

Chapter 2

Social Environment and DNA Methylation: A Mechanism for Linking Nurture and Nature

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Abstract During cellular differentiation cell type-specific DNA methylation patterns are formed which are conserved during life maintaining cell type identity. Cellular differentiation is an innate process. It was therefore believed that DNA methylation patterns remain stable after cellular differentiation and mitosis are completed. Recent data suggests that DNA methylation patterns could also differentiate in response to external social signals in post-mitotic cells after birth and in adults. This raises the attractive possibility that DNA methylation can serve as a mechanism for adapting genomes to changing social environments conferring upon DNA an identity that is “environment-context” specific. DNA methylation is proposed to serve as a genome adaptation mechanism, adapting genome function to changing environmental contexts including social environments. A critical time point for this process is early life when cues from the social and physical environments define lifelong trajectories of physical and mental health. We suggest that we broaden the definition of DNA methylation as a mechanism of conferring differential identities to similar gene sequences. This expands the role that DNA methylation could play beyond the traditional boundaries of cellular differentiation.

Keywords Early life • Epigenetics • DNA demethylation • DNA methylation • Maternal care • Nonhuman primates • Social environment • T cells

Abbreviations

5-HT	5-hydroxytryptamine
AID	Activation-induced cytidine deaminase

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AVP	Arginine vasopressin
BDNF	Brain-derived nerve growth factor
CAMKII	Calmodulin-dependent protein kinase II
DNMT	DNA methyltransferase
ELS	Early life stress
GABRA1	Gamma-aminobutyric acid A receptor alpha 1 subunit
GADD45A	Growth arrest and DNA-damage-inducible alpha
GC	Glucocorticoids
GR	Glucocorticoid receptor
HDAC	Histone deacetylase
HPA	Hippocampal–pituitary–adrenal
LG	Licking and grooming
MBD	Methyl-CpG-binding domain protein
MeCP2	Methyl-CpG-binding protein 2
PCDH	Protocadherin
RRNA	Ribosomal RNA
SAM	S-adenosylmethionine
TSA	Trichostatin A

2.1 Introduction

The social and physical environment influence human development after birth and during different life cycle stations. For example, social adversity early in life has a profound impact on lifelong physical health and behavior (Hertzman et al. 2001; Power et al. 1997, 2006). Maternal behavior plays a cardinal role in the behavioral development of mammals. Models of maternal deprivation in primates and rodents and natural variation in maternal care in rodents have demonstrated the significant impact of maternal care on a panel of phenotypes in the offspring that last into adulthood (Ruppenthal et al. 1976; Suomi et al. 1976). Human mental development occurs in the first years of life and is heavily influenced by external signals derived from the social environment. In addition, many studies have indicated that there is a strong environmental interaction with genetic inheritance. The first example reported is the short 5-HT transporter polymorphism. Individuals that carry two copies of this allele show depressive symptoms, diagnosable depression, and suicidality only if exposed to stressful life events (Caspi et al. 2003). This observation was repeated in nonhuman primate rhesus monkeys. Monkeys bearing the short 5-HT transporter showed lower serotonin levels only when exposed to deleterious early rearing experiences (Bennett et al. 2002). This well-established effect of external environmental signals on gene function and the phenotype begs the following questions: What is the mechanism? Why would the same gene variant

express different phenotypes because of a different history of social environments? What are the mechanisms that embed differential social environments in genome function?

Several animal models have been developed to study the impact of the environment on behavioral phenotypes later in life. The hippocampal glucocorticoid receptor (GR) controls the negative feedback of the hippocampal–pituitary–adrenal (HPA) axis by glucocorticoids. In the rat, 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 (CRF) expression, and more modest HPA stress responses compared to animals reared by low-LG mothers (Francis et al. 1999; Liu et al. 1997). Cross-fostering experiments demonstrated that the long-term behavioral phenotype of the offspring was determined by the fostering mother LG behavior, supporting a post-birth-mediated mechanism that is not germ line transmitted. Similarly, in nonhuman primates maternal deprivation early in life results in profound phenotypic effects later in life (Cirulli et al. 2009; Corcoran et al. 2011; Stevens et al. 2009; Suomi 1991). These studies provide strong evidence that early life experience could shape lifelong phenotypes. These studies beg the question of what mechanism might be mediating these effects of the early environment on the phenotype.

2.2 DNA Methylation Patterns and Cellular Identity of DNA

DNA methylation is part of the DNA molecule chemical entity. It is thus clearly differentiated from other epigenetic mechanisms such as chromatin modification and noncoding RNA which are associated with, but separate from, DNA. Cell-specific DNA methylation patterns that are formed during cellular differentiation by innate developmental programs were described almost two decades ago (Benvenisty et al. 1985; Razin and Szyf 1984) and were recently confirmed by whole-genome methylome mapping (Lister et al. 2009). The DNA molecule has thus two identities, the ancestral identity encoded in the sequence and the cell-specific identity encoded in the pattern of DNA methylation. DNA methylation is therefore a mechanism that can confer differential chemical identities to similar DNA sequences and as a consequence alter gene expression and the phenotype.

There are potentially multiple mechanisms by which DNA methylation confers different functionality on the genome. The best studied function is the impact that DNA methylation in critical regulatory regions has on regulating gene expression. There is an overall inverse correlation between DNA methylation in regulatory regions of genes and gene expression, which was discovered in the early 1980s (Razin and Riggs 1980; Razin and Szyf 1984) and was confirmed by whole-genome approaches (Rauch et al. 2009). However, the role of differential intergenic methylation and gene body methylation (Hellman and Chess 2007; Rauch et al. 2009) is still unclear, but it might be positively associated with gene expression. It is clear

nevertheless that DNA methylation confers specific functional identity to genomes at multiple levels.

The most established role of DNA methylation is in regulation of promoter activity (Stein et al. 1982). At least two mechanisms are well established for inhibition of gene activity by DNA methylation. A methyl group positioned in a recognition element for a transcriptional factor can block binding of the transcription factor to the promoter (Comb and Goodman 1990; Inamdar et al. 1991). Alternatively, methylated DNA attracts methylated DNA-binding proteins (MBD) such as the Rett syndrome protein, methyl-CpG-binding protein 2 (MeCP2), which in turn precipitate an inactive gene-silencing chromatin configuration through recruitment of chromatin-silencing proteins (Nan et al. 1997).

2.3 DNA Methylation and Cellular Differentiation and the Impact of the Early Environment

It is obvious that the differential methylation that accompanies cellular differentiation during gestation is a highly organized process and that it is driven by innate developmental programs. Faithful epigenetic inheritance is critical for DNA methylation to play a role in cellular differentiation as maintenance of the differentiated state requires accurate copying of the DNA methylation pattern (Razin and Riggs 1980). Knockdown of DNA methyltransferase 1 and the resulting demethylation could change the state of differentiation of mammalian cells (Szyf et al. 1992). If indeed DNA methylation is important for cellular differentiation, it stands to reason that changes in DNA methylation should not occur once a terminal pattern of DNA methylation is formed. Nevertheless, there is extensive data to suggest that DNA methylation patterns could be affected by external environments as well (detailed below). The main question is whether mechanisms evolved that result in an organized response of DNA methylation patterns to external signals and whether it is an adaptive response or whether the pattern of methylation is organized exclusively by innate developmental programs. Intrauterine environmental exposures, for example, could result in inhibition of DNA methylation/demethylation enzymes during critical stages when DNA methylation patterns are laid down in a stochastic manner that does not imply the presence of a specific mechanism that responds to external environmental signals.

One of the finest examples of the impact of intrauterine exposures on DNA methylation was provided by the Jirtle lab who demonstrated an effect of maternal diet during gestation on the agouti color phenotype in viable yellow agouti (A^{vy}) mice, which was mediated through methylation of a transposable element in the A^{vy} transposable element (Waterland and Jirtle 2003). The impact of methyl-rich diets during gestation or the impact of other chemicals such as bisphenol A during gestation that inhibit DNA methylation (Dolinoy et al. 2007) could be explained

just as a stochastic chemical interference in enzymatic DNA methylation reactions that are actively laying down the DNA methylation pattern during embryogenesis.

Whereas stochastic mechanisms could nicely explain effects of the environment during gestation, they do not provide explanation for the profound impact that early life experiences during the first years of life has on the development of behavior and mental capacity. If DNA methylation is involved in the response to social experience, mechanisms that translate external signals from the environment into organized DNA methylation patterns must exist.

2.4 Hypothesis: DNA Methylation Is a Genome Adaptation Mechanism That Confers Environmental Exposure-Specific Identity to DNA

DNA methylation is a mechanism for diversification of DNA identity by providing within the same chemical entity *two layers* of information: the ancestral identity encoded in the sequence and the cellular identity encoded in the DNA methylation pattern. We propose that DNA methylation pattern can respond to external as well as innate programs and therefore the same mechanism that is involved in conferring cellular identity upon DNA during cellular differentiation is also involved in the response to external experimental and experiential signals. It is hypothesized here that external signals triggered by the environment can modulate the DNA methylation pattern in an organized way to generate differential “environmental-history” DNA methylation identities. This process could occur at different time points in life and act at different timescales ranging from proximal physiological timescale to lifelong as well as trans-generational timescales if DNA methylation is reversible after birth and in adult-differentiated tissue (Szyf 2011).

2.5 A Dynamic DNA Methylation Pattern: Reversibility of DNA Methylation in Post-mitotic Tissue

In order for DNA methylation to act as a response to external environment in post-mitotic tissue, it has to be reversible. As the targets of the social environment are neurons which are predominantly post-mitotic, the DNA methylation reaction must be reversible in order to respond to these signals (Ramchandani et al. 1999). The main issue is whether the pattern of DNA methylation that is fashioned by innate developmental processes is a final state or is it in a dynamic state which is responsive to external signals and can be further programmed by these signals.

Although there was resistance in the field to accept the possibility of a reversible DNA methylation in post-mitotic tissues, there is nevertheless significant evidence for this hypothesis (Bruniquel and Schwartz 2003; Lucarelli et al. 2001; Oswald

et al. 2000; Szyf et al. 1995; Wilks et al. 1984). It has been shown that brain extracts are capable of demethylating “naked” DNA substrate *in vitro* (Dong et al. 2008; Fuso et al. 2011; Mastronardi et al. 2007). The strongest evidence for dynamic methylation–demethylation comes from several studies showing active demethylation in post-mitotic neurons (Feng et al. 2010; Levenson et al. 2006; Miller and Sweatt 2007; Weaver et al. 2004). Conditional knockout of DNMT1 in post-mitotic neurons results in DNA demethylation suggesting the presence of demethylation activity in nondividing neurons which is critical for a dynamic methylation pattern in the brain (Feng and Fan 2009).

The main issue in the field remains however whether DNA methylation is truly a reversible reaction that involves removal of the methyl moiety and its release as has been previously proposed (Bhattacharya et al. 1999; Ramchandani et al. 1999) or whether DNA demethylation requires excision of the methylated base and its replacement by an unmethylated cytosine through a process of DNA repair (Jost 1993; Razin et al. 1986). The predominant opinion is that DNA demethylation in post-mitotic cells is a repair process rather than a true demethylation event. Several possible mechanisms were proposed. First, DNMTs were proposed to deaminate the methyl cytosine to thymidine creating a C/T mismatch, which is then corrected by a mismatch-repair mechanism (Kangaspeska et al. 2008). Second, growth arrest and DNA-damage-inducible, alpha (GADD45A), a DNA repair protein, was proposed to participate in catalysis of active DNA demethylation by an unknown DNA repair-based mechanism (Barreto et al. 2007). However, this was disputed (Jin et al. 2008). Other studies have suggested involvement of GADD45B in demethylation in the brain (Ma et al. 2009). Third, a complex sequence of coupled enzymatic reactions of deamination and mismatch repair was shown to be involved in demethylation in zebra fish: activation-induced cytidine deaminase (AID, which converts 5-meC to thymine), a G:T mismatch-specific thymine glycosylase methyl-CpG-binding domain protein 4 (MBD4), and repair promoted by GADD45A (Rai et al. 2008). AID has been implicated in the global demethylation in mouse primordial germ cells as well (Popp et al. 2010). An open question is the role of the newly discovered modification 5-hydroxymethylcytosine as a potential intermediate in the DNA demethylation reaction (Kriaucionis and Heintz 2009). Recent data suggest that TET1, the enzyme that catalyzes the hydroxylation of 5-methylcytosine, is present and required for stem cell maintenance of inner cell mass specification (Ito et al. 2010) and for activity-driven demethylation in neurons (Guo et al. 2011). 5-Hydroxymethylation catalyzed by TET1 is followed by deamination of the 5-hydroxymethylated base by the AID/APOBEC (apolipoprotein B mRNA-editing enzyme complex) family of cytidine deaminases and base excision repair enzymes replace the deaminated base with an unmethylated cytosine (Guo et al. 2011). More recently, it has been proposed that 5-hydroxymethylcytosine is further carboxylated and this serves as a substrate for yet unknown decarboxylases that release the entire modified methyl moiety (Ito et al. 2011). Notwithstanding the precise biochemistry, the fact that DNA methylation is reversible even in post-mitotic tissue provides justification for examining the possibility that DNA methylation patterns are adapted to environmental signals including social signals in early life.

2.6 DNA Methylation Association with Early Life Social Experience: Lessons from Candidate Genes

The first line of data that showed association of early life experience with long-term changes in DNA methylation came from a candidate gene approach. Weaver et al. showed that variations in maternal care result in differences in DNA methylation and histone acetylation in the GR/NR3C1 gene encoding the glucocorticoid receptor (GR exon 1₇ promoter) that emerge early in life and remain stable into adulthood (Weaver et al. 2004). Cross-fostering experiments showed a causal relationship between maternal care and the DNA methylation differences, and reversal of the phenotypes with epigenetic drug treatments supported a causal relationship between DNA methylation differences and phenotypic variation (Weaver et al. 2005, 2006). Exposure of infant rats to stressed caretakers that displayed abusive behavior produced persisting changes in methylation of BDNF gene promoter in the adult prefrontal cortex (Roth et al. 2009). Similarly, early life stress (ELS) in mice caused sustained DNA hypomethylation of an important regulatory region of the arginine vasopressin (AVP) gene (Murgatroyd et al. 2009).

Although it is impossible to provide causal evidence for early life experience altering DNA methylation states in humans as it is ethically unfeasible to randomize in humans early life abuse, it is nevertheless possible to associate DNA variations with differences in early life experience. The state of methylation of rRNA gene promoters and NR3C1 promoter in the hippocampus was examined in a cohort of suicide victims in Quebec who were abused as children and their control group. Ribosomal RNA (rRNA) forms the skeleton of the ribosome, the protein synthesis machinery. We have previously demonstrated a critical role for DNA methylation in regulating expression of rRNA genes (Brown and Szyf 2007). Our results showed that the suicide victims who experienced childhood abuse had higher overall methylation in their rRNA genes and expressed less rRNA in a brain region-specific manner (McGowan et al. 2008). We also examined in this cohort the same promoter of NR3C1 gene that was affected by maternal care in rats. Site-specific differences in DNA methylation in the NR3C1 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 was implicated in suicide: the gamma-aminobutyric acid A receptor alpha 1 subunit (GABRA1) promoter (Linthorst et al. 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.

2.7 Reversibility of the DNA Methylation Pattern Programmed by Early Life Social Environment Using Pharmacological Agents

DNA methylation is a biochemical reaction and is therefore potentially responsive to interference with pharmacological activators or inhibitors even in adult post-mitotic cells as long as the pattern of DNA methylation remains defined by an active balance of methylation and demethylation enzymatic activities. If DNA methylation is fixed after birth as has originally been the understanding, then the pattern of DNA methylation should be resistant to any change in post-mitotic neurons. We therefore tested whether it is possible to pharmacologically reverse a phenotype that was determined by early life experience. Our results demonstrate that it is possible to reverse the phenotype of adult offspring of high-LG mothers by treating them with methionine, a precursor of the methyl donor in DNA methyltransferase reactions dependent on S-adenosylmethionine. Moreover, we can also reverse the phenotype of adult offspring of low-LG mothers by treating them with TSA, a histone deacetylase inhibitor that we have shown can trigger DNA demethylation (Cervoni and Szyf 2001; Weaver et al. 2004, 2005, 2006). The dynamic model of DNA methylation after birth is consistent with the concept that DNA methylation patterns are responsive to external experiential signals in early life as well as with the possibility that these phenotypes defined by experience are potentially reversible by pharmacology. It is tempting to speculate that not only pharmacology but cognitive and behavioral therapies as well could reverse phenotypes defined by DNA methylation.

In summary, the dynamic model of DNA methylation explains how on one hand experience could define long-term DNA methylation patterns and phenotypes as well as how this stable pattern is potentially reversible.

2.8 DNA Methylation Association with Early Life Social Experience: Involvement of Broad Genomic Regions in a Clustered and Organized Response

The response to early life experience involves multiple phenotypes suggesting a system-wide response that involves multiple genes and multiple physiological systems. Studies looking at the phenotypes associated with early life socioeconomic positioning revealed multiple phenotypes associated with early life adversity including obesity and asthma (Hertzman et al. 2001; Power et al. 1997, 2006). If indeed the response of DNA methylation states to early life adversity is an adaptation rather than a stochastic disruption, it should involve an organized change in DNA methylation across the genome. We tested this hypothesis in several studies. All studies point to the conclusion that the impact of early life adversity on the

epigenome is broad and that it involves multiple systems and is not limited to the brain. This has diagnostic and mechanistic implications. Most importantly it supports the idea that it might be worthwhile to study behavioral epigenetics in peripheral tissues. It also supports the idea that genome-wide methylation patterns should be examined for understanding of how experience shapes the methylome. We have documented several examples that support this hypothesis.

First, natural variations in maternal care in the rat are associated with coordinate changes in DNA methylation, chromatin, and gene expression spanning over a hundred kilobase pairs. Interestingly, a chromosomal region containing a cluster of the protocadherin- α , protocadherin- β , and protocadherin- γ (*Pcdh*) gene families implicated in synaptogenesis shows the highest differential response to maternal care. The entire cluster reveals epigenetic and transcriptional changes in response to maternal care (McGowan et al. 2011). Second, we showed that a similar pattern of response to childhood abuse is associated with DNA methylation differences throughout the genomic region spanning the six and a half million base-pair region centered at the *NR3C1* gene in the hippocampus of adult humans suggesting evolutionary conservation of this adaptation (Suderman et al. 2012). Third, similar to the rat and human, the changes in DNA methylation associated with differences in rearing in rhesus monkeys are widespread in the genome, they are not limited to the brain, and occur in T cells as well (Provençal et al. 2012). Fourth, we have initiated a study of the impact of socioeconomic positioning on DNA methylation that examined blood DNA from the British birth cohort of 1958. This study detected a signature of DNA methylation that is associated with early life adversity (Borghol et al. 2011) supporting the hypothesis that social environment DNA methylation signatures are found system wide and could be examined in peripheral blood cells. The study also revealed highly organized association with DNA methylation and socioeconomic positioning that clustered across broad genomic regions (Borghol et al. 2011).

Three other studies have demonstrated that epigenetic effects associated with behavioral adversity could be detected in blood cells. First, the *NR3C1* promoter was more methylated in lymphocytes in newborns exposed prenatally to maternal depression than control newborns (Oberlander et al. 2008). Second, pituitary adenylate cyclase-activating polypeptide (PACAP), 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). Third, telomere length differences were identified between orphans in the Bucharest Early Intervention Project who were placed under high-quality foster care compared with those subjected to continued care in institutions (Drury et al. 2011). As discussed above, a long line of data have established that the physiological response to early life socioeconomic adversity is not limited to the brain (Cunha and Heckman 2009; Power et al. 2007, 1997). There is no reason therefore to believe that DNA methylation changes in response to adversity should not occur in the periphery as well as the brain.

2.9 Molecular Mechanisms of Experience-Driven DNA Methylation Changes

It is clear that DNA methylation patterns could be altered stochastically during gestation when DNA methylation patterns are formed by enzymatic reactions that are inherently susceptible to inhibition or activation by chemicals as no enzymatic reaction is completely free from certain degree of error. Since maintenance DNMT1 will copy new DNA methylation events from the template strand to the daughter strands, such stochastic DNA methylation events could be potentially maintained across multiple cell divisions. Stochastic alterations in DNA methylation similar to single-nucleotide polymorphisms could lead to alterations in gene function if they happen to be at critical positions, altering phenotype and causing disease. However, the data that I presented suggests that the high predictability and organization of the DNA methylation differences associated with early life adversity are inconsistent with a purely stochastic mechanism. Mechanisms should exist that mediate between the experience and targeted changes in DNA methylation. It is hypothesized here that social experience triggers signaling pathways in the brain that target DNA methylation/demethylation activities to specific genomic targets resulting in changes in DNA methylation (Fig. 2.1). For example, it was proposed that maternal LG triggered serotonergic pathways in the hippocampus turning on cAMP-mediated signaling, that in turn activated a transcription factor NGFIA which bound specific NR3C1 promoter regions and targeted CBP, a histone acetyltransferase, to the gene resulting in changes in histone acetylation and DNA methylation (Weaver et al. 2007).

A different mechanism that involved direct signaling events was proposed to explain the demethylation of the regulatory region of the arginine vasopressin (AVP) gene in response to maternal stress (Murgatroyd et al. 2009). In this case, neuronal activity leads to alteration in the binding of MeCP2 to the promoter of the gene (Murgatroyd et al. 2009). It was previously shown that MeCP2 was phosphorylated by CAMKII kinase in response to neuronal activation and that this phosphorylation event altered the affinity of binding of MeCP2 to a BDNF promoter resulting in transcription activation (Zhou et al. 2006); this reduced affinity of MeCP2 to the promoter could be followed by DNA demethylation (Chen et al. 2003), perhaps through increased accessibility of the promoter to demethylases. Deciphering the mechanisms that lead from experience to DNA methylation changes is a challenge that needs to be addressed if we want to understand how experience shapes phenotype.

2.10 Summary

The scope of involvement of DNA methylation in long-lasting regulation of genome function is broader than has originally been thought. DNA methylation acts as a mechanism for providing differential identities to similar DNA sequences. Originally, it has been thought that such a mechanism is exclusive for cellular differentiation when an identical genome acquires different identities expressing different

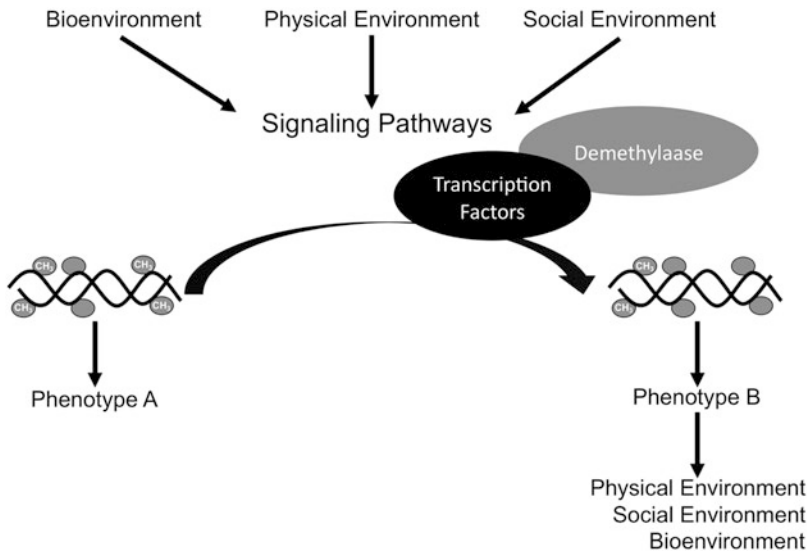


Fig. 2.1 DNA methylation, a genome adaptation mechanism: a model. Signals from the social, bioenvironmental, and physical environment act on signaling pathways to trigger changes in DNA methylation through recruitment of transcription factors and DNA-modifying enzymes to specific targets in the genome. This adapts the genome and the phenotype to the anticipated lifelong environment

phenotypes. It is proposed here that DNA methylation can also act as a mechanism for adaptation of the genome to different environments and experiences. A misfit between programmed DNA methylation in response to an anticipated environment and the real environment is a maladaptation and could result in disease.

Early life experience plays a cardinal role in these adaptations of genome function and the phenotype (Fig. 2.1) However, such a mechanism can be functional at any point in life and act in different kinds and levels of genome adaptation. There is data in both animals and humans that supports this hypothesis. However, the mechanisms that mediate between external social signals and DNA methylation changes that seem to cluster across the genome are largely unknown. Future studies are required to unravel these mechanisms.

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