Please cite this article in press as: Liu and Jaenisch, Editing the Epigenome to Tackle Brain Disorders, Trends in Neurosciences (2019), https://doi.org/10.1016/j.tins.2019.10.003

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Opinion

Editing the Epigenome to Tackle Brain Disorders

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Genetic studies of epigenetic modifiers such as DNA methyltransferases and histone acetyltransferases have revealed a critical role for epigenetic regulation during brain development and function. Alteration of epigenetic modifications have been documented in a variety of brain disorders, including neurodevelopmental, psychiatric, and neurodegenerative diseases. Development of epigenome editing tools enables a functional dissection of the link between altered epigenetic changes and disease outcomes. Here, we review the development of epigenome editing tools, summarize proof of concept applications focusing on brain disease-associated genes, and discuss the promising application and challenges of epigenome editing to tackle brain disorders.

Highlights

Current understanding of the epigenome is based on the systematic profiling of the epigenetic landscape in multiple tissues during development and pathogenesis of diseases.

Development and application of epigenome editing tools has been accelerated by the discovery of the CRISPR/Cas9 system, allowing for locus-specific targeting of epigenetic effectors to any given genomic locus, through easy design and assembly of single guide RNA.

Epigenome editing tools enable us to distinguish correlation and causality of epigenetic events associated with brain diseases.

Off-target effects and stability of epigenome editing are two major considerations for any therapeutic application.

Expansion of editing tools by introduction of other orthologs in the CRISPR/Cas systems will allow for simultaneous modifications of multiple genomic loci at different layers of epigenetic marks, enabling us to tackle polygenic disorders.

The Concept of the Epigenome

Traditionally, epigenetics referred to the study of heritable changes of gene expression in the absence of altering the DNA sequence during cell proliferation and development. This definition is rapidly evolving with the progression in the understanding of molecular mechanisms, including but not limited to DNA methylation, histone modifications, noncoding RNA, and 3D chromatin structures, responsible for a variety of epigenetic phenotypes observed in monocellular organisms such as yeast to multicellular organisms like humans [1]. It was proposed that epigenetic mechanisms enable the genome to integrate both developmental and environmental signals [2]. Emerging evidence suggests a critical role of epigenetic regulation in the nervous system [3]. For instance, DNA methylation and chromatin structure undergo dynamic changes upon neuronal activity, contributing to a variety of brain functions such as learning and memory [4–7]. Therefore, the scope of epigenetics is no longer limited to dividing cells. With advances in next-generation sequencing technologies, the study of epigenetics has been expanded into epigenome analyses focused on a multitude of chemical modifications and physical properties of the genome that control the functions of the genome [8]. To systemically study the function and contribution of the epigenome during development and pathogenesis, the Roadmap Epigenomics Mapping Consortium was launched by the National Institutes of Health (NIH) [9], and an international effort to assay epigenomes commenced in 2010 in the form of the International Human Epigenome Consortium [10]. Comprehensive molecular maps of the epigenome for many cell types is the basis towards understanding the role of the epigenome. A major challenge in the field remains in the establishment of a causal relationship between epigenetic maps and phenotypes.

In this article, we summarize recent progress on the development of epigenome editing tools, discuss several proof of concept applications of these tools to study the epigenetic events in brain disorders, and highlight key challenges and opportunities in the field.

Development of Epigenome Editing Tools

Epigenome editing enables a mechanistic dissection of the functional significance of individual epigenetic events. Over the years, a number of different epigenetic editing tools were used to change DNA methylation at specific sites. In 1997, soon after the application of zinc finger proteins (ZFP) in genome engineering, the fusion of ZFP with a bacterial DNA methylation enzyme was developed to edit DNA methylation *in vitro* [11] (Figure 1). In 2013 and 2015, the transcription activator-like effector (TALE) was used to fuse with the Ten-eleven translocation (TET1) hydroxylase catalytic domain or the DNA methyl transferase 3a (DNMT3a) catalytic domain to mediate targeted demethylation or methylation, respectively, in cells [12,13]. Since CRISPR (clustered regularly interspaced short palindromic repeats) Cas (CRISPR associated) became available for genetic manipulations [14,15], our laboratory pioneered a CRISPR-based DNA methylation editing system using the fusion of a catalytically dead Cas9 (dCas9) with DNMT3a or TET1 catalytic domains, allowing for writing or erasing

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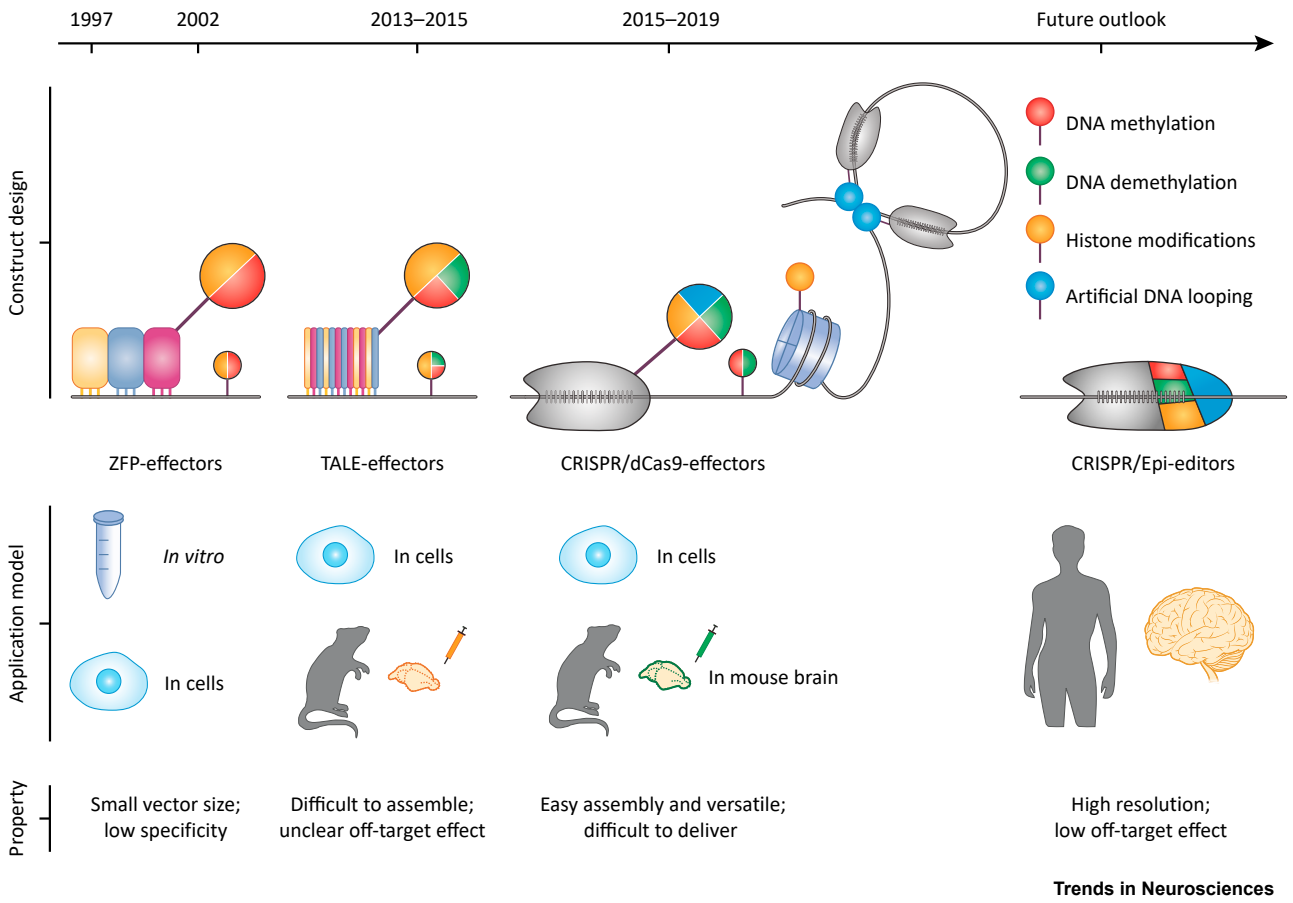

Figure 1. The Development and Application of Epigenome Editing Tools.

The first sequence-specific DNA methylation tool was the fusion of a bacterial methyl transferase with zinc finger proteins (ZFP), recognizing a nine-nucleotide DNA sequence *in vitro* in 1997 [11]. ZFP was also fused with catalytical domains of histone methyl transferases to edit histone H3K9 methylation in cells in 2002 [65] and in mouse brains in 2014 [28]. Then the catalytical domains of DNA methyl transferase 3a (DNMT3a) and Ten-eleven translocation hydroxylase (TET1) or histone demethylase (LSD1) were fused after transcription activator–like effector (TALE), which normally consist of 12–31 arrays to mediate DNA methylation or histone methylation editing in cells [12,13,26]. Introduction of the catalytically dead clustered regularly interspaced short palindromic repeats (CRISPR) associated (Cas9), namely dCas9, tremendously expanded the epigenome editing toolbox, including DNA methylation [16,19,38,66–68], histone modifications [20,21,25,26], DNA looping mediated by dimerization protein pairs [30–32], and nuclear organization [33]. We predict that a conformationally activatable CRISPR/Epi-editor, in which the epigenetic enzymatic domains are functionally integrated into one of the nuclease domains of Cas9, will have higher resolution and lower off-target effect to possibly enable clinical applications in humans. It should be noted that the ZFP-based method was also used to induce DNA loops [69,70] (details not shown).

DNA methylation in the mammalian genome [16,17]. The ease of assembly and versatility of the CRISPR/dCas9 system to target epigenetic effector protein domains to any given locus allowed this approach to become available to the broad scientific community. As summarized in Table 1, the epigenome editing toolbox has been expanded to histone modifications [18–29], DNA looping [30–32], and nuclear organization [33] in the last few years. Comprehensive review articles were published elsewhere [34,35]. In addition, RNA editing has recently been enabled by the application of type VI CRISPR-Cas systems containing the programmable RNA-guided ribonuclease Cas13 [36]. Fusion of a catalytically dead Cas13 (dCas13) with adenosine deaminase acting on RNA (ADAR) or derivative as an effector protein allows for conversion of adenosine to inosine (guanosine-like complementarity) or cytosine to uracil [36,37]. It is feasible to apply dCas13-ADAR to edit noncoding RNAs, another important class of epigenetic regulation.

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DNA targeting module	Effector proteins	Applications	Refs
ZFP (zinc finger protein)	M. SssI (bacterial DNA methyltransferase)	Targeted DNA methylation *in vitro*	[11]
	SUV39H1 and G9A catalytic domains (histone methyltransferases)	Targeted histone H3K9 methylation at the *VEGF-A* locus in cells	[65]
	G9A catalytic domain (histone methyltransferase) and p65 of NF-κB (to induce histone acetylation)	Targeted histone modifications at *FosB* locus in nucleus accumbens of mouse brain	[28]
	Full length or the self-association domain of Ldb1 (transcription cofactor)	To induce chromatin looping at *β-globin* locus or fetal *ϒ-globin* locus	[69,70]
TALE (transcription activator–like effector)	TET1 catalytic domain (Ten-eleven translocation methylcytosine dioxygenase)	Targeted DNA demethylation in cells	[12]
	DNMT3a catalytic domain and DNMT3l (mammalian DNA methyltransferase complex)	Targeted DNA methylation of *CDKN2A* gene in cells	[13]
	LSD1 (histone demethylase)	Targeted histone H3K4 demethylation at multiple enhancers in cells	[26]
CRISPR/dCas9 (clusters of regularly interspaced short palindromic repeats)	TET1 catalytic domain or DNMT3a full length protein or catalytic domain	Targeted DNA methylation and demethylation at multiple genomic loci; Targeted DNA demethylation of a reporter in mouse brain	[16]
	DNMT3a catalytic domain	Targeted DNA methylation in multiple genome loci in cells	[67]
	TET1 catalytic domain with modified sgRNA containing two MS2 RNA elements	Targeted DNA demethylation towards activation of transcriptions in cells	[68]
	TET1 catalytic domain	Targeted DNA demethylation at *BRCA1* locus	[66]
	dCas9-GCN4 and scFv-DNMT3a (SunTag system)	Targeted DNA methylation in cells with high specificity and low off-target effect	[19]

Table 1. Development and Application of Epigenome Editing Tools

(Continued on next page)

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DNA targeting module	Effector proteins	Applications	Refs
	p300 (the core enzymatic domain of histone acetyl transferase)	Targeted histone H3K27 acetylation at multiple loci for activation in cells	[18]
	HDAC3 (histone deacetylase)	Targeted histone deacetylation in cells	[21]
	PRDM9 and DOT1L (histone methyltransferase)	Targeted H3K4me3 and H3K79me in cells	[23]
	LSD1 (histone demethylase)	Targeted histone deacetylation to identify enhancers in cells	[25]
	Ezh2; FOG1; SUV39H1 (histone modifiers)	Targeted H3K27me; histone deacetylation; H3K9me3 in cells	[20]
	KRAB (Krüppel-associated box)	Induced heterochromatin-like state in cells	[27,71]
	Leucine zipper proteins	Artificial DNA looping in bacteria	[30]
	PYL1 and ABI1 (dimerization protein pair from the plant phytohormone S-(+)-abscisic acid pathway)	Artificial DNA looping at *β-globin* and *Oct4* loci in cells	[31]
	CIB (dimerization induced by CRY2)	Light-inducible DNA looping to redirect an enhancer from *Klf4* to *Zfp462* in cells	[32]
	BAF (chromatin remodeler)	Targeted chromatin remodeling towards gene activation in cells	[22]
	dCas9-ABI1 and Emerin-PYL1, or -Coilin, or -PML	Targeted 3D genome positioning to the nuclear envelop, Cajal body, and PML body	[33]

Table 1. *Continued*

Why Brain Disorders?

Brain, the most complex organ in the human body, is vulnerable to genetic mutations as well as environmental assaults. As the coordinator between the genome and the environment, the epigenome undergoes dynamic changes during brain activities [3]. Many epigenetic modifier genes have been shown to play critical roles in brain functions by orchestrating the transcription network in response to environmental stimuli [38,39]. Alterations of the epigenome have been observed in many types of brain disorders [40] and the reversibility of epigenetic events has triggered substantial enthusiasm to tackle these brain disorders via epigenome editing approaches. In the following, we will discuss several applications of epigenome editing tools to study brain disorders and highlight some of the key challenges and opportunities in the field.

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Proof of Concept

We discuss next, two proof of concept studies demonstrating the application of epigenome editing tools to dissect the epigenetic mechanism of brain disorders and to develop potential therapeutic strategies.

Awaken the Silenced Synaptic Gene

Abnormal regulation of synaptic genes has been documented in numerous neurological disorders [41]. One such example is fragile X syndrome (FXS), in which the *FMR1* gene is silenced by the CGG trinucleotide repeat expansion mutation in its 5′ untranslated region (UTR) [42]. Repression of *FMR1* is associated with a heterochromatin-like epigenetic status at the promoter region, including DNA hypermethylation of the CGG repeats, decrease of active histone modifications such as H3K27Ac and H3K4me3, and increase of suppressive histone modifications such as H3K9me3 [42]. What was not fully clear is the functional significance of these epigenetic cascade events. We applied the CRISPR/dCas9-TET1 tool to specifically demethylate the CGG repeats in FXS induced pluripotent stem cells (iPSCs) and showed that demethylation of the CGG repeats triggered a switch from a heterochromatin-like to an active chromatin state in the *FMR1* promoter region within a 10-day period, resulting in reactivation of the silenced *FMR1* [17]. Importantly, reactivation of *FMR1* restored the spontaneous hyperactivity of FXS neurons to wild type (WT) levels. This study allowed two main conclusions: (i) demethylation of the CGG repeats is sufficient to activate the silenced *FMR1* allele; (ii) reactivation of *FMR1* at the stage of differentiated neurons is not too late to rescue the abnormal phenotype of FXS neurons. Epigenetic editing tools enable us to distinguish the causal and correlative role of epigenetic events associated with neurological diseases and is not limited to DNA methylation, but is also applicable to study histone modifications in brain disorders. It is worth noting that different types of epigenetic modifications can interact. For instance, editing of the CGG repeat methylation at the *FMR1* locus triggered changes in histone modifications [17]. Therefore, it will be important to identify the upstream epigenetic modifications, which then can be edited for an optimal effect on the regulation of the targeted gene.

Reverse Addiction/Depression by Sculpting the Histones

Histone modifications represent another major epigenetic mechanism, with possibly faster dynamics as compared with DNA methylation in response to environmental stimuli. Many brain disorders are associated with alterations of histone modifications occurring in distinct brain areas [40] but to establish a causal link between histone modifications and phenotype required epigenetic editing. In a seminal study from Eric Nestler's laboratory, ZFP- and TALE-based approaches were used to introduce histone modifications at the *FosB* locus [28]. Cocaine administration triggers induction of ΔFosB (a *FosB* product) in the nucleus accumbens (NAc), whereas certain forms of chronic stress reduce ΔFosB expression in rodents and humans, with both conditions being correlated with histone acetylation (H3K9 and H3K14) and methylation (H3K9me2) at the *FosB* gene. Targeted histone H3K9 acetylation at *FosB* enhanced cocaine sensitivity, and targeted histone H3K9 methylation at *FosB* blocked cocaine-induced locomotor sensitization. A subsequent study demonstrated the ability to control H3K9 acetylation or methylation at *FosB* in a cell type-specific manner in NAc, with downstream effects on stress responses [29]. These studies provide the first demonstration of histone modification editing in a neuropsychiatric disorder to establish a causal link of locus-specific histone modification to promote behavioral susceptibility.

How Versatile Is Epigenome Editing?

In principle, brain disorders associated with dysregulation of epigenetic mechanisms may be potential targets for an epigenome editing therapy, either by silencing disease-causing genes or activating neuroprotective genes.

Reactivation of the Silenced WT Allele in X-linked Diseases

Rett syndrome (RTT) is an X-linked neurological disorder, mainly observed in girls, caused by heterozygous loss-of-function mutations of methyl-CpG-binding protein 2 (*MECP2*) on the X chromosome

[43]. Studies in mice showed that brain-specific re-expression of *Mecp2* during the adult stage rescued many of the RTT-symptom relevant phenotypes [44–46]. Because each neuron in a heterozygous RTT patient carries a WT *MECP2* copy, either on the active or on the inactive X chromosome, a potential therapeutic approach would be to reactivate the WT allele that is silenced in about 50% of the patient's cells. In contrast to FXS, where the silenced *FMR1* gene resides on an active X chromosome [42], the silent *MECP2* allele resides on the inactive X chromosome and its reactivation may need to overcome several layers of suppressive epigenetic mechanisms, including DNA methylation, histone modifications, 3D chromatin structure, and X-inactive specific transcript (XIST) decoration. Thus, it may be necessary to combine multiple epigenetic editing tools targeted at DNA methylation as well as histone modification and others to establish a stable active state of the *MECP2* gene in the heterochromatic environment of the inactive X chromosome. Our preliminary results (unpublished) suggest that such a combinational approach may be promising. Another important consideration regarding gene reactivation is the dose of gene expression, as proper brain function requires a fine regulation of gene expression. For instance, duplication of the *MECP2* gene causes serious brain dysfunction (*MECP2* duplication syndrome) [47], which poses complications to therapeutic approaches that use gene transfer of *MECP2* into patients' neurons, as this will likely result in overexpression of the protein in some neurons.

Targeting Epimutations in Imprinting Disorders

Genomic imprinting refers to situations where only one allele either from the paternal or the maternal chromosome is expressed. Genomic imprinting is mainly mediated by DNA methylation. Some imprinting-related disorders are caused by abnormal DNA methylation, called epimutations, without underlying genetic mutations. One such example is Angelman syndrome (AS), which mainly affects the nervous system [48]. Loss of methylation in an imprinting center on the maternal chromosome 15 results in silencing of both alleles of the *UBE3a* gene in AS patients [48]. This epigenetic disorder is a promising target for an epigenome editing approach to rewrite DNA methylation, specifically at the maternal imprinting center, in order to restore the normal imprinting pattern. In addition, other imprinting disorders such as Beckwith-Wiedemann syndrome are potential targets for epigenome editing. However, it is an unresolved question whether the pathology of imprinting disorders that have a fetal origin are reversible after birth.

Is the Prospect of Treating Neurodegenerative Diseases beyond the Reach of Epigenome Editing?

Neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) are caused by both genetic and environmental factors and thus their etiology is complex. Characterization of patient samples from AD and PD reveals a global alteration of the epigenome, not only in neurons but also glial cell populations such as astrocytes and microglia [49–52]. Dissection of the functional significance of numerous epigenetic alternations observed in neurodegenerative diseases faces technical and conceptual challenges. For example, the interpretation of epigenetic alteration is complicated by the multiple neuronal cell types present in clinical samples and thus requires a cell type-specific stratification of epigenetic changes. Recent advances in single-cell sequencing technology such as single-cell whole genome bisulfite sequencing [53] provides a possible solution. In addition, it is not clear whether any of the observed epigenetic changes are causal or represent passenger alterations caused by environmental factors. For instance, while a change in DNA methylation at the ankyrin 1 (*ANK1*) gene was identified in AD patients' brain samples in two independent studies [52,54], its pathological relevance for disease remains to be established. Finally, because the progression of most neurodegenerative diseases takes decades, it is possible that some epigenetic modifications such as oxidation-induced DNA methylation changes are the result of naturally accumulated damages to the genome but are not causally involved in disease progression. Even if pathological relevance can be established for a set of epigenetic signatures, current epigenome editing tools may not be efficient enough to edit so many changes simultaneously. Undoubtedly, these challenges reflect our relatively shallow understanding of the regulation of the epigenome as well as methodological limitations of existing tools.

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Challenges and Opportunities in Therapeutic Applications

Is the Disease Reversible?

For any therapeutic application, a key aspect to consider is whether the disease at hand is primarily developmental, which implies that the phenotype may be difficult to reverse after a certain developmental timepoint. In animal models of RTT, it has been demonstrated that the disease phenotype is largely reversible even in the adult, making this disease an attractive target for postnatal epigenetic therapy [44–46]. Similarly, some evidence in animal models suggests a possible therapeutic effect of re-expression of the *Fmr1* gene in FXS [55]. For many other diseases such as imprinting disorders, relevant information based on rescue studies in mice is lacking.

Off-Target Effects

One key parameter for a given editing system targeting the genome or epigenome is the degree of off-target effects. For CRISPR/dCas9-based epigenome editing systems, off-target effects can occur for two reasons. The first is suboptimized single guide RNA (sgRNA). This is mainly due to the limited sgRNA choices determined by available protospacer adjacent motif (PAM) sequences within the targeted genomic locus and thus the suboptimized sgRNAs contribute to the off-target effect. This type of off-target effect could be minimized by introduction of other orthologs for Cas9 with a different PAM sequence, which may enable more sgRNA design options [14,56]. A second cause for off-target effects is the enzymatic domain fused with dCas9 to mediate epigenome editing. Constitutive expression of a CRISPR/dCas9 fusion construct may modify the epigenome in a nonspecific way. An inducible promoter that controls the expression level and duration of dCas9 fusion protein may reduce such off-target effects. One future solution would be to develop a conformationally activatable dCas9-effector system in which the epigenetic effector functionally replaces one of the nuclease domains of Cas9 and adapts its conformationally inducible activation property upon locating its genomic target, as illustrated in the right-hand column of Figure 1.

Stability of Induced Epigenetic Alterations

The stability of induced epigenetic changes induced by epigenome editing is another issue that needs to be addressed. Because of the dynamic nature of epigenetic changes, an important issue is whether changes, once established, are stable in the absence of continuous expression of the editing tools. While the demethylation-induced *FMR1* activation in FXS iPSCs was maintained for at least 4 weeks *in vitro* after inhibition of dCas9-TET1, other epigenetic changes may be less stable and may require continuous expression of the editing tools. Alternatively, combining DNA methylation with histone modification editing or DNA looping may stabilize a new epigenetic state independently of continuous expression of the editing tools. For instance, Amabile *et al.* demonstrated that persistent gene repression can be achieved by combination editing of DNA methylation with H3K9me3 modification [24].

Delivery of Editing Tools

Any therapeutic effect will depend on the fraction of cells that are subject to epigenetic editing for correcting a given phenotype. For example, the fraction of cells that need to be successfully edited in RTT is high, as RTT females expressing MECP2 protein in 50% of their cells have the disease. Thus, to have a therapeutic effect would require highly efficient gene delivery to a substantial fraction of cells. While adeno-associated virus (AAV) vector variants have been shown to be efficient in infecting a large fraction of cells in the brain [57], the packaging capacity of AAV (about 4.5 kb) is insufficient to transduce the Cas9 fusion construct and the sgRNA in a single vector. Thus, two vectors would need to co-infect one cell to be effective in editing, which would reduce the editing efficiency. Recently, CIRTS (a CRISPR-Cas-inspired RNA targeting system made entirely of human proteins) was developed to reduce vector sizes, enabling AAV package and delivery [58]. Another alternative approach would be to use vectors with a higher capacity, such as lentivirus, adenovirus, or herpes simplex virus vectors that can accommodate a larger payload [59,60].

To achieve temporal control of epigenome editing, the light-inducible protein components were coupled with current epigenetic editing system (Cryptochrom2-dCas9 binding to CIBI-effector) [61]. Illumination of transduced cells in the brain of mice with blue light lead to an assembly of functional epigenetic editing tools and triggered epigenome editing.

Concluding Remarks and Future Perspectives

Genetic studies of a handful epigenetic modifiers such as DNMTs and histone acetyl transferase (p300) revealed the critical role of epigenetic mechanisms in brain functions and diseases [38,62]. Currently, a key challenge in the field is to establish a causal link between altered epigenetic events and disease outcome, which requires application of epigenome editing tools to write and erase the disease-associated epigenetic events followed by phenotypic analysis of edited neurons, organoids, and animals as the basis for the development of therapeutic strategies for human brain disorders. A proof of concept study targeting the monogenic disease FXS has shown promising results for functional rescue of neuronal phenotypes and reasonable duration of *FMR1* activation in edited FXS cells transplanted in mouse brains [17]. However, targeting polygenic diseases would necessitate development of epigenome editing tools that have the capacity to edit multiple genomic loci at different layers of epigenetic marks simultaneously. This could be achieved by employing different CRISPR/Cas9 orthologs with distinct DNA recognition sequences. Discovery of RNA-targeting Cas13 in the CRISPR family allows for editing of RNAs [36,37] and modifying the epitranscriptome [63] by a similar approach as the dCas9-effector fusion proteins, providing another set of tools to manipulate noncoding RNAs and to tackle diseases. Efficient delivery of epigenome editing tools *in vivo* is a not as yet solved technical barrier towards clinical application. Besides the herpes simplex or adeno-viral vector systems mentioned above, nanoparticle-based delivery has shown a remarkable ability to cross the blood–brain barrier [64], which may lay a foundation for possible future therapeutic applications. Last but not least, our current understanding of the epigenome in brain functions is just in its infancy. A network of epigenetic events can be triggered by brain activity or under psychiatric conditions [3]. Although epigenome editing tools represent an important approach to dissect the functional significance of specific epigenetic events, manipulation of the epigenetic network, which often displays multiple simultaneous changes, requires a deeper understanding of epigenetic regulation. What is the upstream signal triggering a network event, including multiple epigenetic changes, in response to brain activity? Is there any DNA sequence specificity for these changes? If so, what is the endogenous molecular machinery, rather than CRISPR-based tools, enabling such a specificity in these neurons (see Outstanding Questions)? Tackling these basic questions might not lead to a treatment immediately, but the new knowledge can form a foundation for understanding neural circuit miswiring in disease and pave the way for the development of novel therapeutic avenues.

Outstanding Questions

How long can an edited epigenetic event be maintained *in vivo* after inhibition of the editors?

What is the maximal number of epigenetic editing events that can be achieved by one delivery of current epigenome editing tools?

Is there a driver epigenetic event within a network of epigenetic alterations in brain disorders such as post-traumatic stress disorder and AD?

What is the endogenous machinery that mediates the network of epigenetic events upon brain functions and is this machinery the primary target under pathological conditions?

What type of information is stored in the epigenome of neurons? Transcriptional responses, neuronal status, or possibly synaptic connections?

Acknowledgments

We thank Michael Gallagher, Anthony Flamier, Xin Tang, Andrew Khalil, Emile Wogram, Malkiel Cohen in our laboratory, and Minglu Wang from Princeton University for comments on the manuscript. R.J. was supported by NIH grants HD045022, MH104610, R01NS088538, and R01GM123511. X.S.L. is supported by a Damon Runyon Cancer Foundation Postdoctoral Fellowship, K99 Pathway to Independent Award from National Institute of Mental Health (NIMH), and funding from Rett Syndrome Research Trust. R.J. is cofounder of Fate Therapeutics, Fulcrum Therapeutics, and Omega Therapeutics and an advisor to Dewpoint Therapeutics. We apologize for not being able to cite other exciting works in this field due to the limited space.

References

1. Deichmann, U. (2016) Epigenetics: the origins and evolution of a fashionable topic. *Dev. Biol.* 416, 249–254
2. Jaenisch, R. and Bird, A. (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet.* 33, 245–254
3. Sweatt, J.D. (2013) The emerging field of neuroepigenetics. *Neuron* 80, 624–632
4. Martinowich, K. *et al.* (2003) DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. *Science* 302, 890–893
5. Guo, J.U. *et al.* (2011) Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. *Cell* 145, 423–434
6. Zovkic, I.B. *et al.* (2014) Histone H2A.Z subunit exchange controls consolidation of recent and remote memory. *Nature* 515, 582–586

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7. Yamada, T. *et al.* (2019) Sensory experience remodels genome architecture in neural circuit to drive motor learning. *Nature* 569, 708–713
8. Bernstein, B.E. *et al.* (2007) The mammalian epigenome. *Cell* 128, 669–681
9. Bernstein, B.E. *et al.* (2010) The NIH Roadmap Epigenomics Mapping Consortium. *Nat. Biotechnol.* 28, 1045–1048
10. Bae, J.B. (2013) Perspectives of international human epigenome consortium. *Genomics Inform.* 11, 7–14
11. Xu, G.L. and Bestor, T.H. (1997) Cytosine methylation targetted to pre-determined sequences. *Nat. Genet.* 17, 376–378
12. Maeder, M.L. *et al.* (2013) Targeted DNA demethylation and activation of endogenous genes using programmable TALE-TET1 fusion proteins. *Nat. Biotechnol.* 31, 1137–1142
13. Bernstein, D.L. *et al.* (2015) TALE-mediated epigenetic suppression of CDKN2A increases replication in human fibroblasts. *J. Clin. Invest.* 125, 1998–2006
14. Knott, G.J. and Doudna, J.A. (2018) CRISPR-Cas guides the future of genetic engineering. *Science* 361, 866–869
15. Komor, A.C. *et al.* (2017) CRISPR-based technologies for the manipulation of eukaryotic genomes. *Cell* 168, 20–36
16. Liu, X.S. *et al.* (2016) Editing DNA methylation in the mammalian genome. *Cell* 167, 233–247
17. Liu, X.S. *et al.* (2018) Rescue of fragile X syndrome neurons by DNA methylation editing of the *FMR1* gene. *Cell* 172, 979–992
18. Hilton, I.B. *et al.* (2015) Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. *Nat. Biotechnol.* 33, 510–517
19. Pflueger, C. *et al.* (2018) A modular dCas9-SunTag DNMT3A epigenome editing system overcomes pervasive off-target activity of direct fusion dCas9-DNMT3A constructs. *Genome Res.* 28, 1193–1206
20. O'Geen, H. *et al.* (2017) dCas9-based epigenome editing suggests acquisition of histone methylation is not sufficient for target gene repression. *Nucleic Acids Res.* 45, 9901–9916
21. Kwon, D.Y. *et al.* (2017) Locus-specific histone deacetylation using a synthetic CRISPR-Cas9-based HDAC. *Nat. Commun.* 8, 15315
22. Braun, S.M.G. *et al.* (2017) Rapid and reversible epigenome editing by endogenous chromatin regulators. *Nat. Commun.* 8, 560
23. Cano-Rodriguez, D. *et al.* (2016) Writing of H3K4Me3 overcomes epigenetic silencing in a sustained but context-dependent manner. *Nat. Commun.* 7, 12284
24. Amabile, A. *et al.* (2016) Inheritable silencing of endogenous genes by hit-and-run targeted epigenetic editing. *Cell* 167, 219–232
25. Kearns, N.A. *et al.* (2015) Functional annotation of native enhancers with a Cas9-histone demethylase fusion. *Nat. Methods* 12, 401–403
26. Mendenhall, E.M. *et al.* (2013) Locus-specific editing of histone modifications at endogenous enhancers. *Nat. Biotechnol.* 31, 1133–1136
27. Thakore, P.I. *et al.* (2015) Highly specific epigenome editing by CRISPR-Cas9 repressors for silencing of distal regulatory elements. *Nat. Methods* 12, 1143–1149
28. Heller, E.A. *et al.* (2014) Locus-specific epigenetic remodeling controls addiction- and depression-related behaviors. *Nat. Neurosci.* 17, 1720–1727
29. Hamilton, P.J. *et al.* (2018) Cell-type-specific epigenetic editing at the Fosb gene controls susceptibility to social defeat stress. *Neuropsychopharmacology* 43, 272–284
30. Hao, N. *et al.* (2017) Programmable DNA looping using engineered bivalent dCas9 complexes. *Nat. Commun.* 8, 1628
31. Morgan, S.L. *et al.* (2017) Manipulation of nuclear architecture through CRISPR-mediated chromosomal looping. *Nat. Commun.* 8, 15993
32. Kim, J.H. *et al.* (2019) LADL: light-activated dynamic looping for endogenous gene expression control. *Nat. Methods* 16, 633–639
33. Wang, H. *et al.* (2018) CRISPR-mediated programmable 3D genome positioning and nuclear organization. *Cell* 175, 1405–1417
34. Holtzman, L. and Gersbach, C.A. (2018) Editing the epigenome: reshaping the genomic landscape. *Annu. Rev. Genomics Hum. Genet.* 19, 43–71
35. Rots, M.G. and Jeltsch, A. (2018) Editing the epigenome: overview, open questions, and directions of future development. *Methods Mol. Biol.* 1767, 3–18
36. Cox, D.B.T. *et al.* (2017) RNA editing with CRISPR-Cas13. *Science* 358, 1019–1027
37. Abudayyeh, O.O. *et al.* (2019) A cytosine deaminase for programmable single-base RNA editing. *Science* 365, 382–386
38. Edwards, J.R. *et al.* (2017) DNA methylation and DNA methyltransferases. *Epigenetics Chromatin* 10, 23
39. Mansuy, I.M. and Mohanna, S. (2011) Epigenetics and the human brain: where nurture meets nature. *Cerebrum* 2011, 8
40. Landgrave-Gomez, J. *et al.* (2015) Epigenetic mechanisms in neurological and neurodegenerative diseases. *Front. Cell Neurosci.* 9, 58
41. Lepeta, K. *et al.* (2016) Synaptopathies: synaptic dysfunction in neurological disorders - a review from students to students. *J. Neurochem.* 138, 785–805
42. Jin, P. and Warren, S.T. (2000) Understanding the molecular basis of fragile X syndrome. *Hum. Mol. Genet.* 9, 901–908
43. Amir, R.E. *et al.* (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet.* 23, 185–188
44. Garg, S.K. *et al.* (2013) Systemic delivery of MeCP2 rescues behavioral and cellular deficits in female mouse models of Rett syndrome. *J. Neurosci.* 33, 13612–13620
45. Giacometti, E. *et al.* (2007) Partial rescue of MeCP2 deficiency by postnatal activation of MeCP2. *Proc. Natl. Acad. Sci. U. S. A.* 104, 1931–1936
46. Guy, J. *et al.* (2007) Reversal of neurological defects in a mouse model of Rett syndrome. *Science* 315, 1143–1147
47. Sztainberg, Y. *et al.* (2015) Reversal of phenotypes in MECP2 duplication mice using genetic rescue or antisense oligonucleotides. *Nature* 528, 123–126
48. Buiting, K. *et al.* (2016) Angelman syndrome - insights into a rare neurogenetic disorder. *Nat. Rev. Neurol.* 12, 584–593
49. Liu, X. *et al.* (2018) The epigenetics of Alzheimer's disease: factors and therapeutic implications. *Front. Genet.* 9, 579
50. Pavlou, M.A.S. and Outeiro, T.F. (2017) Epigenetics in Parkinson's disease. *Adv. Exp. Med. Biol.* 978, 363–390
51. Cheray, M. and Joseph, B. (2018) Epigenetics control microglia plasticity. *Front. Cell Neurosci.* 12, 243
52. De Jager, P.L. *et al.* (2014) Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. *Nat. Neurosci.* 17, 1156–1163
53. Karemaker, I.D. and Vermeulen, M. (2018) Single-cell DNA methylation profiling: technologies and biological applications. *Trends Biotechnol.* 36, 952–965
54. Lunnon, K. *et al.* (2014) Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. *Nat. Neurosci.* 17, 1164–1170

Please cite this article in press as: Liu and Jaenisch, Editing the Epigenome to Tackle Brain Disorders, Trends in Neurosciences (2019), https://doi.org/10.1016/j.tins.2019.10.003

55. Gantois, I. *et al.* (2001) Restoring the phenotype of fragile X syndrome: insight from the mouse model. *Curr. Mol. Med.* 1, 447–455
56. Komor, A.C. *et al.* (2017) CRISPR-based technologies for the manipulation of eukaryotic genomes. *Cell* 169, 559
57. Lykken, E.A. *et al.* (2018) Recent progress and considerations for AAV gene therapies targeting the central nervous system. *J. Neurodev. Disord.* 10, 16
58. Rauch, S. *et al.* (2019) Programmable RNA-guided RNA effector proteins built from human parts. *Cell* 178, 122–134
59. Latchman, D.S. (2005) Herpes simplex virus-based vectors for the treatment of cancer and neurodegenerative disease. *Curr. Opin. Mol. Ther.* 7, 415–418
60. Gray, S.J. *et al.* (2010) Viral vectors and delivery strategies for CNS gene therapy. *Ther. Deliv.* 1, 517–534
61. Konermann, S. *et al.* (2013) Optical control of mammalian endogenous transcription and epigenetic states. *Nature* 500, 472–476
62. Roelfsema, J.H. *et al.* (2005) Genetic heterogeneity in Rubinstein-Taybi syndrome: mutations in both the CBP and EP300 genes cause disease. *Am. J. Hum. Genet.* 76, 572–580
63. Rauch, S. *et al.* (2018) Targeted m(6)A reader proteins to study epitranscriptomic regulation of single RNAs. *J. Am. Chem. Soc.* 140, 11974–11981
64. Saraiva, C. *et al.* (2016) Nanoparticle-mediated brain drug delivery: overcoming blood-brain barrier to treat neurodegenerative diseases. *J. Control. Release* 235, 34–47
65. Snowden, A.W. *et al.* (2002) Gene-specific targeting of H3K9 methylation is sufficient for initiating repression *in vivo*. *Curr. Biol.* 12, 2159–2166
66. Choudhury, S.R. *et al.* (2016) CRISPR-dCas9 mediated TET1 targeting for selective DNA demethylation at BRCA1 promoter. *Oncotarget* 7, 46545–46556
67. Vojta, A. *et al.* (2016) Repurposing the CRISPR-Cas9 system for targeted DNA methylation. *Nucleic Acids Res.* 44, 5615–5628
68. Xu, X. *et al.* (2016) A CRISPR-based approach for targeted DNA demethylation. *Cell Discov.* 2, 16009
69. Deng, W. *et al.* (2012) Controlling long-range genomic interactions at a native locus by targeted tethering of a looping factor. *Cell* 149, 1233–1244
70. Deng, W. *et al.* (2014) Reactivation of developmentally silenced globin genes by forced chromatin looping. *Cell* 158, 849–860
71. Gilbert, L.A. *et al.* (2013) CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell* 154, 442–451