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Environmental Influences on Epigenetic Profiles

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Abstract

Studies of environmental challenges, such as hazardous air pollutants, nonmutagenic toxins, diet choice, and maternal behavioral patterns, reveal changes in gene expression patterns, DNA methylation, and histone modifications that are in causal association with exogenous exposures. In this article we summarize some of the recent advances in the field of environmental epigenetics and highlight seminal studies that implicate in utero exposures as causative agents in altering not only the epigenome of the exposed gestation, but that of subsequent generations. Current studies of the effects of maternal behavior, exposure to environmental toxins, and exposure to maternal diet and an altered gestational milieu are summarized.

Keywords

Environmental epigenetics; behavioral epigenetics; toxins and epigenetics; diet and epigenetics

Studies of the wide range of beneficial as well as potentially harmful environmental challenges reveal changes in gene expression patterns, DNA methylation, and histone modifications that likely result from interactions with the in utero environment. The stability of these modifications, and indeed the pattern of their inheritance, is of great interest within the field of epigenetics. In this article we describe some of the recent advances in the field of environmental epigenetics and highlight seminal studies that implicate the possibility that exposure to the environment not only affects the exposed individual but may affect up to four subsequent generations.

ENVIRONMENTAL EXPOSURES: BALANCING GENOMICS WITH EPIGENOMICS

Discussions regarding alterations in the fetal epigenome in response to in utero exposures, whether maternal or environmental, cannot ignore the genomic background in which they occur. Although outside the scope of this article, it bears mention that although many fetuses are exposed to common known harmful substances (such as alcohol and tobacco) in utero, not all experience adverse outcomes as a result of this exposure.^{1–4} This discrepancy cannot be accounted for by dose effect alone.^{5–8} Thus current efforts aimed at understanding the

potential genetic and metabolic basis of susceptibility for the more common exposures such as tobacco are of equal importance in understanding the range of effects on developing offspring in response to environmental exposures.

To illustrate this point, it is worthwhile to consider the genomic basis of adverse fetal outcomes in response to maternal tobacco use. Maternal tobacco use has been identified in multiple population-based studies as the largest modifiable risk factor for intrauterine growth restriction and small for gestational age births.^{10–13} Mechanisms leading to fetal growth restriction following in utero tobacco exposure are poorly understood but have generally often been attributed to chronic fetal hypoxia. Nicotine, a principal alkaloid of tobacco smoke, has been shown to mediate constriction of the intrauterine vessels leading to diminished placental blood flow, as well as result in increased apoptosis of placental syncytiotrophoblasts.^{9,12} Carbon monoxide binds fetal hemoglobin with high affinity, and a direct correlation between fetal oxygen consumption and diminished birthweight has been established in animal models.^{9,12} However, nicotine, cotinine, and potentially harmful DNA adducts (metabolic products of polycyclic aromatic hydrocarbons [PAH]) are known to cross or collect in the placenta of smokers.^{10–17} Thus, although it is possible that chronic hypoxia is a primary mediator of fetal growth restriction in response to in utero tobacco exposure, it is equally plausible that the discrepant variation in fetal susceptibility to smoking-related growth restriction results from differential fetal and/or maternal expression of metabolic gene polymorphisms. As discussed throughout this article, there are both genomic and epigenomic bases that underlie this susceptibility.

Of the >4000 substances in tobacco smoke, PAH compounds together with nitrosamines comprise the most important carcinogenic species in tobacco smoke.^{18–22} An individual difference in susceptibility in patients with chemically induced carcinomas has been ascribed to allelic differences of metabolic activity in the activation or detoxification of carcinogens.^{19–22} The majority of chemical carcinogens are metabolized in a sequential series of two-phase enzymatic metabolic reactions. Phase I enzymes such as cytochrome P450 metabolically activate PAH compounds into oxidized derivatives, resulting in reactive oxygen intermediates capable of covalently binding DNA to form adducts.^{22–26} In turn, these reactive electrophilic intermediates can be detoxified by phase II enzymes, such as the glutathione-S-transferase family, via conjugation with endogenous species to form hydrophilic glutathione conjugates that are then readily excretable. Thus the coordinated expression of these enzymes and their enzymatic balance may determine the extent of cellular DNA damage and related development of adverse outcomes.

CYP1A1 is a phase I metabolic enzyme that encodes the aryl hydrocarbon hydroxylase enzymes responsible for the activation of the PAH compounds to their potentially harmful reactive intermediates. The *CYP1A1 Ile₄₆₂ Val* (AA>AG/GG) allele carriers exhibit higher levels of CYP1A1 enzymatic activity and inducibility, and smokers who carry this variant have increased cellular PAH-DNA adducts.^{18,21–23} Following high-affinity binding of PAH compounds to their intracellular aryl hydrocarbon (AH) ligands, the complex is translocated to the nucleus where it dissociates and then heterodimerizes to form a DNA binding complex (AH:ARNT) to modulate chromatin disruption and regulate induction of *CYP1A1* expression in a DNA methylation-sensitive fashion.¹⁸ Of further interest to the study

reported here, maternal smoking induces placental expression of *CYP1A1*.²⁴ Thus *CYP1A1* expression is modified via both genetic and epigenetic means and has been shown in human lung and placental tissue to be amenable in its regulation to the influence of smoking.^{18,21–24}

Polymorphisms leading to enzymatic inactivity in the phase II metabolic enzyme *GSTT1* (*GSTT1*del) are prevalent (i.e., prevalence approximating 20% of whites) and have been extensively studied in the context of individual susceptibility to tobacco-mediated carcinogenesis, albeit with variable attributable risk associations.^{23,25–27} Theoretically, any combinatorial association of increase phase I activity (e.g., increased expression of *CYP1A1* via functional polymorphisms or epigenetic dysregulation of the promoter) in combination with decreased phase II activity (e.g., decreased *GSTT1* expression) may yield increased susceptibility to tobacco-related adverse outcomes.

Attempts to associate metabolic gene polymorphisms with tobacco consumption in healthy controls have also been attempted in large population-based sample banks, with no associative effect of either phase I or phase II enzymes.²³ However, variable expression of alternate cytochrome P450 enzymes (e.g., *CYP2A6*) have been shown to modify daily cigarette consumption.^{20,25,26} *CYP2A6* is a highly polymorphic allele and functions as the rate-limiting enzyme in the metabolism of nicotine to cotinine.^{20,25,26} Individuals with diminished activity of *CYP2A6* activity at the *CYP2A6**2 allele (*CYP2A6*Lys₁₆₀His T>A) are proposed to inherit the slowest metabolism of nicotine and therefore have been (variably) associated with a decreased risk for smoking, lower cigarette consumption, shorter duration of smoking, and increased ability to quit smoking.^{20,25–27}

For these reasons, we and others hypothesized that it was biologically plausible that maternal and/or fetal metabolic gene polymorphisms would alter the tobacco-related risk of adverse pregnancy outcomes. In our upcoming publication, we used prospectively acquired biological samples from a multi-institutional study and examined both maternal and fetal gene–tobacco exposure interactions among common and relevant metabolic gene polymorphisms. Specifically, we assessed the relationship for known functional allelic variants of *CYP1A1* (Ile₄₆₂Val AA>AG/GG), *GSTT1*(del), and *CYP2A6* (Lys₁₆₀His T>A) in 502 smokers and their conceptuses alongside 1:1 controls in an effort to characterize better the individual susceptibility to tobacco-mediated adverse pregnancy outcomes. We demonstrate that fetal homozygous deletion of a phase II PAH gene integral to excretion of DNA adduct-forming reactive intermediates (*GSTT1*) is significantly and specifically associated with modified fetal growth patterns in response to maternal smoking. Moreover, these findings persisted in multiple allelic interaction models to suggest an interaction between the fetal metabolic gene *GSTT1*, maternal smoking, and modification of birthweight.

Of further interest to the discussion here, other authors have previously observed that *CYP1A1* is inducible in its placental expression among smokers²⁸ and that well-characterized XRE elements in the proximal promoter are differentially methylated at cytosine followed by a guanine (CpG) islands in lung tissue of smokers.²² Because hypermethylation in key gene regulatory sequences at CpG islands is generally associated with gene silencing, we also examined whether the percentage of methylated CpG sites in

the proximal *CYP1A1* promoter would differ among smokers and nonsmokers using the well-established methodology of bisulfite modification and sequencing.²² In the first core primed region containing the XRE element, we observed a unique differential methylation in response to maternal smoking (Aagaard-Tillery, unpublished data, 2009).

In sum, exposure to tobacco in utero is perhaps a classical example of recent advances in human genome research, pharmacogenetics, medical genetics, and the evolving field of epigenetics. Studying such intersections of epigenomics and genomics will further our understanding of the interactions of the heritable genome and chromatin structure in the causal pathways employed in the development of human disease. In light of these observations, we devote the remainder of this article to summarizing epigenetic responses to environmental exposures.

EPIGENETIC MODIFICATIONS

Before embarking further on the epigenetics of environmental exposure, it is worthwhile to briefly review the primary epigenetic modifications at a molecular level. All eukaryotes maintain their genome as a nucleoprotein complex, which consists of DNA wrapped around four histone proteins. The basic repeating unit of chromatin is the nucleosome. The central core of the nucleosome consists of two copies each of four histone proteins. Two copies of H3 and H4 join to form a histone tetramer. In addition to the tetramer are two histone H2A/H2B dimers, which form the histone octamer. Around the octamer, 147 base pairs of DNA wraps ~1.7 times in a left-handed superhelix to form the nucleosome.²⁸

The crystal structure of the nucleosome has provided much insight into the organization of chromatin. Each of the four histones contain a globular histone-fold domain, which is involved in dimer–tetramer interfaces within the nucleosome. Each histone also contains an N-terminal domain, called the histone “tail,” which extrudes from the nucleosome surface. The histone tails do not contribute to the structure of the individual nucleosomes but help contribute to the structure of chromatin as a whole. Histones H2A and H2B contain C-terminal domains that also extrude from the surface of the nucleosome (reviewed in²⁹).

The role of histone modifications in every DNA-templated event has been studied. Histone proteins can be acetylated, ubiquitylated, methylated, phosphorylated, and sumoylated. These modifications have been implicated in replication, transcription, heterochromatin formation, chromatin compaction, and DNA damage repair, and they are believed to recruit effector proteins to chromatin, alter chromatin structure, and disrupt histone-DNA contacts. More than 40 years of work support the paradigm that highly acetylated histones along with methylation of selected lysine and arginine residues are markers of transcriptionally active genes, whereas methylation of distinct lysine and arginine residues along with hypoacetylation of lysine residues are markers of transcriptionally silent genes.^{30–32}

The acetylation of histones as well as other nuclear and cytoplasmic proteins is dynamically regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Histone methylation occurs on select lysine (K) and arginine (R) residues.^{33,34} More than 50 SET domain-containing proteins are known to methylate lysine residues of histones.^{33,34} Data

suggest that histone modifications are extremely dynamic and highly regulated. Much additional work is necessary to demystify the histone code.^{31,32,35}

In addition to histone modifications, many additional proteins have been reported that can potentially serve as epigenetic markers. For example, CTCF functions as an insulator binding protein that marks the boundary of histone methylation domains, and PolII binds to the promoter region of genes.^{36,37} Furthermore, histone chaperones, variants of the canonical replicative histone proteins, changes in higher order chromatin structure, and activity of chromatin remodeling enzymes can also regulate gene expression by modulating the accessibility of DNA.³⁸ Combined with histone modifications, these markers will provide a comprehensive view of the chromatin status across the entire genome.

DNA METHYLATION

Most methylation events in mammals occur at the number-five carbon of the cytosine pyrimidine ring. Genomic methylation patterns are propagated during cell division by DNA methyltransferases. Generally these enzymes are categorized into maintenance (DNA methyltransferase 1, Dnmt1) and de novo (Dnmt3a and Dnmt3b) methyltransferases, but all three enzymes can act as in both modes.^{39–45} One of the most important sites of gene regulation by DNA methylation are CpG-enriched regions associated with promoters (called “CpG islands”).^{46,47} In this context, DNA methylation acts with other enzymes to covalently modify histones to cause gene silencing and to maintain a repressive chromatin state. It is thought that DNA methylation acts as a memory to faithfully maintain gene expression profiles after cell division, thus defining the state of cellular differentiation.^{48,49} DNA methylation is inversely related to both the expression of developmentally regulated genes and the potency of cells. The genome of mammals is progressively demethylated during the preimplantation period, which is believed to be important for reprogramming the genome to a pluripotent state in the preimplantation embryo.^{50,51} After implantation, DNA is progressively remethylated, and cell-type-specific DNA methylation patterns are established as cells differentiate.⁵² Imprinted genes represent a small subset of methylated genes in which allele-specific expression correlates with allele-specific methylation, with some genes showing methylation on the paternal allele and others on the maternal allele.^{53,54} The methylation of these imprinted genes does not change during development, and correct imprinting is believed to be essential for normal development and differentiation.

EPIGENETIC RESPONSE TO THE ENVIRONMENT: THE CANALIZATION HYPOTHESIS

The theory of canalization was proposed by Conrad Waddington in 1942 to explain the consistency of a wild-type phenotype regardless of varying environmental conditions and genetic backgrounds (reviewed in⁵⁵). He proposed that within the genotype, a buffering system makes the phenotype resistant to modification. He postulates that canalization allows for one consistent phenotype, regardless of minor variations over the course of development.⁵⁶

In a seminal study on the molecular mechanism of the buffering capacity of the genotype, Rutherford and Lindquist proposed Hsp90 to be a central player in canalization.⁵⁷ They showed that in *Drosophila melanogaster*, when Hsp90 is impaired, variation arises in every adult structure in the fly. A similar role for Hsp90 has been proposed in *Arabidopsis*, in which mutation of Hsp90 disrupts development and induces morphological changes in response to the environment.^{58,59}

This hypothesis of a buffering system also accounts for rapid phenotypic changes that occur over a time period too short to be accounted for by evolution, if the buffering capacity of the system is disrupted or overextended. A disruption of this phenotypic buffering system has been proposed to explain the rapid rise of obesity and type 2 diabetes in developed nations.⁵⁵ In 1962, J. V. Neel proposed that natural selection had favored “thrifty genes” that made an individual exceptionally efficient at food utilization.⁶⁰ This would allow for a selective advantage in times of famine, allowing for a healthy phenotype in periods during which food access was limited. Yet, in an environment with an abundance of hypercaloric sustenance, this thrift would become disadvantageous, causing reserves of adipose stored to prepare for potential periods of famine to be deleterious.

Although the theory is controversial,^{61,62} it is only one of many trying to explain the rapid rise of diabetes in developed nations. A recent hypothesis put forth by Stoger postulates a thrifty epigenotype.⁵⁵ He argues against a strictly genetic mechanism because there is a dearth of alleles identified to explain the etiology of type 2 diabetes and argues for an epigenetic model to explain the apparent heritability of adult onset of this disease.

The idea that the environment works in concert with genotype to produce a phenotype is not novel. But the study of the environmental influence on molecular mechanisms of gene expression, stability, and heredity is an exciting new field. Together with classical genetics, the study of epigenetics will help to elucidate the mechanism behind the onset of new apparently heritable diseases that do not have a strictly genetic explanation.

ENVIRONMENTAL INFLUENCE AND THE EPIGENETICS OF BEHAVIOR

In humans, although decades separate the time between receiving and giving maternal care to one’s own offspring, maternal behavior is stably transmitted over this extended time period. How this information is stored and transmitted has been postulated to be due to epigenetic mechanisms. Epigenetics is also being invoked to explain other aspects of behavior, such as drug addiction, fear conditioning, as well as the behavioral aspects associated with mental illnesses such as depression and schizophrenia (Fig. 1).

A model system used to study the influence of epigenetics on the transmission of maternal care is the licking and grooming (LG) behavior of maternal rats toward their young pups within the first week of life. Female rats can be characterized as high-, mid- or low-LG mothers.⁶³ Pups raised by high LG mothers exhibit high LG behavior toward their own pups. Cross fostering studies, in which a pup born to a low-LG mother is raised by a high-LG mother, show that programming of maternal behavior is mediated by an epigenetic,

rather than genetic mechanism, because these rats exhibit the behavior of the foster rather than the biological mother.⁶⁴

Initial studies to elucidate aspects of the epigenetic mechanisms of behavioral transmission focused on the differential expression of the glucocorticoid receptor (GR) in the rat hippocampus between pups from low- and high-LG mothers.^{65,66} Pups from high-LG mothers have higher hippocampal expression of GR. Members of the Meaney laboratory showed that this differential expression is due to methylation at a single CpG site within the GR promoter in the low-LG pups, concomitant with decreased acetylation of lysine 9 of histone H3 (H3K9ac) within the promoter.⁶⁷ They showed that this methylation was stable throughout adulthood. They also showed that treatment with trichostatin A (TSA), an HDAC inhibitor, reversed the H3K9ac and DNA methylation levels in the low-LG group, and they determined that the behavioral programming of maternal care is indeed reversible.

This reversibility was further shown using intra-cerebroventricular administration of methionine, a member of the one-carbon metabolism pathway essential for DNA methylation.⁶⁸ Methionine administration increased the site-specific CpG methylation in the high-LG pups, which made promoter methylation indistinguishable from the low-LG pups. The methionine administration only affected expression of 300 genes within the hippocampus, and it did not alter global patterns of DNA methylation.⁶⁸

Expression of the α subunit of the estrogen receptor (ER α) has also been implicated in transmission of maternal behavior.⁶⁹ Low ER α expression is seen in the hippocampus of low-LG pups, which correlates with increased methylation of 7 of 14 CpG islands in the promoter region.

The importance of DNA methylation and its relation to behavior is seen in studies of schizophrenic (SZ) patients. It is known that in SZ patients, expression of reelin and GAD67 is downregulated in GABAergic neurons and the expression of DNMT1 is increased.^{70,71} Studies of SZ patients throughout the 1960s showed that a daily dose of methionine over a 2-week period exacerbated the psychotic state in 40 to 60% of the SZ patients, whereas administration to healthy controls did not affect behavior.⁷² Human brain tissue from SZ and normal patients show that SZ patients have higher than normal concentration of a cofactor in the one-carbon metabolism pathway, S-adenosyl methionine (SAM), implying that the pathway in SZ patients could be deregulated.⁷³

Histone acetylation is also involved in behavior associated with clinical depression. In a mouse model of chronic social defeat, transcripts of brain-derived neurotrophic factor (BDNF) are reduced in defeated mice.⁷⁴ This reduction is accompanied by an increase in the repressive chromatin mark, dimethylation of lysine 27 of histone H3 (H3K27me2) within the promoter region. Administration of the antidepressant imipramine reverses the repression of gene expression and induces hyperacetylation of histones within the promoter region. Importantly, chronic administration of imipramine induces long-lasting hyperacetylation of the promoter region of BDNF.

Epigenetic changes have been implicated in sustaining fear-conditioned behaviors. In a mouse model of fear conditioning, phosphorylation of histone H3 is phosphorylated on

serine 10 in area CA1 of the hippocampus within 1 hour of training.⁷⁵ Hyperacetylation of histone H3 but not H4 occurs after contextual fear conditioning, within area CA1,^{76,77} and inhibition of DNA methylation blocks freezing behavior in the fear-conditioning paradigm.⁷⁶

Epigenetic changes have also been implicated in the behaviors governing drug addiction. In fact it has been shown that cocaine induces specific histone modifications at specific promoters after either acute or chronic cocaine exposure.⁷⁸ An acute cocaine treatment induces hyperacetylation of histone H4 at the *c-fos* promoter, and the promoters of BDNF and *cdk5* are hyperacetylated on histone H3 after chronic cocaine treatment. Interestingly, coadministration of cocaine with the HDAC inhibitor butyrate significantly increases the locomotor behavior seen with cocaine administration.⁷⁸ Administration of the HDAC inhibitor TSA attenuates the motivation to seek cocaine in a mouse model self-administration paradigm.⁷⁹ These studies show that epigenetic mechanisms are at work that influence the behavioral aspects of drug use.

The study of the epigenetics of behavior even encompasses the potential heredity of feeding and drinking behavior.⁸⁰ Consumption of alcohol during pregnancy can influence the appetite for alcohol in the offspring in rats.⁸¹ The offspring of pregnant rats who were subjected to dehydration that increases salt appetite showed an increase in salt-motivated behavior compared with their control counterparts.⁸² As the molecular mechanisms of behavior are further studied, scientists are turning to the field of epigenetics to help explain the stability of the influence of early environmental stimuli on behaviors exhibited later in life.

ENVIRONMENTAL TOXINS

Exposure to toxins known to cause DNA mutations has been determined to be carcinogenic. In a seminal 2005 *Science* paper from the Skinner laboratory, it is shown that nongenotoxic compounds can be carcinogenic (Fig. 2). Their results revealed that exposure of a gestating rat during fetal sex determination to an endocrine disruptor (ED) will affect four generations of male rats.⁸³ Using the EDs vinclozolin, a common fungicide used in the wine industry, and methoxychlor, a common pesticide, they studied the effects of ED exposure through the F4 generation. Pregnant rats (F0) were injected daily with a dose of one of the EDs during the period of fetal sex differentiation. Subsequent offspring (F1) of the treated animals were mated with rats from untreated mothers, and this outcrossing continued for three more generations (F2 to F4). Males from the lineage of the ED-treated mothers showed a twofold increase in spermatogenic sperm apoptosis, reduced sperm numbers, and reduced sperm motility. These phenotypes were transmitted specifically through the male germ cell line. Vinclozolin was shown to disrupt DNA methylation in rat testes. In later studies the Skinner laboratory showed that the male offspring (F1 to F4) had epithelial cell atrophy, glandular dysgenesis, prostatitis, and hyperplasia of the ventral prostate.⁸⁴ Further follow-up studies on the effect on female rats by Nilsson in 2008 revealed the vinclozolin offspring had significantly more incidence of uterine hemorrhage, anemia, or both in late pregnancy than their control counterparts.⁸⁵

These reports show that an environmental insult cannot only affect the current generation but the impact can be felt for generations to come. The reports of the female offspring of women who were administered diethylstilbestrol (DES), a synthetic estrogen, during gestation show a similar inheritance of cancer from a nongenotoxic compound.⁸⁶ Although DES was administered to the mother, it was their daughters who, decades later, presented with cervical-vaginal carcinoma. Women who were exposed in utero to DES also have a higher incidence of uterine fibroids.⁸⁷ Members of the McLachlan laboratory, using a mouse model, reported there is a single CpG island in the promoter of the estrogen-responsive lactoferrin gene that remains unmethylated in the uterine epithelial cells of those offspring from DES-treated dams.⁸⁸ This gene exhibits abnormal expression in adulthood of offspring of treated mothers.

Environmental exposures to nickel have been shown to be carcinogenic, but standard assays of mutagenesis yielded low results for the genotoxicity of nickel.⁸⁹ This led to a hypothesis that the carcinogenic properties of nickel were due to epigenetic mechanisms. Studies in human cell lines show that nickel exposure deregulates ubiquitylation of histones H2B.⁹⁰ Using a human cell line with an *Escherichia coli* transgene inserted into a heterochromatic region, members of the Costa laboratory showed that nickel ions caused reversible transgene silencing due to changes in DNA methylation patterns and chromatin condensation.⁹¹ Nickel was found to decrease global H3 and H4 acetylation in human cell lines^{92,93} and to inhibit HAT activity in vitro.⁹² Nickel treatment of cells increases global levels of H3K9me2, increases levels of an HMTase (G9a) and decreases the activity of a Fe(II)-2-oxoglutarate-dependent histone K9me2 demethylase.⁹⁴

Arsenic is another toxin believed to cause cancer through epigenetic mechanisms. Millions of people are exposed to arsenic through their drinking water, mostly those in developing countries.⁹⁵ Poor nutrition is thought to be a susceptibility factor to developing cancer via arsenic exposure. Arsenic metabolism requires methylation, with a transfer of methyl groups from SAM. Therefore, both arsenic metabolism and DNA methylation require the one-carbon metabolism pathway. Indeed, in liver from newborn mice exposed to arsenic in utero, DNA hypomethylation changes at guanine-cytosine-rich regions and is associated with changes in the gene expression patterns in the newborn mouse.⁹⁶ In human lung carcinoma cells, exposure to inorganic arsenic increased H3K9me2 and H3K4me2 and decreased H3K27me3.⁹⁷ In studies of Bangladeshi adults who have been chronically exposed to arsenic levels that exceed World Health Organization standards, arsenic exposure was positively correlated with DNA methylation in peripheral blood leukocytes, and this hypermethylation required folate.⁹⁸

Cadmium is a metal that mimics the hepatocarcinogenic effects of methyl deficiency in the diet. It is a potent carcinogen that causes a wide range of changes in gene expression profiles in exposed cells.⁹⁹ It is widely used in industry and can affect humans through environmental exposures. In vitro DNA methylation assays using hepatic nuclei harvested from rats showed that treatment with cadmium inhibited overall DNA methylation levels in this assay.¹⁰⁰ Treatment of the chronic myelogenous leukemia cell line (K562) with cadmium leads to global hypomethylation, which leads to cell proliferation and presumably carcinogenesis.¹⁰¹

EPIGENETIC VARIATION AND DIET

Insight into the hypothesis that diet can influence epigenetics came from studies of children born during the Dutch Hunger Winter (also known as the Dutch Winter Famine) of World War II. During the winter of 1944–1945, food rations for Dutch citizens dropped from 1400 calories per day to 1000 calories per day, and then dropped as low as 400 to 800 calories per day, due to an imposed embargo of food deliveries by the Germans.¹⁰² Once the embargo was lifted, the daily ration of calories rose to almost 2000 calories per person per day.¹⁰² It is the phenomenon of imposed famine followed by an abundance of food that makes the studies of children born from the Dutch Hunger Winter unique. The increase and specificity of the health problems of children conceived during this hunger winter has fortified the developmental origins of disease hypothesis (Fig. 3).¹⁰³

A cohort study of 2414 people born during the Dutch famine show that exposure to famine at any stage during gestation causes a propensity toward glucose intolerance.¹⁰² Children exposed to famine early during gestation show increased incidence of coronary heart disease, obesity, and an increased stress response, among other morbidities.¹⁰³ Studies between adults who were exposed to the famine in utero and same-sex siblings who were not exposed show that the exposed siblings have less DNA methylation at the imprinted insulinlike growth factor 2 locus 60 years following the famine.¹⁰⁴ Exposure to famine has been linked to an increased incidence of schizophrenia,¹⁰⁵ drug addiction,¹⁰⁶ and breast cancer¹⁰⁷ as well as many other disorders. These studies imply that calorie restriction during pregnancy imposes constraints that can lead to diseases in adulthood.

Studies of the transgenerational influence of nutrition have shown that the nutritional status of the grandfather from ages 8 to 12 years, also known as the slow growth period (SGP), will affect the lifespan and propensity for diabetes in his grandson. A study of the population of the Överkalix parish of people born in 1890, 1905, and 1920 showed that if nutrition was not readily available during the grandfathers' SGP, the incidence of cardiovascular disease mortality was low.¹⁰⁸ An excess of food availability caused an increase in the incidence of diabetes in the grandson. This study hints that a memory of the grandfather's nutritional status is programmed and transmitted through two generations, likely due to an epigenetic mechanism.

The effects of a maternal high-fat diet on epigenetic changes in the fetus have been characterized in a nonhuman primate model. Exposure to a maternal high-fat diet increases the global amount of acetylation of lysine 14 of histone H3 in fetal liver tissue, harvested at the beginning of the third trimester. This increase in H3 acetylation is accompanied by a decrease in levels of the HDAC1 histone deacetylase enzyme, as well as a decrease in HDAC activity of fetal liver extracts.

The requirement for essential nutrients in the one-carbon metabolism pathway for healthy development of the fetus has been well characterized. A good model system to test the molecular interactions and DNA methylation is using the viable yellow agouti mouse (A^{vy}). In this model system a transposon is associated with the promoter region of the agouti gene (reviewed in¹¹⁰). Transposons are traditionally silenced via DNA methylation, but this locus

encodes a metastable allele. This allele will be differentially expressed in genetically identical cells based on epigenetics mechanisms. Ectopic expression of the agouti gene results in yellow fur, obesity, diabetes, and increased susceptibility to tumors.¹¹¹ The degree of methylation of this locus changes the degree of transcription, which changes the color of the mouse. A completely silenced locus will yield a completely black mouse, whereas an expressed locus will yield a yellow (agouti) mouse.

The distribution of coat color, from agouti, to pseudo-agouti, to black, depends on the biological mother and is not the result of the maternal environment.¹¹² Fertilized oocytes from yellow agouti mice were transplanted in black mice. The resultant offspring were consistent with those born from the biological mother (i.e., agouti yellow). To test if DNA methylation within the promoter of the agouti gene was responsible for this pattern of inheritance, methylation levels within the paternal and maternally contributed alleles were measured at the zygote, two-cell, and blastocyst stage. The Whitelaw group showed that methylation was completely erased at both alleles during embryogenesis.¹¹³ Therefore, it is not DNA methylation alone that causes the inheritance of the coat color of the biological mother.

Although methylation does not appear to be inherited, dietary supplementation of the maternal diet with methyl donors within the one-carbon metabolism pathway (folic acid, choline, methionine, arginine) will change the coat color of the agouti mouse in a manner that correlates with the level of methylation of the DNA within the promoter.^{114,115} The effect of supplementation, however, is also not inherited transgenerationally.¹¹⁶

The agouti mice have also been used to study the transgenerational inheritance of obesity because they have a propensity toward obesity and diabetes. Methyl supplementation of the maternal diet prevents inheritance of obesity.¹¹⁷ Supplementation of the maternal diet with genistein, an isoflavone found in soy, was found to increase methylation of the CpG sites in the offspring, early in embryonic development, and protected the offspring from obesity.¹¹⁸

ENVIRONMENTAL EPIGENETICS: FUTURE DIRECTIONS

Studies in the field of environmental epigenetics in the past few years have pointed to a direct effect of the environment in the establishment of stable gene expression patterns. The elucidation of the apparent heritability and stability of these DNA and histone modifications will certainly prove to be an exciting challenge in the field of epigenetics. Already, vitamins fortified with cofactors of the one-carbon metabolism pathway are a part of standard prenatal care. The potential for diet supplementation in the care of diabetes and metabolic syndrome is becoming apparent. As the contribution of epigenetics to behavior in mental illness becomes clearer, therapeutics that disrupt HDAC activity, and are a standard treatment in cancer patients, may prove useful in the treatment of psychiatric disorders. The dynamic nature of chromatin yields potential for both preventive medicine and treatment, which the study of classical genetics did not address.

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Behavior	Epigenetic Changes	Ref
Maternal Care; Licking, Grooming Behavior	•Site Specific changes of CpG methylation and H3K9ac levels within GR promoter. •Changes in CpG methylation in ER α promoter.	11-13 15
Depression and Social Defeat	•Increase in H3K27me2 in BDNF promoter	20
Fear Conditioning	•Changes in H3 phosphorylation and acetylation in hippocampus	21-23
Drug Abuse	•Changes in H3 and H4 acetylation at site specific promoters in response to either acute or chronic cocaine exposure	24

Figure 1.

Behavioral influences, such as fear and depression, cause specific epigenetic changes.

BDNF, brain-derived neurotrophic factor; CpG, cytosine followed by a guanine; ER α , α subunit of the estrogen receptor; GR, glucocorticoid receptor.

Toxins	Epigenetic Changes	Ref
	Vinclozolin	•Disruption of DNA methylation in rat testes four generations after maternal vinclozolin exposure	29
	Synthetic estrogen (diethylstilbestrol)	•Women exposed <i>in utero</i> have higher incidence of uterine fibroids. •Changes in CpG methylation in mouse model in promoter of lactoferrin gene.	33 34
	Nickel	•Changes in DNA methylation and chromatin structure. •Changes in global H3 and H4 acetylation. •Increased global levels of H3K9me2.	37 38, 39 40
	Arsenic	•DNA hypomethylation at CpG rich regions. •Increased H3K9me2 and H3K4me2 and decreased H3K27me3.	42 43

Figure 2.

Exposure to environmental toxins alters DNA methylation and histone modification profiles.
CpG, cytosine followed by a guanine.

Diet	Epigenetic Changes	Ref
Calorie Restriction: the Dutch Hunger Winter	<ul style="list-style-type: none"> •Propensity for glucose intolerance, coronary heart disease and obesity. •Increased incidence of cancer, drug abuse and schizophrenia. •Lower CpG methylation at IGF-2 locus in exposed offspring. 	48 50 51-53
Maternal High Fat Diet	<ul style="list-style-type: none"> •Changes in H3K14ac and overall HDAC activity in fetal liver. 	55
One Carbon Metabolism Supplementation	<ul style="list-style-type: none"> •Differential methylation at the Agouti locus. •Prevention of inheritance of obesity. 	60, 61 64

Figure 3.

Maternal diet can cause in utero epigenetic changes in the fetus thought to reprogram genes and increase the susceptibility to adult disease. CpG, cytosine followed by a guanine; IGF, HDAC, histone deacetylase.