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CONTEMPORARY REVIEW

Linking the Epigenome with Exposure Effects and Susceptibility: The Epigenetic Seed and Soil Model

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ABSTRACT

The epigenome is a dynamic mediator of gene expression that shapes the way that cells, tissues, and organisms respond to their environment. Initial studies in the emerging field of "toxicoepigenetics" have described either the impact of an environmental exposure on the epigenome or the association of epigenetic signatures with the onset or progression of disease; however, the majority of these pioneering studies examined the relationship between discrete epigenetic modifications and the effects of a single environmental factor. Although these data provide critical blocks with which we construct our understanding of the role of the epigenome in susceptibility and disease, they are akin to individual letters in a complex alphabet that is used to compose the language of the epigenome. Advancing the use of epigenetic data to gain a more comprehensive understanding of the mechanisms underlying exposure effects, identify susceptible populations, and inform the next generation risk assessment depends on our ability to integrate these data in a way that accounts for their cumulative impact on gene regulation. Here we will review current examples demonstrating associations between the epigenetic impacts of intrinsic factors, such as such as age, genetics, and sex, and environmental exposures shape the epigenome and susceptibility to exposure effects and disease. We will also demonstrate how the "epigenetic seed and soil" model can be used as a conceptual framework to explain how epigenetic states are shaped by the cumulative impacts of intrinsic and extrinsic factors and how these in turn determine how an individual responds to subsequent exposure to environmental stressors.

Key words: epigenetics; chromatin; susceptibility; DNA methylation; seed and soil; developmental toxicity, prenatal; reproductive and developmental toxicology; toxicoepigenetics.

The structure and function of cells, tissues, and organs is determined by the differential expression of approximately 20 000 genes (Pruitt *et al.*, 2009), which must be regulated in a carefully choreographed manner. The epigenome—a suite of covalent modifications to DNA and its histone protein scaffolding—dictates chromatin structure, interactions between the transcriptional machinery and DNA, and ultimately gene expression (Figure 1). Epigenetic modification of DNA is limited to methylation; however, while most commonly associated with gene silencing (Baylin, 2005; Clark and Melki, 2002; Esteller, 2007; Thienpont, *et al.*, 2016; Venolia and Gartler, 1983), DNA methylation has a diverse range of roles in regulating gene expression that vary with its genomic context (reviewed in Jones, 2012; Law and Jacobsen, 2010; Smith and Meissner, 2013). In contrast to DNA, histone proteins are decorated with a broad range of covalent modifications, including methylation, acetylation, phosphorylation, ubiquitination, and many others (Kouzarides, 2007). In all, at least 130 unique epigenetic modifications have been identified to date (Tan *et al.*, 2011). Akin to the arrangement of letters to form words in a language, these modification

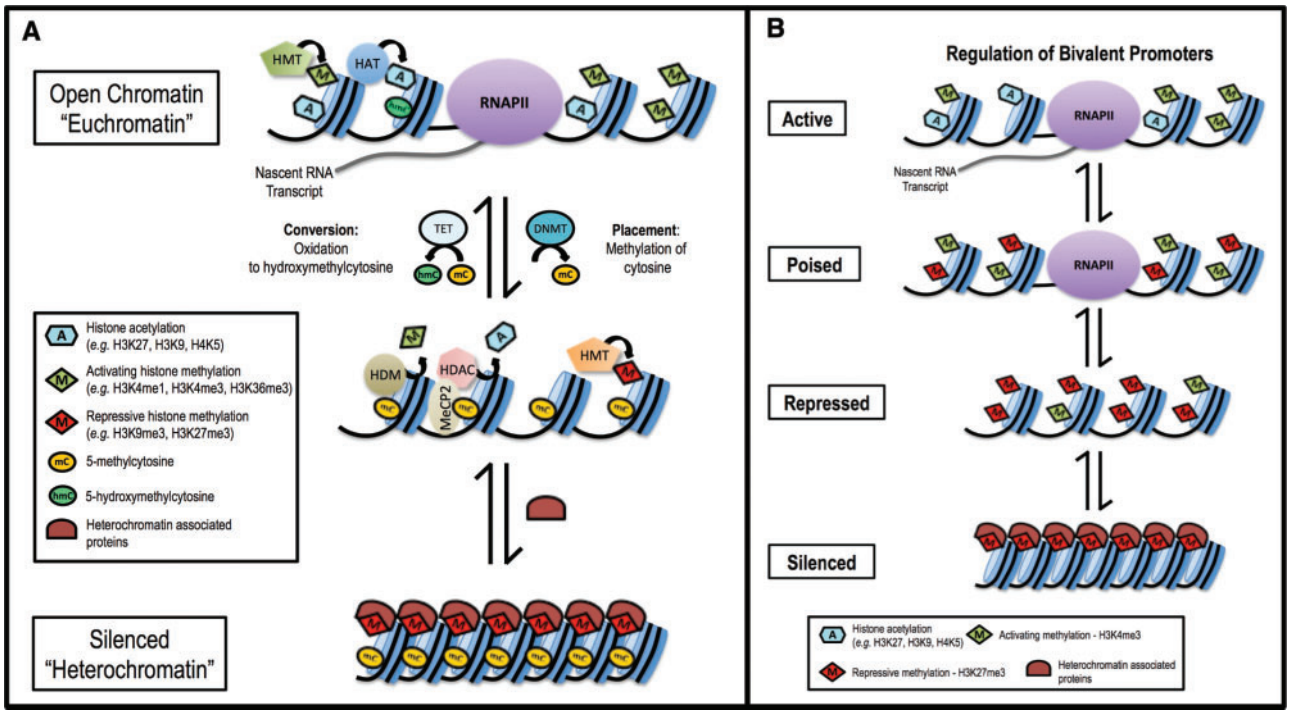

FIG. 1. Epigenetic modifications function in concert to regulate gene expression. **A,** Histone modifications and DNA methylation function cooperatively to regulate chromatin structure, accessibility to transcription factors, and gene expression. DNA methylation is the addition of a methyl group by a DNMT to the cytosine residue of CpG dinucleotides in DNA. Methylation of DNA in gene regulatory regions (promoters and enhancers) often results in transcriptional repression; however, the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine by the 10–11 translocation family of methylcytosine dioxygenases is associated with the activation of gene expression. The genome is packaged on a protein scaffolding composed of histone proteins arranged into repeating units known as nucleosomes. The unstructured tails of these histones extend outside of the core nucleosome and are subject to numerous modifications such as acetylation, methylation, phosphorylation, ubiquitination, *et cetera*. These modifications can be activating (eg, H3K4me3 and acetylation) or repressive/silencing (eg, H3K9me3 and H3K27me3). Activating histone acetylation and methylation, modifications made by histone acetyltransferases and histone methyltransferases (HMTs), facilitate chromatin accessibility (euchromatin), recruitment of the transcriptional machinery, including RNA polymerase II, and initiation/elongation of transcription. DNA methylation and repressive histone modifications function cooperatively, through proteins such as methyl-CpG binding protein 2, histone deacetylases, histone demethylases, and repressive HMTs, in the recruitment of transcriptional co-repressors and the formation of repressed and inactive (heterochromatin) epigenetic states. **B,** Bivalent gene promoters regulate expression based on the balance of activating and repressive histone modifications. Bivalent modifications occur in gene promoters (H3K4me3/H3K27me3) and enhancers (H3K27ac/5mC) in both stem and somatic cells. The balance of otherwise opposing modifications determines whether a gene is repressed, poised (contains a paused polymerase ready to initiate transcription), or actively expressed. This figure is a representation of the generalized functions of certain epigenetic factors; however, the functionality of epigenetic modifications can vary based on the specific context in which they exist.

patterns function cooperatively as an epigenetic code that is "written" through the enzymatic activities of epigenetic modifying enzymes and "read" by specialized binding domains in transcription factors and other chromatin-associated proteins (Cedar and Bergman, 2009; Jenuwein and Allis, 2001; Strahl and Allis, 2000). Patterns of activating modifications facilitate an open chromatin structure ("euchromatin") where DNA is accessible to transcription factors, while patterns of repressive modifications lead to compaction of chromatin structure ("heterochromatin") that obstructs binding of the transcriptional machinery (Jenuwein and Allis, 2001).

The epigenetic code functions as a form of biological memory at the cellular level that directs both basal gene expression and stimulus/exposure-responsive gene induction based on the persistent epigenetic impacts of an individual's chemical and non-chemical environment. These patterns of epigenetic modifications are both inherited, mitotically and meiotically, and acquired as a result of intrinsic and extrinsic environmental factors. As a result, the epigenome acts as a biosensor of an individual's environment. The use of epigenetic data has the potential to refine traditional methods for identifying at-risk populations by providing a biomarker of how the cumulative impact of an individual's environmental history influences their response to future exposures. Although promising, this prospect has yet to be validated for practical application; however, it has fueled the rapid expansion of toxicoepigenetics research and led to the identification of novel putative links between environmental exposures, disease susceptibility, and public health (Bollati and Baccarelli, 2010; Cortessis *et al.*, 2012).

Early toxicoepigenetic studies have played a pivotal role in demonstrating that environmental factors can alter the epigenome thus revising the epigenetic language that regulates gene expression and susceptibility; however, these studies have typically focused on individual epigenetic markers, the equivalent of a single letter in an alphabet containing at least 130 characters. Although each of these studies provides a letter in this regulatory language, the complexity of the epigenome requires the incorporation of additional information to assemble a clear picture of how the environment shapes the regulation of gene expression and susceptibility to exposure-related disease. Unfortunately, this complexity also engenders significant technical and practical challenges to simultaneously examining multiple epigenetic markers within the regulatory regions of a range of genes. Traditional approaches to identifying susceptible populations consider intrinsic factors such as age, sex, and genotype. In recent years, greater consideration has been given

to the role of the aggregate effects of extrinsic exposures (referred to as the "exposome") in the alteration of physiological processes and susceptibility to disease. Although these individual approaches provide valuable insight into aspects of susceptibility, considering intrinsic and extrinsic factors separately does not faithfully reflect the effects of an individual's environment on health and disease susceptibility. Since both intrinsic and extrinsic factors (collectively referred to as "environmental factors") impact the epigenome, we proposed the "epigenetic seed and soil" model (Figure 2; adapted from McCullough *et al.*, 2016) as a conceptual framework that describes the cumulative effects of environmental factors on susceptibility and exposure-related disease by integrating their impact on the epigenome. Here we will review literature that demonstrates the effects of individual environmental factors on the epigenome and discuss how the epigenetic seed and soil model can be used to explain how the cumulative impact of environmental factors on the epigenome shapes exposure effects and susceptibility.

ENVIRONMENTAL FACTORS INFLUENCE THE EPIGENOME

Age

An individual's epigenome is constantly being reshaped throughout his or her lifetime by two related processes known as the epigenetic clock and epigenetic drift. Although epigenetic changes associated with the epigenetic clock are programmed, those associated with epigenetic drift result from the accumulation of errors in epigenome maintenance. Common trends in specific age-related DNA methylation changes across individuals have been described as the "epigenetic clock" (Wilson *et al.*, 1987; reviewed in Jones *et al.*, 2015). Although this epigenetic clock correlates with chronological age (Hannum *et al.*, 2013; Horvath *et al*, 2012; Horvath, 2013; Smith *et al.*, 2014), it advances more slowly in those with relatively "healthier" lifestyles and greater longevity (Gentilini *et al.*, 2013; Marioni *et al.*, 2015b) and accelerated epigenetic age is associated with toxic exposures, obesity, disease, and early mortality (Christiansen *et al.*, 2016; Faulk *et al.*, 2014; Horvath, 2013; Horvath *et al.*, 2014; Levine *et al.*, 2015; Marioni *et al.*, 2015a).

The age-dependent accumulation of changes in epigenetic modification states correlates with age-related changes in the expression of key metabolic enzymes, such as the cytochrome p450 (CYP) enzyme family (Giebel *et al.*, 2016; Li *et al.*, 2009) and may play a critical role in lifestage-dependent windows of susceptibility (de Magalhães *et al.*, 2009). The diversity and abundance of expressed CYPs changes as a function of age (Hakkola *et al.*, 1998; Parkinson *et al.*, 2004; Stevens *et al.*, 2003) and is an important determinant of inter-individual variability in xenobiotic metabolism (Hughes *et al.*, 1996; Johnson, 2003; Tréluyer et al., 2001). *CYP3A7* is highly expressed in the fetal liver, yet by two years of age its expression is barely detectable. Conversely, hepatic *CYP3A4* expression is low at birth but increases through childhood and plateaus by adulthood (Stevens *et al.*, 2003). Giebel *et al.* (2016) attributed the ontogeny of *CYP3A7 and 3A4* expression to changes in the balance of activating histone H3 lysine 4 trimethylation (H3K4me3) and repressive (H3K27me3) modification levels within the regulatory regions of these genes. The post-natal increase in *CYP3A4* expression corresponds to an increase in H3K4me3 abundance, and thus a shift in the balance of H3K4me3/H3K27me3 in favor of gene expression after birth. Similarly, the post-natal increase in H3K27me3 abundance at the *CYP3A7* locus in post-natal liver shifted the H3K4me3/H3K27me3 balance in favor of repression. This "bivalency", the co-occupancy of both activating and repressive epigenetic modifications, serves as a biological "switch" to regulate gene expression (described in Figure 1B). The age-dependent changes in the H3K4me3/H3K27me3 balance regulate the expression of specific metabolic enzymes during development and have significant consequences on xenobiotic metabolism, which are particularly well documented for pediatric use of pharmaceutical drugs (de Wildt, *et al.*, 1999).

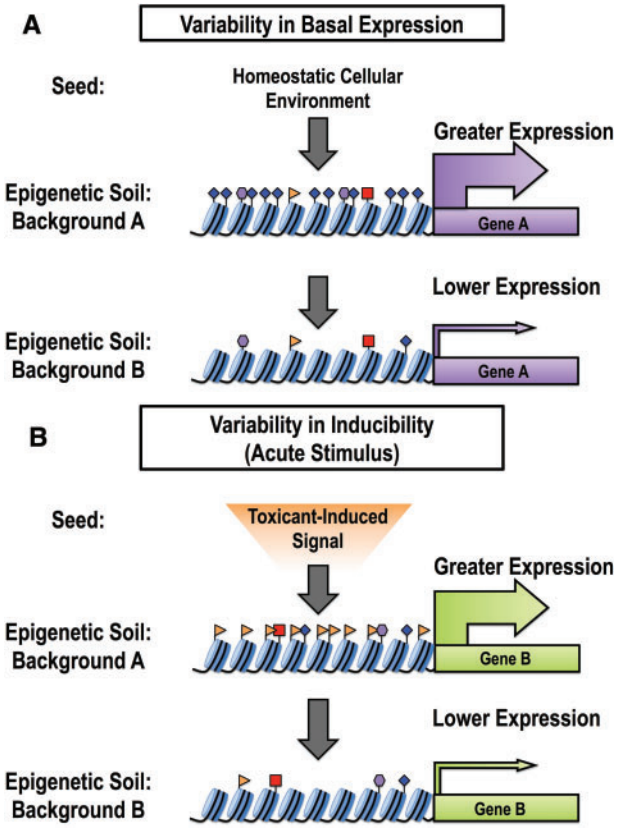

FIG. 2. The epigenetic seed and soil model. Individuals with different backgrounds, representing unique combinations of intrinsic and extrinsic factors, will have differing "epigenetic soil." The "seed" represents the cellular signaling arriving at a gene promoter, either homeostatic signaling or signaling arising from an acute stimulus (*eg*, a toxicant exposure). In more responsive individuals, the epigenetic soil is more receptive to the incoming seed, resulting in increased gene transcription. Increases in gene transcription may either occur at baseline (a result of homeostatic signaling) or in response to an acute stimulus.

Genetics

Genetic variation between individuals is often assessed by examining single nucleotide polymorphisms (SNPs). These variations in DNA sequence can have functional consequences on the epigenome and can be important arbiters of health and disease. SNPs within the regulatory regions of a gene, such as the promoter or enhancer, can influence its expression by altering CpG sites that are subject to DNA methylation or transcription factor binding sites. Further, SNPs within the protein-coding regions of epigenetic effector proteins—such as histone and DNA modifying-enzymes and transcription factors—can alter the epigenetic landscape across the epigenome by influencing binding and/or catalytic activity (Lemire *et al.*, 2015; Tehranchi *et al.*, 2016). As a result, genetic polymorphisms play a notable role in

shaping inter-individual variability in the epigenome (Gertz *et al.*, 2011).

Genome-wide association and quantitative trait loci (QTL) studies have identified important associations between SNPs and disease phenotypes; however, these traditional approaches often lack the ability to provide a mechanistic connection when identified SNPs are located in noncoding regions (Visscher *et al.*, 2012). The incorporation of epigenetic information into genetic analysis has provided insight into the role of SNPs in a range of disease studies (Gamazon *et al.*, 2013; Heyn *et al.*, 2014; Jaffe *et al.*, 2016; Liu *et al.*, 2013). Similar integrative approaches have been used to describe the role of non-coding SNPs in the modulating expression of susceptibility genes such as paraoxonase 1 (*PON1*), an arylesterase enzyme involved in lipid biodisposition, antioxidant defense, and the hydrolysis of organophosphate compounds. Individuals with reduced expression or activity of *PON1* are at an increased risk of a wide range of diseases including vascular diseases, metabolic syndrome, Alzheimer's disease, various cancers, and reduced capacity to metabolize xenobiotics (Camps *et al.*, 2009; Costa *et al.*, 2003; Furlong *et al.*, 2010). Of the various *PON1* polymorphisms, the SNP $PON1_{T\text{-}108}$ is the best predictor of gene expression; however, it is located in a non-coding promoter region and genetic analyses were not sufficient to identify the mechanism by which this SNP influenced *PON1* expression. By integrating epigenetic data, Huen *et al.* (2015) determined that $PON1_{T\text{-}108}$ was located in a CpG site and reduced *PON1* expression by increasing local DNA methylation. Subsequent studies demonstrated that $PON1_{T\text{-}108}$ disrupted the binding of the transcription factor specificity protein 1 (Deakin *et al.*, 2003; Osaki *et al.*, 2004) and reduced promoter activity, which led to increased local DNA methylation. These studies, demonstrate the capacity for genetic polymorphisms to impact the epigenome within the regulatory regions of genes that play important roles in the response to toxic exposures.

Sex

Males and females exhibit sex-specific expression of a wide range of genes, including metabolic enzymes, which impact both basic physiology and the response to environmental exposures (reviewed in Anderson 2005; Rademaker 2001; Soldin and Mattison, 2009; Tran *et al.*, 1998). Sexually dimorphic gene expression is largely a function of endocrine differences between males and females, especially in the liver where approximately 1000 genes, including many CYPs, exhibit sexually biased expression (Waxman and O'Connor 2006; Zhang *et al.*, 2011). Differences in the secretion of hormones, such as growth hormone (GH), control the expression of many transcription factors, particularly signal transducer and activator of transcription 5 (STAT5). The role of the GH-STAT5 axis, which has been implicated in the regulation of as many as 75–82% of hepatic sex-biased genes (Clodfelter *et al.*, 2006), was recently linked to sex-dependent differences in DNase hypersensitivity (a measure of chromatin accessibility), 6 histone modifications (activating H3K4me3, K27ac, K4me1, K36me3; repressive K27me3, K9me), and the binding of five GH-regulated transcription factors (Sugathan and Waxman, 2013). Enhancer regions of male-biased genes were enriched for the "pioneer" transcription factors forkhead box proteins A1 and/or A2 (FOXA1, FOXA2), which facilitate the opening of chromatin. Increased accessibility was followed by the recruitment of additional transcription factors such as STAT5 and deposition of activating histone modifications (H3K27ac and H3K4me1). Aberrant expression of female-biased genes in males was prevented by the enrichment of both the repressive histone modification H3K27me3 and the male-biased transcriptional repressor B-Cell CLL/Lymphoma 6 (BCL6). Female-specific gene expression was strongly driven by the transcription factor cut-like homeobox 2 (CUX2), which can interact with distinct factors to act as either a repressor or an activator. To activate female-biased genes, CUX2 functions cooperatively with STAT5 and FOXA2 to facilitate the removal of repressive H3K27me3 at gene enhancers. Conversely, CUX2 suppresses male-biased genes by binding enhancer regions to promote repressive chromatin structure. Although further studies are required to integrate the contributions of DNA methylation and other histone modifications, the epigenome plays a clear role in the sexually dimorphic gene expression.

Toxicant Exposures and Developmental Reprogramming

The aforementioned intrinsic factors are intractable qualities that influence an individual's susceptibility to toxicant exposure effects; however, environmental exposures also alter the epigenome and impact susceptibility and disease. These factors range from daily nutrition to overt toxicant exposures, but share the potential to alter the epigenome. Broadly speaking, these extrinsic factors can impact health through phenomena such as epigenetic carcinogenesis, regulation of inflammation, and developmental reprogramming, among others. Carcinogenesis is a frequently studied toxicological outcome, yet many carcinogens are not directly genotoxic. Although the underlying mechanisms have not yet been thoroughly described, the "epigenetic model of carcinogenesis" (Feinberg, 2004; Koturbash *et al.*, 2011) postulates that epigenetic changes at cancer-associated genes impair the normal cellular mechanisms that prevent carcinogenic transformation. This model is supported by data demonstrating that exposure to tobacco smoke, benzene, arsenic, or nickel (reviewed in Koturbash *et al.*, 2011; Ray *et al.*, 2014) alters the epigenome at cancer-associated genes such as *p53*, *p16*, and Ras association domain family member 1 (*Rassf1*), making exposed cells more susceptible to subsequent carcinogenic stimuli. Similarly, exposure to a range of air pollutants alters the epigenome and expression of genes that are critical in regulating the balance between host defense and inflammatory disease (Bellavia *et al.*, 2013; Bind *et al.*, 2014; Madrigano *et al.*, 2012; Nadeau *et al.*, 2010). Further, epigenetic modifications play key roles in modulating responses to multiple exposures, such as inflammatory adaptation and priming (Foster *et al.*, 2007; Gazzar *et al.*, 2007). Many further examples exist and each theme could constitute its own review; however, here we will focus on endocrine disruptor exposure studies because they offer some of the most promising evidence for the potential use of the epigenome as an indicator of long-term susceptibility due to the persistent reprogramming that results from exposure. The studies discussed below provide examples of how multiple lines of evidence can be integrated to assemble a more complete understanding of the impacts of environmental exposures on the epigenome. Further, the studies by Bredfeldt *et al.* (2010), Greathouse *et al.* (2012), and Jefferson *et al.* (2013) adopt a target gene-centered approach to evaluating the relationship between exposure-induced epigenetic changes and the alternative regulation of outcome-associated genes.

Developmental Reprogramming

Exposures that occur during sensitive periods of epigenetic remodeling, such as during pregnancy and early childhood, have

been shown to reprogram the epigenome and predispose offspring to diseases later in life. Strong associations between developmental exposures, the epigenome, and adult disease come from early exposure to endocrine disrupting chemicals (EDCs). Endogenous hormones, such as estrogen, play an integral and carefully choreographed role in regulating gene expression programs that are critical for normal development and reproductive function. These processes can be disrupted and reprogrammed by early exposure to exogenous estrogen mimetic compounds (xenoestrogens), such as the synthetic estrogen diethylstilbestrol (DES), dietary phytoestrogen genistein, and the ubiquitous consumer goods plasticizer, bisphenol-A (BPA). Although DES was removed from the American pharmaceutical market due to adverse health effects from *in utero* exposures, BPA and genistein are commonplace in the developed world. Unlike analogous adult exposures, DES, BPA, and genistein reprogram hormone-responsive gene expression and increase the incidence of uterine abnormalities and neoplasia in rodent developmental exposure models (Greathouse *et al.*, 2008; Jefferson *et al.*, 2013; Li *et al.*, 1997; Markey *et al.*, 2005; Murray *et al.*, 2007; Newbold *et al.*, 2007, 2012; Suen *et al.*, 2016; Wang *et al.*, 2014). This reprogramming is thought to occur through various changes in DNA methylation and histone modifications (reviewed in Walker, 2011), which alter both basal gene expression and hormone-dependent gene induction. These changes in transcriptional programs are thought to underlie developmental abnormalities (Jefferson *et al.*, 2011) and the development of neoplasia later in life, especially after the initiation of menses in female mice when endogenous estrogen levels increase.

To explore the relationship between xenoestrogen exposure, neoplasia, and the epigenome, Bredfeldt *et al.*, (2010) and Greathouse *et al.*, (2008, 2012) modeled developmental DES, genistein, and BPA exposure in the Eker rats, a strain that is predisposed to a common hormone-responsive uterine tumor known as leiomyoma (Walker and Stewart, 2005). Although the transcriptional reprogramming induced by all 3 EDCs required estrogen receptor-α (ERα), only DES and genistein induced activation of "pre-genomic" ERα signaling through the PI3K/Akt kinase pathway. The resulting phosphorylation and inactivation of the histone methyltransferase enhancer of zeste homolog 2 (Ezh2) led to a global reduction in abundance of the repressive histone modification H3K27me3 in the uteri of DES- and genistein-exposed animals. Unlike DES and genistein, BPA exposure increased global abundance of H3K27me3 and did not increase the incidence of leiomyoma, despite being associated with reproductive anomalies and other types of neoplasia in other studies (Murray *et al.*, 2007; Newbold *et al.*, 2007; Wang *et al.*, 2014). As the catalytic component of the histone-modifying polycomb repressive complex 2 Ezh2 plays a critical role in embryonic development, differentiation, and the control of bivalent gene promoters. Although the mechanisms through which different xenoestrogens exert their effects on the epigenome varies, dysregulation of key regulatory histone modification, such as H3K27me3, is likely to play an integral role in developmental reprogramming.

A similar study in mice demonstrated that developmental exposure to DES reprogrammed expression of a range of histone modifying enzymes (Hdac1, Hdac2, Hdac3, Kat2a, Kat2b, Myst2, and Kmt2b), DNA methyltransferases (Dnmt1 and Dnmt3a), and a methylcytosine dioxygenase (Tet1) (Jefferson *et al.*, 2013). These changes in epigenetic modification enzymes coincided with alterations in the abundance of the activating histone modifications H3K9ac, H4K5ac, and H3K4me3 within the regulatory regions of cancer-associated genes that were permanently up-regulated following developmental genistein exposure (Suen *et al.*, 2016). By examining multiple epigenetic aspects these studies provide a more comprehensive perspective on the mechanisms responsible for the xenoestrogen-induced persistent reprogramming of gene expression and associated increase in developmental abnormalities and cancer susceptibility later in life.

CUMULATIVE EFFECTS OF ENVIRONMENTAL FACTORS ON THE EPIGENOME

Relating Cumulative Epigenetic States to Exposure Outcomes and Susceptibility

Unlike traditional approaches that rely on discrete factors such as age, genotype, and disease state, using the epigenome as a biomarker has the potential to provide a more comprehensive perspective on susceptibility by integrating the cumulative impacts of environmental factors. An individual's epigenome is composed of patterns of DNA methylation and histone modifications that are both inherited and acquired as a result of intrinsic and extrinsic environmental factors. Due to practical limitations, the influence of environmental factors on the epigenome is typically studied individually; however, translating their impact on exposure-mediated disease requires consideration of the cumulative impacts of environment on the epigenome. We recently hypothesized that baseline epigenetic modification states, an "epigenetic snapshot" in time reflecting the cumulative influences of intrinsic and extrinsic factors on the epigenome, could predict both basal and toxicant-induced gene expression (McCullough *et al.*, 2016). To test this hypothesis we compared the relative baseline (pre-exposure) abundance of specific epigenetic modifications in gene promoters with the basal and pollutant-induced (ozone) expression of target genes in a panel of donors using a primary bronchial epithelial cell air-liquid interface exposure model. We found that distinct epigenetic signatures were associated with the magnitude of basal and ozone-induced expression. Although not encompassing all epigenetic modifications or genes, our findings demonstrate that cumulative epigenetic states correlate with both basal and toxicant-induced gene expression.

The Seed and Soil Model

Here we expand upon our previously proposed "epigenetic seed and soil model" (McCullough *et al.*, 2016), a conceptual framework that describes the cumulative effects of environmental factors on susceptibility and exposure-related disease by integrating the cumulative impact of environmental exposures on the epigenome (Figure 2). In this model, the "seed" represents incoming cellular signal (either homeostatic or toxicant-induced) and the "soil" represents the mosaic of epigenetic modifications within the regulatory region of a given gene. The epigenetic "soil" influences gene expression by either altering basal gene expression or modulating the magnitude of gene induction in response to an acute stimulus. In the first scenario, environmental factors shape the epigenome within the regulatory region(s) of a gene, which alters basal expression by reprograming its response to normal homeostatic signals. In the second scenario, environmentally mediated epigenetic changes reprogram how inducible a gene will be in response to an acute stimulus, such as toxicant exposure. If the cumulative effects of intrinsic and extrinsic forces result in a more receptive epigenetic soil (ie, contains modifications favoring gene

TABLE 1. "Seed and Soil Table"

Authors	Intrinsic or Extrinsic Factor	Driver	Epigenetic Soil	"Seed" ('–'indicates homeostatic signaling)	Functional Outcome	Health and Disease Implications	Expression change: Basal (B) Induced (I)
Bredfeldt *et al.* (2010), Greathouse *et al.* (2008, 2012)	Extrinsic (rat)	Neonatal exposure: DES, BPA, genistein	Genistein and DES activated the PI3K/AKT pathway, inhibiting EZH2 via phosphorylation thereby reducing global H3K27me3; BPA increased H3K27me3	— (Endogenous estrogen)	BPA, Genistein and DES reprogrammed both basal and estrogen-induced gene expression	Uterine neoplasia	B, I
Burdge *et al.* (2007); Lillycrop et al. (2008)	Extrinsic (mice)	Maternal protein-restricted diet	*Pparα*, *Gr* promoter hypomethylation; altered expression	—	Metabolic phenotype; increased *Gr*, *Pparα* expression	Metabolic syndrome and associated diseases	B
Cui et al. (2006)	Extrinsic (mice)	Arsenic	Hypomethylation of *Rassf1a* and *p16*	—	Reduced gene expression found in lung tumors	Carcinogenesis	B
Dave *et al.* (2015)	Baseline (human)	Baseline	Altered methylation of *PPARG1* and *ADIPOR1*	—	Altered expression of *PPARG1* and *ADIPOR1*	Obesity; Metabolic syndrome	B
Foster *et al.* (2007)	Extrinsic (*in vitro* human)	LPS	Different patterns of H4Ac and H3K4me3 at genes that show LPS tolerance (pro-inflammatory genes) and those that do not (antimicrobial genes)	Secondary LPS exposure	Reduced pro-inflammatory gene expression and increased antimicrobial gene expression	Inflammation; Host defense	I
Giebel *et al.* (2016)	Intrinsic (human)	Age	In postnatal liver, alteration at bivalent promoters: enrichment of repressive H3K27me3 at *CYP3A7*; less H3K27me3 enrichment at *CYP3A4* promoter	—	CYP ontogeny; predominant expression of fetal form (*CYP3A7*) switches to adult form (*CYP3A4*)	Adverse drug reactions	B
Ho *et al.* (2006), Tang *et al.* (2012)	Extrinsic (rat; *in vitro* rat)	Neonatal estradiol, BPA	Hypomethylation of *Pde4d4*; hypomethylation of *Nsbp1*; hypermethylation of *Hpcal1*	— (xenoestrogen exposures)	Continual elevated expression of *Pde4d4* demonstrated to precede pathological change; also noted aberrant expression of epigenetic regulators	Prostate cancer	B
Huen *et al.* (2015)	Intrinsic (human)	Genome	Promoter polymorphisms→ Differential methylation of *PON1*	—	Reduced expression of PON1	Various diseases; reduced ability to metabolize certain drugs and pollutant exposures	B
Lemire *et al.* (2015)	Intrinsic (human)	Genome	meQTLs: SNPS associated with methylation of CpGs; Various effects	—	Various effects	Various health implications	B, I

(Continued)

TABLE 1. (continued)

Authors	Intrinsic or Extrinsic Factor	Driver	Epigenetic Soil	"Seed" ('–'indicates homeostatic signaling)	Functional Outcome	Health and Disease Implications	Expression change: Basal (B) Induced (I)
Li *et al.* (2009)	Intrinsic (mouse)	Age	Alterations in H3K4me2 and H3K27me2 in hepatic gene promoters at various developmental ages	—	CYP ontogeny: Increased H3K4me2 in neonates and adults leads to increases in Cyp3a16 and Cyp3a11 expression, respectively. Suppressed expression of neonatal form (Cyp3a16) in adults corresponded with increases in H3K27me3 and reduction of H3K4me2	Adverse drug reactions	B
McCullough *et al.* (2016)	Baseline (*in vitro* human)	Baseline	Baseline chromatin motifs at ozone-responsive genes	Ozone	H3K4me3, H3K27me, H4Ac are associated with inducibility of *HMOX-1*, *COX2*. Basal expression of *IL-8* and *IL-6* are related to H4Ac and H3K4me3, among others.	Susceptibility to ozone exposure	B, I
Nadeau *et al.* (2010)	Extrinsic (human)	Ambient air pollution	Hypermethylation of *FOXP3*	—	Altered *FOXP3* expression; Altered T-Reg Cell function	Asthma	B
Rojas *et al.* (2015)	Extrinsic (human)	Pre-natal arsenic exposure	Altered DNA methylation patterns in six genes	—	Genes with altered methylation patterns also have altered expression. Associated with birth outcomes: gestational age, placental weight, head circumference	Pregnancy complications; Arsenic-associated diseases	B
Salam *et al.* (2012)	Extrinsic and Intrinsic (human)	Genome: *NOS2* promoter haplotype	Differential methylation status	PM Exposure	Haplotype interacts with exposure to dictate the level of *NOS2* detected in exhaled NO	Lung inflammation and pulmonary disease	I
Sugathan and Waxman (2013)	Intrinsic (mouse)	Sex	The regulatory regions of sex-biased genes have particular chromatin landscapes characterized by DNAse hypersensitivity, histone modifications, and the binding of GH-mediated transcription factors	—	Approximately 900 genes in the liver exhibit sexually dimorphic gene expression	Sex-based differences in drug pharmacokinetics	B, I

(Continued)

TABLE 1. (continued)

Authors	Intrinsic or Extrinsic Factor	Driver	Epigenetic Soil	"Seed" ('–'indicates homeostatic signaling)	Functional Outcome	Health and Disease Implications	Expression change: Basal (B) Induced (I)
Susiarjo *et al.* (2013)	Extrinsic (mouse)	BPA	Altered methylation at six imprinted genes	—	Altered expression of imprinted genes in placenta; abnormal placental development	Fetal and post-natal health; imprinting disorders	B
Tang *et al.* (2012)	Extrinsic (human *in vivo*; *in vitro*)	PAH (BaP)	Hypermethylation of *IFNγ*	—	Reduced expression of *IFNγ in vitro*; Association between *IFNγ* methylation and PAH exposure *in vivo*	Dysregulation of T-cell response-asthma	B
Tehranchi *et al.* (2016)	Intrinsic	Genotype	Altered TF binding and chromatin architecture	Various effects	Various effects	Various health implications	B, I
Tserel et al. (2015)	Intrinsic (human)	Age	Altered methylation of genes involved in T-cell immune responses and differentiation	—	Expression changes in genes related to T-cell function	"Inflamm-aging;" Impaired T-cell function	B
Zeybel *et al.* (2012)	Extrinsic (rat)	CCl_4 (fibrogenic hepatotoxin)	Altered DNA methylation at *Pparγ* and *Tgfβ1*; Enriched H2A.Z and H3K27me3 at *Pparγ* locus in sperm	Subsequent CCl_4 exposure in F1-3 generations	Altered expression of *Pparγ* and *Tgfβ1*; suppressed fibrotic response in offspring with ancestral liver fibrosis	Liver fibrosis-carcinogenesis	B

Selected studies linking epigenetic alterations with functional changes and health outcomes.

transcription) then the stimulus induced-signal will be robust; however, a less-receptive epigenetic soil will result in modest induction. Thus the seed and soil model serves as a streamlined approach to conceptualizing the functional consequences resulting from the cumulative impact of environment-induced epigenetic changes with respect to health, exposure effects, and susceptibility (examples given in Table 1).

CONCLUSIONS AND FUTURE DIRECTIONS

Toxicoepigenetics is a rapidly emerging field of study that has made great advances in associating a broad range of environmental exposures with changes to the epigenome. The impact of toxicoepigenetic studies within the basic science and risk assessment communities will continue to grow as they evolve to include a broader range of epigenetic modifications to develop a more comprehensive understanding of how environmental factors shape the epigenome and thus exposure effects and susceptibility. Further, the identification of more complete epigenetic susceptibility profiles will provide targets to facilitate the exploration of causal links between environmental factors, the epigenome, and health outcomes. The application of toxicoepigenetic data will rely heavily on forthcoming studies bridging current knowledge gaps (Figure 3), which will involve addressing the following:

1. DNA methylation and histone modifications are often studied independently; however, future studies will benefit from the integration of data related to both types of epigenetic modifications due to their concerted roles in regulating gene expression (Roadmap Epigenetics Consortium, 2015). Further, current high-throughput methods for analyzing global DNA methylation do not distinguish between 5-methylcytosine and its relatively abundant oxidation product 5-hydroxymethylcytosine, which can play an opposing role in gene regulation. Future studies would benefit from the incorporation of methods that distinguish the contributions of these two types of DNA methylation with divergent functions.
2. Most toxicoepigenetics studies have examined the relationship between epigenetic states and basal gene expression; however, this approach may overlook inducible genes that also play critical roles in response to external insults. The inclusion of both of these types of gene expression will give a more comprehensive perspective on how differences in epigenetic states shape exposure effects and susceptibility. Current studies typically examine the effects of environmental exposures on the epigenome at a single dose and time. The expansion of these studies to include a range of doses and exposure durations will facilitate the identification of threshold doses and response times.
3. The majority of toxicoepigenomic studies have succeeded in observing associations between environmental factors, epigenetic changes, and health outcomes. The impact of these studies will be increased by further studies that directly assess causal relationships between these factors (Figure 3) (Birney *et al.*, 2016).
4. High-throughput data have been instrumental in generating hypotheses regarding the role of the epigenome in exposure effects and susceptibility. The utility of these data will be increased by complimentary studies that test the hypotheses generated through focused approaches that determine whether the identified epigenetic states are causative of associated health outcomes.
5. Controlling for cell populations, especially in blood samples, is a major complicating factor as inadvertently measuring different proportions of cell types could mislead the identification of environmentally induced epigenetic changes (Reinius *et al.*, 2012). Ideally, cell sub-types should be separated by biochemical or immunologically based techniques prior to epigenetic analysis to avoid this potential flaw in the information obtained; however, when not possible (eg, when using previously stored samples), the development, validation, and application of emerging *post-hoc* computational methods, such as those described by Houseman *et al.* (2012), may allow for the interrogation of cell type specific epigenetic changes in samples that contain mixtures of cell types.

This review has provided a brief overview of how intrinsic and extrinsic factors can influence the epigenome and thus modulate exposure effects and disease susceptibility; however, we have only described a subset here. This rapidly evolving field has the potential to fundamentally change our understanding of the mechanisms underlying exposure effects and how cumulative environmental history shapes susceptibility. Reaching this potential will require continued innovation by

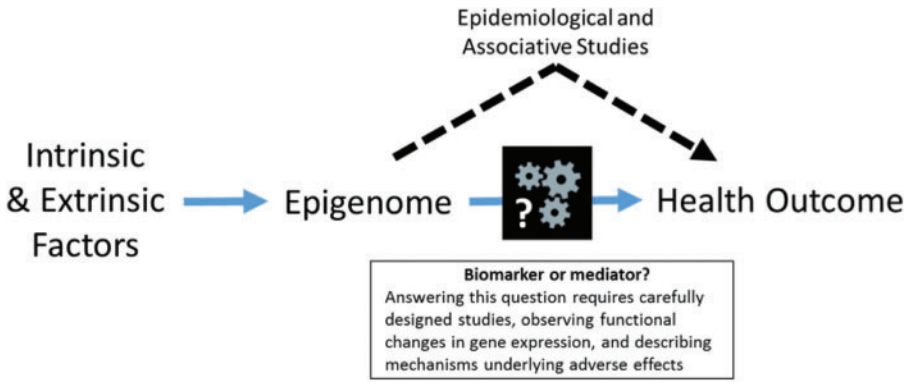

FIG. 3. Need for causal in addition to associative evidence in defining the relationship between epigenetic state, exposures, and health outcomes. The majority of current epigenetics studies in public health demonstrate that intrinsic or extrinsic forces shape the epigenome, or describe associations between the epigenome and disease. Based on this information it is difficult to distinguish whether the epigenome has a role in disease development or is a biomarker of effect. In order to link the epigenome with health outcomes, it is necessary to implement study designs that may be able distinguish these roles. In addition to high-throughput screening, additional experiments should identify functional changes in gene expression and how these changes lead to the resulting outcome or phenotype.

researchers to overcome both technical and scientific challenges to definitively define causal roles for the epigenome in exposure-related outcomes. Doing so will ultimately allow for validation of the utility of epigenetic endpoints as indicators of exposure, modulators of susceptibility, and predictors of adverse health effects.

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