

The Use of Animals Models to Advance Epigenetic Science

Dana C. Dolinoy and Christopher Faulk

Changes in the epigenome induced by the environment have been documented in diverse animal phyla, ranging from insects to rodents to humans. These include chromatin remodeling, histone tail modifications, and DNA methylation, and more recently the list has expanded to encompass noncoding RNA and microRNA gene regulation (Matzke and Birchler 2005). Thus, it is increasingly recognized that exposure to chemical, nutritional, behavioral, and physical factors alters gene expression and affects health and disease not only through mutation of but also through modification of the epigenome. Moreover, such exposures have been directly linked with subsequent disease formation through deregulation of epigenetic mechanisms. Unlike genetic mutations, these epigenetic changes are potentially reversible, providing a unique avenue to improve human health. Consequently research in epigenetics has increased dramatically in the last few years (Figure 1).

The term “epigenetics” was popularized in the early 1940s by developmental biologist Conrad Waddington (1940) to explain “the interactions of genes with their environment, which bring the phenotype into being.” In the 1970s, Holliday and Pugh (1975) first proposed covalent chemical DNA modifications, including methylation of cytosine-guanine (CpG) dinucleotides, as the molecular mechanism to explain Waddington’s hypothesis. The revelations several decades later that X inactivation in mammals and genomic imprinting are regulated by complex and multifactorial mechanisms (Monk 1988; Willard et al. 1993) resulted in an updated definition, describing epigenetics as heritable changes in gene expression that occur without a change in DNA sequence, including the modification of DNA methylation and chromatin remodeling (Wolfe and Matzke 1999). The genomics revolution inspired the investigation of genome-wide rather than local gene analyses, and the term “epigenomics” was

coined as the study of the “effects of chromatin structure including the higher order of chromatin folding and attachment to the nuclear matrix, packaging of DNA around nucleosomes, covalent modifications of histone tails (acetylation, methylation, phosphorylation, ubiquitination), and DNA methylation” (Murrell et al. 2005). Finally, evidence that demonstrated the resistance of certain gene loci to methylation reprogramming during embryogenesis revealed that epigenetic modifications can be inherited not only mitotically but also transgenerationally (Lane et al. 2003; Morgan et al. 1999; Rakyan et al. 2003).

DNA methylation is the most widely studied form of epigenetic modification and occurs within the one-carbon metabolism pathway, which is dependent upon several enzymes in the presence of micronutrient cofactors, including folate, choline, and betaine derived through the diet. In mammals, DNA methylation is primarily a stable repressive mark found at cytosines in CpG dinucleotides; however, its regulation is more dynamic than previously believed (Maunakea et al. 2010). For example, recent evidence for methylation of non-CpG cytosines in human embryonic stem cells suggests that methylation at non-CpG sites may be important to developmental homeostasis (Lister et al. 2011). It has been documented that CpG dinucleotides are greatly underrepresented in mammalian genomes because of spontaneous deamination of 5-methylcytosine to thymine and subsequent fixation in a population over evolutionary timescales (Holliday and Grigg 1993). Thus, the majority of unmethylated CpG sites occur within CpG islands, defined as

Dana C. Dolinoy, MSc, PhD, is the John G. Searle Assistant Professor of Environmental Health Sciences, and Christopher Faulk, PhD, is a research fellow in the Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor.

Address correspondence and reprint requests to Dr. Dana C. Dolinoy, Department of Environmental Health Sciences, School of Public Health, University of Michigan, 1415 Washington Heights, Ann Arbor, MI 48109-2200 or email ddolinoy@umich.edu.

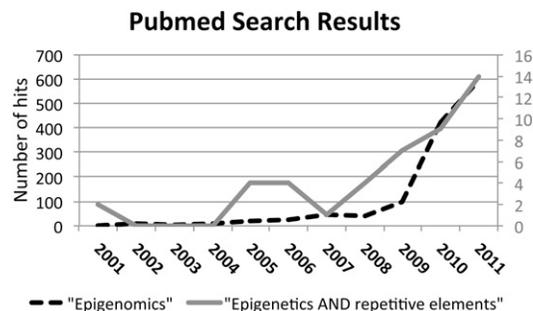


Figure 1 Literature search results show increasing interest, albeit at different growth rates, in both epigenomics (left axis) and repetitive elements (right axis) over time.

discreet regions containing a preponderance of CpG content (Deaton and Bird 2011). The resulting uneven distribution of CpG islands is thought to result from uniform genomic CpG site deamination and conversion coupled with the regeneration of new CpG islands found in repetitive elements with expansion by retrotransposition (Xing et al. 2004). Normally, CpG islands are located within or near gene promoters or in the first exons of housekeeping genes. In contrast, the body and regulatory elements of repetitive DNA sequences, such as transposable elements, are methylated, consequently inhibiting the parasitic transposable and repetitive elements from replicating by transcription. Of important note, however, not all animals use DNA methylation as a gene repression mechanism; for example, the model organisms fruit fly (*Drosophila melanogaster*) and roundworm (*Caenorhabditis elegans*) lack appreciable DNA methylation, whereas other insects and nematodes do retain DNA methylation machinery (Gutierrez and Sommer 2004; Maleszka 2008).

Epigenetic manipulation of cellular phenotype is also driven by alteration of chromatin structure by covalent histone modifications and incorporation of histone variants into the nucleosome (Saha et al. 2006). Chromatin is a nucleoprotein complex that packages linear genomic DNA by means of an array of nucleosomes. Each nucleosome consists of about 147 base pairs of DNA coiled around an octamer of histone proteins. Each octamer contains two copies each of the four core histones, H2A, H2B, H3, and H4. Chromatin may be further modified by association with linker histones, histone variants, and nonhistone proteins as well as myriad posttranslational modifications of histone proteins, including histone acetylation, methylation, ubiquitination, phosphorylation, and ADP-ribosylation (Caiafa and Zampieri 2005; Cheung and Lau 2005). Histone acetylation is usually associated with transcriptional activation because the affinity of histone proteins for DNA is reduced and chromatin packaging is relaxed. Histone methylation results in various transcriptional consequences depending on histone number and the lysine residue modified (Kouzarides 2007). Each lysine residue may be methylated in the form of mono-, di-, or trimethylation, adding enormous complexity to the histone code (Jenuwein and Allis 2001).

Furthermore, histone modifications interact with DNA methylation patterns to recruit multi-subunit chromatin–protein complexes, adding yet another layer of complexity to epigenetic gene regulation. For example, in this issue, Kim and Kim (2012) examine protein complexes affecting epigenetic mark placement. Two histone marks in particular, H3K27 trimethylation and H3K9 trimethylation, are well-characterized repressive chromatin marks important in genic and nongenic regions of the metazoan genomes, but the mechanisms by which these marks are targeted are not wholly understood. Herein Kim and Kim provide evidence that in mammals H3K27 and H3K9 trimethylation mark distinct regions of the genome, whereas the repressive polycomb repressive complex 2 histone-modifying complex works in concert with DNA-binding proteins such as JARID2, AEBP2, and YY1 to target histone modifications. Specifically, deep sequencing

approaches, including chromatin immunoprecipitation–sequencing, are employed to evaluate the genome-wide distribution of histone modification marks in mammals.

Vulnerable Time Points

DNA methylation and other epigenetic patterns are prone to change throughout the life course, especially during reprogramming events associated with normal development and aging (Fraga et al. 2005; Hajkova et al. 2002; Martin 2005). For example, the epigenome is particularly dynamic during embryogenesis because of extensive DNA synthesis, and the elaborate DNA methylation patterning required for normal tissue development is established during early development (Faulk and Dolinoy 2011). As individuals age, gradual DNA hypomethylation occurs at the genome-wide level, concurrent with locus-specific promoter increases in DNA methylation at normally unmethylated CpG islands, leading, for example, to genome instability or gene-specific suppression, respectively (Mugatroyd et al. 2010). Additionally, compared with normal tissue, cancer is often associated with hypomethylated DNA and notable hypermethylation of tumor suppressor genes (Feinberg 2007). These reprogramming events throughout the life course result in tissue-specific DNA methylation patterning (Hajkova et al. 2002; Reik et al. 2001). Differences in these epigenetic patterns are important to cellular differentiation and tissue homeostasis.

The developmental origins of health and disease hypothesis posits that increased susceptibility to disease after early life experiences is shaped by epigenetic modifications such as DNA methylation and chromatin modifications (Bateson et al. 2004; Gabory et al. 2011). In this issue, Ganu and colleagues (2012) describe diverse approaches for investigating epigenetic marks as a mechanism linking early origins to adult disease in rodent models, nonhuman primates, and humans. Focusing on both in utero constraint (i.e., famine) and overabundance (i.e., high-fat and caloric-dense diets), they review recent and provocative data supporting a role for histone modifications in particular to mediate the effects of early experiences and adult metabolic disease. As an alternative approach, Seelan and colleagues (2012) focus on a specific time period of vulnerability linked to epigenetic mechanisms. Orofacial clefts occur in approximately 1 to 2 of every 100 live births and are associated with a complex etiology involving both genetic and epigenetic mechanisms. Specifically, they review the literature supporting the hypothesis that the early embryonic palatal methylome, transcriptome, and repertoire of microRNAs act in concert, resulting in normal orofacial ontogeny, which, when deregulated, can lead to secondary palate defects.

Nutritional and Environmental Epigenetics

Nutri-epigenomics is an emerging discipline examining the role of dietary influences on gene expression. Ultimately,

DNA methylation and other epigenetic events, as well as dietary practices, particularly micronutrient intake, may influence disease phenotypes. We have previously highlighted the importance of an interspecies approach to synthesize the existing nutri-epigenomic literature to identify sensitive periods throughout the life course where diet may substantially alter epigenetic marks (Anderson et al. 2012). Now, Niculescu (2012) puts forth the intriguing platform that, through comprehensive investigation of varying levels of nutrient exposure during vulnerable time points, researchers can grasp the magnitude and degree of impact that each nutrient has on one-carbon metabolism and, subsequently, DNA methylation and other epigenetic events. Focusing on life-course environmental exposures, Ho and colleagues (2012) characterize timing, dose, duration, and chemical composition and important factors leading to epigenetic consequences affecting disease risk. These epigenetic “memories,” once elucidated, can serve as important biomarkers for not only chemical risk assessment and historical exposure but also identification of individuals at risk for future disease.

Behavioral and Social Epigenetics

Behavioral- and stress-induced epigenetic alterations are widespread from insects to mammals. For example, the desert locust, *Schistocerca gregaria*, produces more offspring of the gregarious swarming phenotype when breeding in crowded conditions (Maeno and Tanaka 2010), and the pea aphid, *Acyrtosiphon pisum*, when under stress from crowded conditions or predators, will produce more winged offspring (Weisser et al. 1999), both of which are hypothesized to be linked to epigenetic adaptations. Similarly, rodents exhibit persistent DNA methylation alterations of the glucocorticoid receptor and many other loci in the hippocampus associated with high versus low levels of maternal grooming in the first week of life (McGowan et al. 2011). Herein, Jašarević and colleagues (2012) focus on sexually selected traits, including female choice and male–male competition, as a fundamental conceptual framework to best assess behavioral epigenetics. They propose an expansion to the traditionally used model organisms to capture a wider range of behavioral modification in regards to mate choice. Because sexually selected behaviors are programmed during early embryonic and postnatal development by means of endogenous hormone exposure and because xenobiotic endocrine-disrupting chemicals such as bisphenol A have been shown to affect the fetal epigenome, this provocative approach may help elucidate the origins of steroid-induced epigenetic programming. Also in this issue, Gudsnuk and Champagne (2012) examine animal models of early-life stress and social experience across the lifespan, focusing on laboratory rodents and the associations among epigenetic marks and prenatal stress, maternal separation, maternal care, abusive caregiving, and social stress. The importance of stress in mediating the effects of early environmental exposures is also discussed.

Diseases of Epigenetic Origins

Epigenetic systems in mammals may have developed as a consequence of totipotency and the need to activate genes in only certain cell types despite the fact that all cells share the same genetic components (Jablonka and Lamb 2002). One of the most extensively studied epigenetic phenomena in mammals is genomic imprinting, in which one parental allele is epigenetically altered, resulting in parent-of-origin modification of gene transcription (Murphy and Jirtle 2003; Reik and Walter 2001). Abnormal developmental expression of imprinted genes results in a number of severe pediatric disorders, such as Prader-Willi syndrome, Angelman syndrome, and Beckwith-Wiedemann syndrome, and is suspected to play a role in many neurological disorders (Murphy and Jirtle 2003). Herein, Skaar and colleagues (2012) review emerging evidence supporting alterations in the epigenome as important contributory or causative roles in human disease. Focusing on the transition from animal models to human investigation, they examine numerous epigenetic mechanisms regulating the “imprintome” and advocate for the systematic identification of the full human imprintome using emerging technologies.

Although numerous disease phenotypes have been associated with epigenetic etiology, including metabolic syndrome and obesity, neurologic dysfunction and carcinogenesis remain two of the most actively studied diseases of epigenetic origins. In this issue, Schaevitz and Berger-Sweeney (2012) focus on the roles of nutrition and epigenetics in autism and autism spectrum disorders. They focus on the role of one-carbon metabolism and the important cofactors driving this pathway, including methyl donors, such as folate, and vitamins, such as essential B vitamins (e.g., riboflavin). Similar to autism spectrum disorders, cancer is a heterogeneous disease, displaying both genetic and epigenetic etiologies as well as inconsistent methylation profiles; however, in general, the epigenome is widely hypomethylated compared with normal tissue, with notable hypermethylation of tumor suppressor genes (Feinberg 2004). Virani and colleagues (2012) explore animal models of specific pathways of carcinogenesis as critical to understanding mechanisms and discuss the integration of laboratory and epidemiologic approaches as a cogent approach to best translate data to human clinical and population approaches to better prevent and treat cancer. Both Schaevitz and Berger-Sweeney and Virani and colleagues stress that if nutritional or environmental factors play a critical role in altering epigenetic marks and predisposing individuals to disease, animal models will be invaluable in identifying prevention and treatment options to reduce or eliminate disease.

Animal Ethics Considerations Related to Animal Models of Epigenetics

The use of animals is critical to understanding the mechanisms of epigenetics and central to this issue of the *Journal*. Animal welfare is forefront in the mind of laboratory workers as they seek to minimize their use while at the same time

maximize the irreplaceable epigenetic and other biologic data resulting from their use in research. Harris (2012) provides thoughtful insight into institutional animal care and use committees' (IACUCs) perspectives on the use of animal models. Of particular note is the rapid emergence of this field over the last one to two decades. Harris explains that the "dynamic epigenome and the many epigenetic mechanisms that regulate phenotypic expression stand poised to attract the causal blame for many of the diseases, health disparities, and abnormalities now existing in living organisms." Harris focuses on the role of epigenetic mechanisms in the developmental origins of disease and hence the ethical considerations surrounding observing an animal across the entire lifespan. Further, as indicated in this brief perspective, a number of factors contribute to epigenetic dysregulation, and IACUCs must make important decisions about the types of stimuli employed to induce modifications to the epigenome. This article should be a useful perspective for not only researchers but also IACUC members.

Concluding Thoughts on the Value of Animal Models in Epigenetic Research and the Translation to Human Clinical and Population Approaches

To ultimately succeed in identifying the role of epigenetic mechanisms leading to complex phenotype and disease, researchers must integrate the various animal models, human clinical approaches, and human population approaches, paying attention to the times of sensitivity and model system of evaluation. As highlighted above, it is increasingly recognized that chemical, nutritional, behavioral, social, and physical factors alter gene expression and affect health and disease by not only mutating promoter and coding regions of genes but also modifying the epigenome. The use of animal models in these investigations has informed the fields of molecular biology and toxicology by elucidating the mechanisms underlying developmental exposure and adult disease. Candidate gene approaches have recently been enhanced by concomitant whole epigenome technologies. Thus, the evaluation of epigenetic mechanisms in health and disease is now poised for enhanced investigation in animal models as well as expansion into clinical and population health approaches. Animal models will continue to help inform the evaluation of vulnerable time periods and multigenerational studies that are not feasible in human populations. Additionally, the epigenome, in contrast with the genome, is particularly affected by cell-type specificity. Thus, animal model studies, in which cell type specificity is more readily evaluated than in humans, can serve as important proof-of-principle approaches to evaluate the use of peripheral tissue (e.g., blood, saliva) in human epigenetic epidemiology studies. Ultimately, to fully succeed in elucidating epigenetic mechanisms underlying disease susceptibility, researchers must integrate animal models and human approaches to generate the best prescriptions for human health evaluation and disease prevention.

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