FEATURE ARTICLE

Epigenetics: A Fascinating Field with Profound Research, Clinical, & Public Health Implications

RICHARD A. STEIN, DEVRA LEE DAVIS

Abstract

Epigenetics is emerging as one of the most dynamic and vibrant biomedical areas. Multiple lines of evidence confirm that inherited genetic changes alone cannot fully explain all phenotypic characteristics of live organisms, and additional factors, which are not encoded in the DNA sequence, are involved. The contribution of non-genetic factors is perhaps best illustrated by monozygotic twins, which, despite sharing nearly identical DNA sequences, are often discordant for diseases they develop. Even when twins develop the same condition, they may experience different clinical manifestations or clinical onset at different ages. Epigenetic mechanisms explain how a zygote can differentiate into >220 different cell types that form an adult organism and, with rare exceptions, share the same DNA. Increasingly, epigenetic factors emerge, in addition to genetic ones, as important contributors to carcinogenesis. Epigenetic modifications also explain the biological impact of environmental factors, including chemical and dietary compounds, physical agents, pathogens linked to cancer, and social–emotional interactions. Unlike genetic changes, epigenetic changes are reversible, a characteristic that opens unprecedented therapeutic avenues, exemplified by the first epigenetic drugs that were recently approved. Understanding the combined contribution of genetic and epigenetic factors to gene expression will be essential to dissect the biological networks shaping development and disease, and to develop a new array of prophylactic, diagnostic, and therapeutic applications.

Key Words: *Gene expression; epigenetics; development; carcinogenesis; arsenic; tobacco.*

Genetics Does Not Explain Everything: Discordance in Monozygotic Twins

For some time, researchers have appreciated that only 5–10% of all cancers can be attributed to genetic defects, whereas the vast majority, 90–95%, are linked to environmental factors and lifestyle (Anand et al., 2008). The contribution of non-genetic factors to the phenotype is most apparent in monozygotic twin pairs, who often develop different diseases, despite being genetically nearly identical. A high discordance rate between monozygotic twin pairs, sometimes >50%, was reported for many medical conditions, including schizophrenia and type 2 diabetes mellitus, and even for diseases previously considered to be highly heritable (Cardno & Gottesman, 2000; Poulsen et al., 2007; Bell & Spector, 2011). Friedman et al. (2005) described, for the first time, a monozygotic twin pair with clinical onset of Huntington's disease at least 7 years apart. All the twin pairs described until then showed clinical onset within 1 year from each other. In the twin with earlier onset of the disease, the authors identified smoking and industrial toxins as potential explanations for the discordance. Until this report, Huntington's disease was considered a genetic condition for which environmental influences were thought to have little or no impact. Many of these observations point to the contribution, in addition to genetic factors, of non-genetic influences that shape the phenotype.

Epigenetics is the study of heritable and potentially reversible gene-expression changes that do not involve structural alterations in the DNA sequence, such as mutations.

Epigenetic Mechanisms

Epigenetics is the study of heritable and potentially reversible gene-expression changes that do not involve structural alterations in the DNA sequence, such as mutations. This term was coined to describe changes that could not be explained by genetic mechanisms. Three major types of epigenetic changes have been described: DNA methylation, covalent post-translational histone modification, and small inhibitory RNA-mediated signaling pathways.

DNA methylation is the most extensively studied epigenetic modification in humans. It consists of the covalent addition of a methyl group to the 5 position of a cytosine to generate 5-methylcytosine and occurs mostly in the context of cytosines that precede guanines, known as 5'-CpG-3' dinucleotides (Portela & Esteller, 2010). To a lesser extent, methylation was also found in CpA and CpT sequences from embryonic stem cells, but not somatic cells

The American Biology Teacher, Vol. 74, No. 4, pages 213–216, 219–223. ISSN 0002-7685, electronic ISSN 1938-4211. DOI: 10.1525/abt.2012.74.4.3

(Kulis & Esteller, 2010). Two types of CpG methylation changes relevant to carcinogenesis were described. One involves regions at repetitive chromosomal sequences, where DNA is normally methylated. Global methylation loss causes chromosomal instability, which is linked to cancer development (Portela & Esteller, 2010). Feinberg and Vogelstein (1983) found that four of five cancer patients they examined had significant hypomethylation in cancer cells as compared with adjacent cancer-free cells. One patient exhibited even more accentuated hypomethylation in a metastatic tumor as compared with the primary tumor of origin. This also represented the first time that the link between DNA methylation and human cancer was reported. The second context in which DNA methylation is important is at CpG islands, which are regions of ~200 base pairs (bp) with a G+C content >50% and a ratio between the observed and statistically expected CpG dinucleotide frequency ≥0.6 (Portela & Esteller, 2010). CpG islands are frequently found in the 5' regulatory region of the genes, where normally they are not methylated. In general, methylation is associated with gene silencing (Esteller, 2008; Kulis & Esteller, 2010; Portela & Esteller, 2010). In 1989, Greger et al. (1989) reported, for the first time, gene silencing that may occur in diploid somatic cells (apart from the X-chromosome inactivation) by methylation. They found that *RB*, the retinoblastoma suppressor gene, was hypermethylated in a sporadic unilateral retinoblastoma tumor. Many other tumor-suppressor genes have since been shown to be silenced by epigenetic mechanisms (Kulis & Esteller, 2010).

Histone post-translational changes represent the second type of epigenetic modifications. Histones are small basic DNA-binding proteins that have globular cores and ≤40 amino-acid-long N-terminal unstructured tails, which are rich in basic amino acids and represent the sites that are covalently modified (Kouzarides, 2007; Campos & Reinberg, 2009). Until the early 1990s, histones were thought to serve exclusively structural functions, and their regulatory roles emerged only later. In recent years, at least eight different types of covalent modifications, such as acetylation, methylation, or ubiquitylation, have been described on histone tails. These modifications can disrupt their interactions with DNA and other proteins, affect chromosome compaction, and change gene function (Suganuma & Workman, 2011). Histone acetylation, for example, emerges as a central factor that shapes chromatin dynamics and gene accessibility (Görisch et al., 2005; Roh et al., 2005).

Different types of epigenetic modifications influence each other in a process known as "epigenetic crosstalk." In the fungus *Neurospora crassa*, Tamaru and Selker (2001) noticed that histone H3 methylation affects DNA methylation, and other studies revealed that in higher organisms, DNA methylation influences histone acetylation. In several model organisms, an intimate bi-directional interplay has been described between histone post-translational modifications and DNA methylation (Eden et al., 1998; Vaissière et al., 2008).

A third type of epigenetic modifications is mediated by microRNAs (miRNA), which are ~22 nucleotide-long, single-stranded, noncoding RNA molecules that negatively regulate gene expression at the post-transcriptional level and participate in many biological processes, including development, differentiation, and cell proliferation (Calin & Croce, 2006; Lujambio & Esteller, 2009). Each miRNA can target hundreds of different messenger RNA (mRNA) molecules, and each mRNA can be targeted by several miRNAs (Fabbri & Calin, 2010). Computational predictions have estimated that >60% of human mRNA molecules are targeted by microRNAs (Friedman et al., 2009). Several studies have reported that cancer cells exhibit aberrant miRNA expression profiles. Some miRNAs can be up-regulated, whereas others are down-regulated, and miRNA profiling promises important clinical applications (Calin & Croce, 2006; Nakajima & Yokoi, 2011).

○ Epigenetic Reprogramming during Mammalian Development

In at least two developmental stages in mammals, gametogenesis and pre-implantation development, the DNA methylation pattern is reprogrammed genome-wide. This epigenetic reprogramming is believed to be necessary for return to pluripotency and for lineage commitment (Corry et al., 2009).

Primordial germ cell development is accompanied by a wave of genomic demethylation that is completed in both the male and the female mouse germ cells by embryonic days 13 to 14, a time by which the primordial cells have entered the gonadal anlagen (Reik et al., 2001; Corry et al., 2009). This DNA methylation erasure affects most but not all the genome, and certain regions remain highly methylated. This is followed, days later, by the gradual *de novo* remethylation that occurs at different rates in the male and female germ cells. In the mouse, remethylation in males begins on embryonic days 15 to 16 in the prospermatogonia arrested in the G1 phase of mitosis and is completed before birth (Reik et al., 2001). In females, in which the primary oocytes are arrested in the diplotene stage of meiotic prophase I, this process is initiated only after birth, during the follicular growth phase.

In mammals, the genomes of mature sperm cells and eggs remain highly methylated until fertilization. During pre-implantation development, a second round of demethylation takes place in the zygote, occurring at different rates in the two parental genomes (Reik et al., 2001; Corry et al., 2009; Meissner, 2010). Demethylation of the paternal genome occurs after fertilization but before cell division, it is more rapid than in the maternal genome, and involves an active mechanism (Feng et al., 2010). Demethylation of the maternal genome occurs gradually over the first few cleavage divisions of the embryo, involves a passive mechanism, and is dependent on DNA replication. Both the paternal and the maternal genomes are remethylated around the time of implantation. This *de novo* methylation silences genes that are involved in maintaining pluripotency and is more accentuated in the inner cell mass of the blastocyst, which generates the adult tissues, than in the outer layer of cells (trophectoderm), which develops into the extraembryonic tissues, such as the placenta.

○ The Epigenetic Landscape in Embryonic Stem Cells & during Differentiation

Adult organisms contain >220 different cell types that, with very few exceptions, share the same genetic information. Establishing different gene expression patterns is crucial for shaping the identity of individual cell types, a task that is accomplished by epigenetic mechanisms. In 1957, Conrad Waddington proposed the term "epigenetic landscape" to refer to the process of cellular decision-making during development

(Waddington, 1957; Goldberg et al., 2007). Waddington depicted the cell as a ball rolling downhill through a landscape of ridges and valleys that branch at different points, to illustrate the possible choices that it can make during development. As the ball moves downward, its options are progressively narrowed until it reaches the valley, where it becomes a differentiated cell.

During early development, the inner cell mass of the blastocyst forming from the zygote develops into the embryo. A thin outer layer of cells, the trophoblast, develops into the fetal portion of the placenta. Embryonic stem cells, derived from the inner cell mass of the developing blastocyst, are distinguished by two main characteristics: an unlimited self-renewing capacity and pluripotency. Self-renewal is the ability of embryonic stem cells to proliferate and remain in the same state; pluripotency is their ability, under the appropriate signals, to differentiate and generate all cell lineages and cell types from the adult organism (Young, 2011). Pluripotent cells can differentiate into many cell types, and during this differentiation process they progressively narrow their potential. Differentiation is accompanied by specific gene-expression pattern changes, orchestrated by epigenetic programs that silence genes responsible for multipotency and activate genes governing tissue-specific functions.

In embryonic stem cells, most genes that encode transcription factors related to pluripotency are hypomethylated, whereas the promoters of lineage-specific genes are hyper- or heavily methylated. Several transcription factors maintain the pluripotent state. Of these, the core transcription factors Oct4, Sox2, and Nanog establish an interconnected autoregulatory loop that positively regulates their expression. At the same time, they also regulate genes that maintain the embryonic stem-cell state while repressing those that encode cell-lineage-specific transcription factors (Young, 2011).

Transcription factors, together with cofactors, chromatin regulators such as histone-modifying enzymes, and noncoding RNAs, regulate the embryonic stem-cell state. During differentiation, changes often occur in all these classes of regulators. For example, a microRNA cluster – miRNA 290 to miRNA 295 – that contributes to stem-cell identity was recently characterized. These microRNAs are abundant in mouse embryonic stem cells, where their promoters are occupied by several transcription factors, including Oct4, Sox2, and Nanog, and their expression decreases early during differentiation (Marson et al., 2008). Bernstein et al. (2006) reported a unique histone methylation pattern in embryonic stem cells, in which genes harbor both the repressive H3K27 methylation mark and the activating H3K4 methylation mark. The authors referred to these apparently conflicting combinations of activating and repressive modifications as "bivalent domains." These are characteristic of pluripotent cells and are associated with a low level of expression that changes as cells differentiate along a specific lineage. When embryonic stem cells differentiate, for example into a neuronal cell lineage, H3K27 methylation disappears from neuron-specific promoters, where only the H3K4 methylation persists. At the same time, promoters of genes that remain silent in neurons lose the activating H3K4 methylation and retain the repressive H3K27 methylation marks. Similarly, many genes that are methylated in hematopoietic stem cells, which represent the multipotent and self-renewing cells from which the entire mammalian blood system develops, undergo selective demethylation in a cell-linage-dependent manner during hematopoietic cell development (Cedar & Bergman, 2011). For example, *Mpo*, the gene encoding myeloperoxidase, an important enzyme responsible for the microbicidal activity of neutrophils, and *Cxcr2*, which encodes a chemokine receptor responsible for neutrophil chemotaxis, are selectively demethylated in granulocyte-macrophage progenitor cells that develop into several cell types, including neutrophils (Cedar & Bergman, 2011).

In 2006, Takahashi and Yamanaka (2006) discovered that the overexpression of four transcription factors, Oct4, Sox2, Klf4, and c-Myc, is sufficient to reprogram terminally differentiated mouse fetal or adult fibroblasts into induced pluripotent stem (iPS) cells, which resemble embryonic stem cells. These iPS cells have similar, but not identical, molecular and morphological characteristics as embryonic stem cells, exhibiting self-renewal and the ability to differentiate into many cell types. This finding not only helped clarify the molecular basis of embryonic stem-cell differentiation, but also opened important novel research and therapeutic opportunities, including the possibility of generating patient-specific embryonic stem cells to treat a broad range of medical conditions or for regenerative-medicine purposes.

○ Epigenetics & Cancer

For many years, the genetic origin of cancer was a centrally held dictum among theories of carcinogenesis and the topic of intense scrutiny. Considerable research has focused on evaluating the mutational activation of oncogenes and inactivation of tumor-suppressor genes. But in many instances, it was evident that carcinogenic phenomena did not fit this dictum. Recently, several lines of evidence have revealed the importance of non-genotoxic epigenetic processes for cancer onset, progression, metastatic dissemination, and subsequent inheritance. Cancer cells often show global DNA hypomethylation, a modification associated with chromosomal instability, hypermethylation at promoter CpG sites, which causes aberrant gene silencing, and disruption in the normal pattern of covalent histone modifications. The global loss of histone H4K20 trimethylation and histone H4K16 acetylation are among the frequently described changes that occur in cancer cells (Fraga et al., 2005). The gain or loss of microRNAs that function as oncogenes or tumor suppressor genes are additional modifications that were reported, and the different types of epigenetic changes often influence each other.

Epigenetic modifications promise to reshape many of the current concepts that guide cancer therapy. In the first comprehensive analysis of promoter methylation in normal tissues adjacent to cancer tissues in patients with breast cancer, Yan et al. (2006) described DNA hypermethylation extending as far as 4 cm from the primary tumors. In certain patients, hypermethylation was present even in normal tissues from the contralateral breast, an observation that provides a valuable tool with applications in cancer prophylaxis and therapy. Kitago et al. (2009) revealed, for the first time, that the mRNA for *RUNX3*, a tumor suppressor gene involved in several cancers, is suppressed in primary cutaneous melanoma and is further suppressed in metastatic tumors, as compared with normal tissue; and Nobeyama et al. (2007) reported hypermethylation of the *TFPI2* tumor suppressor gene in 29% of the metastatic melanomas examined, but in none of their primary tumors of origin. These findings point toward the involvement of hypermethylation and gene inactivation in tumor metastasis.

The first epigenetic drugs were recently introduced in clinical medicine, based on the reversibility of epigenetic changes. Two

hypomethylating compounds, azacitidine and decitabine, were approved by the FDA in 2004 and 2006, respectively. More recently, two additional compounds that inhibit histone acetyltransferases were approved (Boumber & Issa, 2011). These therapeutic agents exhibit great efficiency in hematopoietic malignancies, and additional compounds are currently at various stages of preclinical development and clinical trials.

○ An Epigenetic Switch

By transiently activating with tamoxifen a fusion construct between the Src kinase oncoprotein (v-Src) and the ligand-binding domain of the estrogen receptor, Iliopoulos et al. (2009) revealed that the inflammatory response initiated an epigenetic switch and, as a result, a non-transformed epithelial cell line was converted, within 24–36 hours, to a transformed state. Tamoxifen induction for as short as 5 minutes was sufficient to achieve the transformed state. During this process, the NF-κB-mediated inflammatory response activates a positive feedback loop, which maintains a stable epigenetically transformed state for many generations, even in the absence of the initial signal that started the process. The authors subsequently identified two microRNAs, miR-21 and miR-181b-1, which are known to be involved in human cancers, and are differentially regulated as part of this positive feedback loop. The transient expression of either of these microRNAs was sufficient to activate the epigenetic switch and cause transformation (Iliopoulos et al., 2010).

○ Case Studies: Epigenetics & Environmental Exposures

For many years, it was erroneously assumed that, to cause cancer, a compound has to cause mutations in the double-stranded DNA, and mutations were viewed as the only way that could lead to carcinogenesis. Consequently it was assumed, equally erroneously, that a compound cannot cause cancer as long as it does not mutate the DNA. Nevertheless, animal models and human epidemiological studies have revealed that several chemicals that are not mutagens *in vitro* could be causally linked to cancer. To illustrate the fallacy of having envisioned all carcinogens as being mutagens while failing to realize that cancer may also occur in the absence of mutations through other mechanisms such as epigenetic modifications, Trosko and Upham (2005) referred to the "lamp post effect": when we drop keys in the dark, we always look for them under the lamp, where the light reaches, but we do not search the dark places, where they may have fallen and could also be found.

1. Arsenic

Arsenic, a human carcinogen causally linked to skin, liver, lung, and bladder cancer, does not appear to be mutagenic and does not induce point mutations in standard mutagenesis assays. It is estimated that >100 million people worldwide are exposed to carcinogenic levels of arsenic (Reichard & Puga, 2010). Several authors have found that arsenic causes cancer by epigenetic mechanisms, and DNA methylation was most extensively studied (Reichard & Puga, 2010). Mass and Wang (1997) found a dose-dependent hypermethylation in the p53 gene promoter in human lung adenocarcinoma cells exposed to sodium arsenite or arsenate, but not to the inert dimethylarsenic acid. In the first study to examine genome-wide DNA methylation changes in a population with ongoing inorganic arsenic exposure, Smeester et al. (2011) surveyed CpG islands from >14,000 genes in individuals from Zimapan, Hidalgo State, Mexico, who were exposed to relatively high levels of arsenic from drinking water, and in whom the extent of exposure was known from urinary arsenic levels. The authors identified 183 genes that were epigenetically modified as a result of exposure. They were able to characterize a "tumor suppressorome," a group of 17 confirmed or putative tumor suppressor genes that are silenced in human cancers, some of which had previously been implicated in arsenic-associated cancers.

2. Infectious Diseases

The link between infectious diseases and cancer was first reported over a century ago. The human papilloma virus, hepatitis B virus, and certain *Helicobacter pylori* strains are among the most extensively studied microorganisms, causally linked, respectively, to cancers of the cervix and esophagus, liver, and stomach. Fernandez et al. (2009) reported for the first time a dynamic methylation pattern that occurs in several double-stranded cancer-causing DNA viruses. At progressive stages of disease, ranging from asymptomatic carriage to chronic infection, premalignant lesions, and malignant tumors, the authors reported that the DNA methylation status of the viral genome ranged from unmethylated to highly methylated, and these modifications have functional implications for the behavior of these viruses.

The epigenetic changes that explain the causal link between infectious diseases and cancer were most extensively explored for *H. pylori*, a pathogen declared a class I human carcinogen by the World Health Organization in 1994. Certain *H. pylori* strains, which contain virulence factors such as the ones encoded by the *cagA* or *vacA* genes, were causally linked to stomach cancer, and this pathogen was estimated to be responsible for >60% of stomach cancers worldwide (Wen & Moss, 2009).

Higher CpG methylation was found in the gastric mucosa of healthy volunteers infected with *H. pylori* than in uninfected ones (Maekita et al., 2006), and this increased methylation is correlated with a higher risk of developing gastric cancer, which indicates that the dynamics of methylation could provide a very early marker to assess the risk of malignant transformation (Nakajima et al., 2006). The experimental infection of Mongolian gerbils revealed that methylation levels in the gastric mucosa start to increase 5–10 weeks after the infection, reach maximal levels by 50 weeks, and decrease after eradicating the infection, but remain higher than in uninfected animals (Niwa et al., 2010). Cyclosporin A, which suppresses inflammation without affecting bacterial colonization, blocked this aberrant methylation, which indicates that DNA methylation is induced not by the pathogen itself, but by the inflammatory reaction that it initiates during the infection.

3. Polycyclic Aromatic Hydrocarbons & Chemicals from Cigarette Smoke

One of the most extensively studied environmental toxins is tobacco smoke, a mixture of >4000 chemicals, at least 62 of which show sufficient evidence of human or animal carcinogenicity (Chen et al., 2011). Smoking increases the risk of at least 19 types of cancer and many other chronic diseases (Anand et al., 2008; Secretan et al., 2009). Yet the precise mechanisms through which tobacco induces these diseases are not yet fully understood. Certain components of cigarette smoke

THE [AllinOneBOX] CONCEPT

One-box CMOS imaging solutions

MOTICAM

Convert almost any microscope into a Digital Microscope

Integrate interactive white boards with your microscope

Find the camera that suits your needs as well as your budget

NEWmoticamGENERATION

Canada I China I Germany I Spain I USA

www.motic.com

are mutagens, but increasing numbers of studies describe epigenetic perturbations as a result of exposure. Polycyclic aromatic hydrocarbons (PAHs) are among the most extensively studied chemicals from cigarette smoke. While much attention was focused on genotoxicity, their epigenetic effects were largely ignored (Upham et al., 1998).

For several years, prenatal exposure to polycyclic aromatic hydrocarbons has been linked to an increased risk of respiratory problems by age 1 to 2, with symptoms worsened by postnatal exposure to environmental tobacco smoke (Miller et al., 2004). Perera et al. (2009) performed a DNA methylation analysis in a group of children from urban, low-income minority communities in New York City where the asthma rates exceed 25% and are among the highest in the country. They found a positive and significant correlation between monitored maternal PAH exposure and methylation of a CpG island in acyl-CoA synthetase long-chain family member 3 (*ACSL3*), a gene expressed in the lung and thymus that encodes a key enzyme involved in fatty acid metabolism.

In the first study to show that maternal smoking changes DNA methylation and gene expression in the placenta, Suter et al. (2010) found that placental expression of *CYP1A1* was more than 4× greater in smokers than in nonsmokers. *CYP1A1* encodes a protein that converts polycyclic aromatic hydrocarbons into mutagenic and carcinogenic compounds that can initiate malignant transformation (Anttila et al., 2003; Suter et al., 2010). The authors also reported that this increased expression was associated with decreased CpG methylation in a *CYP1A1* promoter region in smokers as compared with nonsmokers, pointing toward a potential epigenetic signature that develops as a result of maternal smoking (Suter et al., 2010).

Breton et al. (2009) found gene-specific and global DNA methylation changes in children exposed *in utero* to maternal smoking. The authors examined the effect of maternal smoking on two repetitive elements, a long interspersed nucleotide element (LINE1) and a short interspersed nucleotide element (AluYb8), which are markers for global DNA methylation and, in addition, surveyed 1505 CpG sites in 807 promoters for gene-specific methylation changes. Exposed children showed lower overall methylation in the AluYb8 elements, and higher CpG methylation in eight genes examined. Two genes, encoding a receptor tyrosine kinase involved in cell survival and a protein tyrosine phosphatase receptor important during central and peripheral nervous system development, showed consistent changes. The altered methylation visualized in these genes in the offspring of maternal smokers opens thought-provoking public health questions.

DNA methylation is not the only epigenetic change linked to smoking. Maccani et al. (2010) found three miRNAs (miR-16, miR-21, and miR-146a) that were significantly down-regulated in the placenta of pregnant smokers. This finding is supported by the observation that in a placental cell line, nicotine and benzpyrene exposure showed a dose-dependent down-regulation of miR-146a, which was previously implicated in cancer and inflammation (Williams et al., 2008).

4. Social & Emotional Factors

Social interactions were recently linked to epigenetic changes, possibly via hormonal pathways. One of the most informative systems for studying the influence of maternal care on gene expression in rodent offspring is the licking and grooming (LG) and arched-back nursing (ABN) behaviors that certain rats exhibit during the first week after their pups are born. Weaver et al. (2004) reported that increased LG-ABN induces epigenetic changes in the pups at the glucocorticoid receptor promoter in the hippocampus. Offspring born to high-LG-ABN mothers had lower methylation at certain CpG sites in the promoter and increased histone acetylation, and showed higher transcription-factor binding and increased gene expression. Increased expression of this receptor is associated with a more moderate hypothalamic–pituitary–adrenal response to stress during adulthood (McGowan et al., 2009), which explains the fact that the offspring of high-LG-ABN mothers are less fearful of stress than the offspring of low-LG-ABN mothers (Szyf et al., 2005). Cross-fostering produced a methylation pattern that reflected the behavior of the rearing mother, reinforcing the causal link between maternal care and DNA methylation in the offspring (Szyf et al., 2005). L-methionine, a precursor of the methyl group donor S-adenosyl-methionine, reversed this methylation pattern, indicating that epigenetic changes induced by maternal behavior, even though stable throughout the life of the offspring, are nevertheless reversible (Weaver et al., 2005).

The glucocorticoid receptor is not the only target of epigenetic modifications induced by maternal care. Weaver et al. (2006) analyzed the rat hippocampal transcriptome and reported that transcriptional changes in response to maternal care occur in >900 genes, a finding that could provide the molecular basis to explain the relationship between early life experiences and stress response during adulthood. The survey of a 7-million-bp region on rat chromosome 18, which contains the glucocorticoid receptor gene, also unveiled epigenetic changes that occur in response to maternal care and are clustered in broad genomic areas (McGowan et al., 2011).

McGowan et al. (2009) examined epigenetic modifications in the promoter of *NR3C1*, which encodes the human glucocorticoid receptor in the hippocampus. In a stunning indication of the impact that child abuse has on chromatin, they found increased CpG methylation and decreased mRNA levels in suicide victims with a history of childhood abuse, as compared with suicide victims without a history of abuse. This suggested that a history of childhood adversity is associated with long-lasting and possibly heritable changes in the glucocorticoid receptor expression. These changes could shape individual differences in psychopathology later in life.

Several recent findings reveal that epigenetic changes could help understand the biological basis of posttraumatic stress disorder (PTSD). Koenen et al. (2011) examined the link between methylation of *SLC6A4*, which encodes the serotonin transporter, and PTSD. *SLC6A4* polymorphisms were previously linked to PTSD, but several authors reported that the relationship is very complex and cannot be explained by genetic factors alone. After controlling for the genotype, the authors revealed that *SLC6A4* methylation changes the relationship between the number of traumatic events and the subsequent risk of developing PTSD, but only at lower methylation levels, offering a potential molecular signature (Koenen et al., 2011). Renthal et al. (2007) reported, in a mouse model, that chronic (but not acute) stress regulates histone deacetylase 5 (HDAC5) in nucleus accumbens, a brain region involved in cocaine and stress response. Histone deacetylases, by removing acetyl groups from the histone tails, establish a less accessible chromatin conformation and repress gene expression. HDAC5 emerges, thus, as an epigenetic regulator that is involved in the transition between short-term physiological responses and long-term pathological states, with implications for the understanding of biological changes that occur during addiction and emotional stress.

5. Dietary Factors

The intrauterine environment is instrumental for offspring development, and perturbations during this stage may have long-lasting consequences in the adult (Tamashiro & Moran, 2010). Both under- and overnutrition during pregnancy have been linked to increased risk of diabetes and other metabolic conditions later in life. The mechanisms appear to involve epigenetic changes induced in response to nutritional perturbations, which are "remembered" later in life and shape health and disease (McKay & Mathers, 2011). In addition to the type of nutritional perturbation, the consequences also depend on its timing during development (Burdge et al., 2007; Simmons, 2011).

The effects of maternal nutritional deprivation during pregnancy on the risk of adult-onset disease are illustrated by studies from the Dutch Hunger Winter, also known as the Dutch Famine, the period at the end of World War II characterized by several months of extreme food shortage (Painter et al., 2005). Between December 1944 and April 1945, official daily food rations in Amsterdam varied between 400 and 800 calories. The situation improved after 5 May 1945 with the liberation of the Netherlands, and by June 1945 the daily food rations exceeded 2000 calories (Painter et al., 2005). Medical records that were carefully made during this period revealed that nutritional deprivation during intrauterine development increased the risk of several adult-onset conditions, including coronary heart disease and obstructive airway disease, but timing of the nutritional deprivation played a crucial role in determining the organ(s) and system(s) predominantly affected (Painter et al., 2005).

The "Barker hypothesis," also known as the "developmental origins of health and disease," is a concept used to describe the increased risk of developing adult-onset chronic diseases by adults exposed to unfavorable conditions during intrauterine development (Barker, 2004). Langley-Evans et al. (1999) showed that maternal diet affects the health of the offspring even when the dietary insult is brief in duration or relatively mild in intensity. The authors found that feeding a low-protein diet to pregnant rats programmed the number of nephrons in the offspring and, in the mature animals, shaped blood pressure and the risk of developing renal disease.

A recent topic for which epigenetics provided a thought-provoking perspective is the biology of folic acid. Folic acid decreases the incidence of neural tube defects, which are the second most frequent type of serious birth defects, when administration to women starts before pregnancy (Czeizel & Dudas, 1992). In addition, an inverse link between the folate status and the risk of developing several types of cancer has been known for many years. Animal models show that folate depletion is causally linked to colorectal cancer, and folate supplementation exerts a dose-dependent protective effect (Kim, 2004). Many studies found a 20–40% lower risk of colorectal cancer, the most extensively studied malignant tumor in this context, among individuals with the highest dietary intake of folate or the highest blood levels of folate (Kim, 2005). However, recent studies observed that folate might have a paradoxical effect in colorectal cancer, depending on dose and timing. Although folate supplementation initiated before the development of malignant tumors suppresses cancer development, it appears to accelerate the progression of already existing preneoplasic lesions (Smith et al., 2008; Lee et al., 2011). In this context, it is important to consider the findings reported by Waterland and Jirtle (2003), who used a mouse model to study the phenotypic effects of dietary supplementation with methyl group donors, including folic acid. The authors revealed that in addition to its positive effects, diet supplementation with methyl donors may also have unintended consequences by inducing CpG methylation changes. This illustrates the need to better understand epigenetic changes caused by nutritional factors and environmental exposures. In addition, these examples indicate that contrary to the conventional view of toxicology – that dose makes the poison – the timing can be more important than dose alone (Axelrod et al., 2001). In determining the biological importance of any given exposure, the period of development or the critical window during which exposure occurs, and the rate at which a dose is absorbed, can be even more important than the total quantity of exposure.

○ Multigenerational Inheritance

In recent years, several investigators have revealed that epigenetic changes caused by certain compounds shape the phenotype not only in the offspring, but also in subsequent generations. An example of multigenerational effect is provided by diethylstilbestrol (DES), a synthetic hormone with strong estrogenic properties that was prescribed from the late 1930s to the 1970s to prevent miscarriages and complications in high-risk pregnancies (Newbold et al., 2006; Kalfa et al., 2011). Herbst et al. (1971) reported a rare condition, adenocarcinoma of the vagina, in several young women born in New England hospitals between 1946 and 1951, and found that it developed in a small percentage (<0.1%) of adolescent women whose mothers used DES during pregnancy (Newbold et al., 2006). Later, DES was shown to affect the development of several organs and systems, including the male and female reproductive tracts (Ma, 2009). Miscarriages, ectopic pregnancies, and poor reproductive outcome were more common among the daughters of exposed women, whereas hypospadias, cryptorchidism, and testicular hypoplasia were reported in their sons (Mittendorf, 1995; Newbold et al., 1998; Brouwers et al., 2006; Kalfa et al., 2011).

Kalfa et al. (2011) examined the grandsons of women exposed to DES and, after ruling out other environmental and genetic factors, found that the prevalence of hypospadias in sons born to DES daughters, which represented the third generation, was higher (8%, or 8/97) than in sons born to unexposed women (0/360). This finding was supported by animal experiments in which the effects of DES persisted not only in the offspring, but in one additional generation (Walker & Haven, 1997).

Illustrating the multigenerational epigenetic effects of another environmental exposure, Anway et al. (2005) found that exposing pregnant rats to the fungicide vinclozolin, used in the fruit industry, at a critical time during gonadal sex determination, decreased spermatogenesis in the offspring, not only in the first generation, but in subsequent generations as well, including the fourth, which was the last one examined in the study (Anway et al., 2005). Subsequently, Guerrero-Bosagna et al. (2010) found 52 different chromosomal regions, corresponding to 48 different promoters, that showed statistically significant DNA methylation changes in the sperm epigenome in the third generation (F_3) of rats whose F_0 generation gestating mother was exposed to vinclozolin, confirming that transgenerational epigenetic modifications occur after exposure. A bioinformatics tool identified a consensus DNA sequence that was present in 75% of these promoters, but only in 16.8% of a set of 125 random promoters. This consensus region, called EDM1 (Environmental

Induced Differential Methylation Consensus Sequence 1), has up to three potential CpG repeats, depending on the sequence. These examples reveal that epigenetic modifications may persist for several generations, even in the absence of the environmental exposure that initially caused them, a concept that has profound and far-reaching medical and public health implications.

Thus, recent studies reveal that mutations are not the sole pathway to cancer, reproductive health impairment, and other chronic health problems. Nutritional and other interventions that modulate epigenetic responses, and are synchronized with critical windows of vulnerability, may well have broad applications in promoting health and preventing disease, and should be seriously investigated.

References

Anand, P., Kunnumakkara, A.B., Sundaram, C., Harikumar, K.B., Tharakan, S.T., Lai, O.S. & others. (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharmaceutical Research, 25*, 2097–2116.

Ang, Y.S., Gaspar-Maia, A., Lemischka, I.R. & Bernstein, E. (2011). Stem cells and reprogramming: breaking the epigenetic barrier? *Trends in Pharmacological Sciences*, *32*, 394–401.

Anttila, S., Hakkola, J., Tuominen, P., Elovaara, E., Husgafvel-Pursiainen, K., Karjalainen, A. & others. (2003). Methylation of cytochrome P4501A1 promoter in the lung is associated with tobacco smoking. *Cancer Research*, *63*, 8623–8628.

Anway, M.D., Cupp, A.S., Uzumcu, M. & Skinner, M.K. (2005). Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science*, *308*, 1466–1469.

Axelrod, D., Davis, D.L., Hajek, R.A. & Jones, L.A. (2001). It's time to rethink dose: the case for combining cancer and birth and developmental defects. *Environmental Health Perspectives*, *109*, A246–A249.

Barker, D.J.P. (2004). The developmental origins of chronic adult disease. *Acta Paediatrica*, *93*(Supplement s 446), 26–33.

Bell, J.T. & Spector, T.D. (2011). A twin approach to unraveling epigenetics. *Trends in Genetics*, *27*, 116–125.

Bernstein, B.E., Mikkelsen, T.S., Xie, X., Kamal, M., Huebert, D.J., Cuff, J. & others. (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell*, *125*, 315–326.

Boumber, Y. & Issa, J.P. (2011). Epigenetics in cancer: what's the future? *Oncology (Williston Park)*, *25*, 220–226.

Breton, C.V., Byun, H.M., Wenten, M., Pan, F., Yang, A. & Gilliland, F.D. (2009). Prenatal tobacco smoke exposure affects global and gene-specific DNA methylation. *American Journal of Respiratory and Critical Care Medicine*, *180*, 462–467.

Brouwers, M.M., Feitz, W.F., Roelofs, L.A., Kiemeney, L.A., de Gier, R.P. & Roeleveld, N. (2006). Hypospadias: a transgenerational effect of diethylstilbestrol? *Human Reproduction*, *21*, 666–669.

Burdge, G.C., Hanson, M.A., Slater-Jefferies, J.L. & Lillycrop, K.A. (2007). Epigenetic regulation of transcription: a mechanism for inducing variations in phenotype (fetal programming) by differences in nutrition during early life? *British Journal of Nutrition*, *97*, 1036–1046.

Calin, G.A. & Croce, C.M. (2006). MicroRNA signatures in human cancers. *Nature Reviews Cancer*, *6*, 857–866.

Campos, E.I. & Reinberg, D. (2009). Histones: annotating chromatin. *Annual Review of Genetics*, *43*, 559–599.

Cardno, A.G. & Gottesman, I.I. (2000). Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. *American Journal of Medical Genetics*, *97*, 12–17.

Cedar, H. & Bergman, Y. (2011). Epigenetics of haematopoietic cell development. *Nature Reviews Immunology*, *11*, 478–488.

Chen, R.J., Chang, L.W., Lin, P. & Wang, Y.-J. (2011). Epigenetic effects and molecular mechanisms of tumorigenesis induced by cigarette smoke: an overview. *Journal of Oncology*, 654931.

Corry, G.N., Tanasijevic, B., Barry, E.R., Krueger, W. & Rasmussen, T.P. (2009). Epigenetic regulatory mechanisms during preimplantation development. *Birth Defects Research C*, *87*, 297–313.

Czeizel, A.E. & Dudas, I. (1992). Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *New England Journal of Medicine*, *327*, 1832–1835.

Eden, S., Hashimshony, T., Keshet, I., Cedar, H. & Thorne, A.W. (1998). DNA methylation models histone acetylation. *Nature*, *394*, 842.

Esteller, M. (2008). Epigenetics in evolution and disease. *Lancet*, *372*, S90–S96.

Fabbri, M. & Calin, G.A. (2010). Epigenetics and miRNAs in human cancer. *Advances in Genetics*, *70*, 87–99.

Feinberg, A.P. & Vogelstein, B. (1983). Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature*, *301*, 89–92.

Feng, S., Jacobsen, S.E. & Reik, W. (2010). Epigenetic reprogramming in plant and animal development. *Science*, *330*, 622–627.

Fernandez, A.F., Rosales, C., Lopez-Nieva, P., Graña, O., Ballestar, E., Ropero, S. & others. (2009). The dynamic DNA methylomes of double-stranded DNA viruses associated with human cancer. *Genome Research*, *19*, 438–451.

Fraga, M.F., Ballestar, E., Villar-Garea, A., Boix-Chornet, M., Espada, J., Schotta, G. & others. (2005). Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nature Genetics*, *37*, 391–400.

Friedman, J.H., Trieschmann, M.E., Myers, R.H. & Fernandez, H.H. (2005). Monozygotic twins discordant for Huntington disease after 7 years. *Archives of Neurology*, *62*, 995–997.

Friedman, R.C., Farh, K.K., Burge, C.B. & Bartel, D.P. (2009). Most mammalian mRNAs are conserved targets of microRNAs. *Genome Research*, *19*, 92–105.

Goldberg, A.D., Allis, C.D. & Bernstein, E. (2007). Epigenetics: a landscape takes shape. *Cell*, *128*, 635–638.

Görisch, S.M., Wachsmuth, M., Tóth, K.F., Lichter, P. & Rippe, K. (2005). Histone acetylation increases chromatin accessibility. *Journal of Cell Science*, *118*, 5825–5834.

Greger, V., Passarge, E., Hopping, W., Messmer, E. & Horsthemke, B. (1989). Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. *Human Genetics*, *83*, 155–158.

Guerrero-Bosagna, C., Settles, M., Lucker, B. & Skinner, M.K. (2010). Epigenetic transgenerational actions of vinclozolin on promoter regions of the sperm epigenome. *PLoS One*, *5*, e13100.

Herbst, A.L., Ulfelder, H. & Poskanzer, D.C. (1971). Adenocarcinoma of the vagina – Association of maternal stilbestrol therapy with tumor appearance in young women. *New England Journal of Medicine*, *284*, 878–881.

Iliopoulos, D., Hirsch, H.A. & Struhl, K. (2009). An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell*, *139*, 693–706.

Iliopoulos, D., Jaeger, S.A., Hirsch, H.A., Bulyk, M.L. & Struhl, K. (2010). STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. *Molecular Cell*, *39*, 493–506.

Kalfa, N., Paris, F., Soyer-Gobillard, M.O., Daures, J.P. & Sultan, C. (2011). Prevalence of hypospadias in grandsons of women exposed to diethylstilbestrol during pregnancy: a multigenerational national cohort study. *Fertility and Sterility*, *95*, 2574–2577.

Kim, Y.-I. (2004). Folate, colorectal carcinogenesis, and DNA methylation: lessons from animal studies. *Environmental and Molecular Mutagenesis*, *44*, 10–25.

Kim, Y.-I. (2005). Nutritional epigenetics: impact of folate deficiency on DNA methylation and colon cancer susceptibility. *Journal of Nutrition*, *135*, 2703–2709.

Kitago, M., Martinez, S.R., Nakamura, T., Sim, M.S. & Hoon, D.S. (2009). Regulation of RUNX3 tumor suppressor gene expression in cutaneous melanoma. *Clinical Cancer Research*, *15*, 2988–2994.

Koenen, K.C., Uddin, M., Chang, S.C., Aiello, A.E., Wildman, D.E. & Galea, S. (2011). SLC6A4 methylation modifies the effect of the number of traumatic events on risk for posttraumatic stress disorder. *Depression and Anxiety*, *28*, 639–647.

Kouzarides, T. (2007). Chromatin modifications and their function. *Cell*, *128*, 693–705.

Kulis, M. & Esteller, M. (2010). DNA methylation and cancer. *Advances in Genetics*, *70*, 27–56.

Langley-Evans, S.C., Welham, S.J.M. & Jackson, A.A. (1999). Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. *Life Sciences*, *64*, 965–974.

Lee, J.E., Willett, W.C., Fuchs, C.S., Smith-Warner, S.A., Wu, K., Ma, J. & others. (2011). Folate intake and risk of colorectal cancer and adenoma: modification by time. *American Journal of Clinical Nutrition*, *93*, 817–825.

Lujambio, A. & Esteller, M. (2009). How epigenetics can explain human metastasis: a new role for microRNAs. *Cell Cycle*, *8*, 377–382.

Ma, L. (2009). Endocrine disruptors in female reproductive tract development and carcinogenesis. *Trends in Endocrinology and Metabolism*, *20*, 357–363.

Maccani, M.A., Avissar-Whiting, M., Banister, C.E., McGonnigal, B., Padbury, J.F. & Marsit, C.J. (2010). Maternal cigarette smoking during pregnancy is associated with downregulation of miR-16, miR-21, and miR-146a in the placenta. *Epigenetics*, *5*, 583–589.

Maekita, T., Nakazawa, K., Mihara, M., Nakajima, T., Yanaoka, K., Iguchi, M. & others. (2006). High levels of aberrant DNA methylation in *Helicobacter pylori*-infected gastric mucosae and its possible association with gastric cancer risk. *Clinical Cancer Research*, *12*, 989–995.

Marson, A., Levine, S.S., Cole, M.F., Frampton, G.M., Brambrink, T., Johnstone, S. & others. (2008). Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells. *Cell*, *134*, 521–533.

Mass, M.J. & Wang, L. (1997). Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene p53 in human lung cells: a model for a mechanism of carcinogenesis. *Mutation Research*, *386*, 263–277.

McGowan, P.O., Sasaki, A., D'Alessio, A.C., Dymov, S., Labonté, B., Szyf, M. & others. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nature Neuroscience*, *12*, 342–348.

McGowan, P.O., Suderman, M., Sasaki, A., Huang, T.C., Hallett, M., Meaney, M.J. & others. (2011). Broad epigenetic signature of maternal care in the brain of adult rats. *PLoS One*, *6*, e14739.

McKay, J.A. & Mathers, J.C. (2011). Diet induced epigenetic changes and their implications for health. *Acta Physiologica*, *202*, 103–118.

Meissner, A. (2010). Epigenetic modifications in pluripotent and differentiated cells. *Nature Biotechnology*, *28*, 1079–1088.

Miller, R.L., Garfinkel, R., Horton, M., Camann, D., Perera, F.P., Whyatt, R.M. & others. (2004). Polycyclic aromatic hydrocarbons, environmental tobacco smoke, and respiratory symptoms in an inner-city birth cohort. *Chest*, *126*, 1071–1078.

Mittendorf, R. (1995). Teratogen update: carcinogenesis and teratogenesis associated with exposure to diethylstilbestrol (DES) *in utero*. *Teratology*, *51*, 435–445.

Nakajima, T., Maekita, T., Oda, I., Gotoda, T., Yamamoto, S., Umemura, S. & others. (2006). Higher methylation levels in gastric mucosae significantly correlate with higher risk of gastric cancers. *Cancer Epidemiology, Biomarkers & Prevention*, *15*, 2317–2321.

Nakajima, M. & Yokoi, T. (2011). MicroRNAs from biology to future pharmacotherapy: regulation of cytochrome P450s and nuclear receptors. *Pharmacology & Therapeutics*, *131*, 330–337.

Newbold, R.R., Hanson, R.B., Jefferson, W.N., Bullock, B.C., Haseman, J. & McLachlan, J.A. (1998). Increased tumors but uncompromised fertility in the female descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis*, *19*, 1655–1663.

Newbold, R.R., Padilla-Banks, E. & Jefferson, W.N. (2006). Adverse effects of the model environmental estrogen diethylstilbestrol are transmitted to subsequent generations. *Endocrinology*, *147*, S11–S17.

Niwa, T., Tsukamoto, T., Toyoda, T., Mori, A., Tanaka, H., Maekita, T. & others. (2010). Inflammatory processes triggered by *Helicobacter pylori* infection cause aberrant DNA methylation in gastric epithelial cells. *Cancer Research*, *70*, 1430–1440.

Nobeyama, Y., Okochi-Takada, E., Furuta, J., Miyagi, Y., Kikuchi, K., Yamamoto, A. & others. (2007). Silencing of tissue factor pathway inhibitor-2 gene in malignant melanomas. *International Journal of Cancer*, *121*, 301–307.

Painter, R.C., Roseboom, T.J. & Bleker, O.P. (2005). Prenatal exposure to the Dutch famine and disease in later life: an overview. *Reproductive Toxicology*, *20*, 345–352.

Perera, F., Tang, W.Y., Herbstman, J., Tang, D., Levin, L., Miller, R. & others. (2009). Relation of DNA methylation of 5'-CpG island of ACSL3 to transplacental exposure to airborne polycyclic aromatic hydrocarbons and childhood asthma. *PLoS One*, *4*, e4488.

Portela, A. & Esteller, M. (2010). Epigenetic modifications and human disease. *Nature Biotechnology*, *28*, 1057–1068.

Poulsen, P., Esteller, M., Vaag, A. & Fraga, M.F. (2007). The epigenetic basis of twin discordance in age-related diseases. *Pediatric Research*, *61*, 38R–42R.

Reichard, J.F. & Puga, A. (2010). Effects of arsenic exposure on DNA methylation and epigenetic gene regulation. *Epigenomics*, *2*, 87–104.

Reik, W., Dean, W. & Walter, J. (2001). Epigenetic reprogramming in mammalian development. *Science*, *293*, 1089–1093.

Renthal, W., Maze, I., Krishnan, V., Covington, H.E., Xiao, G., Kumar, A. & others. (2007). Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. *Neuron*, *56*, 517–529.

Roh, T.Y., Cuddapah, S. & Zhao, K. (2005). Active chromatin domains are defined by acetylation islands revealed by genome-wide mapping. *Genes & Development*, *19*, 542–552.

Secretan, B., Straif, K., Baan, R., Grosse, Y., El Ghissassi, F., Bouvard, V. & others. (2009). A review of human carcinogens – part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. *Lancet Oncology*, *10*, 1033–1034.

Simmons, R. (2011). Epigenetics and maternal nutrition: nature v. nurture. *Proceedings of the Nutrition Society*, *70*, 73–81.

Smeester, L., Rager, J.E., Bailey, K.A., Guan, X., Smith, N., García-Vargas, G. & others. (2011). Epigenetic changes in individuals with arsenicosis. *Chemical Research in Toxicology*, *24*, 165–167.

Smith, A.D., Kim, Y.I. & Refsum, H. (2008). Is folic acid good for everyone? *American Journal of Clinical Nutrition*, *87*, 517–533.

Suganuma, T. & Workman, J.L. (2011). Signals and combinatorial functions of histone modifications. *Annual Review of Biochemistry*, *80*, 473–499.

Suter, M., Abramovici, A., Showalter, L., Hu, M., Shope, C.D., Varner, M. & Aagaard-Tillery, K. (2010). In utero tobacco exposure epigenetically modifies placental CYP1A1 expression. *Metabolism*, *59*, 1481–1490.

Szyf, M., Weaver, I.C., Champagne, F.A., Diorio, J. & Meaney, M.J. (2005). Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. *Frontiers in Neuroendocrinology*, *26*, 139–162.

Takahashi, K. & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, *126*, 663–676.

Tamaru, H. & Selker, E.U. (2001). A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. *Nature*, *414*, 277–283.

Tamashiro, K.L. & Moran, T.H. (2010). Perinatal environment and its influences on metabolic programming of offspring. *Physiological Behavior*, *100*, 560–566.

Trosko, J.E. & Upham, B.L. (2005). The emperor wears no clothes in the field of carcinogen risk assessment: ignored concepts in cancer risk assessment. *Mutagenesis*, *20*, 81–92.

Upham, B.L., Weis, L.M. & Trosko, J.E. (1998). Modulated gap junctional intercellular communication as a biomarker of PAH epigenetic toxicity: structure–function relationship. *Environmental Health Perspectives*, *106*(Supplement 4), 975–981.

Vaissière, T., Sawan, C. & Herceg, Z. (2008). Epigenetic interplay between histone modifications and DNA methylation in gene silencing. *Mutation Research*, *659*, 40–48.

Waddington, C.H. (1957). *The Strategy of the Genes: A Discussion of Some Aspects of Theoretical Biology.* London, U.K.: Allen & Unwin.

Walker, B.E. & Haven, M.I. (1997). Intensity of multigenerational carcinogenesis from diethylstilbestrol in mice. *Carcinogenesis*, *18*, 791–793.

Waterland, R.A. & Jirtle, R.L. (2003). Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Molecular and Cellular Biology*, *23*, 5293–5300.

Weaver, I.C.G., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R. & others. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience*, *7*, 847–854.

Weaver, I.C.G., Champagne, F.A., Brown, S.E., Dymov, S., Sharma, S., Meaney, M.J. & Szyf, M. (2005). Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. *Journal of Neuroscience*, *25*, 11045–11054.

Weaver, I.C., Meaney, M.J. & Szyf, M. (2006). Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proceedings of the National Academy of Sciences, USA*, *103*, 3480–3485.

Wen, S. & Moss, S.F. (2009). *Helicobacter pylori* virulence factors in gastric carcinogenesis. *Cancer Letters*, *282*, 1–8.

Williams, A.E., Perry, M.M., Moschos, S.A., Larner-Svensson, H.M. & Lindsay, M.A. (2008). Role of miRNA-146a in the regulation of the innate immune response and cancer. *Biochemical Society Transactions*, *36*, 1211–1215.

Yan, P.S., Venkataramu, C., Ibrahim, A., Lui, J.C., Shen, R.Z., Diaz, N.M. & others. (2006). Mapping geographic zones of cancer risk with epigenetic biomarkers in normal breast tissue. *Clinical Cancer Research*, *12*, 6626–6636.

Young, R.A. (2011). Control of the embryonic stem cell state. *Cell*, *144*, 940–954.

RICHARD A. STEIN is an Associate Research Scholar in Molecular Biology at Princeton University, One Washington Road, 320 LTL, Princeton, NJ 08544; e-mail: richardastein@gmail.com. DEVRA LEE DAVIS, an epidemiologist and toxicologist, is President of Environmental Health Trust (http://www.ehtrust.org), PO Box 58, Teton Village, WY 83025; e-mail: ddavis@ehtrust.org.