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Small molecule-inducible gene regulatory systems in mammalian cells: progress and design principles

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Small molecule-inducible gene circuits are some of the most important tools in biology because they provide a convenient way to exert precise regulation of biological systems. These systems typically are designed to govern gene activation, repression, or disruption at multiple levels, such as through genome modification, transcription, translation, or post-translational regulation of protein activity. Due to their importance, many new systems have been created in the past few years to address different needs or afford orthogonality. They can be broadly characterized based on the inducer used, the mode of regulation, and the effector protein enabling the regulation. Furthermore, each synthetic circuit has varying performance metrics and design considerations. Here, we provide a concise comparison of recently developed tools and recommend standardized metrics for evaluating their performance and potential as biological interrogators or therapeutics.

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Introduction

Small molecule-inducible genetic circuits have revolutionized our ability to fine-tune protein levels and temporally control cellular output states. This advanced precision instrumentally impacts our understanding of cellular behavior and expands our toolset to address biotech challenges. An inducible system typically consists of two main protein components — a ligand binding domain that senses the inducer and the effector domain that enacts a change on the genetic target. These components can be combined to develop complex, multilayered circuits with temporal control over gene expression. Using small molecules as inducers is desirable because they tend to have high bioavailability, membrane permeability, and are also easy to manufacture. These tools have been developed within every aspect of regulation surrounding the central dogma of biology with effectors that modulate DNA, RNA, and protein activity. Designing inducible switches at each of these regulatory levels comes with its own unique design considerations and circuit performance. In this review, we will briefly summarize circuits developed in the past 3 years and follow up with a discussion of suggested standardized metrics for evaluating these systems.

Small molecule-inducible genetic switches: modes of regulation and their design considerations

Chemically inducible gene switches can be categorized according to the effector's mechanism of action. This effector protein can be a nuclease that induces DNA or RNA modification or a transcription factor (TF) that controls the expression of the gene of interest (GOI). Some systems directly control effector protein activity through dimerization domains, destabilization domains, or localization tags that bind to the inducer [1–7]. More complex systems include multiple layers of induction by controlling transcriptional or translational regulators linked to the expression of the effector or GOI [8–16]. To this end, a digitizer circuit that integrates both chemically inducible site-specific recombinase (SSR) activity and shRNA regulation to control protein expression over a wide dynamic range has been developed [17]. Finally, some systems bypass the use of an effector protein and instead directly control the functional gene product through induced dimerization, degradation or synthetic blocking of the active region of the protein [18–22]. We break down these systems based on their main mode of regulation: inducible DNA modification, transcriptional, post-transcriptional, or post-translational regulation. Within each category, we highlight the main design elements that determine the activity of each genetic switch, the inducer and effector protein, and the advantages and disadvantages of these circuit designs.

Inducible DNA modification via nucleases

A powerful method of gene regulation is direct genetic manipulation through DNA modification. Sequence-specific nucleases are a class of enzymes capable of

cleaving DNA (or RNA) at precise locations based on recognition domains. There are three main DNA nuclease-based systems that continue to advance over the past few years: zinc-finger (ZFN) and transcription activator-like effector nuclease (TALEN) systems, recombinase systems, and clustered regularly interspaced short palindromic repeat (CRISPR)-Cas systems (Figure 1a).

ZFNs and TALENs are fusion proteins composed of a DNA binding domain that confers sequence targeting and a catalytic domain, FokI, that enables DNA cleavage. These nucleases can be fused with chemical-inducible dimerization (CID) domains to design inducible gene editing. However, both ZFNs and TALENs have inherent limitations in their specificity. ZFNs have proven difficult to design, and while TALENs have overcome this limitation to some extent, they both suffer from off-target activity at DNA sequences with high similarity [23]. To overcome this limitation, most inducible designs incorporate the dimerization of two distinct Fokl domains targeting adjacent regions of the target gene, such as a system developed by Matsumoto et al. that combines dCas9-mediated gene targeting to improve specificity [2].

In contrast to ZFNs and TALENs which must be designed to include specificity to the GOI, SSRs such as Cre and Flp are enzymes that recognize their own specific set of recombination sites that can be placed around a GOI or termination sequence. Specific recognition sites ensure high-fidelity recombination, the possibility of orthogonality, and straightforward circuit construction which we see extensively *in vitro* and further in animal model development [4,24]. While SSRs on their own are sensitive to basal activity, our lab and others have combined SSRs with CIDs to induce a variety of outputs such as chimeric antigen receptor (CAR) expression in T cells [25], tumor neoantigen expression [11•], or *in vivo* tissue-specific gene editing [4]. To further expand the potential of SSRs, we have also curated a library of orthogonal, inducible, split recombinase systems that can be combined to perform complex logic in mammalian cells [26].

The CRISPR-Cas system is a revolutionary gene-editing tool that simplifies design considerations to a guide RNA (gRNA) that targets the GOI and a protospacer adjacent motif recognized by the Cas nuclease. The most well-characterized version, CRISPR-Cas9, targets DNA, but there are other classes of CRISPR-Cas enzymes that target RNA or both DNA and RNA. While it has proven to be a powerful tool, constitutively active CRISPR-Cas9 exhibits significant off-target effects as well as toxicity at high activity levels [27]. It has therefore been critical to design temporal control through exogenous inducibility of the Cas9 activity. In a similar pattern to the previously described nuclease systems, CRISPR-Cas9 has also been fused with CIDs to confer inducibility [3]. More recently, Kundert et al. employed a novel strategy of engineering ligand-inducible gRNAs to control CRISPR/Cas9 activity [28], and previous work has utilized post-synthetic masking to control gRNA activity [29].

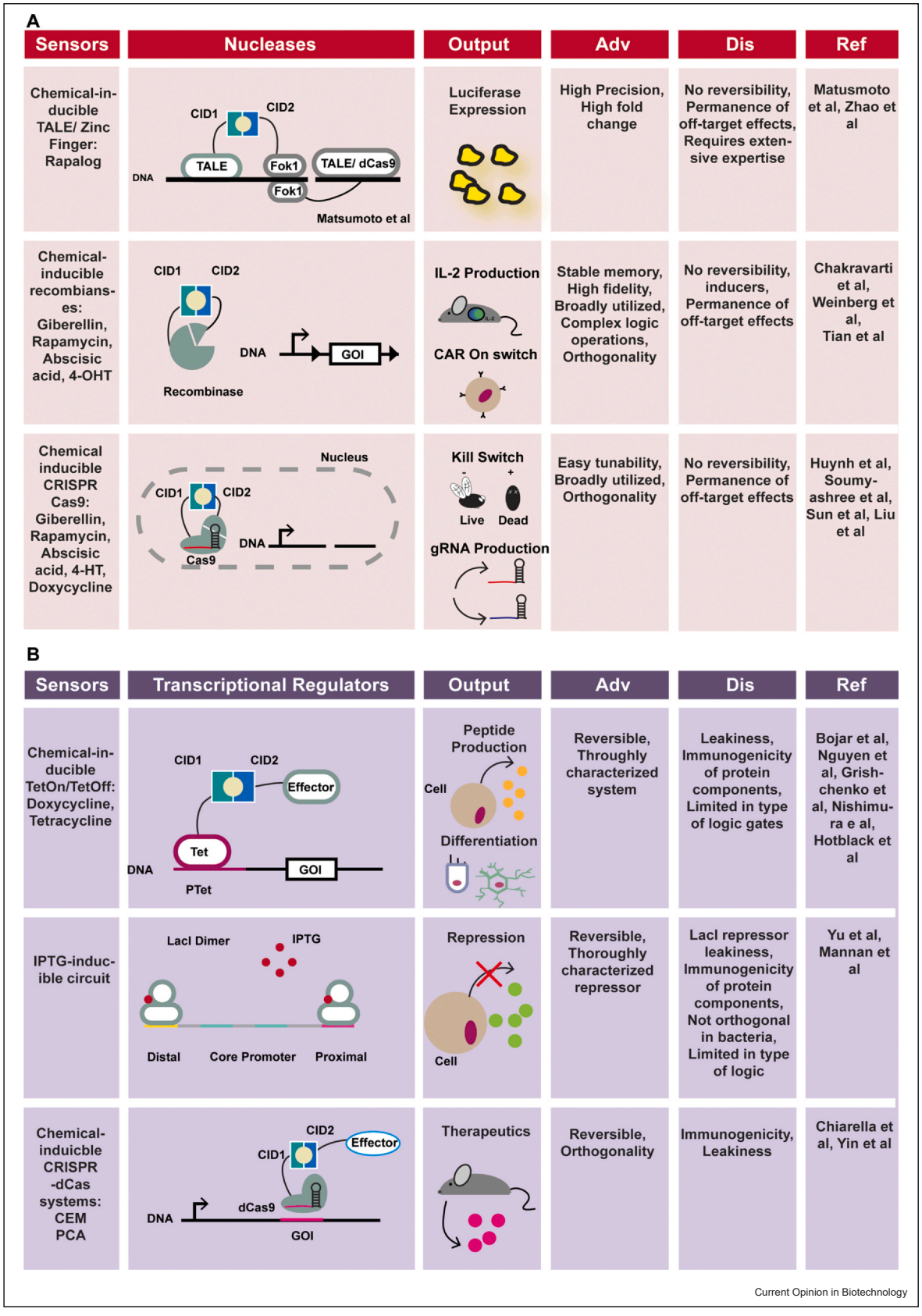
Transcription regulation-based systems

Many important reviews have been dedicated to describing seminal small-molecule inducible TF-based systems [30–32]. Here, we will highlight some of the more recent explorations (Figure 1b). TFs are modular proteins composed of a DNA binding domain, transcription regulatory domain, and optionally a ligand-binding domain. For example, a powerful collection of transcription factors called synZiFTRs (synthetic zinc finger transcription regulators) fuses zinc fingers programmed as DNA binding domains to various transcriptional regulators [33]. This platform is sensibly optimized for induction via Federal Drug Administration (FDA)-approved small molecules with a focus on compact design, orthogonal regulation, tunable activity, and human-based origins. The modular structure of transcription factors has been leveraged to co-opt natural inducible pathways as well as synthetic programmable circuits.

There exists a wide range of TFs that naturally respond to small molecule inducers and more are being discovered and characterized every year [34]. Perhaps the most widely used TF system is the Tet-On/Tet-Off tetracycline-inducible system which has been widely utilized to design multilayered genetic circuits [8,35,36], such as induced differentiation of human induced pluripotent stem cells (hiPSCs) into dopaminergic neurons achieved by placing the expression of proneuronal and ventral midbrain-specific TFs under the control of the tetracycline responsive element promoter [7]. Similarly foundational are IPTG (isopropyl-β-D-1-thiogalactopyranoside)-inducible transcriptional regulators, which have been studied extensively to identify design criteria for reduced leakiness and increased inducible fold change [37•]. Despite their broad use, the IPTG-binding lacI repressor and reverse tetracycline-controlled transactivator, rtTA, have shown immunogenicity in mammalian systems due to their bacterial origin, necessitating the exploration of other inducible TF systems to leverage for therapeutic use [38].

A more recent subset of inducible transcriptional-based tools uses catalytically dead Cas proteins fused with TFs to activate or repress the GOI. In one example, catalytically dead Cas9 (dCas9) was fused to an FKBP (FK506 binding protein) domain to impart transcriptional activation in the presence of chemical epigenetic modifier (CEM) small molecules [12]. In another example, gene

Figure 1


A
Sensors
Nucleases
Output
Adv
Dis
Ref
Chemical-inducible TALE/ Zinc Finger: Rapalog
CID1
CID2
TALE
Fok1
TALE/ dCas9
Fok1
DNA
Matsumoto et al
Luciferase Expression
High Precision, High fold change
No reversibility, Permanence of off-target effects, Requires extensive expertise
Matusmoto et al, Zhao et al
Chemical-inducible recombianses: Giberellin, Rapamycin, Abscisic acid, 4-OHT
CID1
CID2
DNA
GOI
Recombinase
IL-2 Production
CAR On switch
Stable memory, High fidelity, Broadly utilized, Complex logic operations, Orthogonality
No reversibility, inducers, Permanence of off-target effects
Chakravarti et al, Weinberg et al, Tian et al
Chemical inducible CRISPR Cas9: Giberellin, Rapamycin, Abscisic acid, 4-HT, Doxycycline
Nucleus
CID1
CID2
Cas9
DNA
Kill Switch
Live
Dead
gRNA Production
Easy tunability, Broadly utilized, Orthogonality
No reversibility, Permanence of off-target effects
Huynh et al, Soumyashree et al, Sun et al, Liu et al
B
Sensors
Transcriptional Regulators
Output
Adv
Dis
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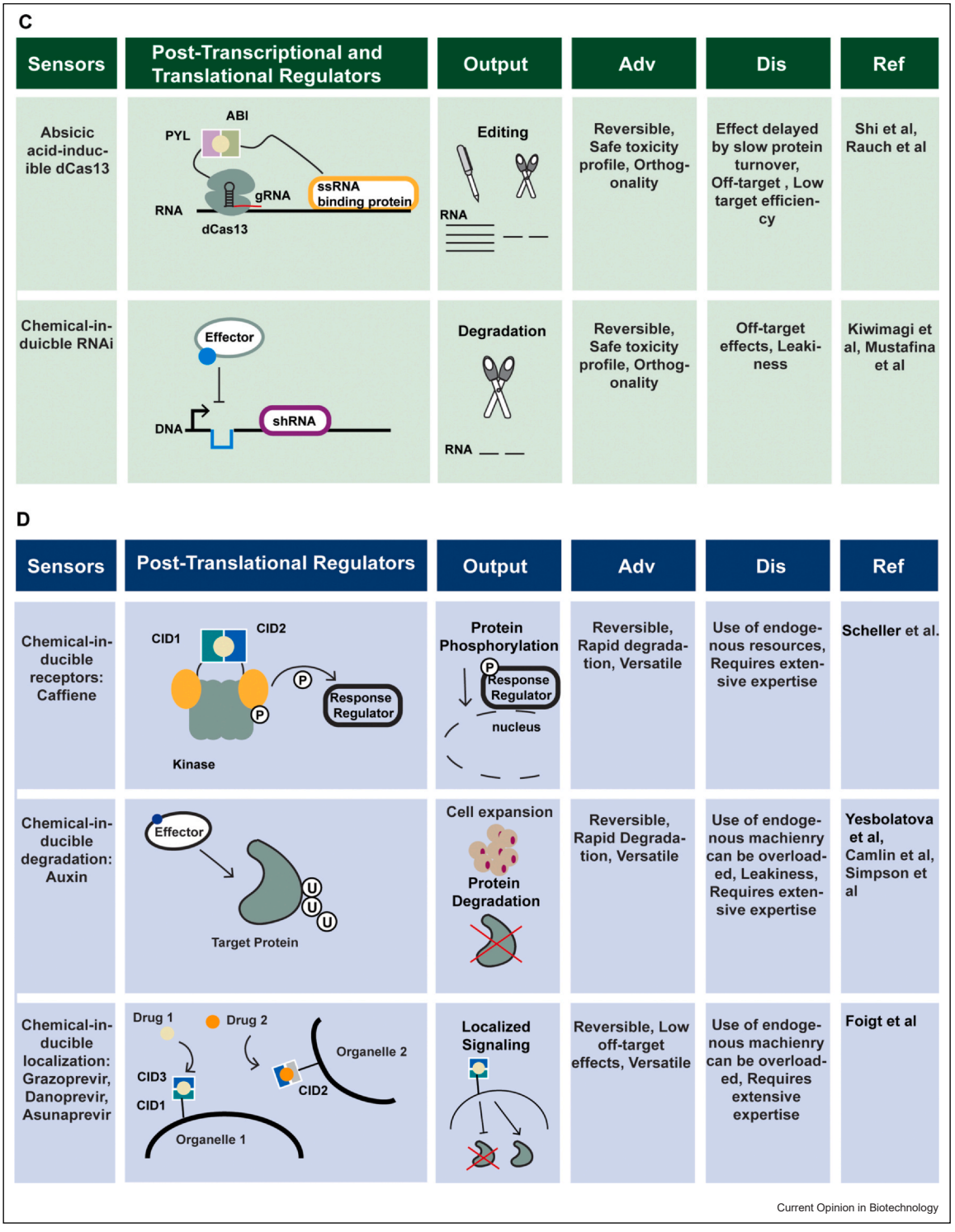
Chemical-inducible TetOn/TetOff: Doxycycline, Tetracycline
CID1
CID2
Effector
Tet
DNA
GOI
PTet
Peptide Production
Cell
Differentiation
Reversible, Throughly characterized system
Leakiness, Immunogenicity of protein components, Limited in type of logic gates
Bojar et al, Nguyen et al, Grishchenko et al, Nishimura e al, Hotblack et al
IPTG-inducible circuit
LacI Dimer
IPTG
Distal
Core Promoter
Proximal
Repression
Cell
Reversible, Thoroughly characterized repressor
LacI repressor leakiness, Immunogenicity of protein components, Not orthogonal in bacteria, Limited in type of logic
Yu et al, Mannan et al
Chemical-induicble CRISPR -dCas systems: CEM PCA
CID2
CID1
Effector
DNA
dCas9
GOI
Therapeutics
Reversible, Orthogonality
Immunogenicity, Leakiness
Chiarella et al, Yin et al
Current Opinion in Biotechnology

Classification of chemical-inducible gene circuits. Inducible gene circuits can be categorized into **(a)** nuclease-based systems **(b)** transcriptional regulators **(c)** post-transcriptional and translational regulators and **(d)** post-translational regulators discussed throughout the text.

Figure 1



(*continued*)

repression was initiated via dCas9 fused to the Krüppel-associated box (KRAB) repressor in the presence of protocatechuic acid (PCA), a metabolite found in green tea [10]. Compared with TF systems that rely on inducible promoters of varying strength, CRISPR/Cas systems advance our ability to multiplex gene control with facile tunability and orthogonal guide RNAs for different orthologs.

While split TF systems are indispensable in activating and repressing versatile gene targets with large fold inductions, this class cannot establish permanent output memory, which has limited the possible logic operations [39]. Additionally, as more TFs are discovered and added to our synthetic toolbox, each one requires characterization of its DNA binding affinity and regulatory dynamics in model systems before it can be integrated into gene circuit designs.

Post-transcriptional regulation-based systems

Post-transcriptional regulation encompasses control over mRNA processing, RNA stability, and translational regulation. RNA modifications have been implicated in a variety of human diseases [40], making strategies for specific and inducible control of mRNA modification particularly powerful. RNA interference (RNAi) is the most pervasively used tool to regulate target RNA activity. This strategy uses endogenous machinery to either repress translation or degrade RNA depending on the degree of sequence complementarity (Figure 1c). Some drawbacks of RNAi have been off-target knockdown due to partial complementarity-driven repression and global interference of RNA degradation due to competition of endogenous resources [41]. To mitigate the first drawback, small molecule inducible RNAi has improved tool specificity [42]. To this end, shRNA expression has been driven by inducible promoters [17] and RNA aptamers have been fused to a ribozyme to trigger gene expression [13•].

Inducible RNA-regulating effectors have also been gaining more momentum recently. One example is CRISPR-Cas13, an RNA-guided RNA nuclease (Figure 1c). Cas13 has been fused to RNA effectors to more precisely control RNA editing in a dose-dependent manner [5•]. An abscisic acid (ABA) inducible and reversible m^6A editing platform developed by Shi et al. combines chemically inducible proximity domains with dCas13b [15•]. This system can also be expanded to a light-inducible system by incorporating a photo-caged version of the inducer, ABA. In comparison to RNAi, Cas13 is lauded for its ability to target nuclear and circular RNA and has been claimed to have a higher specificity due to its requirement of a near-perfect match to its target [16,43]. Some downsides to Cas13 include its size and its display of nonspecific, off-target cleavage in bacteria, although this has not been demonstrated in mammalian cells [44].

Despite the challenge of off-target effects and slower time scales compared with post-translational regulation, post-transcriptional regulation benefits from reversible dynamics [18]. This contrasts with nucleases, where the permanence of off-target effects weighs more heavily. Overall, the transience of post-transcriptional regulation is incredibly appealing to therapeutics and has great potential to shed light on RNA regulation mechanisms.

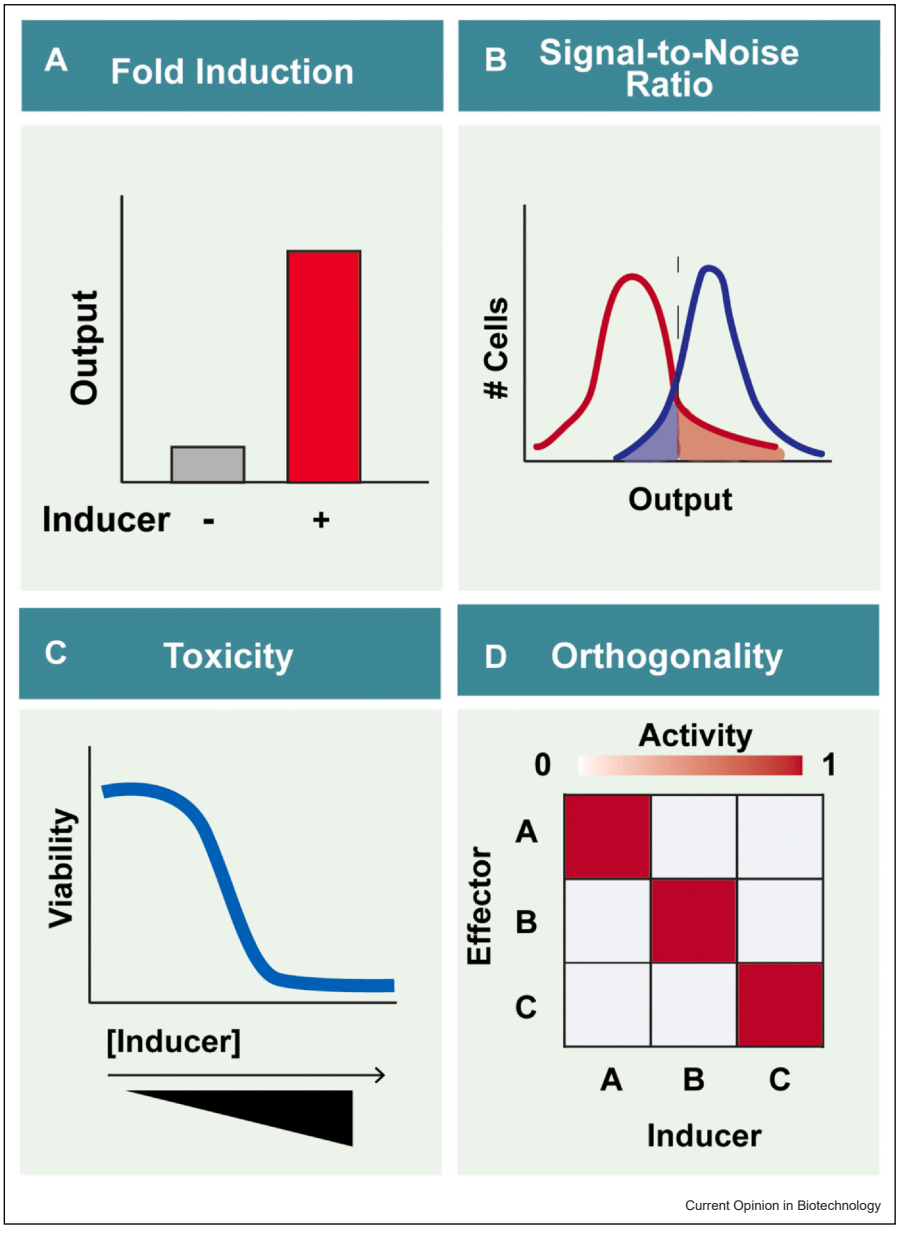
Post-translational regulation of protein activity

The protein products of gene expression can also be the targets of inducible regulation. This can occur through steric blocking of protein activity, localization of the protein product to hinder or facilitate its function [1,20], induced degradation [19,22], and induced dimerization/oligomerization (Figure 1d) [21••,22]. For example, dimerization strategies can be employed to control active protein targets such as kinases, phosphatases, and glycosidases. To investigate the endogenous signaling pathways controlled by these proteins, Scheller's group designed and optimized an inducible kinase for signal transduction in the generation of protein circuits [6•].

In contrast with dimerization strategies, a common inducible degradation mechanism utilizes the ubiquitination pathway via inducible protein binders or chemical-inducible domains. For instance, Simpson et al. developed a reversible protein degradation system that activates only in the presence of their novel HaloPROTAC technology [45••]. The HaloPROTAC tool includes a Halo-tag that simultaneously binds to a protein of interest and to a protein degradation complex in the presence of a ligand (HaloPROTAC-E). Other methods utilize an auxin-inducible degron that functions by recruiting endogenous ubiquitin-mediated protein degradation machinery in response to the presence of the plant hormone auxin. However, these strategies have shown both basal degradation as well as incomplete degradation of the protein target. Recently developed systems have focused on improving this leakiness and lowering the dose of inducer required for functional degradation, resulting in induced rapid depletion of protein targets for fast phenotype changes [19].

Localization of proteins to various cellular compartments is a critical element to their endogenous function. Leveraging this design element, Foight et al. designed PROCISiR: a multi-input, multioutput inducible NS3a reader-based analog switch [1]. This system utilizes NS3a, ahepatitus C virus protease inhibited by danoprevir, grazoprevir, and asunaprevir, as one half of a CID system that dimerizes with a variety of engineered

Figure 2



Metrics to evaluate and optimize within inducible gene circuits. Chemical-inducible systems can be characterized in various ways. **(a)** Fold induction calculates the ratio of an induced output to a negative control. **(b)** Signal-to-noise ratio accounts for overlap between on and off populations. **(c)** Cellular viability can be plotted against concentration of inducer as a measure of toxicity. **(d)** Orthogonality matrices measure cross-talk as each effector should ideally have high activity only with its intended pair.

'readers' to produce distinct analog outputs depending on the inducer used. This multiplexed architecture expands the potential of chemically controlled systems to integrate a variety of input signals to produce not only digital, but graded analog responses. While these examples show the promise of post-translational regulation strategies, the design of these systems can tend to have a high genetic payload due to the additional regulatory elements, and implementation of these strategies towards native protein products requires extensive understanding of protein activity.

Performance metrics to compare inducible genetic systems

Ideal chemical-inducible genetic circuits should have robust fold induction, high signal-to-noise ratio (SNR), low toxicity, and orthogonal effectors for multiplexed control (Figure 2). Most groups report fold induction as a

metric of switch activity, but we propose that each new inducible system should be characterized on all four metrics we have listed. Small molecule-inducible systems that meet these criteria will play an indispensable role in interrogating native biological systems and developing the next generation of safe, effective therapeutics.

Fold induction

Fold induction is the most common metric evaluated for small molecule inducible genetic circuits. This value is measured as the ratio of average activity with inducer added to average basal activity without inducer [9]. The basal expression level of a circuit used to calculate fold induction is also quantified as leakiness, which Yeoh et al. identify as a major impediment to the successful design of inducible promoter-based genetic circuits [46]. Lower leakiness improves fold induction metrics, since induced expression levels are compared with basal expression levels, and is also critical for therapeutic purposes where high basal expression can impact the safety of a switch.

Signal-to-noise ratio

In contrast to fold-induction, SNR relays variance in addition to amplitude of single-cell flow cytometry data. While the signal measures on/off populations, noise estimates the standard deviation between populations as well as the inability of the circuit to perform ideally due to overlap between on and off states [17]. Although SNR historically applies to information technology, this metric can be adapted to inducible genetic circuits to measure and improve differentiation between on/off output states [14]. SNR is critical to characterize in multilayered genetic circuits because obstacles such as cross-talk between genetic parts or limited strong regulatory elements can lead to degradation of signals [47].

Toxicity

A rigorous safety profile of each inducer is necessary to facilitate the clinical translation of small molecule-inducible systems. Commonly used FDA-approved small molecules such as tamoxifen, grazoprevir, and doxycycline have extensive data supporting a lack of toxicity in mammalian and bacterial cells at appropriate doses. Small molecules that are not FDA-approved will face more challenges translating into therapeutics, and circuits that are designed to respond to these molecules should consider including toxicity assays in their system evaluation.

Orthogonality

Orthogonal chemical-inducible effectors allow control of multiple variables in a genetic circuit with minimal cross-talk. Our lab has measured wild-type recombinase activity against the recognition sites of other recombinases to evaluate SSR orthogonality [26]. This characterization establishes that multiple recombinases can be implemented into the same circuit to modulate multiple reporters in an inducible manner. Orthogonality of chemically inducible dCas9 is possible with the design of guide RNAs towards different target genes to ultimately enable multiplex control [48]. Evaluating the orthogonality of genetic circuit components will greatly improve the complexity and versatility of synthetic circuits.

Conclusion and future outlook

We anticipate that the future of chemical-inducible circuit design will focus on multiplexing orthogonal systems for control over a greater number of components. This will enable more complex temporal and sequential gene switching and interrogation of native regulation pathway dynamics. Additionally, we emphasize the need to more thoroughly characterize these systems as they are developed with the metrics we have outlined to allow a direct comparison of genetic circuit designs. Finally, we have highlighted some recent advances in the development of small molecule inducible systems, but a vast collection of these tools exist beyond those mentioned here. To facilitate further creation and exploration, an international repository should be created that organizes, validates, and standardizes these systems as they are developed and provides a collection of tools for future research. This will overcome the challenge of tool standardization that we have outlined and enable future iterations that continue to improve our genetic regulatory toolkit.

Conflict of interest statement

W.W.W. is a cofounder and shareholder of Senti Biosciences and 4Immune.

Data Availability

No data were used for the research described in the article.

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References and recommended reading

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

- • of special interest
- •• of outstanding interest.

1. Foight GW, *et al.*: **Multi-input chemical control of protein dimerization for programming graded cellular responses**. *Nat*

Biotechnol 2019, **37**:1209-1216, https://doi.org/10.1038/S41587-019-0242-8 (Oct.).

2. Matsumoto D, Tamamura H, Nomura W: **TALEN-based chemically inducible, dimerization-dependent, sequence-specific nucleases**. *Biochemistry* 2020, **59**:197-204, https://doi.org/10.1021/ACS.BIOCHEM.9B00798/ASSET/IMAGES/LARGE/BI9B00798_0004.JPEG (Jan.).

3. Huynh N, Wang S, King-Jones K: **Spatial and temporal control of gene manipulation in Drosophila via drug-activated Cas9 nucleases**. *Insect Biochem Mol Biol* 2020, **120**:103336, , https://doi.org/10.1016/J.IBMB.2020.103336 (May).

4. Tian X, *et al*.: **Generation of a self-cleaved inducible Cre recombinase for efficient temporal genetic manipulation**. *EMBO J* 2020, **39**:e102675, , https://doi.org/10.15252/EMBJ.2019102675 (Feb.).

5. • Rauch S, Jones KA, Dickinson BC: **Small molecule-inducible RNA-targeting systems for temporal control of RNA regulation**. *ACS Cent Sci* 2020, **6**:1987-1996, https://doi.org/10.1021/ACSCENTSCI.0C00537 (Nov.).
This work describes an ABA-inducible RNA base editor biosensor platform that can perform RNA editing, degradation, or induced translation. Furthermore, they demonstrate this system *in vivo*, expanding the toolbox for studying dynamic RNA regulation.

6. • Scheller L, Schmollack M, Bertschi A, Mansouri M, Saxena P, Fussenegger M: **Phosphoregulated orthogonal signal transduction in mammalian cells**. *Nat Commun* 2020, **11**:1-10, https://doi.org/10.1038/s41467-020-16895-1 (Jun.).
Building upon the design of the native bacterial two-component system, the authors design a chemically induced phototransfer system for orthogonal signal transduction in mammalian cells. This system, named POST, serves as a proof of concept for using bacterially derived components as modular building blocks in mammalian protein circuits.

7. Bojar D, Scheller L, el Hamri GC, Xie M, Fussenegger M: **Caffeine-inducible gene switches controlling experimental diabetes**. *Nat Commun* 2018, **9**:1-10, https://doi.org/10.1038/s41467-018-04744-1 (Jun.).

8. Sun N, *et al*.: **Development of drug-inducible CRISPR-Cas9 systems for large-scale functional screening**. *BMC Genom* 2019, **20**:1-15, https://doi.org/10.1186/s12864-019-5601-9

9. Kang K, *et al*.: **An improved Tet-on system in microRNA overexpression and CRISPR/Cas9-mediated gene editing**. *J Anim Sci Biotechnol* 2019, **10**:1-12, https://doi.org/10.1186/s40104-019-0354-5

10. Yin J, *et al*.: **A green tea-triggered genetic control system for treating diabetes in mice and monkeys**. *Sci Transl Med* 2019, **11**:1-15, https://doi.org/10.1126/scitranslmed.aav8826

11. • Damo M, *et al*.: **Inducible de novo expression of neoantigens in tumor cells and mice**. *Nat Biotechnol* 2020, **39**:64-73, https://doi.org/10.1038/s41587-020-0613-1 (Jul.).
The NINJA mouse introduces tissue-specific inducible expression of genetically encoded neoantigens that overcomes the common issue of thymic antigen tolerance in inducible mouse models. This systems utilizes multiple layers of gene regulation to enable the study of endogenous antigen-specific T cell activity.

12. 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, https://doi.org/10.1038/s41587-019-0296-7

13. • Mustafina K, Fukunaga K, Yokobayashi Y: **Design of mammalian ON-riboswitches based on tandemly fused aptamer and ribozyme**. *ACS Synth Biol* 2020, **9**:19-25, https://doi.org/10.1021/acssynbio.9b00371.
Few synthetic riboswitches have been developed to include chemically induced ON-switch behavior. This system describes a guanine-inducible riboswitch composed of a tandem-fused aptamer and ribozyme, expanding the current toolkit for inducible RNA regulation.

14. Greco FV, Pandi A, Gorochowski TE, Erb TJ, Grierson CS: **Harnessing the central dogma for stringent multi-level control of gene expression**. *Nat Commun* 2021, **12**:1-11.

15. • Shi H, Xu Y, Tian N, Yang M, Liang F-S, Xu Y ✉: **Inducible and reversible RNA N6-methyladenosine editing**. *Nat Commun* 2022, **13**:1-10, https://doi.org/10.1038/s41467-022-29665-y (Apr.).
This system combines ABA-inducible CIP domains to dCas13b to create a chemically induced, reversible RNA m6A editing platform using either the m6A writer METTL3 or eraser ALKBH5. The temporal dynamics of both editing induction and reversal are evaluated as well as the performance compared with noninducible editing strategies. Furthermore, the system can incorporate photo-caged ABA inducers to improve the spatial control by adding UV light induction.

16. Xu D, *et al*.: **A CRISPR/Cas13-based approach demonstrates biological relevance of vlinc class of long non-coding RNAs in anticancer drug response**. *Sci Rep* 2020, **10**:1-13, https://doi.org/10.1038/s41598-020-58104-5

17. Kiwimagi KA, *et al*.: **Quantitative characterization of recombinase-based digitizer circuits enables predictable amplification of biological signals**. *Commun Biol* 2021, **4**:1-12, https://doi.org/10.1038/s42003-021-02325-5 (Jul.).

18. Camlin NJ, Evans JP: **Auxin-inducible protein degradation as a novel approach for protein depletion and reverse genetic discoveries in mammalian oocytes**. *Biol Reprod* 2019, **101**:704-718, https://doi.org/10.1093/biolre/ioz113

19. Yesbolatova A, *et al*.: **The auxin-inducible degron 2 technology provides sharp degradation control in yeast, mammalian cells, and mice**. *Nat Commun* 2020, **11**:1-13, https://doi.org/10.1038/s41467-020-19532-z (Nov.).

20. Mahameed M, *et al*.: **Engineering a rapid insulin release system controlled by oral drug administration**. *Adv Sci* 2022, **9**:2105619, , https://doi.org/10.1002/ADVS.202105619 (Mar.).

21. •• Wu HD, *et al*.: **Rational design and implementation of a chemically inducible heterotrimerization system**. *Nat Methods* 2020, **17**:928-936, https://doi.org/10.1038/s41592-020-0913-x (Aug.).
First demonstration of chemically inducible trimerization by splitting rapamycin-binding FRB and FKBP domains. This system trimerizes quickly (seconds to minutes) and was shown to induce tri-organellar junctions in Cos-7 cells. This introduces a new tool for probing cell signaling with spatiotemporal control and interrogating the significance of trimerization in nature.

22. Wu M, *et al*.: **Conditional gene knockout and reconstitution in human iPSCs with an inducible Cas9 system**. *Stem Cell Res* 2018, **29**:6-14, https://doi.org/10.1016/J.SCR.2018.03.003 (May).

23. Gaj T, Gersbach CA, Barbas CF: **ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering**. *Trends Biotechnol* 2013, **31**:397-405, https://doi.org/10.1016/j.tibtech.2013.04.004 (Jul.).

24. Tian X, Zhou B: **Strategies for site-specific recombination with high efficiency and precise spatiotemporal resolution**. *J Biol Chem* 2021, **296**:100509, , https://doi.org/10.1016/J.JBC.2021.100509 (Jan.).

25. Chakravarti D, Caraballo LD, Weinberg BH, Wong WW: **Inducible gene switches with memory in human T cells for cellular immunotherapy**. *ACS Synth Biol* 2019, **8**:1744-1754, https://doi.org/10.1021/ACSSYNBIO.8B00512/SUPPL_FILE/SB8B00512_SI_001.PDF (Aug.).

26. Weinberg BH, *et al*.: **High-performance chemical- and light-inducible recombinases in mammalian cells and mice**. *Nat Commun* 2019, **10**:1-10, https://doi.org/10.1038/s41467-019-12800-7 (Oct.).

27. Gangopadhyay SA, *et al*.: **Precision control of CRISPR-Cas9 using small molecules and light**. *Biochemistry* 2019, **58**:234-244, https://doi.org/10.1021/ACS.BIOCHEM.8B01202/ASSET/IMAGES/LARGE/BI-2018-01202Z_0002.JPEG (Jan.).

28. Kundert K, *et al*.: **Controlling CRISPR-Cas9 with ligand-activated and ligand-deactivated sgRNAs**. *Nat Commun* 2019, **10**:1-11, https://doi.org/10.1038/s41467-019-09985-2 (May).

29. Wang SR, *et al*.: **Conditional control of RNA-guided nucleic acid cleavage and gene editing**. *Nat Commun* 2020, **11**:91, , https://doi.org/10.1038/S41467-019-13765-3 (Dec.).

30. Doshi A, Sadeghi F, Varadarajan N, Cirino PC: **Small-molecule inducible transcriptional control in mammalian cells**. *Crit Rev Biotechnol* 2020, **40**:1131-1150, https://doi.org/10.1080/07388551.2020.1808583 (Nov.).

31. Kim NM, Sinnott RW, Sandoval NR: **Transcription factor-based biosensors and inducible systems in non-model bacteria: current progress and future directions**. *Curr Opin Biotechnol* 2020, **64**:39-46, https://doi.org/10.1016/j.copbio.2019.09.009 (Aug.).

32. Ding N, Zhou S, Deng Y: **Transcription-factor-based biosensor engineering for applications in synthetic biology**. *ACS Synth Biol* 2021, **10**:911-922, https://doi.org/10.1021/acssynbio.0c00252 (May).

33. Israni D v, *et al*.: **Clinically-driven design of synthetic gene regulatory programs in human cells**. *bioRxiv* 2021, (2021.02.22.432371), ⟨https://www.biorxiv.org/content/10.1101/2021.02.22.432371v2%0Ahttps://www.biorxiv.org/content/10.1101/2021.02.22.432371v2.abstract⟩.

34. Hanko EKR, Paiva AC, Jonczyk M, Abbott M, Minton NP, Malys N: **A genome-wide approach for identification and characterisation of metabolite-inducible systems**. *Nat Commun* 2020, **11**:1213, , https://doi.org/10.1038/s41467-020-14941-6 (Dec.).

35. Nguyen DH, *et al*.: **Optimized doxycycline-inducible gene expression system for genetic programming of tumor-targeting bacteria**. *Mol Imaging Biol* 2022, **24**:82-92, https://doi.org/10.1007/s11307-021-01624-x

36. Nishimura K, Nitta T, Doi K, Takata K: **Rapid conversion of human induced pluripotent stem cells into dopaminergic neurons by inducible expression of two transcription factors**. *Stem Cells Dev* 2022, **31**:1-9, https://doi.org/10.1089/scd.2021.0363

37. • Yu TC, *et al*.: **Multiplexed characterization of rationally designed promoter architectures deconstructs combinatorial logic for IPTG-inducible systems**. *Nat Commun* 2021, **12**:1-14, https://doi.org/10.1038/s41467-020-20094-3 (Jan.).
Profiling of over 8000 IPTG inducible promoters to fit a statistical model that accurately identifies design elements that minimize leakiness and maximize fold change in the IPTG repressor system. This combination of rational design and large-scale multiplexed assays describes a method for exploring promoter variants and mechanisms that could potentially be applied towards other inducible systems.

38. Doshi A, Sadeghi F, Varadarajan N, Cirino PC: **Small-molecule inducible transcriptional control in mammalian cells**. *Crit Rev Biotechnol* 2020, **40**:1131-1150, https://doi.org/10.1080/07388551.2020.1808583

39. Stanton BC, Nielsen AAK, Tamsir A, Clancy K, Peterson T, Voigt CA: **Genomic mining of prokaryotic repressors for orthogonal logic gates**. *Nat Chem Biol* 2014, **10**:99-105, https://doi.org/10.1038/nchembio.1411

40. Shi H, Chai P, Jia R, Fan X: **Novel insight into the regulatory roles of diverse RNA modifications: re-defining the bridge between transcription and translation**. *Mol Cancer* 2020, **19**:78, , https://doi.org/10.1186/s12943-020-01194-6 (Dec.).

41. Zhang S, Cheng Z, Wang Y, Han T: **The risks of miRNA therapeutics: in a drug target perspective**. *Drug Des, Dev Ther* 2021, **15**:721-733, https://doi.org/10.2147/DDDT.S288859

42. Liao Y, Tang L: **Inducible RNAi system and its application in novel therapeutics**. *Crit Rev Biotechnol* 2016, **36**:630-638, https://doi.org/10.3109/07388551.2014.1003030 (Jul.).

43. Abudayyeh OO, *et al*.: **RNA targeting with CRISPR-Cas13**. *Nature* 2017, **550**:280-284, https://doi.org/10.1038/nature24049

44. Xu C, *et al*.: **Programmable RNA editing with compact CRISPR–Cas13 systems from uncultivated microbes**. *Nat Methods* 2021, **18**:499-506, https://doi.org/10.1038/s41592-021-01124-4

45. •• Simpson LM, *et al*.: **Inducible degradation of target proteins through a tractable affinity-directed protein missile system**. *Cell Chem Biol* 2020, **27**:1164-1180.e5, https://doi.org/10.1016/j.chembiol.2020.06.013 (Sep.).
This ligand-inducible affinity-directed protein missile (L-AdPROM) system combines the Halo-tag/VHL-recruiting proteolysis-targeting chimera (HaloPROTAC) technology with an antiGFP nanobody conjugated to the Halo-tag to achieve degradation of GFP-tagged proteins of interest. They demonstrate that this targeted degradation leads to loss of function of the tagged protein, expanding the currently available targeted protein technologies.

46. J. W. Yeoh, et al., **An automated biomodel selection system (BMSS) for gene circuit designs,** *ACS Synthetic Biology,* 8(7), 2019, 1484-1497, doi: 10.1021/acssynbio.8b00523.

47. Beal J: **Signal-to-noise ratio measures efficacy of biological computing devices and circuits**. *Front Bioeng Biotechnol* 2015, **3**:1-13, https://doi.org/10.3389/fbioe.2015.00093

48. Zhang J, Chen L, Zhang J, Wang Y: **Drug inducible CRISPR/Cas systems**. *Comput Struct Biotechnol J* 2019, **17**:1171-1177, https://doi.org/10.1016/J.CSBJ.2019.07.015 (Jan.).