

Review

Recent Synthetic Biology Approaches for Temperature- and Light-Controlled Gene Expression in Bacterial Hosts

Jongdoo Choi , Jiyeun Ahn, Jieun Bae  and Minseob Koh * 

Department of Chemistry, Pusan National University, Busan 46241, Korea

* Correspondence: minseob.koh@pusan.ac.kr; Tel.: +82-51-510-2278

Abstract: The expression of genes of interest (GOI) can be initiated by providing external stimuli such as temperature shifts and light irradiation. The application of thermal or light stimuli triggers structural changes in stimuli-sensitive biomolecules within the cell, thereby inducing or repressing gene expression. Over the past two decades, several groups have reported genetic circuits that use natural or engineered stimuli-sensitive modules to manipulate gene expression. Here, we summarize versatile strategies of thermosensors and light-driven systems for the conditional expression of GOI in bacterial hosts.

Keywords: thermosensor; photocaged inducer; photoswitch; genetic code expansion; optogenetics



Citation: Choi, J.; Ahn, J.; Bae, J.; Koh, M. Recent Synthetic Biology Approaches for Temperature- and Light-Controlled Gene Expression in Bacterial Hosts. *Molecules* **2022**, *27*, 6798. <https://doi.org/10.3390/molecules27206798>

Academic Editor: Alessia Ligresti

Received: 30 August 2022

Accepted: 10 October 2022

Published: 11 October 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Organisms have evolved the ability to sense external stimuli, including changes in temperature and light irradiation. Temperature affects the growth rate and pathogenesis of certain microbes [1]. External temperature changes may trigger cellular mechanisms involving homeostasis, such as the chaperone system, which is responsible for protein folding [2], and virulence genes of pathogenic bacteria, which promote survival mechanisms when they are in the host [3]. Light sources, however, can be directly utilized as energy sources in the photosynthetic processes of plants, algae, and cyanobacteria [4]. In addition to this, organisms have evolved to sense light, as exemplified by vision [5].

From a synthetic biology perspective, nature's mechanisms for sensing temperature and light have provided powerful insights for designing tools to achieve conditional gene expression. At the molecular level, researchers have utilized the heat shock response mechanism to construct temperature-sensitive gene expression systems such as the temperature-sensitive dimeric repressor [6–13], RNA polymerase (RNAP) [14–17], replication machinery [18–22], and oligonucleotide topology [23–33]. Photocaged [34–43] or photoswitchable [44,45] small-molecule inducers and photocaged transcription factors [46] have been used to develop light-driven gene expression systems. Additionally, light-sensitive proteins have been widely used as essential modules to control diverse cellular functions in optogenetics [5] (Figure 1).

In this review, we discuss recent advances in synthetic biology that utilize temperature- and light-sensitive modules for conditional gene expression in bacterial hosts.

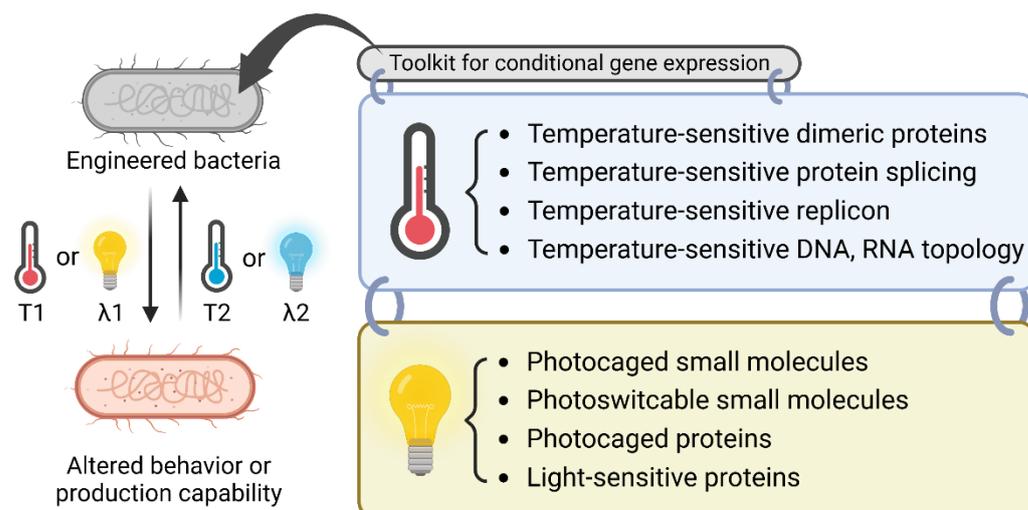


Figure 1. Overview of the molecular biology toolkit for conditional gene expression. A bacterium can be engineered to be sensitive to a temperature shift or specific light irradiation for the dynamic regulation of bacterial behavior or the production of chemicals or proteins. The toolkit for thermoregulation includes temperature-sensitive dimeric proteins, protein splicing, replicons, and oligonucleotide topology. For light-sensitive regulation, one can choose from the toolkit of photocaged small molecules, photoswitchable small molecules, photocaged proteins, and light-sensitive proteins. T1, T2: different temperatures; $\lambda 1$, $\lambda 2$: different wavelengths.

2. Temperature-Controlled Gene Expression

The induction of gene expression by temperature has many advantages, including being low-cost, requiring simple instrumentation, being non-invasive, having minimal contamination risk, and being reversible. Diverse regulatory systems in cells that utilize proteins, RNA, and DNA are responsible for sensing temperature inputs [1,47,48]. In this section, we provide the temperature-sensing modules found in nature and discuss how they can be utilized in genetic circuit design.

2.1. Engineering of Dimeric Repressor

The bacteriophage lambda cI homodimer binds to its cognate operator sites (oR1, oR2, and oR3) in the pR/pRM promoter [49]. In the bacteriophage, the wild-type cI is temperature-insensitive, and the induction of downstream gene expression is enabled by the RecA-mediated autocatalytic cleavage of cI in the lytic growth stage [50]. The mutant protein cI857 gains temperature sensitivity due to unstable dimer formation at higher temperatures ($>37^\circ\text{C}$), caused by the Ala66Thr mutation at the N-terminus [51] (Figure 2A). The pL/pR-cI857 thermoinduced expression system is useful for the expression of heterologous proteins [52] in phages, viruses, and humans as well as high-value metabolites [10] in *Escherichia coli*. In addition, the cI857 repression system is useful in constructing genetic circuits for bifunctional dynamic control [7] and kill switches [13]. cI857 variants can also be used for temperature-sensitive transcriptional activation [6]. Recently, a genetic circuit for a cold-inducible switch was developed [11]. A cold-inactivated variant of the bacteriophage 434 cI repressor containing a TEV recognition site was coupled with a heat-inactivated TEV protease and a *Mycoplasma florum* Lon protease for tight control.

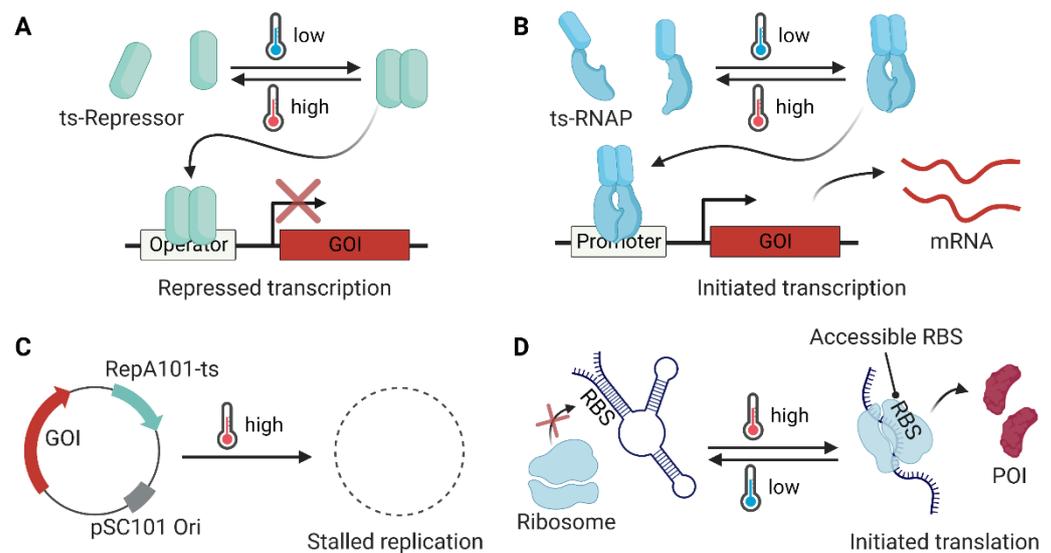


Figure 2. Temperature-sensitive modules utilized in bacterial engineering. (A) A temperature-sensitive (ts) dimeric repressor (e.g., cI857 and TlpA) forms a dimer and binds to the operator, thereby inhibiting gene expression at low temperatures. (B) A temperature-sensitive dimer module (e.g., the coiled-coil domain of TlpA) can be fused to split RNA polymerase (RNAP) to generate a temperature-sensitive transcription system. (C) A temperature-sensitive replication system restricts the replication of a plasmid that contains the genes of interest (GOI) at the defined temperature. (D) An RNA thermometer can be designed to have a buried ribosome binding site (RBS) by forming a stable hairpin structure at low temperatures (<30 °C) to inhibit translation, whereas the RBS is exposed at high temperatures (>37 °C) for ribosome entry and the translation of the GOI to generate the protein of interest (POI).

Another useful dimeric repressor is TlpA from *Salmonella* [53], which possesses an intrinsic temperature sensitivity in the formation of its coiled-coil structure. TlpA binds to its cognate promoter and represses downstream gene expression at temperatures below 37 °C, whereas its structural instability at higher temperatures (>42 °C) confers a loss of repression [47] (Figure 2A). Recently, the Shapiro group reported engineered TlpA variants that shifted working temperatures relative to the wild-type. They also developed similar versions of the cI857 variants to produce orthogonal thermal bioswitches. It is noteworthy that such a fine-tuned thermal switch can be utilized for the spatiotemporal control of microbial therapeutics using focused ultrasound [12].

2.2. Regulation of RNAP

RNAP can be engineered directly to achieve temperature sensitivity. Liang et al. adopted a temperature-sensitive *Saccharomyces cerevisiae* VMA intein (intein^{TS}) [17] to engineer temperature-sensitive T7 RNAP for conditional gene expression at 18 °C in *E. coli* [16]. The intein^{TS} fused in the middle of the T7 RNAP (between Ala491 and Cys492) can be cleaved by an autonomous protein splicing process and can generate full-length T7 RNAP only at a low temperature (18 °C). The same idea was utilized in metabolic engineering for lycopene biosynthesis in *E. coli*, which resulted in a 15% improvement in productivity [15]. Recently, the Poh research group reported a reversibly controlled temperature-sensitive gene expression system by the fusion of the C-terminal coiled-coil domain of TlpA (Ala94–Ala371 region, 278 amino acids, TlpA coil) with split-T7 RNAP. To generate the Thermal T7RNAP, N-terminal fragment of T7 RNAP (Met1–Ser564)–TlpA coil and TlpA coil–C-terminal fragment of T7 RNAP (Glu565–Ala883) fusions were used [14] (Figure 2B).

2.3. Conditional Replication of Plasmid

Conditional gene expression was achieved using a plasmid containing a temperature-sensitive replicon. The temperature-sensitive plasmid pSC101-ts has been widely used for conditional gene expression at low temperature (30 °C) in *E. coli* [22,54]. The mutation (Ala56Val) in the RepA protein, which is responsible for the replication initiation of pSC101 [55], confers temperature sensitivity to plasmid replication [56] (Figure 2C). The temperature-sensitive replicon of pSC101-ts has been utilized in many recombineering efforts [18,21]. Recently, efficient gene inactivation in *Pseudomonas aeruginosa* was reported using a low-temperature (30 °C) adopted replicon [19], which contains Gly100Cys and Ser204Arg mutations in the replication protein [20].

2.4. Regulation of Oligonucleotide Topology

2.4.1. RNA Thermometer (RNAT)

Thermosensing using RNA has advantages over other biomolecular tools for temperature-sensitive gene expression. Plausible reasons may include the quick rearrangement of its secondary and tertiary structures upon temperature change. To regulate the expression of pathogenesis-related genes and heat shock genes, bacteria utilize an RNA-based thermoregulation system [47], such as the ROSE element (repression of heat shock gene expression element) [57]. The Shine–Dalgarno (SD) region of the mRNA is buried at low temperatures and exposed at higher temperatures. At high temperatures, the downstream gene can be expressed (Figure 2D). Synthetic RNATs have been reported, which localize anti-SD sequences upstream of the SD region for heat-induced gene expression [27] or repression [26]. RNATs can also be utilized in the design of temperature-responsive transcription terminators [24] and temperature-sensing protocells [23].

The conditional expression of sigma factor 32 (σ^{32}) is also regulated by an RNAT. The mRNA of the σ^{32} coding gene (*rpoH*) has a distinct secondary structure, where the SD sequence and AUG start codon are inaccessible to the 30S ribosome for translation at low temperatures [58]. Elevated temperatures promote the unfolding of such inhibitory structures and facilitate ribosome binding for σ^{32} expression in response to heat stimuli [59]. The three-way junction of the highly structured mRNA of *rpoH* can be targeted by small-molecule stabilizers to disrupt the temperature-sensing mechanism of bacteria [25].

2.4.2. Histone-Like Nucleoid Structuring Protein (H-NS)

H-NS, a chromatin-structuring protein in bacteria, responds to environmental temperature changes. In total, 69% of the temperature-regulated genes of *E. coli* K-12 [32] and 77% of thermoregulated genes of *Salmonella typhimurium* [33] are associated with H-NS. H-NS binds to a specific site on DNA and forms higher-order oligomers, which cause DNA bending and inhibit RNA polymerase access. The oligomerization status of H-NS is temperature-dependent. Gene transcription is re-initiated when bacteria are exposed to a permissive temperature [60]. In *E. coli* K-12, genes related to nutrient acquisition are highly expressed at 37 °C, while genes related to stress response, biofilm formation, and cold shock are highly expressed at 23 °C. This is regulated by H-NS [32]. The molecular mechanism of H-NS de-oligomerization at low temperatures remains largely unknown. However, the regulation of the post-translational modification of H-NS may contribute to gene expression at low temperatures, following a similar mechanism as the xenogeneic silencing of *Shewanella oneidensis* [28]. Despite its versatile use in nature, there have been no successful applications of H-NS in synthetic biology. This is possibly due to the challenges in finding an orthogonal system relative to the other strategies described in this review.

3. Light-Driven Regulation of Gene Expression

Light is the perfect choice as a stimulus for inducing gene expression because it is non-invasive, highly controllable, orthogonal, and precise, and can be used to achieve spatiotemporal control [5]. In this section, transcription systems using light-sensitive small

molecules or proteins and optogenetic systems using photosensitive proteins, focusing on the flavin-binding light oxygen and voltage (LOV) domains, are discussed.

3.1. Photocaged Small-Molecule Inducers

To address the low spatiotemporal precision of small-molecule inducers, caging strategies have been used to activate gene expression at specific times and regions [61]. The activity of the caged inducers is initially inhibited and is regained upon light irradiation at the appropriate wavelength (Figure 3A). Small-molecule inducers such as isopropyl β -D-1-thiogalactopyranoside (IPTG) [34–36,38,39,42,43] and L-arabinose [34,40] are usually targeted for caging. Among the collection of many photocleavable protecting groups (PPGs) [62], limited caging groups including *ortho*-nitrobenzyl (ONB) derivatives and coumarin derivatives have been employed to develop photocaged small-molecule inducers. Caged IPTG [43] was utilized for light-triggered gene expression at the single-cell level [42] for the production of the commodity compound (+)-valencene [39], for a parallelized gene expression setup [38], and for optochemical control of the microbial consortium [36]. In addition to the caged inducer, gene expression can also be triggered by the caged riboswitch systems, such as the caged synthetic riboswitch ligand theophylline [41,63] and caged guanine [37] for photocontrolled gene expression in *E. coli*.

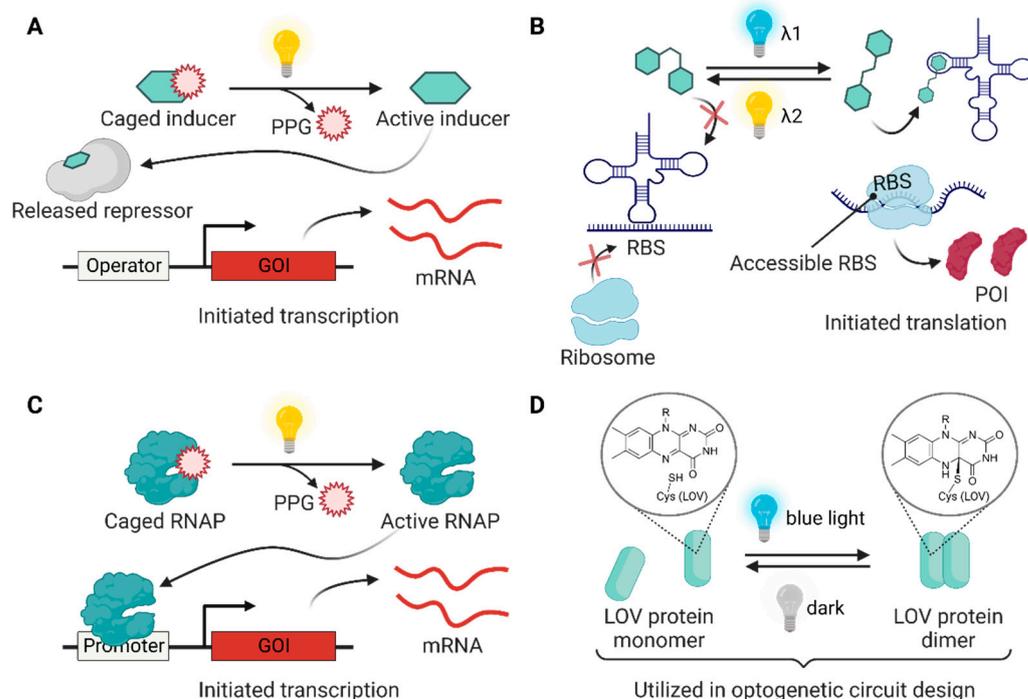


Figure 3. Light-driven regulation modules utilized in bacterial engineering. (A) Chemical inducers can be inactivated by installing a photocleavable protecting group (PPG) and activated by irradiating light to unmask the PPG for the induction of gene expression. (B) A photoswitchable small molecule (e.g., stiff-stilbene and azobenzene) non-covalently binds to riboswitches, leading to the reversible expression of GOI under the controlled exposure of two different light signals. (C) A genetically encoded photocaged non-canonical amino acid (ncAA) allows the construction of photocaged RNAP, whose function can be triggered by light for GOI expression. (D) Some LOV domains (e.g., VVD, EL222, and Magnets) dimerize when flavin cofactor forms a covalent bond with the active-site cysteine under blue light irradiation. In the dark, the structures return to their original state through thermal processes. The photocycle of the LOV domains can be utilized in optogenetic circuit design.

3.2. Photosensitive Small-Molecule Switch

One of the limitations of light sensing with photocaged small molecules is their irreversibility. Once the PPG is deprotected, the forward reaction cannot be stopped, and the reverse reaction cannot be induced. Photoswitchable molecules have two isomeric states

that can be interconverted through irradiation with different light inputs at different wavelengths [64], which enables reversible genetic control. Although challenging, controlling gene expression with a photoswitchable riboswitch ligand [65,66] offers the opportunity to reversibly regulate artificial genetic circuits [67]. Recently, *in vivo* demonstrations of stiff-stilbene- [45] and azobenzene-based [44] photoswitchable ligands were reported, showing the regulation of RNA-controlled gene expression in *E. coli* (Figure 3B).

3.3. Photocaged Transcription Factor

Advances in genetic code expansion strategies have enabled the incorporation of a non-canonical amino acid (ncAA) to the tailored site of proteins *in vivo* [68]. A requirement of this strategy is an orthogonal amino acyl tRNA synthetase/tRNA pair that lacks cross-reactivity with the host translational machinery [69]. Among the large libraries of ncAAs that are encoded in the genome, photocaged amino acids and azobenzene-containing amino acids have been reported in many photoresponsive bioapplications [70]. In 2010, the Deiters research group reported photocaged T7 RNAP for light-activated gene expression in *E. coli*. The Tyr639 position of T7 RNAP was mutated to ONBY, the ONB-protected tyrosine, to inactivate T7 RNAP by inhibiting the NTP binding site. The irradiation of 365 nm UV removed the ONB group and restored T7 RNAP function for gene expression [46] (Figure 3C).

3.4. Engineering Light-Sensitive Proteins

Recently developed optogenetic approaches commonly utilize natural or engineered photoreceptor-regulated systems because they are highly reversible and genetically encodable without additional synthetic components [5]. The essential chromophores of photoreceptors include tryptophan (in UVR8 [71]), flavins (in LOV domains [72], BLUF domains [73], and cryptochromes [74]), cobalamins (in cobalamin-binding domains [75]), and tetrapyrroles (in phytochromes [76]).

LOV domains have higher versatility in circuit design for bacterial gene expression compared to other photoreceptors due to their small sizes and naturally available flavin chromophores [77]. LOV domains are classified in the PAS (period circadian protein–aryl hydrocarbon receptor nuclear translocator protein–single-minded protein) [78,79] family. When irradiated with blue light, a thioether bond between flavin and a nearby cysteine residue of the PAS core is formed, resulting in structural changes in the LOV domain [72,77]. The light-induced structural changes reverted to the ground state through the thermal relaxation process in the dark [80] (Figure 3D).

In this subsection, we review how LOV domains are utilized in optogenetic circuit designs in bacteria by categorizing them into two-component and one-component systems.

3.4.1. Two-Component Systems

In a two-component system (TCS), for example, the blue-light-regulated LOV-domain-containing histidine kinase (HK) is autophosphorylated and transfers a phosphate group to a cognate response regulator (RR), which in turn triggers the expression of the corresponding gene [81] (Figure 4A). In 2012, Ohlendorf et al. reported a pDusk/pDawn system based on HK YF1 and RR FixJ for light-driven gene expression [82]. In the dark, YF1 [83] phosphorylates FixJ, resulting in gene expression from the FixK2 promoter. The above process is largely hampered by blue light irradiation, and the expression of genes of interest (GOI) is repressed (pDusk). Similarly, GOI expression can be activated by light (pDawn) by inserting the cI repressor and lambda promoter pR into the pDusk system. The pDusk/pDawn system is a widely used LOV-based TCS. For example, pDawn has been used in biofilm lithography with *E. coli* [84], light-induced biofilm inhibition in *P. aeruginosa* [85], the production of drug-releasing living material [86], and the optogenetic control of the *lac* operon (OptoLAC) for chemical and protein production in *E. coli* [87]. Recently, the Avalos research group reported a light-regulated toxin–antitoxin system (OptoTA) using the pDusk/pDawn system for the dynamic regulation of bacterial populations [88].

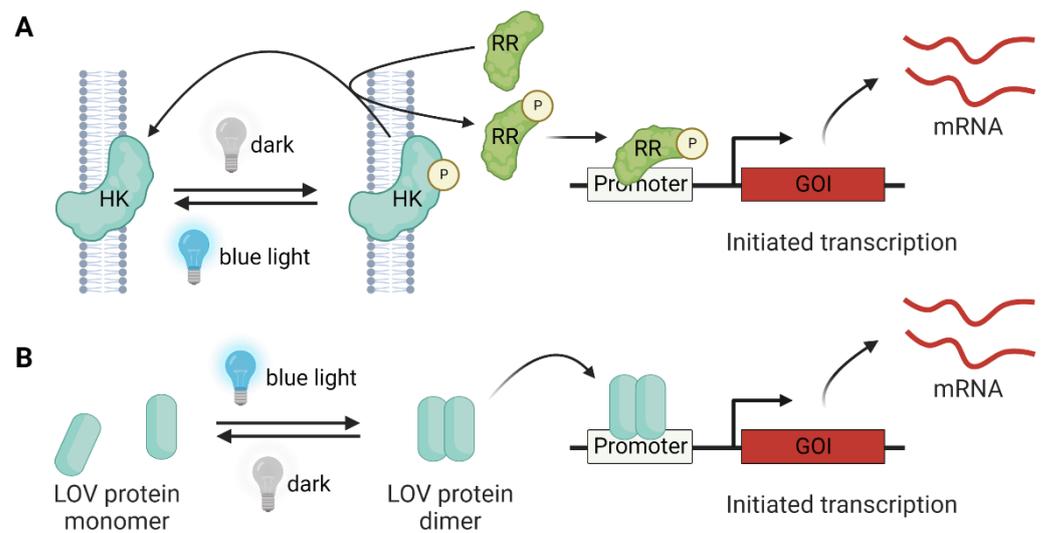


Figure 4. Light-controlled gene expression by LOV proteins. **(A)** The two-component system consists of histidine kinase (HK) and a response regulator (RR) for signal relay. In the absence of light, the HK is phosphorylated, and the phosphate group is transferred to the RR, leading to GOI expression. The blue light induces the dephosphorylation of HK and RR, and the GOI expression is inhibited. **(B)** In the one-component system, the blue-light-driven LOV dimerization (e.g., EL222) can directly trigger GOI expression.

3.4.2. One-Component Systems

One-component systems (OCSs) are relatively faster than TCSs in reversible signaling because of the more direct and simpler designs of the genetic circuits [81] (Figure 4B). The OCSs utilized in bacteria largely rely on LOV-domain-containing modules, including VVD [89], EL222 [90], and Magnets [91]. The light-regulated homodimer VVD has been used for light-induced gene repression (LEVI) [92] and expression (LexRO) [93]. Recently, Romano et al. reported a VVD-AraC fusion for a light-inducible system of the *araBAD* operon (BLADE) [94]. In 2017, the Liu research group reported photoactivatable T7 RNAP by creating fusion constructs of split T7 RNAP and VVD or its heterodimeric variant Magnets [95]. Later, the Khammash group reported an improved system using split T7 RNAP-Magnets fusions with inducible promoters (Opto-T7RNAP) [96]. In 2020, they demonstrated a new chemo-optogenetic control system with an improved dynamic range (from 65- to 700-fold) of Opto-T7RNAP by providing additional units of photodegradable anhydrotetracycline and a reverse Tet repressor [97]. The blue-light-induced homodimerization and DNA-binding properties of EL222 were utilized in the construction of a programmable transcriptional activator (pBLind) and repressor (pBLrep) in *E. coli* [98,99]. Recently, the Yuan research group reported a light-regulated CRISPR interference system (opto-CRISPRi) using EL222 and its cognate-binding sequence [100]. The opto-CRISPRi enabled the dynamic control of metabolic flux for optimal chemical production in *E. coli* by alternating the blue-light-induced growth phase and the dark-induced recovered production phase.

4. Conclusions and Perspectives

Modules for constructing a temperature-regulated gene expression system target all stages of gene expression, including replication (ts-replicon), transcription (ts-dimeric repressor and ts-RNAP), and translation (RNAT). As RNAT targets the late stage of gene expression, one can choose the RNAT system when a quick response is needed. Utilizing the ts-dimer module has advantages because of its relatively easy tunability at the working temperature [6,12] and modularity as a fusion partner, which broadens the scope of its applications. In contrast to the other ts modules, gene expression with the ts-replicon is

irreversible. This limits the utility of the ts-replicon; however, it has advantages when permanent gene removal is required [18,21].

The LOV domain has proven useful in many light-controlled applications. However, there are some considerations when utilizing the LOV domains in optogenetics. One should consider the dynamic range and the leakiness of the system due to the imperfection in the association and dissociation dynamics of the LOV proteins [77]. Although flavin chromophores are naturally abundant, the amount in the cytosol may not be sufficient for generating fully loaded LOV domains [101]. This limits the efficiency of signal relay due to the unloaded non-functional apo-LOV proteins. This issue may be resolved by genetically encoding a photoswitchable amino acid that replaces flavin.

Systems employing non-invasive external signals, such as gene expression with TlpA-split T7 RNAP fusion [14] or Opto-T7RNAP [96], do not rely on chemical inducers, such as IPTG. This makes temperature- or light-regulated systems useful for reducing the cost of production when high-volume microbial cultures are used for large-scale chemical or protein production [7,99,102]. Although heat equilibrium and light penetration conditions need to be optimized at large industrial scales (>250 L), appropriate bioreactor designs, such as flow setups, may help in achieving success [103]. In this regard, the temperature- and light-controlled gene expression systems discussed in this review can be part of a valuable toolkit for the construction of microbial factories of chemicals or proteins required for industrial and biomedical applications.

Author Contributions: Conceptualization, M.K.; writing and review, J.C., J.A., J.B., and M.K.; funding acquisition, M.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Research Foundation of Korea (grant No. 2021R1A4A1021950) and a Pusan National University Research Grant, 2020.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: All figures were edited and created with BioRender.com.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Samtani, H.; Unni, G.; Khurana, P. Microbial mechanisms of heat sensing. *Indian J. Microbiol.* **2022**, *62*, 175–186. [[CrossRef](#)]
2. Schumann, W. Regulation of bacterial heat shock stimulons. *Cell Stress Chaperones* **2016**, *21*, 959–968. [[CrossRef](#)]
3. Fang, F.C.; Frawley, E.R.; Tapscott, T.; Vazquez-Torres, A. Bacterial stress responses during host infection. *Cell Host Microbe* **2016**, *20*, 133–143. [[CrossRef](#)]
4. Kerfeld, C.A.; Krogmann, D.W. Photosynthetic cytochromes c in cyanobacteria, algae, and plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1998**, *49*, 397–425. [[CrossRef](#)]
5. Baumschlager, A.; Khammash, M. Synthetic biological approaches for optogenetics and tools for transcriptional light-control in bacteria. *Adv. Biol.* **2021**, *5*, 2000256. [[CrossRef](#)]
6. Xiong, L.L.; Garrett, M.A.; Buss, M.T.; Kornfield, J.A.; Shapiro, M.G. Tunable temperature-sensitive transcriptional activation based on lambda repressor. *ACS Synth. Biol.* **2022**, *11*, 2518–2522. [[CrossRef](#)]
7. Wang, X.; Han, J.-N.; Zhang, X.; Ma, Y.-Y.; Lin, Y.; Wang, H.; Li, D.-J.; Zheng, T.-R.; Wu, F.-Q.; Ye, J.-W.; et al. Reversible thermal regulation for bifunctional dynamic control of gene expression in *Escherichia coli*. *Nat. Commun.* **2021**, *12*, 1411. [[CrossRef](#)]
8. Versace, G.; Palombo, M.; Menon, A.; Scarlato, V.; Roncarati, D. Feeling the Heat: The *Campylobacter jejuni* HrcA Transcriptional Repressor Is an Intrinsic Protein Thermosensor. *Biomolecules* **2021**, *11*, 1413. [[CrossRef](#)]
9. Fu, L.X.; Gong, J.S.; Gao, B.; Ji, D.J.; Han, X.G.; Zeng, L.B. Controlled expression of lysis gene E by a mutant of the promoter pL of the thermo-inducible lambdacl857-pL system. *J. Appl. Microbiol.* **2021**, *130*, 2008–2017. [[CrossRef](#)]
10. Fang, Y.; Wang, J.; Ma, W.; Yang, J.; Zhang, H.; Zhao, L.; Chen, S.; Zhang, S.; Hu, X.; Li, Y.; et al. Rebalancing microbial carbon distribution for L-threonine maximization using a thermal switch system. *Metab. Eng.* **2020**, *61*, 33–46. [[CrossRef](#)]
11. Zheng, Y.; Meng, F.; Zhu, Z.; Wei, W.; Sun, Z.; Chen, J.; Yu, B.; Lou, C.; Chen, G.Q. A tight cold-inducible switch built by coupling thermosensitive transcriptional and proteolytic regulatory parts. *Nucleic Acids Res.* **2019**, *47*, e137. [[CrossRef](#)] [[PubMed](#)]
12. Piraner, D.I.; Abedi, M.H.; Moser, B.A.; Lee-Gosselin, A.; Shapiro, M.G. Tunable thermal bioswitches for in vivo control of microbial therapeutics. *Nat. Chem. Biol.* **2017**, *13*, 75–80. [[CrossRef](#)] [[PubMed](#)]

13. Pearce, S.C.; McWhinnie, R.L.; Nano, F.E. Synthetic temperature-inducible lethal gene circuits in *Escherichia coli*. *Microbiology* **2017**, *163*, 462–471. [[CrossRef](#)] [[PubMed](#)]
14. Chee, W.K.D.; Yeoh, J.W.; Dao, V.L.; Poh, C.L. Highly reversible tunable thermal-repressible split-T7 RNA polymerases (Thermal-T7RNAPs) for dynamic gene regulation. *ACS Synth. Biol.* **2022**, *11*, 921–937. [[CrossRef](#)] [[PubMed](#)]
15. Korvin, D.; Yada, V.G. A molecular switch that enhances productivity of bioprocesses for heterologous metabolite production. *Mol. Syst. Des. Eng.* **2018**, *3*, 550–559. [[CrossRef](#)]
16. Liang, R.; Liu, X.; Liu, J.; Ren, Q.; Liang, P.; Lin, Z.; Xie, X. A T7-expression system under temperature control could create temperature-sensitive phenotype of target gene in *Escherichia coli*. *J. Microbiol. Methods* **2007**, *68*, 497–506. [[CrossRef](#)]
17. Zeidler, M.P.; Tan, C.; Bellaiche, Y.; Cherry, S.; Hader, S.; Gayko, U.; Perrimon, N. Temperature-sensitive control of protein activity by conditionally splicing inteins. *Nat. Biotechnol.* **2004**, *22*, 871–876. [[CrossRef](#)]
18. Koh, M.; Yao, A.; Gleason, P.R.; Mills, J.H.; Schultz, P.G. A general strategy for engineering noncanonical amino acid dependent bacterial growth. *J. Am. Chem. Soc.* **2019**, *141*, 16213–16216. [[CrossRef](#)]
19. Prathapam, R.; Uehara, T. A temperature-sensitive replicon enables efficient gene inactivation in *Pseudomonas aeruginosa*. *J. Microbiol. Methods* **2018**, *144*, 47–52. [[CrossRef](#)]
20. Silo-Suh, L.A.; Elmore, B.; Ohman, D.E.; Suh, S.J. Isolation, characterization, and utilization of a temperature-sensitive allele of a *Pseudomonas* replicon. *J. Microbiol. Methods* **2009**, *78*, 319–324. [[CrossRef](#)]
21. Datsenko, K.A.; Wanner, B.L. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 6640–6645. [[CrossRef](#)] [[PubMed](#)]
22. Hashimoto, T.; Sekiguchi, M. Isolation of temperature-sensitive mutants of R plasmid by in vitro mutagenesis with hydroxylamine. *J. Bacteriol.* **1976**, *127*, 1561–1563. [[CrossRef](#)] [[PubMed](#)]
23. Jia, H.; Heymann, M.; Hartel, T.; Kai, L.; Schwille, P. Temperature-sensitive protein expression in protocells. *Chem. Commun.* **2019**, *55*, 6421–6424. [[CrossRef](#)] [[PubMed](#)]
24. Rossmannith, J.; Weskamp, M.; Narberhaus, F. Design of a temperature-responsive transcription terminator. *ACS Synth. Biol.* **2018**, *7*, 613–621. [[CrossRef](#)] [[PubMed](#)]
25. Barros, S.A.; Yoon, I.; Chenoweth, D.M. Modulation of the *E. coli* rpoH temperature sensor with triptycene-based small molecules. *Angew. Chem. Int. Ed.* **2016**, *55*, 8258–8261. [[CrossRef](#)] [[PubMed](#)]
26. Hoynes-O'Connor, A.; Hinman, K.; Kirchner, L.; Moon, T.S. De novo design of heat-repressible RNA thermosensors in *E. coli*. *Nucleic Acids Res.* **2015**, *43*, 6166–6179. [[CrossRef](#)]
27. Neupert, J.; Karcher, D.; Bock, R. Design of simple synthetic RNA thermometers for temperature-controlled gene expression in *Escherichia coli*. *Nucleic Acids Res.* **2008**, *36*, e124. [[CrossRef](#)]
28. Liu, X.; Lin, S.; Liu, T.; Zhou, Y.; Wang, W.; Yao, J.; Guo, Y.; Tang, K.; Chen, R.; Benedik, M.J.; et al. Xenogeneic silencing relies on temperature-dependent phosphorylation of the host H-NS protein in *Shewanella*. *Nucleic Acids Res.* **2021**, *49*, 3427–3440. [[CrossRef](#)]
29. Baomo, L.; Lili, S.; Moran, R.A.; van Schaik, W.; Chao, Z. Temperature-Regulated IncX3 Plasmid Characteristics and the Role of Plasmid-Encoded H-NS in Thermoregulation. *Front. Microbiol.* **2021**, *12*, 765492. [[CrossRef](#)]
30. Brambilla, L.; Moran-Barrio, J.; Viale, A.M. Expression of the *Escherichia coli* ompW colicin S4 receptor gene is regulated by temperature and modulated by the H-NS and StpA nucleoid-associated proteins. *FEMS Microbiol. Lett.* **2014**, *352*, 238–244. [[CrossRef](#)]
31. Yun, S.H.; Ji, S.C.; Jeon, H.J.; Wang, X.; Kim, S.W.; Bak, G.; Lee, Y.; Lim, H.M. The CnuK9E H-NS complex antagonizes DNA binding of DicA and leads to temperature-dependent filamentous growth in *E. coli*. *PLoS ONE* **2012**, *7*, e45236. [[CrossRef](#)]
32. White-Ziegler, C.A.; Davis, T.R. Genome-wide identification of H-NS-controlled, temperature-regulated genes in *Escherichia coli* K-12. *J. Bacteriol.* **2009**, *191*, 1106–1110. [[CrossRef](#)] [[PubMed](#)]
33. Ono, S.; Goldberg, M.D.; Olsson, T.; Esposito, D.; Hinton, J.C.; Ladbury, J.E. H-NS is a part of a thermally controlled mechanism for bacterial gene regulation. *Biochem. J.* **2005**, *391*, 203–213. [[CrossRef](#)] [[PubMed](#)]
34. Hogenkamp, F.; Hilgers, F.; Bitzenhofer, N.L.; Ophoven, V.; Haase, M.; Bier, C.; Binder, D.; Jaeger, K.E.; Drepper, T.; Pietruszka, J. Optochemical control of bacterial gene expression: Novel photocaged compounds for different promoter systems. *ChemBioChem* **2022**, *23*, e202100467. [[CrossRef](#)]
35. Hogenkamp, F.; Hilgers, F.; Knapp, A.; Klaus, O.; Bier, C.; Binder, D.; Jaeger, K.E.; Drepper, T.; Pietruszka, J. Effect of photocaged isopropyl beta-d-1-thiogalactopyranoside solubility on the light responsiveness of lacI-controlled expression systems in different bacteria. *ChemBioChem* **2021**, *22*, 539–547. [[CrossRef](#)]
36. Burmeister, A.; Akhtar, Q.; Hollmann, L.; Tenhaef, N.; Hilgers, F.; Hogenkamp, F.; Sokolowsky, S.; Marienhagen, J.; Noack, S.; Kohlheyer, D.; et al. (Optochemical) control of synthetic microbial coculture interactions on a microcolony level. *ACS Synth. Biol.* **2021**, *10*, 1308–1319. [[CrossRef](#)]
37. Dhamodharan, V.; Nomura, Y.; Dwidar, M.; Yokobayashi, Y. Optochemical control of gene expression by photocaged guanine and riboswitches. *Chem. Commun.* **2018**, *54*, 6181–6183. [[CrossRef](#)]
38. Wandrey, G.; Bier, C.; Binder, D.; Hoffmann, K.; Jaeger, K.E.; Pietruszka, J.; Drepper, T.; Buchs, J. Light-induced gene expression with photocaged IPTG for induction profiling in a high-throughput screening system. *Microb. Cell Fact.* **2016**, *15*, 63. [[CrossRef](#)]

39. Binder, D.; Frohwitter, J.; Mahr, R.; Bier, C.; Grünberger, A.; Loeschcke, A.; Peters-Wendisch, P.; Kohlheyer, D.; Pietruszka, J.; Frunzke, J.; et al. Light-controlled cell factories: Employing photocaged isopropyl-beta-d-thiogalactopyranoside for light-mediated optimization of lac promoter-based gene expression and (+)-valencene biosynthesis in *Corynebacterium glutamicum*. *Appl. Environ. Microbiol.* **2016**, *82*, 6141–6149. [[CrossRef](#)]
40. Binder, D.; Bier, C.; Grünberger, A.; Drobiez, D.; Hage-Hülsmann, J.; Wandrey, G.; Büchs, J.; Kohlheyer, D.; Loeschcke, A.; Wiechert, W.; et al. Photocaged arabinose: A novel optogenetic switch for rapid and gradual control of microbial gene expression. *ChemBioChem* **2016**, *17*, 296–299. [[CrossRef](#)]
41. Walsh, S.; Gardner, L.; Deiters, A.; Williams, G.J. Intracellular light-activation of riboswitch activity. *ChemBioChem* **2014**, *15*, 1346–1351. [[CrossRef](#)] [[PubMed](#)]
42. Binder, D.; Grünberger, A.; Loeschcke, A.; Probst, C.; Bier, C.; Pietruszka, J.; Wiechert, W.; Kohlheyer, D.; Jaeger, K.E.; Drepper, T. Light-responsive control of bacterial gene expression: Precise triggering of the lac promoter activity using photocaged IPTG. *Integr. Biol.* **2014**, *6*, 755–765. [[CrossRef](#)] [[PubMed](#)]
43. Young, D.D.; Deiters, A. Photochemical activation of protein expression in bacterial cells. *Angew. Chem. Int. Ed.* **2007**, *46*, 4290–4292. [[CrossRef](#)]
44. Paul, A.; Huang, J.; Han, Y.; Yang, X.; Vukovic, L.; Kral, P.; Zheng, L.; Herrmann, A. Photochemical control of bacterial gene expression based on trans encoded genetic switches. *Chem. Sci.* **2021**, *12*, 2646–2654. [[CrossRef](#)]
45. Rotstan, K.A.; Abdelsayed, M.M.; Passalacqua, L.F.; Chizzolini, F.; Sudarshan, K.; Chamberlin, A.R.; Misek, J.; Luptak, A. Regulation of mRNA translation by a photoriboswitch. *Elife* **2020**, *9*, e51737. [[CrossRef](#)]
46. Chou, C.; Young, D.D.; Deiters, A. Photocaged τ 7 RNA polymerase for the light activation of transcription and gene function in pro- and eukaryotic cells. *ChemBioChem* **2010**, *11*, 972–977. [[CrossRef](#)] [[PubMed](#)]
47. Mandin, P.; Johansson, J. Feeling the heat at the millennium: Thermosensors playing with fire. *Mol. Microbiol.* **2020**, *113*, 588–592. [[CrossRef](#)]
48. Rodrigues, J.L.; Rodrigues, L.R. Potential applications of the *Escherichia coli* heat shock response in synthetic biology. *Trends Biotechnol.* **2018**, *36*, 186–198. [[CrossRef](#)]
49. Ptashne, M.; Jeffrey, A.; Johnson, A.D.; Maurer, R.; Meyer, B.J.; Pabo, C.O.; Roberts, T.M.; Sauer, R.T. How the lambda repressor and cro work. *Cell* **1980**, *19*, 1–11. [[CrossRef](#)]
50. Anderson, L.M.; Yang, H. DNA looping can enhance lysogenic CI transcription in phage lambda. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 5827–5832. [[CrossRef](#)]
51. Lieb, M. A fine structure map of spontaneous and induced mutations in the lambda repressor gene, including insertions of IS elements. *Mol. Gen. Genet.* **1981**, *184*, 364–371. [[CrossRef](#)] [[PubMed](#)]
52. Valdez-Cruz, N.A.; Caspeta, L.; Perez, N.O.; Ramirez, O.T.; Trujillo-Roldan, M.A. Production of recombinant proteins in *E. coli* by the heat inducible expression system based on the phage lambda pL and/or pR promoters. *Microb. Cell Fact.* **2010**, *9*, 18. [[CrossRef](#)] [[PubMed](#)]
53. Hurme, R.; Berndt, K.D.; Normark, S.J.; Rhen, M. A proteinaceous gene regulatory thermometer in *Salmonella*. *Cell* **1997**, *90*, 55–64. [[CrossRef](#)]
54. Hashimoto-Gotoh, T.; Franklin, F.C.; Nordheim, A.; Timmis, K.N. Specific-purpose plasmid cloning vectors. I. Low copy number, temperature-sensitive, mobilization-defective pSC101-derived containment vectors. *Gene* **1981**, *16*, 227–235. [[CrossRef](#)]
55. Kues, U.; Stahl, U. Replication of plasmids in gram-negative bacteria. *Microbiol. Rev.* **1989**, *53*, 491–516. [[CrossRef](#)]
56. Armstrong, K.A.; Acosta, R.; Ledner, E.; Machida, Y.; Pancotto, M.; McCormick, M.; Ohtsubo, H.; Ohtsubo, E. A 37×10^3 molecular weight plasmid-encoded protein is required for replication and copy number control in the plasmid pSC101 and its temperature-sensitive derivative pHS1. *J. Mol. Biol.* **1984**, *175*, 331–348. [[CrossRef](#)]
57. Abduljalil, J.M. Bacterial riboswitches and RNA thermometers: Nature and contributions to pathogenesis. *Noncoding RNA Res.* **2018**, *3*, 54–63. [[CrossRef](#)]
58. Kortmann, J.; Narberhaus, F. Bacterial RNA thermometers: Molecular zippers and switches. *Nat. Rev. Microbiol.* **2012**, *10*, 255–265. [[CrossRef](#)]
59. Nonaka, G.; Blankschien, M.; Herman, C.; Gross, C.A.; Rhodius, V.A. Regulon and promoter analysis of the *E. coli* heat-shock factor, sigma32, reveals a multifaceted cellular response to heat stress. *Genes Dev.* **2006**, *20*, 1776–1789. [[CrossRef](#)]
60. Klinkert, B.; Narberhaus, F. Microbial thermosensors. *Cell. Mol. Life Sci.* **2009**, *66*, 2661–2676. [[CrossRef](#)]
61. Josa-Cullere, L.; Llebaria, A. In the search for photocages cleavable with visible light: An overview of recent advances and chemical strategies. *Chemphotochem* **2021**, *5*, 298–316. [[CrossRef](#)]
62. Hansen, M.J.; Velema, W.A.; Lerch, M.M.; Szymanski, W.; Feringa, B.L. Wavelength-selective cleavage of photoprotecting groups: Strategies and applications in dynamic systems. *Chem. Soc. Rev.* **2015**, *44*, 3358–3377. [[CrossRef](#)] [[PubMed](#)]
63. Lynch, S.A.; Gallivan, J.P. A flow cytometry-based screen for synthetic riboswitches. *Nucleic Acids Res.* **2009**, *37*, 184–192. [[CrossRef](#)] [[PubMed](#)]
64. Kathan, M.; Hecht, S. Photoswitchable molecules as key ingredients to drive systems away from the global thermodynamic minimum. *Chem. Soc. Rev.* **2017**, *46*, 5536–5550. [[CrossRef](#)] [[PubMed](#)]
65. Berdnikova, D.V. Photoswitches for controllable RNA binding: A future approach in the RNA-targeting therapy. *Chem. Commun.* **2021**, *57*, 10819–10826. [[CrossRef](#)] [[PubMed](#)]

66. Jaschke, A. Genetically encoded RNA photoswitches as tools for the control of gene expression. *FEBS Lett.* **2012**, *586*, 2106–2111. [[CrossRef](#)]
67. Serganov, A.; Nudler, E. A decade of riboswitches. *Cell* **2013**, *152*, 17–24. [[CrossRef](#)]
68. Young, D.D.; Schultz, P.G. Playing with the molecules of life. *ACS Chem. Biol.* **2018**, *13*, 854–870. [[CrossRef](#)]
69. Wang, L.; Brock, A.; Herberich, B.; Schultz, P.G. Expanding the genetic code of *Escherichia coli*. *Science* **2001**, *292*, 498–500. [[CrossRef](#)]
70. Courtney, T.; Deiters, A. Recent advances in the optical control of protein function through genetic code expansion. *Curr. Opin. Chem. Biol.* **2018**, *46*, 99–107. [[CrossRef](#)]
71. Rizzini, L.; Favory, J.-J.; Cloix, C.; Faggionato, D.; O'Hara, A.; Kaiserli, E.; Baumeister, R.; Schäfer, E.; Nagy, F.; Jenkins, G.I.; et al. Perception of UV-B by the arabidopsis UVR8 protein. *Science* **2011**, *332*, 103–106. [[CrossRef](#)] [[PubMed](#)]
72. Pudasaini, A.; El-Arab, K.K.; Zoltowski, B.D. LOV-based optogenetic devices: Light-driven modules to impart photoregulated control of cellular signaling. *Front. Mol. Biosci.* **2015**, *2*, 18. [[CrossRef](#)] [[PubMed](#)]
73. Braatsch, S.; Gomelsky, M.; Kuphal, S.; Klug, G. A single flavoprotein, AppA, integrates both redox and light signals in *Rhodobacter sphaeroides*. *Mol. Microbiol.* **2002**, *45*, 827–836. [[CrossRef](#)] [[PubMed](#)]
74. Devlin, P.F.; Kay, S.A. Cryptochromes-bringing the blues to circadian rhythms. *Trends Cell Biol.* **1999**, *9*, 295–298. [[CrossRef](#)]
75. Takano, H.; Kondo, M.; Usui, N.; Usui, T.; Ohzeki, H.; Yamazaki, R.; Washioka, M.; Nakamura, A.; Hoshino, T.; Hakamata, W.; et al. Involvement of CarA/LitR and CRP/FNR family transcriptional regulators in light-induced carotenoid production in *Thermus thermophilus*. *J. Bacteriol.* **2011**, *193*, 2451–2459. [[CrossRef](#)]
76. Butler, W.L.; Norris, K.H.; Siegelman, H.W.; Hendricks, S.B. Detection, assay, and preliminary purification of the pigment controlling photoresponsive development of plants. *Proc. Natl. Acad. Sci. USA* **1959**, *45*, 1703–1708. [[CrossRef](#)] [[PubMed](#)]
77. Hoffmann, M.D.; Bubeck, F.; Eils, R.; Niopek, D. Controlling cells with light and LOV. *Adv. Biosyst.* **2018**, *2*, 1800098. [[CrossRef](#)]
78. Taylor, B.L.; Zhulin, I.B. PAS domains: Internal sensors of oxygen, redox potential, and light. *Microbiol. Mol. Biol. Rev.* **1999**, *63*, 479–506. [[CrossRef](#)]
79. Moglich, A.; Ayers, R.A.; Moffat, K. Structure and signaling mechanism of Per-ARNT-Sim domains. *Structure* **2009**, *17*, 1282–1294. [[CrossRef](#)]
80. Christie, J.M.; Corchnoy, S.B.; Swartz, T.E.; Hokenson, M.; Han, I.S.; Briggs, W.R.; Bogomolni, R.A. Steric interactions stabilize the signaling state of the LOV2 domain of phototropin 1. *Biochemistry* **2007**, *46*, 9310–9319. [[CrossRef](#)]
81. Liu, Z.; Zhang, J.; Jin, J.; Geng, Z.; Qi, Q.; Liang, Q. Programming bacteria with light-sensors and applications in synthetic biology. *Front. Microbiol.* **2018**, *9*, 2692. [[CrossRef](#)] [[PubMed](#)]
82. Ohlendorf, R.; Vidavski, R.R.; Eldar, A.; Moffat, K.; Moglich, A. From dusk till dawn: One-plasmid systems for light-regulated gene expression. *J. Mol. Biol.* **2012**, *416*, 534–542. [[CrossRef](#)] [[PubMed](#)]
83. Moglich, A.; Ayers, R.A.; Moffat, K. Design and signaling mechanism of light-regulated histidine kinases. *J. Mol. Biol.* **2009**, *385*, 1433–1444. [[CrossRef](#)] [[PubMed](#)]
84. Jin, X.; Riedel-Kruse, I.H. Biofilm Lithography enables high-resolution cell patterning via optogenetic adhesin expression. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 3698–3703. [[CrossRef](#)] [[PubMed](#)]
85. Pu, L.; Yang, S.; Xia, A.; Jin, F. Optogenetics manipulation enables prevention of biofilm formation of engineered *Pseudomonas aeruginosa* on surfaces. *ACS Synth. Biol.* **2018**, *7*, 200–208. [[CrossRef](#)] [[PubMed](#)]
86. Sankaran, S.; Becker, J.; Wittmann, C.; Del Campo, A. Optoregulated drug release from an engineered living material: Self-replenishing drug depots for long-term, light-regulated delivery. *Small* **2019**, *15*, 1804717. [[CrossRef](#)]
87. Lalwani, M.A.; Ip, S.S.; Carrasco-Lopez, C.; Day, C.; Zhao, E.M.; Kawabe, H.; Avalos, J.L. Optogenetic control of the lac operon for bacterial chemical and protein production. *Nat. Chem. Biol.* **2021**, *17*, 71–79. [[CrossRef](#)]
88. Lalwani, M.A.; Kawabe, H.; Mays, R.L.; Hoffman, S.M.; Avalos, J.L. Optogenetic control of microbial consortia populations for chemical production. *ACS Synth. Biol.* **2021**, *10*, 2015–2029. [[CrossRef](#)]
89. Heintzen, C.; Loros, J.J.; Dunlap, J.C. The PAS protein VIVID defines a clock-associated feedback loop that represses light input, modulates gating, and regulates clock resetting. *Cell* **2001**, *104*, 453–464. [[CrossRef](#)]
90. Nash, A.I.; McNulty, R.; Shillito, M.E.; Swartz, T.E.; Bogomolni, R.A.; Luecke, H.; Gardner, K.H. Structural basis of photosensitivity in a bacterial light-oxygen-voltage/helix-turn-helix (LOV-HTH) DNA-binding protein. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 9449–9454. [[CrossRef](#)]
91. Kawano, F.; Suzuki, H.; Furuya, A.; Sato, M. Engineered pairs of distinct photoswitches for optogenetic control of cellular proteins. *Nat. Commun.* **2015**, *6*, 6256. [[CrossRef](#)] [[PubMed](#)]
92. Chen, X.; Liu, R.; Ma, Z.; Xu, X.; Zhang, H.; Xu, J.; Ouyang, Q.; Yang, Y. An extraordinary stringent and sensitive light-switchable gene expression system for bacterial cells. *Cell Res.* **2016**, *26*, 854–857. [[CrossRef](#)] [[PubMed](#)]
93. Li, X.; Zhang, C.; Xu, X.; Miao, J.; Yao, J.; Liu, R.; Zhao, Y.; Chen, X.; Yang, Y. A single-component light sensor system allows highly tunable and direct activation of gene expression in bacterial cells. *Nucleic Acids Res.* **2020**, *48*, e33. [[CrossRef](#)] [[PubMed](#)]
94. Romano, E.; Baumschlager, A.; Akmeric, E.B.; Palanisamy, N.; Houmani, M.; Schmidt, G.; Ozturk, M.A.; Ernst, L.; Khammash, M.; Di Ventura, B. Engineering AraC to make it responsive to light instead of arabinose. *Nat. Chem. Biol.* **2021**, *17*, 817–827. [[CrossRef](#)] [[PubMed](#)]
95. Han, T.; Chen, Q.; Liu, H. Engineered photoactivatable genetic switches based on the bacterium phage T7 RNA polymerase. *ACS Synth. Biol.* **2017**, *6*, 357–366. [[CrossRef](#)]

96. Baumschlager, A.; Aoki, S.K.; Khammash, M. Dynamic blue light-inducible T7 RNA polymerases (Opto-T7RNAPs) for precise spatiotemporal gene expression control. *ACS Synth. Biol.* **2017**, *6*, 2157–2167. [[CrossRef](#)]
97. Baumschlager, A.; Rullan, M.; Khammash, M. Exploiting natural chemical photosensitivity of anhydrotetracycline and tetracycline for dynamic and setpoint chemo-optogenetic control. *Nat. Commun.* **2020**, *11*, 3834. [[CrossRef](#)]
98. Jayaraman, P.; Devarajan, K.; Chua, T.K.; Zhang, H.; Gunawan, E.; Poh, C.L. Blue light-mediated transcriptional activation and repression of gene expression in bacteria. *Nucleic Acids Res.* **2016**, *44*, 6994–7005. [[CrossRef](#)]
99. Ding, Q.; Ma, D.; Liu, G.-Q.; Li, Y.; Guo, L.; Gao, C.; Hu, G.; Ye, C.; Liu, J.; Liu, L.; et al. Light-powered *Escherichia coli* cell division for chemical production. *Nat. Commun.* **2020**, *11*, 2262. [[CrossRef](#)]
100. Wu, P.; Chen, Y.; Liu, M.; Xiao, G.; Yuan, J. Engineering an optogenetic CRISPRi platform for improved chemical production. *ACS Synth. Biol.* **2021**, *10*, 125–131. [[CrossRef](#)]
101. Arinkin, V.; Granzin, J.; Rollen, K.; Krauss, U.; Jaeger, K.E.; Willbold, D.; Batra-Safferling, R. Structure of a LOV protein in apo-state and implications for construction of LOV-based optical tools. *Sci. Rep.* **2017**, *7*, 42971. [[CrossRef](#)]
102. Zhao, E.M.; Lalwani, M.A.; Chen, J.M.; Orillac, P.; Toettcher, J.E.; Avalos, J.L. Optogenetic amplification circuits for light-induced metabolic control. *ACS Synth. Biol.* **2021**, *10*, 1143–1154. [[CrossRef](#)]
103. Pouzet, S.; Banderas, A.; Le Bec, M.; Lautier, T.; Truan, G.; Hersen, P. The promise of optogenetics for bioproduction: Dynamic control strategies and scale-up instruments. *Bioengineering* **2020**, *7*, 151. [[CrossRef](#)]