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Editing the Epigenome: Technologies for Programmable Transcriptional Modulation and Epigenetic Regulation

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Abstract

Gene regulation is a highly complex and tightly controlled process that defines cell identity, health, and response to environmental signals. Technologies for precisely perturbing gene regulation are critical for improving our understanding of its coordination and for manipulating pathways for applications in biotechnology and medicine. Recently developed DNA-targeting platforms, including zinc finger proteins, TALEs, and CRISPR/Cas9, have enabled the recruitment of transcriptional modulators and epigenome-modifying factors to any genomic site. These technologies are generating novel insights into the function of epigenetic marks and the role of genome structure in gene expression. Additionally, custom transcriptional and epigenetic regulation is facilitating refined control over cell function and decision-making. The unique properties of the CRISPR/Cas9 system have also created new opportunities for multiplexing targets and manipulating complex gene expression patterns, as well as high-throughput genetic screens. This review summarizes recent technology developments in this area and their applications to modern biomedical challenges. We also discuss remaining limitations and necessary future directions for this field.

Introduction

The recent evolution of customizable epigenome engineering tools is driving biomedical research into a new era of unprecedented control over gene expression and epigenetic regulation. Multiple platforms now exist for targeting virtually any DNA sequence with engineered biomolecules, and a variety of effectors have been identified that can regulate gene expression by modifying transcription and/or epigenetic state. Creating site-specific alterations to the epigenomic landscape in eukaryotic cells is a powerful strategy to interrogate the mechanistic relationships between chromatin state, gene regulation, and cell phenotype. Furthermore, control over gene regulation is a valuable tool for applications such

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as reprogramming cell fate and gene therapy. Here, we provide an overview of current tools for transcriptional regulation and epigenetic manipulation and present their diverse applications for biological research and regenerative medicine (Figure 1).

Principles of Transcriptional and Epigenetic Regulation in Mammalian Cells

Eukaryotic transcription is guided by interactions between the RNA polymerase II holoenzyme (Pol II), associated transcription factors, and genomic regulatory elements[1]. Transcription by Pol II is initiated from the proximal promoter region directly upstream of the gene. Actively transcribed promoters are generally characterized by an accessible chromatin state that is amenable to binding by activating transcription factors[2]. Promoter activity can be also affected by local and distal regulatory elements[1]. Distal enhancers can form long-range interactions with target gene promoters that facilitate the recruitment of transcription factors and epigenetic modifiers for gene activation[1, 3, 4]. Chemical modifications to DNA and associated histone proteins govern chromatin accessibility, and regulatory elements demonstrate dynamic signatures of these modifications that correlate with their activity in different cell states and types[4–7]. The eukaryotic epigenome thus encompasses the regulatory element interactions, chemical modifications to DNA, post-translational modifications of histone proteins, and the tightly controlled positioning of these histones that determines chromatin structure and transcription[3, 4].

The causal relationships between histone modifications and transcription are complex and incompletely understood, and exhaustive mapping of the eukaryotic histone code has only just begun[4]. Acetylation of lysine residues 9 and/or 27 of the histone subunit H3 (H3K9ac and H3K27ac respectively; Table 1) is generally associated with active promoters and enhancers[2, 4, 7, 8]. Although methylation of histone subunit H3 lysine 4 (H3K4me) is linked to transcriptional activity[2, 7], methylation at histone subunit H3 lysine residues 9 and/or 27 (H3K9me and H3K27me, respectively) is commonly associated with gene repression[9]. Moreover, the degree of methylation observed can be an indicator for different regulatory elements. For instance, H3K4me is frequently observed at enhancer elements, whereas H3K4me3 is often enriched near active transcription start sites (TSSs)[2]. Histone acetylation in human cells is primarily coordinated by interplay between histone acetyltransferases (HATs) and histone deacetylases (HDACs), while methylation of histone lysine residues occurs via catalytic activity of histone methyltransferases (HMTs) and histone demethylases (HDMs) (Table 1)[8, 10, 11].

Gene expression is also regulated by variable levels of cytosine methylation of genomic DNA[12, 13]. High levels of CpG methylation are most frequently associated with inactive promoter and enhancer elements[13]. However, high CpG methylation is also found within actively transcribed regions of the genome, indicating that the local context of CpG methylation is related to its regulatory capacity[14]. CpG methylation is catalyzed by the DNA methyltransferases (DNMT; Table 1)[12] and can be actively removed by enzymes from the TET family of proteins[12] and by thymine-DNA glycosylase (TDG)[15].

Coordinated efforts to annotate eukaryotic epigenomes have revealed the complex layer of regulation that orchestrates diverse phenotypes from the same underlying genomic sequence

in multicellular organisms[3, 4, 7]. These findings have generated correlations between transcriptional activity and the dynamic modification of DNA and histone subunits[10, 12, 16] that can be perturbed to test function through the use of programmable DNA-targeting technologies coupled with epigenetic effectors.

Programmable DNA-Binding Domains

Synthetic epigenome engineering tools typically consist of a protein-based programmable DNA-binding domain (DBD) genetically fused to an enzymatic or scaffolding effector domain. Commonly employed DBDs include zinc finger proteins (ZFPs), transcription activator-like effectors (TALEs), and the type II clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 system (Figure 2). ZFPs and TALEs are modular DNA-binding proteins that can be engineered to form specific interactions between amino acid side chains of the DBD and the nucleotides of target DNA sequences. In contrast, the CRISPR/Cas9 system targets DNA by exploiting RNA:DNA base pair complementarity.

The ZFP structure is a common DNA-binding motif found in many natural mammalian transcription factors and was the first programmable DBD used in epigenome engineering applications[17–19]. Each zinc finger module is approximately 30 amino acids in length and contains an α-helix that interacts with three or four nucleotides in the major groove of DNA (Figure 2A)[20, 21]. Multiple zinc fingers can be concatenated to form a polydactyl targeting domain[21]. In contrast to ZFPs, subunits in engineered TALE arrays each bind a single nucleotide. Adapted from their native function as a plant pathogen from the bacteria genus *Xanthamonas*, TALEs contain subunits of 33 to 35 amino acid repeats (Figure 2B). The 12th and 13th positions form the repeat variable di-residue (RVD), which determines single nucleotide binding[22, 23]. In engineered TALEs, the amino acids in the RVD can be exchanged in a simple code to program sequence specificity. Open-access tools are available to facilitate the choice of target sites and design of corresponding ZFPs[24] and TALEs[25]. However, finding a highly active ZFP or TALE requires custom protein engineering and a sometimes arduous screening process. Nonetheless, ZFPs and TALEs have been used extensively as targeting platforms with epigenome editing effectors (Table 2).

The recent development of the RNA-guided CRISPR/Cas9 system has greatly simplified programmable DNA-targeting[26–30]. Cas9 is an endonuclease isolated from the type II CRISPR-Cas bacterial adaptive immune systems[26]. Inactivating mutations in the RuvC and HNH domains of Cas9 generate a deactivated Cas9 (dCas9) that no longer cleaves DNA but retains function as a DBD. dCas9 is localized to target sequences by a guide RNA (gRNA), an engineered nucleic acid consisting of an 18–25 nucleotide custom protospacer followed by a constant region that complexes with dCas9[31]. dCas9 binds target genomic sequences that are complementary to the protospacer sequence (Figure 2C)[32]. Target site flexibility for CRISPR/Cas9 is determined by the protospacer-adjacent motif (PAM), a Cas9 recognition site found immediately following the protospacer target site in the genome[33]. The size of the Cas9 protein and sequence of the PAM recognition site differ based on the originating species[27, 34, 35]. The simplicity and effectiveness of this system have facilitated its implementation as a common DBD linked to epigenetic effectors (Table 2).

Site-Specific Epigenome Editing

Epigenome editing, the precise placement of epigenetic marks with programmable DBD fusions, is a valuable method for research, biotechnology, and medicine by enabling control over gene regulation without changing DNA sequence[16]. Mechanisms of epigenome editing include regulating transcription, altering post-translational histone modifications, modifying DNA methylation, and modulating regulatory element interactions. Several epigenome editing effectors have been fused with synthetic DBDs to modify target loci (Table 1).

Targeted Transcriptional Activation

The direct fusion of potent transcriptional effector domains to designed DBDs can induce transcriptional activation when targeted to endogenous genes. Shortly after the modular nature of ZFP DNA recognition was identified[36], ZFPs were linked to VP16[37, 38] to create a programmable transcriptional activator[24, 25]. VP16 is a viral activation domain that recruits Pol II transcriptional machinery[39]. VP64, a tetramer of VP16 domains, has been linked to DBDs to activate coding and non-coding genes by targeting promoters and regulatory elements[18, 40–48]. Though VP64 does not directly modify chromatin, it recruits remodeling factors and has been linked to the deposition of activating histone marks, including as H3K27ac and H3K4me, and to increased chromatin accessibility[46, 48, 49]. Another commonly used activator is the p65 subunit of the human NF-κB complex[50] that has been tethered to ZFPs[51], TALEs[41, 42], and dCas9[45]. Gene induction by VP64 and p65 is generally strongest when effector domains are targeted upstream of transcription start sites and within promoter regions[45], although targeting downstream of TSSs and at distal enhancers can also be effective[46, 47, 52–54].

Recruiting multiple TALE- and dCas9-VP64 fusions to a single target locus is often required to elicit a robust transcriptional response[20, 30, 41–44, 55, 56]. Recently, next-generation activators have been developed that outperform the original dCas9-VP64 fusion by recruiting multiple effector domains to a single dCas9-gRNA complex[44, 45, 47, 57–59]. For example, the SunTag system recruits multiple VP64 activators to dCas9, resulting in stronger activation with a single gRNA compared to dCas9-VP64 fusions[60]. Repurposing the gRNA as a scaffold for recruitment of activation domains p65 and HSF1 via MS2-targeting aptamers also improved transcriptional activation[45]. The tandem fusion of VP64, p65 and Rta, a transactivation domain from gammaherpesviruses[61], (VPR) to the C terminus of dCas9 improved transcriptional activation in human cells compared to dCas9-VP64[58]. These improved methods enable the use of single gRNAs to achieve robust activation, potentiating genome-wide gene activation screens[45, 57]. Further work is required to understand the differences between these next generation activators, assess the epigenetic marks indirectly deposited by these domains, and elucidate the mechanisms through which co-recruitment of orthogonal domains synergistically enhances transcription. Epigenetic effectors that directly catalyze covalent modifications to DNA or histones can also activate gene expression. Engineered ZFP- and TALE-based TET and TDG fusions can demethylate CpGs at target promoters, leading to transcriptional induction[53, 62–64]. Additionally, dCas9, TALEs and ZFPs have been fused to the catalytic core of the p300 HAT(p300 Core) to deposit H3K27ac and activate gene expression from promoters and distal enhancers[48]. p300 Core fusions are

particularly promising for transcriptional activation, as they do not require multiplexing and can activate distal enhancers that were unresponsive to dCas9-VP64[48]. DBD-p300 Core fusions were the first reported targetable HAT, and future engineered activators may include effectors that recruit other histone marks associated with active gene expression, such as H3K4me.

Targeted Transcriptional Repression

Site-specific gene silencing with engineered DBD-repressor fusions offers an alternative to traditional gene repression methods such as RNA interference (RNAi) and nuclease-mediated gene knockout. RNAi silences target protein-coding genes by mRNA transcript degradation and translational inhibition mediated by small interfering RNAs. RNAi, however, has been limited by inefficient knockdown, off-target effects, and toxicity associated with oversaturation of endogenous microRNA pathways[65–67]. Furthermore, programmable DBDs can target anywhere in the genome, facilitating silencing of regulatory elements and non-coding genes that cannot be targeted by RNAi. Genes and regulatory elements can also be disrupted with site-specific nucleases, but nuclease-mediated genome editing is a stochastic and often inefficient process that also alters DNA sequence permanently, precluding dynamic epigenetic regulation.

Endogenous gene repression can be achieved with engineered repressor fusions through a variety of mechanisms. Localizing a DBD without an effector domain to promoter regions or downstream of the transcription start site can silence gene expression[32, 37, 68, 69]. In these strategies, repression is caused by steric interference of transcription factor binding and RNA polymerase elongation[69]. For example, dCas9 targeted to the Nanog enhancer disrupted binding of endogenous activating transcription factors and silenced Nanog expression[47]. However, gene repression by steric hindrance alone is often not sufficient for robust silencing. Effectors that recruit endogenous epigenetic modifiers of histone marks and DNA methylation, leading to chromatin condensation, typically generate more potent silencing[70–73]. The silencing domain most commonly used with DBDs is the Kruppel associated box (KRAB), a naturally occurring motif in mammalian zinc finger transcription factors[17, 57, 74]. Localizing KRAB to DNA initiates a heterochromatin-forming complex that includes the HMT SETDB1 and the HDAC NuRD complex[75–78]. In contrast to engineered activator platforms that benefit from multiplexing for potent activation, KRAB fusions do not appear to act synergistically and can readily achieve ten-fold or greater repression of endogenous genes with recruitment of a single effector[47, 57, 70, 71]. In addition to silencing genes from promoters, KRAB is an effective repressor of distal and proximal gene regulatory elements including enhancers[73, 79]. Fusions of TALE DBDs to SID4X, the interaction domain of mSin3a, also co-recruit histone deacetylase activity in order to silence target genes[74, 80].

Alternatively, effector domains that directly catalyze repressive DNA marks or histone modifications can be fused to DBDs to create a custom epigenetic silencing protein. Synthetic ZFPs tethered to DNMT3a catalyze DNA methylation and suppress transcription from endogenous gene promoters[64, 82–85]. LSD1 has been tethered to TALE and dCas9 DBDs to remove H3K4me from active enhancers and suppress downstream target

expression[73, 86]. A variety of ZFP- and TALE-based HMT fusions have also been created that repress endogenous gene transcription by depositing H3K9 mono-[81], di-[72, 87, 88], and tri-methylation[88] at promoter regions (Table 2)[81].

Each type of epigenetic repressor provides unique advantages conferred by its mechanism of action. The KRAB domain acts as a recruiter, and the complex of heterochromatin-modifying enzymes localized to target DNA by KRAB likely contributes to its versatility and potency for silencing protein-coding genes, non-coding RNA, and regulatory elements[47, 57, 70, 74, 79]. For applications investigating the role of specific histone marks or DNA methylation states, the use of enzymatic epigenetic effectors that catalyze a particular type of epigenetic modification may be desired. Lastly, the temporal stability and heritability of silencing is an important consideration. Silencing induced by the Kap1 complex associated with the KRAB domain can persist through cell replication,[89, 90] and H3K9 methylation and HP1 localization are properties of constitutive heterochromatin[91]. Silencing with KRAB- and H3K9 methyltransferase-based repressors, however, has been reversible following removal of the DBD-repressor fusion in some studies[57, 88]. Alternatively, targeted DNA methylation marks have the potential to be inherited by daughter cells and persist long-term, a potential benefit for applications in which stable and heritable suppression is desired[82, 85]. An important area for future work is generating a better understanding of how the heritability and persistence of the gene modulation and epigenetic marks is affected by the duration and magnitude of the activity of the epigenetic effector, the local chromatin environment, the cell type and presence of endogenous co-factors, and the identify of epigenetic marks being created.

Eukaryotic cells host a variety of potential epigenetic modifying domains, only a small fraction of which have been utilized for epigenome engineering applications thus far. Generating new active DBD-effector combinations can be challenging, as genetically fusing effectors to synthetic DBDs may affect epigenome editing activity. Optimizing linkers for epigenome editing proteins or developing alternative recruitment strategies could address this issue and lead to an extended toolbox of novel effector fusions.

Specificity of Epigenome Editing

The rapid and widespread application of designer epigenome editing proteins necessitates further study of the specificity of these tools for binding target sequences, modulating transcription, and altering chromatin structure. Genome-wide mapping of DNA-binding by ChIP-seq has revealed substantial off-target localization of ZFP-, TALE-, and dCas9-fusions in many cases[49, 79, 92–96]. For dCas9, off-target binding correlates with the presence of a 5–7 bp protospacer seed sequence followed by a PAM[49, 79, 93–96]. The functional consequences of these off-target binding events are unclear, as off-target localization does not always result in changes in gene transcription or chromatin accessibility, as measured by RNA-sequencing (RNA-seq) and DNase I hypersensitivity sequencing[49, 55, 79]. In fact, analysis of global gene expression via microarray analysis and RNA-seq has revealed near-perfect specificity with transcription-modulating proteins[49, 55, 71, 79, 97]. TALE- and dCas9-VP64 activators have demonstrated high specificity with a robust transcriptional response[42, 45, 49]. Similarly, dCas9-p300 Core fusions have demonstrated highly specific gene activation[48],. dCas9-

KRAB repressor fusions caused no off-target gene silencing events when targeting a gene reporter[71] and was highly specific with respect to genome-wide gene regulation, DNA-binding, creation of targeted H3K9me3 marks, and formation of heterochromatin when targeted to an endogenous enhancer[79]. Furthermore, dCas9-KRAB silencing is highly sensitive to mismatches in the PAM-proximal region of the protospacer[57].

Small-scale targeted assessments, such as ChIP-qPCR for histone modifications, suggest that programmable epigenetic editing proteins have limited off-target effects[48, 53, 73, 86]. However genome-wide studies of histone marks or DNA methylation have been reported for a limited subset of epigenetic effector domains[49, 79, 92]. For example, reports of the distance of silencing activity, spreading of H3K9me3 signal, or chromatin condensation induced by KRAB domains attached to different DBDs vary significantly[76, 79, 89, 90]. The specificity of engineered epigenome editing proteins for altering DNA and histone structure is critical to their further application for high throughput screens, mechanistic studies of epigenome modifications, and guiding cell behavior.

Dosing Cas9 expression, implementing truncated gRNAs, and engineering more stringent requirements for Cas9-PAM interactions have mitigated off-target Cas9 nuclease activity and may potentially reduce the likelihood of off-target dCas9 binding events in epigenome editing proteins[98–100] (reviewed in[101]). Several open-access platforms exist for designing gRNA protospacer targets that are specific genome-wide[98, 102–104]. An important area of future research is improving the ability of these tools to predict potential off-target sites, which may be facilitated by the incorporation of experimental observations into existing models[105].

Conditional Gene Regulation Systems

Temporal control of epigenetic regulation facilitates the dissection of complex gene networks and aids in recapitulating the natural dynamics of gene expression. Inducible epigenome editing systems can be used to study the inheritability and stability of specific epigenetic marks Conditional gene regulation systems also provide solutions for research and therapeutic applications in which sustained activity of epigenome tools is unnecessary or permanent modification of gene expression may not be desirable.

Chemically inducible promoters, such as the doxycycline-controlled expression system, are widely used for temporal control of transcription[32, 57, 106]. Steroid hormone receptor ligand-binding domains that control protein conformation and cellular trafficking have also been combined with ZFP- and TALE- activators to achieve conditional transcriptional modulation[107]. Recently, a split dCas9-VP64 system has been combined with chemically inducible domains that dimerize and activate gene expression in response to an inducer molecule[100]. For the CRISPR/Cas9 system, inducible gRNA expression may provide more responsive control over transcriptional modulation, as generation and degradation of functional gRNA molecules is likely faster than that of dCas9 protein. For this purpose, methods to adapt gRNA expression to chemically inducible RNA Pol II promoters have been developed using introns and ribozymes[108, 109].

Light-inducible systems decouple DBDs from the transcriptional regulatory domain and induce their association with exposure to light for spatiotemporal control of gene expression[81, 110–113]. Light-inducible gene regulation systems have been employed to spatially pattern expression of a transgene[111–113], induce expression of endogenous genes in mammalian cells in culture[81, 112, 113] and *in vivo*[81], and induce specific epigenetic modifications at target loci.[81] Light-inducible epigenetic regulation is a promising strategy for recapitulating the precisely timed and spatially organized gene expression patterns that emerge during natural tissue development.

Modulating Regulatory Elements and Higher Order Chromatin Organization

A primary challenge in the study of gene regulation lies in elucidating the function of the millions of putative regulatory elements that orchestrate the complex control of tens of thousands of genes in a highly coordinated and context-specific manner[114]. Defining the function of these elements is a particular challenge because their target genes can vary in number and distance from the regulatory region. It is also a critical area of future research since the vast majority of genetic variation associated with complex disease lies in these regions[5]. The programmable nature of epigenome editing tools uniquely enables the interrogation of regulatory regions to uncover the biological role of unique genomic elements in the native genomic context[46–48, 54, 73, 79, 86].

Targeting enhancers is an efficient strategy to modulate multiple genes with a single epigenome editing protein[48, 79]. Furthermore, regulating genes via their associated enhancers may achieve more effective control over transcription than targeting the promoter alone[46, 73, 115]. The lysine demethylase LSD1 has been coupled with TALEs[86] and dCas9[73] to silence putative enhancers by removing H3K4me. Loss-of-function studies with dCas9-LSD1 were used to identify novel enhancers involved in embryonic stem cell pluripotency[73]. For gain-of-function studies, dCas9-p300 Core fusions have been shown to activate potent gene transcription from a variety of regulatory elements[48]. Importantly, these studies show that altering specific histone modifications can directly modulate transcription from distal regulatory elements, illustrating how epigenome editing technologies can be used to dissect mechanisms of gene regulation. Future studies that combine targeted epigenetic alterations with genome-wide analysis of transcriptional and epigenetic consequences can facilitate the discovery and characterization of novel downstream targets of candidate non-coding genes, enhancers, and insulators.

Engineered epigenome editing proteins can be used to investigate how gene regulation is linked to higher order chromatin structure. Enhancers activate distal genes by physically interacting with target promoters via a chromatin loop. As an example of using these tools to reorganize chromatin structure, fusions of an engineered ZFP to the self-association domain (SAD) of Ldb1 were designed to initiate looping between the globin locus control region and the silenced γ-globin promoter[116]. Targeting the ZFP-Ldb1 SAD fusions to the γ-globin promoter induced recruitment of endogenous Ldb1 located in enhancer-associated protein complexes at the globin LCR. Notably, this led to potent activation of γ-globin and concomitant reduction of adult β-globin expression, suggesting that physical rearrangement of chromatin is causal to globin activation[116]. Ldb1 is an erythroid-specific enhancer-

associated factor, but generalized strategies for engineering chromatin looping could potentially be developed by creating chemically inducible DBD dimers or linking DBDs to CTCF-mediated looping activity[117]. To better understand the role of nuclear positioning on gene regulation, further studies could investigate silencing gene expression programs by sequestering the target region in a loop or subnuclear compartment such as the nuclear lamina, or by blocking regulatory elements that participate in looping.

Design and Modulation of Complex Gene Regulatory Networks

Synthetic biology seeks to understand complex gene regulatory relationships by recreating these networks in controlled environments. The development of synthetic programmable transcription factors has greatly expanded the toolbox for generating regulatory networks of increasing complexity. Synthetic gene promoters offer further flexibility in designing multi-component systems with tunable input-output relationships and temporal control[118]. These advances have led to a surge in the development of gene circuits in eukaryotic cells. Recently, targeted epigenetic modulators have been used to construct autoregulatory feedback loops, signaling cascades, genetic switches, and Boolean logic systems[68, 108, 119]. Furthermore, genetic circuits have been constructed in eukaryotic cells to model biological phenomena such as synergy, cooperativity, competition, and repressive epigenetic memory[42, 118, 120, 121].

As our understanding grows through *de novo* construction of gene regulatory networks, epigenome editing also enables multifaceted modulation of endogenous gene regulatory networks. For these applications, orthogonal tools that mediate different epigenome editing activities at distinct loci within cells are needed. As single-component systems, ZFPs and TALEs are inherently orthogonal due to their unique programmable protein-DNA interactions. In contrast, two distinct dCas9-effector fusions cannot discriminate between co-expressed gRNAs. However, orthogonal Cas9 species can be used to mediate transcriptional activity via unique associated gRNAs and PAM recognition sites[34, 35], thus providing a means to engineer diverse transcriptional behaviors (Figure 3a). Although most gene regulation work to date has been performed with *S. pyogenes* dCas9-fusions[30, 43–45, 55, 56, 71], orthogonal dCas9s from *N. meningitidis* and *S. aureus* have been described for gene editing and regulation applications[34, 35, 73, 122]. Additionally, recent studies have shown that *S. pyogenes* Cas9 can be re-engineered via directed evolution to recognize alternatives to the canonical 5’-NGG-3’ PAM[123].

Alternatively, orthogonality can be incorporated directly into the gRNA molecule (Figure 3b). Integrating protein-binding hairpin structures into the 3’ stem loop region of the gRNA can mediate recruitment of distinct functional effector proteins at individual target loci by association with a single dCas9 species[10646, 47, 106]. Advances in understanding the molecular basis of the dCas9-gRNA interaction will enable the further design of engineered gRNA scaffolds with novel functions[124]. For example, long RNAs have recently been integrated into the gRNA molecule for locus-specific targeting, expanding potential dCas9 effectors to long non-coding RNA, aptamers, and other functional RNA motifs[125]. Conditional expression of gRNAs combined with gRNA-specific effector recruitment would allow for complex programs of gene activation or repression to influence cellular

reprogramming and multifaceted behaviors such as tissue development, cell migration, and inflammatory response. Additionally, a functional Cas9 nuclease fused to effector domains can be used simultaneously for gene editing and gene regulation depending on gRNA length, which determines the conformational change of the Cas9 enzyme necessary for DNA cleavage[126–128]. This approach may be used to devise even more complex cell engineering strategies that combine changes to the genome sequence and gene expression.

High-Throughput Screens with Epigenome Editing Proteins

Gene regulatory pathways are often complex, involving distal regulatory elements or trans-acting regulatory molecules. Forward genetic screens with libraries of targeted epigenome editing proteins can be employed to reveal as yet unknown upstream regulators of differentiation programs, critical members of signaling pathways, or genes that become misregulated in disease progression. Highly flexible, programmable platforms have led to the development of libraries of DBDs to target all potential DNA sequences in a given genome[45, 57, 67, 129, 130]. The CRISPR/Cas9 system is particularly well-suited to pooled screening approaches because targeting is oligonucleotide-based, allowing for commercial synthesis of custom libraries and recovery of library targets by next-generation sequencing approaches[45, 57, 67] (Figure 4). gRNA libraries have already been designed to activate or repress all coding genes in the human genome[45, 57], and custom libraries can be obtained from commercial vendors. For gain-of-function screens,[45, 57, 12945, 57, 129] second generation dCas9-based activator systems were developed to ensure a robust increase in gene expression with a single gRNA[45, 57]. Alternatively, dCas9-KRAB repressors directed by a single gRNA library have been employed for genome-wide silencing screens[57].

These seminal studies provide optimized guidelines for gRNA library design, although recommendations may evolve as future studies extend screening strategies to different genomic targets, epigenetic effectors, and cell types. A better fundamental understanding of the factors that determine optimal interactions of gRNAs with their genomic target sites will also advance these methods. For VP64-based dCas9 effectors, gRNA targets were designed in the region approximately −400 to +1 bp from the target transcription start site (TSS)[45, 57], and for dCas9-KRAB, gRNA targets covered the region approximately −50 to +300 bp relative to the TSS[57]. Selecting gRNAs that are highly specific is important when designing libraries, as off-target effects could convolute interpretation of screens. However this is largely addressed by the redundancy that was incorporated into the gRNA libraries, with coverage of up to 10 gRNAs per TSS.

Robust delivery methods are required to ensure complete library coverage in pooled-library screens. Lentiviral gene delivery of gRNAs is particularly advantageous because the delivered genes are integrated into the host cell genome, providing stable expression and a means to track delivered gRNAs through cell progeny[45, 57]. Viral production is also highly scalable, and the multiplicity of infection can be optimized for complete coverage of the gRNA library at approximately a single gRNA per cell in order to minimize interactions between gRNAs and facilitate isolation of individual gene targets.

Fluorescence-activated cell sorting, drug-based selection, or competitive growth are all potential strategies to enrich gene targets of interest within a library screen. gRNA libraries can be employed to identify as yet unknown upstream regulators of cellular pathways or phenotypes, such as factors that activate differentiation programs or genes that become misregulated in disease progression. If selecting for regulation of a gene product, screening could be achieved by a reporter system in which expression of a fluorescent protein or antibiotic resistance gene is contingent on modulation of the target gene. Response to drug treatment is also a phenotype that is particularly conducive to screening and has been used to understand mechanisms of antibiotic resistance, drug sensitivity, and escape mechanisms of cancer cells[45, 57].

Genome-scale libraries with epigenome editing proteins have thus far focused on protein-coding genes, but high-throughput screening approaches can be envisioned to target other types of genomic element spaces, including enhancers, insulators, and non-coding RNA. For instance, dCas9 fusions to the p300 Core for activation and LSD1 for repression may be appropriate for screening activity of putative enhancers. These future directions will benefit from publicly available data on genome-wide epigenetic marks[4] to customize libraries to the epigenetic state of the cell and phenotype of interest.

Guiding Cellular Reprogramming

The forced direct conversion of somatic cells to diverse cell types has emerged as a prominent approach to generating cell sources for disease modeling, drug discovery, and gene and cell therapies. A common strategy to direct cellular reprogramming is through the ectopic expression of fate-specifying master regulatory transcription factors[131]. The acquisition of stably reprogrammed cells is contingent on the silencing of exogenously delivered master transcription factors and the concurrent activation of endogenous transcription factors through positive feedback networks. Many endogenous transcription factors required for reprogramming are contained within a cis-repressive chromatin state that can preclude transcription factor binding and pose an epigenetic barrier to their reactivation[132, 133]. The epigenetic landscape of the starting cell type can thus determine its permissiveness to reprogramming by exogenous factors[134], and insufficient levels and/or duration of expression of the exogenous factors can lead to lowered efficiency and incomplete reprogramming[135].

Customizable epigenome editing proteins have recently been used to address some of the intrinsic limitations of exogenous transcription factors for direct cell reprogramming. Using TALE-based transcription factors targeted to the distal enhancer of *Oct4* in concert with *SOX2, KLF4*, and *C-MYC* transgenes, Gao et al. reprogrammed mouse embryonic fibroblasts to induced pluripotent stem cells (iPSCs)[46]. Compared to delivery of the *OCT4* transgene, targeted activation of *Oct4* induced rapid chromatin remodeling that more closely resembled the epigenetic landscape of the native *Oct4* locus in mouse embryonic stem cells (ESCs). This work provided insight into the value of targeting enhancers to regulate gene expression and facilitate epigenetic remodeling. A similar approach could be employed to assess the importance of putative enhancers in reactivating cell type-specific gene regulatory

networks for cellular reprogramming applications or to interrogate residual or aberrant 'epigenetic memory' observed in reprogrammed cells[136, 137].

Recent improvements made to CRISPR/Cas9-based transcriptional activators have also been implemented for cellular reprogramming. Targeting dCas9 fused to two VP64 effectors to the *Myod1* gene induced the transdifferentiation of murine embryonic fibroblasts to skeletal myocytes at an efficiency comparable to that achieved through the overexpression of *MYOD1* cDNA[59]. Direct differentiation of human iPSCs into induced neurons has also been demonstrated with one of the next generation dCas9-based activators, dCas9-VPR[58]. Efficient generation of the neuronal cells was contingent on the higher levels of gene induction achieved with dCas9-VPR compared to the first generation dCas9-VP64 activator.

These proof-of-principle examples demonstrate the feasibility and potential advantages of using targeted epigenome editing proteins for cellular reprogramming. Future work may apply these programmable transcription factors to reprogramming applications that require multiplexed gene activation and repression. Furthermore, genome-wide interrogation of non-coding transcripts and gene regulatory elements could reveal factors, that when modulated, improve the kinetics, efficiency, and fidelity of reprogramming.

Harnessing Epigenetic Regulation to Treat Disease

Aberrant genetic regulation is often associated with pathological states, either as a symptom or cause of underlying disease[138]. Site-specific epigenome editing provides the opportunity to study the contributions of gene regulation to disease and is an exciting potential avenue for treatment.

Targeted activation of compensatory genes can mitigate symptoms of diseases that otherwise have no cure. For instance, engineered activators can induce expression of developmentally silenced fetal γ-globin and counteract the loss of functional β-globin in sickle cell anemia or β-thalassemia[55, 139, 140]. ZFP-based activators targeted to glial cell line-derived neurotrophic factor (GDNF) have shown promise in rat models for protecting against neural damage as a potential treatment for neurodegeneration associated with Parkinson's disease[141]. Laganiere et al hypothesized that endogenous gene activation limited GDNF expression to physiological levels, thus potentially avoiding the toxic side-effects observed in ectopic GDNF factor treatments. Transcriptional modulation strategies can also generate protective effects for regenerative medicine applications. Activating endogenous VEGF with engineered ZFPs has been proposed to generate neovasculature for diabetic neuropathy and peripheral arterial disease, as well as enhancing wound healing[142, 143]. Targeting the VEGF promoter with engineered activators activates all VEGF isoforms, which can result in more mature vasculature formation compared to exogenous delivery of a single VEGF isoform[142]. Similarly, engineered ZFP-p65 fusions upregulated pigment epithelium-derived factor and prevented neovascularization in mouse models, a potential anti-angiogenic treatment for choroidal neovascularization such as that seen in acute macular degeneration[144].

Targeted repressors can also suppress detrimental gene products associated with disease progression. Engineered ZFP repressors have been designed to silence oncogenes and have

been effective at slowing cancer cell growth in mouse models[83, 145]. Synthetic ZFP repressors were also engineered to selectively silence mutant *htt* in a Huntingtin's disease mouse model[70]. Several ZFP lengths were tested to develop a repressor with specific activity at the longer CAG repeats found in disease-causing mutant *htt* alleles. This strategy may also be applicable for treating other gain-of-function genetic diseases, such as Facioscapulohumeral muscular dystrophy (FSHD), Fragile X syndrome, or myotonic dystrophy. Beyond transcriptional control, pharmacologic modulation of DNA methylation and histone modification has shown preclinical promise to treat tumor progression and neurodegenerative disorders, such as Alzheimer's, Parkinson's, and Huntington disease[146–148]. However, the small molecule drugs used in these therapies typically act by broadly inhibiting the enzymatic activity of epigenetic effectors, and doses are often limited by toxicity following systemic administration. Programmable epigenetic modification with designer DBDs could potentiate targeted therapy by treating the specific histone and DNA marks that contribute to the progression of these diseases.

Although programmable gene regulation has engendered innovative therapeutic strategies in preclinical studies, barriers to clinical translation remain. A primary challenge is establishing safe and efficient delivery methods[149]. Adeno-associated virus (AAV) is being widely explored in gene therapy *in vivo* studies and clinical trials, with an AAV product approved for clinical use in Europe. AAV delivery of ZFP- and TALE-based transcriptional regulators have demonstrated promising preclinical results in animal models for Huntington and Parkinson's disease[70, 141]. The recent development of smaller Cas9 systems that are compatible with AAV is a major advance in the development of CRISPR/Cas9-based gene therapy[35].

Another concern for clinical translation is the potential immunogenicity of engineered epigenome editing proteins. ZFPs, which are based on protein motif commonly found in human transcription factors, have been well-tolerated in *in vivo* studies, but the potential immunogenicity of TALE and dCas9 DBDs, which are not of mammalian origin, has yet to be explored. The addition of effector domains that are derived from non-mammalian systems, such as VP64, may exacerbate any potential immunogenicity. Furthermore, introduction of ectopic small RNAs for directing CRISPR/dCas9 factors has the potential to incite innate immune responses, although this may be combated with chemical modification of the delivered gRNAs[150, 151]. Therefore further studies that improve delivery methods and characterize immune responses to engineered epigenome editing proteins are needed before these technologies can be applied clinically.

Conclusions

Site-specific epigenome editing technologies provide custom control over gene regulation. The recent development of simple and economical DNA-targeting with the CRISPR/Cas9 system has enabled widespread use of targeted epigenome engineering tools and enabled platforms for screening large numbers of genomic targets. However, several of the epigenome editing effectors that have been successful with ZFPs and TALEs have not been reproduced with dCas9. Thus users of the technologies need to balance the simplicity and ease of use of CRISPR/dCas9 with the validated efficacy of earlier tools when choosing a

platform to use for specific applications. Custom epigenome editing proteins have the potential to become a standard method for probing interactions between specific chromatin modifications and gene expression. This will require the continued incorporation of new functions into DNA-targeting platforms for catalyzing specific epigenetic marks. Furthermore, manipulating gene expression and epigenetic marks is emerging as a powerful method to direct cellular phenotype for reprogramming that can be applied to disease modeling, drug discovery, and regenerative medicine. Thus far these studies have focused on gene targets that have already been characterized, but high-throughput strategies are enabling forward genetic screens to identify novel genes, regulatory elements, and chromatin modifications that control cell phenotypes.

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Competing Interest Statement

The authors are inventors on patent applications related to genome engineering. C.A.G. is a scientific advisor to Editas Medicine, a company engaged in therapeutic development of genome engineering technologies.

References

1. Maston GA, Evans SK, Green MR. Transcriptional regulatory elements in the human genome. Annu Rev Genomics Hum Genet. 2006; 7:29–59. [PubMed: 16719718]
2. Heintzman ND, et al. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. Nat Genet. 2007; 39:311–318. [PubMed: 17277777]
3. Consortium EP, et al. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012; 489:57–74. [PubMed: 22955616]
4. Roadmap Epigenomics C, et al. Integrative analysis of 111 reference human epigenomes. Nature. 2015; 518:317–330. [PubMed: 25693563]
5. Hnisz D, et al. Super-enhancers in the control of cell identity and disease. Cell. 2013; 155:934–947. [PubMed: 24119843]
6. Ong CT, Corces VG. Enhancer function: new insights into the regulation of tissue-specific gene expression. Nat Rev Genet. 2011; 12:283–293. [PubMed: 21358745]
7. Heintzman ND, et al. Histone modifications at human enhancers reflect global cell-type-specific gene expression. Nature. 2009; 459:108–112. [PubMed: 19295514]
8. Rada-Iglesias A, et al. A unique chromatin signature uncovers early developmental enhancers in humans. Nature. 2011; 470:279–283. [PubMed: 21160473]
9. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. Nat Rev Genet. 2012; 13:343–357. [PubMed: 22473383]
10. Verdin E, Ott M. 50 years of protein acetylation: from gene regulation to epigenetics, metabolism and beyond. Nat Rev Mol Cell Biol. 2015; 16:258–264. [PubMed: 25549891]
11. Jin Q, et al. Distinct roles of GCN5/PCAF-mediated H3K9ac and CBP/p300-mediated H3K18/27ac in nuclear receptor transactivation. EMBO J. 2011; 30:249–262. [PubMed: 21131905]
12. Schubeler D. Function and information content of DNA methylation. Nature. 2015; 517:321–326. [PubMed: 25592537]

13. Ziller MJ, et al. Charting a dynamic DNA methylation landscape of the human genome. Nature. 2013; 500:477–481. [PubMed: 23925113]
14. Baubec T, et al. Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation. Nature. 2015; 520:243–247. [PubMed: 25607372]
15. He YF, et al. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. Science. 2011; 333:1303–1307. [PubMed: 21817016]
16. Keung AJ, Joung JK, Khalil AS, Collins JJ. Chromatin regulation at the frontier of synthetic biology. Nat Rev Genet. 2015; 16:159–171. [PubMed: 25668787]
17. Beerli RR, Segal DJ, Dreier B, Barbas CF 3rd. Toward controlling gene expression at will: specific regulation of the erbB-2/HER-2 promoter by using polydactyl zinc finger proteins constructed from modular building blocks. Proc Natl Acad Sci U S A. 1998; 95:14628–14633. [PubMed: 9843940]
18. Beerli RR, Dreier B, Barbas CF 3rd. Positive and negative regulation of endogenous genes by designed transcription factors. Proc Natl Acad Sci U S A. 2000; 97:1495–1500. [PubMed: 10660690]
19. Gersbach CA, Gaj T, Barbas CF 3rd. Synthetic zinc finger proteins: the advent of targeted gene regulation and genome modification technologies. Acc Chem Res. 2014; 47:2309–2318. [PubMed: 24877793]
20. Cheng AW, et al. Multiplexed activation of endogenous genes by CRISPR-on, an RNA-guided transcriptional activator system. Cell Res. 2013; 23:1163–1171. [PubMed: 23979020]
21. Klug A. The discovery of zinc fingers and their applications in gene regulation and genome manipulation. Annu Rev Biochem. 2010; 79:213–231. [PubMed: 20192761]
22. Boch J, et al. Breaking the code of DNA binding specificity of TAL-type III effectors. Science. 2009; 326:1509–1512. [PubMed: 19933107]
23. Moscou MJ, Bogdanove AJ. A simple cipher governs DNA recognition by TAL effectors. Science. 2009; 326:1501. [PubMed: 19933106]
24. Maeder ML, Thibodeau-Beganny S, Sander JD, Voytas DF, Joung JK. Oligomerized pool engineering (OPEN): an 'open-source' protocol for making customized zinc-finger arrays. Nat Protoc. 2009; 4:1471–1501. [PubMed: 19798082]
25. Cermak T, et al. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Res. 2011; 39:e82. [PubMed: 21493687]
26. Gasiunas G, Barrangou R, Horvath P, Siksnys V. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci U S A. 2012; 109:E2579–E2586. [PubMed: 22949671]
27. Mali P, et al. RNA-guided human genome engineering via Cas9. Science. 2013; 339:823–826. [PubMed: 23287722]
28. Cong L, et al. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013; 339:819–823. [PubMed: 23287718]
29. Cho SW, Kim S, Kim JM, Kim JS. Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. Nat Biotechnol. 2013; 31:230–232. [PubMed: 23360966]
30. Jinek M, et al. RNA-programmed genome editing in human cells. Elife. 2013; 2:e00471. [PubMed: 23386978]
31. Jinek M, et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012; 337:816–821. [PubMed: 22745249]
32. Qi LS, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell. 2013; 152:1173–1183. [PubMed: 23452860]
33. Sternberg SH, Redding S, Jinek M, Greene EC, Doudna JA. DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. Nature. 2014; 507:62–67. [PubMed: 24476820]
34. Esvelt KM, et al. Orthogonal Cas9 proteins for RNA-guided gene regulation and editing. Nat Methods. 2013; 10:1116–1121. [PubMed: 24076762]
35. Ran FA, et al. In vivo genome editing using Staphylococcus aureus Cas9. Nature. 2015; 520:186–191. [PubMed: 25830891]

36. Pavletich NP, Pabo CO. Zinc finger-DNA recognition: crystal structure of a Zif268-DNA complex at 2.1 A. Science. 1991; 252:809–817. [PubMed: 2028256]
37. Choo Y, Sanchez-Garcia I, Klug A. In vivo repression by a site-specific DNA-binding protein designed against an oncogenic sequence. Nature. 1994; 372:642–645. [PubMed: 7990954]
38. Liu Q, Segal DJ, Ghiara JB, Barbas CF 3rd. Design of polydactyl zinc-finger proteins for unique addressing within complex genomes. Proc Natl Acad Sci U S A. 1997; 94:5525–5530. [PubMed: 9159105]
39. Ingles CJ, Shales M, Cress WD, Triezenberg SJ, Greenblatt J. Reduced binding of TFIID to transcriptionally compromised mutants of VP16. Nature. 1991; 351:588–590. [PubMed: 1646402]
40. Zhang F, et al. Efficient construction of sequence-specific TAL effectors for modulating mammalian transcription. Nat Biotechnol. 2011; 29:149–153. [PubMed: 21248753]
41. Maeder ML, et al. Robust, synergistic regulation of human gene expression using TALE activators. Nat Methods. 2013; 10:243–245. [PubMed: 23396285]
42. Perez-Pinera P, et al. Synergistic and tunable human gene activation by combinations of synthetic transcription factors. Nat Methods. 2013; 10:239–242. [PubMed: 23377379]
43. Maeder ML, et al. CRISPR RNA-guided activation of endogenous human genes. Nat Methods. 2013; 10:977–979. [PubMed: 23892898]
44. Mali P, et al. CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. Nat Biotechnol. 2013; 31:833–838. [PubMed: 23907171]
45. Konermann S, et al. Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. Nature. 2015; 517:583–588. [PubMed: 25494202]
46. Gao X, et al. Reprogramming to Pluripotency Using Designer TALE Transcription Factors Targeting Enhancers. Stem Cell Reports. 2013; 1:183–197. [PubMed: 24052952]
47. Gao X, et al. Comparison of TALE designer transcription factors and the CRISPR/dCas9 in regulation of gene expression by targeting enhancers. Nucleic Acids Res. 2014; 42:e155. [PubMed: 25223790]
48. Hilton IB, et al. Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. Nat Biotechnol. 2015
49. Polstein L, et al. Genome-wide specificity of DNA-binding, gene regulation, and chromatin remodeling by TALE- and CRISPR/Cas9-based transcriptional activators. Genome Res. 2015
50. Schmitz ML, Baeuerle PA. The p65 subunit is responsible for the strong transcription activating potential of NF-kappa B. EMBO J. 1991; 10:3805–3817. [PubMed: 1935902]
51. Liu PQ, et al. Regulation of an endogenous locus using a panel of designed zinc finger proteins targeted to accessible chromatin regions. Activation of vascular endothelial growth factor A. J Biol Chem. 2001; 276:11323–11334. [PubMed: 11145970]
52. Ji Q, et al. Engineered zinc-finger transcription factors activate OCT4 (POU5F1), SOX2, KLF4, c-MYC (MYC) and miR302/367. Nucleic Acids Res. 2014; 42:6158–6167. [PubMed: 24792165]
53. Maeder ML, et al. Targeted DNA demethylation and activation of endogenous genes using programmable TALE-TET1 fusion proteins. Nat Biotechnol. 2013; 31:1137–1142. [PubMed: 24108092]
54. Frank CL, et al. Regulation of chromatin accessibility and Zic binding at enhancers in the developing cerebellum. Nat Neurosci. 2015
55. Perez-Pinera P, et al. RNA-guided gene activation by CRISPR-Cas9-based transcription factors. Nat Methods. 2013; 10:973–976. [PubMed: 23892895]
56. Farzadfard F, Perli SD, Lu TK. Tunable and multifunctional eukaryotic transcription factors based on CRISPR/Cas. ACS Synth Biol. 2013; 2:604–613. [PubMed: 23977949]
57. Gilbert LA, et al. Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation. Cell. 2014; 159:647–661. [PubMed: 25307932]
58. Chavez A, et al. Highly efficient Cas9-mediated transcriptional programming. Nat Methods. 2015; 12:326–328. [PubMed: 25730490]
59. Chakraborty S, et al. A CRISPR/Cas9-Based System for Reprogramming Cell Lineage Specification. Stem Cell Reports. 2014; 3:940–947. [PubMed: 25448066]

60. Tanenbaum ME, Gilbert LA, Qi LS, Weissman JS, Vale RD. A protein-tagging system for signal amplification in gene expression and fluorescence imaging. Cell. 2014; 159:635–646. [PubMed: 25307933]
61. Staudt MR, Dittmer DP. The Rta/Orf50 transactivator proteins of the gamma-herpesviridae. Curr Top Microbiol Immunol. 2007; 312:71–100. [PubMed: 17089794]
62. Gregory DJ, Zhang Y, Kobzik L, Fedulov AV. Specific transcriptional enhancement of inducible nitric oxide synthase by targeted promoter demethylation. Epigenetics. 2013; 8:1205–1212. [PubMed: 24008769]
63. Chen H, et al. Induced DNA demethylation by targeting Ten-Eleven Translocation 2 to the human ICAM-1 promoter. Nucleic Acids Res. 2014; 42:1563–1574. [PubMed: 24194590]
64. Li K, et al. Manipulation of prostate cancer metastasis by locus-specific modification of the CRMP4 promoter region using chimeric TALE DNA methyltransferase and demethylase. Oncotarget. 2015; 6:10030–10044. [PubMed: 25888628]
65. Jackson AL, et al. Widespread siRNA "off-target" transcript silencing mediated by seed region sequence complementarity. RNA. 2006; 12:1179–1187. [PubMed: 16682560]
66. Grimm D, et al. Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways. Nature. 2006; 441:537–541. [PubMed: 16724069]
67. Shalem O, Sanjana NE, Zhang F. High-throughput functional genomics using CRISPR-Cas9. Nat Rev Genet. 2015; 16:299–311. [PubMed: 25854182]
68. Li Y, et al. Modular construction of mammalian gene circuits using TALE transcriptional repressors. Nat Chem Biol. 2015; 11:207–213. [PubMed: 25643171]
69. Larson MH, et al. CRISPR interference (CRISPRi) for sequence-specific control of gene expression. Nat Protoc. 2013; 8:2180–2196. [PubMed: 24136345]
70. Garriga-Canut M, et al. Synthetic zinc finger repressors reduce mutant huntingtin expression in the brain of R6/2 mice. Proc Natl Acad Sci U S A. 2012; 109:E3136–E3145. [PubMed: 23054839]
71. Gilbert LA, et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. Cell. 2013; 154:442–451. [PubMed: 23849981]
72. Snowden AW, Gregory PD, Case CC, Pabo CO. Gene-specific targeting of H3K9 methylation is sufficient for initiating repression in vivo. Curr Biol. 2002; 12:2159–2166. [PubMed: 12498693]
73. Kearns NA, et al. Functional annotation of native enhancers with a Cas9-histone demethylase fusion. Nat Methods. 2015
74. Cong L, Zhou R, Kuo YC, Cunniff M, Zhang F. Comprehensive interrogation of natural TALE DNA-binding modules and transcriptional repressor domains. Nat Commun. 2012; 3:968. [PubMed: 22828628]
75. Sripathy SP, Stevens J, Schultz DC. The KAP1 corepressor functions to coordinate the assembly of de novo HP1-demarcated microenvironments of heterochromatin required for KRAB zinc finger protein-mediated transcriptional repression. Mol Cell Biol. 2006; 26:8623–8638. [PubMed: 16954381]
76. Groner AC, et al. KRAB-zinc finger proteins and KAP1 can mediate long-range transcriptional repression through heterochromatin spreading. PLoS Genet. 2010; 6:e1000869. [PubMed: 20221260]
77. Schultz DC, Ayyanathan K, Negorev D, Maul GG, Rauscher FJ 3rd. SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes Dev. 2002; 16:919–932. [PubMed: 11959841]
78. Reynolds N, et al. NuRD-mediated deacetylation of H3K27 facilitates recruitment of Polycomb Repressive Complex 2 to direct gene repression. EMBO J. 2012; 31:593–605. [PubMed: 22139358]
79. Thakore PI, et al. Highly specific epigenome editing by CRISPR-Cas9 repressors for silencing of distal regulatory elements. Nat Methods. 2015
80. David G, et al. Specific requirement of the chromatin modifier mSin3B in cell cycle exit and cellular differentiation. Proc Natl Acad Sci U S A. 2008; 105:4168–4172. [PubMed: 18332431]
81. Konermann S, et al. Optical control of mammalian endogenous transcription and epigenetic states. Nature. 2013; 500:472–476. [PubMed: 23877069]

82. Rivenbark AG, et al. Epigenetic reprogramming of cancer cells via targeted DNA methylation. Epigenetics. 2012; 7:350–360. [PubMed: 22419067]

83. Stolzenburg S, et al. Targeted silencing of the oncogenic transcription factor SOX2 in breast cancer. Nucleic Acids Res. 2012; 40:6725–6740. [PubMed: 22561374]

84. Siddique AN, et al. Targeted methylation and gene silencing of VEGF-A in human cells by using a designed Dnmt3a-Dnmt3L single-chain fusion protein with increased DNA methylation activity. J Mol Biol. 2013; 425:479–491. [PubMed: 23220192]

85. Stolzenburg S, et al. Stable oncogenic silencing in vivo by programmable and targeted de novo DNA methylation in breast cancer. Oncogene. 2015

86. Mendenhall EM, et al. Locus-specific editing of histone modifications at endogenous enhancers. Nat Biotechnol. 2013; 31:1133–1136. [PubMed: 24013198]

87. Heller EA, et al. Locus-specific epigenetic remodeling controls addiction- and depression-related behaviors. Nat Neurosci. 2014; 17:1720–1727. [PubMed: 25347353]

88. Kungulovski G, et al. Targeted epigenome editing of an endogenous locus with chromatin modifiers is not stably maintained. Epigenetics Chromatin. 2015; 8:12. [PubMed: 25901185]

89. Hathaway NA, et al. Dynamics and memory of heterochromatin in living cells. Cell. 2012; 149:1447–1460. [PubMed: 22704655]

90. Ayyanathan K, et al. Regulated recruitment of HP1 to a euchromatic gene induces mitotically heritable, epigenetic gene silencing: a mammalian cell culture model of gene variegation. Genes Dev. 2003; 17:1855–1869. [PubMed: 12869583]

91. Bhaumik SR, Smith E, Shilatifard A. Covalent modifications of histones during development and disease pathogenesis. Nat Struct Mol Biol. 2007; 14:1008–1016. [PubMed: 17984963]

92. Grimmer MR, et al. Analysis of an artificial zinc finger epigenetic modulator: widespread binding but limited regulation. Nucleic Acids Res. 2014; 42:10856–10868. [PubMed: 25122745]

93. Wu X, et al. Genome-wide binding of the CRISPR endonuclease Cas9 in mammalian cells. Nat Biotechnol. 2014; 32:670–676. [PubMed: 24752079]

94. Kuscu C, Arslan S, Singh R, Thorpe J, Adli M. Genome-wide analysis reveals characteristics of off-target sites bound by the Cas9 endonuclease. Nat Biotechnol. 2014

95. O'Geen H, Henry IM, Bhakta MS, Meckler JF, Segal DJ. A genome-wide analysis of Cas9 binding specificity using ChIP-seq and targeted sequence capture. Nucleic Acids Res. 2015; 43:3389–3404. [PubMed: 25712100]

96. Duan J, et al. Genome-wide identification of CRISPR/Cas9 off-targets in human genome. Cell Res. 2014; 24:1009–1012. [PubMed: 24980957]

97. Tan S, et al. Zinc-finger protein-targeted gene regulation: genomewide single-gene specificity. Proc Natl Acad Sci U S A. 2003; 100:11997–12002. [PubMed: 14514889]

98. Hsu PD, et al. DNA targeting specificity of RNA-guided Cas9 nucleases. Nat Biotechnol. 2013; 31:827–832. [PubMed: 23873081]

99. Fu Y, Sander JD, Reyon D, Cascio VM, Joung JK. Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. Nat Biotechnol. 2014; 32:279–284. [PubMed: 24463574]

100. Zetsche B, Volz SE, Zhang F. A split-Cas9 architecture for inducible genome editing and transcription modulation. Nat Biotechnol. 2015; 33:139–142. [PubMed: 25643054]

101. Bolukbasi MF, Gupta A, Wolfe SA. Creating Precise Scalpels for Genomic Surgery: Strategies to Evaluate and Improve CRISPR/Cas9 Nuclease Performance. Nat Methods. 2016

102. Aach J, Mali P, Church GM. CasFinder: Flexible algorithm for identifying specific Cas9 targets in genomes. bioRxiv. 2014

103. Heigwer F, Kerr G, Boutros M. E-CRISP: fast CRISPR target site identification. Nat Methods. 2014; 11:122–123. [PubMed: 24481216]

104. Doench JG, et al. Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation. Nat Biotechnol. 2014; 32:1262–1267. [PubMed: 25184501]

105. Singh R, Kuscu C, Quinlan A, Qi Y, Adli M. Cas9-chromatin binding information enables more accurate CRISPR off-target prediction. Nucleic Acids Res. 2015

106. Zalatan JG, et al. Engineering complex synthetic transcriptional programs with CRISPR RNA scaffolds. Cell. 2015; 160:339–350. [PubMed: 25533786]

107. Mercer AC, Gaj T, Sirk SJ, Lamb BM, Barbas CF 3rd. Regulation of endogenous human gene expression by ligand-inducible TALE transcription factors. ACS Synth Biol. 2014; 3:723–730. [PubMed: 24251925]
108. Nissim L, Perli SD, Fridkin A, Perez-Pinera P, Lu TK. Multiplexed and Programmable Regulation of Gene Networks with an Integrated RNA and CRISPR/Cas Toolkit in Human Cells. Mol Cell. 2014; 54:698–710. [PubMed: 24837679]
109. Tsai SQ, et al. Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing. Nat Biotechnol. 2014; 32:569–576. [PubMed: 24770325]
110. Kennedy MJ, et al. Rapid blue-light-mediated induction of protein interactions in living cells. Nat Methods. 2010; 7:973–975. [PubMed: 21037589]
111. Polstein LR, Gersbach CA. Light-inducible spatiotemporal control of gene activation by customizable zinc finger transcription factors. J Am Chem Soc. 2012; 134:16480–16483. [PubMed: 22963237]
112. Nihongaki Y, Yamamoto S, Kawano F, Suzuki H, Sato M. CRISPR-Cas9-based photoactivatable transcription system. Chem Biol. 2015; 22:169–174. [PubMed: 25619936]
113. Polstein LR, Gersbach CA. A light-inducible CRISPR-Cas9 system for control of endogenous gene activation. Nat Chem Biol. 2015; 11:198–200. [PubMed: 25664691]
114. Hilton IB, Gersbach CA. Enabling functional genomics with genome engineering. Genome Res. 2015; 25:1442–1455. [PubMed: 26430154]
115. Crocker J, Stern DL. TALE-mediated modulation of transcriptional enhancers in vivo. Nat Methods. 2013; 10:762–767. [PubMed: 23817068]
116. Deng W, et al. Reactivation of developmentally silenced globin genes by forced chromatin looping. Cell. 2014; 158:849–860. [PubMed: 25126789]
117. Phillips JE, Corces VG. CTCF: master weaver of the genome. Cell. 2009; 137:1194–1211. [PubMed: 19563753]
118. Khalil AS, et al. A synthetic biology framework for programming eukaryotic transcription functions. Cell. 2012; 150:647–658. [PubMed: 22863014]
119. Kiani S, et al. CRISPR transcriptional repression devices and layered circuits in mammalian cells. Nat Methods. 2014; 11:723–726. [PubMed: 24797424]
120. Lienert F, et al. Two- and three-input TALE-based AND logic computation in embryonic stem cells. Nucleic Acids Res. 2013; 41:9967–9975. [PubMed: 23982518]
121. Keung AJ, Bashor CJ, Kiriakov S, Collins JJ, Khalil AS. Using targeted chromatin regulators to engineer combinatorial and spatial transcriptional regulation. Cell. 2014; 158:110–120. [PubMed: 24995982]
122. Hou Z, et al. Efficient genome engineering in human pluripotent stem cells using Cas9 from Neisseria meningitidis. Proc Natl Acad Sci U S A. 2013; 110:15644–15649. [PubMed: 23940360]
123. Kleinstiver BP, et al. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. Nature. 2015
124. Briner AE, et al. Guide RNA functional modules direct Cas9 activity and orthogonality. Mol Cell. 2014; 56:333–339. [PubMed: 25373540]
125. Shechner DM, Hacisuleyman E, Younger ST, Rinn JL. Multiplexable, locus-specific targeting of long RNAs with CRISPR-Display. Nat Methods. 2015; 12:664–670. [PubMed: 26030444]
126. Josephs EA, et al. Structure and specificity of the RNA-guided endonuclease Cas9 during DNA interrogation, target binding and cleavage. Nucleic Acids Res. 2015; 43:8924–8941. [PubMed: 26384421]
127. Dahlman JE, et al. Orthogonal gene knockout and activation with a catalytically active Cas9 nuclease. Nat Biotechnol. 2015; 33:1159–1161. [PubMed: 26436575]
128. Kiani S, et al. Cas9 gRNA engineering for genome editing, activation and repression. Nat Methods. 2015; 12:1051–1054. [PubMed: 26344044]
129. Blancafort P, Magnenat L, Barbas CF 3rd. Scanning the human genome with combinatorial transcription factor libraries. Nat Biotechnol. 2003; 21:269–274. [PubMed: 12592412]

130. Park KS, et al. Phenotypic alteration of eukaryotic cells using randomized libraries of artificial transcription factors. Nat Biotechnol. 2003; 21:1208–1214. [PubMed: 12960965]
131. Graf T, Enver T. Forcing cells to change lineages. Nature. 2009; 462:587–594. [PubMed: 19956253]
132. Vierbuchen T, Wernig M. Direct lineage conversions: unnatural but useful? Nat Biotechnol. 2011; 29:892–907. [PubMed: 21997635]
133. Hanna J, et al. Direct cell reprogramming is a stochastic process amenable to acceleration. Nature. 2009; 462:595–601. [PubMed: 19898493]
134. Wapinski OL, et al. Hierarchical mechanisms for direct reprogramming of fibroblasts to neurons. Cell. 2013; 155:621–635. [PubMed: 24243019]
135. Brambrink T, et al. Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. Cell Stem Cell. 2008; 2:151–159. [PubMed: 18371436]
136. Kim K, et al. Epigenetic memory in induced pluripotent stem cells. Nature. 2010; 467:285–290. [PubMed: 20644535]
137. Lister R, et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. Nature. 2011; 471:68–73. [PubMed: 21289626]
138. Kelly TK, De Carvalho DD, Jones PA. Epigenetic modifications as therapeutic targets. Nat Biotechnol. 2010; 28:1069–1078. [PubMed: 20944599]
139. Graslund T, Li X, Magnenat L, Popkov M, Barbas CF 3rd. Exploring strategies for the design of artificial transcription factors: targeting sites proximal to known regulatory regions for the induction of gamma-globin expression and the treatment of sickle cell disease. J Biol Chem. 2005; 280:3707–3714. [PubMed: 15537646]
140. Wilber A, et al. A zinc-finger transcriptional activator designed to interact with the gamma-globin gene promoters enhances fetal hemoglobin production in primary human adult erythroblasts. Blood. 2010; 115:3033–3041. [PubMed: 20190190]
141. Laganiere J, et al. An engineered zinc finger protein activator of the endogenous glial cell line-derived neurotrophic factor gene provides functional neuroprotection in a rat model of Parkinson's disease. J Neurosci. 2010; 30:16469–16474. [PubMed: 21147986]
142. Rebar EJ, et al. Induction of angiogenesis in a mouse model using engineered transcription factors. Nat Med. 2002; 8:1427–1432. [PubMed: 12415262]
143. Dai Q, et al. Engineered zinc finger-activating vascular endothelial growth factor transcription factor plasmid DNA induces therapeutic angiogenesis in rabbits with hindlimb ischemia. Circulation. 2004; 110:2467–2475. [PubMed: 15477407]
144. Yokoi K, et al. Gene transfer of an engineered zinc finger protein enhances the anti-angiogenic defense system. Mol Ther. 2007; 15:1917–1923. [PubMed: 17700545]
145. Magnenat L, Schwimmer LJ, Barbas CF 3rd. Drug-inducible and simultaneous regulation of endogenous genes by single-chain nuclear receptor-based zinc-finger transcription factor gene switches. Gene Ther. 2008; 15:1223–1232. [PubMed: 18528430]
146. Jakovcevski M, Akbarian S. Epigenetic mechanisms in neurological disease. Nat Med. 2012; 18:1194–1204. [PubMed: 22869198]
147. Fierz B, Muir TW. Chromatin as an expansive canvas for chemical biology. Nat Chem Biol. 2012; 8:417–427. [PubMed: 22510649]
148. Azad N, Zahnow CA, Rudin CM, Baylin SB. The future of epigenetic therapy in solid tumours--lessons from the past. Nat Rev Clin Oncol. 2013; 10:256–266. [PubMed: 23546521]
149. Kay MA. State-of-the-art gene-based therapies: the road ahead. Nat Rev Genet. 2011; 12:316–328. [PubMed: 21468099]
150. Burnett JC, Rossi JJ. RNA-based therapeutics: current progress and future prospects. Chem Biol. 2012; 19:60–71. [PubMed: 22284355]
151. Hendel A, et al. Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. Nat Biotechnol. 2015
152. Bernstein BE, et al. Genomic maps and comparative analysis of histone modifications in human and mouse. Cell. 2005; 120:169–181. [PubMed: 15680324]

153. Wang Z, et al. Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. Cell. 2009; 138:1019–1031. [PubMed: 19698979]

154. Wang F, Marshall CB, Ikura M. Transcriptional/epigenetic regulator CBP/p300 in tumorigenesis: structural and functional versatility in target recognition. Cell Mol Life Sci. 2013; 70:3989–4008. [PubMed: 23307074]

155. Dou Y, et al. Regulation of MLL1 H3K4 methyltransferase activity by its core components. Nat Struct Mol Biol. 2006; 13:713–719. [PubMed: 16878130]

156. Shi Y, et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell. 2004; 119:941–953. [PubMed: 15620353]

157. Agger K, et al. UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. Nature. 2007; 449:731–734. [PubMed: 17713478]

158. Bernstein BE, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell. 2006; 125:315–326. [PubMed: 16630819]

159. Di Croce L, Helin K. Transcriptional regulation by Polycomb group proteins. Nat Struct Mol Biol. 2013; 20:1147–1155. [PubMed: 24096405]

160. Cao R, et al. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science. 2002; 298:1039–1043. [PubMed: 12351676]

161. Peters AH, et al. Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. Mol Cell. 2003; 12:1577–1589. [PubMed: 14690609]

162. Rea S, et al. Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature. 2000; 406:593–599. [PubMed: 10949293]

163. Tachibana M, et al. G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. Genes Dev. 2002; 16:1779–1791. [PubMed: 12130538]

164. Chen T, Ueda Y, Dodge JE, Wang Z, Li E. Establishment and maintenance of genomic methylation patterns in mouse embryonic stem cells by Dnmt3a and Dnmt3b. Mol Cell Biol. 2003; 23:5594–5605. [PubMed: 12897133]

165. Liao J, et al. Targeted disruption of DNMT1, DNMT3A and DNMT3B in human embryonic stem cells. Nat Genet. 2015; 47:469–478. [PubMed: 25822089]

166. Tahiliani M, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 2009; 324:930–935. [PubMed: 19372391]

167. Bernstein DL, Le Lay JE, Ruano EG, Kaestner KH. TALE-mediated epigenetic suppression of CDKN2A increases replication in human fibroblasts. J Clin Invest. 2015; 125:1998–2006. [PubMed: 25866970]

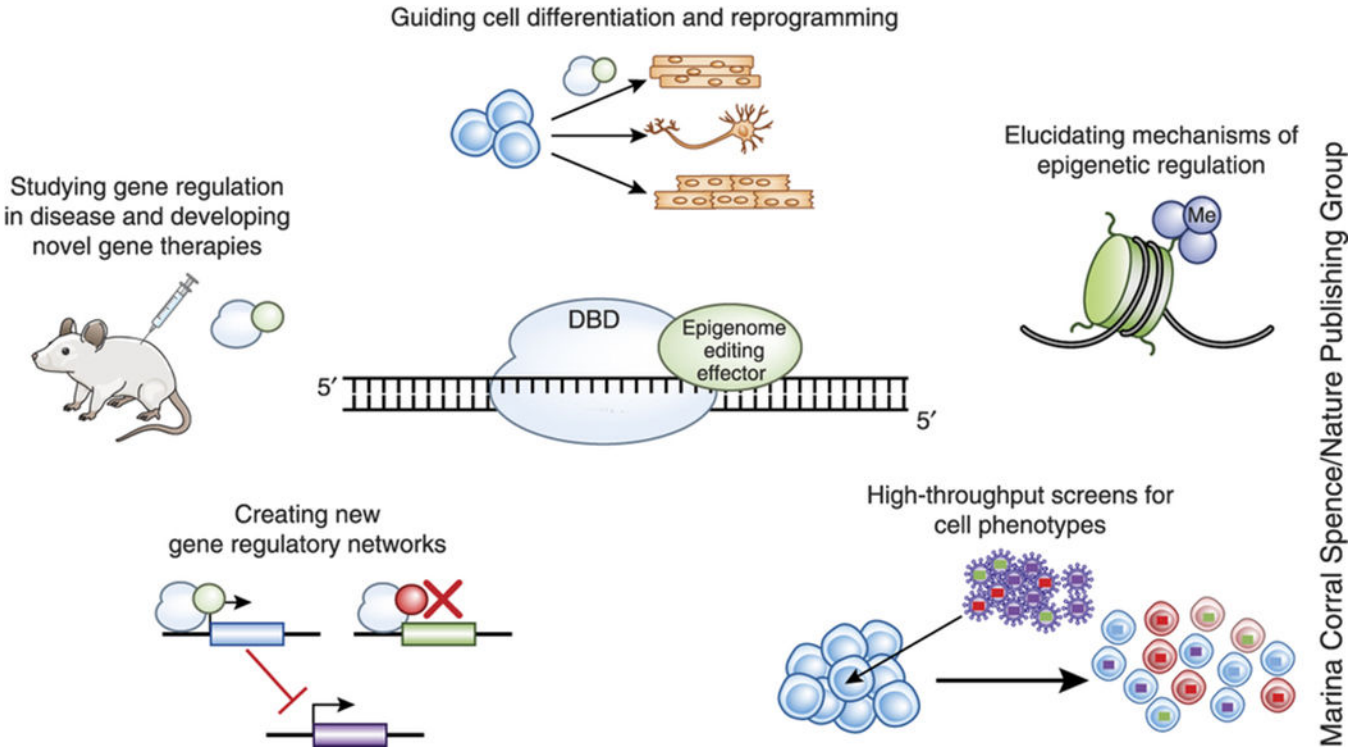

Figure 1. Applications of epigenome editing

Targeted control over epigenetic regulation is achieved by fusing programmable DNA-binding domains (DBDs) to epigenome editing effectors. Engineered epigenome editing proteins can be used to study mechanisms of epigenetic regulation and the contributions of gene regulation to cellular function and disease. Novel gene regulation relationships can be discovered through high-throughput screens performed with gRNA libraries, and new gene regulatory networks can be constructed with orthogonal epigenome editing proteins. Therapeutic applications of epigenome editing include cellular reprogramming and gene therapies that correct aberrant gene expression.

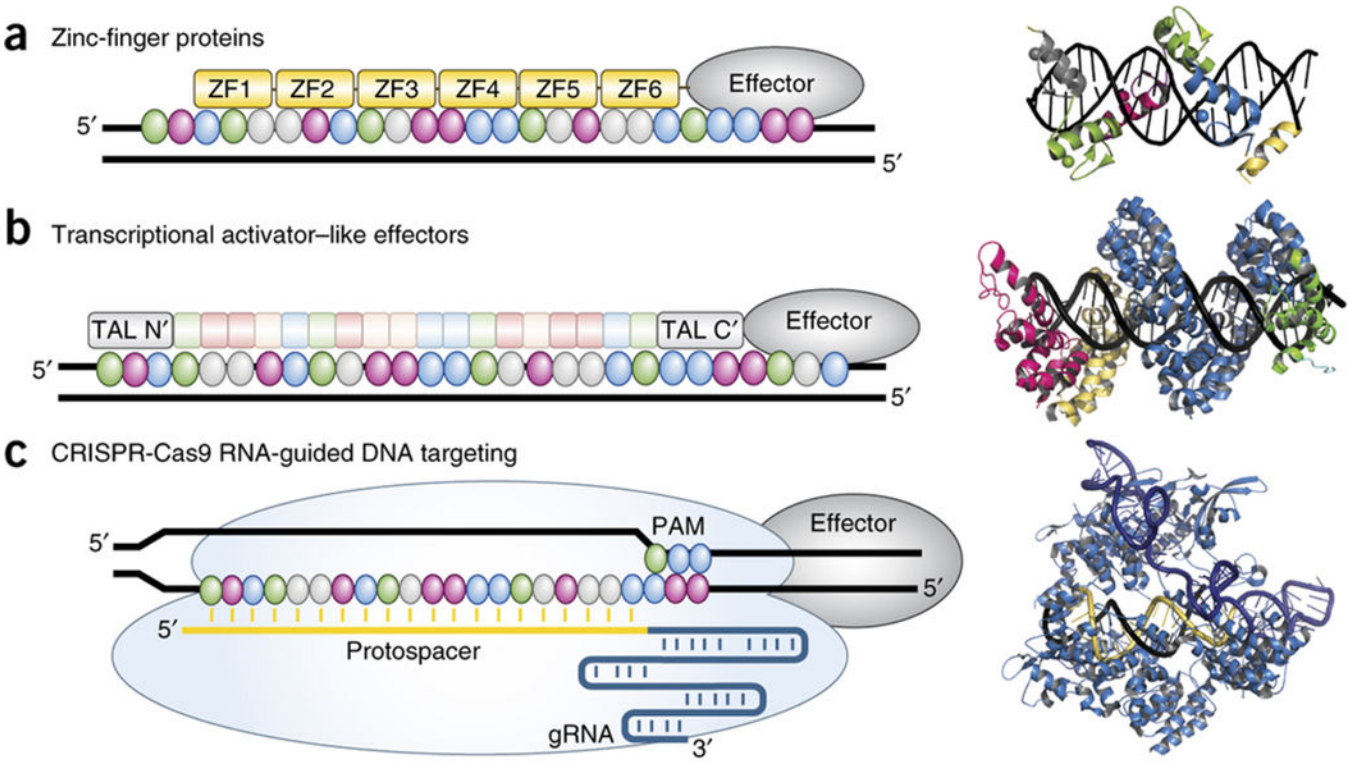

Figure 2. Programmable DNA-binding domains

A) Zinc fingers and B) TALEs are DNA-targeting platforms consisting of protein modules that bind within the major groove of DNA to recognize specific DNA base pairs. Zinc fingers domains recognize 3–4 nucleotide sequences, whereas TALE modules recognize single nucleotides according to a specific code. C) Cas9 is directed to the target site by an engineered guide RNA (gRNA). The gRNA consists of a 20 base pair targeting sequence, which recognizes its complementary genomic sequence via Watson-Crick base-pairing, and a constant region that interacts with the Cas9 protein. In order to bind target DNA, Cas9 also requires the presence of a protospacer-adjacent motif (PAM) immediately following the target sequence. Cas9 derived from *S. pyogenes* recognizes a 5'-NGG-3' PAM. PDB files 2I13, 3UGM, and 4OO8 for the zinc finger protein, TALE, and CRISPR/Cas9 structures, respectively

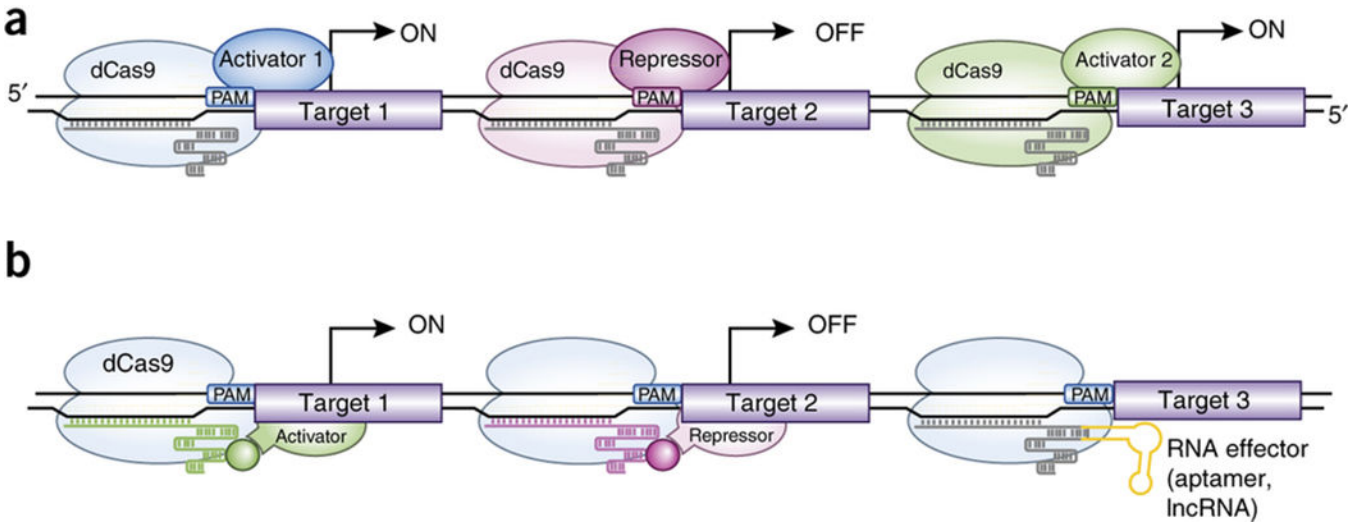

Figure 3. Orthogonal CRISPR/dCas9 systems for complex regulation of distinct genomic targets

A) dCas9 orthologs with distinct PAM requirements can be adapted from different host species or engineered via directed evolution. Through unique gRNAs and PAM recognition sites, dCas9 ortholog-fusions can be used to effect distinct gene regulation events at multiple targets in a single host genome simultaneously. **B)** Alternatively, complex gene regulation events can be coordinated by recruiting epigenetic effectors directly to the gRNA molecule. Protein-binding motifs and long RNAs can be incorporated directly in the stem-loop structure of the gRNA.

Table 1

Representative correlations between epigenetic modifications and gene regulatory activity.

Modification	Associated Genomic Regulatory Elements	Depositing Enzymes	Erasing Enzymes
H3K9ac	Active Promoters/TSSs[4, 152]	GCN5, PCAF[11]	HDACs[10, 153]
H3K27ac	Active Promoters/TSSs; active Enhancers[4, 8]	p300, CBP[8, 11, 154]	HDACs[10, 78, 153]
H3K4me	Active/Bivalent Enhancers[2, 4]	SETDB1[77], MLL family[153, 155]	LSD1[156], UTX, JMJD3[157]
H3K4me3	Active Promoters/TSSs[4, 152]	SETDB1[77], MLL family[155]	LSD1[156], UTX, JMJD3[157]
H3K27me3	Repressed and Bivalent Promoters/Enhancers and some gene bodies[4, 158]	EZH2[159, 160]	LSD1[156], UTX, JMJD3[157]
H3K9me	Repressed loci[4, 161]	SETDB1[77], SUV39H1[162], G9a[163]	LSD1[156], UTX, JMJD3[157]
Low/Absent CpG methylation	Active/Bivalent Promoters and Enhancers[4]	DNMT3A, DNMT3B, DNMT3L, DNMT1 (maintenance)[14, 164, 165]	TET family[166], TDG[15]
High CpG Methylation	Repressed Promoters and Enhancers[4] and often within transcribed gene bodies[14]	DNMT3A, DNMT3B, DNMT3L, DNMT1 (maintenance)[14, 164, 165]	TET family[166], TDG[15]

Table 2

Epigenome editing effectors for gene regulation.

Gene Regulation	Effector	Mechanism of Effect	DNA Binding Domain	Targeted Locus	Epigenomic Modifications
Activation	VP64	Recruitment of transcriptional activators	ZFP[18, 51, 52], TALE[40–42], dCas9[20, 43, 44, 55, 56]	Promoters and enhancers	DNA demethylation; Increased H3K27ac and H3K4me
	p65	Recruitment of transcriptional activators	ZFP[51], TALE[41, 42], dCas9[45, 58]	Promoters	Not evaluated
	p300 catalytic domain	Histone acetyltransferase	ZFP[48], TALE[48], dCas9[48]	Promoters and enhancers	Increased H3K27ac
	TET1 catalytic domain	DNA demethylase	ZFP[53], TALE[53]	Promoters	DNA demethylation
	TDG	DNA demethylase	ZFP[62]	Promoters	DNA demethylation
	Ldb1 self-association domain	Recruits enhancer-associated endogenous Ldb1	ZFP[116]	Promoter and enhancers	Forced looping between promoter and enhancer
	SAM activator (VP64, p65, HSF1)	Recruits transcriptional activators	dCas9[45]	Promoters	Not evaluated
	VPR (VP64, p65, Rta)	Recruits transcriptional activators	ZFP[58], TALE[58], dCas9[58]	Promoters	Not evaluated
Repression	KRAB	Recruitment of histone methyltransferases and deacetylases	ZFP[17], TALE[74], dCas9[71]	Promoters and enhancers	Increased H3K9me3
	Sin3a	Recruitment of histone deacetylases	TALE[74], dCas9[81]	Promoters	Reduced H3K9ac
	LSD1	Histone demethylase	TALE[86], dCas9[73]	Enhancers	Decreased H3K4me
	SUV39H1	Histone methyltransferase	ZFP[72]	Promoters	Increased H3K9me3

Gene Regulation	Effector	Mechanism of Effect	DNA Binding Domain	Targeted Locus	Epigenomic Modifications
	G9A (EHMT2)	Histone methyltransferase	ZFP[72]	Promoters	Increased H3K9me2
	DNMT3a	DNA methyltransferase	ZFP[82]	Promoters	DNA methylation
	DNMT3a-DNMT3L	DNA methyltransferase	ZFP[84], TALE[167]	Promoters	DNA methylation

A variety of activators and repressors have been fused to ZFP, TALE, and dCas9 DNA-targeting platforms in order to regulate gene expression from promoters and regulatory elements.