) nucleases, which are selective towards target DNA sequences. Essential residues in catalytic sites of Cre and ZF nucleases have been modified with 2-nitrobenzyl [86,87] and coumarin [88] to activate expression upon irradiation. Alternatively, incorporation of an azobenzene into the backbone of an epigenetic regulating peptide has allowed photocontrol over its activity in mammalian cells [89].

Naturally light-sensitive proteins

In addition to modifying proteins with photocages, naturally light-responsive proteins from plants, cyanobacteria, and algae, have also been incorporated into proteins involved in gene expression (Figure 2B). The mechanisms of these engineered proteins fall into two categories: direct modulation of gene expression using light-activated transcription factors or enzymes, and indirect modulation through light-activated signalling cascades that modulate downstream effectors (Figure 4). While many different photoreceptors have been used to control gene expression, this review focuses primarily on three of the most common classes; phytochromes, Light-Oxygen-Voltage (LOV)-domain proteins, and cryptochromes.

Phytochromes (red and NIR light-responsive)

Phytochromes are photoreceptors that utilise tetrapyrrole chromophores such as biliverdin IXα (BV) or phycocyanobilin (PCB) (Figure 2B) to absorb red and NIR light and induce reversible conformational changes in the protein structure. The first photoreceptor used to control gene expression was Phytochrome protein B (PhyB), and its interaction partner Phytochrome-Interacting Factor 3 (PIF3), which dimerise under red light and dissociate under far-red light. By fusing DNA-Binding Domains (DBDs) and transActivation Domains (ADs) to the distinct PhyB and PIF modules, red light inducible two-hybrid systems have been used to activate gene expression in yeast [90] and mammalian cells [91] (Figure 4A-i).

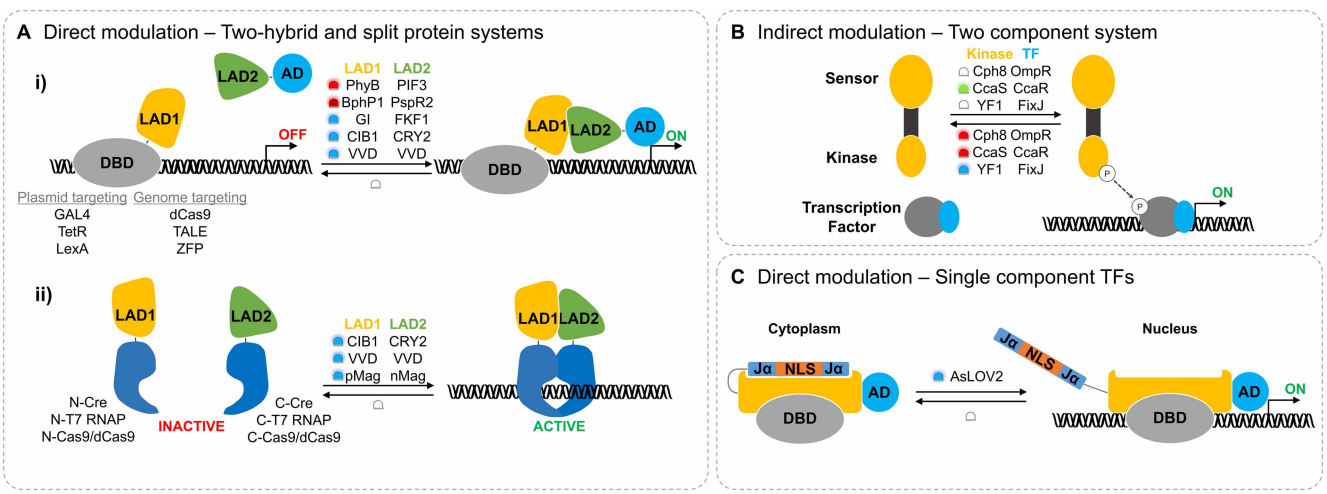

Figure 4. Controlling gene expression by using naturally light-sensitive proteins.

(**A**) Protein modules that dimerise in response to light, represented here as Light-Activated Domains (LADs), have been fused to effector domains to create transcription factors that activate gene expression in the presence of light, but are inactivate in the absence of light. Gene expression is activated upon co-localisation of DNA-Binding Domains (DBD) with transActivation Domains (ADs) via a light-activated two-hybrid system (i), or via the co-localisation of inactive C- and N-terminal domains of a split protein and reconstitution of the active protein (ii). (**B**) Light-responsive two component systems (TCSs) are initiated when light is absorbed by the sensory domain of a histidine kinase, which stimulates/represses autophosphorylation of the kinases domains. Phosphorylated kinase domains transfer phosphate groups to downstream Transcription Factors (TF), which can then bind to consensus promoter sequences and activate gene expression. (**C**) Uncaging of the Jα helix in AsLOV2, when exposed to blue light, is used to reveal a shielded Nuclear Localisation Signal (NLS). The exposed NLS is recognised by the importin complex and AsLOV2 is transported into the nucleus. Gene expression can be regulated by fusing DBDs and ADs to AsLOV2, and controlling their nuclear localisation by exposing or shielding the NLS.

In bacteria, Two-Component signalling Systems (TCSs) comprising of a natural or engineered light-responsive kinase and a downstream response regulator are more prevalent. Cph8 is a light-responsive kinase that was engineered by replacing the osmosensory domain of a membrane-bound histidine kinase, with the Cph1 phytochrome. In the absence of light, Cph8 phosphorylates the transcription factor OmpR and promotes gene expression, while in the presence of red light the kinase activity and gene expression are inactivated [92] (Figure 4B). The engineered Cph8/OmpR TCS is analogous to the natural cyanobacteriochrome TCS, CcaS/CcaR, which regulates gene expression according to green and red light [93] (Figure 4B). In their most basic forms, these systems were used in bacterial edge detection algorithms [94] and for dual-colour control over gene expression [95]. More efficient versions were later developed by genetic refactoring and mutagenesis [96,97] and have been used to tightly control metabolic flux [98], cell division [99] and feedback loops in bacteria [100].

Most of the red light-responsive gene expression systems require a PCB chromophore to function. Although PCB can be added to the growth media and taken up by cells [90,91], gene cassettes encoding enzymes for PCB biosynthesis are more commonly used to enable cells to synthesise PCB from intracellular heme [92,101,102]. NIR-responsive phytochromes on the other hand utilise BV chromophores, which are produced endogenously by mammalian cells. These NIR-responsive gene expression systems are derived from BphP1 and PpsR2 proteins. PpsR2 is sequestered by BphP1 in NIR light and then released in the presence of red light or absence of light. NIR-responsive transcription factors were created by fusing BphP1 and PpsR2 to DBDs and ADs, and have been shown to activate reporter gene expression in bacteria [103], mammalian cells, and mice [104,105] (Figure 4A-i).

LOV domain proteins and Cryptochromes (blue-light responsive)

LOV domain proteins and cryptochromes are two distinct protein families that differ in their protein architecture, yet both use blue light absorbing flavin chromophores (Figure 2B) to induce conformational changes in the protein. Whereas many different LOV domain systems have been developed for blue light-activated gene expression, cryptochrome-based systems are based primarily on the interaction of Cryptochrome protein 2 (CRY2) with Cryptochrome-Interacting Basic-helix-loop-helix protein (CIB1).

The first blue light-responsive transcription system was a TCS implementing an engineered light-sensitive kinase, YF1, created by replacing an oxygen-sensing domain of a natural kinase with the YvtA LOV domain. In the absence of light, YF1 phosphorylated and activated a transcription factor, whereas in the presence of blue-light, kinase activity and gene expression were inactivated [106] (Figure 4B). Based on this system, a bacterial repression switch, pDusk, and activation switch, pDawn [107], were developed. Blue light-regulated gene expression in eukaryotic cells, however, is typically controlled with photoactivatable transcription factors. Flavin-binding, Kelch domain, F-box protein (FKF1) and its interaction partner GIGANTEA (GI) were the first photoreceptor pair to be fused to ADs and DBDs and demonstrated blue light-activated transcription in mammalian cells [108] (Figure 4A-i). Similar light-activated effector proteins based on CRY2/CIB1, and smaller LOV domain proteins also function in this way. Amongst these is Vivid (VVD), a photoreceptor that rapidly and reversibly forms homodimers. CRY2/CIB1 and VVD have been fused to a variety of DBDs and effector domains to control both transcription [109,110] and translation [111,112] in mammalian cells, bacteria [113], and yeast [110,114,115]. They have been applied in various fields including the study of oscillating gene expression [116] and in cell-based immunotherapies [117]. Blue light-induced dimerisation of CRY2/CIB1, VVD, and 'Magnets', improved VVD mutants [118], have also been used to control gene expression by reconstituting split enzymes, such as Cre recombinases [110,119,120] and RNA polymerases [121,122] (Figure 4A-ii).

In most cases, light-inducible transcription factors depend on the heterodimerisation of two different proteins to modulate gene expression. However, single component blue light-activated transcription factors also exist, and are typically derived from AsLOV2 or EL222. Upon exposure to blue light, both AsLOV2 and EL222 undergo conformational changes involving the release of an alpha helix from the LOV domain. By inserting nuclear localisation signals (NLS) or nuclear export signals (NES) within the Jα helix of AsLOV2, its cellular location can be switched with blue light (Figure 4C). In this way, genes have been expressed via the import of AsLOV2-based transactivators into the nucleus [123,124], or export of AsLOV2-based repressors into the cytoplasm [125,126]. Alternatively, uncaging of the 4α helix in EL222 is accompanied by the release of a DBD and exposure of a dimerization interface. EL222 fused to an AD has been shown to rapidly induce gene expression in mammalian cells [127] and zebrafish embryos following irradiation [128], and has also been used in yeast to

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improve their chemical production capabilities [129]. Single component gene expression systems in bacteria [130] and cell-free expression systems [131] have used the transcription factor activity of wild type EL222.

Genome targeting for light-activated expression

Technologies that target specific sites on the genome allow for precise control of endogenous genes. Genome targeting has been achieved using several types of DNA binding proteins, that can be tailored to recognise specific DNA sequences [132]. These include the Clustered, Regularly Interspaced, Short Palindromic Repeat (CRISPR)-associated nuclease Cas9 system, ZF proteins, Transcription Activator-Like Effectors (TALEs), and recombinases. Photocages and naturally light-sensitive proteins have been incorporated into these technologies for spatiotemporal control of gene knockouts and transcription.

CRISPR-Cas9

CRISPR-Cas systems are found in bacteria and archaea as a means of adaptive immunological protection against phages. The bacterial Cas9 nuclease is targeted to the genome using a non-coding guide RNA (gRNA) and cleaves the double stranded DNA causing either a gene knockout or mutagenesis [132]. By mutating Cas9, a catalytically inactive protein (dCas9) can serve as an RNA-guided DNA-binding protein. Both the gRNA and Cas9 have been modified to create light-activated CRISPR-Cas9 systems. A protector DNA, containing 2-nitrobenzyls in the backbone has been bound to gRNA to control gene knockout in mammalian cells [133]. More efficient control was achieved by attaching 2-nitrobenzyls to the Watson–Crick face of nucleobases in the gRNA, which was used to control gene editing in zebrafish embryos [134,135]. To control the Cas9 nuclease, a 2-nitrobenzyl-modified amino acid was installed using an orthogonal tRNA/tRNA synthetase pair [136]. Cas9 has also been covalently linked to UCNPs, via a 2-nitrobenzyl photocage, allowing NIR-activated gene editing and reduction of tumour size in mice [137].

Naturally light-sensitive proteins have been used with both the Cas9 nuclease and dCas9 to achieve light-activated gene knockout and transcriptional control, respectively (Figure 4A). These rely on the dimerization of split Cas9 domains and/or dCas9 with ADs via photoreceptors, particularly CRY2/CIB1 [138–140] and magnet proteins [140,141]. Other light-activated CRISPR-Cas9 systems depend on light-activated phosphorylation [142] or cyclic diguanylate monophosphate (c-di-GMP) signalling cascades [143], as well as a dimeric green fluorescent protein, pdDronpa [144].

Zinc finger and transcription activator-like effector proteins

ZF proteins recognise a specific 3-base pair DNA sequence and individual TALE proteins each recognise a single base pair. Hence, effector proteins can be targeted to specific locations on a genome by fusing them to contiguous ZF and TALE domains [132]. By fusing ZFs to GI and an AD to FKF1, gene expression in mammalian cells has been controlled [145] (Figure 4A-i). TALEs and ADs using the CRY2/CIB1 pair have been used to regulate gene expression in mouse brain cortex [146] and recruitment of epigenetic modifiers in rat neuronal stem cells [147] (Figure 4A-i).

Recombinases

In recombinase-mediated gene editing, DNA flanked by LoxP recognition sites is commonly excised from the genome of transgenic organisms to study the response of gene knockouts [148]. By incorporating these artificial recognition sites in the genome, light-activated recombinases have been used to control the expression of targeted genes. Light-activated Cre systems have been developed using 2-nitrobenzyl-photocaged tamoxifen [149], which controlled light-dependent recombination and gene expression in mice [150]. CRY2/CIB1, magnet, or VVD pairs have also been fused to split Cre and Flp recombinase domains to control gene expression in mouse brains [151–154] and zebrafish embryos [153] (Figure 4A-ii). This has been applied to reconstruct the morphology of single neurons across an entire mouse brain [153].

Outlook

Controlling gene expression with light has led to a wide range of applications in cell-free to *in vivo* systems. Chemical modification of nucleic acids with photocages has been used to create conductive pathways in synthetic tissues [37,38] and control the development of zebrafish [7,55,60,134,135] and cancer therapeutics [75,76,79,137] with light. While naturally light-sensitive proteins have been used to control cell division [99],

bioreactors [129], and genome activation in zebrafish and mice [146,151,153]. Identification of new applications is vital to realising the full potential of light-controlled gene expression.

As we have focused on discussing methods and applications within this review, there has been a limited discussion of the efficiencies of each system. To allow general use of these technologies an efficient 'ON' and 'OFF' state is required, where minimal activity is observed without light, and maximal with light. It is worth noting the efficiencies vary wildly for the methods discussed. More efficient and easily accessible systems are required to allow more general use.

Photocages and naturally light-sensitive proteins each have their own advantages and limitations. Plasmid DNA encoding light-sensitive proteins and some simple photocages attached to DNA are commercially available. Most photocaged small molecules and the more advanced photocages must be synthesised in house. However, this is also an advantage as more diverse systems have been generated through the chemical synthesis of photocages with small molecules, nucleic acids, and proteins. Another advantage of photocages is their immediate generation of an active species, compared with the requirement that cells first generate the light-sensitive proteins from plasmid DNA, prior to their application. Most chemical photocages absorb in the UV, which can damage cells. Chemical photocages that absorb at longer wavelengths of light, which minimises cellular damage and increases tissue penetration [155], are becoming more popular. However, more easily accessible NIR photocages would give a major boost to this area. In contrast with this, naturally light-sensitive proteins regularly absorb UV, visible or NIR light. Hence, more orthogonal systems also exist for light-sensitive proteins, where expression of different genes can be put under the control of different wavelengths of light, even inside the same cell. Additionally, many reversible naturally light-sensitive proteins exist, compared with only a few chemical photoswitches. Regarding cell delivery, small molecules are highly advantageous as they are cell permeable. Plasmid DNA encoding naturally light-sensitive proteins and photocaged nucleic acids must be transfected. Naturally light-sensitive proteins also tend to require multiple or complex plasmids to be delivered. However, in bacteria and yeast these systems can be controlled over multiple generations as the plasmid system encoding the light-sensitive proteins is replicated. An important goal for this field is to combine the light-activation systems with cell delivery systems, where only limited examples exist [53,72,73,78,146,151,153], to produce far-reaching technologies.

Perspective

- **Importance:** Using light to control gene expression opens the door to myriad biotechnology applications in therapeutics, biomanufacturing, and emerging fields such as cell-free systems.
- **Summary:** Control of exogenous and endogenous gene expression has been achieved in many different ways. Chemical tools rely on attaching photocages to small molecules, nucleic acids, and proteins, while biological tools use engineered naturally light-sensitive proteins.
- **Direction:** Beyond identifying new applications, the three main areas of research that still require attention are: increased usage of longer wavelength photocages, more efficient and general systems, and combining light-activation with cell delivery.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations
AD, transactivation domain; ASO, antisense oligonucleotide; ATP, adenosine triphosphate; AuNP, gold nanoparticle; BV, biliverdin Ixα; c-di-GMP, cyclic diguanylate monophosphate; CIB1, cryptochrome-interacting basic helix-loop-helix protein; CREB, cAMP response element-binding protein; CRISPR, clustered, regularly interspaced, short palindromic repeat; CRY2, cryptochrome protein 2; DBD, DNA binding domain; dCas9, deactivated Cas9; DNA, deoxyribonucleic acid; FKF1, Flavin-binding, Kelch domain, F-box protein; G4, G-quadruplex; GI, GIGANTEA; gRNA, guide RNA; hm, hydroxymethyl; IPTG, isopropyl-β-D-thiogalactopyranoside; LAD, light-activated domain; LOV, light-oxygen-voltage; miRNA, microRNA; mRNA, messenger RNA; NIR, near-infrared; NLS, nuclear localisation signal; NP, nanoparticle; PCB, phycocyanobilin; PCR, polymerase chain reaction; PhyB, phytochrome protein B; PIF3, phytochrome interacting factor 3; RNA, ribonucleic acid; RNAi, RNA interference; siRNA, small interfering RNA; TALE, transcription activator-like effectors; TCS, two component system; Tet, tetracycline; TF, transcription factor; tRNA, transfer RNA; UCNP, upconverting nanoparticle; UV, ultraviolet; VVD, Vivid; ZF, zinc finger.

References
1 Devor, A., Sakadžić, S., Srinivasan, V.J., Yaseen, M.A., Nizar, K., Saisan, P.A. et al. (2012) Frontiers in optical imaging of cerebral blood flow and metabolism. *J. Cereb. Blood Flow Metab.* **32**, 1259–1276 https://doi.org/10.1038/jcbfm.2011.195
2 Hughes, R.M. (2018) A compendium of chemical and genetic approaches to light-regulated gene transcription. *Crit. Rev. Biochem. Mol. Biol.* **53**, 453–474 https://doi.org/10.1080/10409238.2018.1487382
3 Deiters, A. (2009) Light activation as a method of regulating and studying gene expression. *Curr. Opin. Chem. Biol.* **13**, 678–686 https://doi.org/10.1016/j.cbpa.2009.09.026
4 de Mena, L., Rizk, P. and Rincon-Limas, D.E. (2018) Bringing light to transcription: the optogenetics repertoire. *Front. Genet.* **9**, 518 https://doi.org/10.3389/fgene.2018.00518
5 Klán, P., Šolomek, T., Bochet, C.G., Blanc, A., Givens, R., Rubina, M. et al. (2013) Photoremovable protecting groups in chemistry and biology: reaction mechanisms and efficacy. *Chem. Rev.* **113**, 119–191 https://doi.org/10.1021/cr300177k
6 Bojtár, M., Kormos, A., Kis-Petik, K., Kellermayer, M. and Kele, P. (2019) Green-light activatable, water-soluble red-shifted coumarin photocages. *Org. Lett.* **21**, 9410–9414 https://doi.org/10.1021/acs.orglett.9b03624
7 O'Connor, M.J., Beebe, L.L., Deodato, D., Ball, R.E., Page, A.T., Vanleuven, A.J. et al. (2019) Bypassing glutamic acid decarboxylase 1 (Gad1) induced craniofacial defects with a photoactivatable translation blocker morpholino. *ACS Chem. Neurosci.* **10**, 266–278 https://doi.org/10.1021/acschemneuro.8b00231
8 Griepenburg, J.C., Rapp, T.L., Carroll, P.J., Eberwine, J. and Dmochowski, I.J. (2015) Ruthenium-caged antisense morpholinos for regulating gene expression in zebrafish embryos. *Chem. Sci.* **6**, 2342–2346 https://doi.org/10.1039/C4SC03990D
9 Gorka, A.P., Nani, R.R., Zhu, J., Mackem, S. and Schnermann, M.J. (2014) A near-IR uncaging strategy based on cyanine photochemistry. *J. Am. Chem. Soc.* **136**, 14153–14159 https://doi.org/10.1021/ja5065203
10 Beharry, A.A. and Woolley, G.A. (2011) Azobenzene photoswitches for biomolecules. *Chem. Soc. Rev.* **40**, 4422–4437 https://doi.org/10.1039/c1cs15023e
11 Görner, H. and Kuhn, H.J. (2007) *Cis-trans* photoisomerization of stilbenes and stilbene-like molecules. In *Advances in Photochemistry* (Neckers, D.C., Volman, D.H. and von Bünau, G., eds), pp. 1–117, John Wiley & Sons Ltd
12 Dong, M., Babalhavaeji, A., Collins C, V., Jarrah, K., Sadovski, O., Dai, Q. et al. (2017) Near-infrared photoswitching of azobenzenes under physiological conditions. *J. Am. Chem. Soc.* **139**, 13483–13486 https://doi.org/10.1021/jacs.7b06471
13 Cruz, F.G., Koh, J.T. and Link, K.H. (2000) Light-activated gene expression. *J. Am. Chem. Soc.* **122**, 8777–8778 https://doi.org/10.1021/ja001804h
14 Faal, T., Wong, P.T., Tang, S., Coulter, A., Chen, Y., Tu, C.H. et al. (2015) 4-Hydroxytamoxifen probes for light-dependent spatiotemporal control of Cre-ER mediated reporter gene expression. *Mol. Biosyst.* **11**, 783–790 https://doi.org/10.1039/C4MB00581C
15 Wong, P.T., Roberts, E.W., Tang, S., Mukherjee, J., Cannon, J., Nip, A.J. et al. (2017) Control of an unusual photo-Claisen rearrangement in coumarin caged tamoxifen through an extended spacer. *ACS Chem. Biol.* **12**, 1001–1010 https://doi.org/10.1021/acschembio.6b00999
16 Gorka, A.P., Yamamoto, T., Zhu, J. and Schnermann, M.J. (2018) Cyanine photocages enable spatial control of inducible Cre-mediated recombination. *ChemBioChem* **19**, 1239–1243 https://doi.org/10.1002/cbic.201800061
17 Zhang, W., Hamouri, F., Feng, Z., Aujard, I., Ducos, B., Ye, S. et al. (2018) Control of protein activity and gene expression by cyclofen-OH uncaging. *ChemBioChem* **19**, 1232–1238 https://doi.org/10.1002/cbic.201700630

18 Fournier, L., Gauron, C., Xu, L., Aujard, I., Le Saux, T., Gagey-Eilstein, N. et al. (2013) A blue-absorbing photolabile protecting group for in vivo chromatically orthogonal photoactivation. *ACS Chem. Biol.* **8**, 1528–1536 https://doi.org/10.1021/cb400178m
19 Cambridge, S.B., Geissler, D., Keller, S. and Cürten, B. (2006) A caged doxycycline analogue for photoactivated gene expression. *Angew. Chem. Int. Ed. Engl.* **45**, 2229–2231 https://doi.org/10.1002/anie.200503339
20 Cambridge, S.B., Geissler, D., Calegari, F., Anastassiadis, K., Hasan, M.T., Stewart, A.F. et al. (2009) Doxycycline-dependent photoactivated gene expression in eukaryotic systems. *Nat. Methods* **6**, 527–531 https://doi.org/10.1038/nmeth.1340
21 Link, K.H., Cruz, F.G., Ye, H.F., O'Reilly, K.E., Dowdell, S. and Koh, J.T. (2004) Photo-caged agonists of the nuclear receptors RARγ and TRβ provide unique time-dependent gene expression profiles for light-activated gene patterning. *Bioorganic Med. Chem.* **12**, 5949–5959 https://doi.org/10.1016/j.bmc.2004.08.022
22 Lin, W., Albanese, C., Pestell, R.G. and Lawrence, D.S. (2002) Spatially discrete, light-driven protein expression. *Chem. Biol.* **9**, 1347–1353 https://doi.org/10.1016/S1074-5521(02)00288-0
23 Imoto, T., Kawase, A., Minoshima, M., Yokoyama, T., Bito, H. and Kikuchi, K. (2020) Photolytic release of a caged inhibitor of an endogenous transcription factor enables optochemical control of CREB-mediated gene expression. *Org. Lett.* **22**, 22–25 https://doi.org/10.1021/acs.orglett.9b03568
24 Young, D.D. and Deiters, A. (2007) Photochemical activation of protein expression in bacterial cells. *Angew. Chem. Int. Ed. Engl.* **119**, 4368–4370 https://onlinelibrary.wiley.com/doi/abs/10.1002/anie.200700057
25 Binder, D., Frohwitter, J., Mahr, R., Bier, C., Grünberger, A., Loeschcke, A. et al. (2016) Light-controlled cell factories: employing photocaged isopropyl-β-D-thiogalactopyranoside for light-mediated optimization of lac-based gene expression and (+)-valencene biosynthesis in corynebacterium glutamicum. *Appl. Environ. Microbiol.* **82**, 6141–6149 https://doi.org/10.1128/AEM.01457-16
26 Binder, D., Bier, C., Grünberger, A., Drobietz, D., Hage-Hülsmann, J., Wandrey, G. et al. (2016) Photocaged arabinose: a novel optogenetic switch for rapid and gradual control of microbial gene expression. *ChemBioChem* **17**, 296–299 https://doi.org/10.1002/cbic.201500609
27 Pinheiro, A.V., Baptista, P. and Lima, J.C. (2008) Light activation of transcription: photocaging of nucleotides for control over RNA polymerization. *Nucleic Acids Res.* **36**, e90 https://doi.org/10.1093/nar/gkn415
28 Chan, V., Novakowski, S.K., Law, S., Klein-Bosgoed, C. and Kastrup, C.J. (2015) Controlled transcription of exogenous mRNA in platelets using protocells. *Angew. Chem. Int. Ed. Engl.* **54**, 13590–13593 https://doi.org/10.1002/anie.201506500
29 Ohno, K., Sugiyama, D., Takeshita, L., Kanamori, T., Masaki, Y., Sekine, M. et al. (2017) Synthesis of photocaged 6-O-(2-nitrobenzyl)guanosine and 4-O-(2-nitrobenzyl) uridine triphosphates for photocontrol of the RNA transcription reaction. *Bioorganic Med. Chem.* **25**, 6007–6015 https://doi.org/10.1016/j.bmc.2017.09.032
30 Young, D.D., Garner, R.A., Yoder, J.A. and Deiters, A. (2009) Light-activation of gene function in mammalian cells via ribozymes. *Chem. Commun.*, 568–570 https://doi.org/10.1039/b819375d
31 Walsh, S., Gardner, L., Deiters, A. and Williams, G.J. (2014) Intracellular light-activation of riboswitch activity. *ChemBioChem* **15**, 1346–1351 https://doi.org/10.1002/cbic.201400024
32 Murat, P., Gormally M, V., Sanders, D., Di, A.M. and Balasubramanian, S. (2013) Light-mediated in cell downregulation of G-quadruplex-containing genes using a photo-caged ligand. *Chem. Commun.* **49**, 8453–8455 https://doi.org/10.1039/c3cc44737e
33 Estévez-Torres, A., Crozatier, C., Diguet, A., Hara, T., Saito, H., Yoshikawa, K. et al. (2009) Sequence-independent and reversible photocontrol of transcription/ expression systems using a photosensitive nucleic acid binder. *Proc. Natl Acad. Sci. U.S.A.* **106**, 12219–12223 https://doi.org/10.1073/pnas.0904382106
34 Kröck, L. and Hechel, A. (2005) Photoinduced transcription by using temporarily mismatched caged oligonucleotides. *Angew. Chem. Int. Ed. Engl.* **44**, 471–473 https://doi.org/10.1002/anie.200461779
35 Hemphill, J., Govan, J., Uprety, R., Tsang, M. and Deiters, A. (2014) Site-specific promoter caging enables optochemical gene activation in cells and animals. *J. Am. Chem. Soc.* **136**, 7152–7158 https://doi.org/10.1021/ja500327g
36 Stafforst, T. and Stadler, J.M. (2013) Photoactivation of a psoralen-blocked luciferase gene by blue light. *Angew. Chem. Int. Ed. Engl.* **52**, 12448–12451 https://doi.org/10.1002/anie.201306150
37 Booth, M.J., Restrepo Schild, V., Graham, A.D., Olof, S.N. and Bayley, H. (2016) Light-activated communication in synthetic tissues. *Sci. Adv.* **2**, e1600056 https://doi.org/10.1126/sciadv.1600056
38 Booth, M.J., Restrepo Schild, V., Box, S.J. and Bayley, H. (2017) Light-patterning of synthetic tissues with single droplet resolution. *Sci. Rep.* **7**, 9315 https://doi.org/10.1038/s41598-017-09394-9
39 Vaníková, Z., Janoušková, M., Kambová, M., Krásný, L. and Hocek, M. (2019) Switching transcription with bacterial RNA polymerase through photocaging, photorelease and phosphorylation reactions in the major groove of DNA. *Chem. Sci.* **10**, 3937–3942 https://doi.org/10.1039/C9SC00205G
40 Heimes, M., Kolmar, L. and Brieke, C. (2018) Efficient cosubstrate enzyme pairs for sequence-specific methyltransferase-directed photolabile caging of DNA. *Chem. Commun.* **54**, 12718–12721 https://doi.org/10.1039/C8CC05913F
41 Zhang, D., Zhou, C.Y., Busby, K.N., Alexander, S.C. and Devaraj, N.K. (2018) Light-activated control of translation by enzymatic covalent mRNA labeling. *Angew. Chem. Int. Ed. Engl.* **57**, 2822–2826 https://doi.org/10.1002/anie.201710917
42 Zhang, D., Jin, S., Piao, X. and Devaraj, N.K. (2020) Multiplexed photoactivation of mRNA with single-Cell resolution. *ACS Chem. Biol.* https://doi.org/10.1021/acschembio.0c00205
43 Yamaguchi, S., Chen, Y., Nakajima, S., Furuta, T. and Nagamune, T. (2010) Light-activated gene expression from site-specific caged DNA with a biotinylated photolabile protection group. *Chem. Commun.* **46**, 2244–2246 https://doi.org/10.1039/b922502a
44 Ando, H., Kobayashi, M., Tsubokawa, T., Uyemura, K., Furuta, T. and Okamoto, H. (2005) Lhx2 mediates the activity of Six3 in zebrafish forebrain growth. *Dev. Biol.* **287**, 456–468 https://doi.org/10.1016/j.ydbio.2005.09.023
45 Ando, H., Furuta, T., Tsien, R.Y. and Okamoto, H. (2001) Photo-mediated gene activation using caged RNA/DNA in zebrafish embryos. *Nat. Genet.* **28**, 317–325 https://doi.org/10.1038/ng583
46 Monroe, W.T., McQuain, M.M., Chang, M.S., Alexander, J.S. and Haselton, F.R. (1999) Targeting expression with light using caged DNA. *J. Biol. Chem.* **274**, 20895–20900 https://doi.org/10.1074/jbc.274.30.20895
47 Schroeder, A., Goldberg, M.S., Kastrup, C., Wang, Y., Jiang, S., Joseph, B.J. et al. (2012) Remotely activated protein-producing nanoparticles. *Nano Lett.* **12**, 2685–2689 https://doi.org/10.1021/nl2036047

48 Liang, X., Wakuda, R., Fujioka, K. and Asanuma, H. (2010) Photoregulation of DNA transcription by using photoresponsive T7 promoters and clarification of its mechanism. *FEBS J.* **277**, 1551–1561 https://doi.org/10.1111/j.1742-4658.2010.07583.x
49 Kamiya, Y., Takagi, T., Ooi, H., Ito, H., Liang, X. and Asanuma, H. (2015) Synthetic gene involving azobenzene-tethered T7 promoter for the photocontrol of gene expression by visible light. *ACS Synth. Biol.* **4**, 365–370 https://doi.org/10.1021/sb5001092
50 Ogasawara, S. (2018) Transcription driven by reversible photocontrol of hyperstable G-Quadruplexes. *ACS Synth. Biol.* **7**, 2507–2513 https://doi.org/10.1021/acssynbio.8b00216
51 Ogasawara, S. (2014) Control of cellular function by reversible photoregulation of translation. *ChemBioChem* **15**, 2652–2655 https://doi.org/10.1002/cbic.201402495
52 Deiters, A., Garner, R.A., Lusic, H., Govan, J.M., Dush, M., Nascone-Yoder, N.M. et al. (2010) Photocaged morpholino oligomers for the light-regulation of gene function in zebrafish and xenopus embryos. *J. Am. Chem. Soc.* **132**, 15644–15650 https://doi.org/10.1021/ja1053863
53 Govan, J.M., Uprety, R., Thomas, M., Lusic, H., Lively, M.O. and Deiters, A. (2013) Cellular delivery and photochemical activation of antisense agents through a nucleobase caging strategy. *ACS Chem. Biol.* **8**, 2272–2282 https://doi.org/10.1021/cb400293e
54 Sakamoto, T., Shigeno, A., Ohtaki, Y. and Fujimoto, K. (2014) Photo-regulation of constitutive gene expression in living cells by using ultrafast photo-cross-linking oligonucleotides. *Biomater. Sci.* **2**, 1154–1157 https://doi.org/10.1039/C4BM00117F
55 Tallafuss, A., Gibson, D., Morcos, P., Li, Y., Seredick, S., Eisen, J. et al. (2012) Turning gene function ON and OFF using sense and antisense photo-morpholinos in zebrafish. *Development* **139**, 1691–1699 https://doi.org/10.1242/dev.072702
56 Tang, X., Swaminathan, J., Gewirtz, A.M. and Dmochowski, I.J. (2007) Regulating gene expression in human leukemia cells using light-activated oligodeoxynucleotides. *Nucleic Acids Res.* **36**, 559–569 https://doi.org/10.1093/nar/gkm1029
57 Tang, X.J., Maegawa, S., Weinberg, E.S. and Dmochowski, I.J. (2007) Regulating gene expression in zebrafish embryos using light-activated, negatively charged peptide nucleic acids. *J. Am. Chem. Soc.* **129**, 11000–11001 https://doi.org/10.1021/ja073723s
58 Ouyang, X., Shestopalov, I.A., Sinha, S., Zheng, G., Pitt, C.L.W., Li, W.H. et al. (2009) Versatile synthesis and rational design of caged morpholinos. *J. Am. Chem. Soc.* **131**, 13255–13269 https://doi.org/10.1021/ja809933h
59 Yang, L., Kim, H.B., Sul, J.-Y., Yeldell, S.B., Eberwine, J.H. and Dmochowski, I.J. (2018) Efficient synthesis of light-triggered circular antisense oligonucleotides targeting cellular protein expression. *ChemBioChem* **19**, 1250–1254 https://doi.org/10.1002/cbic.201800012
60 Yamazoe, S., Liu, Q., McQuade, L.E., Deiters, A. and Chen, J.K. (2014) Sequential gene silencing using wavelength-selective caged morpholino oligonucleotides. *Angew. Chemie Int. Ed. Engl.* **53**, 10114–10118 https://doi.org/10.1002/anie.201405355
61 Blidner, R.A., Svoboda, K.R., Hammer, R.P. and Monroe, W.T. (2008) Photoinduced RNA interference using DMNPE-caged 2′-deoxy-2′- fluoro substituted nucleic acids in vitro and in vivo. *Mol. Biosyst.* **4**, 431–440 https://doi.org/10.1039/b801532e
62 Zhang, L., Chen, C., Fan, X. and Tang, X. (2018) Photomodulating gene expression by using caged siRNAs with single-aptamer modification. *ChemBioChem* **19**, 1259–1263 https://doi.org/10.1002/cbic.201700623
63 Yu, L., Jing, N., Yang, Z., Zhang, L. and Tang, X. (2018) Caged siRNAs with single folic acid modification of antisense RNA for photomodulation of exogenous and endogenous gene expression in cells. *Org. Biomol. Chem.* **16**, 7029–7035 https://doi.org/10.1039/C8OB01952E
64 Kala, A., Jain, P.K., Karunakaran, D., Shah, S. and Friedman, S.H. (2014) The synthesis of tetra-modified RNA for the multidimensional control of gene expression via light-activated RNA interference. *Nat. Protoc.* **9**, 11–20 https://doi.org/10.1038/nprot.2013.165
65 Kala, A., Jain, P.K. and Friedman, S.H. (2014) Patterning of cells through patterning of biology. *Mol. Biosyst.* **10**, 1689–1692 https://doi.org/10.1039/C3MB70587K
66 Jain, P.K., Shah, S. and Friedman, S.H. (2011) Patterning of gene expression using new photolabile groups applied to light activated RNAi. *J. Am. Chem. Soc.* **133**, 440–446 https://doi.org/10.1021/ja107226e
67 Meyer, A. and Mokhir, A. (2014) RNA interference controlled by light of variable wavelength. *Angew. Chem. Int. Ed. Engl.* **53**, 12840–12843 https://doi.org/10.1002/anie.201405885
68 Hammill, M.L., Islam, G. and Desaulniers, J. (2020) Synthesis, derivatization and photochemical control of ortho-functionalized tetrachlorinated azobenzene-modified siRNAs. *ChemBioChem* https://doi.org/10.1002/cbic.202000188
69 Mikat, V. and Heckel, A. (2007) Light-dependent RNA interference with nucleobase-caged siRNAs. *RNA* **13**, 2341–2347 https://doi.org/10.1261/rna.753407
70 Govan, J.M., Young, D.D., Lusic, H., Liu, Q., Lively, M.O. and Deiters, A. (2013) Optochemical control of RNA interference in mammalian cells. *Nucleic Acids Res.* **41**, 10518–10528 https://doi.org/10.1093/nar/gkt806
71 Zheng, G., Cochella, L., Liu, J., Hobert, O. and Li, W.H. (2011) Temporal and spatial regulation of microRNA activity with photoactivatable cantimirs. *ACS Chem. Biol.* **6**, 1332–1338 https://doi.org/10.1021/cb200290e
72 Braun, G.B., Pallaoro, A., Wu, G., Missirlis, D., Zasadzinski, J.A., Tirrell, M. et al. (2009) Laser-activated gene silencing via gold nanoshell-siRNA conjugates. *ACS Nano.* **3**, 2007–2015 https://doi.org/10.1021/nn900469q
73 Huang, X., Hu, Q., Braun, G.B., Pallaoro, A., Morales, D.P., Zasadzinski, J. et al. (2015) Light-activated RNA interference in human embryonic stem cells. *Biomaterials* **63**, 70–79 https://doi.org/10.1016/j.biomaterials.2015.06.006
74 Riley, R.S., Dang, M.N., Billingsley, M.M., Abraham, B., Gundlach, L. and Day, E.S. (2018) Evaluating the mechanisms of light-triggered siRNA release from nanoshells for temporal control over gene regulation. *Nano Lett.* **18**, 3565–3570 https://doi.org/10.1021/acs.nanolett.8b00681
75 Zhang, P., Wang, C., Zhao, J., Xiao, A., Shen, Q., Li, L. et al. (2016) Near infrared-Guided smart nanocarriers for microRNA-controlled release of doxorubicin/siRNA with intracellular ATP as fuel. *ACS Nano.* **10**, 3637–3647 https://doi.org/10.1021/acsnano.5b08145
76 Wang, F., Huang, Q., Wang, Y., Shi, L., Shen, Y. and Guo, S. (2018) NIR-light and GSH activated cytosolic p65-shRNA delivery for precise treatment of metastatic cancer. *J. Control. Release* **288**, 126–135 https://doi.org/10.1016/j.jconrel.2018.09.002
77 Yang, Y., Liu, F., Liu, X. and Xing, B. (2013) NIR light controlled photorelease of siRNA and its targeted intracellular delivery based on upconversion nanoparticles. *Nanoscale* **5**, 231–238 https://doi.org/10.1039/C2NR32835F
78 Foster, A.A., Greco, C.T., Green, M.D., Epps, T.H. and Sullivan, M.O. (2015) Light-Mediated activation of siRNA release in diblock copolymer assemblies for controlled gene silencing. *Adv. Healthc. Mater.* **4**, 760–770 https://doi.org/10.1002/adhm.201400671
79 Huo, S., Gong, N., Jiang, Y., Chen, F., Guo, H., Gan, Y. et al. (2019) Gold-DNA nanosunflowers for efficient gene silencing with controllable transformation. *Sci. Adv.* **5**, eaaw6264 https://doi.org/10.1126/sciadv.aaw6264

80 Kamiya, Y., Arimura, Y., Ooi, H., Kato, K., Liang, X.-G. and Asanuma, H. (2018) Development of visible-light-responsive RNA scissors based on a 10-23 DNAzyme. *ChemBioChem* **19**, 1305–1311 https://doi.org/10.1002/cbic.201800020
81 Govan, J.M., Lively, M.O. and Deiters, A. (2011) Photochemical control of DNA decoy function enables precise regulation of nuclear factor κb activity. *J. Am. Chem. Soc.* **133**, 13176–13182 https://doi.org/10.1021/ja204980v
82 Govan, J.M., Uprety, R., Hemphill, J., Lively, M.O. and Deiters, A. (2012) Regulation of transcription through light-activation and light-deactivation of triplex-forming oligonucleotides in mammalian cells. *ACS Chem. Biol.* **7**, 1247–1256 https://doi.org/10.1021/cb300161r
83 Endoh, T., Sisido, M. and Ohtsuki, T. (2009) Spatial regulation of specific gene expression through photoactivation of RNAi. *J. Control. Release.* **137**, 241–245 https://doi.org/10.1016/j.jconrel.2009.04.015
84 Oliveira, S., Fretz, M.M., Høgset, A., Storm, G. and Schiffelers, R.M. (2007) Photochemical internalization enhances silencing of epidermal growth factor receptor through improved endosomal escape of siRNA. *Biochim. Biophys. Acta Biomembr.* **1768**, 1211–1217 https://doi.org/10.1016/j.bbamem.2007.01.013
85 Hemphill, J., Chou, C., Chin, J.W. and Deiters, A. (2013) Genetically encoded light-activated transcription for spatiotemporal control of gene expression and gene silencing in mammalian cells. *J. Am. Chem. Soc.* **135**, 13433–13439 https://doi.org/10.1021/ja4051026
86 Chou, C. and Deiters, A. (2011) Light-activated gene editing with a photocaged zinc-finger nuclease. *Angew. Chem. Int. Ed. Engl.* **50**, 6839–6842 https://doi.org/10.1002/anie.201101157
87 Luo, J., Arbely, E., Zhang, J., Chou, C., Uprety, R., Chin, J.W. et al. (2016) Genetically encoded optical activation of DNA recombination in human cells. *Chem. Commun.* **52**, 8529–8532 https://doi.org/10.1039/C6CC03934K
88 Brown, W. and Deiters, A. (2019) Light-activation of Cre recombinase in zebrafish embryos through genetic code expansion. *Methods Enzymol.* **624**, 265–281 https://doi.org/10.1016/bs.mie.2019.04.004
89 Albert, L., Xu, J., Wan, R., Srinivasan, V., Dou, Y. and Vázquez, O. (2017) Controlled inhibition of methyltransferases using photoswitchable peptidomimetics: towards an epigenetic regulation of leukemia. *Chem. Sci.* **8**, 4612–4618 https://doi.org/10.1039/C7SC00137A
90 Shimizu-Sato, S., Huq, E., Tepperman, J.M. and Quail, P.H. (2002) A light-switchable gene promoter system. *Nat. Biotechnol.* **20**, 1041–1044 https://doi.org/10.1038/nbt734
91 Müller, K., Engesser, R., Metzger, S., Schulz, S., Kämpf, M.M., Busacker, M. et al. (2013) A red/far-red light-responsive bi-stable toggle switch to control gene expression in mammalian cells. *Nucleic Acids Res.* **41**, e77 https://doi.org/10.1093/nar/gkt002
92 Levskaya, A., Chevalier, A.A., Tabor, J.J., Simpson, Z.B., Lavery, L.A., Levy, M. et al. (2005) Engineering *Escherichia coli* to see light. *Nature* **438**, 441–442 https://doi.org/10.1038/nature04405
93 Hirose, Y., Shimada, T., Narikawa, R., Katayama, M. and Ikeuchi, M. (2008) Cyanobacteriochrome CcaS is the green light receptor that induces the expression of phycobilisome linker protein. *Proc. Natl Acad. Sci. U.S.A.* **105**, 9528–9533 https://doi.org/10.1073/pnas.0801826105
94 Tabor, J.J., Salis, H.M., Simpson, Z.B., Chevalier, A.A., Levskaya, A., Marcotte, E.M. et al. (2009) A synthetic genetic edge detection program. *Cell* **137**, 1272–1281 https://doi.org/10.1016/j.cell.2009.04.048
95 Tabor, J.J., Levskaya, A. and Voigt, C.A. (2011) Multichromatic control of gene expression in *Escherichia coli*. *J. Mol. Biol.* **405**, 315–324 https://doi.org/10.1016/j.jmb.2010.10.038
96 Ong, N.T. and Tabor, J.J. (2018) A miniaturized *Escherichia coli* green light sensor with high dynamic range. *ChemBioChem* **19**, 1255–1258 https://doi.org/10.1002/cbic.201800007
97 Schmidl, S.R., Sheth, R.U., Wu, A. and Tabor, J.J. (2014) Refactoring and optimization of light-switchable *Escherichia coli* two-component systems. *ACS Synth. Biol.* **3**, 820–831 https://doi.org/10.1021/sb500273n
98 Fernandez-Rodriguez, J., Moser, F., Song, M. and Voigt, C.A. (2017) Engineering RGB color vision into *Escherichia coli*. *Nat. Chem. Biol.* **13**, 706–708 https://doi.org/10.1038/nchembio.2390
99 Mushnikov N, V., Fomicheva, A., Gomelsky, M. and Bowman, G.R. (2019) Inducible asymmetric cell division and cell differentiation in a bacterium. *Nat. Chem. Biol.* **15**, 925–931 https://doi.org/10.1038/s41589-019-0340-4
100 Milias-Argeitis, A., Rullan, M., Aoki, S.K., Buchmann, P. and Khammash, M. (2016) Automated optogenetic feedback control for precise and robust regulation of gene expression and cell growth. *Nat. Commun.* **7**, 12546 https://doi.org/10.1038/ncomms12546
101 Uda, Y., Goto, Y., Oda, S., Kohchi, T., Matsuda, M. and Aoki, K. (2017) Efficient synthesis of phycocyanobilin in mammalian cells for optogenetic control of cell signaling. *Proc. Natl Acad. Sci. U.S.A.* **114**, 11962–11967 https://doi.org/10.1073/pnas.1707190114
102 Kyriakakis, P., Catanho, M., Hoffner, N., Thavarajah, W., Hu, V.J., Chao, S.S. et al. (2018) Biosynthesis of orthogonal molecules using ferredoxin and ferredoxin-NADP+ reductase systems enables genetically encoded phyB optogenetics. *ACS Synth. Biol.* **7**, 706–717 https://doi.org/10.1021/acssynbio.7b00413
103 Ong, N.T., Olson, E.J. and Tabor, J.J. (2018) Engineering an *E. coli* near-Infrared light sensor. *ACS Synth. Biol.* **7**, 240–248 https://doi.org/10.1021/acssynbio.7b00289
104 Kaberniuk, A., Shemetov, A.A. and Verkhusha V, V. (2016) A bacterial phytochrome-based optogenetic system controllable with near-infrared light. *Nat. Methods* **13**, 591–597 https://doi.org/10.1038/nmeth.3864
105 Redchuk, T.A., Omelina, E.S., Chernov, K.G. and Verkhusha V, V. (2017) Near-infrared optogenetic pair for protein regulation and spectral multiplexing. *Nat. Chem. Biol.* **13**, 633–639 https://doi.org/10.1038/nchembio.2343
106 Möglich, A., Ayers, R.A. and Moffat, K. (2009) Design and signaling mechanism of light-Regulated histidine kinases. *J. Mol. Biol.* **385**, 1433–1444 https://doi.org/10.1016/j.jmb.2008.12.017
107 Ohlendorf, R., Vidavski, R.R., Eldar, A., Moffat, K. and Möglich, A. (2012) From dusk till dawn: one-plasmid systems for light-regulated gene expression. *J. Mol. Biol.* **416**, 534–542 https://doi.org/10.1016/j.jmb.2012.01.001
108 Yazawa, M., Sadaghiani, A.M., Hsueh, B. and Dolmetsch, R.E. (2009) Induction of protein-protein interactions in live cells using light. *Nat. Biotechnol.* **27**, 941–945 https://doi.org/10.1038/nbt.1569
109 Wang, X., Chen, X. and Yang, Y. (2012) Spatiotemporal control of gene expression by a light-switchable transgene system. *Nat. Methods* **9**, 266–269 https://doi.org/10.1038/nmeth.1892
110 Taslimi, A., Zoltowski, B., Miranda, J.G., Pathak, G.P., Hughes, R.M. and Tucker, C.L. (2016) Optimized second-generation CRY2-ClB dimerizers and photoactivatable Cre recombinase. *Nat. Chem. Biol.* **12**, 425–430 https://doi.org/10.1038/nchembio.2063

111 Cao, J., Arha, M., Sudrik, C., Schaffer D, V. and Kane, R.S. (2014) Bidirectional regulation of mRNA translation in mammalian cells by using PUF domains. *Angew. Chem. Int. Ed. Engl.* **53**, 4900–4904 https://doi.org/10.1002/anie.201402095
112 Kim, N.Y., Lee, S., Yu, J., Kim, N., Won, S.S., Park, H. et al. (2020) Optogenetic control of mRNA localization and translation in live cells. *Nat. Cell Biol.* **22**, 341–352 https://doi.org/10.1038/s41556-020-0468-1
113 Chen, X., Liu, R., Ma, Z., Xu, X., Zhang, H., Xu, J. et al. (2016) An extraordinary stringent and sensitive light-switchable gene expression system for bacterial cells. *Cell Res.* **26**, 854–857 https://doi.org/10.1038/cr.2016.74
114 Xu, X., Du, Z., Liu, R., Li, T., Zhao, Y., Chen, X. et al. (2018) A single-component optogenetic system allows stringent switch of gene expression in yeast cells. *ACS Synth. Biol.* **7**, 2045–2053 https://doi.org/10.1021/acssynbio.8b00180
115 An-Adirekkun, J.M., Stewart, C.J., Geller, S.H., Patel, M.T., Melendez, J., Oakes, B.L. et al. (2020) A yeast optogenetic toolkit (yOTK) for gene expression control in *Saccharomyces cerevisiae*. *Biotechnol. Bioeng.* **117**, 886–893 https://doi.org/10.1002/bit.27234
116 Yoshioka-Kobayashi, K., Matsumiya, M., Niino, Y., Isomura, A., Kori, H., Miyawaki, A. et al. (2020) Coupling delay controls synchronized oscillation in the segmentation clock. *Nature* **580**, 119–123 https://doi.org/10.1038/s41586-019-1882-z
117 Huang, Z., Wu, Y., Allen, M.E., Pan, Y., Kyriakakis, P., Lu, S. et al. (2020) Engineering light-controllable CAR T cells for cancer immunotherapy. *Sci. Adv.* **6**, eaay9209 https://doi.org/10.1126/sciadv.aay9209
118 Kawano, F., Suzuki, H., Furuya, A. and Sato, M. (2015) Engineered pairs of distinct photoswitches for optogenetic control of cellular proteins. *Nat. Commun.* **6**, 6256 https://doi.org/10.1038/ncomms7256
119 Polstein, L.R., Juhas, M., Hanna, G., Bursac, N. and Gersbach, C.A. (2017) An engineered optogenetic switch for spatiotemporal control of gene expression, cell differentiation, and tissue morphogenesis. *ACS Synth. Biol.* **6**, 2003–2013 https://doi.org/10.1021/acssynbio.7b00147
120 Sheets, M.B., Wong, W.W. and Dunlop, M.J. (2020) Light-inducible recombinases for bacterial optogenetics. *ACS Synth. Biol.* **9**, 227–235 https://doi.org/10.1021/acssynbio.9b00395
121 Han, T., Chen, Q. and Liu, H. (2017) Engineered photoactivatable genetic switches based on the bacterium phage T7 RNA polymerase. *ACS Synth. Biol.* **6**, 357–366 https://doi.org/10.1021/acssynbio.6b00248
122 Baumschlager, A., Aoki, S.K. and Khammash, M. (2017) Dynamic blue light-inducible T7 RNA polymerases (Opto-T7RNAPs) for precise spatiotemporal gene expression control. *ACS Synth. Biol.* **6**, 2157–2167 https://doi.org/10.1021/acssynbio.7b00169
123 Niopek, D., Benzinger, D., Roensch, J., Draebing, T., Wehler, P., Eils, R. et al. (2014) Engineering light-inducible nuclear localization signals for precise spatiotemporal control of protein dynamics in living cells. *Nat. Commun.* **5**, 4404 https://doi.org/10.1038/ncomms5404
124 Yumerefendi, H., Dickinson, D.J., Wang, H., Zimmerman, S.P., Bear, J.E., Goldstein, B. et al. (2015) Control of protein activity and cell fate specification via light-mediated nuclear translocation. *PLoS ONE* **10**, e0128443 https://doi.org/10.1371/journal.pone.0128443
125 Niopek, D., Wehler, P., Roensch, J., Eils, R. and Di Ventura, B. (2016) Optogenetic control of nuclear protein export. *Nat. Commun.* **7**, 10624 https://doi.org/10.1038/ncomms10624
126 Yumerefendi, H., Lerner, A.M., Zimmerman, S.P., Hahn, K., Bear, J.E., Strahl, B.D. et al. (2016) Light-induced nuclear export reveals rapid dynamics of epigenetic modifications. *Nat. Chem. Biol.* **12**, 399–401 https://doi.org/10.1038/nchembio.2068
127 Motta-Mena, L.B., Reade, A., Mallory, M.J., Glantz, S., Weiner, O.D., Lynch, K.W. et al. (2014) An optogenetic gene expression system with rapid activation and deactivation kinetics. *Nat. Chem. Biol.* **10**, 196–202 https://doi.org/10.1038/nchembio.1430
128 Reade, A., Motta-Mena, L.B., Gardner, K.H., Stainier, D.Y., Weiner, O.D. and Woo, S. (2017) TAEL: A zebrafish-optimized optogenetic gene expression system with fine spatial and temporal control. *Development* **144**, 345–355 https://doi.org/10.1242/dev.139238
129 Zhao, E.M., Zhang, Y., Mehl, J., Park, H., Lalwani, M.A., Toettcher, J.E. et al. (2018) Optogenetic regulation of engineered cellular metabolism for microbial chemical production. *Nature* **555**, 683–687 https://doi.org/10.1038/nature26141
130 Jayaraman, P., Devarajan, K., Chua, T.K., Zhang, H., Gunawan, E. and Poh, C.L. (2016) Blue light-mediated transcriptional activation and repression of gene expression in bacteria. *Nucleic Acids Res.* **44**, 6994–7005 https://doi.org/10.1093/nar/gkw548
131 Jayaraman, P., Yeoh, J.W., Jayaraman, S., Teh, A.Y., Zhang, J. and Poh, C.L. (2018) Cell-Free optogenetic gene expression system. *ACS Synth. Biol.* **7**, 986–994 https://doi.org/10.1021/acssynbio.7b00422
132 Guha, T.K., Wai, A. and Hausner, G. (2017) Programmable genome editing tools and their regulation for efficient genome engineering. *Comput. Struct. Biotechnol. J.* **15**, 146–160 https://doi.org/10.1016/j.csbj.2016.12.006
133 Jain, P.K., Ramanan, V., Schepers, A.G., Dalvie, N.S., Panda, A., Fleming, H.E. et al. (2016) Development of light-activated CRISPR using guide RNAs with photocleavable protectors. *Angew. Chem. Int. Ed. Engl.* **55**, 12440–12444 https://doi.org/10.1002/anie.201606123
134 Zhou, W., Brown, W., Bardhan, A., Delaney, M., Ilk, A., Rauen, R. et al. (2020) Spatiotemporal control of CRISPR/Cas9 function in cells and zebrafish using light-Activated guide RNA. *Angew. Chem. Int. Ed. Engl.* **59**, 8998–9003 https://doi.org/10.1002/anie.201914575
135 Moroz-Omori E, V., Satyapertiwi, D., Ramel, M.C., Høgset, H., Sunyovszki, I.K., Liu, Z. et al. (2020) Photoswitchable gRNAs for spatiotemporally controlled CRISPR-Cas-Based genomic regulation. *ACS Cent. Sci.* **6**, 695–703 https://doi.org/10.1021/acscentsci.9b01093
136 Hemphill, J., Borchardt, E.K., Brown, K., Asokan, A. and Deiters, A. (2015) Optical control of CRISPR/Cas9 gene editing. *J. Am. Chem. Soc.* **137**, 5642–5645 https://doi.org/10.1021/ja512664v
137 Pan, Y., Yang, J., Luan, X., Liu, X., Li, X., Yang, J. et al. (2019) Near-infrared upconversion-activated CRISPR-Cas9 system: A remote-controlled gene editing platform. *Sci. Adv.* **5**, eaav7199 https://doi.org/10.1126/sciadv.aav7199
138 Nihongaki, Y., Yamamoto, S., Kawano, F., Suzuki, H. and Sato, M. (2015) CRISPR-Cas9-based photoactivatable transcription system. *Chem. Biol.* **22**, 169–174 https://doi.org/10.1016/j.chembiol.2014.12.011
139 Polstein, L.R. and Gersbach, C.A. (2015) A light-inducible CRISPR-Cas9 system for control of endogenous gene activation. *Nat. Chem. Biol.* **11**, 198–200 https://doi.org/10.1038/nchembio.1753
140 Nihongaki, Y., Furuhata, Y., Otabe, T., Hasegawa, S., Yoshimoto, K. and Sato, M. (2017) CRISPR-Cas9-based photoactivatable transcription systems to induce neuronal differentiation. *Nat. Methods* **14**, 963–966 https://doi.org/10.1038/nmeth.4430
141 Nihongaki, Y., Kawano, F., Nakajima, T. and Sato, M. (2015) Photoactivatable CRISPR-Cas9 for optogenetic genome editing. *Nat. Biotechnol.* **33**, 755–760 https://doi.org/10.1038/nbt.3245
142 Nguyen, N.T., He, L., Martinez-Moczygemba, M., Huang, Y. and Zhou, Y. (2018) Rewiring calcium signaling for precise transcriptional reprogramming. *ACS Synth. Biol.* **7**, 814–821 https://doi.org/10.1021/acssynbio.7b00467

143 Shao, J., Wang, M., Yu, G., Zhu, S., Yu, Y., Heng, B.C. et al. (2018) Synthetic far-red light-mediated CRISPR-dCas9 device for inducing functional neuronal differentiation. *Proc. Natl Acad. Sci. U.S.A.* **115**, E6722–E6730 https://doi.org/10.1073/pnas.1802448115
144 Zhou, X.X., Zou, X., Chung, H.K., Gao, Y., Liu, Y., Qi, L.S. et al. (2018) A single-chain photoswitchable CRISPR-Cas9 architecture for light-inducible gene editing and transcription. *ACS Chem. Biol.* **13**, 443–448 https://doi.org/10.1021/acschembio.7b00603
145 Polstein, L.R. and Gersbach, C.A. (2012) Light-inducible spatiotemporal control of gene activation by customizable zinc finger transcription factors. *J. Am. Chem. Soc.* **134**, 16480–16483 https://doi.org/10.1021/ja3065667
146 Konermann, S., Brigham, M.D., Trevino, A.E., Hsu, P.D., Heidenreich, M., Cong, L. et al. (2013) Optical control of mammalian endogenous transcription and epigenetic states. *Nature* **500**, 472–476 https://doi.org/10.1038/nature12466
147 Lo, C.L., Choudhury, S.R., Irudayaraj, J. and Zhou, F.C. (2017) Epigenetic editing of Ascl1 gene in neural stem cells by optogenetics. *Sci. Rep.* **7**, 42027 https://doi.org/10.1038/srep42047
148 Nagy, A. (2000) Cre recombinase: The universal reagent for genome tailoring. *Genesis* **26**, 99–109 https://doi.org/10.1002/(SICI)1526-968X(200002)26:2<99::AID-GENE1>3.0.CO;2-B
149 Inlay, M.A., Choe, V., Bharathi, S., Fernhoff, N.B., Baker, J.R., Weissman, I.L. et al. (2013) Synthesis of a photocaged tamoxifen for light-dependent activation of Cre-ER recombinase-driven gene modification. *Chem. Commun.* **49**, 4971–4973 https://doi.org/10.1039/c3cc42179a
150 Lu, X., Agasti, S.S., Vinegoni, C., Waterman, P., Depinho, R.A. and Weissleder, R. (2012) Optochemogenetics (OCG) allows more precise control of genetic engineering in mice with CreER regulators. *Bioconjug. Chem.* **23**, 1945–1951 https://doi.org/10.1021/bc300319c
151 Schindler, S.E., McCall, J.G., Yan, P., Hyrc, K.L., Li, M., Tucker, C.L. et al. (2015) Photo-activatable Cre recombinase regulates gene expression in vivo. *Sci. Rep.* **5**, 13627 https://doi.org/10.1038/srep13627
152 Jung, H., Kim, S.W., Kim, M., Hong, J., Yu, D., Kim, J.H. et al. (2019) Noninvasive optical activation of Flp recombinase for genetic manipulation in deep mouse brain regions. *Nat. Commun.* **10**, 314 https://doi.org/10.1038/s41467-018-08282-8
153 Yao, S., Yuan, P., Ouellette, B., Zhou, T., Mortrud, M., Balaram, P. et al. (2020) Recv recombinase system for in vivo targeted optogenomic modifications of single cells or cell populations. *Nat. Methods* **17**, 422–429 https://doi.org/10.1038/s41592-020-0774-3
154 Morikawa, K., Furuhashi, K., de Sena-Tomas, C., Garcia-Garcia, A.L., Bekdash, R., Klein, A.D. et al. (2020) Photoactivatable Cre recombinase 3.0 for in vivo mouse applications. *Nat. Commun.* **11**, 2141 https://doi.org/10.1038/s41467-020-16030-0
155 Olejniczak, J., Carling, C.J. and Almutairi, A. (2015) Photocontrolled release using one-photon absorption of visible or NIR light. *J. Control. Release* **219**, 18–30 https://doi.org/10.1016/j.jconrel.2015.09.030