NRC Research Press

Biological embedding in mental health: An epigenomic perspective[1]

Aya Sasaki, Wilfred C. de Vega, and Patrick O. McGowan

Abstract: Human epidemiological studies and studies of animal models provide many examples by which early life experiences influence health in a long-term manner, a concept known as biological embedding. Such experiences can have profound impacts during periods of high plasticity in prenatal and early postnatal life. Epigenetic mechanisms influence gene function in the absence of changes in gene sequence. In contrast to the relative stability of gene sequences, epigenetic mechanisms appear, at least to some extent, responsive to environmental signals. To date, a few examples appear to clearly link early social experiences to epigenetic changes in pathways relevant for mental health in adulthood. Our recent work using high-throughput epigenomic techniques points to large-scale changes in gene pathways in addition to candidate genes involved in the response to psychosocial stress and neuroplasticity. Elucidation of which pathways are epigenetically labile under what conditions will enable a more complete understanding of how the epigenome can mediate environmental interactions with the genome that are relevant for mental health. In this mini-review, we provide examples of nascent research into the influence of early life experience on mental health outcomes, discuss evidence of epigenetic mechanisms that may underlie these effects, and describe challenges for research in this area.

Key words: epigenetics, DNA methylation, early life adversity, psychiatric disorders, animal models.

Résumé : Des études épidémiologiques réalisées chez l'humain et des études portant sur des modèles animaux fournissent plusieurs exemples dans lesquels des expériences vécues tôt dans la vie influencent la santé à long terme, un concept connu sous l'appellation de « conditionnement biologique ». De telles expériences peuvent avoir de profonds impacts durant les périodes de haute plasticité lors de la vie prénatale et tôt après la naissance. Les mécanismes épigénétiques influencent la fonction des gènes en absence de changements dans leur séquence. À la différence de la relative stabilité des séquences géniques, les mécanismes épigénétiques semblent, du moins dans une certaine mesure, répondre aux signaux environnementaux. Jusqu'à présent, quelques exemples semblent lier clairement des expériences sociales vécues précocement à des changements épigénétiques dans des sentiers pertinents à la santé mentale à l'âge adulte. Nos récents travaux réalisés à l'aide de techniques épigénomiques à haut débit indiquent la présence de changements à grande échelle dans des sentiers géniques en plus des gènes candidats impliqués dans la réponse au stress psychosocial et la neuroplasticité. De savoir quels sont les sentiers labiles au plan épigénétique et sous quelles conditions ils le sont nous permettra de mieux comprendre comment l'épigénome peut intervenir dans les interactions de l'environnement avec le génome, qui sont pertinentes à la santé mentale. Dans cette mini-revue, nous donnons des exemples de la recherche émergente portant sur les expériences vécues tôt dans la vie et leurs conséquences sur la santé mentale, nous discutons des données qui révèlent que des mécanismes épigénétiques peuvent sous-tendre ces effets, et nous décrivons les défis que pose la recherche dans ce domaine. [Traduit par la Rédaction]

Mots-clés : épigénétique, méthylation d'ADN, épreuves vécues dans l'enfance, maladies psychiatriques, modèles animaux.

Biological embedding and mental health

The concept of biological embedding has gained substantial traction as a framework for understanding the roots of complex multifactorial phenomena in health and disease (McGowan 2012). A body of research over several decades indicates that early life environmental experiences have enduring consequences for health in adulthood, including mental health, as a consequence of establishing long-term health gradients (Hertzman and Boyce 2010). Early social experiences exert among the most profound influences on mental health (Davidson and McEwen 2012). Such experiences can be partitioned in a variety of ways and inevitably involve multiple factors. A common example is that of low socio-economic status (SES) in early life, a measure of relative financial, educational, and social position, which strongly predicts a wide range of mental health problems in adulthood, including schizophrenia and depression (McEwen 2003; Hackman et al. 2010; Borghol et al. 2011). Likewise, early adverse experiences such as

physical abuse or neglect are well-known risk factors for mental health problems later in life (Turecki et al. 2012). Children who experience parental neglect as a result of institutionalization in early life show profound intellectual impairment and failure to completely catch-up even with social intervention (Beckett et al. 2006). Childhood physical and sexual abuse also impairs intellectual function and increases the risk of affective disorders and suicide (Mann and Currier, 2010). Environmental experiences during early life have been suggested to exert enhanced impact on health trajectories in part because early postnatal development is a time of enhanced plasticity (Hackman et al. 2010; Hanson et al. 2010; McGowan 2012).

Elucidating the biological mechanisms underlying effects of early social experiences on later mental health is challenging in humans for reasons that include limited access to relevant biological material. More is known about the pathways altered by adversity than other forms of early social experience. Studies in animal models have suggested that early life stress impairs neuroplastic-

A. Sasaki, W.C. Vega, and P.O. McGowan. Department of Biological Sciences, University of Toronto, Scarborough, and Department of Cell and Systems Biology, University of Toronto, 1265 Military Trail, Toronto, ON M1C 1A4, Canada.
Corresponding author: Patrick O. McGowan (e-mail: patrick.mcgowan@utoronto.ca).

ity in brain regions such as the hippocampus and has a lasting impact on endocrine systems underlying the response to psychosocial stressors (Meaney 2001; McEwen 2003). Studies in humans also indicate that the endocrine response to stress is altered in a long-term manner by early abuse. These alterations occur concomitantly with changes in the expression of genes involved in neuroplasticity and affective disorders, such as brain-derived neurotrophic factor (BDNF). Altered gene expression of both central and peripheral regulators of the hypothalamic–pituitary–adrenal (HPA) axis such as the glucocorticoid receptor (GR) has also been extensively documented (Davidson and McEwen 2012).

Epigenetics as a potential mechanism of biological embedding

By definition, biological embedding occurs when environmental factors influence developmental phenotypes relevant for health in a stable and long-term manner. This criterion is compatible with epigenetic effects; long-term changes in gene function that are mitotically though not necessarily meiotically heritable without a change in gene sequence (McGowan et al. 2008a; McGowan and Szyf, 2010a, 2010b). Mechanisms regulating epigenetic effects consist of modifications to histone proteins, modifications regulated by noncoding RNA, and modifications to the DNA itself through DNA methylation (Razin 1998; Mehler and Mattick, 2007). Modifications to histone proteins determine accessibility of the transcription machinery to the DNA. Small noncoding RNAs termed microRNA (miRNA) (Bergmann and Lane 2003) have been linked to behavioural pathologies in humans and regulate gene function through a variety of mechanisms, as has been extensively reviewed elsewhere (Vo et al. 2005; Mehler and Mattick 2006, 2007; Qureshi and Mehler 2009). These mechanisms are currently the subject of intense investigation. To our knowledge, only one paper has reported links between altered miRNA expression in adulthood and early life environment (stress) in rats (Uchida et al. 2010). DNA methylation is the best studied epigenetic modification and is associated with gene silencing in regulatory elements. DNA methylation also plays an important role in exonic regions, where increased DNA methylation is associated with increased transcriptional abundance, putatively by silencing retrotransposon and alternative transcription start site activity (Flanagan et al. 2009).

From the description above, it is clear that there are many ways in which cells can respond epigenetically to environmental factors (that is, regulate gene expression) both within and from outside the organism. As they concern outcomes related to biological embedding, epigenetic changes of particular interest are assumed to be relatively stable throughout the lifespan. Candidate cell types for such effects include neurons and some leukocytes of the adaptive immune system, which are relatively long-lived and postmitotic in adult tissues. Such cells show epigenetic alterations during development that could, at least in theory, remain relatively stable throughout the lifespan. Recent studies, however, have challenged this assumption at least for some genomic loci. We will focus on chromatin remodeling and DNA methylation — epigenetic mechanisms most closely linked to examples that meet the criterion for long-term gene regulation as a function of early experience.

Histones and chromatin remodeling

The basic building block of chromatin is the nucleosome, made up of an octamer of histone proteins. The N-terminal tails of these histones are extensively modified by methylation, phosphorylation, acetylation, and ubiquitination. The state of modification of these tails plays an important role in defining the accessibility of the DNA wrapped around the nucleosome core McGowan and Szyf 2010a, 2010b). The amino terminal tails of H3 and H4 histones that are positively charged form tight interac-

tions with the negatively charged DNA backbone, thus blocking the interaction of transcription factors with the DNA. The most investigated histone modifying enzymes are histone acetyltransferases (HAT), which acetylate histone H3 at the K9 residue as well as other residues and H4 tails at a number of residues, and histone deacetylases (HDAC), which deacetylate histone tails (Kuo and Allis 1998). Histone acetylation is believed to be a predominant signal for an active chromatin configuration, whereas deacetylated histones signal inactive chromatin and chromatin associated with inactive genes (Perry and Chalkley 1982; Lee et al. 1993). Histone acetylation neutralizes the charge and relaxes the tight grip of the histone tails on the DNA, thereby enhancing the accessibility of a gene to the transcription machinery. In contrast, deacetylated tails are highly charged and tightly associate with the DNA, limiting accessibility of genes to transcription factors (Kuo and Allis 1998). Some specific histone methylation events are associated with gene silencing and some with gene activation (Lachner et al. 2001). Histone demethylases remove the methylation mark, causing either activation or repression of gene expression (Shi et al. 2004; Tsukada et al. 2006). Particular proteins such as chromatin remodeling complexes, which are ATP dependent, recognize histone modifications and can further stabilize the transcriptional status of genes by altering the position of nucleosomes around the transcription initiation site, thereby defining accessibility of regulatory regions to the transcription machinery. For example, the heterochromatin associated protein HP1 binds H3-histone tails methylated at the K9 residue and precipitates an inactive chromatin structure (Lachner et al. 2001). The specific pattern of histone modifications was proposed to form a histone code that delineates the parts of the genome to be expressed at a given point in time in a given cell type (Jenuwein and Allis 2001).

It is now becoming clear that there is an interrelationship between chromatin modification and chromatin remodeling (Bultman et al. 2005). Chromatin remodeling complexes, which are ATP-dependent, alter the position of nucleosomes around the transcription initiation site and define its accessibility to the transcription machinery. Different histone variants that replace the standard isoforms also play a regulatory role and serve to mark active genes in some instances (Henikoff et al. 2004). As such, both chromatin modification and chromatin remodeling are highly dynamic in response to inter- and intra-cellular signals that modify transcriptional output (McGowan and Szyf 2010a, 2010b).

DNA methylation

The DNA molecule itself can be chemically modified by methyl residues, typically at the 5′ position of the cytosine rings in the dinucleotide sequence CG in vertebrates (Razin 1998), thus offering a mode of direct interaction between the environment and the genome itself. Recently, 5-hydroxymethylcytosine was rediscovered to be present at high levels in the brain and is enriched in some neuronal cell types such as cerebellar Purkinje cells (Penn et al. 1972; Kriaucionis and Heintz 2009). The functional role of this modification is currently not known. It may play a role as an intermediary between 5-methylcytosine and unmethylated DNA (Guo et al. 2011). Importantly, so-called gold standard methods of DNA methylation quantification that rely on bisulfite conversion of DNA cannot distinguish between 5-methylcytosine and 5-hydroxymethylcytosine. During development, DNA is methylated at distinct loci in different cell types, generating cell-type specific patterns of DNA methylation. Thus, the DNA methylation pattern confers upon the genome its cell type identity (Razin, 1998). Since DNA methylation is part of the chemical structure of the DNA itself, it has been considered more stable than other epigenetic marks and thus of extremely important diagnostic potential in humans (Beck et al. 1999; McGowan and Szyf 2010a, 2010b; Wu and Zhang 2010).

The DNA methylation machinery in vertebrates has 2 main roles. First, it establishes new cell-type specific DNA methylation patterns during development and possibly during adulthood in response to new signals as discussed below. Second, it maintains these patterns during downstream cell divisions and after DNA repair. The DNA methylation pattern is not copied by the DNA replication machinery, but by independent enzymatic machinery, the DNA methyltransferase(s) (DNMT) (Razin and Cedar, 1977). The methylation of DNA occurs immediately after replication by a transfer of a methyl moiety from the donor S-adenosyl-L-methionine (AdoMet) in a reaction catalyzed by DNMTs. In effect, this reaction consists of the first mechanism by which the environment can directly interact with the genome, as levels of AdoMet are regulated by diet (McGowan and Szyf 2010a, 2010b). In mammals, 3 distinct enzymes have been identified as functional DNA methyltransferases. DNMT1 shows preference for hemimethylated DNA in vitro, consistent with its role as a maintenance DNMT, whereas DNMT3a and DNMT3b methylate unmethylated and methylated DNA at an equal rate, consistent with a de novo DNMT role (Okano et al. 1998). Other proteins exist that share an evolutionary origin with the DNMTs but do not appear to play a role in actively methylating DNA. DNMT2 functions as a tRNA methyltransferase and DNMT3 L contributes to maternal genomic imprinting during gametogenesis but lacks a DNA-binding catalytic domain (Hermann et al. 2004).

DNA methylation in critical regulatory regions typically, but not always, serves as a signal to silence gene expression (McGowan et al. 2008a; McGowan and Szyf 2010a, 2010b). There are 2 main mechanisms by which cytosine methylation suppresses gene expression. The first mechanism involves direct interference of the methyl residue with the binding of a transcription factor to its recognition element in the gene. The interaction of transcription factors with genes is required for activation of the gene; lack of binding of a transcription factor would result in silencing of gene expression. This form of inhibition of transcription by methylation requires that the methylation events occur within the recognition sequence of a transcription factor. Thus, aberrant methylation will silence a gene resulting in loss of function, which will have a similar consequence to a loss of function by genetic mechanisms such as mutation, deletion, or rearrangement. A second mechanism is indirect. A certain density of DNA methylation moieties in the region of the gene attracts the binding of methylated DNA binding proteins such as MeCP2 (Nan et al. 1997). MeCP2 recruits other proteins such as SIN3A and histone modifying enzymes, which lead to the formation of a closed chromatin configuration and silencing of gene expression (Nan et al. 1997). Thus, there is crosstalk between DNA methylation and chromatin structure, whereby active regions of the chromatin, which enable gene expression, are associated with hypomethylated DNA, and hypermethylated DNA is packaged in inactive chromatin (Razin and Cedar 1977; Razin 1998). In some cases, DNA methylation of regulatory elements is linked to gene activation. For example, there is evidence that methylated DNA can recruit MeCP2 and CREB, a transcriptional activator (Chahrour et al. 2008; Zachariah and Rastegar 2012).

We have proposed that the DNA methylation pattern is a balance of methylation and demethylation reactions that are responsive to physiological and environmental signals, and thus serve as a platform for gene–environment interactions (McGowan and Kato 2008; McGowan et al. 2008a; McGowan and Szyf 2010a, 2010b). Indeed, evidence accumulated over the last 10 years indicates that, at least in some contexts, the DNA methylation status of some genes may not be as static as was once thought. It is well known that DNA can be passively demethylated during replication by, for example, disruption of DNMT activity (Wu and Zhang 2010). There are also now convincing examples of active, replication-independent DNA demethylation during development as well as in somatic tissues (Lucarelli et al. 2001; Kersh et al. 2006). The mechanism whereby DNA is actively demethylated remains an area of some controversy (Ooi and Bestor 2008).

Sources of individual differences in mental health via stochastic epigenetic mechanisms

Epigenetic differences among individuals are thought to arise from 3 principle causes: genetic, stochastic, and environmental factors. During fertilization and oocyte maturation, epigenetic modifications are predominantly reset, though it is now known that some epigenetic marks survive this resetting (Reik et al. 2001; Youngson and Whitelaw 2008). Stochastic changes in DNA methylation during cell division, when enzymatic machinery must faithfully copy the DNA methylation pattern during replication, have been estimated to exceed variation in gene sequence by 3 orders of magnitude (Petronis 2010). Inter- and intra-individual differences in DNA methylation are apparent in human sperm, though it is unknown which differences survive resetting or their function (Flanagan et al. 2006). As described above, there is extensive crosstalk between DNA methylation and histone modifications that tightly regulate gene function, which may mitigate some of these effects. It is currently not clear to what extent genetically influenced and stochastic epigenetic differences contribute to phenotypic plasticity.

Monozygotic twin pairs appear to provide a natural experiment to examine these questions, as they share virtually identical genomes and similar environments, yet frequently differ in their prevalence of mental disorders (e.g., Petronis 2006)). For example, many studies have reported DNA methylation differences between monozygotic twins discordant for schizophrenia and bipolar disorder (Tsujita et al. 1998; McDonald et al. 2003; Petronis et al. 2003; Iwamoto et al. 2005; Tochigi et al. 2007; McGowan and Kato 2008). A large-scale study of DNA methylation discordance in adolescent twins using whole-genome microarray found substantial variability across the genome in DNA methylation between monozygotic twin pairs (Kaminsky et al. 2009). These data suggest that wide-spread differences in DNA methylation in the absence of substantial genetic divergence may underlie some of the variability associated with divergent incidences of mental disorders. A recent study of twins' epigenomes at birth revealed detectable differences in DNA methylation in cord blood (Ollikainen et al. 2010). However, the contribution of stochastic compared with environmental factors to differences in DNA methylation is still unclear in these studies. For example, even among monochorionic monozygotic twins, divergent growth between twin pairs is more the rule than the exception even at birth, possibly indicating a divergent prenatal (nutritional) environment (Lewi et al. 2007). In addition, DNA methylation differences between monozygotic twin pairs were reported to increase with age (Fraga et al. 2005), suggesting the potential for continued interaction with environmental factors throughout the life-course. In the following sections, evidence for environmental contributions to mental health via epigenetic mechanisms in animal and human studies will be discussed. The examples provided below are not meant to be an exhaustive list of research in this fast-moving area, but to connect work in animal models that provide foundational or mechanistic studies of processes linked to biological embedding in humans.

Sources of individual differences in mental health via environmental epigenetic mechanisms

Animal studies

In rodents, the adult offspring of mothers that exhibit increased levels of pup licking/grooming (i.e., high LG mothers) over the first week of life show increased hippocampal GR expression, enhanced glucocorticoid feedback sensitivity, decreased hypothalamic corticotrophin releasing factor expression, and more modest HPA axis stress responses compared with animals reared by

low LG mothers (Francis et al. 1999). Such differences occur naturally in populations of rodents living in research settings, and cross-fostering studies suggest direct effects of maternal care on both gene expression and stress responses (Francis et al. 1999). These studies showing sustained effects of early care that persist until adulthood implicate the involvement of an epigenetic mechanism, because the fostering mother and not the biological genetic mother define the stress response of its adult offspring. There is evidence that early maternal care induces differences in histone 3 lysine 9 (H3K9) acetylation, DNA methylation, and the occupancy of the promoter with the transcription factor nerve growth factor-inducible protein A (NGFI-A) of the GR 1_7 splice variant in the hippocampus and the GAD67 gene in the prefrontal cortex (Weaver et al. 2004; Zhang et al. 2010). Building on these previous results, we recently conducted a whole-genome microarray analysis of DNA methylation, H3K9 acetylation, and gene expression in a 7 million bp region containing the GR gene in the rat hippocampus (McGowan et al. 2011). We found that epigenetic differences in adulthood that were associated with early maternal care occurred in clustered regions of up to 100 kb but were nonetheless exquisitely patterned, whereby increased transcription occurred in conjunction with hyperacetylation and hypermethylation of exons and hypomethylation of promoters. We found epigenetic differences in association with altered transcription as a function of maternal care predominate within the clustered protocadherin (PCDH) gene locus (McGowan et al. 2011). Clustered PCDHs are a class of cell-adhesion molecules largely expressed in the central nervous system. They exist as both a clustered superfamily composed of 58 genes in 3 groupings (α, β, and γ) across ~1 Mb on chromosome 18p11 in rats (Zou et al. 2007), as well as ~20 nonclustered genes on several chromosomes (Takeichi 2007). A body of literature indicates that PCDH gene clusters regulate neuronal morphology and synaptic plasticity (Yagi 2012). For example, dysregulation of PCDH-α genes are required for proper serotonergic innervation of the hippocampus (Katori et al. 2009) and are implicated in learning dysfunction (Fukuda et al. 2008). The expression of nonclustered PCDH during early postnatal life has also been proposed to play a role in hippocampal plasticity (Kim et al. 2010). These data are the first to link epigenetic changes across both coding and noncoding regions in the mammalian brain and implicate a nonrandom epigenetic programming across large-scale loci in response to differences in early care. Other groups have provided evidence that additional genes in neural pathways mediating the stress response are epigenetically regulated in association with early life stress, including arginine vasopressin in the hypothalamus (Murgatroyd et al. 2009) and BDNF in the prefrontal cortex and hippocampus (Roth et al. 2009).

Human studies

There has been a flurry of reports in the scientific literature pointing to a role for epigenetic alterations in mental health in humans, including complex diseases such as schizophrenia, bipolar disorder, and depression (Tsankova et al. 2007; McGowan and Szyf 2010a, 2010b). Because access to neural tissues that directly underlie behavioural processes is often limited in human studies, many studies have relied on the characterization of peripheral tissues to assess the contribution of environmental factors to pathological processes related to mental disorders. For example, there is substantial evidence of altered HPA axis regulation, including cortisol response to stress, in the offspring of mothers exposed to stress during pregnancy (e.g., Entringer et al. 2009). DNA methylation of the GR promoter in infants' cord blood was found to differ as a function of maternal mood during pregnancy and correlate with infants' cortisol response (Oberlander et al. 2008). These data suggest that GR promoter methylation in the brain and in lymphocytes is under epigenetic control as a function of the pre- and postnatal factors. A more recent study indicated

that DNA methylation of the GR promoter in placenta was associated with birth weight, implicating GR methylation in placental function and suggesting that environmental factors alter metabolic processes in part via epigenetic changes in GR (Filiberto et al. 2011).

We undertook a series of investigations of postmortem brain tissue from adults with well-characterized life histories to investigate the effects of early adversity in humans. Using established forensic psychiatric analyses, we focused on individuals with a history of severe physical or sexual abuse or neglect during childhood, which is common among suicide victims. Data in the literature suggest that suicide may have a developmental origin, and it was known that adversity in early life is an important risk factor for suicide (Turecki et al. 2012). In a first published report of aberrant DNA methylation associated with suicide, we showed that promoters of the genes encoding ribosomal RNA (rRNA) are heavily methylated in hippocampi from subjects who committed suicide relative to controls (McGowan et al. 2008b). Methylation of rRNA defines the fraction of rRNA molecules that are active in a cell, and the output of rRNA transcription defines to a large extent the protein synthesis capacity of a cell. We found that the genetic sequence of rRNA was identical in all subjects, and there was no difference in methylation between suicide victims and controls in the cerebellum, a brain region that is not normally associated with psychopathology. These data suggest that epigenetic effects associated with psychopathology likely target particular neural pathways. Because all of the suicide victims and none of the controls had a history of severe abuse or neglect in childhood, the data suggest that severe adversity during early childhood may have been a contributing factor to the observed epigenetic differences. But it was unclear in this study whether the observed abnormalities were a result of early adversity or whether they had emerged during adulthood as a result of the mental disorders associated with suicide (McGowan et al. 2008a, 2008b). Therefore, we undertook another study to address this question.

We examined the GR promoter in the hippocampus of human suicide victims and controls (McGowan et al. 2009). Family dysfunction and childhood adversity are linked to altered HPA stress responses and an increased risk for suicide. As in the study mentioned above, all of the suicide victims and none of the controls in this study had a history of childhood abuse or severe neglect. A third group was composed of suicide victims with a history that was negative for childhood abuse or neglect. We found evidence that, as in the animal model described above, the GR was epigenetically regulated in the human brain and associated with altered GR gene expression. Hypermethylation of GR was found among suicide victims with a history of abuse in childhood, but not among controls or suicide victims with a negative history of childhood abuse. The data suggest that epigenetic processes might mediate the effects of the social environment during childhood on hippocampal gene expression and that stable epigenetic marks such as DNA methylation might then persist into adulthood and influence vulnerability for psychopathology through effects on intermediate levels of function such as activity of the HPA axis that regulates the stress response. However, we should remain cautious about this interpretation, because it is still unclear whether the epigenetic aberrations were present in the germ line, whether they were introduced during embryogenesis, or whether they were truly changes occurring during early childhood (McGowan and Szyf 2010a, 2010b).

Using high-throughput DNA microarray, we recently analyzed the GR gene locus by interrogating a 7 Mb region containing the GR gene in hippocampi of adult suicide victims who were abused early in life compared with controls (Suderman et al. 2012). In an interesting parallel to the data for rats described above (McGowan et al. 2011), we found large-scale correlations in differences in DNA methylation between abused suicide victims compared with controls. We also found that, again similar to the data for rats de-

scribed above (McGowan et al. 2011), the clustered PCDH gene showed the largest alterations in DNA methylation within the locus examined. Alterations in PCDH genes are most closely associated with intellectual impairment in humans. For example, autism is associated with profound deficits in learning, especially learning concerning social information, and has been linked to PCDH gene mutations (Morrow et al. 2008) though, to our knowledge, the potential role of epigenetic alterations in PCDH in autism has not been examined. DNA methylation of PCDH was also found to be altered in whole blood from low SES individuals, with childhood SES more predictive of adult DNA methylation patterns than adulthood SES (Borghol et al. 2011). The function of these differences in PCDH DNA methylation remains unknown. These data suggest that PCDH genes are epigenetically labile in response to the early life social environment in both rodents and humans (Suderman et al. in press). Nevertheless, the data indicate that the animal model of parental care may have broad applicability for understanding the consequences of epigenetic modification of PCDH gene pathways in humans.

Chromatin modification has been less frequently studied in association with the early life social environment in humans (McGowan and Szyf 2010a, 2010b). Evidence that chromatin modifications influence mental health in humans comes from studies of the effects of pharmacological manipulations known to alter histones. Valproic acid, a long established antiepileptic and mood stabilizer, is also a histone deacetylase inhibitor (HDACi) (Phiel et al. 2001), suggesting a possible role for HDACi in treating mental disorders such as schizophrenia and bipolar disorder. Valproic acid has some effect in alleviating psychotic agitation as an adjunct to antipsychotics in schizophrenia (Bowden 2007; Yoshimura et al. 2007). One recent study found that valproate, when used during pregnancy, was associated with a 6–9 point lower average IQ in offspring at 3 years of age (Meador et al. 2009). Although biological and behavioural effects of HDACi in the brain are somewhat characterized, the specific gene targets of HDACi in the brain and their function in mental disorders are not well delineated. One question that needs to be addressed is whether the observed defects in histone acetylation in mental disorders are a consequence of aberrant deregulation of the overall levels of certain HDAC isotypes or HATs or whether it involves the aberrant targeting of HDAC to a selection of promoters. The fact that inhibition of a general enzyme such as HDAC results in exquisite positive changes in the brain implies some specificity, even for a general inhibitor of a specific class of HDACs. It will be important to delineate the response of the transcriptomes of different brain regions to HDACi and to map the genes that are critically involved in the molecular pathology of mental disorders.

Altering early life epigenetic signaling in adulthood

As described above, the covalent modifications that constitute epigenetic signaling are thought to be relatively stable, particularly in nondividing tissue such as neurons. However, there is some evidence that epigenetic signaling continues to be, at least in part, responsive to environmental intervention in the postdevelopmental period. One example is that of the response of adult animals to injections of the HDACi trichostatin A (TSA) and the methyl donor methionine a precursor of S-adenosyl-methionine (SAM) as a function of levels of maternal care received in early life (McGowan et al. 2010a, 2010b; McGowan and Kato 2008). If the DNA methylation and chromatin state is in a dynamic equilibrium even in adult neurons, it should be possible to alter the epigenetic programming, leading to a reversal of the maternal programming of GR expression and HPA responses to stress. TSA injected into brains of adult offspring of low LG maternal care mothers increased acetylation, reduced methylation, activated GR exon 1_7 promoter at levels indistinguishable from those of adult offspring of high LG maternal care mothers, and reduced stress responsivity to the levels of high LG offspring (Weaver et al.

2004). In contrast, injections of methionine into the brain of the adult offspring of high LG mothers changed the DNA methylation state of GR exon 1_7 promoter and expression of GR in the hippocampus as well as increased their stress responsiveness and reduced the time that these animals spent in the centre of an open field, a measure of anxiety (Weaver et al. 2005). Because methionine alone does not methylate DNA but is converted to SAM in the DNA methylation reaction, the data suggest that the enzymatic machineries required to generate new methylation patterns are present in adult tissue. Taken together, the TSA and methionine experiments support the basic hypothesis that epigenetic programs in the brain are maintained by a dynamic equilibrium of methylation and demethylation, a balance that could be shifted by agents that either inhibit demethylation reactions or stimulate DNMTs. Other evidence comes from a study of the effect of zebularine, a thymidine analogue and inhibitor of DNMT activity, that normalizes the hypermethylation of BDNF promoters observed in a model of early abuse in rats (Roth et al. 2009). Thus, despite the remarkable stability of epigenetic programs, they appear to be, at least in some cases, reversible.

Very little is known about the ability of the behavioural, pharmacological, or social environment to reverse epigenetic changes influencing mental health in humans. What little we do know comes primarily from studies of the effects of drugs that tap in to the epigenetic machinery and are used in the treatment of mental disorders (McGowan and Kato 2008; McGowan et al. 2008a). One example is the effect of the HDACi valproate on mood disorders (Phiel et al. 2001) and psychosis (Yoshimura et al. 2007), as mentioned above. Another example is the effect of SAM in mood disorders. As mentioned above, central infusion of L-methionine, a precursor of SAM, increases DNA methylation of the promoter of the GR gene in rodents. The fact that SAM, which similarly enhances DNA methylation, is effective in the treatment of depression is apparently contradictory to this effect of methionine. However, SAM is a methyl residue donor not only for the DNA methylation reaction but also for other enzymatic reactions. For example, creatine is produced from SAM and guanidinoacetate, and SAM treatment increases phosphocreatine levels in the brain. This effect may also contribute to the antidepressive effect of SAM because decreased phosphocreatine levels have been reported in bipolar depression (Kato et al. 1994). It is becoming clear that we need to consider these issues in the future when assessing the safety of drugs, nutraceuticals, and dietary interventions. As noted in the examples provided above, HDACi used in the treatment of psychiatric disorders, either in combination with other psychiatric drugs or alone, lack specificity for particular genes or neural networks. In the same manner as classical drugs used in psychiatric therapy, it is unlikely that any epigenetic drug by itself will be entirely effective in treating mental disorders. It will be important to develop HDACi that are specific for particular chromatin modifications as well as animal models lacking particular HDAC activities to directly test evidence for their molecular and behavioural mechanisms (Tsankova et al. 2007). Such studies will aid in the development of pharmacological therapies to mitigate the influence of early life environment on epigenetic changes associated with mental disorders.

Challenges and prospective

Progress in the coming years will involve grappling with several specific challenges in this area. First, it is likely that epigenetic patterns are specific to not only to cell-type (e.g., Iwamoto et al. 2011) but also to distinct neuronal pathways within the same anatomically defined tissue. For example, several groups have reported relatively modest changes in DNA methylation levels in the GR gene in a variety of tissue types in response to environmental factors (Mueller and Bale 2008; Oberlander et al. 2008; McGowan et al. 2009; Radtke et al. 2011; Labonte et al. 2012). How-

ever, it remains unclear whether the absolute change in levels of DNA methylation reflects a relatively small change across the majority of cells or a relatively large change in a minority of critical cell populations. This may be especially true in the brain, where optogenetic methods have identified distinct behaviourally relevant subpopulations of anatomically proximate neurons based upon distinct gene expression profiles (e.g., Kim et al. 2009). A second important issue for future studies of early life adversity in living humans is the correspondence between epigenetic alterations in the brain and those in peripheral tissues, which would enable resampling over time and after intervention. In this regard, the GR appears epigenetically sensitive across multiple tissues to alterations in the early life environment that affect risk for psychopathology (Oberlander et al. 2008; McGowan et al. 2009). Third, selecting the right study populations is paramount. Genome-wide sequencing approaches yield substantial power in the analysis of individual epigenomes, yet such approaches need to be counterbalanced with analogously sensitive phenotypic screens to identify populations of interest. Fourth, appropriate animal models of early life influences on epigenetic signaling pathways will help elucidate epigenetic mechanisms, and several have emerged. For example, transcription factors like GR target other genes, and it will be important to examine downstream genomic targets of this altered transcriptional activity. Fifth, we, along with others, have hypothesized that the social environment early in life has a long-lasting impact on mental and physical health trajectories via epigenetic marking of specific genes (McGowan and Kato 2008; Murgatroyd et al. 2009; Roth and Sweatt 2009). However, an important aspect of the basic epigenetic mechanisms reviewed here is that although the epigenetic markings are long-lasting, they are nevertheless potentially reversible. The studies described above indicate that DNA methylation can be altered through a blockade of enzymes involved in DNA methylation and changes in levels of substrate of the methylation reaction. The studies also indicate that DNA methylation can be altered by pharmacologically induced changes in chromatin structure such as HDACi. The role of environmental influences later in life in altering epigenetic programming is a question of intense study. For example, studies of posttraumatic stress disorder, the hallmark of which is a transformational change in patients' response to trauma, indicate that extreme aversive events in adulthood can induce long-lasting alterations in HPA stress response genes in the brain (Yehuda and Bierer 2009; Clinchy et al. 2010). Other data indicate that individual variation in susceptibility to posttraumatic stress disorder is influenced by early life experiences (Yehuda and Bierer 2009). Studies in animal models have shown that adverse experiences in adulthood can alter the same neural pathways implicated in the aforementioned studies of early maternal care (Clinchy et al. 2010). Epigenetic research in this area is in its infancy and offers an important avenue to study the extent to which epigenetic mechanisms remain labile in adulthood and can interact with developmental influences. Newly accessible technologies for genome-wide epigenomic mapping in appropriate contexts are providing powerful methods towards a mechanistic understanding of these processes. Clearly, future studies will move from candidate genes to candidate pathways as areas of the genome that are epigenetically labile in response to early life social experience are defined (McGowan 2012).

Acknowledgments

References

Beck, S., Olek, A., and Walter, J. 1999. From genomics to epigenomics: a loftier view of life. Nat. Biotechnol. **17**: 1144. . doi:10.1038/70651. PMID:10585673.

Beckett, C., Maughan, B., Rutter, M., Castle, J., Colvert, E., Groothues, C., et al. 2006. Do the effects of early severe deprivation on cognition persist into early adolescence? Findings from the English and Romanian adoptees study. Child Dev. **77**: 696–711. doi:10.1111/j.1467-8624.2006.00898.x. PMID:16686796.

Bergmann, A., and Lane, M.E. 2003. HIDden targets of microRNAs for growth control. Trends Biochem. Sci. **28**: 461–463. doi:10.1016/S0968-0004(03)00175-0. PMID:13678953.

Borghol, N., Suderman, M., McArdle, W., Racine, A., Hallett, M., Pembrey, M., et al. 2011. Associations with early-life socio-economic position in adult DNA methylation. Int. J. Epidemiol. **41**: 62–74. doi:10.1093/ije/dyr147. PMID:22422449.

Bowden, C.L. 2007. Spectrum of effectiveness of valproate in neuropsychiatry. Expert Rev. Neurother. **7**: 9–16. doi:10.1586/14737175.7.1.9. PMID:17187491.

Bultman, S.J., Gebuhr, T.C., and Magnuson, T. 2005. A Brg1 mutation that uncouples ATPase activity from chromatin remodeling reveals an essential role for SWI/SNF-related complexes in beta-globin expression and erythroid development. Genes Dev. **19**: 2849–2861. doi:10.1101/gad.1364105. PMID:16287714.

Chahrour, M., Jung, S.Y., Shaw, C., Zhou, X., Wong, S.T., Qin, J., and Zoghbi, H.Y. 2008. MeCP2, a key contributor to neurological disease, activates and represses transcription. Science, **320**: 1224–1229. doi:10.1126/science.1153252. PMID:18511691.

Clinchy, M., Schulkin, J., Zanette, L.Y., Sheriff, M.J., McGowan, P.O., and Boonstra, R. 2010. The Neurological Ecology of Fear: Insights Neuroscientists and Ecologists Have to Offer one Another. Front. Behav. Neurosci. **4**: 21. . doi:10.3389/fnbeh.2011.00021. PMID:21629856.

Davidson, R.J., and McEwen, B.S. 2012. Social influences on neuroplasticity: stress and interventions to promote well-being. Nat. Neurosci. **15**: 689–695. doi:10.1038/nn.3093. PMID:22534579.

Entringer, S., Kumsta, R., Hellhammer, D.H., Wadhwa, P.D., and Wust, S. 2009. Prenatal exposure to maternal psychosocial stress and HPA axis regulation in young adults. Horm. Behav. **55**: 292–298. doi:10.1016/j.yhbeh.2008.11.006. PMID:19084531.

Filiberto, A.C., Maccani, M.A., Koestler, D., Wilhelm-Benartzi, C., Avissar-Whiting, M., Banister, C.E., et al. 2011. Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. Epigenetics, **6**: 566–572. doi:10.4161/epi.6.5.15236. PMID:21521940.

Flanagan, J.M., Popendikyte, V., Pozdniakovaite, N., Sobolev, M., Assadzadeh, A., Schumacher, A., et al. 2006. Intra- and interindividual epigenetic variation in human germ cells. Am. J. Hum. Genet. **79**: 67–84. doi:10.1086/504729. PMID:16773567.

Flanagan, J.M., Munoz-Alegre, M., Henderson, S., Tang, T., Sun, P., Johnson, N., et al. 2009. Gene-body hypermethylation of ATM in peripheral blood DNA of bilateral breast cancer patients. Hum. Mol. Genet. **18**: 1332–1342. doi:10.1093/hmg/ddp033. PMID:19153073.

Fraga, M.F., Ballestar, E., Paz, M.F., Ropero, S., Setien, F., Ballestar, M.L., et al. 2005. Epigenetic differences arise during the lifetime of monozygotic twins. Proc. Natl. Acad. Sci. U.S.A. **102**: 10604–10609. doi:10.1073/pnas.0500398102. PMID:16009939.

Francis, D., Diorio, J., Liu, D., and Meaney, M.J. 1999. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. Science, **286**: 1155–1158. doi:10.1126/science.286.5442.1155. PMID:10550053.

Fukuda, E., Hamada, S., Hasegawa, S., Katori, S., Sanbo, M., Miyakawa, T., et al. 2008. Down-regulation of protocadherin-alpha A isoforms in mice changes contextual fear conditioning and spatial working memory. Eur. J. Neurosci. **28**: 1362–1376. doi:10.1111/j.1460-9568.2008.06428.x. PMID:18973563.

Guo, J.U., Su, Y., Zhong, C., Ming, G.L., and Song, H. 2011. Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. Cell, **145**: 423–434. doi:10.1016/j.cell.2011.03.022. PMID:21496894.

Hackman, D.A., Farah, M.J., and Meaney, M.J. 2010. Socioeconomic status and the brain: mechanistic insights from human and animal research. Nat. Rev. Neurosci. **11**: 651–659. doi:10.1038/nrn2897. PMID:20725096.

Hanson, M., Godfrey, K.M., Lillycrop, K.A., Burdge, G.C., and Gluckman, P.D. 2010. Developmental plasticity and developmental origins of non-communicable disease: theoretical considerations and epigenetic mechanisms. Prog. Biophys. Mol. Biol. **106**: 272–280. doi:10.1016/j.pbiomolbio.2010.12.008. PMID:21219925.

Henikoff, S., McKittrick, E., and Ahmad, K. 2004. Epigenetics, histone H3 variants, and the inheritance of chromatin states. Cold Spring Harb. Symp. Quant. Biol. **69**: 235–243. doi:10.1101/sqb.2004.69.235. PMID:16117654.

Hermann, A., Gowher, H., and Jeltsch, A. 2004. Biochemistry and biology of mammalian DNA methyltransferases. Cell. Mol. Life Sci. **61**: 2571–2587. doi:10.1007/s00018-004-4201-1. PMID:15526163.

Hertzman, C., and Boyce, T. 2010. How experience gets under the skin to create gradients in developmental health. Annu. Rev. Public Health, **31**: 329–347. doi:10.1146/annurev.publhealth.012809.103538. PMID:20070189.

Iwamoto, K., Bundo, M., Yamada, K., Takao, H., Iwayama-Shigeno, Y., Yoshikawa, T., and Kato, T. 2005. DNA methylation status of SOX10 correlates with its downregulation and oligodendrocyte dysfunction in schizophrenia. J. Neurosci. **25**: 5376–5381. doi:10.1523/JNEUROSCI.0766-05.2005. PMID:15930386.

Iwamoto, K., Bundo, M., Ueda, J., Oldham, M.C., Ukai, W., Hashimoto, E., et al.

2011. Neurons show distinctive DNA methylation profile and higher interindividual variations compared with non-neurons. Genome Res. 21: 688–696. doi:10.1101/gr.112755.110.. PMID:21467265.

Jenuwein, T., and Allis, C.D. 2001. Translating the histone code. Science, 293: 1074–1080. doi:10.1126/science.1063127. PMID:11498575.

Kaminsky, Z.A., Tang, T., Wang, S.C., Ptak, C., Oh, G.H., Wong, A.H., et al. 2009. DNA methylation profiles in monozygotic and dizygotic twins. Nat. Genet. 41: 240–245. doi:10.1038/ng.286. PMID:19151718.

Kato, T., Takahashi, S., Shioiri, T., Murashita, J., Hamakawa, H., and Inubushi, T. 1994. Reduction of brain phosphocreatine in bipolar II disorder detected by phosphorus-31 magnetic resonance spectroscopy. J. Affect. Disord. 31: 125–133. doi:10.1016/0165-0327(94)90116-3. PMID:8071475.

Katori, S., Hamada, S., Noguchi, Y., Fukuda, E., Yamamoto, T., Yamamoto, H., et al. 2009. Protocadherin-alpha family is required for serotonergic projections to appropriately innervate target brain areas. J. Neurosci. 29: 9137–9147. doi:10.1523/JNEUROSCI.5478-08.2009. PMID:19625505.

Kersh, E.N., Fitzpatrick, D.R., Murali-Krishna, K., Shires, J., Speck, S.H., Boss, J.M., and Ahmed, R. 2006. Rapid Demethylation of the IFN-{gamma} Gene Occurs in Memory but Not Naive CD8 T Cells. J. Immunol. 176: 4083–4093. PMID:16547244.

Kim, J.C., Cook, M.N., Carey, M.R., Shen, C., Regehr, W.G., and Dymecki, S.M. 2009. Linking genetically defined neurons to behavior through a broadly applicable silencing allele. Neuron, 63: 305–315. doi:10.1016/j.neuron.2009.07.010. PMID:19679071.

Kim, S.Y., Mo, J.W., Han, S., Choi, S.Y., Han, S.B., Moon, B.H., et al. 2010. The expression of non-clustered protocadherins in adult rat hippocampal formation and the connecting brain regions. Neuroscience, 170:189–199. doi:10.1016/j.neuroscience.2010.05.027. PMID:20541594.

Kriaucionis, S., and Heintz, N. 2009. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science, 324: 929–930. doi:10.1126/science.1169786. PMID:19372393.

Kuo, M.H., and Allis, C.D. 1998. Roles of histone acetyltransferases and deacetylases in gene regulation. Bioessays, 20: 615–626. doi:10.1002/(SICI)1521-1878(199808)20:8<615::AID-BIES4>3.0.CO;2-H. PMID:9780836.

Labonte, B., Yerko, V., Gross, J., Mechawar, N., Meaney, M.J., Szyf, M., and Turecki, G. 2012. Differential Glucocorticoid Receptor Exon 1(B), 1(C), and 1(H) Expression and Methylation in Suicide Completers with a History of Childhood Abuse. Biol. Psychiatry, 72: 41–48. doi:10.1016/j.biopsych.2012.01.034. PMID:22444201.

Lachner, M., O'Carroll, D., Rea, S., Mechtler, K., and Jenuwein, T. 2001. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature, 410: 116–120. doi:10.1038/35065132. PMID:11242053.

Lee, D.Y., Hayes, J.J., Pruss, D., and Wolffe, A.P. 1993. A positive role for histone acetylation in transcription factor access to nucleosomal DNA. Cell, 72: 73–84. doi:10.1016/0092-8674(93)90051-Q. PMID:8422685.

Lewi, L., Cannie, M., Blickstein, I., Jani, J., Huber, A., Hecher, K., et al. 2007. Placental sharing, birthweight discordance, and vascular anastomoses in monochorionic diamniotic twin placentas. Am. J. Obstet. Gynecol. 197: 587 e581–e588. doi:10.1016/j.ajog.2007.05.009. PMID:18060944.

Lucarelli, M., Fuso, A., Strom, R., and Scarpa, S. 2001. The dynamics of myogenin site-specific demethylation is strongly correlated with its expression and with muscle differentiation. J. Biol. Chem. 276: 7500–7506. doi:10.1074/jbc.M008234200. PMID:11096088.

Mann, J.J., and Currier, D.M. 2010. Stress, genetics and epigenetic effects on the neurobiology of suicidal behavior and depression. Eur. Psychiatry, 25: 268–271. doi:10.1016/j.eurpsy.2010.01.009. PMID:20451357.

McDonald, P., Lewis, M., Murphy, B., O'Reilly, R., and Singh, S.M. 2003. Appraisal of genetic and epigenetic congruity of a monozygotic twin pair discordant for schizophrenia. J. Med. Genet. 40: E16. . doi:10.1136/jmg.40.2.e16. PMID:12566534.

McEwen, B.S. 2003. Early life influences on life-long patterns of behavior and health. Ment. Retard. Dev. Disabil. Res. Rev. 9: 149–154. doi:10.1002/mrdd.10074. PMID:12953293.

McGowan, P.O. 2012. Epigenetic clues to the biological embedding of early life adversity. Biol. Psychiatry, 72: 4–5. doi:10.1016/j.biopsych.2012.04.017. PMID:22682394.

McGowan, P.O., and Kato, T. 2008. Epigenetics in mood disorders. Environ. Health Prev. Med. 13: 16–24. doi:10.1007/s12199-007-0002-0. PMID:19568875.

McGowan, P.O., and Szyf, M. 2010a. The epigenetics of social adversity in early life: implications for mental health outcomes. Neurobiol. Dis. 39: 66–72. doi:10.1016/j.nbd.2009.12.026. PMID:20053376.

McGowan, P.O., and Szyf, M. 2010b. Environmental epigenomics: understanding the effects of parental care on the epigenome. Essays Biochem. 48: 275–287. doi:10.1042/bse0480275. PMID:20822499.

McGowan, P.O., Meaney, M.J., and Szyf, M. 2008a. Diet and the epigenetic (re)programming of phenotypic differences in behavior. Brain Res. 1237: 12–24. doi:10.1016/j.brainres.2008.07.074. PMID:18694740.

McGowan, P.O., Sasaki, A., Huang, T.C., Unterberger, A., Suderman, M., Ernst, C., et al. 2008b. Promoter-wide hypermethylation of the ribosomal RNA gene promoter in the suicide brain. PLoS One, 3: e2085. doi:10.1371/journal.pone.0002085. PMID:18461137.

McGowan, P.O., Sasaki, A., D', Alessio, A.C., Dymov, S., Labonte, B., Szyf, M., et al. 2009. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat. Neurosci. 12: 342–348. doi:10.1038/nn.2270. PMID:19234457.

McGowan, P.O., Suderman, M., Sasaki, A., Huang, T.C., Hallett, M., Meaney, M.J., and Szyf, M. 2011. Broad epigenetic signature of maternal care in the brain of adult rats. PLoS One, 6: e14739. doi:10.1371/journal.pone.0014739. PMID:21386994.

Meador, K.J., Baker, G.A., Browning, N., Clayton-Smith, J., Combs-Cantrell, D.T., Cohen , M, et al. 2009. Cognitive function at 3 years of age after fetal exposure to antiepileptic drugs. N. Engl. J. Med. 360: 1597–1605. doi:10.1056/NEJMoa0803531. PMID:19369666.

Meaney, M.J. 2001. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu. Rev. Neurosci. 24: 1161–1192. doi:10.1146/annurev.neuro.24.1.1161. PMID:11520931.

Mehler, M.F., and Mattick, J.S. 2006. Non-coding RNAs in the nervous system. J. Physiol. 575: 333–341. doi:10.1113/jphysiol.2006.113191. PMID:16809366.

Mehler, M.F., and Mattick, J.S. 2007. Noncoding RNAs and RNA editing in brain development, functional diversification, and neurological disease. Physiol. Rev. 87: 799–823. doi:10.1152/physrev.00036.2006. PMID:17615389.

Morrow, E.M., Yoo, S.-Y., Flavell, S.W., Kim, T.-K., Lin, Y., Hill, R.S., et al. 2008. Identifying autism loci and genes by tracing recent shared ancestry. Science, 321: 218–223. doi:10.1126/science.1157657. PMID:18621663.

Mueller, B.R., and Bale, T.L. 2008. Sex-specific programming of offspring emotionality after stress early in pregnancy. J. Neurosci. 28: 9055–9065. doi:10.1523/JNEUROSCI.1424-08.2008. PMID:18768700.

Murgatroyd, C., Patchev, A.V., Wu, Y., Micale, V., Bockmuhl, Y., Fischer, D., et al. 2009. Dynamic DNA methylation programs persistent adverse effects of early-life stress. Nat. Neurosci. 12: 1559–1566. doi:10.1038/nn.2436. PMID:19898468.

Nan, X., Campoy, F.J., and Bird, A. 1997. MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell, 88: 471–481. doi:10.1016/S0092-8674(00)81887-5. PMID:9038338.

Oberlander, T.F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., and Devlin, A.M. 2008. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. Epigenetics, 3: 97–106. doi:10.4161/epi.3.2.6034. PMID:18536531.

Okano, M., Xie, S., and Li, E. 1998. Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. Nat. Genet. 19: 219–220. doi:10.1038/890. PMID:9662389.

Ollikainen, M., Smith, K.R., Joo, E.J., Ng, H.K., Andronikos, R., Novakovic, B., et al. 2010. DNA methylation analysis of multiple tissues from newborn twins reveals both genetic and intrauterine components to variation in the human neonatal epigenome. Hum. Mol. Genet. 19: 4176–4188. doi:10.1093/hmg/ddq336. PMID:20699328.

Ooi, S.K., and Bestor, T.H. 2008. The colorful history of active DNA demethylation. Cell, 133: 1145–1148. doi:10.1016/j.cell.2008.06.009. PMID:18585349.

Penn, N.W., Suwalski, R., O'Riley, C., Bojanowski, K., and Yura, R. 1972. The presence of 5-hydroxymethylcytosine in animal deoxyribonucleic acid. Biochem. J. 126: 781–790. PMID:4538516.

Perry, M., and Chalkley, R. 1982. Histone acetylation increases the solubility of chromatin and occurs sequentially over most of the chromatin. A novel model for the biological role of histone acetylation. J. Biol. Chem. 257: 7336–7347. PMID:7085629.

Petronis, A. 2006. Epigenetics and twins: three variations on the theme. Trends Genet. 22: 347–350. doi:10.1016/j.tig.2006.04.010. PMID:16697072.

Petronis, A. 2010. Epigenetics as a unifying principle in the aetiology of complex traits and diseases. Nature, 465: 721–727. doi:10.1038/nature09230. PMID:20535201.

Petronis, A., Gottesman,, I.I., Kan, P., Kennedy, J.L., Basile, V.S., Paterson, A.D., and Popendikyte, V. 2003. Monozygotic twins exhibit numerous epigenetic differences: clues to twin discordance? Schizophr. Bull. 29: 169–178. doi:10.1093/oxfordjournals.schbul.a006988. PMID:12908672.

Phiel, C.J., Zhang, F., Huang, E.Y., Guenther, M.G., Lazar, M.A., and Klein, P.S. 2001. Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. J. Biol. Chem. 276: 36734–36741. doi:10.1074/jbc.M101287200. PMID:11473107.

Qureshi, I.A., and Mehler, M.F. 2009. Regulation of non-coding RNA networks in the nervous system–what's the REST of the story? Neurosci. Lett. 466: 73–80. doi:10.1016/j.neulet.2009.07.093. PMID:19679163.

Radtke, K.M., Ruf, M., Gunter, M., Dohrmann, K., Schauer, M., Meyer, A., and Elbert, T. 2011. Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor. Transl. Psychiatry, 1. doi:10.1038/tp.2011.21. PMID:2283253.

Razin, A. 1998. CpG methylation, chromatin structure and gene silencing-a three-way connection. EMBO J. 17: 4905–4908. doi:10.1093/emboj/17.17.4905. PMID:9724627.

Razin, A., and Cedar, H. 1977. Distribution of 5-methylcytosine in chromatin. Proc. Natl. Acad. Sci. U.S.A. 74: 2725–2728. doi:10.1073/pnas.74.7.2725. PMID:268622.

Reik, W., Dean, W., and Walter, J. 2001. Epigenetic reprogramming in mammalian development. Science, 293: 1089–1093. doi:10.1126/science.1063443. PMID:11498579.

Roth, T.L., and Sweatt, J.D. 2009. Regulation of chromatin structure in memory

formation. Curr. Opin. Neurobiol. **19**: 336–342. doi:10.1016/j.conb.2009.05.011. PMID:19539459.

Roth, T.L., Lubin, F.D., Funk, A.J., and Sweatt, J.D. 2009. Lasting epigenetic influence of early-life adversity on the BDNF gene. Biol. Psychiatry, **65**: 760–769. doi:10.1016/j.biopsych.2008.11.028. PMID:19150054.

Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J.R., Cole, P.A., et al. 2004. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell, **119**: 941–953. doi:10.1016/j.cell.2004.12.012. PMID:15620353.

Suderman, M., McGowan, P.O., Sasaki, A., Huang, T.C., Hallett, M.T., Meaney, M.J., Turecki, G., and Szyf, M. 2012. Conserved epigenetic sensitivity to early life experience in the rat and human hippocampus. Proc Natl Acad Sci USA. Suppl., **2**: 17266–17272. doi:10.1073/pnas.1121260109. PMID:23045659.

Takeichi, M. 2007. The cadherin superfamily in neuronal connections and interactions. Nat. Rev. Neurosci. **8**: 11–20. doi:10.1038/nrn2043. PMID:17133224.

Tochigi, M., Iwamoto, K., Bundo, M., Komori, A., Sasaki, T., Kato, N., and Kato, T. 2007. Methylation Status of the Reelin Promoter Region in the Brain of Schizophrenic Patients. Biol. Psychiatry. doi:10.1016/j.biopsych.2007.07.003. PMID:17870056.

Tsankova, N., Renthal, W., Kumar, A., and Nestler, E.J. 2007. Epigenetic regulation in psychiatric disorders. Nat. Rev. Neurosci. **8**: 355–367. doi:10.1038/nrm2162, 10.1038/nrn2132. PMID:17453016.

Tsujita, T., Niikawa, N., Yamashita, H., Imamura, A., Hamada, A., Nakane, Y., and Okazaki, Y. 1998. Genomic discordance between monozygotic twins discordant for schizophrenia. Am. J. Psychiatry, **155**: 422–424. PMID:9501757.

Tsukada, Y., Fang, J., Erdjument-Bromage, H., Warren, M.E., Borchers, C.H., Tempst, P., and Zhang, Y. 2006. Histone demethylation by a family of JmjC domain-containing proteins. Nature, **439**: 811–816. doi:10.1038/nature04433. PMID:16362057.

Turecki, G., Ernst, C., Jollant, F., Labonte, B., and Mechawar, N. 2012. The neurodevelopmental origins of suicidal behavior. Trends Neurosci. **35**: 14–23. doi:10.1016/j.tins.2011.11.008. PMID:22177979.

Uchida, S., Hara, K., Kobayashi, A., Funato, H., Hobara, T., Otsuki, K., et al. 2010. Early life stress enhances behavioral vulnerability to stress through the activation of REST4-mediated gene transcription in the medial prefrontal cortex of rodents. J. Neurosci. **30**: 15007–15018. doi:10.1523/JNEUROSCI.1436-10.2010. PMID:21068306.

Vo, N., Klein, M.E., Varlamova, O., Keller, D.M., Yamamoto, T., Goodman, R.H.,

and Impey, S. 2005. A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. Proc. Natl. Acad. Sci. U.S.A. **102**: 16426–16431. doi:10.1073/pnas.0508448102. PMID:16260724.

Weaver, I.C., Cervoni, N., Champagne, F.A., D', Alessio, A.C., Sharma, S., Seckl, J.R., et al. 2004. Epigenetic programming by maternal behavior. Nat. Neurosci. **7**: 847–854. doi:10.1038/nn1276. PMID:15220929.

Weaver, I.C., Champagne, F.A., Brown, S.E., Dymov, S., Sharma, S., Meaney, M.J., and Szyf, M. 2005. Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. J. Neurosci. **25**: 11045–11054. doi:10.1523/JNEUROSCI.3652-05.2005. PMID:16306417.

Wu, S.C., and Zhang, Y. 2010. Active DNA demethylation: many roads lead to Rome. Nat. Rev. Mol. Cell Biol. **11**: 607–620. doi:10.1038/nrm2950. PMID:20683471.

Yagi, T. 2012. Molecular codes for neuronal individuality and cell assembly in the brain. Front. Mol. Neurosci. **5**: 45. doi:10.3389/fnmol.2012.00045. PMID:22518100.

Yehuda, R., and Bierer, L.M. 2009. The relevance of epigenetics to PTSD: implications for the DSM-V. J. Trauma Stress, **22**: 427–434. doi:10.1002/jts.20448. PMID:19813242.

Yoshimura, R., Shinkai, K., Ueda, N., and Nakamura, J. 2007. Valproic acid improves psychotic agitation without influencing plasma risperidone levels in schizophrenic patients. Pharmacopsychiatry, **40**: 9–13. doi:10.1055/s-2007-958521. PMID:17327954.

Youngson, N.A., and Whitelaw, E. 2008. Transgenerational epigenetic effects. Annu. Rev. Genomics Hum. Genet. **9**: 233–257. doi:10.1146/annurev.genom.9.081307.164445. PMID:18767965.

Zachariah, R.M., and Rastegar, M. 2012. Linking epigenetics to human disease and Rett syndrome: the emerging novel and challenging concepts in MeCP2 research. Neural. Plast. **2012**: 415825. doi:10.1155/2012/415825. PMID:22474603.

Zhang, T.Y., Hellstrom, I.C., Bagot, R.C., Wen, X., Diorio, J., and Meaney, M.J. 2010. Maternal care and DNA methylation of a glutamic acid decarboxylase 1 promoter in rat hippocampus. J. Neurosci. **30**: 13130–13137. doi:10.1523/JNEUROSCI.1039-10.2010. PMID:20881131.

Zou, C., Huang, W., Ying, G., and Wu, Q. 2007. Sequence analysis and expression mapping of the rat clustered protocadherin gene repertoires. Neuroscience, **144**: 579–603. doi:10.1016/j.neuroscience.2006.10.011. PMID:17110050.