

Epigenetics at the Epicenter of Modern Medicine

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What Is Epigenetics?

Although epigenetics is considered a relatively new area of medicine, the term is more than 60 years old. Waddington first used the term *epigenetics* to describe what is now called developmental biology, the idea that phenotype, or the morphologic and functional properties of an organism, arises sequentially under a program defined by the genome under the influence of the organism's environment.¹ The modern definition of epigenetics is modifications of the DNA or associated proteins, other than DNA sequence variation, that carry information content during cell division.² The best understood example of epigenetic modification is DNA methylation, a covalent addition of a methyl (CH₃) group to the nucleotide cytosine. DNA methylation is maintained during cell division in mammals only at dinucleotide C-G (CpG), by virtue of the enzyme DNA methyltransferase I. This occurs because during semiconservative DNA replication, a methylated CpG on the parent DNA strand is partnered with a newly synthesized unmethylated CpG on the daughter strand. DNA methyltransferase I searches out this hemimethylated DNA and places a new methyl group on the daughter CpG.² An important environmental connection to epigenetics is that the source of methyl groups in this reaction is methionine, an essential amino acid, that is converted to a biologically active methyl donor state through a well-understood pathway that involves folic acid (FIGURE 1).³

Epigenetics, the study of non-DNA sequence–related heredity, is at the epicenter of modern medicine because it can help to explain the relationship between an individual's genetic background, the environment, aging, and disease. It can do so because the epigenetic state varies among tissues and during a lifetime, whereas the DNA sequence remains essentially the same. As cells adapt to a changing internal and external environment, epigenetic mechanisms can remember these changes in the normal programming and reprogramming of gene activity. The common disease genetic and epigenetic (CDGE) model provides an epidemiologic framework that can incorporate epigenetic with genetic variation in the context of age-related susceptibility to disease. Under CDGE, the epigenetic program can modify the effects of deleterious genes or may be influenced by an adverse environment. Thus, including epigenetics into epidemiologic studies of human disease may help explain the relationship between the genome and the environment and may provide new clues to modifying these effects in disease prevention and therapy.

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A second well-studied example of epigenetic change is chromatin modification, specifically, covalent modifications of the histone proteins that make up the nucleosomes around which the DNA double helix is coiled, approximately 2 turns of 200 base pairs, including the linker DNA between each nucleosome. These chemical modifications also include methylation but in this case involve the amino acids arginine or lysine, as well as phosphorylation of serine, acetylation of lysine, and ubiquitinylation of lysine.⁴ Unlike DNA methylation, the mechanism of maintaining chromatin modifications during cell division is not well understood because no enzyme has yet been identified that recognizes chromatin modifications from the parent cell and reproduces them in the daughter cell.⁵ Other examples of epigenetic infor-

mation are the density of nucleosome packing along the DNA, the complex of DNA and nucleosomes with specific proteins that recognize methylated DNA or modified histones, and the higher-order topologic organization of all these elements into complex structures that are only beginning to be recognized in the laboratory.⁴

What is the effect of these epigenetic changes? The simplest answer is that they regulate gene expression. For example, DNA methylation has traditionally been thought to be found with silenced genes. More than 100 specific chromatin modifications have been

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discovered, some of which exist in association with actively transcribed genes and others with silenced genes.⁴ The relaxation of condensed nucleosomes is important for gene activity, and a key insight published more than 10 years ago was that proteins that cooperate

with transcription factors in activating or silencing genes act by acetylating or deacetylating histones, respectively.^{6,7} A more subtle change is that epigenetic modifications as a group may define a higher-order structure within the nucleus. Recent studies using new

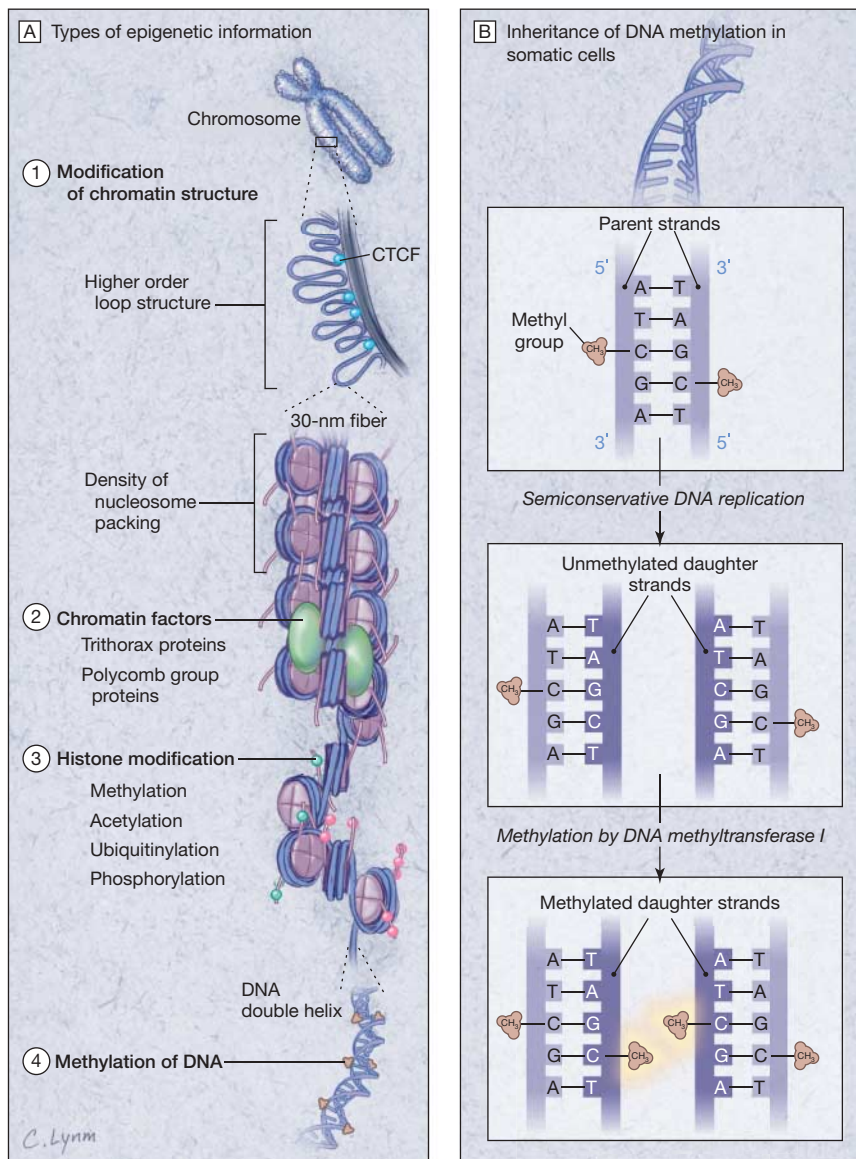
methods, such as chromatin conformation capture to identify long-distance DNA interactions, have revealed that groups of genes may change their physical relationship with one another, depending on their transcriptional state.^{8,9} Similarly, important differences in tissue-specific gene expression are controlled by enhancer sequences on the DNA, and the physical relationship between these enhancers and the promoters, ie, the elements to which the transcriptional machinery binds to activate gene expression, is controlled in part by the methylation of insulator sequences and the resultant folding of gene regions into loops of various sizes, depending on the state of the cell.¹⁰

Modern Epigenetics Is at the Heart of Developmental Biology

Perhaps the most important aspect of epigenetics is that the modern definition and Waddington's definition have converged because the epigenetic state of an organism has a lifecycle, whereas the DNA sequence does not (FIGURE 2). Epigenetic marks distinguish most of the important developmental properties of tissues from one another. For example, stem cell biology has now achieved the point at which differentiated somatic cells can be restored to a pluripotent state, ie, induced progenitor stem cells.¹¹ But clearly, the DNA in these cells has not changed, and without interference from the investigator, the pluripotency of DNA or lack of such capacity is relatively stable, ie, heritable during cell division. Thus, an epigenetic program must underlie these state changes.

Similarly, reprogramming of somatic cells and cancer cells by nuclear transplantation shows that information successfully transmitted during cell division for years or even decades can be erased and reprogrammed epigenetically.¹² This difference between stem and somatic cells extends to individual cell types. After all, how can a liver cell know to divide to form 2 liver cells rather than brain cells or heart cells, without some embedded epigenetic memory? Recently, it was shown that stem cells

Figure 1. Types of Epigenetic Information and Epigenetic Inheritance



A, Types of epigenetic information. The term *epigenetics* refers to modifications of DNA or associated factors—aside from variations in the primary DNA sequence—that carry information content and are maintained during cell division. Examples of epigenetic modifications are DNA methylation, histone modifications, occupancy of chromatin factors, and changes in chromatin structure. CTCF indicates CCCTC-binding factor. B, Inheritance of DNA methylation. In somatic cells, epigenetic information is replicated during mitosis along with the DNA sequence. The mechanism for replication of DNA methylation is well understood but the mechanism for replication of histone modifications is not.

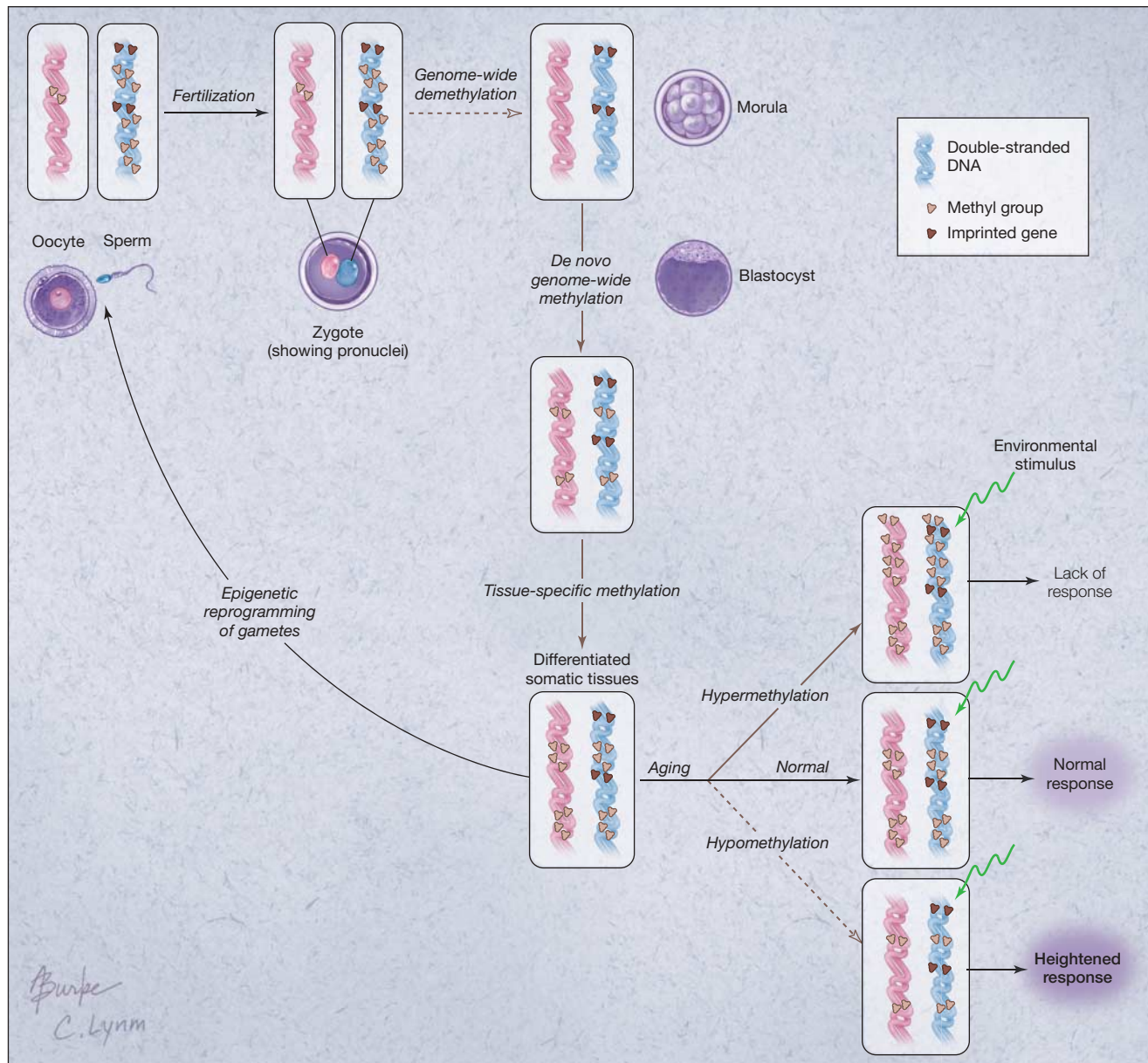
carry “bivalent” chromatin marks, ie, both on and off, on some genes, which then commit to one or the other state in differentiated tissues.¹³ Understanding the epigenetic correlate of tissue-specific differentiation is one of the great challenges of modern developmental biology.

Epigenetics of Disease: Disruption of Normal Phenotypic Plasticity

The first example of a human disease with an epigenetic mechanism was cancer. In 1983, widespread loss of DNA methylation was observed in colorectal cancers compared with matched normal mucosa from the same patients.¹⁴

This hypomethylation has been shown to lead to abnormal activation of genes in cancer, as well as genetic instability and chromosomal rearrangements.² Subsequently, hypermethylation of gene promoters was reported for a number of tumor suppressor genes in cancer.¹⁵⁻¹⁷ Epigenetic activation and si-

Figure 2. Life Cycle of the Epigenome



Unlike the DNA sequence, the epigenetic code changes during one's lifetime in ways specific to a given cell type. Shown here are a sperm, which is highly methylated, and an egg, which is not. After fertilization, there is a wave of demethylation that spares imprinted marks (dark brown). Tissue-specific methylation patterns emerge during later embryonic development. Age-related hypermethylation or hypomethylation could theoretically impair or enhance normal gene responsiveness to environmental signals.

lencing of genes in cancer turn genes on that should be off and vice versa. In fact, each type of normal epigenetic mark described earlier is altered in cancers, including abnormal histone modifications; excess of chromatin factors such as trithorax group proteins that promote gene expression, such as ALL1 in acute lymphocytic leukemia; and polycomb group proteins that repress gene expression, such as EZH2 in metastatic cancers.²

Single gene disorders of the epigenetic machinery also impair normal gene expression. For example, Rett syndrome, which involves progressive loss of developmental milestones caused by abnormal gene expression in the brain, is caused by lack of a normal MeCP2 protein that recognizes methylated DNA and thus helps to repress gene expression.¹⁸ Immunodeficiency, centromeric instability, and facial anomalies syndrome is caused by loss of DNMT3B, a DNA de novo DNA methyltransferase that adds methyl groups to CpGs where they were not present before.¹⁹ Affected cells abnormally express genes involved in immune function, neural function, and development.¹⁹

The unifying theme of epigenetic disease is disruption of normal phenotypic plasticity. Just as epigenetic change is at the heart of normal development, so also do disruptions in epigenetic modification disturb normal developmental programs. Thus, single gene disorders such as Rett syndrome show abnormal brain reprogramming in development, and complex traits such as cancer involve disruptions of the normal commitment of differentiating cells in tumors to specific patterns of active and repressed genes.²⁰

Age-Related Disease and the Common Disease Genetic and Epigenetic Hypothesis

Clinical medicine deals more with delaying and mitigating the effects of aging than reversing and eliminating disease, particularly as the baby boom generation grows older, because all organ systems function more poorly with time among individuals and among tis-

ues within individuals. Dan L. Longo, MD, scientific director of the National Institute on Aging, defines aging as a loss of phenotypic plasticity over time (written communication, February 8, 2008). This loss of responsiveness to stress also exacerbates the effects of underlying genetic variant-associated disease, accounting at least in part for the age dependence of common disorders such as heart disease, diabetes, and acquired intellectual impairment. But what accounts for this loss of responsiveness? Could lack of responsiveness interact at the level of the DNA with disease-predisposing genetic variation?

Fallin, Bjornsson, and Feinberg²¹ have proposed a model that could provide an epigenetic explanation to these questions. The common disease genetic and epigenetic (CDGE) model overlies the genetic variant hypothesis of disease, with an epigenetic component interacting with it. This could occur in several ways, first by environmental factors modifying epigenetic marks on the DNA or chromatin. DNA methylation depends on dietary methionine and folate, both of which are affected by nutritional state. Studies in mice have shown that reduction of dietary methionine can affect coat color by altering DNA methylation of the *agouti* gene.²² Simply feeding rats a low-methionine diet causes them to develop liver cancer at high frequency through hypomethylation of their DNA.²³

Under CDGE, the epigenome could also interact with the genome indirectly. For example, factors such as DNA methyltransferase 1 and MeCP2 are encoded by genes, and variants in their sequence could act generally on disease susceptibility by affecting the fidelity of the DNA methylation machinery. A hint of such a mechanism comes from the worm *Caenorhabditis elegans*, in which genetic variants that affect many signaling pathways appear to encode chromatin-modifying genes.²⁴ Conversely, mutant proteins encoded by conventional DNA variants might not have an effect if their expression is repressed epigenetically. A

striking example of this phenomenon was shown in flies by Rutherford and Lindquist.²⁵ Heat shock, a form of stress, lifted an epigenetic veil, allowing latent mutant genes to be expressed at high frequency.^{25,26}

Thus, the loss of responsiveness that defines aging could easily be one more example of the disruption of phenotypic plasticity that defines epigenetic disease (Figure 2). If this idea is correct, then there should be measurable changes in epigenetic marks throughout an individual's lifetime.

A hint of such an effect comes from a study of identical twins of different ages that suggested a greater discordance in epigenetic marks such as DNA methylation in older twin pairs compared with younger twin pairs.²⁷ However, the study did not examine the same individuals serially over time, so we cannot know from these data whether DNA methylation changed or was different historically. A subsequent study found no age-related variation in DNA methylation²⁸ but also did not track individual patients over time.

A Paradigm for Epigenetic Epidemiology: Beckwith-Wiedemann Syndrome

Epidemiology is the study of disease in populations, and advances in the epigenetics of disease can come only from a new discipline of epigenetic epidemiology. Just as CDGE is layered on traditional models of genetic variation and disease, so also is epigenetic epidemiology layered on traditional epidemiologic tools of case-control design, exposure measurement, and statistical assessment of risk. Added to this are epigenetic measurements and innovations in statistical analysis to deal with the lack of traditional transmission under Mendel's laws. For example, imprinted genes, in which the expression of individual alleles depends on their parent of origin, require new ways to model penetrance.

A first step in this nascent field was a study by DeBaun et al,²⁹ who established a population-based registry for Beckwith-Wiedemann syndrome

(BWS), which is characterized by prenatal overgrowth, midline abdominal wall defects, ear creases or pits, neonatal hypoglycemia, and a high frequency of Wilms and other embryonal tumors, such as rhabdomyosarcoma and hepatoblastoma. BWS is a paradigm for understanding the epigenetics of cancer because it is a rare familial disorder caused by epigenetic changes in several genes, just as Li-Fraumeni³⁰ is a paradigm for understanding the traditional genetics of cancer because it involves familial transmission of mutations in the *P53* gene. The idea for both BWS and Li-Fraumeni is that what occurs constitutionally in these well-defined syndromes may also occur as somatic alterations in common tumors, a principle of conventional cancer epidemiology pioneered by Knudson.³¹

DeBaun et al²⁹ designed a BWS registry, with hundreds of families rigorously examined by a team of specialists and with detailed clinical and family histories, documented with hospital records, allowing to be performed the first epigenotype-phenotype study for a human disease.²⁹ The risk of each of the clinical stigmata of BWS could be determined with respect to the molecular defects. The first of these is loss of imprinting (LOI) of the insulin-like growth factor-II gene (*IGF2*), an imprinted growth factor gene normally expressed only from the paternally inherited allele but in BWS expressed from both paternal and maternal copies. It had been shown earlier that LOI of *IGF2* occurs in about half of embryonal tumors of all types and is also 5-fold more frequent in adult patients with colorectal cancer, suggesting that LOI may confer general cancer risk.³² The frequency of LOI in BWS is approximately 15%.²⁹ Other alterations in BWS involve other imprinted genes in the same chromosomal region of chromosomal band 11p15: rare mutations in *p57^{KIP2}*, a gene that inhibits cell cycle progression; commonly LOI of *LIT1*, an antisense RNA that regulates *p57^{KIP2}*; and uniparental disomy that essentially replaces the maternal alleles of all

of these genes with a second paternal copy.²⁹

The most important result of this study was that cancer was explained specifically by LOI of *IGF2*, even though that epigenetic change accounted for only 15% of the patients.²⁹ Furthermore, virtually all of the patients with LOI of *IGF2* develop premalignant lesions in the kidney, termed *nephrogenic rests*.³³ These were the first data establishing from an epidemiologic perspective that an epigenetic change causes human cancer because in a population of patients, the epigenetic exposure (LOI of *IGF2*) was shown to be specifically associated with cancer risk.²⁹ The other epigenetic changes contributed to the other phenotypes, LOI of *LIT1*, *p57^{KIP2}* mutations to overgrowth and midline abdominal wall defects, and uniparental disomy itself to hypoglycemia, probably because of the involvement of another gene within the region, possibly insulin.²⁹

Bringing the Methylome to Medicine

More extensive studies of the epigenetics of disease are beginning to be performed, including analysis across the genome and of larger populations. These studies depend on methods that can assess the state of the epigenome comprehensively at millions of sites. An approach my collaborators and I are applying to common disease, including neuropsychiatric disorders such as bipolar disorder and autism, is comprehensive high-throughput array-based relative methylation analysis. The method interrogates more than 2 million CpGs throughout the genome, without bias toward assumptions about where methylation differences might arise. Because this method involves hybridizing processed samples to arrays, it is necessary to account for differences in hybridization efficiency that are determined by the CpG density, which is done by genome-weighted smoothing of the raw differences in apparent methylation.³⁴ Other array-based approaches that allow analysis across the genome include *HpaII* tiny fragment en-

richment by ligation-mediated polymerase chain reaction³⁵ and methylated DNA immunoprecipitation,³⁶ and these have already shown the ability to identify methylation differences among tissues and in disease, notably, cancer.

One of the greatest promises of epigenetics for medicine is the possibility of new therapies because epigenetic changes are by definition reversible, unlike sequence mutations in disease. One intensively studied approach to epigenetic therapy involves the use of agents that modify the epigenome globally, such as inhibitors of DNA methylation or histone acetylation. Clinical trials using such agents have been initiated for myelodysplasia.³⁷ One precautionary note in such global approaches is that using drugs that modify the methylome globally can have unexpected effects (or even paradoxical effects) on genes that are not desired targets; for example, 5-aza-2'-deoxycytidine, which inhibits DNA methylation and is being tried clinically to reactivate silenced tumor suppressor genes, can silence as many genes as it can activate.³⁸

An alternative approach to exploiting epigenetic discoveries is targeting the biochemical pathways that are disturbed epigenetically in disease, using "conventional" medicinal chemistry. For example, LOI of *IGF2* enhances signaling through the IGF-II/IGF1 receptor (IGF1R)/Akt phosphorylation pathway, a surprising result because it is an example of positive feedback to the signaling receptor after exposure to the ligand. This abnormality in the signal transduction cascade can be exploited by inhibiting IGF-II signaling at its receptor (IGF1R), which leads to marked inhibition of carcinogenesis in animals.³⁹ Both the epigenome-targeted and pathway-targeted approaches offer opportunities for disease treatment and prevention that could not have been imagined before the epigenetic era.

The most exciting medical idea in epigenetics is that it might be possible to intervene at the junction between the genome and the environment, to modify the effects of deleterious genes, and to influence the effects of the en-

environment on phenotypic plasticity, perhaps influencing aging or mastering tissue reprogramming in regenerative medicine. It is fascinating that the roots of epidemiology derive from the recognition that hidden particles that can travel through the environment cause disease.⁴⁰ So too it might be found that an epigenetic code that is just becoming revealed translates the relationship between the environment and human health.

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The secret of all those who make discoveries is that they regard nothing as impossible.
—Justus Liebig (1803-1873)