

Genetic and epigenetic editing in nervous system

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Numerous neuronal functions depend on the precise spatiotemporal regulation of gene expression, and the cellular machinery that contributes to this regulation is frequently disrupted in neurodevelopmental, neuropsychiatric, and neurological disease states. Recent advances in gene editing technology have enabled increasingly rapid understanding of gene sequence variation and gene regulatory function in the central nervous system. Moreover, these tools have provided new insights into the locus-specific functions of epigenetic modifications and enabled epigenetic editing at specific gene loci in disease contexts. Continued development of clustered regularly interspaced short palindromic repeats (CRISPR)-based tools has provided not only cell-specific modulation, but also rapid induction profiles that permit sophisticated interrogation of the temporal dynamics that contribute to brain health and disease. This review summarizes recent advances in genetic editing, transcriptional modulation, and epigenetic reorganization, with a focus on applications to neuronal systems and potential uses in brain disorders characterized by genetic sequence variation or transcriptional dysregulation.

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Introduction

Gene regulatory mechanisms play an essential role in the nervous system. In the developing brain, intricate and temporally coordinated gene expression programs produce the immense cellular diversity of the nervous system, creating hundreds of distinct cell types. This coordinated regulation is required for the structural, physiological, and functional properties of all neuronal circuits, and dysregulation of these processes is a common feature of neurodevelopmental disorders and intellectual disability. In the adult brain, the same mechanisms remain dynamically responsive to environmental stimuli, and are critical for neuronal plasticity, memory formation and maintenance, and experience-dependent behavioral changes. Moreover, altered epigenetic patterns and transcriptional dysregulation are hallmarks of numerous neuropsychiatric and neurolog-

ical disorders, suggesting that treatments targeting aberrant gene expression are relevant to mental health and neurodegenerative conditions.

One critical aspect of gene regulation and chromatin reorganization in the nervous system is the temporally dynamic nature of this process. For example, rapid experience-dependent changes in gene expression shape network formation in the developing brain,¹ and even “stable” epigenetic modifications like DNA methylation can be rapidly reprogrammed at thousands of sites across the genome within hours of a significant behavioral experience. These changes occur at specific genes and with a high degree of cellular precision.²⁻⁴ However, until recently, approaches used to alter transcriptional and epigenetic states in the nervous system suffered from lack of temporal precision, lack of genetic precision, or both (*Figure 1*). For example, even

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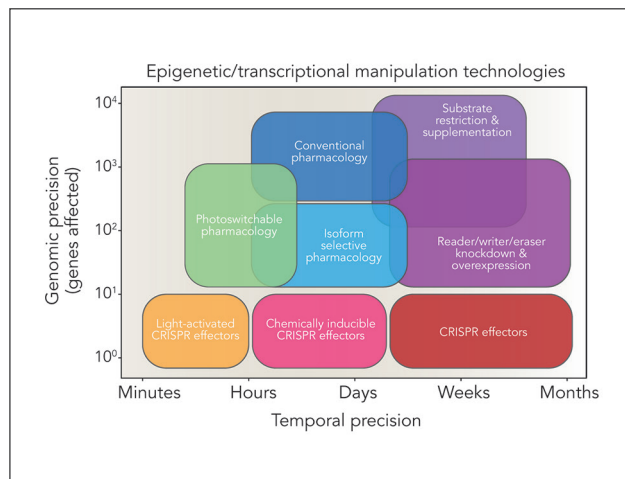


Figure 1. Relationship between temporal precision and genetic precision of available technologies for chromatin and transcriptional regulation. CRISPR-dCas9 tools offer unprecedented genomic precision, and recent adaptations in this technology have also enabled improved temporal control of gene expression and epigenetic editing.

substrate-specific pharmacological approaches alter experience-dependent gene expression and epigenetic profiles in complex ways at hundreds or even thousands of genes,⁵ potentially due to the numerous off-target or compensatory effects that arise with such approaches. Likewise, knockdown or overexpression of chromatin regulators likely affects thousands of loci in the genome, and the temporal resolution of these manipulations is poor. These limitations, together with the central importance of epigenetic regulation in the nervous system highlighted throughout this issue of *Dialogues in Clinical Neuroscience*, highlights the need for site-specific gene modulation and epigenetic editing approaches. This review will summarize recent breakthrough advances in genetic and epigenetic editing approaches, with a specific focus on clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein-9 nuclease (Cas9) systems that have enabled both the genetic and temporal precision required for novel breakthrough discoveries in this field.

CRISPR/Cas9 systems for gene editing and gene regulation

The first approaches to enable customizable DNA sequence editing or localized epigenetic editing were based on zinc-finger nucleases (ZFNs) and transcriptional activator-like

effector nucleases (TALENs).⁶ While ZFNs contain individual zinc finger domains that recognize specific DNA trinucleotide sequences, TALENs contain DNA binding domains that are selective for individual DNA bases. In both cases, these DNA binding domains can be customized into an array to recognize specific (and genetically unique) DNA sequences of interest. Fusion of restriction enzymes or other effector proteins to these sequence-specific arrays has enabled customizable DNA sequence editing, gene activation, gene repression, epigenetic editing, and even splice variant regulation.⁶ However, these systems also harbor several weaknesses, including a laborious synthesis process. To date, these challenges have limited integration of these tools into the neuroscience community.

Cas9-based gene editing

CRISPR/Cas gene editing systems were initially discovered as an RNA-guided endonuclease mechanism used by many species of bacteria and archaea against foreign DNA material from invading viruses and plasmids.^{7,8} Type II CRISPR/Cas innate immunity systems encode sequences of invading DNA as arrays of CRISPR RNAs (crRNAs) in the host genome.⁹ Together with a trans-activating RNA (tracrRNA) and Cas9 nuclease also expressed from the host genome, these crRNAs form a stable complex with Cas9 that seeks out the complementary target sequence in foreign DNA and initiates cleavage of both strands of DNA. One requirement for this double-strand break (DSB) is a protospacer adjacent motif (PAM), a 2-5 nucleotide sequence in DNA that must appear adjacent to the crRNA targeting locus. This motif helps to prevent unintended cleavage of the host DNA, but also imposes some limits in terms of genomic targeting in other species.¹⁰ However, in the most commonly used type II system, from *Streptococcus pyogenes*, the required motif of N-G-G is a relatively common sequence.

Since their discovery in bacteria, CRISPR/Cas9 systems have rapidly been adapted to perform similar roles in eukaryotes.^{8,9,11} In the engineered systems currently used for DNA targeting in mammalian genomes, the tracrRNA and crRNA sequences have been combined into a single guide RNA (sgRNA), which performs both DNA targeting and Cas9 complex formation functions. This arrangement has simplified both the design and generation of CRISPR targeting strategies, as it requires only a 20-nt RNA sequence for targeting instead of long amino acid chains used by ZFN and TALEN approaches. This ease in

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engineering of CRISPR sgRNAs to target selected DNA sequences of interest has become routine and commonplace across nearly all subfields of biology, and CRISPR/Cas9 systems have now outpaced TALEN and ZFN approaches for gene editing applications.

The Cas9 nuclease contains two distinct nuclease domains – the HNH nuclease targets the complementary (sgRNA-targeting) DNA strand, whereas the RuvC nuclease cleaves the non-template strand (*Figure 2a*). The resulting DSB can be repaired by one of two cellular mechanisms – the nonho-

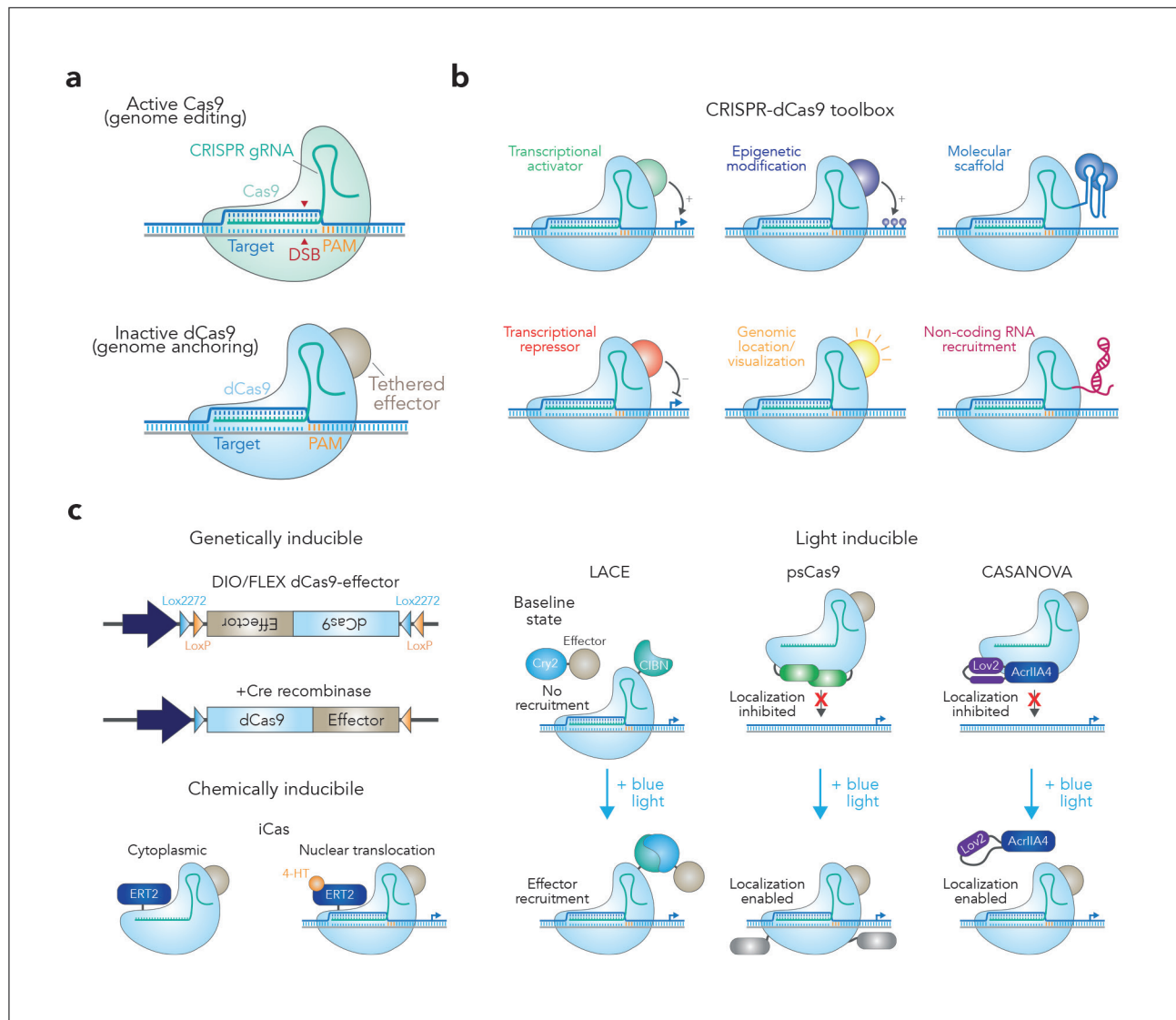


Figure 2. Emerging toolbox for genetic and temporal precision of CRISPR-Cas9 based approaches for studying gene regulation. **a**, Top, use of CRISPR-Cas9 protein to induce double-strand breaks (DSB) at specific sites based on complementarity of the sgRNA target and location of a protospacer-adjacent motif (PAM). Bottom, catalytically inactive dCas9 protein creates a modular genomic anchor for fused effector proteins. **b**, dCas9-effector toolbox enables robust bidirectional transcriptional regulation, epigenetic editing of chromatin or DNA, fusion of modular scaffolds or fluorescent proteins, and recruitment of long non-coding RNAs. **c**, Strategies for genetic, chemical, and light-activated control of dCas9-effector expression and/or localization. See text for details and abbreviations.

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mologous end joining (NHEJ) pathway or the homology directed repair (HDR) pathway.⁸ The NHEJ pathway results in relatively random mutations, including base substitutions, small insertions, and deletions. This pathway is most active in nondividing cells and is frequently used for gene disruption experiments in neuronal systems by creating large deletions, frameshift mutations, or early stop codons. In contrast, the HDR pathway uses a donor DNA fragment as a template for DNA repair, resulting in a precise insertion/correction event. However, this pathway is largely only active in dividing cells, and thus is not commonly employed in terminally differentiated neurons.

dCas9-based transcriptional modulation

In addition to its common use in DNA cleavage, the Cas9 nuclease can be inactivated via two simple amino acid substitutions (D10A in the RuvC nuclease and H840A in the HNH nuclease of the *Streptococcus pyogenes* Cas9). These mutations render the Cas9 catalytically inactive, and thus this version is called “dead” Cas9, or dCas9. However, this does not alter formation of complexes between the sgRNA and dCas9, and does not alter genome-targeting efficiency. Therefore, whereas Cas9 can be used for genome editing, dCas9 can be used as a global genomic anchoring system for stable and site-specific DNA targeting¹²⁻¹⁴ (Figure 2a). This anchor can be used as a modular scaffold for tethered effector proteins with many different functions. Most commonly, these effectors are fused directly to the dCas9, enabling direct genomic targeting as a modular scaffold that can accept nearly any fusion protein (Figure 2b). However, other systems utilize RNA extensions from the sgRNA that contain RNA binding motifs (such as MS2 loops) to direct effectors fused to MS2 proteins directly to the locus of interest.¹⁵

An initial realization after generation of dCas9 variants was that even without a fused protein, targeting of dCas9 to the non-template strand of DNA just downstream of transcription start sites was sufficient to disrupt gene transcription and/or elongation, causing robust decreases in expression of target genes.^{16,17} Subsequently, transcriptional inactivation domains (such as the KRAB domain closely associated with histone 3 lysine 9 trimethylation) were tethered directly to the dCas9, and these dCas9-KRAB fusion variants yielded even more robust decreases in target gene expression than dCas9 alone.^{18,19} This CRISPR “interference” (CRISPRi) strategy has now been employed with large sgRNA libraries to identify key regulatory elements and screen for individual

genes involved in critical cellular processes.^{18,20} More relevant to this review, this approach has also successfully been adapted for neuronal systems,^{21,22} revealing nearly complete gene silencing and extremely high on-target specificity that is sensitive to even a single nucleotide substitution in the sgRNA sequence. Moreover, CRISPRi is more effective at gene knockdown than previous gold-standard technology (RNAi), enabling complex dissociation of single gene contributions to neurotransmission as well as multiplexed gene regulation in the adult brain.²²

While previous strategies for gene repression relied on expensive knockout technology or RNAi approaches that are subject to off-target effects, available tools for gene overexpression have been even more limited. Typical approaches rely on driving exogenous expression of cDNA from a viral vector, which makes titration of gene expression profiles or exploration of specific transcript variants difficult. In addition, these approaches are limited by viral vector capacity, and thus longer neuronal genes have not been amenable to this overexpression approach. Fortunately, CRISPR/dCas9 strategies can also be employed for this goal via fusion of transcriptional activators such as the viral protein VP64 to achieve gene induction from the endogenous gene locus.^{19,23,24} Like CRISPRi, this CRISPR “activation” (CRISPRa) approach is highly selective at induction of the target gene, and has increasingly been used for cellular reprogramming, genome-scale CRISPR screens, and dissection of functional elements in the genome²⁵⁻²⁸ (Carullo et al, unpublished). While initial gene regulation levels with CRISPRa fusion approaches were somewhat modest, recent development of multi-part activators (such as VP64-p65-Rta [VPR], a fusion of three distinct transcriptional activation domains) has dramatically increased the efficiency of this strategy.²⁸ CRISPRa has now been adapted for effective use in primary neuronal cultures and the adult brain,^{21,29} demonstrating the ability to target many different genes regardless of length or cellular function, as well as upregulation of specific transcript variants from complex genes such as brain-derived neurotrophic factor (*Bdnf*). Likewise, targeted transcriptional activation of the circadian and activity-responsive *Per1* gene with dCas9-VP64 rescued age-related loss of this gene in the hippocampus and restored normal long-term memory.³⁰ Similarly, CRISPRa targeting to an enhancer near an obesity-linked gene in a haploinsufficiency mouse model was shown to rescue deficient *Sim1* gene expression and reverse obesity phenotypes.³¹

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CRISPR epigenetic editing

As highlighted in the previous section, epigenetic modifications are highly dynamic in the central nervous system, where they respond to specific activity states and experiences, are altered during aging, and are dysregulated in diseases that affect brain function. Moreover, instead of affecting epigenetic landscapes globally, these processes produce gene-specific changes in epigenetic states. Thus, another goal of developing dCas9-based tools has been centered upon establishing site-specific epigenetic modifications via fusion of chromatin modifiers to dCas9. Typically, these efforts have been focused on either writers or erasers of specific epigenetic modifications, which are the most likely to produce sustained results at the associated gene locus.

Among the first epigenetic CRISPR effectors to be engineered was a dCas9 fusion to the histone acetyltransferase p300, which catalyzes acetylation of H3K27. This mark is commonly found at active gene promoters, but is also enriched at enhancer elements in the genome and has been used as a readout of enhancer activity state.^{32,33} Notably, Gersbach and colleagues found that dCas9-p300 fusions were capable of upregulating H3K27ac at enhancers and promoters, and increasing expression from linked genes with genome-wide specificity.³⁴ Interestingly, enhancer-based gene regulation effects could not be reproduced with previous generations of CRISPRa tools (dCas9-VP64 fusions) and was dependent on the catalytic activity of the p300 histone acetyltransferase domain, suggesting that chromatin remodeling may be a critical event in activation of distal regulatory loci. More recently, this approach was used at activity-regulated enhancers near the *Fos* gene locus in neurons, revealing for the first time that experience-dependent gene regulation in the nervous system is finely tuned by H3K27ac dynamics at genomic enhancers.³⁵

Whereas dCas9-p300 fusions were found to broadly increase gene expression at both promoters and enhancers, site-specific removal of histone acetylation using dCas9-HDAC fusions has led to more mixed results. The initial report demonstrating a dCas9-HDAC3 fusion revealed that effects

CRISPR-based tools provide a versatile, multiplexable, and inexpensive technology for the systematic interrogation of genetic and epigenetic contributions to neuronal function

of local histone deacetylation were highly dependent on the specific target sequence, with some sgRNAs producing robust effects and others failing to alter gene expression at all.³⁶ Likewise, targeting dCas9-HDAC8 to enhancers at the *Fos* gene locus in neurons revealed modest decreases in baseline H3K27ac, but this only altered expression of *Fos* mRNA under stimulation-dependent conditions (not at baseline³⁵). Active enhancers are also marked by H3K4 methylation, and other reports have obtained similar results via targeted recruitment of dCas9-LSD1 (a lysine demethylase) to enhancer loci in the genome, but observed no effects when dCas9 alone or dCas9-KRAB fusions were targeted to the same sites.³⁷ Together, these results suggest that the effect of epigenetic editing is largely dependent on local chromatin landscape and cellular state, and indicates that targeting endogenous modifications at specific regulatory sites may be required for desired readouts.

Perhaps most of the effort in development of dCas9-based epigenetic effectors has focused on cytosine methylation, which is catalyzed by DNA methyltransferases (DNMTs) and removed as part of an oxidation pathway by ten-eleven translocation enzymes (TETs). Fusion of the active methyltransferase domain from DNMT proteins to dCas9 has been reported to result in robust increases in methylation within a defined ~100bp window surrounding the location of the sgRNA. Critically, these changes were not observed with a catalytically inactive dCas-DNMT mutant, and increased cytosine methylation was also associated with decreased expression of target genes.³⁸ Conversely, directing dCas9-TET fusions to specific gene promoters results in decreased methylation and increased expression of target genes in postmitotic neurons, demonstrating bidirectional regulation with this approach.³⁹ While dCas9-DNMT fusions have subsequently been shown to have potential off-target effects in methylation depleted cells,⁴⁰ these tools have already been employed for early stage proof-of-principle experiments in disease models.⁴¹⁻⁴³

A powerful example of the potential for CRISPR-based epigenetic editing is Fragile X Syndrome (FXS), an

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X-linked neurodevelopmental disorder characterized by intellectual disability. FXS is caused by methylation-mediated silencing of the FMR1 gene, which occurs due to a CGG trinucleotide repeat expansion in the FMR1 promoter. FXS has no cure and there are currently no treatments. Remarkably, Rudolf Jaenisch and colleagues demonstrated that recruitment of dCas9-TET fusions to CGG repeats caused robust loss of methylation at the FMR1 promoter in human cells, which was associated with almost complete restoration of FMR1 protein expression and normalization of FXS related physiological and cellular deficits.⁴³ Notably, rescued expression of FMR1 persisted even after removal of active dCas9 binding to the FMR1 locus, suggestive of enduring maintenance of altered DNA methylation status. Finally, these authors also used RNA sequencing and whole genome bisulfite sequencing (to measure DNA methylation changes), demonstrating that transcriptional and epigenetic effects were highly specific to the targeted FMR1 locus. Overall, these results provide a powerful example of the potential for this type of technology in disease states linked to epigenetic dysregulation, setting the stage for investigations into more complex polygenic disorders. Additionally, this general strategy can be used for recruitment of additional effectors, such as long noncoding RNAs that are increasingly being viewed as key regulators of chromatin states and neuronal function⁴⁴⁻⁴⁶ (Carullo et al, unpublished).

Inducible CRISPR/Cas9 gene regulation systems

Constitutively expressed CRISPR/Cas9 gene editing and gene regulation systems have already proven to be useful for understanding the function of specific genes, disease-linked mutations, and regulatory elements. However, these tools come with a central limitation, in that their constitutive expression means that temporally specific gene expression patterns cannot be recapitulated or blocked. This element is crucial in the nervous system, given the overwhelming evidence for rapid and coordinated changes in gene expression or chromatin regulatory dynamics. Additionally, these tools are often driven via robust promoters that are active in all cell types, making it difficult to ascertain the cell-specific functions of gene expression programs. To overcome these challenges, the core CRISPR/Cas9 tools highlighted above have undergone considerable reengineering in the past few years, resulting in unprecedented temporal and cellular precision of genetic, transcriptional, or epigenetic editing.

Cellular specificity

Initial Cas9 and dCas9 tools were driven by robust artificial or nonselective promoters, enabling strong expression in diverse cell lines and mammalian cell types. However, continued refinement of this approach has brought about improved cell type resolution, for example by using cell-type specific promoters. In a recent publication, the human Synapsin (hSyn) promoter was used to drive expression of a dCas9-VPR transgene, revealing selective induction of targeted genes across multiple primary neuron types and neuron-specific expression in the adult brain via lentiviral delivery.²¹ Similarly, unique promoters for other distinct cell types (eg, GFAP for glia, MBP for oligodendrocytes) could be useful for driving dCas9 fusion proteins in a more selective fashion. One advantage of this strategy is that it does not require a specific transgenic animal, and thus is compatible with almost any animal model system, cell line, or induced stem cell.

A more common approach involves combination of CRISPR technology with cell-specific expression of Cre recombinase, either via transgenic Cre driver lines or virally mediated expression of Cre recombinase under the control of a cell-specific promoter. Cre recombinase performs cleavage and ligation reactions at specific DNA elements called “lox” sites, which are most commonly inserted flanking a specific transgene of interest. Lox sequences are directional, and the action of Cre recombinase therefore varies based on the orientation of lox pairs relative to each other. For example, insertion of lox sites facing the same direction will result in excision of DNA between these sites. Likewise, insertion of lox sites facing each other will result in inversion and ligation of DNA between these sites, effectively flipping the transgene. The existence of heterotypic lox sites (eg, loxP and lox2272) increases combinatorial power of this system, as recombination events are specific within heterotypic pairs. Insertion of lox sites flanking CRISPR transgenes can therefore be used to gain Cre-dependent control over expression of CRISPR machinery. This approach has taken two basic strategies. In one strategy, a lox-stop-lox cassette is inserted ahead of the dCas9 fusion transgene (or functional Cas9), which prevents Cas9/dCas9 expression until the lox-stop-lox cassette is excised by Cre recombinase. Similarly, an inverted Cas9/dCas9 cassette can be flanked by heterotypic lox sites, which requires two recombination events for normal Cas9 expression.⁴⁷ A lox-stop-lox approach has been used for generation of mouse and rat

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lines for Cas9-mediated genome editing in specific cell populations,^{48,49} and more recently for development of a mouse line for CRISPRa in targeted cell populations.³⁰ A second, alternate approach is to drive the targeting sgRNA in a Cre-dependent fashion by placing a lox-stop-lox cassette upstream of the sgRNA and enabling ubiquitous expression of the Cas9 or dCas-fusion protein.⁴⁸ Critically, either of these strategies requires access to a cell-specific Cre driver transgenic animal or co-delivery of Cre recombinase vectors driven by cell-specific promoters. However, in all cases these manipulations produce irreversible recombination events, meaning that CRISPR transgenes cannot be controlled in a temporally specific fashion.

Temporal specificity and reversible activation

A classic way to limit transgene expression to a defined temporal window is to use a tetracycline-inducible promoter. Tetracycline expression systems use a bacterial tetracycline response element, which contains repeats of a tetracycline operator sequence. Recent reports have used this strategy for temporally specific control of sgRNA expression by using a constitutively expressed Tet repressor (TetR). In the presence of tetracycline derivatives like doxycycline, TetR cannot bind to tetracycline operator sequences, relieving repression of sgRNA expression.⁵⁰ This inducible sgRNA vector is capable of initiating gene editing both in vitro and in vivo, with effects occurring in the adult brain as soon as 1 day after addition of doxycycline to the diet. Although a similar approach has been used to regulate expression of Cas9 protein in other somatic tissues and cell lines,^{51,52} this approach does not appear to provide temporally specific expression of virally expressed Cas9 in neuronal systems due to leaky baseline expression.⁵⁰ Thus far, neither of these approaches have been developed for dCas9 tools.

A second common chemical approach for temporally restricted transgene localization is the ERT2-tamoxifen system, in which a mutated ligand binding domain from ERT2 is fused to the protein of interest. ERT2 exhibits cytoplasmic sequestration in the absence of its ligand, 4hydroxy-tamoxifen (4HT), but rapidly translocates to the nucleus in the presence of 4HT. Adopting this strategy, a recent report demonstrated that fusion of ERT2 to Cas9 resulted in 4HT-induced gene editing capabilities that were sensitive to as little as 4hr of 4HT treatment and associated with reversible export of Cas9 after 4HT treatment.⁵³ However, the efficiency of this approach was relatively low,

and there were considerable tradeoffs between efficiency of targeting and leakiness of targeting. Similarly, a split Cas9 system that employed fusion of N-terminal and C-terminal components of Cas9 or dCas9 to distinct rapamycin binding domains of the mammalian target of rapamycin (mTOR) enabled rapamycin-dependent gene editing and transcriptional activation.⁵⁴ Like ERT2-based tools, this system also presented substantially lower efficiency than constitutive expression of wild-type Cas9 and was hampered by leaky gene regulation in the absence of rapamycin. Additionally, the reversibility of splitCas9 tools has not been systematically assessed.

Perhaps the most widely used approach for rapid control of CRISPR tools is light delivery, which is not only faster than chemical or genetic manipulations, but also rapidly reversible. These efforts drew on a rich history of using light to control protein interactions and gene regulation in simple cellular systems, but also on rapidly developing advances in optogenetic tools for delivery of light to specific organs, including the mammalian brain.^{55,56} The first demonstration of customizable light-induced gene targeting used TALE systems in which Cryptochrome 2 (Cry2), a blue light-sensitive protein from *Arabidopsis Thaliana*, was fused to a site-specific TALE array, enabling light-independent recruitment of Cry2 to a DNA locus. Instead of fusing an effector directly to the DNA binding TALE, the transcriptional activator VP64 was instead fused to CIB1, a binding partner that interacts with Cry2 only in the presence of blue waveform light (due to a conformational change in Cry2 protein). Remarkably, this system enabled light-induced transcriptional modulation via recruitment of VP64 in the presence of blue light, and similar results were obtained when VP64 was fused to Cry2 and CIB1 was fused to the TALE array.⁵⁷ Moreover, this approach supported light-activated epigenetic editing with fused histone methyltransferases and histone deacetylases, revealing the possibility for light-activated “optoepigenetic” approaches for precise and specific epigenetic editing with temporal and spatial precision.

Following this landmark demonstration, similar results were reported for light-based transcriptional activation using custom zinc finger proteins,⁵⁸ and then for dCas9-based approaches.⁵⁹ This toolbox has rapidly grown in the past 5 years,⁶⁰⁻⁶² giving rise to additional blue-light sensitive effectors for genetic and transcriptional regulation

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(Figure 2c). These include a photoswitchable Cas9 (psCas9) that uses two photo-dissociable fluorescent proteins to obscure the DNA binding domain of Cas9 under normal conditions, preventing gene editing or Cas9 targeting. These domains dissociate in the presence of blue wavelength (500 nm) light, enabling Cas9 targeting or dCas9-effector recruitment for robust regulation of gene expression.⁶² A similar strategy has been employed in the recently described CASANOVA system,⁶⁰ which ingeniously uses anti-CRISPR (Acr) proteins discovered in bacteriophages.⁶³ These naturally occurring proteins bind to Cas9 with subnanomolar affinity in the region that normally interacts with the PAM motif, providing a mechanism for bacteriophages to escape gene editing functions of CRISPR/Cas9. However, these proteins are also active and provide robust inhibition of Cas9 and dCas9 in mammalian cells. In the CASANOVA (which stands for “CRISPR–Cas9 activity switching via a novel optogenetic variant of AcrIIA4”) system, a light-sensitive LOV2 domain from *Avena Sativa* was fused to the anti-CRISPR protein AcrIIA4. The N and C termini of the LOV2 protein are in close proximity in darkness but unravel upon exposure to blue light, disrupting AcrIIA4 interactions with Cas9. This switch allows for nearly complete inhibition of gene editing or gene regulation functions of Cas9 and dCas9 in baseline dark states, but activation in the presence of blue light.⁶⁰

One limitation to blue light sensitive Cas9 or dCas9 tools is that this wavelength of light does not penetrate deeply into tissues, and in some cases can damage tissues (or neurons) with increased light dosage.⁵⁵ In contrast, near infra-red (NIR) light penetrates deeply into mammalian tissues and does not present robust phototoxicity complications. Recently described strategies have adapted light-inducible CRISPR systems for NIR light using either BphS protein (a NIR-sensitive c-di-GMP synthase) to signal to a c-di-GMP responsive transcription factor to initiate transcription of CRISPR effectors,⁶⁴ or by NIR-sensitive nanocarriers of

sgRNA/Cas9 complexes that release CRISPR cargo upon NIR light stimulation.⁶⁵ However, these systems are in the early design phases and have not undergone systematic testing in different tissues and with different dCas9-effectors. Nevertheless, these approaches may eventually provide a path towards noninvasive gene and transcriptional editing in the brain, given the capacity for these wavelengths to penetrate transcranially into deep brain structures.⁶⁶

Conclusions and future directions

The research tools outlined above have enabled rapid transformation of a burgeoning subfield that previously lacked necessary genetic and temporal specificity. These emerging tools set the stage for causal, mechanistic dissection of key regulatory events linked to experience-dependent changes in neuronal function and dysregulation of gene expression in brain disease states. Although these approaches are likely many years away from translation to clinical applications, it is noteworthy that many CRISPR-based approaches are already in early clinical or preclinical trials to treat numerous genetic diseases, including inherited childhood blindness, sickle-cell disease, and Duchenne muscular dystrophy. While numerous hurdles exist for delivery of CRISPR machinery to the CNS,¹³ these examples offer hope that CRISPR-based genetic editing, transcriptional modulation, or epigenetic editing could be used in human diseases that affect brain function. However, regardless of potential clinical applications, these tools provide a versatile, multiplexable, and inexpensive technology for the systematic interrogation of genetic and epigenetic contributions to neuronal function. ■

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References

1. Hardingham GE, Pruunsild P, Greenberg ME, Bading H. Lineage divergence of activity-driven transcription and evolution of cognitive ability. *Nat Rev Neurosci.* 2018;19(1):9-15.
2. Duke CG, Kennedy AJ, Gavin CF, Day JJ, Sweatt JD. Experience-dependent epigenomic reorganization in the hippocampus. *Learn Mem.* 2017;24(7):278-288.
3. Halder R, Hennon M, Vidal RO, et al. DNA methylation changes in plasticity genes accompany the formation and maintenance of memory. *Nat Neurosci.* 2016;19(1):102-110.
4. Guo JU, Ma DK, Mo H, et al. Neuronal activity modifies the DNA methylation landscape in the adult brain. *Nat Neurosci.* 2011;14(10):1345-1351.
5. Kennedy AJ, Rahn EJ, Paulukaitis BS, et al. Tcf4 regulates synaptic plasticity, DNA methylation, and memory function. *Cell Rep.* 2016;16(10):2666-2685.
6. Gaj T, Gersbach CA, Barbas CF, 3rd. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol.* 2013;31(7):397-405.
7. Barrangou R, Fremaux C, Deveau H, et al.

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- CRISPR provides acquired resistance against viruses in prokaryotes. *Science*. 2007;315(5819):1709-1712.
8. Jiang F, Doudna JA. CRISPR-Cas9 structures and mechanisms. *Annu Rev Biophys*. 2017;46:505-529.
9. Charpentier E, Marraffini LA. Harnessing CRISPR-Cas9 immunity for genetic engineering. *Curr Opin Microbiol*. 2014;19:114-119.
10. Anders C, Niewoehner O, Duerst A, Jinek M. Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. *Nature*. 2014;513(7519):569-573.
11. Heidenreich M, Zhang F. Applications of CRISPR-Cas systems in neuroscience. *Nat Rev Neurosci*. 2016;17(1):36-44.
12. Savell KE, Day JJ. Applications of CRISPR/Cas9 in the mammalian central nervous system. *Yale J Biol Med*. 2017;90(4):567-581.
13. Xu SJ, Heller EA. Recent advances in neuroepigenetic editing. *Curr Opin Neurobiol*. 2019;59:26-33.
14. Holtzman L, Gersbach CA. Editing the epigenome: reshaping the genomic landscape. *Annu Rev Genomics Hum Genet*. 2018;19:43-71.
15. Dahlman JE, Abudayyeh OO, Joung J, Gootenberg JS, Zhang F, Konermann S. Orthogonal gene knockout and activation with a catalytically active Cas9 nuclease. *Nat Biotechnol*. 2015;33(11):1159-1161.
16. Larson MH, Gilbert LA, Wang X, Lim WA, Weissman JS, Qi LS. CRISPR interference (CRISPRi) for sequence-specific control of gene expression. *Nat Protoc*. 2013;8(11):2180-2196.
17. Qi LS, Larson MH, Gilbert LA, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*. 2013;152(5):1173-1183.
18. Gilbert LA, Horlbeck MA, Adamson B, et al. Genome-scale CRISPR-mediated control of gene repression and activation. *Cell*. 2014;159(3):647-661.
19. Gilbert LA, Larson MH, Morsut L, et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell*. 2013;154(2):442-451.
20. Liu SJ, Horlbeck MA, Cho SW, et al. CRISPRi-based genome-scale identification of functional long noncoding RNA loci in human cells. *Science*. 2017;355(6320).
21. Savell KE, Bach SV, Zipperly ME, et al. A neuron-optimized CRISPR/dCas9 activation system for robust and specific gene regulation. *eNeuro*. 2019;6(1). doi:10.1523/ENEURO.0495-18.2019.
22. Zheng Y, Shen W, Zhang J, et al. CRISPR interference-based specific and efficient gene inactivation in the brain. *Nat Neurosci*. 2018;21(3):447-454.
23. Maeder ML, Linder SJ, Cascio VM, Fu Y, Ho QH, Joung JK. CRISPR RNA-guided activation of endogenous human genes. *Nat Methods*. 2013;10(10):977-979.
24. Perez-Pinera P, Kocak DD, Vockley CM, et al. RNA-guided gene activation by CRISPR-Cas9-based transcription factors. *Nat Methods*. 2013;10(10):973-976.
25. Liu Y, Yu C, Daley TP, et al. CRISPR activation screens systematically identify factors that drive neuronal fate and reprogramming. *Cell Stem Cell*. 2018;23(5):758-771.
26. Yang J, Rajan SS, Friedrich MJ, et al. Genome-scale CRISPRa screen identifies novel factors for cellular reprogramming. *Stem Cell Reports*. 2019;12(4):757-771.
27. Black JB, Adler AF, Wang HG, et al. Targeted epigenetic remodeling of endogenous loci by CRISPR/Cas9-based transcriptional activators directly converts fibroblasts to neuronal cells. *Cell Stem Cell*. 2016;19(3):406-414.
28. Chavez A, Scheiman J, Vora S, et al. Highly efficient Cas9-mediated transcriptional programming. *Nat Methods*. 2015;12(4):326-328.
29. Zhou H, Liu J, Zhou C, et al. In vivo simultaneous transcriptional activation of multiple genes in the brain using CRISPR-dCas9-activator transgenic mice. *Nat Neurosci*. 2018;21(3):440-446.
30. Kwapis JL, Alagband Y, Kramar EA, et al. Epigenetic regulation of the circadian gene *Per1* contributes to age-related changes in hippocampal memory. *Nat Commun*. 2018;9(1):3323.
31. Matharu N, Rattanasopha S, Tamura S, et al. CRISPR-mediated activation of a promoter or enhancer rescues obesity caused by haploinsufficiency. *Science*. 2019;363(6424).
32. Nord AS, Blow MJ, Attanasio C, et al. Rapid and pervasive changes in genome-wide enhancer usage during mammalian development. *Cell*. 2013;155(7):1521-1531.
33. Gray JM, Kim TK, West AE, Markenscoff-Papadimitriou E, Lomvardas S. Genomic views of transcriptional enhancers: essential determinants of cellular identity and activity-dependent responses in the CNS. *J Neurosci*. 2015;35(41):13819-13826.
34. Hilton IB, D'Ippolito AM, Vockley CM, et al. Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. *Nat Biotechnol*. 2015;33(5):510-517.
35. Chen LF, Lin YT, Gallegos DA, et al. Enhancer histone acetylation modulates transcriptional bursting dynamics of neuronal activity-inducible genes. *Cell Rep*. 2019;26(5):1174-1188 e1175.
36. Kwon DY, Zhao YT, Lamonica JM, Zhou Z. Locus-specific histone deacetylation using a synthetic CRISPR-Cas9-based HDAC. *Nat Commun*. 2017;8:15315.
37. Kearns NA, Pham H, Tabak B, et al. Functional annotation of native enhancers with a Cas9-histone demethylase fusion. *Nat Methods*. 2015;12(5):401-403.
38. Vojta A, Dobrinic P, Tadic V, et al. Repurposing the CRISPR-Cas9 system for targeted DNA methylation. *Nucleic Acids Res*. 2016;44(12):5615-5628.
39. Liu XS, Wu H, Ji X, et al. Editing DNA methylation in the mammalian genome. *Cell*. 2016;167(1):233-247 e217.
40. Galonska C, Charlton J, Mattei AL, et al. Genome-wide tracking of dCas9-methyltransferase footprints. *Nat Commun*. 2018;9(1):597.
41. Kantor B, Tagliafierro L, Gu J, et al. Downregulation of SNCA expression by targeted editing of DNA methylation: a potential strategy for precision therapy in PD. *Mol Ther*. 2018;26(11):2638-2649.
42. Wei Y, Lang J, Zhang Q, et al. DNA methylation analysis and editing in single mammalian oocytes. *Proc Natl Acad Sci U S A*. 2019;116(20):9883-9892.
43. Liu XS, Wu H, Krzisch M, et al. Rescue of fragile X syndrome neurons by DNA methylation editing of the *FMR1* gene. *Cell*. 2018;172(5):979-992.
44. Kim TK, Hemberg M, Gray JM. Enhancer RNAs: a class of long noncoding RNAs synthesized at enhancers. *Cold Spring Harb Perspect Biol*. 2015;7(1):a018622.
45. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. *Annu Rev Biochem*. 2012;81:145-166.
46. Shechner DM, Hacisuleyman E, Younger ST, Rinn JL. Multiplexable, locus-specific targeting of long RNAs with CRISPR-Display. *Nat Methods*. 2015;12(7):664-670.
47. Kumar N, Stanford W, de Solis C, et al. The development of an AAV-based CRISPR SaCas9 genome editing system that can be delivered to neurons in vivo and regulated via doxycycline and cre-recombinase. *Front Mol Neurosci*. 2018;11:413.
48. Back S, Necarsulmer J, Whitaker LR, et al. Neuron-specific genome modification in the adult rat brain using CRISPR-Cas9 transgenic rats. *Neuron*. 2019;102(1):105-119.
49. Platt RJ, Chen S, Zhou Y, et al. CRISPR-Cas9 knockin mice for genome editing and cancer modeling. *Cell*. 2014;159(2):440-455.
50. de Solis CA, Ho A, Holehonnur R, Ploski JE. The development of a viral mediated CRISPR/Cas9 system with doxycycline dependent gRNA expression for inducible in vitro and in vivo genome editing. *Front Mol Neurosci*. 2016;9:70.
51. Dow LE, Fisher J, O'Rourke KP, et al. Inducible in vivo genome editing with CRISPR-Cas9. *Nat Biotechnol*. 2015;33(4):390-394.
52. Gonzalez F, Zhu Z, Shi ZD, et al. An iCRISPR platform for rapid, multiplexable, and inducible genome editing in human pluripotent stem cells. *Cell Stem Cell*. 2014;15(2):215-226.
53. Liu KI, Ramli MN, Woo CW, et al. A chemical-inducible CRISPR-Cas9 system for rapid control of genome editing. *Nat Chem Biol*. 2016;12(11):980-987.
54. Zetsche B, Volz SE, Zhang F. A split-Cas9 architecture for inducible genome editing and transcription modulation. *Nat Biotechnol*. 2015;33(2):139-142.
55. Poleskaya O, Baranova A, Bui S, et al. Optogenetic regulation of transcription. *BMC Neurosci*. 2018;19(Suppl 1):12.
56. Deisseroth K. Optogenetics: 10 years of microbial opsins in neuroscience. *Nat Neurosci*. 2015;18(9):1213-1225.
57. Konermann S, Brigham MD, Trevino A, et al. Optical control of mammalian endogenous transcrip-

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tion and epigenetic states. *Nature*. 2013;500(7463):472-476.

58. Polstein LR, Gersbach CA. Light-inducible gene regulation with engineered zinc finger proteins. *Methods Mol Biol*. 2014;1148:89-107.

59. Polstein LR, Gersbach CA. A light-inducible CRISPR-Cas9 system for control of endogenous gene activation. *Nat Chem Biol*. 2015;11(3):198-200.

60. Bubeck F, Hoffmann MD, Harteveld Z, et al. Engineered anti-CRISPR proteins for optogenetic control of CRISPR-Cas9. *Nat Methods*. 2018;15(11):924-927.

61. Nihongaki Y, Furuhashi Y, Otabe T, Hasegawa S, Yoshimoto K, Sato M. CRISPR-Cas9-based photoactivatable transcription systems to induce neuronal differentiation. *Nat Methods*. 2017;14(10):963-966.

62. Zhou XX, Zou X, Chung HK, et al. A single-chain photoswitchable CRISPR-Cas9 Architecture for light-inducible gene editing and Transcription. *ACS Chem Biol*. 2018;13(2):443-448.

63. Rauch BJ, Silvis MR, Hultquist JF, et al. Inhibition of CRISPR-Cas9 with bacteriophage proteins. *Cell*. 2017;168(1-2):150-158.

64. Shao J, Wang M, Yu G, et al. Synthetic far-red light-mediated CRISPR-dCas9 device for inducing functional neuronal differentiation. *Proc Natl Acad Sci U S A*. 2018;115(29):E6722-E6730.

65. Pan Y, Yang J, Luan X, et al. Near-infrared upconversion-activated CRISPR-Cas9 system: A remote-controlled gene editing platform. *Sci Adv*. 2019;5(4):eaav7199.

66. Lin JY, Knutsen PM, Muller A, Kleinfeld D, Tsien RY. ReaChR: a red-shifted variant of channelrhodopsin enables deep transcranial optogenetic excitation. *Nat Neurosci*. 2013;16(10):1499-1508.