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DNA Methylation: A Mechanism for Embedding Early Life Experiences in the Genome

Moshe Szyf
McGill University

Johanna Bick
Yale University

Although epidemiological data provide evidence that early life experience plays a critical role in human development, the mechanism of how this works remains in question. Recent data from human and animal literature suggest that epigenetic changes, such as DNA methylation, are involved not only in cellular differentiation but also in the modulation of genome function in response to early life experience affecting gene function and the phenotype. Such modulations may serve as a mechanism for life-long genome adaptation. These changes seem to be widely distributed across the genome and to involve central and peripheral systems. Examining the environmental circumstances associated with the onset and reversal of DNA methylation will be critical for understanding risk and resiliency.

Epidemiological data point to the importance of early life experience in setting life-long health and mental health trajectories in humans (Power, Jefferis, Manor, & Hertzman, 2006). Recent evidence suggests that early life is an especially sensitive period in which environmental signals affect the structure and function of the genome. These epigenetic processes seem to be ideal mechanisms for how early life environments may influence life-long trajectories of health and wellness. The current review provides an overview of a specific epigenetic process, DNA methylation, and presents evidence from human and animal research supporting links between early life stress, changes in DNA methylation, and phenotypic variation.

Epigenetics

The modern synthesis of Mendelian genetics and Darwinian evolution culminated with the discovery of the structure of DNA and eventually in the sequencing of the human genome. Until recently, the common belief has been that interindividual differences in sequence (i.e., structural differences in the strings of A, C, G, and T nucleotides) would account for the differences in phenotype, susceptibility to disease, and behavioral differences between individuals. Genome-wide associations have indeed identified rare alleles that associate with differences in behavior and disease susceptibility (Manolio et al., 2009). However, the structural variations in sequence have accounted for only a small fraction of human phenotypic variation and human disease (Manolio et al., 2009). There is some evidence that these interindividual differences in sequences (i.e., allelic variations) do not operate alone but interact with environmental conditions to predict phenotypic outcomes (Caspi et al., 2003). Although the interaction between genotypes and environmental conditions has been an exciting possibility, there is significant controversy regarding the replicability of these original findings (Risch et al., 2009).

The function of DNA depends not only on the variation in sequence but also on the manner by which it is stably programmed. The idea that DNA function could be stably diversified without changing the sequence comes from the study of cellular differentiation during embryonal development. The process that generates and organizes epigenetic patterns is highly programmed and organized while also dynamic and responsive to the environment

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Correspondence concerning this article should be addressed to Moshe Szyf, Department of Pharmacology and Therapeutics, McGill University, 3655 Sir William Osler Promenade, Montreal, QC, Canada H3G1Y6. Electronic mail may be sent to moshe.szyf@mcgill.ca.

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(Szyf, McGowan, & Meaney, 2008). This sensitivity of the epigenetic machinery to the environment can modify the transcriptome (i.e., what is transcribed from the genome) without modifying the genome itself; these transcriptome modifications, in turn, can have a long-term impact on somatic and mental health.

Whereas the transcriptional activity involving mRNA in response to environmental influences has been studied for decades (Schirmer, Fischer, Madureira, & Pillai, 2010), more recent research has examined the specific epigenetic mechanisms associated with genomic alterations resulting from environmental signals. These mechanisms involve chemical changes that affect the transcriptional (or functional) activity of the DNA, without altering the specific sequence of the DNA and include (a) chromatin structure and histone modification that gate the access of transcriptional machinery to genes (Jenuwein & Allis, 2001; Strahl & Allis, 2000); (b) noncoding RNA activities including microRNA that regulate gene expression through altering chromatin configuration, inhibition of translation, and degradation of RNA (Bergmann & Lane, 2003); and (c) remarkably, the DNA molecule itself bears epigenetic information encoded in the DNA methylation pattern (Figure 1; Razin & Riggs, 1980; Razin & Szyf, 1984). Recently, a specific modification of DNA methylation 5-hydroxymethylcytosine (involving an additional chemical change to the DNA methylation pattern) has been found to be particularly abundant in the brain early in embryogenesis (Jin, Wu, Li, & Pfeifer, 2011). This epigenetic process may also be important for further diversifying gene function (Williams et al., 2011). However, its specific role in controlling gene expression and its relation with DNA methylation is still unknown. This review focuses on the third epigenetic process described earlier, in which information encoded in the DNA methylation patterns, whose accessibility profile changes in response to environmental signals, influences the transcriptional activity of DNA, and shapes phenotypic outcomes in the long term.

The Process of DNA Methylation

DNA methylation refers to the process by which the DNA molecule is chemically altered through an addition of methyl groups to a specific nucleotide (cytosine) on the chain of the millions of base pairs of nucleotides that comprise DNA (Adams, 1995; Adams & Burdon, 1982; Adams et al., 1984). By adding information to the DNA molecule without changing the specific sequence of the gene, DNA methylation alters the transcriptional state of DNA, leading to changes in gene expression (Comb & Goodman, 1990; Inamdar, Ehrlich, & Ehrlich, 1991). Additionally, DNA methylation can lead to the recruitment of enzymes that modify the chromatin, or protein structure surrounding the DNA molecule, rendering it into an ''inactive'' state thereby silencing the gene (Figure 2).

Almost three decades ago, it was shown that patterns of DNA methylation vary across cell types (Figure 1; Razin & Szyf, 1984). DNA methylation is involved in the diversification of genome function during embryogenesis and cellular differentiation. For some time, it has been believed that once the DNA methylation patterns are generated during embryogenesis, they remain ''fixed'' throughout life. The biological properties related to the nucleotide sites and enzymes involved in DNA methylation seemed to imply a rigidity in DNA methylation patterns following birth (see Szyf et al., 2008, for a review of this topic). However, it has recently been considered that environmental signals might influence

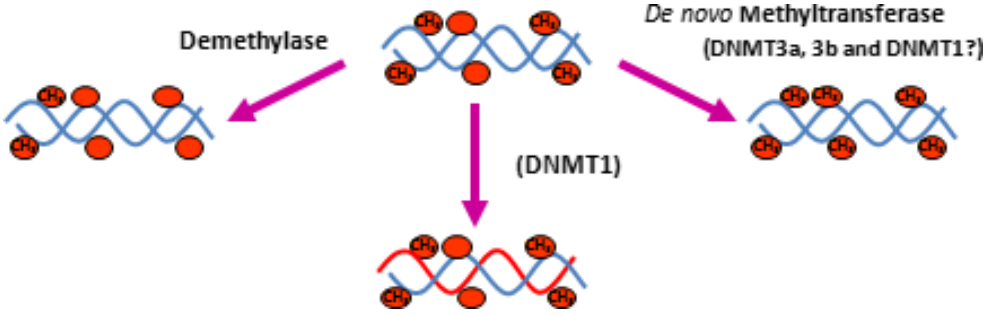

Figure 1. The pattern of DNA methylation is sculpted during gestation.
Note. The DNA methylation pattern is sculpted during embryogenesis by enzymes that add methyl groups (de novo methyltransferases) such as DNMT3a or DNMT3b, enzyme that copies the DNA methylation pattern (maintenance DNMT1) and demethylases. Different patterns of methylation are generated in distinctive cell types creating a cell-type identity.

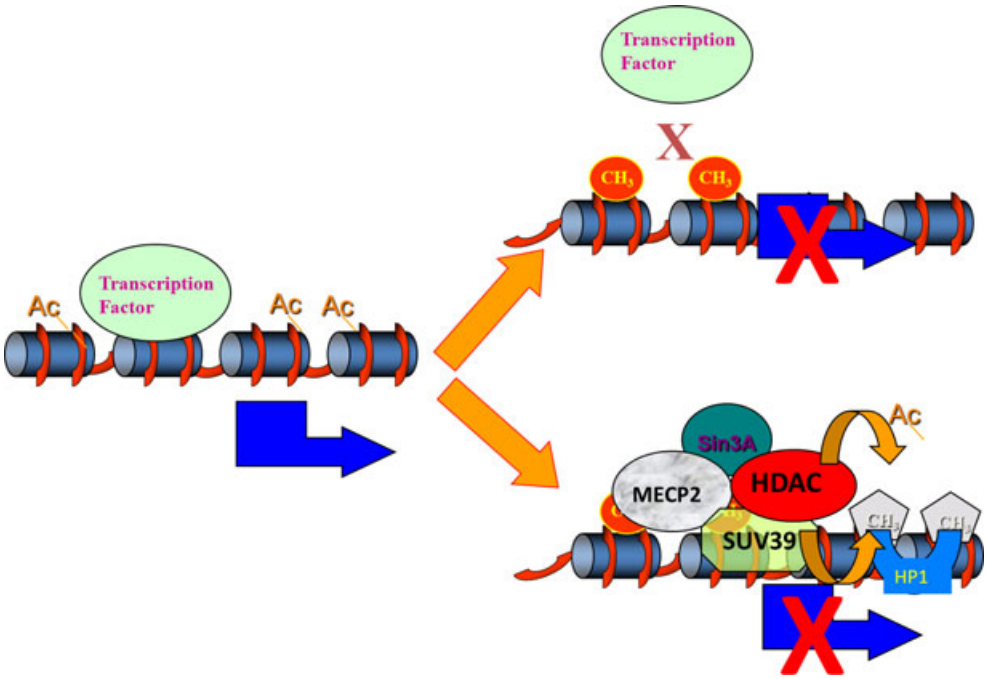

Figure 2. DNA methylation in promoters silences gene expression by different mechanisms.
Note. The first mechanism involves interference with binding of transcription factors (top right). The second mechanism involves recruitment of chromatin modifying enzymes and formation of inactive chromatin (bottom right). (AC = histone acetylation; horizontal arrow = transcription; MeCP2 = a methylCpG-binding protein; HDAC = histone deacetylase; SUV39 = a histone methyltransferase; Sin3A = a co-repressor; HPI = a methylated histone-binding protein).

DNA methylation patterns outside of embryogenesis and cellular differentiation, by occurring after birth, especially during the early stages of development (Szyf et al., 2008).

DNA Methylation as a Mechanism for Diversification of Genome Function in Response to Experience

Given that development related to emotional and behavioral maturation occurs largely after birth, the possibility that DNA methylation could potentially serve as a mechanism for the diversification of genome's functioning in response to external experiential environmental signals following birth has been examined. In recent years, new data point to models in which DNA methylation are dynamic and reflective of altered extra- and intracellular signaling (Figure 3), constituting pathological or adaptive mechanisms that are responsive to environmental stimuli, and leading to changes in either gene silencing or activation (see Szyf et al., 2008, for a review of this topic). The literature refers to the emergence of new DNA methylation sites (i.e., de novo methylation) and the removal of DNA methylation (by demethylation; Bhattacharya, Ramchandani,

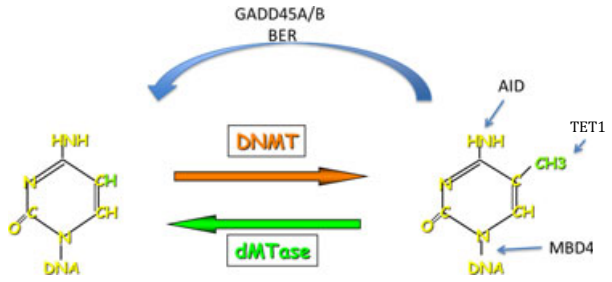

Figure 3. The DNA methylation pattern is an equilibrium between DNA methylation and demethylation reactions.
Note. Several demethylase enzymatic activities were proposed; bona fide demethylation that releases the methyl moiety (MBD2) as well as a repair reaction that involves modification of the methyl group to 5-hydroxymethyl cytosine by TET1, deamination by AID, glycosylation by MBD4 or other glycosylases that is followed by excision and repair with base excision repair enzymes (BER) and insertion of an unmethylated cytosine.

Cervoni, & Szyf, 1999; Detich, Bovenzi, & Szyf, 2003; Detich, Hamm, Just, Knox, & Szyf, 2003; Detich, Theberge, & Szyf, 2002; Ramchandani, Bhattacharya, Cervoni, & Szyf, 1999; see Figure 3 for proposed biochemical mechanisms of DNA demethylation). However, it is important to note that the process by which DNA is demethylated is highly controversial (Ng et al., 1999) and there is

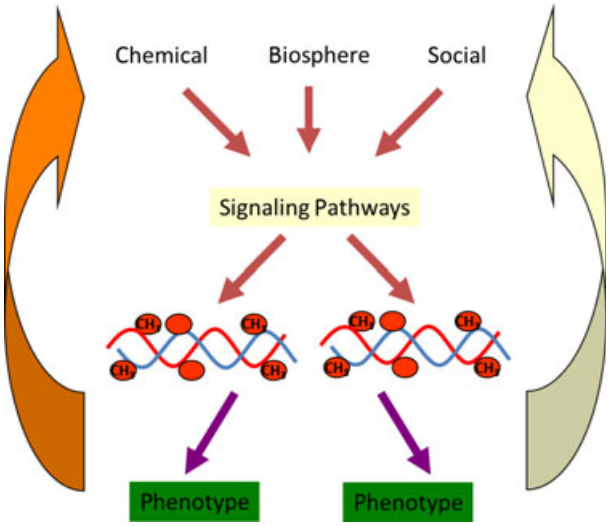

Figure 4. The dynamic relation between DNA methylation pattern and the early environments.
Note. Chemical biosphere and social environments activate signaling pathways that target DNA methylation–demethylation enzymes to multiple targets in the genome in several tissues modulating the DNA methylation pattern. The modulation of the DNA methylation pattern in different tissues alters the phenotype. There is a bilateral relation between the DNA methylation pattern and the environment. The DNA methylation matrix defines the relation with the environment, which in turn continues to modulate the DNA methylation pattern.

reluctance to accept the idea that DNA methylation is reversible (Wolffe, Jones, & Wade, 1999).

DNA Methylation as a Genome Adaptation Mechanism

Recent efforts have examined the dynamics of DNA methylation patterns following birth. It has been proposed that these DNA methylation adaptations early in life are system-wide and that they involve multiple gene circuitries (McGowan et al., 2011; Figure 4). The idea that the adaptations to early life adversity are system-wide has important implications for studying the DNA methylation changes that respond to adversity in living human populations. If indeed the response to social adversity is not limited to the brain, then it should be possible to study, follow, and assess interventions in peripheral cells such as T cells.

DNA Methylation Embedding Early Life Experiences in the Genome

Models of natural variation in maternal care in rodents have been used to demonstrate the profound impact of maternal care and ''nurture'' on a panel of phenotypes in the offspring that last into adulthood (Ruppenthal, Arling, Harlow, Sackett, & Suomi, 1976; Suomi, Collins, Harlow, & Ruppenthal, 1976). In the rat, the adult offspring of mothers that exhibit increased levels of pup licking and grooming over the 1st week of life show increased hippocampal glucocorticoid receptor (GR) expression, enhanced GR feedback sensitivity, decreased hypothalamic corticotrophin-releasing factor expression, and more modest hypothalamus–pituitary–adrenal (HPA) stress responses compared to animals reared by mothers that exhibited lower levels of increased licking and grooming toward their offspring (Francis, Diorio, Liu, & Meaney, 1999; Liu et al., 1997). The *GR/NR3C1* gene encoding the glucocorticoid receptor (GR exon 1_7 promoter) exhibits differences in DNA methylation and histone acetylation (involved in chromatin remodeling) in the hippocampus of the offspring of high and low licking and grooming mothers. Differences in DNA methylation in response to variations in maternal licking and grooming emerged early in life and remained stable into adulthood, illustrating that epigenetic programming early in life could set up life-long behavioral trajectories (Weaver et al., 2004).

The basic concepts of this study were repeated more recently in several other models of early life social adversity. Exposure of infant rats to stressed caretakers that displayed abusive behavior produced persisting changes in methylation of brain-derived neurotropic factor (BDNF) gene promoter in the adult prefrontal cortex (Roth, Lubin, Funk, & Sweatt, 2009). Furthermore, early life stress in mice caused sustained DNA hypomethylation of an important regulatory region of the arginine vasopressin (AVP) gene (Murgatroyd et al., 2009).

There have been several attempts to examine whether the results from animal studies could be translated to humans. Methylation patterns of GR genes were examined among a cohort of suicide victims in Quebec who had experienced abuse in early childhood (McGowan et al., 2009). Individuals with treatment-resistant forms of major depression showed decreased GR expression and increased HPA activity. Site-specific differences in DNA methylation in the GR exon 1f promoter and its expression were detected between suicide completers who had reported social adversity early in life and suicide completers who did not experience social adversity early in life (McGowan et al., 2009). Epigenetic modulation of other candidate genes has been implicated in suicide; the *Gamma-aminobutyric acid A receptor alpha 1* subunit (*GABRA1*) promoter

(Linthorst, Flachskamm, Muller-Preuss, Holsboer, & Reul, 1995) within the frontopolar cortex (Poulter et al., 2008) and *Tropomyosin-related kinase B* (*TRKB*) in the frontal cortex of suicide completers (Ernst et al., 2009). It is unknown yet whether these changes in DNA are also associated with early life adversity.

In addition, the state of methylation of ribosomal RNA (rRNA) gene promoters has also been examined. rRNA forms the skeleton of the ribosome, which is the protein synthesis machinery essential for building new memories and creating new synapses in the brain. One possible way to control the protein synthesis capacity of a cell is through changing the fraction of active rRNA alleles in a cell (Brown & Szyf, 2007). It has previously been shown that the fraction of rRNA genes that is active and associated with relevant transcription machinery is unmethylated whereas the fraction that is inactive (and therefore not associated with transcriptional activity) is methylated (Brown & Szyf, 2007). In the cohort of suicide victims in Quebec who were abused as children and their control group, results showed that the suicide victims who had also experienced childhood abuse had higher overall methylation in their rRNA genes and expressed less rRNA. This difference in methylation was brain-region specific: It was present in the hippocampus and was not observed in the cerebellum. Based on the retrospective design of this study, it is not possible to determine whether early life abuse was directly responsible for DNA methylation profiles. However, ongoing longitudinal work may address this issue (see, e.g., Naumova et al., 2012).

Importantly, although significant methylation differences were observed between the controls and suicide victims, the participants did not vary in their DNA sequence. The fact that the difference in methylation was brain-region specific and that no sequence differences were observed further strengthens the conclusion that this difference in methylation was driven by environmental rather than genetic variation (McGowan et al., 2008).

The Impact of Early Life Adversity Is Not Limited to the Brain or to Candidate Genes: The Effect is System- and Genome-Wide

Early studies examining epigenetic changes associated with early life adversity have focused on selecting candidate genes. However, the large number of phenotypes that are associated with early life adversity both in animals and humans suggest that the impact of early adversity on the DNA methylation pattern is broad and should affect peripheral tissues in addition to specific brain regions. Moreover, it is by now almost self-evident that genes do not act independently but participate in functional gene circuitries (Van Weerd, Koshiba-Takeuchi, Kwon, & Takeuchi, 2011). A candidate gene approach biases us to reiterate what is already well established and could be tedious and frustrating when the ''wrong'' candidate gene is investigated. The advent of genome-wide association studies to DNA methylation and genome function mapping enables us to probe the entire system rather than a limited set of genes. However, given that methylation levels are examined in up to several millions of CPG sites (locations of DNA where methylation activity is high), there is also great risk for Type I errors with the genome-wide approach. Moreover, the differentially methylated sites can be located anywhere in the genome, not necessarily within a gene or its specific parts, thus making interpretations difficult.

In several studies involving both humans and animals, McGowan et al. (2011) have tested whether the epigenetic responses to early life adversity was genome-wide. First, in a study involving adult rats, they examined the state of DNA methylation, histone acetylation (an epigenetic process related to changes in chromatin, the protein structure surrounding the DNA molecule), and gene expression in a 7 million base pair region of chromosome 18, which contains the GR gene, in the hippocampus. Natural variations in maternal care in the rat were associated with coordinate changes in DNA methylation, alterations in the chromatin, and variation in gene expression spanning over a hundred kilobase pairs. Interestingly, a chromosomal region containing a cluster of gene families implicated in synaptogenesis (known as protocadherin α, -β, and -γ [Pcdh]) showed the highest differential response to maternal care. The entire cluster revealed changes in DNA methylation, chromatin, and gene expression in response to variations in maternal care. This finding suggests that the DNA methylation response to early life maternal care is coordinated across gene clusters that cover broad areas in the genome. Furthermore, epigenetic response to early life maternal care involves not only single candidate gene promoters, such as that of the GR gene, but involves regions surrounding the gene, including transcriptional and intragenic sequences, as well as sequences residing distantly from transcription start sites and regions containing noncoding RNAs (McGowan et al., 2011).

The finding from the animal work has also been replicated in humans. Suderman, McGowan, Hallett, Meaney, and Szyf (2012) showed that a similar pattern of response to childhood abuse is associated with DNA methylation differences throughout the genomic region spanning the 6½ million base-pair region centered at the GR (*NR3C1)* gene in the hippocampus of adult humans. The DNA methylation differences associated with child abuse bear a striking resemblance to DNA methylation differences between adult offspring of high and low maternal care rats. This provides evidence for an analogous cross-species epigenetic and transcriptional response to early life environment (Suderman et al., in press).

Epigenetic Changes Due to Early Life Stress Are System-Wide

Primate studies have been important for examining whether the epigenetic response to early life adversity is system-wide, involving parallel central and peripheral systems (involving the brain as well as T cells). For example, Provencal, Suderman, Suomi, and Szyf (2012) showed that, similar to the rat and human findings, the changes associated with differences in rearing are not limited to the brain but occur in T cells as well. Although the vast majority of DNA methylation changes that associate with rearing are different in T cells than the prefrontal cortex, some similarities were detected. These data are consistent with the hypothesis that the response to early life adversity is genome- and system-wide.

These findings have had important implications for examining epigenetic changes among humans. Given that changes in DNA methylation associated with maternal rearing are detectable in T cells, researchers have become interested in performing population DNA methylation studies examining either whole blood or T cells among humans. Evidence from several studies indicates that it is indeed possible to detect differences in DNA methylation in peripheral system. Borghol et al. (2011) examined the impact of socioeconomic positioning on blood DNA methylation from the British birth cohort of 1958. This study detected a signature of DNA methylation that was associated with early life adversity (Borghol et al., 2011). Furthermore, the pituitary adenylate cyclase-activating polypeptide (PACAP) gene *(ADCTAP1)*, a protein known to be involved in stress response in the pituitary, was found to be differentially methylated in peripheral blood cells in humans with posttraumatic stress syndrome (Ressler et al., 2011). Differences in DNA methylation of the GR promoter were also observed in peripheral blood cells among newborns exposed prenatally to maternal depression than a comparison group (Oberlander et al., 2008). Infants who were prenatally exposed to maternal depression showed increased methylation in the GR promoter in lymphocytes in comparison to control newborns (Oberlander et al., 2008). Finally, telomere length differences, measured from buccal swabs, were identified between orphans in the Bucharest Early Intervention Project who were placed under high-quality foster care when compared to those subjected to continued care in institutions (Drury et al., 2011). These few studies are encouraging in that they point to the possibility that a DNA methylation response to early life adversity could be molecularly detected in peripheral systems.

How Is Experience Translated Into DNA Methylation Changes?

One of the most appealing aspects of the hypothesis that DNA methylation embeds experience ''under the skin'' is that it allows us to start to chart a molecular bridge between the world and the DNA that might be responding to predicted rules and mechanisms. Although there is not much that can be done to prevent the sequence changes that randomly occur during the course of human evolution, giving rise to certain risky phenotypes, there may be potential avenues of intervention that lead to changes in the activity of genes. Understanding the ''rules'' governing DNA methylation changes in response to experience might make it possible in the future to design both prevention and intervention strategies that change the expression of genes to promote healthy outcomes.

If indeed the change in DNA methylation is not stochastic, it could be linked to experience through triggering of signaling pathways. Cervoni and Szyf (2001) showed a decade ago that the histone deacetylase inhibitor TSA (an enzyme associated with re-modeling of the chromatin) could induce a DNA demethylation event, beyond that which is found to occur during DNA replication. As histone acetylation could be affected by triggering of signaling pathways by extracellular signals (Magnaghi-Jaulin, Ait-Si-Ali, & Harel-Bellan, 1999) such as cAMP-mediated signaling pathways (Yuan & Gambee, 2001), induction of histone acetylation in response to activation of signaling cascades could serve as a conduit that could lead to demethylation of DNA. These early data were further supported by animal

experiments that showed that it was possible to reverse epigenetic programming by maternal care by treating adult offspring of low licking and grooming mothers with TSA (Weaver et al., 2004).

Although the signaling pathways mediating the relation between external triggers and DNA methylation are as yet unclear, examples that could provide a plausible working hypothesis have been previously reported. Maternal behavior triggers a signaling pathway that involves the serotonin receptor, increase in cAMP, recruitment of the transcription factor nerve growth factor-induced protein A, which in turn recruits the histone acetyltransferase (HATs) CREB-binding protein (CBP), and the methylated DNA-binding protein and candidate DNA demethylase MBD2 to the *GR* promoter (Weaver et al., 2007). It has been hypothesized that that the increased histone acetylation triggered by CBP or by other recruited HATs facilitates the demethylation of the gene by MBD2 or other DNA demethylases (Weaver, Brown, Hellstrom, Meaney, & Szyf, 2012).

A different signaling cascade linking social exposure to DNA demethylation provides a mechanism for how early life stress results in persistent life-long hypomethylation of the *AVP* gene. The *AVP* promoter is methylated and bound by MeCp2. Depolarization of hypothalamic neurons triggers phosphorylation of MeCp2 at Ser438 by calcium-dependent CamKII (calmodulin kinase II; Murgatroyd et al., 2009). This phosphorylation converts MeCp2 from a transcriptional silencer with high affinity to methylated DNA into a transcriptional activator with low affinity to methylated DNA (Zhou et al., 2006). This facilitates demethylation of the *AVP* gene. Phosphorylation of MeCP2 in response to neuronal activation has been shown to facilitate demethylation of the BDNF promoter (Chen et al., 2003). This signaling pathway delineates a direct link between neuronal activation and the phosphorylation state of a protein interacting with methylated genes in the brain. Neuronal activation resulting in signaling through phosphorylation of proteins interacting with methylated DNA might be a general pathway that links social exposure and the activation of neurons. Future studies are required to map the signaling pathways that link early life adversity to the DNA methylation–demethylation of gene circuitries in brain and T cells.

Therefore, scientific knowledge concerning the process by which environmental signals affect DNA methylation or demethylation patterns is progressing. Despite the exciting progress in the field, many questions regarding the process of methylation remain unanswered. For example, given that DNA methylation appears to be dynamic after birth, how is the pattern of methylation that is critical for maintaining the terminal differentiation phenotype maintained? Also, what is protecting the cell-type-specific DNA methylation pattern from drifting? Continued efforts involving human and animal models will be critical for answering these important questions in the future.

Prospective and Summary

DNA methylation is a mechanism for genome diversification; identical genomes could have different phenotypic expression resulting from differences in DNA methylation. The process of cellular differentiation illustrates how identical genomes could have different chemical identities defined by covalent chemical modifications. This review suggests that the same mechanism that provides genomes with different identities during cellular differentiation is involved in conferring differential identities to individuals in response to experience and particularly early life experience. Furthermore, these changes in DNA methylation in response to experience may constitute an adaptive response, which could turn maladaptive if there is a misfit between the DNA methylation pattern and the environment, resulting in human disease. One of the challenges is to understand the mechanisms that direct genome- and system-wide DNA methylation adjustments and to delineate how these changes in DNA methylation could lead to changes in genome function and physiology. Delineating the signaling pathways leading from early life experience to DNA methylation changes is critical.

The hypothesis that DNA methylation changes are driving human disease and health problems bears an optimistic message; it might be possible to reverse either with pharmacological or behavioral interventions maladaptive DNA methylation marks. As several epigenetic drugs are now at different stages in clinical trials for the treatment of cancer (Kramer, Gottlicher, & Heinzel, 2001; Weidle & Grossmann, 2000) and various psychiatric disorders (Simonini et al., 2006), there is a hope for a possible rerouting of deleterious epigenetic effects. Understanding the pathways that lead from specific experiences to phenotypic DNA methylation changes should allow us to design prevention and intervention strategies to decrease disease and increase health and wellness.

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