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Current Opinion in Chemical Biology

Advances of epigenetic editing

Rutger A. F. Gjaltema[1] and Marianne G. Rots[2]

Abstract

Epigenetic editing refers to the locus-specific targeting of epigenetic enzymes to rewrite the local epigenetic landscape of an endogenous genomic site, often with the aim of transcriptional reprogramming. Implementing clustered regularly interspaced short palindromic repeat–dCas9 greatly accelerated the advancement of epigenetic editing, yielding preclinical therapeutic successes using a variety of epigenetic enzymes. ,CRISPR/dCas9 Here, were review the current applications of these epigenetic editing tools in mammalians and shed light on biochemical improvements that facilitate versatile applications.

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Keywords

CRISPR/dCas, Epigenome editing, Targeted DNA methylation, Targeted histone modifications, Expression reprogramming.

Introduction

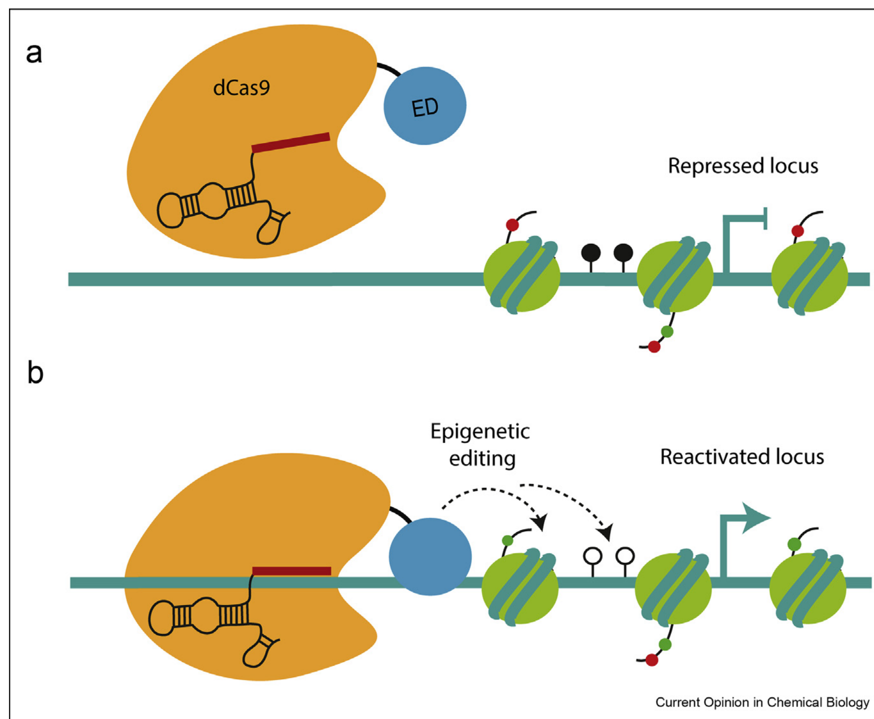
Epigenetic modifications of DNA and histones are known for their multifaceted contributions to transcriptional regulation. As these modifications are faithfully propagated throughout DNA replication [1], they are considered central players in cellular memory of transcriptional states. Many efforts in the last decade have generated a vast understanding of individual epigenetic modifications and their contribution to transcriptional regulation. However, standing questions remain regarding how and which modifications contribute to a certain transcriptional output. Epigenetic editing offers powerful tools to dissect these questions at the endogenous locus level, as well to function as preclinical tools to engineer gene transcription. The foundation of epigenetic editing is formed by the ability to generate fusion proteins of epigenetic enzymes or their catalytic domains (CDs)with programmable DNA-binding platforms such as the clustered regularly interspaced short palindromic repeat (CRISPR) Cas9 to target these to an endogenous locus of choice (Figure 1) [2,3]. The enzymatic fusion protein then dictates the initial deposited modification while subsequent cross-talk within the local chromatin environment likely influences epigenetic and transcriptional output. In this review, we discuss recent advances of epigenetic editing in mammals based on the CRISPR–dCas9 platform, with emphasis on the latest chemical and biotechnological developments to control temporal and on-target activity.

Epigenetic editing of DNA methylation

DNA methylation (5mC) at CpG islands in promoter regions is associated with transcriptional repressive states. Targeting DNA methyltransferases (DNMTs) to those regions would allow target gene repression through inducing *de novo* 5mC. Indeed, the full length or the CD of human or mouse DNMT3A in fusion with dCas9 (dCas9–DNMT3A and dCas9–DNMT3ACD, respectively) introduced *de novo* 5mC up to ∼60% at targeted regions (mostly promoters) which was followed by inhibition of transcription [4]. In a direct comparison between full-length dCas9–DNMT3A and the dCas9–DNMT3ACD, the latter displayed more efficient 5mC activity, whereas dCas9–DNMT3A induced less off-target 5mC [5].

For enhanced targeted 5mC, various approaches have been tested: first, fusions of DNMT3ACD and DNMT3L, a stimulator of DNMT3A catalytic activity [6] (dCas9–DNMT3A3 L), could induce ∼5-fold more 5mC deposition at various target loci than those of dCas9–DNMT3ACD [7], although not nearing a fully methylated state of target regions. Another report confirmed these observations [8]. As a second approach, full-length DNMT3A was applied to an adaptation of the SunTag system [5] to enable the recruitment of multiple copies of scFv-DNMT3ACD fusion proteins (Figure 2B) [9]. Despite multimer recruitment, *de novo* 5mC was lower than that of a dCas9–DNMT3ACD fusion. Alternatively, increasing the nuclear trafficking of dCas9–DNMT3ACD

Figure 1

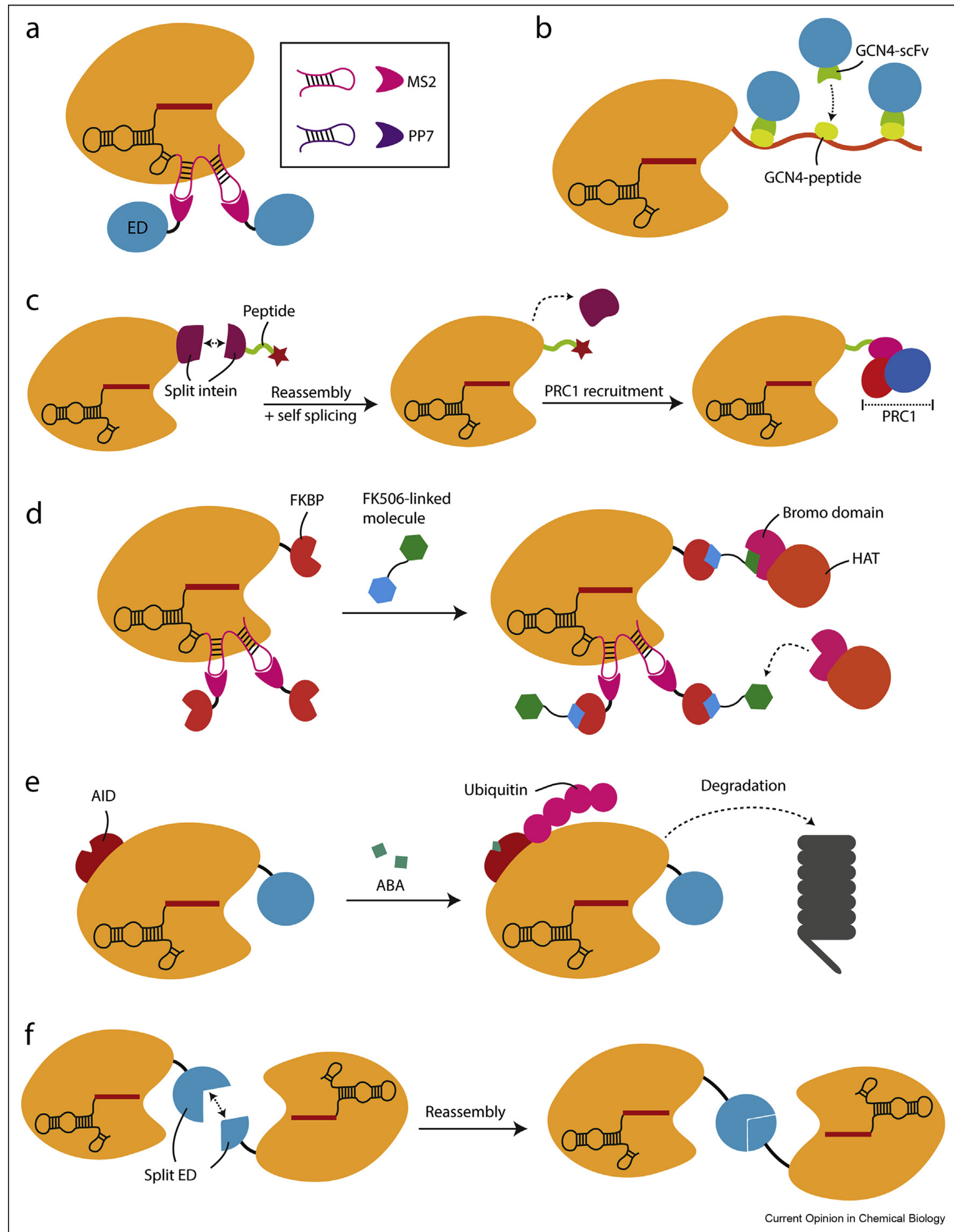


Principle of epigenetic editing. Epigenetic editing with the CRISPR/dCas9 platform involves targeting an effector domain (ED) fused to dCas9 (dCas9-ED) **(a)** Upon sgRNA-mediated recruitment to a target location (e.g. promoter) the dCas9-ED is able to rewrite the local epigenetic state such as histone tails or 5mC (depicted as lollipops) and thereby modify transcriptional activity **(b)**. CRISPR, clustered regularly interspaced short palindromic repeat.

through cloning a nucleoplasmin nuclear localization signal C-terminally of DNMT3ACD improved targeted 5mC from ∼40 to ∼60% [10], suggesting that conventional dCas9−DNMT3ACD fusions experience lower nuclear translocation. In addition, simultaneous targeting of dCas9 fusions with DNMT3A, DNMT3L, and the Krüppel associated box (KRAB) repressor has been successfully applied to induce repressive transcriptional memory [11] and effective epigenetic reprogramming at CCCTC-binding factor (CTCF) binding sites [8]. In addition to mammalian DNMTs, the prokaryotic CpG methyltransferase (M.SssI) has been explored for targeted DNA methylation. Targeting a humanized M.SssI derivative (dCas9−MQ1) introduced high levels of *de novo* 5mC (up to ∼70%) that was widely spread alongside the target region [12]. However, due to extensive off-target 5mC, further modifications to M.SssI are required for it to be exploited for targeted DNA methylation ('Precision epigenetic editing').

As various disease-related genes are repressed by DNA methylation, targeted demethylation would offer unique therapeutic possibilities. Active DNA demethylation is initiated by ten-eleven translocation dioxygenases (TETs) that oxidize 5mC to 5-hydroxymethylcytosine and further intermediates. To initiate targeted demethylation of 5mC, dCas9 fusions with TET1CD were targeted to methylated regions [10,13−17] but with a varied degree of demethylation efficiencies, likely depending on genetic and chromatin context, as well as on delivery efficacy of the dCas9 tools. Even despite partial DNA demethylation of target regions, transcriptional reactivation of the targeted genes was rather weak, likely caused by remaining repressive microenvironment (e.g. deacetylated histones, H3K9me2) [15]. In addition to cultured cells, targeted DNA demethylation has also been applied in preclinical mouse models. For example, targeted demethylation of CGG-repeats within the fragile X mental retardation 1 (FMR1) promoter through lentiviral expression of dCas9-TET1CD in post-mitotic neurons obtained from patient-derived induced pluripotent stem cells (iPSCs) restored *FMR1* expression and neuronal function in culture and was even maintained following engrafting into mouse brains [19]. In another report, *in vivo* lentiviral delivery of dCas9−TET3CD in a kidney fibrosis mouse model resulted in targeted promoter demethylation and subsequent reactivation of two antifibrotic genes, which attenuated kidney fibrosis and restored kidney function [20].

Figure 2



Enhanced CRISPR–dCas9–based epigenetic editors. **(a)** Second-generation CRISPR systems contain RNA aptamers (MS2 or PP7) linked to the sgRNA handle, which recruit their corresponding aptamer coat protein (MCP or PCP, respectively) fused to (epigenetic) effector domains (EDs). **(b)** The SunTag system consists of a dCas9 fusion with GCN4 peptide repeats that enable the recruitment of multiple copies of an anti-GCN4-scFv-effector domain fusion protein. **(c)** Autonomous intein-mediated bioconjugation of a peptide (e.g. UNC3866, a ligand for CBX proteins) to dCas9 (dCas9–UNC3866) allows targeted recruitment of the endogenous PRC1 complex. **(d)** CRISPR-CEM contains either a dCas9-FKBP fusion or recruits FKBP through sgRNA-aptamers (refer **(a)**). By supplementing a FK506 derivative that additionally contains a bromodomain ligand, this system recruits endogenous binding partners (HATs) to the FKBP at CRISPR–dCas9. **(e)** A degron (AID) fusion with dCas9 can be destabilized upon supplementing its ligand ABA. Subsequent ubiquitination of the fusion protein leads to rapid proteasomal degradation. **(f)** The split effector domain approach involves splitting an epigenetic effector domain in minimum of two compatible subdomains that autonomously reassembled based on proximity at their target sites. CRISPR, clustered regularly interspaced short palindromic repeat; HAT, histone acetyltransferase; ABA, abscisic acid.; MCP, MS2 coat protein; PCP, PP7 coat protein; CEM, chemical epigenetic modifier.

To further improve on the targeted demethylation effects, adaptations of the dCas9−TET1 system have been tested. Josipovic et al. [10] performed a side-by-side comparison of the TET1 fusion orientation and found an N-terminal fusion to dCas9 to be ∼2-fold more efficient than the C-terminal fusion. To maximize local DNA demethylation activity, Xu et al. [16] tethered TET1CD to the MS2 coat protein and combined these with MS2−aptamer sgRNAs and the conventional dCas9−TET1CD fusion (Figure 2A). As such, targeting triple TET1CDs to a target location resulted in ∼2-fold more DNA demethylation. In addition, applying TET1CD to a repurposed SunTag system (Figure 2B) to recruit three copies of TET1CD allowed robust demethylation of target genes up to 4-fold higher than that of the conventional dCas9−TET1CD, although transcriptional reactivation was still minimal [18]. Together these reports indicate that single fusions of dCas9−TET1CD are often not effective enough to fully overcome remaining repressive chromatin and reactivate target gene expression. This point has been addressed by a combinatorial approach targeting dCas9−TET1CD together with dCas9−VP64 to *Sox1*, which resulted in synergistic gene reactivation while the individual fusions hardly had any effect [17].

Epigenetic editing of histones

Next to DNA methylation, gene expression is strongly associated with histone modifications (e.g. H3K4me1/H3K27ac for active enhancers; H3K4me3/H3K27ac for active promoters; H3K79me/H3K36me2/3 for transcribed gene bodies; and H3K9me2/3 or H3K27me3 for silenced genes). The causative effects of certain histone modifications in modulating gene expression could clearly be demonstrated by epigenetic editing: targeting histone lysine methyltransferases (HKMTs) of H3K9me2/3 (SUV39H1, G9A) performed particularly well in repressing gene expression, while H3K27me3 (EZH2) performed slightly less [21]. However, the transcriptional effects of these fusions were highly context (gene and cell line) dependent. In proliferating cells, simultaneous targeting of dCas9−DNMT3A3 L and dCas9−EZH2 to *HER2* induced stable repressive chromatin (up to 50 days), which was not observed for the combination dCas9−DNMT3A3 L and dCas−KRAB [22]. Furthermore, targeting EZH2 with the PP7−PCP aptamer recruitment system (Figure 2A) induced strong H3K27me3 and repressed target gene expression up to 65% [23].

Next to writing repressive modifications, removing activating modifications offers another opportunity to repress endogenous loci. Indeed, transiently targeting dCas9−HDAC3 adjacent to H3K27ac peaks at promoters of three genes not only removed histone acetylation [24] but induced low, yet significant, target gene re-pression in a context-dependent manner. Surprisingly, in cells stably expressing dCas9−HDAC3, only one of the three targeted genes showed strong deacetylation of H3K27ac and repression.

To interrogate promoter−enhancer interactions, several laboratories targeted a dCas9 fusion with the histone acetyltransferase p300 (dCas9-p300) to induce H3K27ac at enhancers, either targeting single elements [25−27] or delivered as pooled CRISPR screens [28], which indeed affected transcriptional activation of neighboring loci. Furthermore, Yan et al [29] targeted a dCas9 fusion of the H3K4me1 HKMT MLL3 SET domain (dCas9−MLL3SET) to the *Sox2* super enhancer in MLL3/4 double knockout mouse embryonic stem cells (ESCs) and induced *de novo* H3K4me1. Following this observation, they detected elevated cohesin levels, suggesting that MLL3-catalyzed H3K4me1 facilitates cohesion complex formation at enhancers and subsequent promoter interactions. Alternatively, targeting a H3K4me1/2 demethylase fusion (dCas9−LSD1) to a *Tbx3* upstream enhancer in ESCs lead to reduction of enhancer mark H3K4me2 and a reduced *Tbx3* transcription [30]. Whereas, targeting dCas9−LSD1 to the *Tbx3* promoter did not lead to a repressive chromatin signature, nor *Tbx3* transcriptional repression, suggesting indeed an enhancer-specific mode of action for H3K4me2.

To gain more insight into the role of promoter H3K4me3 in transcriptional activation of repressed genes, we have targeted the H3K4me3 HKMT PRDM9 (dCas9−PRDM9) to the transcription start site of several repressed target genes. Particularly promoters with low levels of 5mC could be reactivated, although to a low extend [31]. Cotargeting with the H3K79 HKMT DOT1L slightly improved target gene expression, indicating an additive behavior of both H3K4me3 and H3K79me2/3 in transcriptional activation.

Small molecule−assisted epigenetic editing

Conditional control over CRISPR−dCas9 activity benefits research related to transcriptional memory and could potentially improve specificity in therapeutic applications. In pioneering work, Liszczak et al [32] implemented bioconjugation of a synthetic PRC1 chromodomain ligand (UNC3866) to dCas9 through intein-directed protein trans-splicing (Figure 2C) to recruit endogenous PRC1 complex members to target genes. Building upon this methodology, Chiarella et al. [33] repurposed the FK506 binding domain of FKBP12 (FKBP) and its ligand FK506 as a chemical recruitment system of endogenous histone acetylation machineries. For instance, a FK506-linked BRD4 ligand (CEM87) could subsequently bind to dCas9−FKBP and through BDR4 binding subsequently recruit p300 to target sites (Figure 2D). Compared with a dCas9-p300 fusion, this

CRISPR–CEM system activated target gene transcription more effectively.

Instead of controlling recruitment, controlling protein stability of dCas9 fusions offers another level of conditional control. As such, the auxin-inducible degron (AID) system has been implemented to dCas9–p300 targeting. Upon supplementing abscisic acid (ABA), AID binds to exogenous expressed plant-specific F-box protein TIR1 and together recruits an E3 ubiquitin ligase complex that targets AID–dCas9–p300 for proteosomal degradation (Figure 2E). With this system, Kuscu et al. [25] reactivated targeted enhancers that upon supplementing ABA realized a rapid (up to 12 h) degradation of AID–dCas9–p300 followed by a decline in H3K27ac and transcriptional activity of the associated gene.

Precision epigenetic editing

For CRISPR/(d)Cas9 to properly bind DNA, it first scans the genome for sgRNA seed complementary sites. Meanwhile, any dCas9-tethered epigenetic enzyme could potentially perform off-target editing, depending on the enzyme and its activity. Indeed, in a 5mC depleted but maintenance competent mouse ESC line, widespread off-target 5mC by dCas9–DNMT3ACD was observed after targeting a selection of CpG islands [34]. It appeared that the DNMT activity of the fusion protein is a key contributor to off-target methylation, as a Dnmt3a mutant (R832E) that affects DNMT multimerization and catalytic activity resulted in lower off-target 5mC and confined methylation to the vicinity of the targeted sites [7]. Analogous to this, targeting a less active variant (Q147L, affecting DNA binding) of M.SssI as a fusion with dCas9 (dCas9–MQ1^{Q147L}) resulted in no obvious off-target 5mC, offering an advantage over its wild-type dCas9-MQ1 both *in vivo* as in cell cultures [12].

Another approach to enhance on-target epigenetic editing is to target split epigenetic enzymes, which are designed and expressed in at least two domains that upon proximity reassemble into a functional enzyme (Figure 2F). As such, two separate dCas9 fusions, with each a split M.SssI domain (dCas9–MN/MC), [35] were targeted to the *SALL2* promoter and induced 5mC as effective as dCas9–DNMT3ACD. Indeed, compared with dCas9–DNMT3ACD and negative controls, background methylation by dCas9–MN/MC splits was barely detected, although this was not assessed genomewide. The split-enzyme approach is limited by a thorough understanding of the 3D structure of an epigenetic enzyme. Only one other split epigenetic enzyme has been recently reported, although not applied yet for epigenetic editing, namely a split-TET2CD system that upon chemical-induced proximity could perform DNA demethylation at approximately the same efficiency as wild-type TET2CD [36].

Conclusions

By repurposing the CRISPR–Cas9 platform, mainstream application of epigenetic editing has become more feasible. However, there are still some hurdles that need to be overcome for epigenetic editing to become a straightforward tool for manipulating the epigenome. For instance, targeting CRISPR–dCas9 to heterochromatin regions is technically challenging due to steric hindrance with nucleosomes and other heterochromatin-associated proteins [37]. In addition, various reports indicated that local (epi)genetic contexts play an important role in successes and failures on rewriting a target locus. Furthermore, suboptimal delivery methods greatly contribute to the variable results of epigenetic editing. Despite these current limitations, application of CRISPR–dCas9-based epigenetic editing has made tremendous progress with several epigenetic domains seem to function fairly well in a variety of tested cell types, as well as *in vivo*. Together this provides a solid framework to further shape the epigenetic editing toolbox for future applications in, for instance, clinical settings.

Declaration of competing interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: M.G.R. reports serving as a consultant to Sangamo Therapeutics, Richmond, CA.

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References

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

- * of special interest
- ** of outstanding interest

1. Alabert C, Groth A: **Chromatin replication and epigenome maintenance**. *Nat Rev Mol Cell Biol* 2012, **13**:153-167.
2. Thakore PI, Black JB, Hilton IB, Gersbach CA: **Editing the epigenome: technologies for programmable transcription and epigenetic modulation**. *Nat Methods* 2016, **13**:127-137.
3. Geel TM, Ruiters MHJ, Cool RH, Halby L, Voshart DC, Andrade Ruiz L, Niezen-Koning KE, Arimondo PB, Rots MG: **The past and presence of gene targeting: from chemicals and DNA via proteins to RNA**. *Philos Trans R Soc Lond B Biol Sci* 2018, **373**.
4. Lei Y, Huang YH, Goodell MA: **DNA methylation and demethylation using hybrid site-targeting proteins**. *Genome Biol* 2018, **19**:187.
5. Huang YH, Su J, Lei Y, Brunetti L, Gundry MC, Zhang X, Jeong M, Li W, Goodell MA: **DNA epigenome editing using CRISPR-Cas SunTag-directed DNMT3A**. *Genome Biol* 2017, **18**:176.

6. Gowher H, Liebert K, Hermann A, Xu G, Jeltsch A: **Mechanism of stimulation of catalytic activity of Dnmt3A and Dnmt3B DNA-(cytosine-C5)-methyltransferases by Dnmt3L**. *J Biol Chem* 2005, **280**:13341–13348.

7. * Stepper P, Kungulovski G, Jurkowska RZ, Chandra T, Krueger F, Reinhardt R, Reik W, Jeltsch A, Jurkowski TP: **Efficient targeted DNA methylation with chimeric dCas9-Dnmt3a-Dnmt3L methyltransferase**. *Nucleic Acids Res* 2017, **45**:1703–1713.
Were the first to apply a chimeric fusion approach by fusing DNMT3A together with its catalytic stimulator (DNMT3L) to dCas9 which further enhanced the quantitative output of targeted DNA methylation.

8. Tarjan DR, Flavahan WA, Bernstein BE: **Epigenome editing strategies for the functional annotation of CTCF insulators**. *Nat Commun* 2019, **10**:4258.

9. 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.

10. Josipovic G, Tadic V, Klasic M, Zanki V, Beceheli I, Chung F, Ghantous A, Keser T, Madunic J, Boskovic M, *et al.*: **Antagonistic and synergistic epigenetic modulation using orthologous CRISPR/dCas9-based modular system**. *Nucleic Acids Res* 2019, **47**:9637–9657.

11. Amabile A, Migliara A, Capasso P, Biffi M, Cittaro D, Naldini L, Lombardo A: **Inheritable silencing of endogenous genes by hit-and-run targeted epigenetic editing**. *Cell* 2016, **167**: 219–232 e214.

12. Lei Y, Zhang X, Su J, Jeong M, Gundry MC, Huang YH, Zhou Y, Li W, Goodell MA: **Targeted DNA methylation in vivo using an engineered dCas9-MQ1 fusion protein**. *Nat Commun* 2017, **8**:16026.

13. Liu XS, Wu H, Ji X, Stelzer Y, Wu X, Czauderna S, Shu J, Dadon D, Young RA, Jaenisch R: **Editing DNA methylation in the mammalian genome**. *Cell* 2016, **167**:233–247. e217.

14. Choudhury SR, Cui Y, Lubecka K, Stefanska B, Irudayaraj J: **CRISPR-dCas9 mediated TET1 targeting for selective DNA demethylation at BRCA1 promoter**. *Oncotarget* 2016, **7**: 46545–46556.

15. Kang JG, Park JS, Ko JH, Kim YS: **Regulation of gene expression by altered promoter methylation using a CRISPR/Cas9-mediated epigenetic editing system**. *Sci Rep* 2019, **9**.

16. Xu XX, Tao YH, Gao XB, Zhang L, Li XF, Zou WG, Ruan KC, Wang F, Xu GL, Hu RG: **A CRISPR-based approach for targeted DNA demethylation**. *Cell Discovery* 2016, **2**.

17. Baumann V, Wiesbeck M, Breunig CT, Braun JM, Koferle A, Ninkovic J, Gotz M, Stricker SH: **Targeted removal of epigenetic barriers during transcriptional reprogramming**. *Nat Commun* 2019, **10**:2119.

18. Morita S, Noguchi H, Horii T, Nakabayashi K, Kimura M, Okamura K, Sakai A, Nakashima H, Hata K, Nakashima K, *et al.*: **Targeted DNA demethylation in vivo using dCas9-peptide repeat and scFv-TET1 catalytic domain fusions**. *Nat Biotechnol* 2016, **34**:1060–1065.

19. Liu XS, Wu H, Krzisch M, Wu X, Graef J, Muffat J, Hnisz D, Li CH, Yuan B, Xu C, *et al.*: **Rescue of fragile X syndrome neurons by DNA methylation editing of the FMR1 gene**. *Cell* 2018, **172**: 979–992. e976.

20. ** Xu X, Tan X, Tampe B, Wilhelmi T, Hulshoff MS, Saito S, Moser T, Kalluri R, Hasenfuss G, Zeisberg EM, *et al.*: **High-fidelity CRISPR/Cas9- based gene-specific hydroxymethylation rescues gene expression and attenuates renal fibrosis**. *Nat Commun* 2018, **9**:3509.
First ever successful *in vivo* delivery of dCas9-mediated epigenetic editing in a pre-clinical setting using a murine disease model. The authors targeted a high fidelity variant of dCas9 fused to TET3CD to several disease-related genes, which enhanced on-target activity and subsequently attenuated disease phenotypes.

21. O'Geen H, Ren C, Nicolet CM, Perez AA, Halmai J, Le VM, Mackay JP, Farnham PJ, Segal DJ: **dCas9-based epigenome editing suggests acquisition of histone methylation is not sufficient for target gene repression**. *Nucleic Acids Res* 2017, **45**:9901–9916.

22. * O'Geen H, Bates SL, Carter SS, Nisson KA, Halmai J, Fink KD, Rhie SK, Farnham PJ, Segal DJ: **Ezh2-dCas9 and KRAB-dCas9 enable engineering of epigenetic memory in a context-dependent manner**. *Epigenet Chromatin* 2019, **12**:26.
Addressed the hit-and-go potential, as well as the context dependency, of epigenetic editing by introducing targeted repressive histone modifications at promoters of target genes. Repression of one target gene was maintained up to 50 days in proliferating cells.

23. Chen X, Wei M, Liu X, Song S, Wang L, Yang X, Song Y: **Construction and validation of the CRISPR/dCas9-EZH2 system for targeted H3K27Me3 modification**. *Biochem Biophys Res Commun* 2019, **511**:246–252.

24. Kwon DY, Zhao YT, Lamonica JM, Zhou Z: **Locus-specific histone deacetylation using a synthetic CRISPR-Cas9-based HDAC**. *Nat Commun* 2017, **8**:15315.

25. ** Kuscu C, Mammeadov R, Czikora A, Unlu H, Tufan T, Fischer NL, Arslan S, Bekiranov S, Kanemaki M, Adli M: **Temporal and spatial epigenome editing allows precise gene regulation in mammalian cells**. *J Mol Biol* 2019, **431**:111–121.
Implemented a small molecule-inducible degron system in order to both quantitatively and temporally control the protein levels of a dCas9-p300 fusion and thereby its epigenetic editing actvitiy.

26. Hilton IB, D'Ippolito AM, Vockley CM, Thakore PI, Crawford GE, Reddy TE, Gersbach CA: **Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers**. *Nat Biotechnol* 2015, **33**. 510-U225.

27. Chen TJ, Gao D, Zhang RS, Zeng GH, Yan H, Lim EJ, Liang FS: **Chemically controlled epigenome editing through an inducible dCas9 system**. *J Am Chem Soc* 2017, **139**:11337–11340.

28. Klann TS, Black JB, Chellappan M, Safi A, Song LY, Hilton IB, Crawford GE, Reddy TE, Gersbach CA: **CRISPR-Cas9 epigenome editing enables high-throughput screening for functional regulatory elements in the human genome**. *Nat Biotechnol* 2017, **35**:561–568.

29. * Yan J, Chen SA, Local A, Liu T, Qiu Y, Dorighi KM, Preissl S, Rivera CM, Wang C, Ye Z, *et al.*: **Histone H3 lysine 4 monomethylation modulates long-range chromatin interactions at enhancers**. *Cell Res* 2018, **28**:204–220.
Used epigenetic editing to enhance the molecular understanding of enhancer–promoter interactions by writing the enhancer mark H3K4me1 at a target enhancer within H3K4me1 depleted cells. This work offers a good example how epigenetic editing can be used to assist in dissecting standing questions in transcriptional regulation and chromatin architecture.

30. Kearns NA, Pham H, Tabak B, Genga RM, Silverstein NJ, Garber M, Maehr R: **Functional annotation of native enhancers with a Cas9-histone demethylase fusion**. *Nat Methods* 2015, **12**:401–403.

31. Cano-Rodriguez D, Gjaltema RA, Jilderda LJ, Jellema P, Dokter-Fokkens J, Ruiters MH, Rots MG: **Writing of H3K4Me3 overcomes epigenetic silencing in a sustained but context-dependent manner**. *Nat Commun* 2016, **7**:12284.

32. Liszczak GP, Brown ZZ, Kim SH, Oslund RC, David Y, Muir TW: **Genomic targeting of epigenetic probes using a chemically tailored Cas9 system**. *Proc Natl Acad Sci USA* 2017, **114**: 681–686.

33. ** Chiarella AM, Butler KV, Gryder BE, Lu D, Wang TA, Yu X, Pomella S, Khan J, Jin J, Hathaway NA: **Dose-dependent activation of gene expression is achieved using CRISPR and small molecules that recruit endogenous chromatin machinery**. *Nat Biotechnol* 2020, **38**:50–55.
Realized a chemically-inducible dCas9 activator system that recruits endogenous histone acetylation complexes to target sites and which furthermore realizes dose-dependent control over transcriptional activation levels.

34. ** Galonska C, Charlton J, Mattei AL, Donaghey J, Clement K, Gu H, Mohammad AW, Stamenova EK, Cacchiarelli D, Klages S, *et al.*: **Genome-wide tracking of dCas9-methyltransferase footprints**. *Nat Commun* 2018, **9**:597.
First extensive investigation of genome-wide off-target DNA methylation by dCas9-DNMT3ACD. For this purpose the authors used DNA methylation depleted but maintenance competent mouse ESCs and observed ubiquitous activity of dCas9-DNMT3ACD with gain of 5mC

predominantly found at prior methylated sites as in wildtype mouse ESCs.

35. Xiong T, Meister GE, Workman RE, Kato NC, Spellberg MJ, Turker F, Timp W, Ostermeier M, Novina CD: **Targeted DNA methylation in human cells using engineered dCas9-methyltransferases**. *Sci Rep* 2017, **7**:6732.

36. Lee M, Li J, Liang Y, Ma G, Zhang J, He L, Liu Y, Li Q, Li M, Sun D, *et al.*: **Engineered split-TET2 enzyme for inducible epigenetic remodeling**. *J Am Chem Soc* 2017, **139**:4659–4662.

37. Verkuijl SA, Rots MG: **The influence of eukaryotic chromatin state on CRISPR-Cas9 editing efficiencies**. *Curr Opin Biotechnol* 2019, **55**:68–73.