

Chapter 2

Epigenetics, Phenotype, Diet, and Behavior

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Abbreviations

CBP	CREB binding protein
DNMT	DNA methyltransferase
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
HDACi	HDAC inhibitor ()
HMT	Histone methyltransferase ()
LG	Licking/Grooming
MBD2	Methylated domain DNA-binding Protein 2
NGFI-A	Nerve growth factor-inducible protein A
SAM	S-adenosyl methionine
TSA	Trichostatin A

2.1 Introduction

Different cell types execute distinctive programs of gene expression, which are highly responsive to developmental, physiological, pathological, and environmental cues. The combinations of mechanisms that confer long-term programming to genes and could bring about a change in gene function without changing gene sequence are termed here as epigenetic changes. We therefore propose here a definition of epigenetics, which includes any long-term change in gene function that does not involve a change in gene sequence or structure. This definition stands in contrast to other classical definitions of epigenetics that emphasize heritability. Epigenetic changes occurring in the germ line would result in heritable and trans-generational transmission of alterations in gene function in the classical sense of epigenetics. In addition, epigenetics changes in dividing cells are heritable from cell to daughter cells but are not inherited through the germ line or in postmitotic cells such as neurons and are therefore

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Table 2.1 Key facts about the epigenome

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1. Almost all cells in the body have the same genetic information, but the cell's epigenetic state determines what genes it expresses, and thus its specific cell type identity (e.g., blood cell, brain cell, etc.)
 2. DNA methylation is believed to mark silent genes, and thus aberrant methylation could have similar consequences as genetic mutations.
 3. There are also extensive epigenetic marks on chromatin that define whether genes are active or silent
 4. The epigenetic status of DNA and chromatin is thought to regulate gene activity by targeting specific molecules to specific sites in the genome
 5. There is thought to be a bilateral relationship between DNA methylation and epigenetic marks on chromatin
 6. DNA methylation is an extremely stable chemical modification of the DNA, with important diagnostic potential for human disease
 7. Both chromatin modifications and DNA methylation are potentially reversible in response to particular environmental conditions
 8. The dietary, social, behavioral, and physiological environment can modify the epigenome, with long-term consequences for gene expression, cell signaling, and thus phenotype
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This table delineates the role of the epigenome in cellular function and its response to signals from the environment

not heritable. Such changes could be environmentally driven, may occur in response to triggers at different points in life and are potentially reversible, whereas genetic differences are germ line transmitted, fixed, and irreversible.

Many of the phenotypic variations seen in human populations might be caused by differences in long-term programming of gene function rather than the sequence per se, and any future study of the basis for interindividual phenotypic diversity should consider epigenetic variations in addition to genetic sequence polymorphisms (Meaney and Szyf 2005). In effect, epigenetic silencing and genetic silencing could have similar phenotypic consequences. Therefore, epigenetic mapping is potentially as important as genetic mapping in our quest to understand phenotypic differences in human behavior.

Thus, identifying epigenetic changes that are associated with behavioral pathologies have important implications for human health, because they are potentially reversible and amenable to therapeutic intervention (Szyf 2001). Drugs that target epigenetic mechanisms are currently being tested in clinical trials in psychiatric disorders (Simonini et al. 2006). Once we understand the rules through which different environmental exposures modify the epigenetic processes, we might also be able to design behavioral strategies to prevent and revert deleterious environmentally driven epigenetic alterations. The dynamic nature of epigenetic regulation in difference from the static nature of the gene sequence provides a mechanism for reprogramming gene function in response to changes in life style trajectories, including diet. Thus, epigenetics could provide an explanation for well-documented gene x environment interactions. In this chapter, we will describe the path, which we have taken to delineate the basic mechanisms involved in epigenetic programming by maternal care in rats as a paradigm for unraveling the epigenetic basis of phenotypic differences in behavior in humans. We will also discuss our studies of epigenetic differences associated with early life adversity in humans and potential dietary contributions to epigenetic regulation (Table 2.1).

2.2 The Epigenome

2.2.1 Chromatin and the Histone Code

The epigenome consists of the chromatin, a protein-based structure around which the DNA is wrapped, as well as a covalent modification of the DNA itself by the methylation of cytosine rings found at CG dinucleotides (Razin 1998). The epigenome determines the accessibility of the

transcription machinery – which transcribes the genes into messenger RNA – to the DNA. Inaccessible genes are therefore silent whereas accessible genes are transcribed. Densely packaged chromatin can be visualized microscopically and is termed heterochromatin while open accessible chromatin is termed euchromatin. Recently, another new level of epigenetic regulation by small noncoding RNAs termed microRNA has been discovered (Bergmann and Lane 2003), which could potentially play an important role in behavioral pathologies in humans (Vo et al. 2005).

The basic building block of chromatin is the nucleosome, which is 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. It was proposed that the amino terminal tails of H3 and H4 histones that are positively charged form tight interactions with the negatively charged DNA backbone, thus blocking the interaction of transcription factors with the DNA. Modifications of the tails neutralize the charge on the tails, thus relaxing the tight grip of the histone tails. Different histone variants, which replace the standard isoforms also play a regulatory role and serve to mark active genes in some instances (Henikoff et al. 2004). 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).

2.2.2 Histone-Modifying Enzymes and Chromatin Remodeling

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 (Perry and Chalkley 1982; Lee et al. 1993). Deacetylated histones signal inactive chromatin and chromatin associated with inactive genes. Histone tail acetylation is believed to enhance the accessibility of a gene to the transcription machinery whereas deacetylated tails are highly charged and believed to be tightly associated with the DNA backbone and thus 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). Particular factors recognize histone modifications and further stabilize an inactive state. Recently described histone demethylases remove the methylation mark causing either activation or repression of gene expression (Shi et al. 2004; Tsukada et al. 2006). 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. It is becoming clear now that there is an interrelationship between chromatin modification and chromatin remodeling (Bultman et al. 2005).

A basic principle in epigenetic regulation is targeting. Histone-modifying enzymes are generally not gene specific. Specific transcription factors and transcription repressors recruit histone-modifying enzymes to specific genes and thus define the gene-specific profile of histone modification (Jenuwein and Allis 2001). Transcription factors and repressor recognize specific *cis*-acting sequences in genes, bind to these sequences and attract the specific chromatin-modifying enzymes to these genes through protein–protein interactions. Signal transduction pathways, which are activated by cell-surface receptors, could serve as conduits for epigenetic change, linking the environmental trigger at cell surface receptors with gene-specific chromatin alterations and reprogramming of gene activity.

2.2.3 DNA Methylation and Gene Expression Silencing

The DNA molecule itself can be chemically modified by methyl residues 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 such as diet and the genome itself (Fig. 2.1). What distinguishes DNA methylation in vertebrate genomes is the fact that not all CGs are methylated in any given cell type (Razin 1998). Distinct CGs are methylated in different cell types, generating cell type-specific patterns of 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 is more stable than other epigenetic marks and thus it has extremely important diagnostic potential in humans (Beck et al. 1999).

Recent data supports the idea that similar to chromatin modification, DNA methylation is also potentially reversible (Ramchandani et al. 1999b) even in predominantly post mitotic tissues such as the brain (Weaver et al. 2004a). The DNA methylation pattern is not copied by the DNA replication machinery, but by independent enzymatic machinery, (Razin and Cedar 1977) the DNA methyltransferase(s) (DNMT) (Figs. 2.2 and 2.3). DNA methylation patterns in vertebrates are distinguished by their correlation with chromatin structure. Active regions of the chromatin, which enable gene expression, are associated with hypomethylated DNA whereas hypermethylated DNA is packaged in inactive chromatin (Razin and Cedar 1977; Razin 1998).

DNA methylation in critical regulatory regions serves as a signal to silence gene expression. There are two main mechanisms by which cytosine methylation suppresses gene expression (Fig. 2.3). 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 the 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. 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 formation of a “closed” chromatin configuration and silencing of gene expression (Nan et al. 1997). 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 mechanism such as mutation, deletion, or rearrangement (Fig. 2.4).

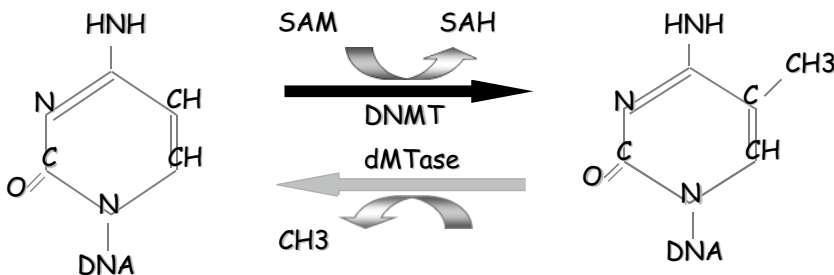


Fig. 2.1 The reversible DNA methylation reaction. DNA methyltransferases (DNMT) catalyze the transfer of methyl groups from the methyl donor *S*-adenosylmethionine to DNA releasing *S*-adenosylhomocysteine. Demethylases release the methyl group from methylated DNA. This is the first mechanism by which the environment can directly interact with the DNA, as levels of *S*-adenosylmethionine are regulated by diet

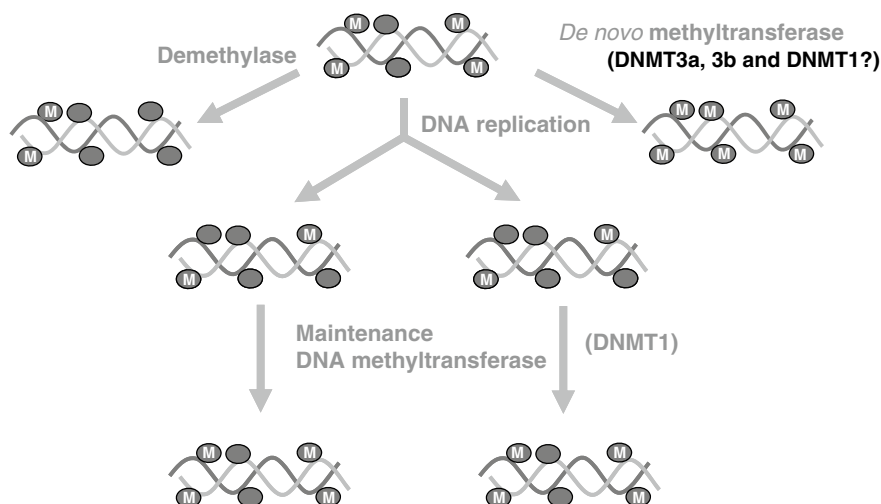


Fig. 2.2 DNA methylation reactions. Early after fertilization many of the methylation marks are removed by demethylases. (methyl groups are indicated by M, potential methylatable sites are indicated by an open circle). De novo DNA methyltransferases (DNMT) add methyl groups. Once a pattern is generated it is inherited by maintenance DNMTs that copy the methylation pattern

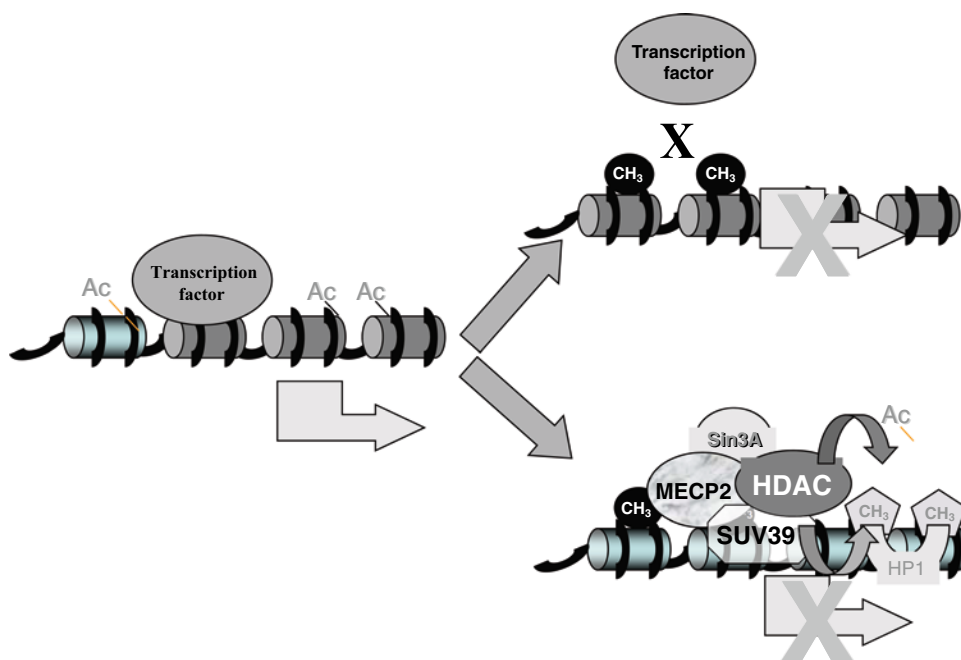


Fig. 2.3 Two mechanisms of silencing gene expression by DNA methylation. An expressed gene (transcription indicated by horizontal arrow) is usually associated with acetylated histones and is unmethylated. An event of methylation would lead to methylation by two different mechanisms. The methyl group (CH₃) interferes with the binding of a transcription factor, which is required for gene expression resulting in blocking of transcription. The second mechanism shown in the bottom right is indirect. Methylated DNA attracts methylated-DNA-binding proteins, which in turn recruit corepressors, histone methyltransferases that methylate histones, and histone deacetylases (HDAC), which remove the acetyl groups from histone tails. Methylated histones recruit heterochromatin proteins, which contribute to a closed chromatin configuration and silencing of the gene

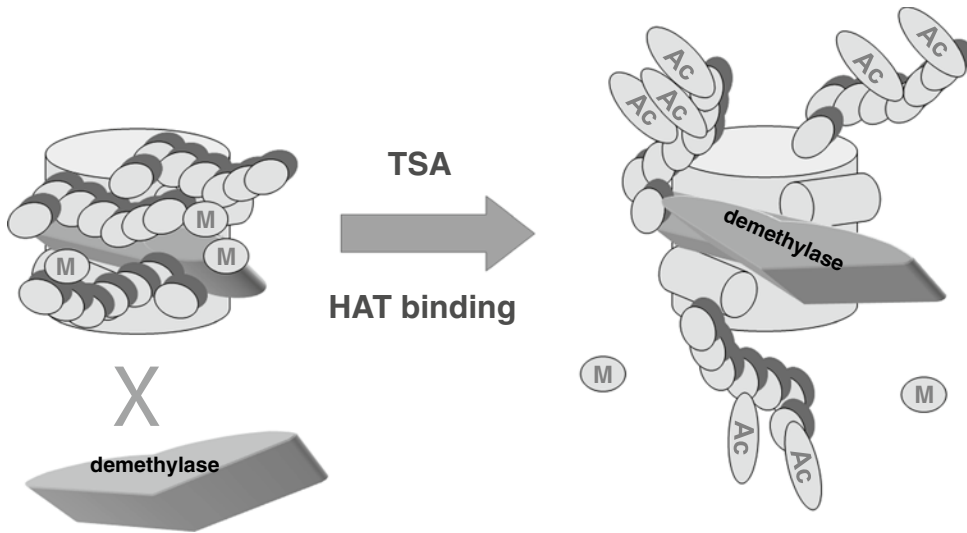


Fig. 2.4 Demethylation is directed by the state of chromatin structure. Histone acetylation (Ac) triggered by a pharmacological inhibitor of histone deacetylase facilitates the interaction of demethylases with methylated DNA allowing for demethylation

2.2.4 The Roles of the DNA Methylation Machinery and the Reversibility of DNA Methylation Patterns

The DNA methylation machinery in vertebrates has two main roles. First, it has to establish new cell-type-specific DNA methylation patterns during development and possibly during adulthood in response to new signals. Second, it has to maintain these patterns during downstream cell divisions and after DNA repair. The different enzymes and proteins of the DNA methylation machinery must address these different tasks. 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 DNA methyltransferases (DNMT) (Fig. 2.2). 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. Three distinct phylogenic DNA methyltransferases were identified in mammals. DNMT1 shows preference for hemimethylated DNA *in vitro*, which is consistent with its role as a maintenance DNMT (Fig. 2.2), whereas DNMT3a and DNMT3b methylate unmethylated and methylated DNA at an equal rate which is consistent with a *de novo* DNMT role (Okano et al. 1998).

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 forms a platform for gene–environment interactions (Ramchandani et al. 1999a) (Fig. 2.1). There are 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). One example we will explain in detail is that of the glucocorticoid receptor gene promoter in adult rat brains upon treatment with the HDAC inhibitor TSA (Weaver et al. 2004a). This finding has implications for humans, because drugs and dietary constituents known to be HDAC inhibitors are currently in widespread use.

We also propose that the direction of the DNA methylation reaction is defined by the state of chromatin. The gene specificity of the state of chromatin is defined by sequence-specific *trans*-acting

factors that recruit chromatin-modifying enzymes to specific genes. Chromatin configuration then gates the accessibility of genes to either DNA methylation or demethylation machineries (Cervoni and Szyf 2001; D'Alessio and Szyf 2006) (Fig. 2.4). We propose the following model: Factors that target specific chromatin modification events to genes define the direction of the DNA methylation equilibrium by either recruiting DNA methylation enzymes or by facilitating demethylation. We will illustrate how this might be working using gene expression programming by maternal care as a paradigm for behavioral programming of DNA methylation.

2.3 Mechanisms of Epigenetic Programming by Maternal Care and Diet

2.3.1 Maternal Care Epigenetically Programs Stress Responses in the Offspring

In the rat, the adult offspring of mothers that exhibit increased levels of pup licking/grooming and arched-back nursing (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 (CRF) expression, and more modest HPA stress responses compared to animals reared by low-LG mothers (Liu et al. 1997; Francis et al. 1999). Cross-fostering studies suggest direct effects of maternal care on both gene expression and stress responses (Liu et al. 1997; Francis et al. 1999). We have previously published evidence to support the hypothesis that epigenetic mechanisms mediate the maternal effect on stress response. Increased maternal LG is associated with demethylation that includes a nerve-growth-factor-inducible protein A (NGFI-A) transcription factor response element located within the exon 1₇ GR promoter (Weaver et al. 2004a) (Fig. 2.5). The difference in the methylation status of this CpG site between the offspring of high- and low-LG mothers emerges over the first week of life, is reversed with cross-fostering, persists into adulthood, and is associated with altered histone acetylation and NGFI-A binding to the GR promoter (Weaver et al. 2004a). Thus maternal care affects the chromatin, DNA methylation, and transcription factor binding to the GR exon 1₇ promoter, illustrating the basic principles of epigenetic regulation discussed above. We have also shown that maternal care early in life affected the expression of hundreds of genes in the adult hippocampus (Weaver et al. 2006), thus illustrating the profound effect of the social environment early in life on gene expression programming throughout life.

2.3.2 Epigenetic Programming by Maternal Care is Reversible in the Adult Animal

Although epigenetic programming by maternal care is highly stable and results in long-term changes in gene expression, it is nevertheless reversible. The possibility that certain adverse gene expression programming of behaviorally relevant genes could be reversed by either epigenetic drugs or perhaps even by behavioral intervention has obvious implications. To test this hypothesis we used the well-documented histone deacetylase (HDAC) inhibitor TSA (Yoshida et al. 1990). Since the state of histone acetylation is a balance of histone deacetylation and histone acetylation reactions, inhibition of HDAC activity would tilt the equilibrium toward acetylation and as a consequence bring

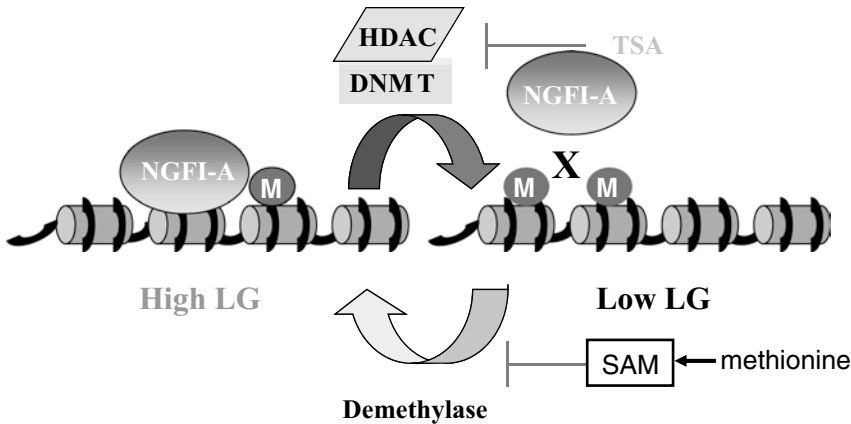


Fig. 2.5 The methylation of the hippocampal glucocorticoid receptor GR₁ promoter blocks the binding of the transcription factor binding NGFI-A. The epigenetic programming of the GR exon 1₇ promoter expression by maternal care is reversible later in life by either the HDAC inhibitor TSA or by the methyl donor SAM

about increased acetylation of histones leading to open chromatin configuration. We have previously proposed as discussed above that chromatin states and DNA methylation states are linked so that opening up of chromatin by increasing histone acetylation would tilt the balance of the DNA methylation equilibrium toward demethylation (Fig. 2.5) (Cervoni et al. 2001; Cervoni and Szyf 2001). Treating adult offspring of low-LG maternal care with TSA reversed the epigenetic marks on the GR exon 1₇ promoter; histone acetylation increased, the gene was demethylated, and there was increased occupancy of the promoter with the transcription factor NGFI-A, resulting in increased GR exon 1₇ promoter expression. The epigenetic reversal was accompanied with a behavioral change so that the stress response of the TSA-treated adult offspring of low LG was indistinguishable from the offspring of high LG (Weaver et al. 2004b). This was the first illustration of reversal of early life behavioral programming by pharmacological modulation of the epigenome during adulthood. TSA is not a DNA methylation inhibitor but nevertheless TSA treatment resulted in demethylation as we predicted. We propose that increased histone acetylation triggered by the HDAC inhibitor facilitated the interaction of a resident demethylase with the GR exon 1₇ promoter (Fig. 2.6). These data illustrate the tight association between the DNA methylation and histone acetylation equilibria in the adult brain and the potential reversibility of the DNA methylation pattern in the nondividing adult neuron.

If the DNA methylation state remains in equilibrium of methylation–demethylation in adult neurons throughout life, it should be possible also to reverse the DNA methylation in the opposite direction by increasing DNA methylation, including manipulations of methyl donors. We have previously demonstrated that the methyl donor *S*-adenosyl methionine (SAM), and amino acid present in the diet, inhibit the demethylation reaction (Detich et al. 2003). Thus, changing SAM levels would alter the DNA methylation equilibrium by either increasing the rate of the DNA methylation reaction or by inhibiting the demethylation reaction or both (Fig. 2.6). Injection of methionine to the brain led to hypermethylation and reduced expression of the GR exon 1₇ expression in the adult hippocampus of offspring of high LG and reversal of its stress response to a pattern that was indistinguishable from offspring of low LG (Weaver et al. 2005). Thus, maternal epigenetic programming could be reversed later in life in both directions.

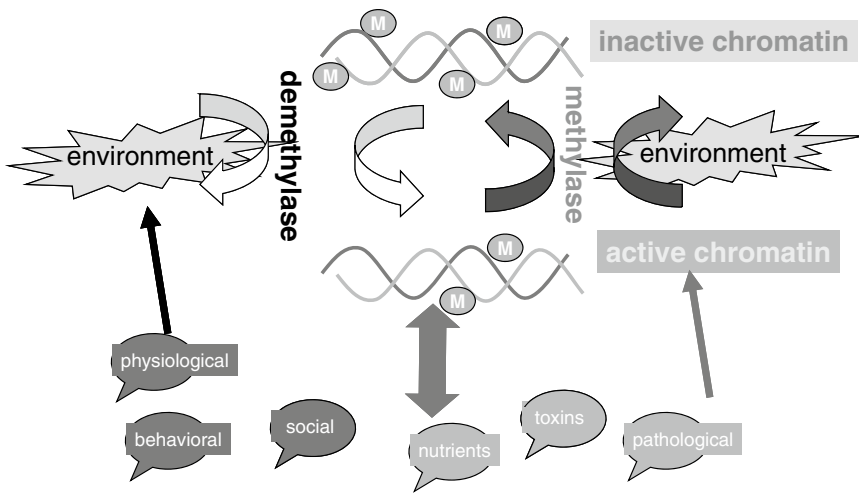


Fig. 2.6 Hypothesis: The steady state methylation pattern is a dynamic equilibrium between methylase and demethylase activities, defined by the state of chromatin. Different environmental exposures could tilt the balance of chromatin state and the DNA methylation state

These studies in rodents offer a model of epigenetic regulation of gene expression in the context of brain, behavior, and nutrition. The data show the regulation of epigenetic mechanisms in the brain (hippocampal glucocorticoid function) by a behavioral mechanism (early life environment) that is susceptible to modulation by L-methionine (a dietary amino acid). The data imply that in animals with a strong parental investment during early development, such as humans, early environment may have a profound effect on later behavioral trajectories. Thus, in humans, it may be expected that analogous mechanisms may exist to those in the animal studies reviewed above, while others may have special relevance in the context of human society.

2.4 Epigenetic Contributions to Mental Health in Humans

2.4.1 Influence of DNA Methylation on Mental Health

Genetic defects in genes encoding the DNA methylation and chromatin machinery exhibit profound effects on mental health in humans. A classic example is RETT syndrome, a progressive neurodevelopmental disorder and one of the most common causes of mental retardation in females, which is caused by mutations in the methylated-DNA-binding protein MeCP2 (Amir et al. 1999). Mutations in MeCP2 and reduced MeCP2 expression were also associated with autism (Nagarajan et al. 2006; Ben Zeev Ghidoni 2007; Herman et al. 2007; Lasalle 2007). ATRX a severe, X-linked form of syndromal mental retardation associated with alpha thalassaemia (ATR-X syndrome) is caused by a mutation in a gene, which encodes a member of the SNF2 subgroup of a superfamily of proteins with similar ATPase and helicase domains, which are involved in chromatin remodeling (Picketts et al. 1996). The ATRX mutation is associated with DNA methylation aberrations (Gibbons et al. 2000). Although these genetic lesions in the methylation machinery were present through development and

are thus fundamentally different from methylation changes after birth, these data nevertheless support the hypothesis that DNA methylation defects could lead to mental pathologies as well. Thus, it is possible that environmental exposures that would affect the activity of the methylation machinery would also lead to behavioral and mental pathologies.

There are some data indicating aberrant methylation in late onset mental pathologies, although it is unclear whether these changes in DNA methylation originated during embryogenesis or later in life as a response to an environmental exposure. The gene encoding *REELIN*, a protein involved in neuronal development and synaptogenesis, which is implicated in long-term memory, was found to be hypermethylated in brains of schizophrenia patients. The methylation of *REELIN* was correlated with its reduced expression and increased DNMT1 expression in GABAergic neurons in the prefrontal cortex (Chen et al. 2002; Costa et al. 2002, 2003; Grayson et al. 2005; Veldic et al. 2007).

We have found in our own work that promoters of the genes encoding rRNA are heavily methylated in hippocampi from subjects who committed suicide relative to controls (McGowan et al. 2008). Methylation of rRNA defines the fraction of rRNA molecules that is active in a cell, and the output of rRNA transcription defines to a large extent the protein synthesis capacity of a cell. Protein synthesis is critical for learning and memory. Thus, a reduced capacity for protein synthesis required for learning and memory in the brains of suicide victims could be epigenetically determined. This might be involved in the pathology leading to suicide. Thus, evidence is emerging that aberrant DNA methylation is involved in psychopathologies, and our study was the first published report of aberrant methylation associated with suicide. In our study, however, we found that the 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 not normally associated with psychopathology. These data imply that epigenetic effects that influence psychopathology likely target particular neural pathways. Standardized forensic psychiatry methods had been used to ascertain that all of the suicide victims in our study had a history of severe abuse or neglect during childhood, which is common among suicide victims. Thus, the data suggest that severe adversity during early childhood may have been a contributing factor to the observed epigenetic pathology. It was unclear 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. We undertook another study to address this question, and to examine whether epigenetic alterations analogous to those observed in rodents with differences in maternal care exist in humans.

As in the previous study, we examined the glucocorticoid receptor gene promoter in the hippocampus of human suicide victims and controls (McGowan et al. 2009). All of the suicide victims, and none of the controls, had a history of childhood abuse or severe neglect. A third group comprised suicide victims with a history that was negative for childhood abuse or neglect. We found that, as in the animal model described above, the glucocorticoid receptor was epigenetically regulated in the brain, and associated with altered glucocorticoid receptor gene expression. In humans, hypermethylation of the glucocorticoid receptor gene 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 are consistent with other data from the literature suggesting that suicide has a developmental origin. They 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 the vulnerability for psychopathology through effects on intermediate levels of function, such as HPA activity. However, it remains unclear whether the epigenetic aberrations documented in brain pathologies were present in the germ line, whether they were introduced during embryogenesis, or whether they were truly changes occurring during early childhood.

2.4.2 Chromatin Modification and Its Role in Mental Health

The fact that histone methylation is reversible provides a wide platform for pharmacological and therapeutic manipulations of the state of histone methylation in both directions. Both histone demethylases and histone methyltransferase are excellent candidates for new drug discovery. Understanding the intricate details of their genomic targets will allow the design of targeted and specific therapeutics.

The epigenetic effects of current clinically used monoamine oxidase inhibitors provide leads to further development of therapies targeting the epigenome. For example, H3K4Me₂ is a hallmark of active genes and the target of the histone demethylase LSD1, which demethylates H3-K4Me₂. Interestingly, certain nonselective monoamine oxidase inhibitors used as antidepressants such as Tranylcypromine that were clinically used for some time and believed to be acting on monoamine oxidases also appear to inhibit LSD1 demethylase (Lee et al. 2006). It is tempting to speculate that the inhibition of LSD1 is part of the mechanism of action of these antidepressants through activation of critical genes suppressed by the H3-K4me₂ demethylating activity of LSD1 in the brain (Shi et al. 2004) or by repressing genes activated by the H3-K9Me₂ demethylation activity of LSD1 (Metzger et al. 2005). Thus, it is possible that LSD1 inhibition is involved in the mechanism of action of antidepressive agents. It is tempting to speculate that selective inhibitors of LSD1 might be effective as antidepressants. This is an idea that might be pursued in the future.

Valproic acid, a long established antiepileptic and mood stabilizer, is also an 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). HDACi were shown to improve memory and induce dendritic sprouting in a transgenic mouse model of neurodegeneration, suggesting that HDACi might be of use in treating neurodegeneration and memory loss as well (Fischer et al. 2007). Although biological and behavioral effects of HDACi in the brain are somewhat characterized, the specific gene targets of HDACi in the brain and their function in mental pathologies are not well delineated. Nevertheless, the limited clinical and animal data suggest a potentially important role for HDACi in treatment of mental disorders. Experiments with a novel HDACi from the benzamide class *N*-(2-aminophenyl)-4-[*N*-(pyridin-3-yl-methoxycarbonyl)aminomethyl]benzamide derivative (MS-275) in mice resulted in brain region specific induction of acetylation in the frontal cortex at two genes involved with schizophrenia pathogenesis, *REELIN* and *GAD(67)* (Simonini et al. 2006). Valproic acid was shown to induce the expression of *REELIN*, which was silenced by methionine treatment in mice (Dong et al. 2007). These studies raise the possibility that treatment of schizophrenics with HDACi might cause activation of expression of critical genes such as *REELIN* and could reverse the course of this disease (Sharma et al. 2006). Several clinical trials have tested valproate as an adjunctive therapy to antipsychotics in schizophrenia. There are indications that valproate might improve violent episodes in a subset of schizophrenia patients (Basan and Leucht 2004) and might have an effect on euphoric mania in combination with antipsychotics (Bowden 2007), as well as, features of manic symptomatology in bipolar disorders (Bowden 2007). It should be noted that many of these trials were of small size and that further clinical trials are needed with valproate and with more potent and selective HDACi to methodically test the therapeutic potential of HDACi in mental pathologies.

One question that needs to be addressed is whether the observed defects in histone acetylation in mental disease 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 as discussed above. How could such specificity be achieved by treatment with nonselective HDACi? 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 the disease. It will also be important to characterize the critical isoforms of HDAC for regulation of these genes. The advent of isotypic-specific HDACi might enhance the efficacy and potency of the treatment and reduce its toxicity.

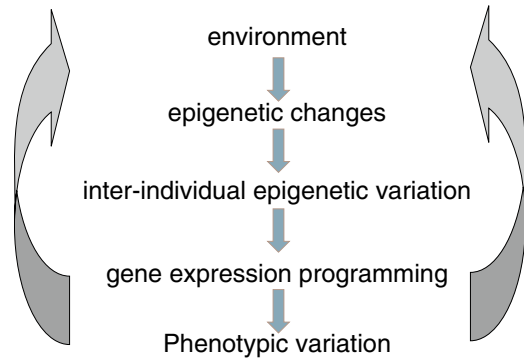
2.5 Applications to Other Areas of Health and Disease: Role of Dietary Epigenetics in Behavior and Mental Health

The experiments described above involving infusion of methionine into the lateral ventricles of the brain raise the possibility that diet can affect the phenotype being studied. Because intracellular levels of methionine can be affected by both dietary intake and polymorphisms of enzymes involved in methionine metabolism, such as methylenetetrahydrofolate-reductase (Friso et al. 2002), it is tempting to consider the possibility that diet could modify epigenetic programming in the brain not only during early development but also in adult life in humans.

Rodent models have been useful in elucidating the mechanisms involved in epigenetic alterations related to diet during development. Several studies have shown that additional dietary factors, including zinc and alcohol, can influence the availability of methyl groups for SAM formation, and thereby influence CpG methylation (Ross 2003; Davis and Uthus 2004; Pogribny et al. 2006; Ross and Milner 2007). Maternal methyl supplements affect epigenetic variation and DNA methylation and positively affect the health and longevity of the offspring (Wolff et al. 1998; Cooney et al. 2002; Waterland and Jirtle 2003). We hypothesize that reversal of epigenetic states in the brain, such as the remethylation of the exon 1₇ GR promoter, could be triggered not only by pharmacological agents but also by stable variations in environmental conditions.

Other studies have shown that certain dietary components may act as an HDACi, including diallyl disulfide, sulforaphane, and butyrate. For example, broccoli, which contains high levels of sulforaphane, has been associated with H3 and H4 acetylation in peripheral blood mononuclear cells in mice 3–6 h after consumption (Dashwood and Ho 2007). The long-term consequences of such epigenetic effects on human health remain to be studied, however HDACis are an active area of research as anti-inflammatory and neuroprotective agents in autoimmune diseases such as lupus and multiple sclerosis (Gray and Dangond 2006), and sodium butyrate has been shown to have antidepressant effects in mice (Schroeder et al. 2007). Thus, it is conceivable that dietary compounds that influence histone acetylation may affect signaling mechanisms that regulate neural function. In light of the aforementioned link between histone modifications and DNA methylation, future studies are needed to address the possibility that sustained exposure to such compounds may affect DNA methylation at susceptible loci, with implications for mental health in humans. Dietary components could act through cellular signaling pathways, leading from cell surface receptors down to *trans*-acting factors that deliver chromatin-modifying enzymes to specific sequences. The dynamic epigenome has obviously adaptive and physiological roles in the crosstalk between our environment and our inherited genome, but could at the same time serve as a target for dietary components (Figs. 2.6 and 2.7). Thus, unraveling the conduits between our diet and our genomes should have an important impact on our health.

Fig. 2.7 A scheme for environmentally driven epigenetic states and interindividual phenotypic variance in behavior and susceptibility to disease in humans. We propose a reciprocal relationship between phenotype and environmental mechanisms leading to the epigenetic programming of gene expression



Summary Points

- We propose that the DNA methylation and chromatin structure are found in a dynamic balance through life, which is maintained and defined by sequence-specific factors that deliver histone modification and DNA modification enzymes to genes.
- We propose that the direction of the DNA methylation reaction is defined by the state of chromatin and, as such, factors that target specific chromatin modification events to genes define the direction of the DNA methylation equilibrium by either recruiting DNA methylation enzymes or by facilitating demethylation.
- Epigenetic programming in the brain of rodents by maternal care during the first week of life is a highly stable yet reversible process that results in long-term changes in gene expression.
- In our studies, we found that aberrant DNA methylation of the ribosomal RNA promoter as well as the glucocorticoid receptor promoter lead to decreased transcription of each gene, and that this effect was associated with a history of early childhood abuse or neglect in humans.
- Many of the phenotypic variations seen in human populations might be caused by differences in long-term programming of gene function rather than the sequence per se, and any future study of the basis for interindividual phenotypic diversity should consider epigenetic variations in addition to genetic sequence polymorphisms.
- The fact that histone methylation, histone acetylation, and DNA methylation are potentially reversible processes provides a wide platform for research into pharmacological and therapeutic manipulations with known epigenetic effects from drugs used to treat mental illness such as valproate to dietary supplements such as l-methionine.

Key Terms

Epigenetics: DNA and chromatin modifications that persist from one cell division to the next, despite a lack of change in the underlying DNA sequence.

Epigenome: The overall epigenetic state of a cell that serves as an interface between the environment and the genome.

DNA methylation/demethylation: A biochemical modification of the DNA involving the transfer of a methyl group (CH_3), typically to the 5' position of the cytosine ring in the dinucleotide combination CG in mammals. In plants and other species DNA methylation may affect other nucleotide pairs.

Histone code: The specific pattern of histone protein modifications that delineate the parts of the genome to be expressed at a given point in time in a given cell type.

Chromatin: Histone proteins associated with the cell's DNA that regulate its accessibility to gene transcription machinery. Chromatin comes in two forms: Heterochromatin, where the DNA is tightly coiled and therefore inaccessible to the transcriptional machinery and euchromatin, where the DNA is more loosely associated with histone proteins.

Phenotype: Any observable characteristic of an organism, including its behavior. An organism's phenotype is a product of its genetics and its environment.

Methyltransferase: Enzymes involved in the transfer of a methyl donor from *S*-Adenosyl Methionine to histone proteins (histone methyltransferases) or DNA (DNA methyltransferases). Epigenetic regulation by histone methyltransferases tends to inhibit transcription.

Histone deacetylase inhibitor (HDACi): A class of chemicals that inhibits the acetylation of histones, leading to a chromatin structure that is more accessible to the transcriptional machinery. A variety of therapeutic chemicals (e.g., valproic acid) and dietary constituents (e.g., sulforaphane) have known HDACi properties.

Psychopathology: The manifestation of mental illness in the form of phenotype, including abnormal behavior and physiology.

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