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Unraveling the epigenetic landscape of depression: focus on early life stress

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Depression is a devastating psychiatric disorder caused by a combination of genetic predisposition and life events, mainly exposure to stress. Early life stress (ELS) in particular is known to “scar” the brain, leading to an increased susceptibility to developing depression later in life via epigenetic mechanisms. Epigenetic processes lead to changes in gene expression that are not due to changes in DNA sequence, but achieved via modulation of chromatin modifications, DNA methylation, and noncoding RNAs. Here we review common epigenetic mechanisms including the enzymes that take part in reading, writing, and erasing specific epigenetic marks. We then describe recent developments in understanding how ELS leads to changes in the epigenome that are manifested in increased susceptibility to depression-like abnormalities in animal models. We conclude with highlighting the need for future studies that will potentially enable the utilisation of the understanding of epigenetic changes linked to ELS for the development of much-needed novel therapeutic strategies and biomarker discovery.

Keywords: chromatin remodeling; histone modification; DNA methylation; noncoding RNA; early life stress

Introduction

Epidemiological and preclinical studies have identified stress as an important risk factor for the onset of an episode of major depressive disorder (MDD), a highly debilitating psychiatric condition characterized by persistent alterations in mood, motivation, or cognitive function.\(^1\) Depression occurs across the lifespan, from childhood through late life, but the peak of incidence is observed during peripubescence.\(^2\)

Early life is a critical period of plasticity for brain development and is highly sensitive to adverse experiences such as stress exposure. Indeed, the consequences of stress on mental health are more severe when experienced early in life. Early life stress (ELS), including child maltreatment, parent neglect, undernutrition, or sexual abuse, increases the risk for depression and other stress-related disorders later in life by two- to fourfold.\(^3,4\) Furthermore, in people subjected to ELS, the early presence of depressive events is a predictor of recurrent episodes and severity of depression.\(^5\)

Susceptibility to MDD is partly mediated by genetic factors with an estimated 35% heritability,\(^6\) mediated by hundreds of genomic variations, each of very small effect. This genetic predisposition, in combination with exposure to stressful life events, can lead to the development of MDD via epigenetic mechanisms, or changes in gene expression that are not due to DNA sequence variation.\(^7\) These epigenetic changes are mediated by several mechanisms, including histone modifications, DNA methylation, noncoding RNAs, and changes in the 3D structure of chromatin,\(^8\) that consequently lead to changes in brain function. Notably, epigenetic changes caused by exposure to ELS in genetically prone
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Individuals can leave a “molecular scar” that leads to the development of MDD, particularly following exposure to additional profound stress throughout life.9

Functional and molecular alterations in the prefrontal cortex (PFC), ventral tegmental area (VTA), nucleus accumbens (NAc), hippocampus (HPC), and amygdala (AMY), collectively known as the mesocorticolimbic system, have been consistently associated with MDD.10 These brain regions are known to be involved in the regulation of cognitive function, emotion, motivation, and mood and are highly sensitive to the effects of stress.11 Indeed, the protracted development of the mesocorticolimbic system renders it highly sensitive to environmental insults during early life.12,13

This review discusses recent advances in the study of epigenetics and depression, with a special focus on ELS. In the first section, we introduce the basic mechanisms of epigenetic regulation, including histone modifications, chromatin remodeling, DNA methylation, and noncoding RNAs. In the second section, we present evidence from rodent and human studies showing alterations in these epigenetic mechanisms that are associated with ELS-induced vulnerability to MDD. We highlight studies exploring the effects of stress from birth to periadolescence and how the consequences of such stress can be observed in adulthood and even subsequent generations. Finally, we suggest future directions in which further work is needed to better understand the deleterious effects of ELS on MDD risk.

Epigenetic mechanisms

Histone modifications
The human genome contains over 3 billion base pairs of DNA reaching ~2 meters in length, that is condensed into the cell nucleus, with a diameter of ~6 µm. This requires intricate organization that is obtained by compacting DNA onto histone proteins, thus creating chromatin. Nucleosomes are the basic repeating unit of chromatin fibers, consisting of ~147 base pairs of DNA wrapped around a single histone octamer comprised of two copies of each of four core histones H3, H4, H2A and H2B (Figure 1). Assembly of nucleosomes is achieved through linker histone H1 and a short linker DNA segment, allowing for interaction between neighboring nucleosomes. This nucleosomal organization of DNA is responsible for the higher-order chromatin structure, thereby facilitating or preventing access of gene regulatory machinery to fine-tune gene expression through post-translational modifications (PTMs; eg, acetylation, methylation, phosphorylation, and ubiquitination) on histone N-terminal tails which face outward from the nucleosome core.14,15

Histone acetylation at lysine residues is generally associated with transcriptional activation by loosening DNA-histone binding and, therefore, increasing spacing between nucleosomes. This state facilitates the binding of transcription factors and other regulatory proteins.15 Histone methylation results from the addition of one, two, or three methyl groups to lysine or arginine residues of histones tails. This PTM can either promote transcriptional repression or activation depending on the modified amino acid.14 Histone acetylation is controlled by two enzymes: histone acetyltransferases and histone deacetylases (HDACs), whereas histone methylation is controlled by lysine or arginine histone methyltransferases (HMTs) and histone demethylases.16 Enzymes that add PTMs are considered “writers,” those that remove PTMs “erasers,” and proteins that recognize these PTMs to direct transcriptional outputs are referred to as “readers.”

Several histone variants have been identified, including H2A.Z, H2A.X, and H3.1–3. These variant histone proteins structurally differ from the canonical histones, ranging from a few amino acids up to entire protein domains. Histone variants are also subjected to PTMs and thus influence chromatin dynamics.17

Chromatin remodeling and 3D chromatin structure
Another mechanism by which chromatin structure can be modified is through the action of proteins known as chromatin remodelers. These protein complexes, including SWI/SNF, CHD, ISWI, and INO80 families, mobilize or reposition histone octamers along the linear DNA18 and thereby regulate the spacing of nucleosomes and DNA accessi-
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There is evidence that functional alternations in chromatin remodeling complexes in the brain are associated with psychiatric disorders, including MDD, schizophrenia, and autism. Likewise, the 3D structure of chromatin is subject to dynamic regulation. This organization allows distant regions of the genome to come into close approximation and contribute to the regulation of gene expression.

DNA methylation
DNA methylation—5-methylcytosine (5mC)—is a well-described epigenetic mechanism classically associated with suppression of gene transcription. This process occurs predominantly within cytosine-guanine (CpG) dinucleotide sequences and is catalyzed by DNA methyltransferases (DNMTs). More recent research has demonstrated 5mC at non CpG sites as well as methylation of other nucleotides. Moreover, variant forms of 5mC, such as 5-hydroxymethylcytosine, have been shown to play important roles in CNS function.

DNMTs are dynamically regulated throughout development and play an important role in guiding DNA methylation patterns in the CNS. DNMT1 is considered a maintenance DNMT as it perpetuates methylation in a newly synthe-

Figure 1. Chromatin structure and epigenetic regulation of gene expression. Gene expression is regulated by DNA methylation, histone post-translational modifications (PTMs), and the actions of non-coding RNAs, among other mechanisms. To fit within the nucleus, DNA is wrapped around histone proteins creating higher order chromatin structure, which can facilitate or prevent access of gene regulatory machinery through steric mechanisms. DNA methylation is canonically associated with suppression of gene transcription. Deposition of methyl groups is catalyzed by DNA methyltransferases (DNMTs). DNMT1 regulates maintenance, while DNMT3a/b deposit de novo methyl groups. Removal of these marks, or demethylation, is facilitated by ten eleven translocation (Tet1-3) proteins, cytidine deaminase (AID), and growth arrest and DNA damage-45 (GADD45). PTMs control chromatin states through modifications, including methylation and acetylation, of histone residues. Histone acetylation is most commonly associated with chromatin relaxation enabling transcriptional activation through greater accessibility of transcriptional machinery. Histone methylation is associated with either transcriptional repression or transcriptional activation, depending on the site undergoing modification. Histone acetylation is mediated by histone acetyltransferases (HATs) and reversed by histone deacetylases (HDACs). Histone methylation is controlled by lysine or arginine histone methyltransferases (HMTs), while histone demethylation is mediated by histone demethylases (HDMs). Lastly, miRNAs are short RNAs capable of regulating gene expression post-transcriptionally. miRNAs target mRNAs to promote their degradation or inhibit their translation. miRNAs also interact with IncRNAs which are regulatory transcripts longer than 200 b that can act as molecular decoys, scaffolds, or guides – adding another layer of epigenetic regulation.
sized complementary DNA strand that is generated during DNA replication. In contrast, DNMT3a and 3b are de novo DNMTs, as they methylate previously unmethylated regions of the DNA. The molecular mechanisms of DNA demethylation have recently been established. They involve enzymes implicated previously in DNA repair, such as ten eleven translocation (TET1-3) proteins, growth arrest and DNA damage-45 (GADD45), and activation-induced cytidine deaminase (AID).

Noncoding RNAs
In the last few decades the central dogma of biology has changed, acknowledging that RNA molecules are key effectors in cells and not simply intermediate messengers in protein generation. Such noncoding RNAs (ncRNAs) are involved in many molecular processes in development and adulthood, in health and disease. ncRNAs are divided into classes by their length, with 200 bases set as an arbitrary cutoff between short (snRNAs) and long noncoding RNAs (lncRNAs). snRNAs include microRNAs (miRNAs), short interfering RNAs, and piwi-interacting RNAs. Of these, miRNAs are the most studied biotype in depression and therefore considered in this review. Notably, multiple classes of ncRNAs can be detected in circulation and body fluids. Indeed, there is evidence that circulating ncRNAs transfer signal between organs, and that their levels in circulation can be used as biomarkers of disease states.

miRNA biogenesis and function
miRNA biogenesis is a multistage process extensively reviewed in ref 29. Briefly, miRNAs arise from RNA hairpin structures and are cleaved into a mature miRNA that forms silencing complex with associated proteins. This complex targets the 3’-untranslated region (3’UTR) of mRNAs to promote their degradation or inhibit their translation. Since miRNAs target mRNAs of complementary sequence, it is possible to use bioinformatics to predict miRNA targets. A single mRNA can be targeted by multiple miRNAs and, conversely, a miRNA can target multiple mRNAs or interact with lncRNAs, which act as sponges of miRNAs.

lncRNA evolution and function
We now know that the majority of the human genome is transcribed and gives rise to more lncRNAs than protein-coding genes. Comparative genomic studies indicate that evolutionarily higher organisms have larger portions of noncoding transcripts in their genome. Specifically, a third of lncRNAs have arisen within the primate lineage and ~40% of lncRNAs are expressed only in brain, suggesting a key role for lncRNAs in the evolution of higher brain function. lncRNAs have similar structural properties to those of protein-coding genes, both at the chromatin level, with similar histone modification patterns, and at the transcript level, as being formed from multiple exons that are subjected to alternative splicing. Functionally, lncRNAs play regulatory roles as decoys, scaffolds, or guides at the transcriptional, post-transcriptional or post-translational levels interacting with DNA, RNAs or proteins. Such interactions can lead to either repression or activation of the interaction patterns. To date, there is no systematic method to predict lncRNA targets or molecular function other than empirically.

Epigenetic alterations and depression in the context of ELS
The use of animal models is a powerful tool for understanding the link between ELS-induced vulnerability to depression and epigenetic molecular adaptations. In rodents, ELS can be modeled by inducing variations to the early caregiving environment, including maternal separation (MS), variations in the levels of maternal care (MC), or periadolescent exposure to several stressors including social isolation (SI) or chronic social defeat stress (CSDS).

These manipulations reprogram numerous brain regions such as the hypothalamus–pituitary–adrenal (HPA) axis, leading to enhanced vulnerability to adult stress, which can be reversed by environmental enrichment or pharmacological manipulations. A comparative study conducted by our group showed that different protocols of ELS can induce an array of phenotypic outputs. In this section, we describe recent implicating epigenetic processes within the mesocorticolimbic reward system underlying the lifelong effects of ELS (Table I).
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| EPIGENETIC MARK | SPECIES | SEX | AGE OF STRESS | STRESS PARADIGM | BRAIN REGION | DIRECTION | SPECIFIC GENES REGULATED | REF |
|-----------------|---------|-----|---------------|-----------------|--------------|-----------|--------------------------|-----|
| H3Ac            | C57BL/6 mice | Male & Female | PND 14 to PND 16 | MS + SI (3 h/day) | HPC | ↑ in both sexes | N/A | 35 |
|                 | C57BL/6 mice | Male | PND 14 to PND 16 | MS (3 h/day) | HPC | ↑ in males | ↑ Binding at promoter region of DARPP-32 | 38 |
|                 | Sprague-Dawley rats | Male & Female | PND3 to PND21 | MS (3 h/day) | mPFC | ↑ in both sexes | Myelinated associated genes | 44 |
| H3K9ac          | Long-Evans rats | Male & Female | PND1 to PND7 | 30 min of maltreatment | mPFC | ↑ in females | Bdnf – Exon IV | 37 |
|                 | Long-Evans rats | Male | Postnatal | Low versus high MC | HPC | ↑ in high MC | Nr3c1, Pcdh-α, -β, -γ and Grm1 | 40,41 |
|                 | Sprague-Dawley rats | Male | PND9 | 24 h MS | VTA | ↑ in males | AKAP79/1150 and Bdnf | 42,43 |
| H3K14ac         | Long-Evans rats | Male & Female | PND1 to PND 7 | 30 min of maltreatment | mPFC | ↑ in females | Bdnf – Exon IV | 37 |
|                 | Wistar rats | Male | PND5 to PND10 | MS (3 h/day) | AMY | ↑ in males | Bdnf | 54 |
|                 | Sprague-Dawley rats | Males | PND2 to PND10 | MS (3 h/day) | CeA & BNST | ↑ in both males | N/A | 48 |
| H4Ac            | C57BL/6 mice | Male & Female | PND14 to PND16 | MS + SI (3 h/day) | HPC | ↑ in both sexes | ↑ Binding at Arc and Egr1 promoters | 35 |
| H4K5ac          | BALB/CJ | Male | PND2 to PND15 | MS (3 h/day) | Fore-brain | ↑ in adult males | N/A | 48 |
| H4K8ac          | BALB/CJ | Male | PND2 to PND15 | MS (3 h/day) | Fore-brain | ↑ in adult males | N/A | 48 |
| H4K12ac         | BALB/CJ | Male | PND2 to PND15 | MS (3 h/day) | Fore-brain | ↑ in adult males | N/A | 48 |
| HDAC1, 3, 7, 8, and 10 | BALB/CJ | Male | PND2 to PND15 | MS (3 h/day) | Fore-brain | ↑ in adult males | N/A | 48 |
| HDAC1/2         | Sprague-Dawley rats | Male & Female | PND3 to PND21 | MS (3 h/day) | mPFC | ↑ in both sexes | Myelinated associated genes | 44 |
| HDAC2           | Sprague-Dawley rats | Male | PND9 | 24 h MS | VTA | ↑ in males | AKAP79/150 and Bdnf | 42,43 |
| H3me2           | BALB/CJ | Male | PND2 to PND15 | MS (3 h/day) | Fore-brain | ↑ in adult males | N/A | 48 |
| H3K4me2         | Wistar rats | Male | PND5 to PND10 | MS (3 h/day) | AMY | ↑ in males | N/A | 54 |
|                 | C57BL/6 mice | Male & Female | PND1 to PND14 | MS (3 h/day) | HPC | ↑ in both sexes | Mineralocorticoid receptor | 56 |

Table I (continued overleaf). Alterations in histone post-translational modifications and associated enzymes in rodent models of early life stress.
### Table I (continued). Alterations in histone post-transcriptional modifications and associated enzymes in rodent models of early life stress.

| EPIGENETIC MARK | SPECIES | SEX | AGE OF STRESS | STRESS PARADIGM | BRAIN REGION | DIRECTION | SPECIFIC GENES REGULATED | REF |
|-----------------|---------|-----|---------------|-----------------|--------------|-----------|--------------------------|-----|
| H3K4me3         | C57BL/6 mice | Male | PND2 to PND14 | MS (3 h/day) | mPFC | ↑ in males | ↑ Binding at promoter regions of Ddias and Pip4k2a | 53  |
|                 | Long-Evans rats | Male | Postnatal | Low versus high MC | HPC | ⇓ in high MC | Grm1 | 41  |
| H3K9me2         | Sprague-Dawley rats | Male | PND2 to PND14 | MS (3 h/day) | HPC | ↑ in young and middle age males | ↑ Binding at exon IV of Bdnf in young and middle age males | 51  |
|                 | Wistar rats | Male | PND5 to PND10 | MS (3 h/day) | HPC | ↑ Binding at exon IV of Bdnf | ↑ Binding at exon IV of Bdnf in old males | 54  |
| H3K9me3         | In-house breed | Male | Postnatal | High versus low responder rats | HPC | ↑ Binding at Nr3c1 and Fyn | 50  |
|                 | Wistar rats | Male | PND5 to PND10 | MS (3 h/day) | AMY | ↑ Binding at Nr3c1 and Fyn | 54  |
| H3K36me3        | C57BL/6 mice | Male and Female | PND1 to PND14 | MS (3 h/day) | HPC | ↑ in both sexes | Mineralocorticoid receptor | 56  |

high corticosterone levels. This effect correlates with elevated expression of the immediate early genes activity-regulated cytoskeleton-associated protein (Arc) and early growth response 1 (Egr1), increased H4 acetylation at the Arc and Egr1 gene promoters and higher dendritic complexity of hippocampal CA3 neurons. In the central AMY and bed nucleus of the stria terminalis, MS elevates oxytocin receptor mRNA and H3 acetylation at lysine (K) 14 (H3K14ac) specifically within the promoter region of the oxytocin receptor gene, coinciding with increased ultrasonic vocalizations in early life as an indicator of enhanced stress-reactivity. In contrast, decreased acetylation of exon IV of the brain-derived neurotrophic factor (Bdnf) gene in rat medial PFC (mPFC) results from early maltreatment. Intriguingly, a brief protocol of MS decreased H3 acetylation at the promoters of genes encoding the dopamine receptor 1 (DRD1) and dopamine- and cAMP-regulated phosphoprotein of 32 kD (DARPP-32) in HPC, effects associated with decreased depression-like behaviors in adult mice, suggesting that the severity of the ELS protocol contributes to the lasting behavioral outputs associated with histone acetylation.

In rodents, the quality of MC and subsequent performance of offspring in stress-related tasks is mediated by sustained epigenetic changes in dorsal HPC (dHPC) and mPFC. Adult offspring from mothers displaying high MC (e.g., high licking and grooming of the pups) show better learning performance, lower stress sensitivity and reduced fearful ness compared with offspring of low MC mothers. These behavioral changes are associated with H3 acetylation in dHPC in stress- and plasticity-associated genes, including those encoding the glucocorticoid receptor (GR; Nr3c1 gene), protocadherins-α, -β, and -γ and metabotropic glutamate receptor 1. Synaptic plasticity in the VTA dopamine (DA) system is sensitive to changes in histone acetylation induced by ELS. For example, a single episode (24 hr) of MS leads to reduced levels of H3 acetylation at lysine 9 (H3K9ac)
and increased expression of HDAC2 in the VTA. These epigenetic changes correlate with high expression of the postsynaptic scaffolding A-kinase anchoring protein 150 (AKAP150), which is known to control GABAε receptor trafficking in VTA DA neurons. Indeed, MS induces long-term depression (LTD) at GABAergic synapses onto VTA DA neurons in an AKAP150-dependent manner. Remarkably, the selective class I HDAC inhibitor, CI-994, reverses MS-induced GABAergic abnormalities both in vitro and in vivo, increasing the levels of H3K9ac and subsequently normalizing expression levels of AKAP150 in VTA. These findings illustrate functional alterations induced by ELS and highlight the therapeutic potential of HDAC inhibitors.

ELS alters acetylation in non-neuronal cells as well. One study showed that MS decreases HDAC1/2 expression and affects myelination of mPFC across the lifespan. The morphological alterations were associated with impaired mPFC-dependent cognitive function in adult rats. Furthermore, MS reduced the number of mature oligodendrocytes via HDAC1/2-induced inhibition of WNT signaling. Postmortem studies in humans with a history of child abuse provide further support for the involvement of epigenetic regulation in oligodendrocytes, showing DNA hypermethylation of genes related to myelin and oligodendrocytes in the anterior cingulate cortex, as well as increased numbers of mature myelinating oligodendrocytes and decreased numbers of oligodendrocyte-lineage cells in ventromedial PFC. Combined, this suggests that epigenetic reprogramming of oligodendrocytes induced by ELS may produce persistent dysregulation to cortical myelination and alter the connectivity within the mesocorticolimbic reward system.

As mentioned, the effects of ELS can vary greatly depending on genetic sensitivity, and there is ample evidence to suggest the involvement of histone acetylation processes when comparing mouse strains. For example, BALB/C mice are behaviorally more sensitive to the effects of ELS and adult stress in comparison to C57BL/6 mice. Adult stress decreases H3 acetylation at the promotier of the glial cell-derived neurotrophic factor gene (Gdnf) in NAc of BALB/C mice, while increases H3 acetylation in C57BL/6 mice at the same locus. Furthermore, BALB/C mice exposed to MS display increased cortical expression of acetylated histone H4 proteins, specifically, H4K5ac, H4K8ac, H4K12ac, and H4K16ac, along with decreased expression of different HDACs.

Collectively, these findings support the idea that the early environment controls activation of synaptic plasticity genes, which in turn disrupts the proper development of stress-sensitive brain structures via mechanisms sustained at least partly via epigenetic mechanisms like histone acetylation. Importantly, pharmacological manipulations using HDAC inhibitors (HDACis) alone or in combination with fluoxetine, regulate both acetylation patterns and stress-related behaviors in rodents exposed to MS, further supporting the potential therapeutic effects of HDACis to prevent or treat the deleterious effects of ELS in vulnerable individuals.

**Histone methylation**

Patterns of histone methylation are influenced by ELS and, similar to acetylation, correlate with genetic-based stress sensitivity. High-stress sensitive rats, which are prone to depression- and anxiety-like behaviors, display a global increase in levels of trimethylation of H3 at lysine 9 (H3K9me3), and in particular a reduction of this mark in proximity to stress-related genes, including those encoding the GR and fibroblast growth factor 2, in HPC, AMY, and NAc. Similarly, stress sensitive BALB/C mice exhibit reduced H3K4me3 expression in NAc following chronic stress. Indeed, alteration in methylation of H3K9 or H3K4 seem to be key players in regulating vulnerability to stress across the lifespan. For example, early MS induces opposite patterns of histone methylation in HPC in early adolescence versus adulthood. Adolescent rats previously exposed to MS display decreased H3K9me2, but increased BDNF, expression and enhanced HPC neurogenesis. By contrast, adult rats exposed to MS exhibit impaired spatial memory, decreased neurogenesis and increased performance in H3K9me2 expression.

MS has also been shown to produce persistent downregulation of numerous HMTs within mPFC, including SMYD3 and SUV420H1, which lasts into adulthood and may underlie some of the altered structural and functional plasticity induced by ELS. Moreover, adult male mice with a history of MS exhibited increased H3K4me3 in the promoter region of DNA damage induced apoptosis suppressor (Ddias) and phosphatidylinositol-5-phosphate 4-kinase type 2 alpha (Pip4k2a) in mPFC. Yet another study using a rat model of ELS found that impaired social
behavior in adulthood was correlated with elevated levels of H3K4me2/me3 and H3K9me2/me3 in AMY. These data complement work mentioned earlier showing that offspring of high MC mothers also display increased levels of H3K4me3 in dHPC. Not all consequences of ELS are deleterious, as potential epigenetic mechanisms underlying the beneficial impact early life adversity have been reported. For instance, male mice exposed to MS along with unpredictable maternal stress (MSUS) show increased performance in anxiety-

Figure 2. Common high-throughput techniques for probing the epigenome. Owing to methodological advances, the toolbox for studying of the dynamics of epigenetic modifications is rich and constantly evolving. Several approaches exist to assess changes in DNA methylation including whole genome bisulfite sequencing (WGBS), methylated DNA immunoprecipitation (MeDIP) and methylation-sensitive restriction enzyme sequencing (MRE-Seq). At the level of RNA: next generation RNA-sequencing (RNA-Seq) is widely used to probe changes in gene expression. Recently m6A/m sequencing has been utilized to test changes in RNA methylation. PTM of histones can be detected using chromatin immunoprecipitation coupled with DNA sequencing (ChIP-Seq) or mass spectrometry (MS) to detect multiple histone PTMs simultaneously. Chromosome conformation capture (Hi-C) can be used to uncover contacts between distant genomic loci that affect gene expression and/or chromatin structure. Assay for transposase-accessible chromatin sequencing (ATAC-Seq) can be used determine genome-wide chromatin accessibility and genome architecture mapping (GAM) allows for the mapping of genome-wide chromosomal interactions. Together, as each tool has its pros and cons, combining multiple assays can enable profiling the changes in the epigenome, allowing a more thorough understanding of potential mechanisms contributing to MDD.
related tasks and goal directed-behavioral assays, as well as increased behavioral flexibility.\textsuperscript{56} This increased overall performance is associated with decreases in H3K4me2 and H3K36me3 at an intragenic region of the gene encoding the mineralocorticoid receptor within HPC. While these data support the possibility that some forms of ELS can promote resilience through epigenetic-based mechanisms, future studies are needed to provide causal evidence, including how, when, and which forms of ELS promote susceptibility/resilience and the specific underlying epigenetic mechanisms involved.

**Histone variants**

The substitution of histone variants for canonical plays a critical role in chromatin function. While these variants are highly conserved across species\textsuperscript{57} little is known about their role in the sustained changes in gene expression induced by ELS. In adults, the histone variant H3.3 is increased in NAc of depressed humans and in adult, susceptible mice exposed to CSDS.\textsuperscript{58} This study analyzed the developmental dynamics of H3.3, and the canonical histones H3.1 and H3.2, in the mouse NAc in response to ELS. ELS induced a sustained elevation of H3.3 and a loss of H3.2 levels in adult mice, indicating that reduced histone turnover may contribute to increased stress susceptibility in adulthood.

In summary, histone modifications are robustly regulated by ELS across brain regions; however, the significance of these changes are region-specific. Existing data suggest that, overall, increased histone acetylation, which would favor an open chromatin conformation and increased transcription, is antidepressant. More work is needed to understand how histone acetylation and methylation patterns regulate expression of specific gene targets implicated in depression and whether this pathway can be targeted for treatment. This may be achieved through future studies geared toward combining genome-wide histone code profiling with RNA-seq (Figure 2) to determine causal relationships between epigenetic modulation and gene expression.

**Chromatin remodeling**

Factors that regulate chromatin remodeling might be associated with depression. For example, the chromatin-remodeling factor, SMARCA3, mediates neurogenesis and the antidepressant effects induced by fluoxetine.\textsuperscript{59} We recently demonstrated that ATP-utilizing chromatin assembly and remodeling factor (ACF) is necessary for stress-induced depressive-like behaviors.\textsuperscript{60} This study shows that upregulation of ACF1, a subunit of the ACF complex, in NAc is linked to stress vulnerability in adult mice and depressed humans. Additionally, altered levels of several ATP-dependent chromatin remodeling factors, including SNF2H in AMY, and CHD3 and CHD5 in ventral HPC, are seen in highly stress-sensitive mice.\textsuperscript{61} However, to date there is no information regarding the role of chromatin remodeling complexes in ELS. Similarly, further work is needed to examine effects of ELS and adult stress on 3D chromatin structure genome-wide.

**DNA methylation**

Several lines of research demonstrate that early life adversity leads to global alterations in DNA methylation and levels of DNMTs that can be observed across the lifespan, and even into subsequent generations (Table II). Indeed, patterns of DNA methylation induced by early adversity alter HPA axis programming and brain structures that regulate stress-induced negative feedback.\textsuperscript{62} For example, early MS induces long-lasting hyperactivity of the HPA axis by decreasing DNA methylation at the enhancer region of the arginine vasopressin gene\textsuperscript{63} and increases its expression in the paraventricular nucleus of the hypothalamus, an effect that persists 1 year after MS.\textsuperscript{64} Similarly, neurotensin receptor-1 (NTSR1) levels are reduced in AMY of adult rats exposed to MS. This effect was linked to increased DNA methylation in the promoter region of Ntsr1, enhanced fear conditioning and HPA axis reactivity.\textsuperscript{64} In addition, FK506 binding protein 5 (FKBP5; also known as FKBP51), a chaperone protein that modulates translocation of GR to the nucleus upon ligand binding, is altered by ELS in humans and rodents.\textsuperscript{65,66} Indeed, individuals with a single nucleotide polymorphism in the intronic region of the FKBP5 gene that were exposed to early trauma exhibit increased risk for post-traumatic stress disorder (PTSD). This polymorphism reduces DNA methylation in the promoter and intronic regions of the FKBP5 gene and increases mRNA expression of FKBP5 in vitro and in blood cells from PTSD subjects. Importantly, the reduced methylation levels correlated with the severity of PTSD.\textsuperscript{67}

Variations of MC or MS alter DNA methylation patterns in the adult HPC and mPFC that result in impaired activation of the stress-induced negative feedback.\textsuperscript{39,68} Offspring experiencing low MC display high levels of hippocampal DNA methylation of the GR gene, concomitant with reduced expression of GR mRNA.\textsuperscript{60,71} This is in line with classic
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| GENE       | GENE REGION | SPECIES                  | SEX   | AGE OF STRESS | STRESS PARADigm | BRAIN REGION | DIRECTION | REF |
|------------|-------------|--------------------------|-------|---------------|-----------------|--------------|-----------|-----|
| Nr3c1      | Exon 1, Nr3c1 promoter | Long-Evans rats          | Male  | Postnatal     | Low versus high MC | HPC          | ↑ in low MC | 41  |
| Nr3c1      | Nr3c1 promoter | C57BL/6N mice           | Male  | PND1 to PND14 | MS (2 h/day)    | HPC          | ↑↑        | 78  |
| Fkbp5      | GRE binding site | Disc1 dominant-negative transgenic mice in C57BL6 background | Male & Female | PND 35 to PND 56 | SI        | VTA        | ↓          | 76  |
| Th         | 2435–2421 from the transcription start site | Disc1 dominant-negative transgenic mice in C57BL6 background | Male & Female | PND 35 to PND 56 | SI        | VTA        | ↑          | 75  |
| Avp        | –0.5 kb downstream of the Avp gene | C57BL/6N mice          | Male  | PND1 to PND 10 | MS (3 h/day)    | PVN         | ↓          | 63  |
| Ntsr1      | Promoter region of Ntsr1 | Sprague–Dawley rats       | Male  | PND 2 to PND 14 | MS (3 h/day)    | AMY         | ↑↑        | 82  |
| Esr1       | Promoter region of Esr1 | Long-Evans rats          | Female | Postnatal       | Low versus high MC | MPOA       | ↓ in high MC | 94  |
| Reelin     | Reelin promoter | Long-Evans rats          | Male & Female | PND 1 to PND 7 | 30 min of maltreatment | PFC | ↓ in infant females | 84  |
| Bdnf       | GRE binding site | Disc1 dominant-negative transgenic mice in C57BL6 background | Male & Female | PND 35 to PND 56 | SI        | VTA        | ↑↑        | 76  |
| Bdnf I exon |                   | Long-Evans rats          | Male & Female | PND 1 to PND 7 | 30 min of maltreatment | PFC | ↓       | 84  |
| Bdnf IV exon |                | Long-Evans rats          | Male & Female | PND 1 to PND 7 | 30 min of maltreatment | PFC | ↑↑ in adult females | 84  |
| Bdnf IV exon |                | Long-Evans rats          | Male & Female | PND 1 to PND 7 | 30 min of maltreatment | AMY vHPC | ↑↑ in adolescent females | 79  |
| Bdnf IX exon |                | Long-Evans rats          | Male & Female | PND 1 to PND 7 | 30 min of maltreatment | dHPC | ↑↑ in adolescent females | 79  |
| Bdnf IX exon |                | Long-Evans rats          | Male & Female | PND 1 to PND 7 | 30 min of maltreatment | PFC | ↑        | 78  |
| Bdnf IX exon |                | Balb/c mice              | Male & Female | PND1 to PND 14 | MS (2 h/day)    | HPC         | ↑↑        | 68  |
| Gdnf       | Gdnf promoter | Sprague-Dawley rats | Male  | PND 1 to PND 14 | MS (6 h/day)    | VTA         | ↑↑        | 83  |

*Table II. Alterations in DNA methylation in rodent models of early life stress.*
findings that associate such changes in GR with heightened HPA activity. Similar alterations have been observed in humans, and in vitro analysis provided causal evidence that methylation of the GR promoter regulates gene transcription.

Social isolation (SI) during peri-adolescence produces glucocorticoid-induced DNA methylation changes in VTA DA neurons. In one study, disrupted-in-schizophrenia 1 (Disc1) mutant mice were subjected to stress by SI in adolescence. Disc1 mice exhibited high-stress sensitivity and developed stress-induced depression-like behaviors. Interestingly, Disc1 mice exhibited decreased extracellular levels of DA in cortex and hypermethylation of the tyrosine hydroxylase (TH) gene promoter in VTA. These epigenetic patterns were observed even 12 weeks post-SI and were specific to DA neurons projecting to PFC. Interestingly, Disc1 mice also show increased methylation in the intronic glucocorticoid response element (GRE) of the Bdnf gene, but decreased methylation in the intronic GRE of the Fkbp5 gene, suggesting involvement of GR as a transcription factor. Indeed, in studies administering the GR antagonist, RU38486, during early adolescence reported reversal of DA alterations induced by SI in Disc1 mice.

ELS also alters methylation of genes that play important roles in neurodevelopment, neurogenesis or synaptic plasticity. For example, variations in MC increased binding of nerve growth factor-inducible protein A (NGFI-A) to the GR gene, which determines its methylation and acetylation patterns, whereas the promoter of the Glial cell line-derived neurotrophic factor (Gdnf) gene, which regulates VTA development, is hypermethylated in MS rats and plasma levels of DA and GDNF inversely correlated with depression-like behaviors. Moreover, several studies report alterations in BDNF signaling and Bdnf methylation in HPC, PFC, AMY, and VTA after ELS. For example, ELS causes long-lasting decreases in Bdnf mRNA levels, along with increased DNA methylation of the Bdnf gene in PFC. These alterations in methylation were observed even one generation after ELS, suggesting persistent and transgenerational changes in DNA methylation. Disruption of the caregiving environment by low nesting material or early maltreatment affect methylation patterns of exon IV of Bdnf in PFC, AMY, and HPC, and it was noted that this is a sex-specific effect that varies across development. Similarly, mice exposed to MS display increased methylation of exon IX of Bdnf in HPC, suggesting that methylation of Bdnf occurs at several regions of the gene. Together, these findings support human studies demonstrating that individuals with Val66Met polymorphism in BDNF are more sensitive to childhood adversity and depression in adulthood.

Early adversity induces age-dependent and sex-specific effects on several regulators of DNA methylation, including DNMTs, and DNMT inhibitors have been used to prevent the deleterious effects of ELS and adult stress in rodents. One study found that MS induces a short-term increase in expression of Dnmt1, Dnmt3a, and Dnmt3b in rat mPFC, an effect that depends on GR binding. Similarly, adolescent males previously exposed to early maltreatment display decreased mRNA levels of MECP2 (a methylated DNA binding protein) in mPFC. In adulthood, levels of Dnmt1, Dnmt3a, Mecp2, Gadd45b, and Hdac1 mRNA were decreased in male rats with early maltreatment, whereas adult females only exhibit a significant decrease in Gadd45b. Gadd45b is highly regulated by maternal interactions and adult stress. Indeed, rewarding maternal contact increases Gadd45b expression, induces β1 adrenergic receptor gene hypomethylation, and enhances noradrenergic signaling in mPFC of adult rats. In HPC, MS increased DNMT1 expression, which is associated with increased methylation at the promoter of the retinoic acid receptor-α (RARα) gene, along with decreased RARα expression. This effect leads to reduced neurogenesis by attenuating neural differentiation of adult neural precursor cells. Adolescent stress (AS) induces global reductions in HPC DNA methylation in females compared with controls, an effect linked to differential regulation of the estrogen receptor 1 (Esr1) gene. Variations in MC also alter DNA methylation of the Esr1 gene and affect maternal behavior in females. Importantly, Esr1 has been causally linked to sex differences in stress responses, suggesting that it may be a promising target for the lasting effects of ELS.

There is also promising evidence that changes in DNA methylation in brain caused by exposure to ELS are associated with alternations in DNA methylation in blood. One study showed that prenatal exposure to bisphenol A (BPA), an agent that disrupts neurodevelopment, induced lasting sex-specific changes in DNA methylation of Bdnf both in mouse HPC and blood. These changes are consistent with methylation alterations at BDNF in cord blood of humans exposed to high maternal BPA levels in utero. These results
Box 1. OTX2 programs lasting stress susceptibility. Adverse early life experiences increase the risk for MDD and other stress-related disorders by inducing long-lasting changes in the function and connectivity of key brain structures. These alterations are mediated in part by master regulators of transcription during this critical period of development. One of these regulators is the transcription factor orthodenticle homeobox 2 (OTX2) that has a key role in development of the ventral tegmental area (VTA) by controlling the differentiation of neuronal progenitors into dopaminergic neurons. In VTA, Otx2 mRNA—which is highly enriched in dopamine neurons in this brain region—is high during early postnatal age, but decreases in adolescence and remains low through adulthood. Our group recently demonstrated that OTX2 in VTA programs long-lasting effects of early life stress (ELS) in mice (panel A). Specifically, Peña et al established a “two-hit” stress model, in which juvenile mice were exposed to ELS first-hit by an adapted protocol of maternal separation. In adulthood, these mice were further exposed to a second-hit of stress, namely, chronic social defeat stress (CSDS). We found that ELS increases susceptibility to adult stress by inducing a transient reduction of OTX2 levels in the juvenile VTA. Viral-mediated manipulations demonstrated that juvenile overexpression or downregulation of Otx2 in VTA prevents or promotes, respectively, susceptibility to adult stress. Reduced OTX2 expression induced by ELS is associated with reduced binding to regulatory regions of critical target genes involved in VTA development, including Sema3c and Wnt1 and thereby leads to a long-lasting transcriptional programming in VTA that renders individuals more susceptible to a second-hit of stress even though OTX2 levels themselves recover. Current research is focused on understanding the nature of the “chromatin scar” that leaves these OTX2 target genes impaired for a lifetime. In a follow up translational study, Kaufman et al. examined whether peripheral markers of OTX2 methylation and OTX2-regulated genes as well as resting-state functional connectivity were associated with vulnerability or resilience to depression in children that experienced maltreatment (panel B). The authors reported that the degree of DNA methylation at the OTX2 gene in blood positively correlated with history of maltreatment and predicted depression in children. Moreover, increased OTX2 methylation (which would be expected to decrease OTX2 expression as seen in mice) was associated with increased functional connectivity between key brain structures implicated in depression, including the right ventromedial PFC and bilateral regions of medial frontal cortex and cingulate gyrus. Future experiments are needed to understand whether and how alterations of OTX2 protein signaling in the juvenile VTA leads to global changes in brain circuitry and connectivity. Collectively, preclinical and clinical evidence support the role of OTX2 as a master developmental regulator of enduring alterations in transcription induced by ELS and a promising predictive biomarker and therapeutic target.

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suggest BDNF DNA methylation in blood as a biomarker for the early detection of psychopathology. Another prospective study profiled CpG methylation genome wide in a large cohort of humans using microarrays on cord blood and on leukocytes later in childhood. The authors identified 38 sites with changed methylation at childhood that are associated with exposure to early life adversity, particularly adversity before the age of 3. Peripheral changes in methylation are of high translational relevance due to their potential use as biomarkers for detecting exposure to stress. Indeed, understanding how alterations in DNA methylation occurring in brain are functionally associated with vulnerability to depression offers novel therapeutic strategies. An example of this approach, focusing on the ELS-regulated gene, orthodenticle homeobox 2 (OTX2), is given in Box 1.

Noncoding RNAs

miRNAs

In the last decade there has been an increased interest in understanding the role of miRNAs in regulating depression and other stress-related disorders. Advances have been possible since large subsets of miRNAs are evolutionarily conserved between rodents and humans. Several studies tested the effects MS on specific miRNA-target interactions in male rat brain. Uchida et al found that MS leads to increased levels of several miRNAs in mPFC when measured in early life, namely miR-132, miR-124, miR-212, miR-9 and miR-29. Upregulation of the first 3 miRNAs was sustained into adulthood. The authors suggest that this may be caused by miR-16-Bdnf interactions. miR-16 has been implicated in depression by regulating the serotonergic system. Zhang et al found that MS and CUS increased DA receptor 2 (DRD2) levels, and inversely decreased miR-9 expression in striatum. The combination of MS and CUS had an augmented effect on this miRNA and target regulation. In vitro assays demonstrated that miR-9 and DRD2 directly interact. In addition, the study showed that miR-326 is downregulated in striatum by MS and that treatment with escitalopram rescues its dysregulation. Stress in adolescence can also alter miRNA expression in brain. Exposure of male rats to AS downregulates miR-135a levels in mPFC, which coincides with increased serotonin receptor 1A levels. This dysregulation was normalized by paroxetine or by a corticotropin-releasing hormone antagonist. This miRNA-target interaction was originally identified in other stress models in raphe nucleus. In another study, Xu et al tested the effects of AS on male rats’ basolateral AMY miRNA expression patterns. They focused on the GR-FKBP5 pathway and miR-18a and miR-124a, which are known regulators of GR. The authors found that AS increased miR-124a expression levels in the basolateral AMY in adolescence, and the change was sustained to adulthood, along with an increase in miR-18a expression during adolescent only. Levels of GR and FKBP5 were inversely downregulated, as expected.

miR-15a was identified in another study as part of the AMY response to chronic stress. The authors reported that miR-15a is elevated in peripheral human blood of patients in response to acute stress and following childhood trauma. This study set an example for the potential use of miRNAs as biomarkers for the long-lasting effects of early life adverse events.

In summary, to date there is evidence for the involvement of central miRNAs in epigenetically mediating the effects of ELS and facilitating the development of stress-related psychopathologies. Yet, there is a need for high-throughput screens profiling the developmental changes in miRNA expression levels caused by exposure to ELS both in brain and blood followed by causal mechanistic studies.

Transgenerational regulation of sperm miRNAs by ELS

Converging evidence suggests a highly controversial concept, that a stress induced phenotype is partly transfer-
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rable to offspring via epigenetic mechanisms.100-112 These studies are focusing on paternal transmission and several of them highlighted sperm miRNAs as the molecular mediators of this effect. Gapp et al showed that exposure of male mice to ELS transgenerationally reduced anxiety-like behavior, increased depression-like behaviors, reduced insulin levels, and blunted glucose tolerance in the offspring. The authors showed that these phenotypic changes are associated with altered miRNA expression in sperm and hippocampus of the stressed mice and their progeny. Injection of several regulated miRNAs into oocytes was sufficient to mimic the stress phenotype, suggesting that sperm miRNAs mediate transgenerational effects of stress.112 These findings are in line with earlier work showing changes in offspring sperm and central miRNAs caused by parental stress exposure, with some overlap in the highlighted miRNAs, for example upregulation of miR-375.111 The same group transgenerationally passed aspects of paternal stress to offspring by manipulating several sperm miRNAs.113

Another study tested whether the effects of ELS on the sperm epigenome is relevant to humans: they assessed miRNA expression in sperm of subjects exposed to adverse childhood experience. They tested several miRNAs based on the literature and noted that members of the miR-449 and miR-34 family were downregulated in men exposed to early life trauma. In the same study, these two miRNAs were downregulated in sperm of ELS-exposed mice and in offspring embryos.114 These findings highlight the translational value of studying regulation of miRNAs by ELS.

LncRNAs
By comparison to miRNAs, research on the function of lncRNAs in stress and depression is in its infancy. This is due to the novelty of this class of ncRNA as well as the challenges in studying them at the conceptual, bioinformatics, and experimental levels. Since many lncRNAs arose in primates, and the majority are not conserved between humans and rodents, it is challenging to perform and interpret causal experiments using animal models. Still, a few studies have looked at stress-induced regulation of lncRNAs in rodent brain115,116 and antidepressant models.117 Few human studies have explored the regulation of lncRNAs in human depression in brain118 and the circulation,119 and notably several of the recent genome-wide association study (GWAS) hits are in lncRNAs genes.120,121 Only one study profiled the effects of MD on the PFC transcriptome including lncRNAs and noted differential expression caused by ELS;122; hence this avenue is open for exploration.

Future directions and concluding remarks
As our understanding of epigenetic regulation has expanded, it has become exceedingly clear that this regulation plays a crucial role in the pathophysiology of neuropsychiatric disorders, including MDD. Notably, alterations to the epigenetic landscape early in life may produce lasting abnormalities that prime the individual for increased susceptibility to stress and depression later in life. While this provides us with a framework for future investigation, there remain many hurdles. For example, nearly all studies to date have assessed epigenetic modifications in heterogeneous populations of cells, but with recent advancement in technology a high priority becomes investigation of specific cell populations. Already the epigenetic regulation of non-neuronal cell populations has been implicated in stress responses and depression, including increased microglial-specific histone modifications123 or increased DNA methylation and histone modifications in astrocytes,124,125 as well as unique global astrocytic DNA methylation patterns in the brains of MDD patients postmortem.126 Even less is known about the contributions of cell-type specific epigenetic modulations after ELS, although a couple of recent studies have implicated epigenome changes in oligodendrocytes as being important.44,45

Women are twice as likely to suffer from depression than men.127,128 There is a dire need to better understand the inherent sex differences associated with epigenetic regulation, during ELS as well as MDD in general, since most research has focused exclusively on males.128 Recent studies conducted in rodents exposed to MS or AS have begun to reveal global epigenetic patterns that are sex-specific.58,90,94 Estrogen receptors emerge as potential therapeutic targets.50,95 In addition, functional studies using more advanced molecular techniques, including viral-mediated CRISPR-Cas9 systems,129 will enable the manipulation of specific epigenetic modifications associated with target genes and assess where and how ELS-induced changes in the epigenetic landscape orchestrate the spatiotemporal organization of neural connectivity throughout life and in a sex-specific manner.

Taken together, improving our understanding of epigenetic mechanisms involved in ELS using genome-wide studies
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with special emphasis on sex- and cell- type specificity across the lifespan can pave the way for the development of therapeutic and diagnostic interventions. Given the complexity of epigenetic regulation resulting from ELS, more advanced analytic models, including machine learning and bioinformatics, are urgently needed.

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