Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Chemical and optical control of CRISPR-associated nucleases

Ashley E. Modell[1,2,a], Sachini U. Siriwardena[1,2,a], Veronika M. Shoba[1,2,a], Xing Li[1,2,a] and Amit Choudhary[1,2,3]

Abstract

The clustered regularly interspaced short palindromic repeats (CRISPR)–Cas system of bacteria has furnished programmable nucleases (e.g., Cas9) that are transforming the field of genome editing with applications in basic and biomedical research, biotechnology, and agriculture. However, broader real-world applications of Cas9 require precision control of its activity over dose, time, and space as off-target effects, embryonic mosaicism, chromosomal translocations, and genotoxicity have been observed with elevated and/or prolonged nuclease activity. Here, we review chemical and optical methods for precision control of Cas9's activity.

Addresses

[1] Chemical Biology and Therapeutics Science, Broad Institute of MIT and Harvard, Cambridge, MA, 02142, USA

[2] Department of Medicine, Harvard Medical School, Boston, MA, 02115, USA

[3] Divisions of Renal Medicine and Engineering, Brigham and Women's Hospital, Boston, MA, 02115, USA

Corresponding author: Choudhary, Amit (achoudhary@bwh.harvard.edu)

[a] These authors contributed equally and listed alphabetically.

Current Opinion in Chemical Biology 2021, **60**:113–121

This review comes from a themed issue on **Omics**

Edited by **Nichollas Scott** and **Laura Edgington-Mitchell**

For a complete overview see the Issue and the Editorial

Available online 27 November 2020

https://doi.org/10.1016/j.cbpa.2020.10.003

Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR)–associated nucleases are programmable RNA-guided DNA endonucleases that are being developed for applications in basic research, biomedicine, biotechnology, and agriculture [1–3]. While the most studied CRISPR-associated nuclease is from *Streptococcus pyogenes* (SpCas9, henceforth called Cas9), several next-generation nucleases (e.g., SaCas9, Cpf1, CasPhi) with several superior attributes have emerged [4,5]. The complex of the nuclease and guide RNA (gRNA) recognizes its substrate DNA sequence via a protospacer adjacent motif and base-pairing of its gRNA to the target DNA. Catalytically dead Cas9 (dCas9) retains its DNA-targeting abilities, but cannot cleave DNA, and has been used to recruit effector domains (e.g., transcriptional activation domains) for controlling gene expression.

Whether using Cas9 or dCas9, there is a need for multidimensional controls over dose, time, and space [6]. As initially described by Paracelsus, "*only the dose permits something not to be poison,*" and therefore, therapeutic agents must exhibit dose and temporal controls [7]. These controls are especially important for Cas9 because enzyme concentrations are much higher than substrate concentrations. Temporal control of Cas9 activity is also important as off-target editing often occurs at a slower rate than on-target editing [8]. Since most delivery systems use constitutively active Cas9, switching on or off the enzyme at the desired time would significantly reduce unwanted side effects [9,10].

The control of Cas9 activity is needed in several other contexts. First, prolonged Cas9 activity is genotoxic to primary and stem cells because double-strand DNA breaks have been shown to induce high levels of apoptosis, leading to a low number of edited cells and potential selection of tumorigenic clones [11,12]. Second, in germline editing, mosaicism (e.g., genotypic heterogeneity in different cells) arises from nonuniform Cas9 activity in dividing cells, which can be tempered by restricting Cas9's activity to a narrow temporal window [13,14]. Third, packaging of Cas9 for adeno-associated virus (AAV)–mediated delivery can be toxic, and this limitation can be addressed by switching off Cas9 [15]. Finally, controls over Cas9 are particularly useful for gene drives in multiple contexts, including controlling the degree of super-Mendelian inheritance and propagation of lethal traits [16]. Small molecules and light are often deployed for precision control of enzyme activity. Here, we review different methods for chemical and optical control of CRISPR–Cas technologies with an emphasis on the underlying molecular mechanisms, their strengths and weaknesses, and the degree of control they afford.

Activation by small molecules

The control of Cas9 with small molecules has been extensively reviewed [17–19]; therefore, here we highlight different classes of chemically induced Cas9/gRNA systems with an emphasis on the most recent advancements with demonstrated therapeutic potential. Small-molecule activation can be grouped into transcriptional control of Cas9/dCas9/gRNA, post-transcriptional/translational control of Cas9/gRNA, or recruitment of transcriptional activation domains.

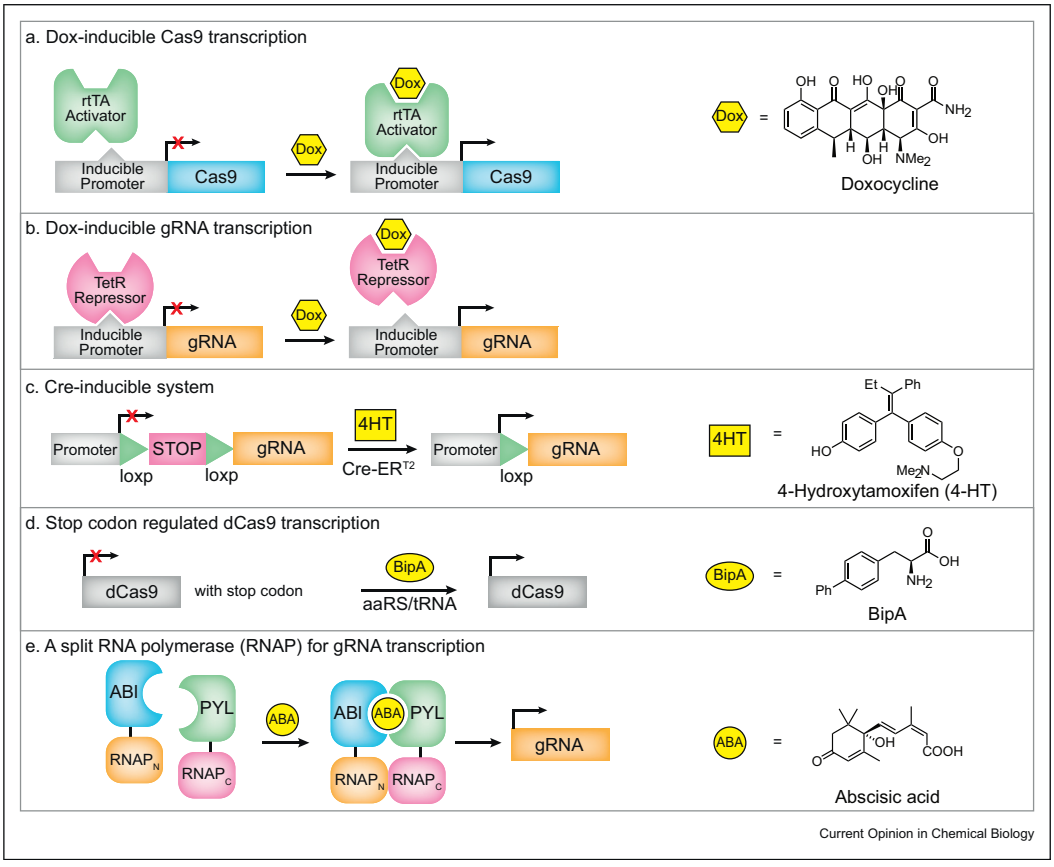
Control of Cas9, dCas9, or gRNA at the transcriptional level

Doxycycline (Dox)-inducible systems are among the earliest methods for controlling the expression of Cas9 (Figure 1**a**) [20,21] or gRNA (Figure 1**b**) [22]. Sun et al. [23] recently used Dox and isopropyl β-D-1-thio-galactopyranoside (IPTG) to induce Tet and Lac systems, respectively, to regulate gRNA expression and induce CRISPR-Cas genome editing in large-scale functional screens. They found minimal leakiness in expression and higher efficiency with 2xTetO and 2xLacO designs, as well as tight regulation of gene knockouts in a range of cell types and a hematopoietic reconstitution mouse model [23]. In addition, the widely used Cre system has been applied to control Cas9/gRNA activity. In the CRISPR-Switch (SgRNA With Induction/Termination by Cre Homologous recombination) system, a tamoxifen-dependent Cre-ER^{T2} rapidly induced gRNA expression only in the presence of the small molecule 4-hydroxytamoxifen (4-HT) (Figure 1**c**) [24]. This method showed superior performance with regards to the leakiness in the OFF state and activity in the ON state compared to other methods for inducible gene editing.

In addition, Van der Oost's [25] group demonstrated transcriptional control with an unnatural amino acid. Introduction of a TAG stop codon in the dCas9 gene leads to functional, full-length dCas9 protein expression only in the presence of L-biphenylalanine with the corresponding aminoacyl synthetase (aaRS) and tRNA to recognize the stop codon. Removal of the small molecule resulted in truncated, nonfunctional nuclease production (Figure 1**d**) [25].

Control at the transcriptional level has been expanded to next-generation CRISPR-associated nucleases such as *Staphylococcus aureus* Cas9 (SaCas9) and Cpf1, also known as Cas12a. Pu et al. [26] regulated gRNA

Figure 1



(a-e) Control of Cas9, dCas9, or gRNA at the transcriptional level with small molecules.

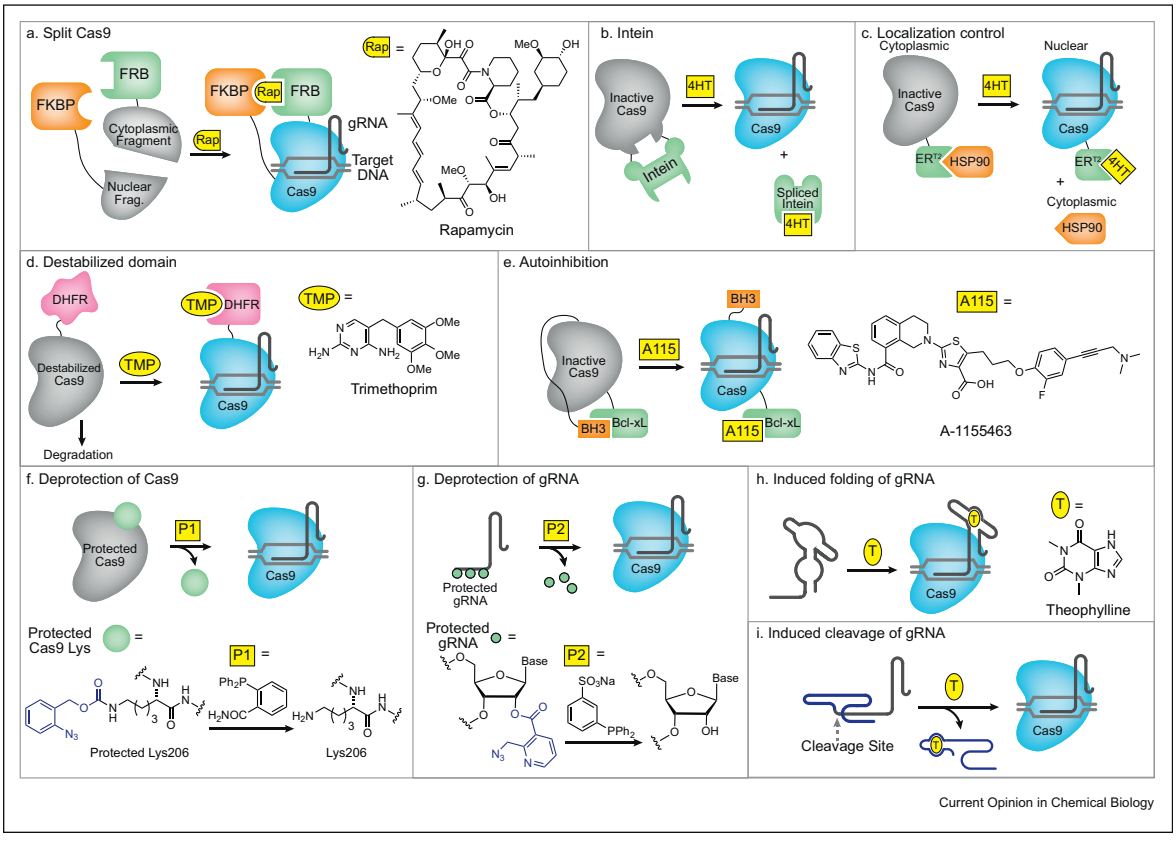
production using abscisic acid (ABA)-inducible split RNA polymerase (RNAP) fused with ABI and PYL proteins to control SaCas9 activity ('ON switch') (Figure 1**e**). This system had high background activity that was reduced by the addition of a rapamycin-inducible 'OFF switch' with a second split RNA polymerase fused to FK506-binding protein 12 (FKBP) and FKBP-rapamycin-binding (FRB) domain to control the gRNA that targets the 'ON switch.' Kempton et al. [27] demonstrated control of a split dead *Lachnospiraceae bacterium* Cpf1 (dLbCpf1) system by cumate and Dox-inducible promoters, as well as crRNA expression under a Dox-inducible RNA polymerase II.

Post-translational/transcriptional control of Cas9 or gRNA

The aforementioned systems have slow response time for activation as they require both transcription and translation, and involve additional elements (e.g., reverse-tTA in the Tet system) for control. Therefore, post-translational control of Cas9 or post-transcriptional control of gRNA by small molecules may provide a more robust way to regulate Cas9's activity. One of the earliest methods in this direction involved a split Cas9 system where rapamycin is used to induce the dimerization of FKBP and FRB (Figure 2**a**) [28]. The C-terminal fragment of split Cas9 is fused to FKBP along with two nuclear localization signals, while the N-terminal fragment is conjugated to FRB with a nuclear export signal. The spatial separation of the split fragments substantially reduces basal activity. Once rapamycin is added, Cas9 assembles and translocates to the nucleus to perform editing. This approach was expanded to Cpf1, where the N- and C-terminal domains of Cpf1 were fused to FRB and FKBP, respectively [29].

In another approach, an inactive Cas9 was generated by inserting a 4-HT–responsive intein derived from the estrogen receptor (ER) [30]. The binding of 4-HT triggered intein splicing and activation of Cas9 (Figure 2**b**). Fusion with an ER variant has also been used to control the localization of the Cas9 [31–33]. The ER is sequestered in the cytoplasm by heat shock protein 90 (Hsp90), and the addition of 4-HT disrupts this ER/Hsp90 interaction, inducing translocation of ER–Cas9 fusion to the nucleus for editing (Figure 2**c**). Nguyen et al. [34] expanded this localization control to a split Cas9 where each domain was tagged with the ER

Figure 2



(a-i) Post-translational/transcriptional control of Cas9/gRNA with small molecules.

variant. The split Cas9 remained in the cytoplasm, segregated from gRNA, until the addition of 4-HT, which induced nuclear localization and generation of active Cas9/gRNA complex.

Cas9's activity can also be controlled by the fusion of a ligand-dependent destabilizing domain to the nuclease. The fusion protein is subject to proteasomal degradation in the absence of its cognate ligand (Figure 2**d**). Examples of destabilizing domain fusions to Cas9 systems include mutant FKBP12, mutant *E. coli* dihydrofolate reductase (DHFR), and estrogen receptor variant (ER50), which are stabilized by Shield1, trimethoprim (TMP), and 4-HT, respectively [9,35]. Some destabilized domains are functional even at 25 °C (vs. 37 °C), a feature that we exploited to develop a TMP-controlled gene drive in *Drosophila* [16]. Using TMP, we established reversibility and dose control of gene drive inheritance allowing us to fine-tune the rate of spread of the engineered gene in a population.

Rose et al. [36] constructed a rapidly inducible Cas9 using an autoinhibitory strategy where the REC2 domain in Cas9 was replaced with Bcl-xL, and its cognate peptide, BH3, was attached to the C-terminus. Disruption of the Bcl-xL/BH3 interaction by small molecule, A-385358, activated Cas9. The use of small molecule A-1155463 (A-115) further improved Bcl-xL/BH3 disruption and lowered background (Figure 2**e**) [37].

In place of ligand-controllable protein fusions, alternative approaches involve chemical modification of Cas9 or the gRNA with inactivating groups that are removed by small-molecule triggers. For example, site-specific (K206) insertion of the unnatural amino acid *ortho*-azidobenzyloxycarbonyl lysine inactivates Cas9, and the reduction of this azide by phosphines reveals the native lysine and activates Cas9 (Figure 2**f**) [38]. Similarly, azide-bearing inactive gRNA was activated by phosphines (Figure 2**g**) [39].

Another approach to controlling Cas9 activity can involve gRNA, which is typically in an 'OFF state' until a small molecule induces an active conformation that can bind to Cas9 (Figure 2**h**) [40]. Tang et al. [41] also developed aptazyme-embedded gRNAs that undergo a self-cleavage of a blocking strand in the presence of small molecules (e.g., theophylline, guanine) (Figure 2**i**).

Recruitment of a transcriptional activation domain to dCas9 or gRNA

There are multiple reported methods for transcriptional regulation of genes with engineered dCas9. Gao et al. [42] applied abscisic acid (ABA)-inducible ABI–PYL1 and gibberellin (GA)-inducible GAI–GID1 heterodimerization systems to dCas9 for transcriptional control of two genes. dCas9 was fused to either ABI or GAI while the transcriptional activation domain VP64-p65-Rta (VPR) system was fused to the recruited PYL1 or GID1 domains, respectively (Figure 3**a**). Similarly, dLbCpf1 was fused to DmrA, which recruited a DmrC–VPR fusion in the presence of the rapamycin analog A/C heterodimerizer [43].

The previous examples rely on fusing ligand-binding domains directly to dCas9, but alternatively, gRNAs can be engineered to include sequences that bind to proteins (e.g., MS2, PP7). These gRNA-binding proteins can either be fused to activation domains or used to recruit transcriptional activation domains via small molecules. For example, we fused these gRNA-binding domains to destabilized domains (e.g., MS2-ER50, PP7-DHFR, controlled by 4-HT and TMP, respectively) to afford dose-dependent, reversible, and orthogonal transcriptional control of two distinct genes (Figure 3**b**) [44]. In a double recruitment strategy, Chiarella et al. [45] designed a system wherein the gRNA (with MS2-binding sequence) recruits MS2-FKBP. The addition of a heterobifunctional molecule that binds to FKBP and endogenous chromatin-modifying enzymes (e.g., BRD4 and CBP/p300) allowed for dose-dependent transcriptional activation (Figure 3**c**) [45].

Recently, stabilizable polypeptide linkages called StaPLs and ligand-inducible connection (LInC) systems in combination with protease inhibitors were used to 'turn ON' dCas9 activity. In the first system, split dCas9 domains were connected to form a functional dCas9–VPR by inhibiting a self-cleaving StaPL^{TI} linker with telaprevir (Figure 3**d**) [46]. The second system contains a hepatitis C virus NS3 protease domain and two self-cleaving linkers: one installed between dCas9 and NS3 and another between NS3 and VPR tagged with a nuclear localization signal sequence. Treatment with protease inhibitors such as telaprevir or BILN-2061 (ciluprevir) prevents self-cleavage and preserves the integrity of these systems, which triggers transcriptional activation (Fig. 3**e**) [47].

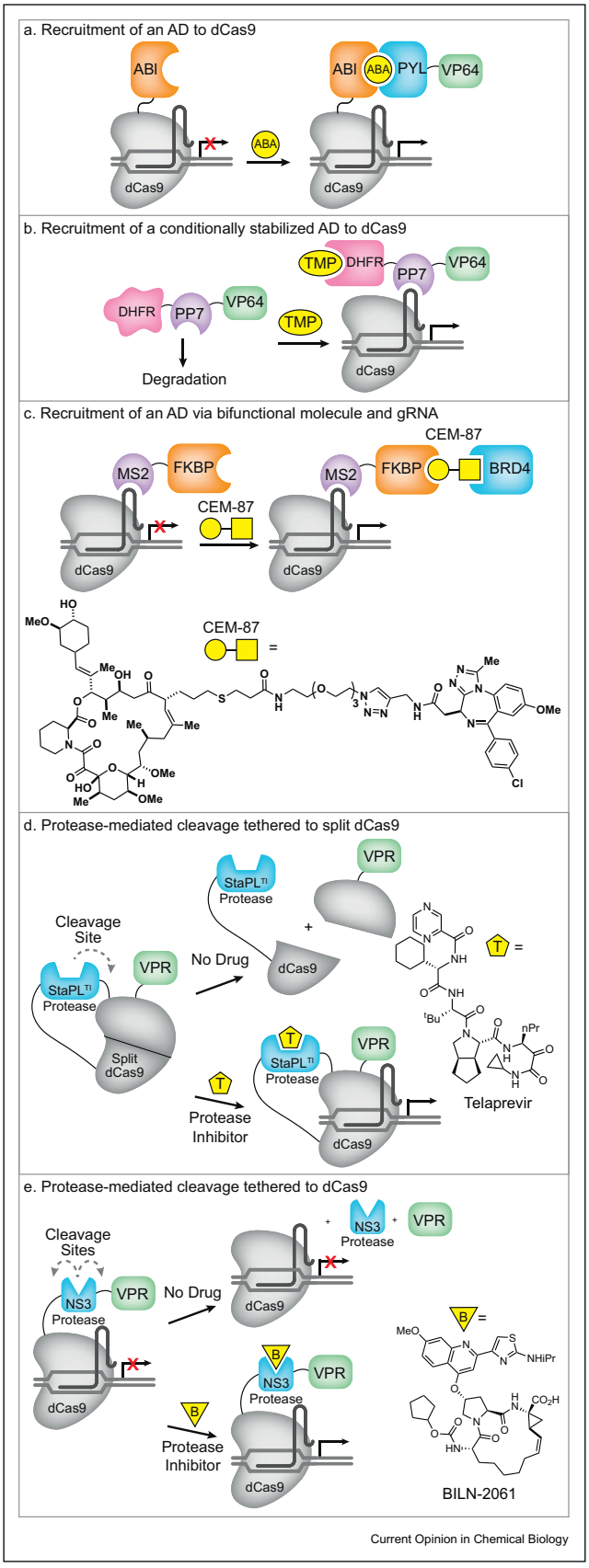
Activation by light

Light is relatively noninvasive and has been widely used for precise spatial and temporal control of protein function [48]. Light activation of Cas9 systems can also be grouped into transcriptional control, post-translational/transcriptional control, and recruitment of a transcriptional activation domain.

Control of Cas9, dCas9, or activation domains at the transcriptional level

Recent advances have allowed for photo-inducible transcription of Cas9, split-Cas9, and transcription activation domains. The Ping group used near-IR to activate full Cas9 expression *in vitro* and *in vivo* [49]. Yu

Figure 3



(a-e) Recruitment of a transcriptional activation domain (AD) to dCas9 or gRNA with small molecules.

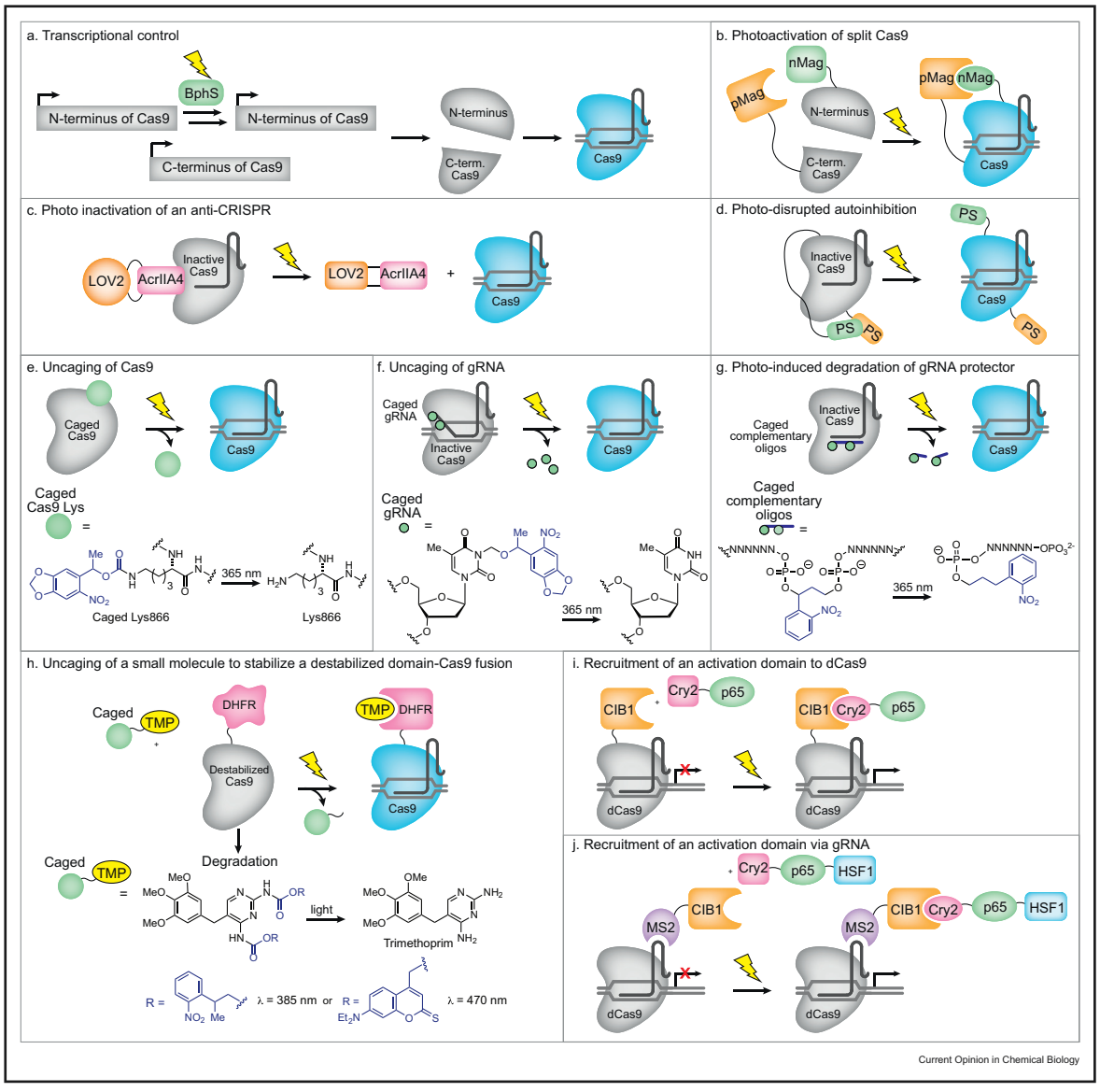
et al. [50] recently created a photo-controlled split Cas9 system that worked successfully in mice. In this system, the C-terminus of Cas9 is constitutively expressed, and when far-red light is added (730 nm), the engineered bacterial photoreceptor BphS triggers a cascade to transcribe the N-terminus, which allows the formation of an active Cas9 (Figure 4**a**) [50]. In another study, far-IR light activated the BphS cascade to express the transcription activation system (MS2-p65-HSF1) [51].

Post-translational/transcriptional control of Cas9, dCas9, or gRNA

Light can be used to control Cas9 systems analogously to small molecules. For example, Nihongaki et al. [52] engineered a photoactivatable split-Cas9 and split-dCas9 systems with each domain fused to photo-inducible dimerization domains pMag and nMag (Figure 4**b**). The dimerization and subsequent activation of these systems can be reversibly controlled by light, and the authors also applied this to split-Cpf1 and split-dCpf1 in a follow-up article [29]. Bubeck et al. [53] recently showed reversible photocontrol over an anti-CRISPR/Cas9 system wherein in the absence of light, the anti-CRISPR, AcrIIA4, can inhibit Cas9. However, in the presence of light, AcrIIA4 is displaced, thus activating Cas9 (Figure 4**c**). An alternative method to control Cas9 activity is through autoinhibition. Photo-dimerizable pdDronpa domains render the nuclease inactive until cyan light disrupts the dimerization of the domains (Figure 4**d**), and this system was extended to dCas9 tethered to VPR [54].

Similar to the small-molecule–triggered deprotection of Cas9 and gRNA described earlier, uncaging of Cas9 and gRNA can be implemented using light. Expansion of the genetic code by site-selective incorporation of a photocaged lysine (K866) into Cas9 inactivates the enzyme [55]. Brief exposure to UV light (365 nm) induces the photolysis of the caging group to generate the active nuclease (Figure 4**e**). Several examples of direct photocaging of nucleotides in gRNA have been reported with success in zebrafish [56,57]. Caged gRNAs are generated by judiciously placing caged nucleobases throughout the gRNA, which suppress the gRNA/target DNA interaction until photouncaging of the gRNA [56]. The Ha group expanded on this caged RNA strategy by moving the protecting group to the protospacer adjacent motif-distal region to develop a very fast CRISPR, which is activated in seconds. In this system, the caged RNA binds to Cas9 and its target DNA, but prevents DNA cleavage until light uncages the RNA (Figure 4**f**) [58]. Alternatively, photocaging of a complementary oligonucleotide protector can be used to prevent gRNA binding to the target DNA. Photodegradation of the protector liberates the gRNA, which allows for the recognition of the DNA target (Figure 4**g**) [59]. We developed a system

Figure 4



Optical control of Cas9-based systems: **(a)** transcriptional control with light, **(b–h)** post-translational/transcriptional control of Cas9/gRNA with light, and **(i–j)** recruitment of a transcriptional activation domain to dCas9 or gRNA with light.

that afforded both light and small-molecule control by photocaging TMP that conditionally stabilizes a Cas9 bearing the destabilized domain DHFR upon photo-uncaging (Figure 4**h**) [9]. This system allowed activation of Cas9 at multiple wavelengths and low-light intensities, but, similar to the other uncaging technologies, it was not reversible [9].

Recruitment of a transcriptional activation domain to dCas9 or gRNA

In addition to the dCas9 examples mentioned previously, transcriptional activators (VP64 or p65) and dCas9 were fused to light-controlled protein dimerizers, CRY2 and CIB (Figure 4**i**) [60,61]. In addition, HSF1 activation domain recruitment by a gRNA was demonstrated (Figure 4**j**) [62]. These light-controlled systems are easily reversible through the removal of the light source.

Conclusions and outlook

We have highlighted and reviewed chemical- and light-based approaches to control Cas9-based technologies over time, dose, and space. Small molecules and light afford precision control, but (un)known side effects or toxicity of many of these systems still need to be

investigated. Some small molecules (e.g., doxycycline and trimethoprim) are United States Food and Drug Administration-approved drugs with known toxicities [63–65]. Other molecules such as abscisic acid and gibberellin are plant hormones whose side effects in humans are not fully understood [66].

Despite these challenges behind the development of small-molecule–based controllers of genome editing, some systems were demonstrated to work *in vivo*. Tamoxifen, rapamycin, and doxycycline were used to switch on Cas9, induce expression of targeted genes, and self-inactivate Cas9 in mice [24,29,67]. Light-based controllers of Cas9-based technologies are relatively less invasive and allow precise spatiotemporal control. However, most light-controlled systems use UV or blue light, which leads to limited penetrability due to the strong absorption and scattering by biological tissues [68]. Thus, it is necessary for *in vivo* applications to use systems that are activated using far-red wavelengths to circumvent these low penetration challenges [50,51]. Notably, light-controlled methods are finding application *in vivo* as exemplified by the development of anti-tumor therapies using infrared activated Cas9 systems [49,50].

For both small molecule and light-controlled systems, a remaining challenge to be addressed is that Cas9 fusions with large proteins or domains may be difficult to package into AAV vectors. In addition, most small molecule or light-based systems discussed here are used in mammalian cells. However, CRISPR-Cas systems are being used for editing genomes in plants, fungi, and prokaryotes as well, raising the question of how many of the methods will be translatable to these organisms. While most of these developments have focused on Cas9 and Cpf1, we envision their expansion into other Cas systems and the development of systems for control of their lifetime.

While we have highlighted the strengths and limitations of each of these inducible systems, the field would greatly benefit from a systematic comparison of inducible systems under a variety of conditions and across multiple species. Similar studies have investigated the efficiencies of Cas9-based transcriptional activation systems in multiple species, including human, mouse, and *Drosophila* [69]. More recently, Jones et al. [70] described NucleaSeq — a massively parallel platform for systematic profiling of cleavage activity and specificity of various nucleases. These studies were focused on constitutively active Cas9 technologies, and it would be beneficial to perform a similar systematic evaluation to investigate small molecule and light-inducible systems. In addition to investigating the aforementioned variables, small molecule concentrations and light intensities should also be varied. Such studies would help to quantify dynamic ranges and maximum activation of these inducible systems to determine which systems and at what conditions are most effective.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Broad Institute has filed patent applications for some of the work described in this article.

Acknowledgements

This work was supported by the Defense Advanced Research Projects Agency (N66001-17-2-4055) and National Institutes of Health (R01GM132825).

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. * Doudna JA: **The promise and challenge of therapeutic genome editing**. *Nature* 2020, **578**:229–236.
Note: In this review, current capabilities, applicability, therapeutic opportunities, and limitations of genome editing are discussed.

2. Anzalone AV, Koblan LW, Liu DR: **Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors**. *Nat Biotechnol* 2020, **38**:824–844.

3. Chen K, *et al.*: **CRISPR/Cas genome editing and precision plant breeding in agriculture**. *Annu Rev Plant Biol* 2019, **70**: 667–697.

4. Chen JS, Doudna JA: **The chemistry of Cas9 and its CRISPR colleagues**. *Nat Rev Chem* 2017, **1**. 0078.

5. Pausch P, *et al.*: **CRISPR-CasPhi from huge phages is a hypercompact genome editor**. *Science* 2020, **369**:333–337.

6. * Gangopadhyay SA, *et al.*: **Precision control of CRISPR-Cas9 using small molecules and light**. *Biochemistry* 2019, **58**: 234–244.
Note: This review discusses advances in the precision control of Cas9 (via controlled activation, inhibition or degradation) over dose, time and space dementions using small molecules and light.

7. Tsatsakis AM, *et al.*: **The dose response principle from philosophy to modern toxicology: the impact of ancient philosophy and medicine in modern toxicology science**. *Toxicol Rep* 2018, **5**:1107–1113.

8. Cho SW, *et al.*: **Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases**. *Genome Res* 2014, **24**:132–141.

9. Manna D, *et al.*: **A singular system with precise dosing and spatiotemporal control of CRISPR-Cas9**. *Angew Chem Int Ed Engl* 2019, **58**:6285–6289.

10. Maji B, *et al.*: **A high-throughput platform to identify small-molecule inhibitors of CRISPR-Cas9**. *Cell* 2019, **177**: 1067–1079.e19.

11. Ihry RJ, *et al.*: **p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells**. *Nat Med* 2018, **24**:939–946.

12. Haapaniemi E, *et al.*: **CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response**. *Nat Med* 2018, **24**: 927–930.

13. Tu Z, *et al.*: **Promoting Cas9 degradation reduces mosaic mutations in non-human primate embryos**. *Sci Rep* 2017, **7**: 42081.

14. Mehravar M, *et al.*: **Mosaicism in CRISPR/Cas9-mediated genome editing**. *Dev Biol* 2019, **445**:156–162.

15. Palmer DJ, Turner DL, Ng P: **Production of CRISPR/Cas9-Mediated self-cleaving helper-dependent adenoviruses**. *Mol Ther Methods Clin Dev* 2019, **13**:432–439.

16. López Del Amo V, *et al.*: **Small-molecule control of super-mendelian inheritance in gene drives**. *Cell Rep* 2020, **31**: 107841.

17. Lo A, Qi L: **Genetic and epigenetic control of gene expression by CRISPR-Cas systems**. *F1000Res* 2017:6.

18. Zhao W, Wang Y, Liang FS: **Chemical and light inducible epigenome editing**. *Int J Mol Sci* 2020, **21**.

19. Zhang J, *et al.*: **Drug inducible CRISPR/Cas systems**. *Comput Struct Biotechnol J* 2019, **17**:1171–1177.

20. González F, *et al.*: **An iCRISPR platform for rapid, multiplexable, and inducible genome editing in human pluripotent stem cells**. *Cell Stem Cell* 2014, **15**:215–226.

21. Cao J, *et al.*: **An easy and efficient inducible CRISPR/Cas9 platform with improved specificity for multiple gene targeting**. *Nucleic Acids Res* 2016, **44**:e149.

22. Aubrey BJ, *et al.*: **An inducible lentiviral guide RNA platform enables the identification of tumor-essential genes and tumor-promoting mutations in vivo**. *Cell Rep* 2015, **10**: 1422–1432.

23. Sun N, *et al.*: **Development of drug-inducible CRISPR-Cas9 systems for large-scale functional screening**. *BMC Genom* 2019, **20**:225.

24. Chylinski K, *et al.*: **CRISPR-Switch regulates sgRNA activity by Cre recombination for sequential editing of two loci**. *Nat Commun* 2019, **10**:5454.

25. Koopal B, *et al.*: **Incorporation of a synthetic amino acid into dCas9 improves control of gene silencing**. *ACS Synth Biol* 2019, **8**:216–222.

26. Pu J, Kentala K, Dickinson BC: **Multidimensional control of Cas9 by evolved RNA polymerase-based biosensors**. *ACS Chem Biol* 2018, **13**:431–437.

27. Kempton HR, *et al.*: **Multiple input sensing and signal integration using a split Cas12a system**. *Mol Cell* 2020, **78**: 184–191.e3.

28. * Zetsche B, Volz SE, Zhang F: **A split-Cas9 architecture for inducible genome editing and transcription modulation**. *Nat Biotechnol* 2015, **33**:139–142.

Note: This work is one of the first examples of Cas9 activation with small molecules based on enginered split nuclease and its rapamycin-trigged reassembly.

29. Nihongaki Y, *et al.*: **A split CRISPR-Cpf1 platform for inducible genome editing and gene activation**. *Nat Chem Biol* 2019, **15**: 882–888.

30. Davis KM, *et al.*: **Small molecule-triggered Cas9 protein with improved genome-editing specificity**. *Nat Chem Biol* 2015, **11**: 316–318.

31. Liu KI, *et al.*: **A chemical-inducible CRISPR-Cas9 system for rapid control of genome editing**. *Nat Chem Biol* 2016, **12**: 980–987.

32. Zhao C, *et al.*: **HIT-Cas9: a CRISPR/Cas9 genome-editing device under tight and effective drug control**. *Mol Ther Nucleic Acids* 2018, **13**:208–219.

33. Oakes BL, *et al.*: **Profiling of engineering hotspots identifies an allosteric CRISPR-Cas9 switch**. *Nat Biotechnol* 2016, **34**: 646–651.

34. Nguyen DP, *et al.*: **Ligand-binding domains of nuclear receptors facilitate tight control of split CRISPR activity**. *Nat Commun* 2016, **7**:12009.

35. Senturk S, *et al.*: **Rapid and tunable method to temporally control gene editing based on conditional Cas9 stabilization**. *Nat Commun* 2017, **8**:14370.

36. Rose JC, *et al.*: **Rapidly inducible Cas9 and DSB-ddPCR to probe editing kinetics**. *Nat Methods* 2017, **14**:891–896.

37. Rose JC, *et al.*: **Rheostatic control of Cas9-mediated DNA double strand break (DSB) generation and genome editing**. *ACS Chem Biol* 2018, **13**:438–442.

38. Luo J, *et al.*: **Small-molecule control of protein function through Staudinger reduction**. *Nat Chem* 2016, **8**:1027–1034.

39. Habibian M, *et al.*: **Reversible RNA acylation for control of CRISPR–Cas9 gene editing**. *Chem Sci* 2020, **11**:1011–1016.

40. Iwasaki RS, *et al.*: **Small molecule regulated sgRNAs enable control of genome editing in E. coli by Cas9**. *Nat Commun* 2020, **11**:1394.

41. Tang W, Hu JH, Liu DR: **Aptazyme-embedded guide RNAs enable ligand-responsive genome editing and transcriptional activation**. *Nat Commun* 2017, **8**:15939.

42. Gao Y, *et al.*: **Complex transcriptional modulation with orthogonal and inducible dCas9 regulators**. *Nat Methods* 2016, **13**:1043–1049.

43. Tak YE, *et al.*: **Inducible and multiplex gene regulation using CRISPR-Cpf1-based transcription factors**. *Nat Methods* 2017, **14**:1163–1166.

44. Maji B, *et al.*: **Multidimensional chemical control of CRISPR-Cas9**. *Nat Chem Biol* 2017, **13**:9–11.

45. * Chiarella AM, *et al.*: **Dose-dependent activation of gene expression is achieved using CRISPR and small molecules that recruit endogenous chromatin machinery**. *Nat Biotechnol* 2020, **38**:50–55.

Note: This paper demonstrated how bifunctional molecules (e.g, FK506-JQ1) could recruit different chemical epigenetic modifiers (e.g., BRD4, BRPF1, and CBP/p300) to dCas9 tethered to FKBP.

46. Jacobs CL, Badiee RK, Lin MZ: **StaPLs: versatile genetically encoded modules for engineering drug-inducible proteins**. *Nat Methods* 2018, **15**:523–526.

47. Tague EP, *et al.*: **Chemogenetic control of gene expression and cell signaling with antiviral drugs**. *Nat Methods* 2018, **15**: 519–522.

48. Gautier A, *et al.*: **How to control proteins with light in living systems**. *Nat Chem Biol* 2014, **10**:533–541.

49. Chen X, *et al.*: **Near-infrared optogenetic engineering of photothermal nanoCRISPR for programmable genome editing**. *Proc Natl Acad Sci U S A* 2020, **117**:2395–2405.

50. Yu Y, *et al.*: **Engineering a far-red light–activated split-Cas9 system for remote-controlled genome editing of internal organs and tumors**. *Sci Adv* 2020, **6**, eabb1777.

51. Shao J, *et al.*: **Synthetic far-red light-mediated CRISPR-dCas9 device for inducing functional neuronal differentiation**. *Proc Natl Acad Sci U S A* 2018, **115**:E6722–E6730.

52. Nihongaki Y, *et al.*: **Photoactivatable CRISPR-Cas9 for optogenetic genome editing**. *Nat Biotechnol* 2015, **33**:755–760.

53. Bubeck F, *et al.*: **Engineered anti-CRISPR proteins for optogenetic control of CRISPR-Cas9**. *Nat Methods* 2018, **15**:924–927.

54. Zhou XX, *et al.*: **A single-chain photoswitchable CRISPR-Cas9 architecture for light-inducible gene editing and transcription**. *ACS Chem Biol* 2018, **13**:443–448.

55. Hemphill J, *et al.*: **Optical control of CRISPR/Cas9 gene editing**. *J Am Chem Soc* 2015, **137**:5642–5645.

56. Moroz-Omori EV, *et al.*: **Photoswitchable gRNAs for spatiotemporally controlled CRISPR-Cas-based genomic regulation**. *ACS Cent Sci* 2020, **6**:695–703.

57. Zhou W, *et al.*: **Spatiotemporal control of CRISPR/Cas9 function in cells and zebrafish using light-activated guide RNA**. *Angew Chem Int Ed* 2020, **59**:8998–9003.

58. ** Liu Y, *et al.*: **Very fast CRISPR on demand**. *Science* 2020, **368**: 1265–1269.

Note: In this work, Liu and co-workers developed a Cas9 system where chemically caged gRNA forms a complex with Cas9 and target DNA, but performs editing only upon activation by light within seconds

59. Jain PK, *et al.*: **Development of light-activated CRISPR using guide RNAs with photocleavable protectors**. *Angew Chem Int Ed* 2016, **55**:12440–12444.

60. Nihongaki Y, *et al.*: **CRISPR-Cas9-based photoactivatable transcription system**. *Chem Biol* 2015, **22**:169–174.

61. Polstein LR, Gersbach CA: **A light-inducible CRISPR-Cas9 system for control of endogenous gene activation**. *Nat Chem Biol* 2015, **11**:198–200.

62. Nihongaki Y, *et al.*: **CRISPR-Cas9-based photoactivatable transcription systems to induce neuronal differentiation**. *Nat Methods* 2017, **14**:963–966.

63. Ermak G, Cancasci VJ, Davies KJ: **Cytotoxic effect of doxycycline and its implications for tet-on gene expression systems**. *Anal Biochem* 2003, **318**:152–154.

64. Bursch W, *et al.*: **Active cell death induced by the anti-estrogens tamoxifen and ICI 164 384 in human mammary carcinoma cells (MCF-7) in culture: the role of autophagy**. *Carcinogenesis* 1996, **17**:1595–1607.

65. Niethammer D, Jackson RC: **The effect of trimethoprim on cellular transport of methotrexate and its cytotoxicity to human lymphoblastoid cells in vitro**. *Br J Haematol* 1976, **32**: 273–281.

66. Chanclud E, Lacombe B: **Plant hormones: key players in gut microbiota and human diseases?** *Trends Plant Sci* 2017, **22**: 754–758.

67. Kelkar A, *et al.*: **Doxycycline-dependent self-inactivation of CRISPR-Cas9 to temporally regulate on- and off-target editing**. *Mol Ther* 2020, **28**:29–41.

68. Clement M, Daniel G, Trelles M: **Optimising the design of a broad-band light source for the treatment of skin**. *J Cosmet Laser Ther* 2005, **7**:177–189.

69. Chavez A, *et al.*: **Comparison of Cas9 activators in multiple species**. *Nat Methods* 2016, **13**:563–567.

70. Jones SK, *et al.*: **Massively parallel kinetic profiling of natural and engineered CRISPR nucleases**. *Nat Biotechnol* 2020. https://www.nature.com/articles/s41587-020-0646-5; 2020.