Epigenetic Epidemiology of the Developmental Origins Hypothesis

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Annu. Rev. Nutr. 2007. 27:363–88

First published online as a Review in Advance on April 27, 2007

The *Annual Review of Nutrition* is online at http://nutr.annualreviews.org

This article's doi: 10.1146/annurev.nutr.27.061406.093705

0199-9885/07/0821-0363$20.00

Key Words

chromatin, DNA methylation, epigenomics, metabolic imprinting, nutrition

Abstract

Extensive human epidemiologic and animal model data indicate that during critical periods of prenatal and postnatal mammalian development, nutrition and other environmental stimuli influence developmental pathways and thereby induce permanent changes in metabolism and chronic disease susceptibility. The biologic mechanisms underlying this "developmental origins hypothesis" are poorly understood. This review focuses on the likely involvement of epigenetic mechanisms in the developmental origins of health and disease (DOHaD). We describe permanent effects of transient environmental influences on the developmental establishment of epigenetic gene regulation and evidence linking epigenetic dysregulation with human disease. We propose a definition of "epigenetic epidemiology" and delineate how this emerging field provides a basis from which to explore the role of epigenetic mechanisms in DOHaD. We suggest strategies for future human epidemiologic studies to identify causal associations between early exposures, long-term changes in epigenetic regulation, and disease, which may ultimately enable specific early-life interventions to improve human health.

Contents

INTRODUCTION

DOHaD: developmental origins of health and disease

The developmental origins hypothesis (37) proposes that during critical periods of prenatal and postnatal mammalian development, nutrition and other environmental stimuli influence developmental pathways and thereby induce permanent changes in metabolism and chronic disease susceptibility. Although extensive human epidemiologic and animal model data support this thesis (65, 70, 120), the underlying biologic mechanisms are poorly understood. This review focuses on the likely involvement of epigenetic mechanisms in the developmental origins of health and disease (DOHaD). Epigenetic dysregulation causes human disease; animal model data demonstrating that transient environmental influences during development alter the establishment of epigenetic gene regulation underscore the likely role of epigenetic mechanisms in DOHaD.

As much as to review existing literature, the overall goal of this article is to delineate the parameters of epigenetic epidemiology. We propose a definition of epigenetic epidemiology, compare this new field with the relatively established discipline of genetic epidemiology, and review human studies illustrating the potential of epigenetic epidemiology. Finally, we suggest strategies for future human epidemiologic studies aimed at identifying causal associations between early environmental exposures and long-term changes in epigenetic regulation and disease. Without such studies, potential opportunities to improve human health by specific nutritional interventions targeted to early life will go unrealized.

THE DEVELOPMENTAL ORIGINS HYPOTHESIS

Awareness of the importance of the intrauterine environment for lifelong health and disease emerged as recently as 40 years ago. The seminal observations of Rose (97) described a family pattern of coronary heart disease (CHD), stillbirth, and infant mortality. Forsdahl (33) was the first to geographically correlate infant mortality with cardiovascular disease (CVD). The hypothesis that CVD originates in utero was subsequently investigated extensively by Barker and colleagues (1). Barker & Osmond (3) found high rates of

death due to CHD in areas with high neonatal mortality in England and Wales and proposed that intrauterine deprivation was an important mediator. Retrospective studies of women and men born in Hertfordshire, United Kingdom, found an inverse association between birth weight and adult CHD mortality, supporting this hypothesis (82). Numerous studies subsequently documented associations between low birth weight and increased incidence of heart disease (93), hypertension (61), and type 2 diabetes (40), as well as relevant markers such as abnormal glucose-insulin metabolism (40) and serum cholesterol concentrations (2).

Parallel to the advancement of the Barker thesis, which centered around CHD, hypertension, and type 2 diabetes, a similar hypothesis arose around the fetal origins of cancer. In 1990, Trichopoulos (110) proposed that breast cancer may originate in utero. Indeed, high birth weight is associated with an increase in breast cancer risk (71, 72). Childhood leukemia and testicular cancer have also been related to high birth weight (43). Intrauterine exposure to high levels of growth hormones was initially proposed as an underlying mechanism, increasing both birth weight and cellular proliferation, setting the stage for cancer in later life. Recently, a unifying concept has linked mechanisms for the developmental origins of cardiovascular diseases, cancer, and other chronic diseases (72).

Various terminologies have been proposed to describe biological phenomena relevant to DOHaD. Lucas (65) proposed the term "programming" to refer to permanent or long-term effects of a stimulus or insult at a critical or sensitive period. Barker (1) referred to the fetal origins hypothesis. Realization that developmental plasticity extends into the postnatal period (120) led to a change in nomenclature to the developmental origins hypothesis (37). Waterland & Garza (120) proposed the term "metabolic imprinting" to describe adaptive responses to specific nutritional conditions early in life that occur during limited periods of sensitivity and persist to adulthood. Moreover, metabolic imprinting describes phenomena in which both the exposure and outcome are specific and measurable and exhibit a dose-response or threshold relation. These refinements were intended to focus attention on specific biologic phenomena appropriate for mechanistic characterization.

Most human epidemiologic studies of DOHaD have used birth weight as an indicator of fetal nutrition and intrauterine growth. Although birth weight is easily obtainable, it is a crude measure of intrauterine events and is affected by an array of factors. Moreover, birth weight is likely an inappropriate target for preventive measures aiming to optimize early development during diverse critical periods. We now have the capability to design epidemiologic studies to test potential molecular mechanisms of metabolic imprinting. Given the potential for metabolic imprinting via nutritional influences on epigenetic gene regulation (116), it is timely to consider the epigenetic epidemiology of DOHaD, which focuses on the thesis that early environmental influences induce epigenetic variation and thereby permanently affect metabolism and chronic disease risk. We begin our discussion of epigenetic epidemiology by introducing the meaning and mechanisms of epigenetics.

Epigenetic epidemiology: the study of the associations between epigenetic variation and risk of disease

EPIGENETIC GENE REGULATION: HERITABLE CHANGES IN GENE EXPRESSION POTENTIAL

Most of our cells contain the same DNA—our entire genome—yet gene expression varies dramatically among different tissues. Epigenetic mechanisms establish and maintain this tissue- and cell-type-specific gene expression. The term "epigenetics" was coined by Conrad Waddington several decades ago to describe the study of "the interactions between genes and their products which bring phenotype into being" (48, 114). By Waddington's definition, virtually all development is epigenetic. Indeed, as epigenetics has become increasingly popular in recent years, some

researchers have begun to use the term very broadly.

Genomic imprinting: an epigenetic phenomenon resulting in gene expression depending upon parent-of-origin

A clear definition of "epigenetic" is essential in developing a framework for the study of epigenetic epidemiology. Epigenetics is now understood as the study of heritable changes in gene expression that are not caused by changes in DNA sequence (94). We support the adoption of a further refinement recently advanced by Jaenisch & Bird (50): Rather than heritable changes in gene expression, epigenetics encompasses heritable changes in gene expression potential. This subtle distinction is critical; cell-specific gene expression is not cell-autonomous but rather responds to various extracellular signals (e.g., paracrine, endocrine, and nutrient). Thus, epigenetic mechanisms determine not only constitutive gene expression but also the potential to appropriately alter gene expression in response to extracellular signals. This focus on gene expression potential also distinguishes bona fide epigenetic changes from expression changes that, although sustained through mitosis, are actually induced by extracellular signals.

An area of intense interest in epigenetic epidemiology is the potential for epigenetic inheritance to convey the effects of environmental exposures transgenerationally (83). All epigenetic mechanisms are mitotically heritable, enabling the maintenance of cell-type specific gene expression as cells proliferate throughout life. Epigenetic mechanisms may also be meiotically heritable, potentiating transgenerational epigenetic inheritance (13). The best-characterized examples of such transgenerational epigenetic inheritance in mammals are genomically imprinted genes, which are expressed preferentially from either the maternally or paternally inherited allele (91). Genomically imprinted genes have evolved molecular mechanisms to convey epigenetic information across generations. (Note: "Imprinting" here describes genomic imprinting, not metabolic imprinting.) Transgenerational epigenetic inheritance has also been widely demonstrated in plants (92) and at specific nonimprinted genes in mammals (75).

As with the term "epigenetics" itself, the term "epigenetic inheritance" loses its utility if given too broad a scope. In a recent review on epigenetic epidemiology, Jablonka (47) proposed that epigenetic inheritance encompasses all phenomena in which the transgenerational transmission of phenotypic variation occurs without variation in DNA base sequence, including "reconstruction of developmental and behavioral legacies." Although a useful starting point, that definition does not distinguish innate from acquired phenotypic traits. For example, transgenerational perpetuation of language does not occur by epigenetic inheritance. In our view, transgenerational epigenetic inheritance must be reserved to describe actual transmission of epigenetic information across generations.

Clearly, not all developmental plasticity is epigenetic. Rats born to mothers fed a low-protein diet during pregnancy exhibit reductions in vascularization in the endocrine pancreas, with lifelong consequences for glucose homeostasis (18, 103). The hormone leptin plays a critical role in stimulating development of neural projections in the mouse hypothalamus; ob/ob mice (lacking endogenous leptin expression) show permanent deficits in hypothalamic innervation. Transient leptin treatment of ob/ob mice during the early postnatal period stimulates appropriate hypothalamic development, permanently normalizing food intake (8). In both examples, early environmental influences appear to perturb morphological rather than epigenetic development, inducing permanent changes in organ structure and adult metabolism. Nonetheless, epigenetic mechanisms are likely to underlie many examples of metabolic imprinting (123).

EPIGENETIC MECHANISMS

Epigenetic gene regulation requires molecular mechanisms that encode information in addition to the DNA base sequence and can be propagated through mitosis and meiosis.

Our current understanding of epigenetic gene regulation involves three classes of molecular mechanisms: DNA methylation, histone modifications, and DNA-binding proteins. Although RNA interference contributes to epigenetic regulation in plants and lower organisms, its role in mammalian epigenetic regulation remains unclear (5). Epigenetic mechanisms have recently been reviewed in several excellent articles (50, 60, 62); we therefore provide only a brief overview here.

DNA methylation is the best-characterized epigenetic mechanism. In mammals, DNA methylation occurs almost exclusively at cytosines within cytosine-guanine dinucleotides (CpGs) (the "p" denotes the intervening phosphate group), converting cytosine to 5-methylcytosine. Because of the palindromic nature of CpG dinucleotides, a CpG on one DNA strand always pairs with a CpG on the complementary strand (**Figure 1**). Following DNA replication, the DNA methyltransferase 1 (DNMT1) maintenance methylase restores the original pattern of CpG methylation in the daughter strands (**Figure 1**), providing a simple mechanism for the perpetuation of epigenetic information in proliferating cells. CpG methylation regulates gene expression by affecting the binding of methylation-sensitive DNA-binding proteins and interacting with various modifications of the histone proteins that regulate DNA accessibility (50, 60). Although CpG methylation is generally correlated with gene silencing, methylation-sensitive DNA-binding proteins enable CpG methylation to regulate diverse effects on transcription (50).

Nuclear DNA is packaged with histone proteins in a highly complex and dynamic structure called chromatin. Chromatin conformation is differentially regulated in different cell types by a dizzying array of modifications to lysine residues in the tails of histone proteins: acetylation, methylation, phosphorylation, ubiquitination, and sumoylation (84). The vast potential information content of these permutations led to the suggestion that a histone code (analogous to the genetic code) exists in differentiated cells to dictate locus-specific transcriptional competence (51). The code currently defies decryption, however, and appears increasingly intractable as novel histone modifications are discovered and characterized. Since histones are thought to completely detach from the DNA during

CpG: cytosine-guanine dinucleotide

DNA methyltransferase 1 (DNMT1): the "maintenance methylase" responsible for restoring methylation at hemimethylated CpG sites following DNA replication

Chromatin: the dynamic assembly of genomic DNA and histone proteins. The structural unit of chromatin is the nucleosome: approximately 250 bp of DNA wrapped around an octamer of histone proteins

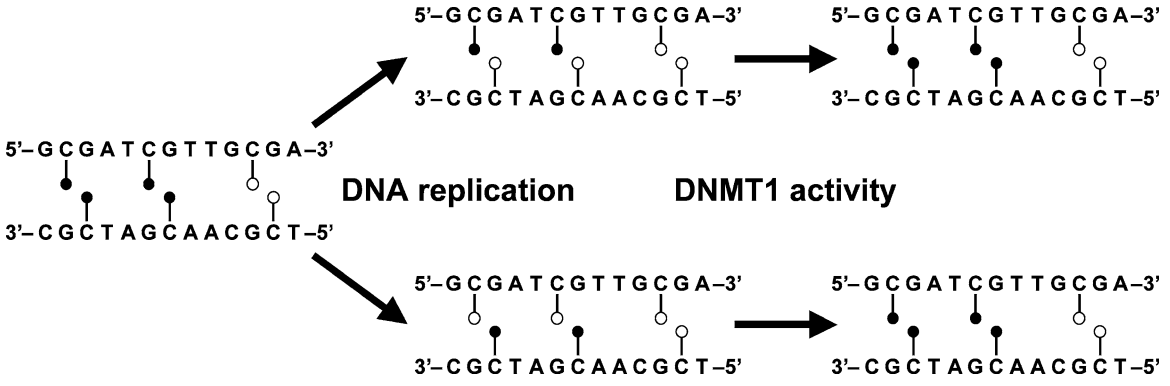


Figure 1

The mechanism for maintenance of site-specific CpG methylation patterns through DNA replication. A short region of DNA is shown; the filled and empty "lollipops" represent methylated and unmethylated CpG sites. Following DNA replication, the newly synthesized strands are unmethylated, resulting in hemimethylation. By preferentially methylating hemimethylated sites, the DNMT1 maintenance methylase restores the original pattern of CpG methylation that existed in the parent DNA molecule. CpG, cytosine-guanine dinucleotide; DNMT1, DNA methyltransferase 1.

DNA replication, it also remains unknown how regional patterns of histone modifications are maintained through mitosis.

The least recognized epigenetic mechanism is feed-forward autoregulation by transcription factors (95). Transcription factor proteins regulate gene expression by binding to recognition sequences within gene promoters. Transcription factors that activate their own gene promoter can perpetuate their expression through cell division. MyoD, an important transcription factor in muscle development, functions in this manner (80). During division of a cell in which *MyoD* has been transcriptionally activated, MyoD protein is partitioned to both daughter nuclei, perpetuating its own transcription while also regulating that of other genes. Thus, contrary to the popular notion that all epigenetic mechanisms involve covalent modification of DNA or histone proteins, autoregulatory transcription factors clearly enable mitotic and potentially meiotic heritability of gene expression potential.

These various mechanisms function in an orchestrated, mutually reinforcing manner to maintain the epigenetic states of differentiated cells throughout life (**Figure 2**). For example, various histone modifications can promote regional CpG methylation, CpG methylation can stimulate specific histone modifications, and the methyl-binding protein MeCP2 appears to facilitate histone deacetylation (50).

EPIGENETIC EPIDEMIOLOGY: CHALLENGES AND OPPORTUNITIES

We define epigenetic epidemiology as the study of the associations between epigenetic variation and risk of disease. Because of genetic-epigenetic interactions, epigenetic epidemiology cannot be completely separated

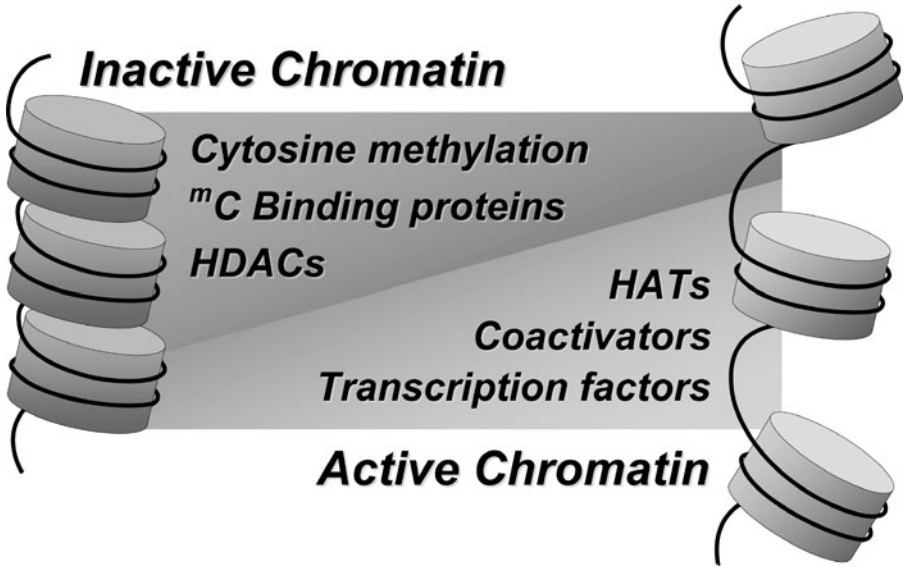


Figure 2

Regional chromatin conformation and transcriptional activity is dynamically regulated by a combination of interacting epigenetic mechanisms. Chromatin is DNA wrapped around nucleosomes, which are composed of histone proteins. Chromatin can exist in either a compact, inactive state or an open, transcriptionally active state. The specific combination of activating and inactivating epigenetic modifications at a genomic locus determines transcriptional competence. ^{m}C, methylcytosine; HATs, histone acetyltransferases; HDACs, histone deacetylases.

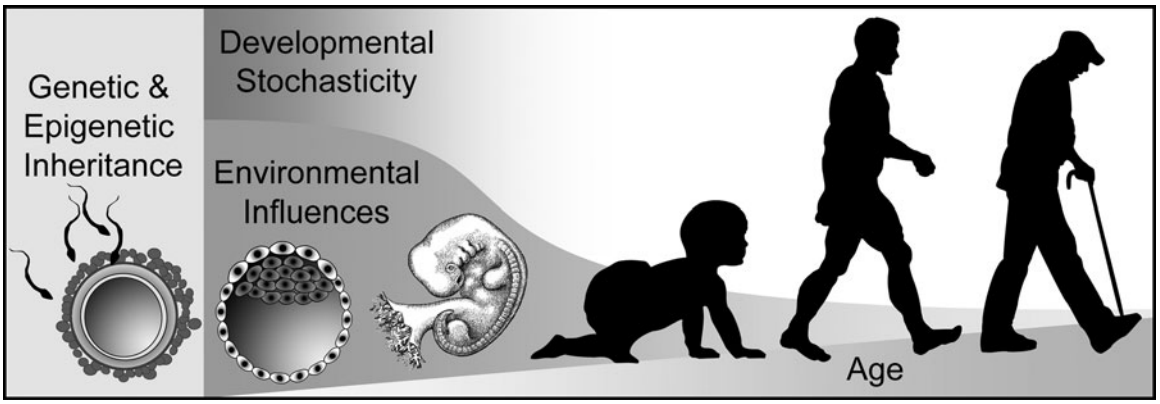


Figure 3

Sources of interindividual epigenetic variation that can contribute to human disease. In addition to genetic and epigenetic inheritance, stochasticity affects the developmental establishment of epigenetic regulation. Environmental influences on epigenetics are likely to be most important during prenatal and early postnatal development, when epigenetic mechanisms undergo establishment and maturation. Cumulative errors in maintenance of epigenetic information will contribute to interindividual epigenetic variation with age.

from genetic epidemiology (7). Nonetheless, it is useful to compare the nascent field of epigenetic epidemiology with the established discipline of genetic epidemiology. Genetic epidemiology focuses on the role of inherited genes in disease etiology; aside from de novo mutations, all genetic variation is inherited. Conversely, epigenetic variation has many sources: genetic and epigenetic inheritance, developmental stochasticity, environmental influences (during development and throughout life), and aging (**Figure 3**) (7, 128). Whereas the starting point for studies of genetic epidemiology is evidence of heritability of disease risk (20), the starting point in epigenetic epidemiology is evidence that interindividual epigenetic variation affects disease risk.

The cornerstone approaches of genetic epidemiology are family-based genetic linkage studies that scan the genome for associations of specific haplotypes with disease, and population-based association studies, in which genetic variation at candidate genes is related to disease (20). Family-based studies are not likely to be of general utility in epigenetic epidemiology. Transgenerational epigenetic inheritance is, in most cases, probably a minor component of interindividual epigenetic variability, and even when familial clustering of epigenotype is detected (11, 101), it will be difficult to determine if it is caused by a genotype-epigenotype interaction (76), shared environment, or epigenetic inheritance. Family-based studies will, however, be critical in epigenetic epidemiology related to genomic imprinting, in which disease risk will depend upon parent of origin. Population-based association studies will be most useful for characterizing relations between epigenetic variability and disease.

Studying monozygotic (MZ) and dizygotic twins to estimate genetic heritability of disease is a classic approach in genetic epidemiology. Twin studies are also an important tool in epigenetic epidemiology: disease discordance within (genetically identical) MZ twin pairs provides support for an epigenetic etiology (85, 132). Just as developmental epigenetics has a stochastic component, so do other developmental processes. Hence, not all phenotypic differences between MZ twins reflect epigenetic differences. Toward understanding the role of epigenetics in DOHaD, studies of

MZ: monozygotic

CpG islands: CpG-rich regions often located at gene promoters

MZ twins may be misleading. Animal studies indicate that effects of maternal nutrition on developmental epigenetics often occur in the preimplantation embryo (122). Before implantation, two twins share the exact same oviduct environment. Thus, although differential placentation and partitioning of placental blood flow often results in discordant nutrient supply within MZ twin pairs, this environmental influence may occur too late to induce systemic epigenetic differences between the twins.

Whereas genetic epidemiology is focused on variation in DNA sequence, epigenetic epidemiology will eventually grapple with the full complexity of interacting epigenetic modifications. Initial studies of epigenetic epidemiology will focus on CpG methylation. Site-specific and regional changes in CpG methylation are often highly correlated with gene expression (27, 50), allowing CpG methylation to serve as an indicator of locus-specific epigenetic regulation. CpG methylation is a relatively simple variable; on a single allele, each CpG site is either methylated or not. Percent methylation within a population of alleles can be measured with high precision (124). Further, CpG methylation is very stable and only small quantities of DNA are required to measure it, so even samples stored for a long time and/or available in limited quantities can be assayed for this epigenetic modification.

Modeling the role of environment will be more challenging in epigenetic as compared to genetic epidemiology. Although researchers often discuss "gene-environment interactions," in most cases they are referring to effects of environment or behavior on the probability that a specific genetic variant will result in disease. The other side of the "interaction"—environment altering genotype—is relatively rare. In epigenetic epidemiology, however, true epigenetic-environment interactions are commonplace. Environment can modify the disease risk associated with a specific epigenotype and also has the vast potential to actually change epigenotype (29, 123). Further, whereas the epigenetic epidemiology of DOHaD will focus on the ability of early nutrition to influence DNA methylation, causing persistent changes in metabolism, it is clear that metabolism will also feed back to affect DNA methylation and potentially other epigenetic modifications (**Figure 4**) (111).

In investigating candidate genes for an epigenetic relation with disease, the specific genomic regions of interest will be more diverse than in genetic epidemiology. Whereas changes in DNA sequence will usually affect health and disease by altering protein function, epigenetic changes will usually affect transcription rather than protein function. Characterization of epigenetic regulatory regions will therefore be a component of epigenetic epidemiology. Many studies of epigenetic regulation have focused on CpG islands, CpG-rich regions often found at gene promoters (55). Recent studies, however, indicate that CpG methylation in intragenic and intergenic regions is critical to tissue-specific gene expression (12, 28, 59). Our ability to predict the genomic region(s) at which epigenetic modifications contribute to gene-specific transcriptional regulation remains rudimentary.

Figure 4

Modeling of epigenetic-environmental interactions will be complicated by the interactions between metabolism and epigenetic regulation.

The inherent tissue-specificity of epigenetic gene regulation adds yet another level of complexity to epigenetic compared to genetic epidemiology. Each individual has only one genotype, but innumerable epigenotypes. Whereas a DNA sample from peripheral blood leukocytes enables us to obtain information about a genetic variant that predisposes to neurodegenerative disease, the epigenetic regulation of this gene may differ markedly between leukocytic and neuronal DNA. Early nutritional influences on epigenetic regulation may likewise be tissue-specific (119).

Clearly, investigators launching epigenetic epidemiologic studies of DOHaD face daunting challenges. However, given the accumulating data linking epigenetic dysregulation to human disease (25, 52, 96), we cannot ignore animal model data showing that subtle environmental influences during development cause persistent changes in epigenetic gene regulation (29, 123). It is of critical public health importance to determine if similar phenomena occur in humans. Recent epigenetic epidemiologic studies show that the challenges are not insurmountable.

EPIGENETIC EPIDEMIOLOGY: EPIGENETIC VARIATION AND HUMAN DISEASE

Assisted Reproduction and Epigenetic Disease

Inappropriate epigenetic reprogramming during early embryonic development can lead to human disease (50). Assisted reproductive technologies (ART), first introduced three decades ago and now accounting for 1%–3% of births in Western countries, may affect epigenetic reprogramming. The most commonly used ART are in vitro fertilization and intracytoplasmic sperm injection. ART-conceived babies are at increased risk for intrauterine growth retardation, low birth weight (24, 102), and, paradoxically, fetal overgrowth. Developmental syndromes associated with aberrant genomic imprinting are more common in children conceived with ART than in naturally conceived children (41). Beckwith-Wiedemann syndrome has been reported to be nine times more common in infants conceived through in vitro fertilization (19). In the small case studies available, all children with Angelman syndrome conceived by ART exhibited hypomethylation of the imprinting control center on the maternal copy of chromosome 15q, in striking contrast with the 3% of Angelman cases associated with this epigenetic abnormality in normally conceived children (15, 81).

ART: assisted reproductive technologies

The direction of causality underlying the associations between ART and epigenetic disease, however, has been questioned (78). In addition to the potential for ART to induce epigenetic changes, these associations could result from extant epigenetic irregularities in the oocyte causing both infertility and epigenetic dysregulation in the offspring (78). Furthermore, most evidence linking ART to imprinting errors comes from case reports and small case-control studies. Nonetheless, all ART involves in vitro culture of the early embryo from the time of fertilization until introduction into the uterus, and animal model data indicate that the artificial environment during this critical period may affect epigenetic reprogramming (57, 58). Conception by ART is becoming increasingly common, and ART programs are extending the period of in vitro culture to optimize implantation rates. Clearly, systematic large-scale studies of the effects of ART on epigenetic regulation and human disease are warranted (78).

Interindividual Epigenetic Variation

An excellent way to identify genomic regions that may contribute to epigenetic disease is to catalog those showing substantial interindividual epigenetic variation. Holliday (44) recently asserted, "[I]f the methylation was variable between individuals, it would probably not be important." To the contrary, only if methylation of important genes differs

between individuals can individual variation in methylation contribute to variation in disease! Population studies of interindividual epigenetic variation are therefore an important part of epigenetic epidemiology.

The Human Epigenome Project aims to "analyze DNA methylation in the regulatory regions of all known genes in most major cell types and their diseased variants" (88). In a pilot project that profiled DNA methylation of the 3.8 Mb major histocompatibility locus, methylation was analyzed in several human tissues, with multiple samples from different individuals for all tissues. Almost half of the amplicons analyzed showed substantial interindividual variation in methylation in at least one tissue (88). A recent study examining the potential for transgenerational epigenetic inheritance (32) used CpG methylation arrays to identify interindividual variation in DNA methylation at several human disease genes in sperm samples from 46 men.

In a study of 48 three-generation families (680 individuals total), Sandovici et al. (101) demonstrated substantial interindividual variation in allelic methylation at the *IGF2/H19* and *IGF2R* imprinted loci in peripheral blood DNA. Interestingly, familial clustering of allelic methylation suggested a genetic component to this epigenetic variation. Temporal changes in individuals over periods up to 20 years indicated influences of environment and aging. A later study on the same population reported dramatic interindividual variation in methylation at specific *Alu* elements (100). *Alu* are short retrotransposon elements present at over a million copies in the human genome. Because of their ability to cause epigenetic dysregulation of neighboring genes (127), interindividual epigenetic variation at retrotransposons could have important implications for human disease.

Together, these studies demonstrate considerable interindividual variation in locus-specific DNA methylation. Loci implicated in human disease are promising candidates for epigenetic association studies. Data are generally lacking, however, regarding potential tissue-specificity of interindividual epigenetic variation.

Epigenetic Discordance in Monozygotic Twins

MZ twins are often discordant for common diseases (85); epigenetic differences provide a potential explanation. In a study of lymphocyte DNA from 40 MZ twin pairs, Fraga et al. (34) employed sophisticated epigenomic approaches to identify loci showing differential DNA methylation within twin pairs. Many of the regions of discordant methylation corresponded to *Alu* sequences, corroborating the findings of Sandovici et al. (100). Highlighting differences between 3-year-old and 50-year-old twin pairs, Fraga et al. (34) concluded that most epigenetic differences between MZ twins are not present in childhood but instead arise over the lifetime. This conclusion is unwarranted, however, since only two 3-year-old twin pairs were studied. Indeed, a study of buccal DNA from twelve 5-year-old MZ twin pairs with discordant birth weights found considerable twin-twin discordance of CpG methylation at the gene encoding catechol-o-methyltransferase (a gene implicated in psychopathology) (73). Notably, birth weight was not associated with catechol-o-methyltransferase methylation, underscoring our previous point that differences in placentation and placental partitioning that result in birth weight differentials among MZ twins may occur too late to affect systemic establishment of epigenetic marks.

Epigenetic differences among MZ twins discordant for disease provide support for the hypothesis that stochastic differences in the establishment of epigenetic gene regulation can lead to human disease. Beckwith-Wiedemann syndrome is a developmental syndrome caused by genetic and epigenetic alterations in an imprinted region on human chromosome 11 (126). Loss of allele-specific methylation and associated biallelic expression of the genomically imprinted gene *LIT1* is the most common epigenetic alteration

associated with Beckwith-Wiedemann syndrome. In skin fibroblast DNA of five MZ twin pairs discordant for Beckwith-Wiedemann syndrome, every affected twin, but no unaffected twin, showed these epigenetic alterations (126). A recent study examined a single MZ twin pair discordant for a caudal duplication anomaly (79). Because of the similarity of the phenotype to that of *Axin Fused* mice, the investigators focused on the human *AXIN1* gene. In DNA from peripheral blood, the *AXIN1* promoter-region CpG island was significantly hypermethylated in the affected twin relative to the unaffected twin. There was lower and individually variable methylation at this region in peripheral blood DNA of healthy control individuals.

Epigenetic Epidemiology of Cancer

Epigenetic epidemiology has been applied in the field of cancer epigenetics. One example relates to epigenetic dysregulation of the insulin-like growth factor 2 (*IGF2*) gene in colon cancer. *IGF2* is a genomically imprinted gene usually expressed in humans only from the paternally inherited allele. A population-based study of peripheral blood DNA from 262 Japanese individuals demonstrated that loss of imprinting (LOI, manifesting as biallelic expression of *IGF2*) occurs in 10% of normal adults (99). In a cross-sectional study of 172 patients undergoing colonoscopy, Cui et al. (17) found that, relative to patients with no personal or family history of colorectal cancer, those with a family history of colorectal cancer had a fivefold elevated odds ratio for *IGF2* LOI in peripheral blood, and those diagnosed with colorectal cancer had a 20-fold elevated odds ratio. These results, if confirmed, suggest that *IGF2* LOI in peripheral blood lymphocytes can serve as an indicator of colorectal cancer risk (17). However, since *IGF2* LOI was assessed concurrently with cancer status, temporality of *IGF2* LOI and cancer development cannot be established; systemic LOI could be a consequence of the disease. An epidemiologic study of the same individuals (16) assessed various environmental exposures (cigarette smoking, alcohol ingestion, and intake of several nutrients) but found none associated with *IGF2* LOI. These findings do not exclude the possibility that environmental factors acting during a critical period of development affect *IGF2* LOI (53).

Age, Genetics, and Environment

Age, genetics, and environment all interact to affect epigenetic regulation. One of the earliest studies that can be characterized as epigenetic epidemiology investigated the role of aging in hypermethylation of the estrogen receptor (ER) gene (46). In 39 healthy control individuals age 20–90, ER CpG island methylation in colonic DNA increased linearly with age ($R^2 = 0.50$). Since ER hypermethylation is found in almost all colorectal tumors, these data suggest that ER hypermethylation could contribute to the increased risk of colorectal cancer with age (46). A population-based study of Italian adults explored the combined effects of plasma folate status and a common polymorphism (C677T) in 5,10-methylenetetrahydrofolate reductase on global DNA methylation (35). In a comparison of 187 C/C and 105 T/T individuals, plasma folate concentrations were directly related to global DNA methylation only in T/T individuals (35). Also, plasma homocysteine concentration in T/T individuals was markedly elevated and correlated inversely with global DNA methylation. Plasma homocysteine was also related to epigenetic alterations in a smaller study of men with severe hyperhomocysteinemia (45). Leukocyte DNA was significantly hypomethylated in hyperhomocysteinemic men relative to controls. In a subset of seven men informative for a polymorphism in the imprinted *H19* gene, the three with the highest plasma homocysteine concentrations all showed aberrant biallelic expression. In every case, monoallelic *H19* expression was restored after eight weeks of daily supplementation with pharmacological levels of 5-methyltetrahydrofolate (45).

Loss of imprinting (LOI): a change in the expression ratio of a genomically imprinted gene away from monoallelic expression. Loss of imprinting can occur either by up-regulation of the normally silenced allele or preferential silencing of the normally expressed allele

Metastable epialleles: alleles at which the developmental establishment of epigenotype occurs stochastically, resulting in wide interindividual variation in DNA methylation

Together, these seminal studies illustrate the potential of epigenetic epidemiology. In these studies of select human populations, significant interindividual epigenetic variation at specific loci has been demonstrated and, in many cases, linked to disease. Epigenetic epidemiology of DOHaD will seek to quantify interindividual epigenetic variation that is both induced by early environmental exposures and associated with risk of chronic disease.

EARLY ENVIRONMENTAL INFLUENCES ON EPIGENETIC REGULATION

Our understanding of the specific mechanisms by which epigenetic gene regulation is first established during mammalian differentiation is fairly rudimentary. Of the various epigenetic mechanisms, developmental changes in DNA methylation are best characterized. Shortly after fertilization, the genome of the early embryo undergoes massive demethylation. Except for specific regions that escape this erasure, the genome of the preimplantation embryo is completely hypomethylated, correlating with its pluripotency (74, 89). Starting around the time of implantation, as the embryo develops into a fetus, lineage-specific re-establishment of DNA methylation occurs, ostensibly restricting the gene expression and developmental fate of differentiating tissues (74, 89).

The intuitive assertion that DNA methylation functions as a component of cellular memory to maintain the differentiated state of mammalian tissues has, however, been controversial. It has been proposed that whereas DNA methylation functions in specialized epigenetic phenomena such as genomic imprinting, X-chromosome inactivation, and silencing of retrotransposons, its role in differentiation and tissue-specific gene expression is unclear (115). Evidence for such a role is now accumulating. Studies of mice engineered with an inducible transgene capable of protecting neighboring sequences from de novo methylation during development indicate that establishment of DNA methylation in the early embryo is critical to setting up the epigenetic profile of open versus closed chromatin states (42). The tissue-specific expression of various genes has been correlated with their tissue-specific hypomethylation. Ehrlich (27) recently reviewed the literature on this topic and employed rigorous criteria to identify mammalian genes at which differentiation-associated DNA methylation controls expression. Genes expressed specifically in diverse tissues including testis, myometrium, liver, brain, and leukocytes show tissue-specific hypomethylation that appears both necessary and sufficient for expression (27). Recent studies of tissue-specific CpG methylation on a genomewide scale suggest an explanation for the failure of many studies to correlate DNA methylation with cellular differentiation. Whereas most previous studies focused on methylation in gene promoter regions, these recent epigenomic analyses indicate that tissue-specific methylation in intragenic regions often contributes to tissue-specific gene expression (12, 104).

Once established during development, epigenetic mechanisms are in most cases maintained with high fidelity throughout life. During development, however, the massive loss and subsequent re-establishment of DNA methylation in the embryo and fetus likely comprise diverse critical periods during which environmental stimuli can affect epigenetic regulation (123). We propose that environmental influences on the developmental establishment of DNA methylation occur via two general mechanisms: (*a*) by affecting the supply of dietary methyl donors and/or activity of DNA methyltransferases to induce either hyper- or hypomethylation at metastable epialleles (see below) or (*b*) by altering transcriptional activity of specific genes during ontogenic periods when DNA methylation is being established (**Figure 5**).

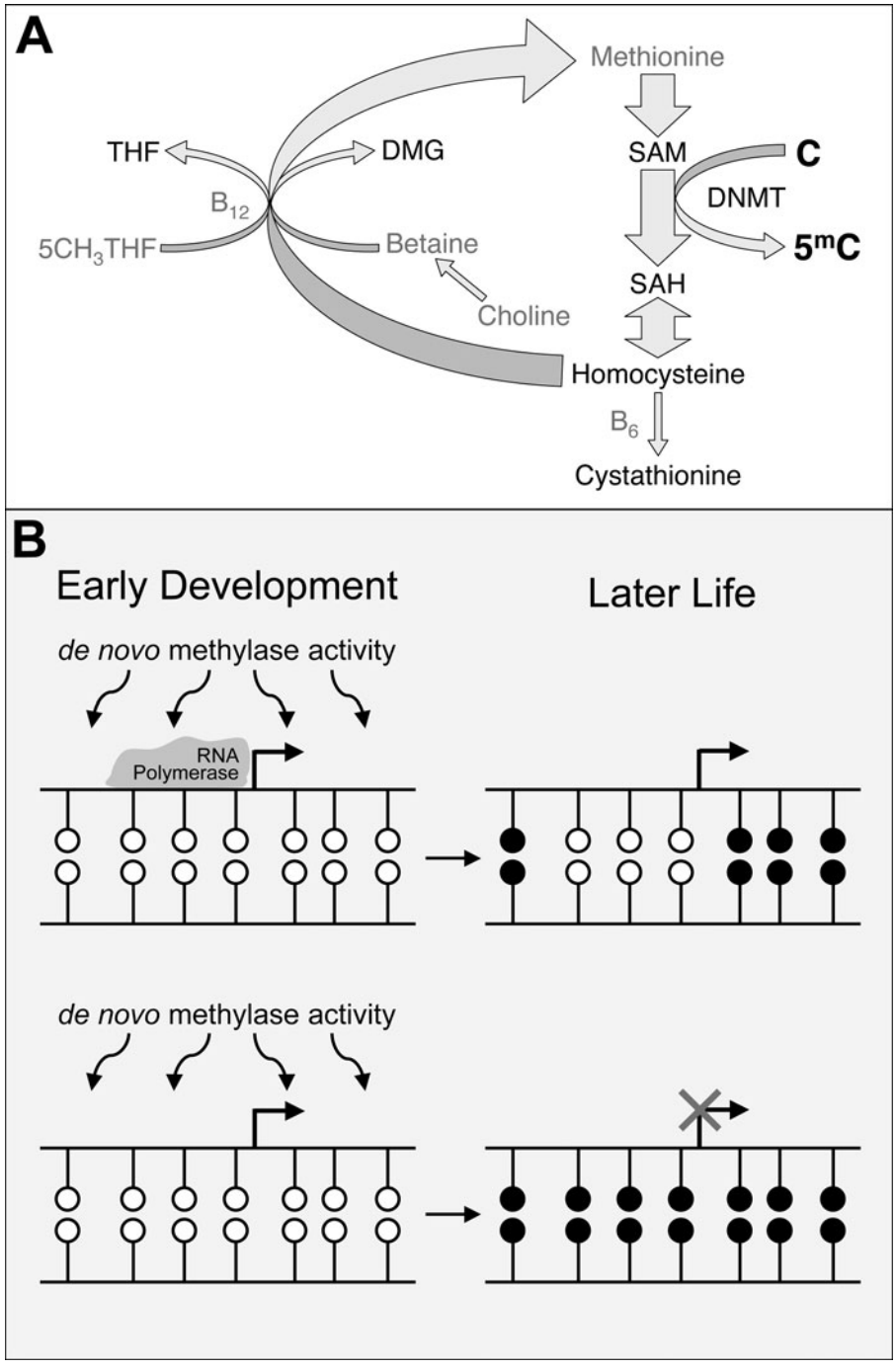
Most genomic regions undergo developmentally programmed establishment of epigenetic regulation and show little

interindividual variability in DNA methylation. Conversely, at regions referred to as metastable epialleles (86), developmental establishment of DNA methylation occurs probabilistically, resulting in dramatic interindividual differences in epigenetic regulation. Nutritional influences on developmental epigenetics were first suggested by studies of the *viable yellow agouti* (A^{vy}) metastable epiallele. The murine A^{vy} mutation resulted from transposition of an IAP retrotransposon upstream of the *agouti* gene, which regulates the production of yellow pigment in fur. Spontaneous variation in CpG methylation of the A^{vy} IAP causes dramatic variation in coat color and other phenotypes among genetically identical A^{vy}/a mice (75). [The *nonagouti* (*a*) allele encodes a nonfunctional *agouti* transcript and therefore does not contribute to phenotypic variation of A^{vy}/a mice.] Wolff et al. (130) found that supplementing mouse dams with the dietary methyl donors and cofactors folic acid, vitamin B_{12}, betaine, and choline shifts the coat color distribution of their A^{vy}/a offspring from yellow to brown, suggesting hypermethylation of the A^{vy} IAP. It was later confirmed that maternal supplementation affects coat color of A^{vy}/a offspring by inducing hypermethylation at A^{vy} (122). The effect of maternal supplementation on establishment of A^{vy} epigenotype apparently occurs prior to gastrulation (122). Interestingly, supplementation of dams with the soy phytoestrogen genistein before and during pregnancy induced offspring A^{vy} hypermethylation comparable to that caused by methyl donor supplementation (22).

Recent studies in *Axin Fused* ($Axin^{Fu}$) mice indicate that epigenetic lability to early nutrition is a general characteristic of metastable epialleles. The $Axin^{Fu}$ metastable epiallele resulted from an IAP insertion into Axin intron 6 and causes a kinky tail phenotype. Similar to A^{vy}, spontaneous variability in CpG methylation at $Axin^{Fu}$ confers dramatic phenotypic variation among isogenic $Axin^{Fu}/+$ mice (87). Maternal supplementation with methyl donors reduces the incidence of tail kinks in $Axin^{Fu}/+$ offspring by inducing hypermethylation at $Axin^{Fu}$ (119), indicating that developmental establishment of DNA methylation at metastable epialleles is, in general, labile to maternal diet. In studies of another *agouti* metastable epiallele (A^{iapy}), haploinsufficiency for the maintenance DNA methyltransferase Dnmt1 (36) shifted the coat-color distribution of A^{iapy}/a mice toward yellow (hypomethylated). Hence, any environmental exposure during early embryonic development that affects either the substrate availability or enzymatic activity of Dnmt1 will likely influence the establishment of DNA methylation at metastable epialleles, inducing permanent changes in gene expression (**Figure 5*A***).

The second general mechanism for early environmental influences on epigenetic regulation potentially encompasses a much broader range of both environmental stimuli and responsive genes. The model states that during limited ontogenic periods when de novo DNA methylation occurs, genes that are actively transcribed in specific cells are protected from hypermethylation (**Figure 5*B***). There is extensive support for this model. Active promoters in CpG island-containing transgenes faithfully remain hypomethylated, but lose their immunity to hypermethylation if promoter function is impaired (9). In cultured cells with a transgene containing binding sites for the inducible metal-responsive transcription factor, induction of this transcription factor caused activation of transgene expression that persisted after the withdrawal of the induction stimulus, showing that transcriptional activation prevents epigenetic silencing (108). Such observations led Bird (6) to propose that, by preventing de novo hypermethylation, the transcriptional activity of embryonic promoters is imprinted for the duration of that somatic lifetime.

This model predicts that any early nutritional or environmental stimulus that alters transcriptional activity when DNA methylation is undergoing developmental changes could result in permanent alterations in epigenetic regulation and related phenotypes.



Studies relating maternal caregiving behavior to epigenetic changes at the rat glucocorticoid receptor (GR) promoter support this model (125). In an inbred strain of rats, there is wide natural variation in the amount of time that dams spend nursing, licking, and grooming their pups. Meaney and colleagues (125) found that the level of maternal care during the suckling period permanently changes offspring physiology and behavior by affecting the ontogeny of methylation at specific CpG sites in the GR promoter. In the hippocampus, a key CpG site in the GR promoter is completely unmethylated just before birth, and then becomes hypermethylated on the first postnatal day (P1). The period from P1 to P6 is a critical window for establishment of CpG methylation and transcriptional regulation of GR in the hippocampus: Methylation at the critical CpG site is almost completely gone by P6 in pups suckled by high-caregiving dams but remains permanently elevated in those suckled by more apathetic dams (125). These observations indicate that high maternal care in the early postnatal period activates GR transcription, inducing permanent GR hypomethylation. This hypomethylation, in turn, causes permanent derepression of GR transcription (125).

Several other examples illustrate that environmental influences during development can induce changes in DNA methylation and epigenetic regulation, but it is unclear whether these effects occur by mass action at metastable epialleles or through transcriptionally mediated effects on the establishment of DNA methylation. In perhaps the earliest such study, Reik et al. (90) transplanted female pronuclei into recipient eggs of a different genotype, allowed the mice to develop to adulthood, and compared hepatic gene expression in these "nucleocytoplasmic hybrids" with that of sham-manipulated control animals. The nucleocytoplasmic hybrids were genetically identical to the control mice, differing only in the cytoplasm to which the early embryonic genome was exposed. Nevertheless, substantial differences in gene expression and CpG methylation in adult liver were induced by this early environmental exposure (90).

More recently, dietary protein restriction of pregnant rats was shown to induce DNA hypomethylation and increased expression of GR and peroxisome proliferator-activated receptor alpha in the livers of offspring at weaning, and these epigenetic changes were completely prevented if the protein-restricted diet was supplemented with folic acid (63). Similarly, dietary choline deficiency during mouse fetal development induced hypomethylation of *Cdkn3* in specific regions of the hippocampus, correlating with increased expression of Kap, the kinase-associated phosphatase encoded by *Cdkn3* (77). Neither of these studies determined whether the epigenetic alterations induced by maternal diet persist to adulthood.

Figure 5

Potential mechanisms for environmental influences on developmental establishment of DNA methylation. (*A*) Nutritional or other stimuli that affect either the efficiency of one-carbon metabolism or the activity of DNMT1 could alter the developmental establishment of DNA methylation at metastable epialleles. Flux through the transmethylation/remethylation pathway is dependent upon nutrients including folate, vitamins B_{12} and B_6, choline, betaine, and methionine. (*B*) Transcriptional activity during critical developmental periods can impair de novo methylation. Any nutritional or other environmental exposure that activates gene transcription during periods of de novo CpG methylation can permanently imprint transcriptional competence by preventing hypermethylation. (Methylated CpG sites are shown as filled "lollipops.") Although a gene promoter region is shown here, similar effects could occur at any genomic region contributing to transcriptional regulation, such as a distal enhancer. $5CH_3$THF, 5-methyl tetrahydrofolate; CpG, cytosine-guanine dinucleotide; DNMT, DNA methyltransferase; SAH, s-adenosylhomocysteine; SAM, s-adenosylmethionine.

Together, these data from animal models indicate that nutrition and other environmental stimuli during development can affect the establishment and/or maturation of epigenetic mechanisms, causing persistent changes in gene expression. Hence, to the extent that epigenetic dysregulation contributes to the etiology of diseases most commonly associated with DOHaD—cardiovascular disease, type 2 diabetes, cancer, and obesity—epigenetic mechanisms may provide a critical link between early environment and adult disease.

EPIGENETIC DYSREGULATION AND HUMAN DISEASE

The role of epigenetic dysregulation in human disease has been reviewed extensively (25, 52, 96). We therefore summarize only the most salient points here. Because the epigenetic basis of several rare developmental diseases is well-established (25), we focus on chronic diseases most often considered in the DOHaD paradigm.

Cancer

A hallmark of tumorigenesis is the coexistence of genomewide hypomethylation and hypermethylation of CpG islands in the promoters of specific genes (26). Promoter-region CpG island hypermethylation associated with inappropriate transcriptional silencing is the most frequently documented epigenetic alteration in tumors. Nearly half of the tumor suppressor genes that cause familial cancer when mutated also undergo epigenetic silencing in sporadic forms of cancer (54). For example, hypermethylation of the mismatch repair gene *MLH1* is associated with tumors exhibiting microsatellite instability, and hypermethylation of the breast cancer gene *BRCA1* is found in 10%–15% of women with nonfamilial breast cancer (54).

Epigenetic dysregulation of genomic imprinting is also implicated in cancer. *IGF2* LOI occurs in several childhood cancers, including a large proportion of Wilms' tumor (30), and in various adult cancers (25). Recent studies suggest that systemic *IGF2* LOI may serve as an indicator of colorectal cancer risk (17). An epigenetic progenitor model of cancer (30) recently highlighted the potential importance of polyclonal epigenetic disruption of stem cells via alterations of tumor progenitor genes including *IGF2*.

As most studies in cancer epigenetics have been cross-sectional, it remains unclear whether the epigenetic dysregulation in tumor tissue or in peripheral blood is a cause or an effect of tumorigenesis. A causal role for *IGF2* LOI was recently supported by a mouse model: Induction of *Igf2* LOI enhanced susceptibility to gastrointestinal cancer (98). Studies of families with strong family histories of cancer have identified apparent germline epimutations in the mismatch repair genes *MLH1* (107) and *MSH2* (11). It is possible, however, that the familial clustering of aberrant epigenetic regulation at these loci is mediated by genetic variation. Regardless of whether they reflect true transgenerational epigenetic inheritance, these epimutations appear to induce carcinogenesis as do genetic mutations at the same loci. Nested case-control studies, in which tissue specimens are collected well before cancer diagnosis, are warranted to determine temporality of the association of epigenetic dysregulation and cancer.

Cardiovascular Disease

CVD, in particular CHD, is now the leading cause of death worldwide, accounting for 27% of deaths in industrialized countries and 21% of deaths in developing countries (64). With genetics explaining less than 5% of CHD, adult lifestyle factors and epigenetics likely explain most of the variation (129). The most obvious link between epigenetics and cardiovascular disease is hyperhomocysteinemia. The basis for the association of hyperhomocysteinemia with cardiovascular disease is unknown, but since elevated

homocysteine concentrations can impair one-carbon metabolism and DNA methylation, epigenetic mechanisms have been postulated (10). Aberrant DNA methylation (both hypo- and hypermethylation) secondary to nutritional factors has been implicated as an early step in atherogenesis (66, 134).

Type 2 Diabetes

An article proposing DNA methylation profiling in diabetes reviewed indirect evidence that epigenetic dysregulation contributes to type 2 diabetes (68). A recent data-mining analysis of more than 12 million Medline records (133) identified epigenetic factors among the strongest statistical associations to type 2 diabetes. The most direct evidence implicating epigenetic dysregulation in human diabetes is from studies of transient neonatal diabetes (TND), a rare form of diabetes that presents within the first few days after birth and, although normally resolving within one year, often recurs later in life. Two studies recently showed that infants with sporadic TND show aberrant methylation at several imprinted genes in peripheral blood leukocytes (21, 67). Effects of parental and grandparental nutrition on diabetes risk in humans has been reported, suggesting transgenerational inheritance of epigenetic alterations that affect diabetes susceptibility (56, 83). Several animal models showing persistent effects of prenatal and early postnatal nutrition on endocrine pancreas function and gene expression suggest an epigenetic basis (112, 121).

Obesity

Prader-Willi syndrome is a developmental syndrome that causes hyperphagic obesity, hypogonadism, and characteristic facial features (38). Whereas the disease most commonly results from genetic abnormalities in an imprinted region of chromosome 15, some sporadic cases result from aberrant epigenetic silencing of that region, providing a clear example of epigenetic dysregulation causing human obesity. Genomewide parent-of-origin linkage analyses suggest that maternally imprinted loci in chromosome regions 10p12 (23) and 2q37 (39) also influence human obesity. Moreover, imprinted genes affect the development of the hypothalamus, which plays a central role in regulating energy homeostasis (14).

Animal models provide further illustrations that epigenetic dysregulation can cause obesity (117). When mice are cloned, they have normal birth weights but often develop adult-onset obesity (109). A similar phenomenon, termed "large offspring syndrome," appears to be related to epigenetic dysregulation in cloned sheep (135). A^{vy} mice provide another animal model of epigenetically based obesity. Agouti protein binds antagonistically to the melanocortin 4 receptor in the hypothalamus. A^{vy}/a mice with A^{vy} hypomethylation therefore develop not only yellow coats but also hyperphagic obesity (131).

Overall, direct evidence for an involvement of epigenetic dysregulation in human cardiovascular disease, type 2 diabetes, and obesity is scant. This contrasts markedly with the compelling body of literature implicating epigenetic dysregulation in human cancer. Compared with these other diseases, however, demonstrating an epigenetic basis for cancer is relatively straightforward. Much of the data implicating epigenetic dysregulation in cancer was obtained by examining epigenetic mechanisms in tumor tissue and adjacent normal tissue. Hence, in cancer, the tissue showing epigenetic dysregulation is both easily identifiable and readily obtainable. The other diseases of greatest relevance to DOHaD are more complex and can result from dysregulation in multiple interacting tissues. As demonstrated by the success of cancer epigenetics, however, any disease with a genetic basis is also likely to have an epigenetic basis. The potential tissue-specificity of epigenetic regulation (and dysregulation) will be the major obstacle to epigenetic epidemiology of DOHaD.

EPIGENETIC EPIDEMIOLOGY OF DOHaD: SUGGESTIONS FOR FUTURE STUDIES

The definition of metabolic imprinting (120) was proposed to guide mechanistic studies into the developmental origins hypothesis. Accordingly, several characteristics of metabolic imprinting are instructive to the design of epidemiologic studies testing the hypothesis that environmental influences on developmental epigenetics contribute to health and disease in humans. A key characteristic of metabolic imprinting is the need to distinguish fundamental mechanisms of metabolic memory, "primary imprints," from secondary physiological alterations that arise in response to primary imprints. The fundamental nature of epigenetic regulatory mechanisms makes them logical primary imprint marks. Demonstrating that the epigenetic regulation of a specific gene in adulthood is correlated with some prenatal exposure does not, however, prove that the epigenetic change serves as a primary imprint. Primary imprint marks must be present directly after the imprinting period as well as in adulthood (120). Hence, incorporating a test of temporality into epigenetic epidemiologic studies will aid in the identification of epigenetically based primary imprint marks. This proposal is most practical in follow-up studies of cohorts in which tissues were collected during early life.

The critical-window nature of metabolic imprinting (120) should also be considered in epigenetic epidemiologic studies of DOHaD. For example, epidemiologic studies seeking to identify systemic epigenetic alterations should focus on periconceptional environmental exposures, since epigenetic alterations occurring in the very early embryo are most likely to be propagated to diverse tissues. Conversely, exposures occurring during late gestation or postnatally are more likely to induce tissue-specific epigenetic changes.

The potential tissue-specificity of epigenetic regulation raises the question of what tissues should be collected in epigenetic epidemiologic studies. Many studies have banked DNA isolated from peripheral blood leukocytes (PBLs), but PBLs represent only one germ layer of the early embryo. Because the different germ layers (endoderm, mesoderm, and ectoderm) are epigenetically divergent, it will be useful to collect tissues representing these different embryonic lineages. Since DNA methylation can be analyzed using very small quantities of DNA, it is feasible to noninvasively collect sufficient quantities of human tissues representing different germ lineages. Buccal cells and hair follicles contain ectodermal DNA. PBLs are of mesodermal origin. Obtaining endodermal tissue in large-scale studies of healthy individuals will pose the greatest challenge. One possible approach, assessing DNA methylation in colonocyte DNA isolated from human stool, was recently validated (4).

Identifying interindividual epigenetic variability that occurs systemically will simplify studies of epigenetic epidemiology because DNA methylation in easily obtainable tissues will correlate with that in tissues of physiological relevance. Here, metastable epialleles are of great interest. In addition to their definitive epigenetic variability among different individuals, metastable epialleles generally exhibit little tissue-specificity in DNA methylation (119, 122). Hence, if metastable epialleles of pathophysiological relevance can be identified in humans, these would be obvious loci at which to focus initial epigenetic epidemiologic studies related to DOHaD. Perhaps the best such candidate metastable epiallele in humans is *IGF2* (99). *IGF2* and other genes found to exhibit epigenetic variation among individuals, such as polymorphic *Alu*s (100) and *IGF2R* (101), should be evaluated to determine if their interindividual epigenetic variability occurs systemically.

Even if specific metastable epialleles exhibit epigenetic variation that appears unrelated to human disease, they may be useful as epigenetic biomarkers, enabling a retrospective measure of environmental exposures

during development. One of the greatest weaknesses of DOHaD has been the reliance upon birth weight as a proxy for fetal nutritional status. Many factors influence birth weight; the effect of maternal nutrition is relatively minor and occurs mainly in the last trimester (106). Just as the coat color of A^{vy} mice may be used as an epigenetic biosensor to gauge the hypermethylating effects of various diets (118), human metastable epialleles may provide a sensitive retrospective indicator of genomic methylating capacity during embryonic development. Such alleles therefore may be used as sentry loci to select individuals likely to have experienced conditions in utero that could predispose to aberrant epigenetic regulation at multiple loci.

Prospective studies testing for effects of maternal nutrition on DNA methylation of offspring would benefit from the validation of robust biomarkers for maternal one-carbon metabolism. Because moderate folate depletion can induce genomewide DNA methylation (49), genomic methylation may be used as an integrative biomarker of methyl donor nutrition (69). Studies, for example, could determine if maternal periconceptional genomic methylation predicts methylation at specific loci in her offspring. Plasma metabolites related to maternal one-carbon metabolism will also be useful. Because s-adenosylmethionine (SAM) and s-adenosylhomocysteine (SAH) are the substrate and product, respectively, of methyltransferase reactions, it has been proposed that the SAM:SAH ratio can be used as a methylation index to identify individuals with high or low capacity for DNA methylation (113). Because SAM does not readily cross the plasma membrane, however, each mammalian cell must synthesize its own SAM from circulating methionine or homocysteine (31). This could explain why dietary exposures that perturb the SAM:SAH ratio often cause paradoxical changes in DNA methylation (111). Homocysteine, which is in equilibrium with SAH (a product inhibitor of methyltransferases), is a key metabolite in mammalian one-carbon metabolism and readily crosses the plasma membrane. Circulating homocysteine therefore shows promise as a systemic indicator of transmethylation capacity (111). Importantly, gene-nutrient interactions will likely complicate the interpretation of all these potential biomarkers. For example, the previously discussed studies of the 5,10-methylenetetrahydrofolate reductase C677T polymorphism (35) suggest that genomic methylation may serve as an effective biomarker for folate status only in T/T individuals.

Most previous studies of epigenetic epidemiology have taken a candidate gene approach. Technologies are rapidly being developed, however, to scan the genome for locus-specific variation in DNA methylation and other epigenetic modifications (12, 59, 104). Once such epigenomic tools are widely available, they will dramatically accelerate the discovery of human loci at which epigenetic regulation is correlated with early environmental exposures.

Perhaps the most pressing research need related to the epigenetic epidemiology of DOHaD is to improve our understanding of the mechanisms by which epigenetic dysregulation contributes to CVD, type 2 diabetes, and obesity. Controlled experiments in animal models will be essential to identify the critical developmental periods, specific tissues, and genomic loci in which epigenetic alterations are induced, affecting metabolic processes and lifelong disease susceptibility. In addition to mouse models, it will be important to extend studies of comparative epigenetics and pathophysiology into more closely related species, including nonhuman primates. The knowledge gained will enable the formulation of specific hypotheses that can be tested in human studies of epigenetic epidemiology.

CONCLUSION

DNA is not destiny. The stochasticity of mammalian development enables one genotype to result in a wide range of phenotypes. Early nutrition may influence this

developmental plasticity to induce metabolic imprinting in humans, with worldwide implications for public health and nutrition policy (105). Overcoming the daunting challenges presented by the epigenetic epidemiology of DOHaD will enable crucial advancements in our understanding of the long-term effects of early nutrition in humans.

SUMMARY POINTS

1. Epigenetic mechanisms are likely to play an important role in the developmental origins of health and disease.
2. Transient environmental influences during development can permanently alter epigenetic gene regulation resulting in metabolic imprinting affecting disease susceptibility.
3. Epigenetic mechanisms include CpG methylation, histone modifications, and autoregulatory DNA-binding proteins.
4. Epigenetic dysregulation is found in developmental diseases and cancer and probably affects cardiovascular disease, diabetes, and obesity.
5. Epigenetic epidemiology provides a basis for future studies of early life exposures, epigenetic mechanisms, and adult disease.
6. Epigenomics will accelerate the discovery of human loci at which epigenetic regulation is correlated with early environmental exposures.
7. Research in animal models is needed to better understand the role of epigenetic dysregulation in disease.

ACKNOWLEDGMENTS

We gratefully acknowledge Jeff Holly, Hannah Landecker, Lanlan Shen, and Lane Strathearn for substantive comments on the manuscript, and Adam Gillum for assistance in developing the figures. RAW is supported by NIH grant 5K01DK070007, research grant #5-FY05-47 from the March of Dimes Birth Defects Foundation, and USDA CRIS #6250-51000-049. KBM is supported in part by research grant R21CA128382 from the National Cancer Institute.

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Errata

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