CHAPTER 4

# Genetic and Epigenetic Processes in Infant Mental Health

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Health, behavior and physiology all change, interdependently, within an individual, as he or she grows and develops. Mental health experts realize that these changes are influenced by the unique environmental and social experiences of the child, and developmental science is devoted to describing the individual as an interplay of within- and between-individual forces that interact across time. Biological and socioemotional domains operating across specific environment and developmental contexts define the developing human being (Gottlieb, Wahlsten, & Lickliter, 1998). Our goal in this chapter is to characterize the way that biological processes, specifically genetic and epigenetic regulatory processes, interact with the environment across development, with a focus on the earliest years. We hope this focus will help to clarify how infant development uniquely unfolds.

## Genetics

The modern study of genetics began in 1953, with the discovery of the double-helical nature of DNA (Watson & Crick, 1953). The discovery of DNA and subsequent unraveling of its structural detail stands as one of the most transformative events in the history of biology. DNA sequencing techniques were developed in the following two decades (Sanger & Coulson, 1975), primarily by Frederick Sanger. DNA sequencing was scaled up and commercialized in the 1980s. During this same time, the groundwork for the Human Genome Project was laid. In 1990, the guidelines for the first 5 years of a proposed 15-year project were laid out to map the human genome. Technological advances sped the rate of scientific discovery to the extent that new governance and an expedited timeline was issued in 1993. In 2001, the Human Genome Project published the results of 90% sequencing of the human genome (Lander et al., 2001).

This historical perspective is more than just interesting anecdote. The roots of genetic inheritance extend back to Gregor Mendel, but in the last 60 years, gene science has transformed exponentially and is currently a revolution in progress. The ideas contained in this chapter represent our best understanding, at present, of the way genes and the environment interact, but this understanding is subject to epistemic calibration as the field continues to mature.

One of the founding goals of the Human Genome Project was to map the sequence of nucleotides that constitute the human genome. *Nucleotide* refers to a nitrogen base (in the case of DNA: adenine, thymine, guanine, cytosine) connected to a phosphate "backbone." Nucleo-

tide bases pair together, forming base pairs (BPs). The hybridization (bases pairing in various combinations) that occurs in base pairing creates the DNA double helix. *Gene* refers to the sequence of nucleotides that inform a protein, but many more nucleotides are needed for making proteins than are present in actual protein coding regions.

Nucleotides are transcribed into proteins through a process of transcription and translation (see Figure 4.1). Genes are characterized, in vertebrates, by short exons and longer introns (De Conti, Baralle, & Buratti, 2013), both referring to sequences of nucleotides. The difference is that exons contain regions of the genome that code for proteins and introns do not. Introns and exons are both read from the genome through a process called *transcription,* which results in a messenger molecule known as pre-mRNA (*pre* because it still contains introns). Introns are removed through a process known as "RNA splicing" (carried out by the spliceosome), and a messenger molecule (mRNA) is left. This messenger can be decoded by a ribosome to form a protein in a process known as "translation."

When the sequence of nucleotides in DNA differs between individuals at a single point, it is known as a single-nucleotide variation (SNV). If this variation at a single nucleotide occurs regularly (traditionally defined as occurring in greater than 1% of the population), it is called a single-nucleotide polymorphism (SNP) (Shaw, 2013; see Figure 4.2). SNPs are the most frequent type of variation in the human genome (Wang et al., 1998). Millions of these variations have been located, characterized, and catalogued since the late 1990s. Where the SNP occurs is important for functionality—variations in nucleotide sequence in introns or intergenic portions of DNA will have different consequences than those that occur in exons. If an SNP occurs within a gene, the gene is said to have *alleles*—alternate forms of the gene.

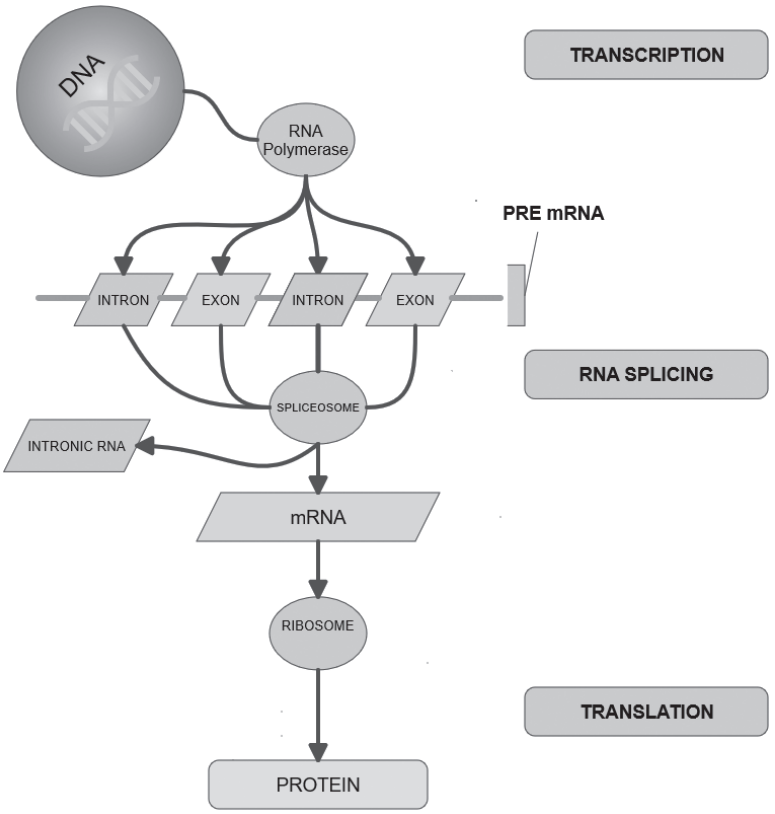


**FIGURE 4.1.** The process of transcription and translation.

Therefore, SNPs are a type (but not the only type) of gene polymorphism, and are utilized in the mapping of *quantitative trait loci (QTLs)*—regions of DNA that correlate with differences in phenotypes.

Mental health practitioners are likely familiar with the association of polymorphisms with health and behavioral outcomes. One frequently cited example is the serotonin transporter. A polymorphism of particular interest is the length of the serotonin transporter gene linked polymorphic region, sometimes referred to as the long and short allele of the serotonin transporter. In a study now cited over 7,000 times, Avashalom Caspi and colleagues (2003) found that participants with the short allele of the serotonin transporter who experienced many stressful life events were more likely to experience depression. However, in a recent meta-analysis of 31 datasets characterizing approximately 40,000 individuals, this finding did not reproduce (Culverhouse et al., 2018), leading the authors to conclude that any interaction between stressful life events, the short allele of the serotonin transporter, and depression must be of "modest effect size and only observable in limited situations" (p. 1). This reservation about the magnitude of effect is broadly applicable given the interactive nature of genes and environment in gene expression and the limited impact of the majority of polymorphisms. The issue of replication has received increased attention since 2011. A 2015 report of 100 replication attempts across three psychology journals by the Open Science Collaboration found that only 39% of studies replicated. Biological science is undergoing an important period of self-evaluation, and direct or conceptual replication attempts are ongoing (Nosek & Errington, 2017).

## Epigenetics

In general, epigenetics can be thought of as nuance in transcription and translation. This chapter explains much of the variance that occurs, from the sequence of nucleotides that makes up DNA to the proteins that comprise and regulate cells.

*Epigenetics* is the study of how genes are regulated, without change to the actual sequence of DNA in a cell. Functionally, epigenetics is the study of changes in gene expression, and sits at the center of the gene–environment balance. We focus on epigenetics in this chapter precisely because the epigenetic layer is susceptible to environmental influence. Epigenetics helps explain the perplexing reality that DNA is unchanging,

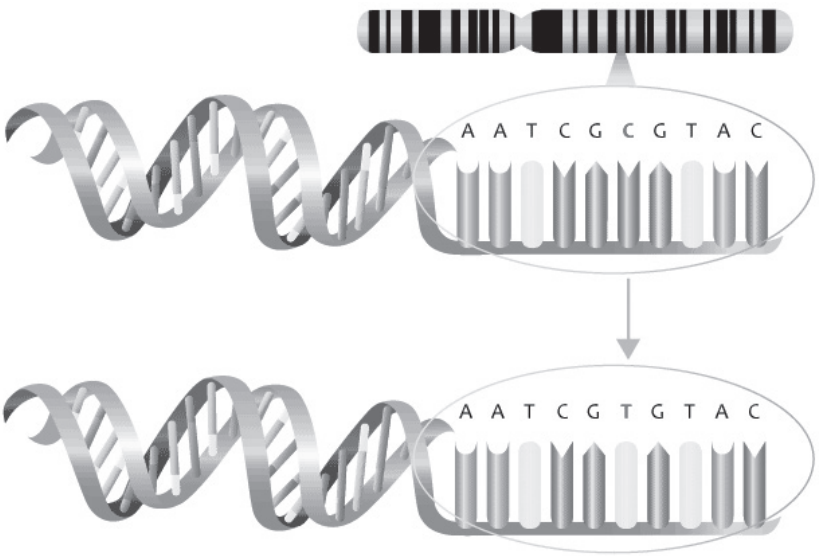


**FIGURE 4.2.** The structure of a single-nucleotide polymorphism. From Wikimedia Commons. Retrieved from *https://commons.wikimedia.org/wiki/File:Single_nucleotide_polymorphism_substitution_mutation_diagram_-_cytosine_to_thymine.png.*

yet each individual develops differently. The genome alone is insufficient to explain such developmental complexity. Epigenetic processes represent a vital line of inquiry for describing between- and within-individual change over time. Given that the core processes that constitute epigenetics can be environmentally mediated, epigenetics is emerging as a key set of biological processes for study in environmentally informed developmental science across the lifespan. We propose that epigenetics is a core part of the underlying mechanism that links development, biology, and the environment, shaping both within- and between-individual differences across the lifespan, and potentially even across generations, and describe how some of the most common epigenetic processes work. It is our hope that this approach will demystify epigenetics and make these processes more approachable and familiar to infant mental health practitioners.

### Definitions and Historical Context

Epigenetics received increased attention in the previous decade, despite the fact that Waddington coined the phrase in the 1940s (Goldberg, Allis, & Bernstein, 2007; Waddington, 1956). Epigenetics was an attempt to reconcile and merge the discrete fields of developmental biology and genetics (Holliday, 2006) into a new theory of human development stipulating that the early embryo was undifferentiated, and species-specific differentiation took place "on top of" genetic programming. Early conceptualizations of epigenetics encapsulated any process that modified the relationship between *genotype,* the specific alleles within an individual inherited from their parents, and *phenotype,* the outward expression of a specific trait or set of traits that defines an individual. Waddington was fond of referring to the "epigenetic landscape," which he drew as a physical plane (defined by genes) shaped into hills and valleys (the epigenetic regulatory layer) that combined to determine how a ball rolling downhill would move (cellular differentiation) (see Figure 4.3). Over the course of development, these hills become grooves as experience "canalizes" development along a particular trajectory. The term (and the field) have evolved over time and added mechanism to this model. *Epigenetics* at present refers to the study of the molecular processes that lead to changes in gene expression but are not the result of alterations in the DNA sequence (Dupont, Armant, & Brenner, 2009; Moore, 2015; Morris, 2001). The scope of this term is relevant for infant mental health professionals, as epigenetics is centrally viewed as the direct interaction between development, genes, and the environment.

### Epigenetic Processes and Infant Mental Health

Infant mental health specialists know that early development is a time for rapid change and

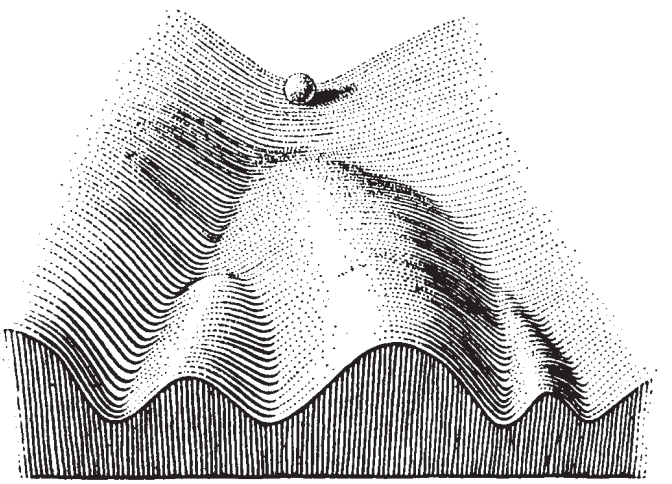

**FIGURE 4.3.** Waddington's epigenetic landscape. From Waddington (1957). Copyright 1957 by Routledge, reprinted by permission of Taylor & Francis Books UK.

molding. Epigenetic processes unfold across the lifespan, but substantial remodeling of both epigenetic marks and neural circuitry occurs early in development, which suggests that the pre- and postnatal windows are valuable targets for setting the trajectory of human development. From this perspective, epigenetics helps solve the conundrum that results from the fact that the same genotype can lead to varied outcomes.

Extending the idea that epigenetics operates at the intersection of social and environmental context, biological adaptation within the individual suggests a potential mechanism explaining the developmental origins of health and disease (Barker, 2004, 2007). This developmental theory suggests that disparities in disease and mortality emerge from early experiences (Godfrey & Barker, 2001). Developmental origins theory extends to infancy and even prenatally, and acknowledges that the environment in the earliest years of life shapes health trajectories. Functional differences in the cardiovascular, neuroendocrine, and immune systems also vary based on epigenetic differences (Mathews et al., 2011; McGowan et al., 2009; Saban, Matthews, DeVon, & Janusek, 2014). These same physiological systems are altered throughout the lifespan, potentially in response to negative early life experiences (Bogdarina, Welham, King, Burns, & Clark, 2007; Miller & Ho, 2008; Seckl & Holmes, 2007). Given these convergent lines of research, it is worthwhile to account for epigenetic changes associated with environmental perturbations that contribute to health and behavioral outcomes. This vantage point highlights the importance of early adversity, and epigenetic processes may provide novel mechanistic insight into the link between early experience and mental and physical health. These epigenetic processes likely precede detectable changes in developmental and health outcomes, setting the stage for targeted interventions long before adverse outcomes have canalized development along an adverse trajectory (Waddington, 2012).

### Mechanisms

DNA is the repository of transferrable information in living organisms, transcribed into messenger RNA (mRNA), and translated into proteins. Protein formation is neither as simple nor as linear as this description suggests. DNA is like a library: The genome represents a profound amount of information that is tightly packaged into chunks that are accessed and read for meaning. Most DNA is not expressed into proteins, just as most library books remain on the shelves. If DNA is a library, epigenetic regulation is the librarian: selecting, organizing, promoting, and restricting access.

Functionally, *epigenetics* refers to numerous discrete but interoperative processes that modulate gene expression at different stages in the pathway from a gene to protein product. This term represents a related, often coordinated, set of processes, *but does not refer to any single molecular process*. These processes control access to DNA, the stability of RNA transcripts, and the efficiency of translation into proteins. Epigenetic processes may exist in equilibrium with each other, as is common in many regulatory and counterregulatory biological processes that promote adaptation within the organism. This is worth emphasizing as our understanding of the diversity and intricate complexity of epigenetic processes continues to grow.

New molecular processes of epigenetic regulation continue to emerge, driven by technological advances in molecular biology. There is already debate about what processes are truly epigenetic. *Telomeres,* the caps at the ends of chromosomes that regulate cellular senescence, may be considered epigenetic because they are modifiable by experience and can influence gene expression. A counterargument suggests that telomeres are not epigenetic because changes in telomere length constitute loss of DNA sequences (remember that we defined *epigenetics* as a change in gene expression without a change in the underlying sequence of nucleotides). Epigeneticists are forced to ask: Are telomeres an epigenetic mechanism in and of themselves? Or are telomeres the outcome of an epigenetic mechanism? This small example illustrates the difficulty of applying simple labels to these complex phenomena, and as the science driving epigenetics matures, it is possible we will discover that both views are true: Telomeres may be epigenetic markers whose length is regulated epigenetically. In summary, epigenetics should be regarded as an emerging discipline that encompasses an ever-increasing variety of molecular processes. For the sake of imposing structure and to characterize the best understood mechanisms to date, we highlight three core processes: methylation, histone modification, and noncoding RNA effects.

### *Methylation*

DNA methylation was first characterized in 1980 by Razin and Riggs. Methylation modulates nucleotides, the basic structural unit of DNA, by adding a methyl group to the nucleotide cytosine. This conversion mostly (but not exclusively) occurs at cytosine–phosphate–guanine dinucleotides, which in mammals tend to be concentrated in the regulatory regions of genes. These concentrations are referred to as "CpG islands." The addition of methyl groups influences DNA structure in a repressive way and also interferes with transcriptional machinery reading DNA to convert the DNA sequence to mRNA. A common analogy is that methylated CpG sites act as a speedbump for transcription or, returning to the library analogy, a librarian restricting access to a book. Methylation outside of CpG islands also occurs, and functions to either suppress or increase gene transcription depending on location. In general, greater than 70% of CpG sites are methylated, but the percent of specific cites methylated depends on tissue type and varies far more than originally thought (Strichman-Almashanu et al., 2002; Suzuki & Bird, 2008).

DNA methylation was initially considered a static process, which means that once methylated, sites would remain methylated forever. However, methylation is now recognized as a dynamic process. The clearest evidence for the dynamic nature of methylation is found in twin studies in which increasing epigenetic divergence is found over the lifespan (Fraga et al., 2005; Kaminsky et al., 2009; Petronis et al., 2003). DNA methylation is highly conserved across species, existing in almost all multicellular organisms (Allis, Jenuwein, & Reinberg, 2007).

Methylation is typically associated with the suppression of gene expression (Jones & Baylin, 2007). However, suppression is not the *only* function of methylation. The eventual impact on gene expression depends on *where* the methyl group is located and how that specific genomic region influences DNA transcription. For example, methylation in a genetic region that silences other regions can functionally increase gene expression. An additional level of complexity arises around methylation of the structure DNA complexes around—the histone. This represents a growing area of active research, with significant implications for multilevel epigenetic regulation (Greer & Shi, 2012).

### *Histone Modification*

Much like books on a shelf in a library, DNA is stored on a scaffolding, termed "chromatin," which functions to organize and increase storage of genetic information. Chromatin, the complex of DNA with specialized histone and nonhistone proteins, is a critical component of epigenetic organization across the genome (Ridley, 2003) and comes in two distinct forms: *euchromatin,* which has a more open structure permissible for gene transcription, and *heterochromatin,* which is a tightly condensed form generally not transcribed into RNA. DNA is wound around four pairs of basic proteins, called *histones.* The specific complex of DNA around a histone is known as a *nucleosome,* a major functional epigenetic unit. The structure of the nucleosome has been compared to beads on a string, with the beads representing a nucleosome and the unwound DNA between the nucleosomes representing the string. Protruding from each of these octamers of histones is a tail that functions as a central site for dynamic changes in nucleosome structure. Modifying histone tails can result in global change to the histone structure and subsequent gene regulation conferred by the nucleosome (Taverna, Li, Ruthenburg, Allis, & Patel, 2007).

Histones are modified through *acetylation* and *deacetylation,* which are controlled by a series of enzymes that attach acetyl groups to, or remove them from, specific amino acids within the histone cores (Moore, 2015). In general, acetylation is associated with transcriptional activation, or "turning on" of a gene, through the local relaxation and unwinding of the chromatin structure and subsequent change in electrostatic charge of the histone tail (Allis, Jenuwein, Reinberg, & Caparros, 2007). Deacetylation is associated with transcriptional silencing through tightening of the chromatin spool. Recently, new histone modifications have been identified that involve the addition of different basic compounds to the histone (Sidoli, Cheng, & Jensen, 2012). The wealth of chromatin-associated catalytic enzymes, as well as the large number of environmental and pharmacological agents (including several used to treat psychological disorders and seizures) that modify chromatin by adding (or subtracting) subgroups represents another area of active research with significant implications for neurodevelopment and developmental psychopathology (Marmorstein & Zhou, 2014).

Histones are not modified in any single way. Instead, these processes represent dynamic and balanced forces that exist in a shifting equilibrium that controls the global level of accessibility of the nucleosome. This shifting equilibrium permits each cell to adjust gene expression and maximize functionality across different developmental stages and to adapt to environmental exposures and metabolic demand. This dynamic interaction of multiple organic subunits is referred to as "writing" and "erasing." For example, while histone acetylation may have a permissive effect on gene expression, a similar enzymatic addition termed *phosphorylation,* the addition of phosphoryl groups, typically condenses chromatin. Thus, phosphorylation may counteract the impact of acetylation, and the regulation of gene expression results from the relative balance between these factors, among others. This balance of competing modification of the histones is labeled the "histone code," as it reflects the molecular sequences that ultimately help to determine where, when, and how much a particular gene is transcribed (Jenuwein & Allis, 2001). For these reasons, histone modification is considered to be one of the more dynamic epigenetic processes.

The field of epigenetics is rapidly changing as scientific exploration reveals additional complexity and sequence variability that ultimately functions to change the scaffolding of the DNA through histone modification. Different primary, secondary, and tertiary structures of the underlying histone can result in greater or lesser degrees of affinity for subgroup binding. There are higher-order arrangements that impose themselves on accessibility: so-called "architectural factors" that inform patterns that affect transcription (Woodcock & Dimitrov, 2001; Zhang & Reinberg, 2001). These patterns expand on the historical "bead on a string" characterization of histones described previously, and are at the forefront of molecular epigenetic investigations. Designer chromatin, capable of revealing greater information into these higher-order structures, offers significant potential for explaining the molecular machinery underlying this additional layer of epigenetic control through histones (Fierz & Muir, 2012). As with many epigenetic processes, histones are conserved across species, but imperfectly, and a growing body of evidence suggests histone modification contributes to the within- and between-individual variation in response to the environment, particularly in infancy.

### *Noncoding RNA*

An emerging epigenetic process involving noncoding RNA (ncRNA) emphasizes the importance of transcription and translation for understanding epigenetic mechanisms. The genome has been called an "RNA machine" (Amaral, Dinger, Mercer, & Mattick, 2008), yet the mRNA that is translated into proteins is not the only type of RNA that influences levels of proteins and cellular functions. Indeed, protein-coding RNA regions are increasingly viewed as only a small portion of the genome. ncRNAs, defined by the fact that they are not translated into proteins, play multiple roles in the process of converting mRNA into protein, with some encouraging conversion, others inhibiting conversion, and still others influencing the rate at which mRNA is degraded and destroyed, thereby preventing the creation of additional protein products. These RNA molecules are coded for by DNA just like mRNA, and often in overlapping regions of the genome. These ncRNAs include transfer RNA (tRNA) and ribosomal RNA (rRNA), as well as an ever-growing collection of novel ncRNAs such as long noncoding RNA (lncRNA) and small RNA (sRNA) species such as micro RNA (miRNA). These RNA species are now thought to be involved with broad regulation of gene expression within specific molecular and/or metabolic pathways.

ncRNAs contribute to changes in gene expression by blocking or enhancing transcription and translation, as well as influencing the stability of existing mRNA and protein species through sequestration and/or targeting for degradation within the cell (Lai et al., 2013; Ørom & Shiekhattar, 2013). lncRNAs especially blur lines between category, mechanism, and site of action, and their study is akin to the search for "patterns in chaos" (Kung, Colognori, & Lee, 2013). Changes in expression of lncRNA can induce biochemical cascades that result in altered histone methylation (e.g., Gupta et al., 2010), and thus work indirectly through histone channels as upstream actors influencing proximal genome availability at the histone. Other pathways for lncRNA exist; for example, lncRNA can ease transcription (Lai et al., 2013) and is critical for the processes of X chromosome inactivation and imprinting (Kung et al., 2013).

In the nucleus, sRNAs operate mainly through *RNA interference* pathways: the process of targeting genes for inactivation at the transcriptional or posttranscriptional level. Thus,

sRNAs can facilitate histone and DNA methylation (Holoch & Moazed, 2015) or influence gene transcription through *cotranscriptional silencing,* the jointly regulated transcription of multiple genes (Moazed, 2009). Although methylation and histone modifications are more site-specific actors, ncRNAs often function to influence multiple genes and gene pathways, which lead to broad regulation.

This set of RNA interference processes is not only important mechanistically for proximal epigenetic impact on transcription and translation, but it can also act as a determinant of a putative "epigenetic memory" made up of self-reinforcing feedback loops. As an example, certain patterns of methylation are dependent on sRNA interference, but expression of the necessary sRNAs is dependent on the methyltransferase that facilitates methylation—forming a mutually dependent feedback loop between methylation and sRNA, which is thought to perpetuate and amplify over time. Several such patterns have been identified (Holoch & Moazed, 2015). These feedback loops, and the consequences they have for development, will likely be a key area of investigation in the future.

miRNAs also provide a valuable example of interdependent processes that operate summatively to change gene expression. These miRNAs are small ncRNAs that influence gene expression posttranscriptionally by binding to mRNA. This makes the mRNA less stable and diminishes translation rates (Chuang & Jones, 2007). The level of binding affinity in the miRNA–mRNA complex likely determines the effectiveness of mRNA degradation. Each miRNA has multiple targets, and each mRNA is likely regulated by multiple miRNAs. Furthermore, miRNA expression itself appears to be regulated epigenetically (Saito & Jones, 2006) and increasing histone acetylation or decreasing DNA methylation has been shown to increase production of miRNA (Han, Witmer, Casey, Valle, & Sukumar, 2007). The point here is not to encourage memorization of the process of ncRNA influence on transcription, but to illustrate that epigenetics is fundamentally a complex network of mutually informative processes, together creating a dynamic, environmentally and developmentally responsive cellular milieu.

In summary, each of these epigenetic processes, and their interaction, works to regulate the molecular machinery that is capable of biologically embedding environmental experiences into the developing child. Very simply, methylation modifies transcription by adding a methyl group to DNA. Histone modification changes how easy it is to transcribe DNA based on how tightly it is wound around histones. ncRNA activity, as a class of processes, is more complicated, but essentially functions to control levels of transcription and translation of the genome into proteins. These influence which proteins are made in each and every cell, and do so as a function of the environment.

### *The Environment Influences Epigenetics*

Understanding *how* common epigenetic processes likely operate is useful, but equally important for the field of infant mental health is to understand how individual differences in these processes emerge. There are two important caveats to this discussion. First, we use the framework that environmental stimuli predict individual differences in epigenetic signatures, but our discussion of this relationship is necessarily oversimplified. The complexity of epigenetic processes makes it clear that the sort of "if this, then that" descriptions necessary for modeling and describing environmental impacts on epigenetic factors is oversimplified. Imprecision stems from our inability to effectively capture all epigenetic processes simultaneously and the challenges of measuring epigenetic factors in peripheral tissues. Second, epigenetic change varies with development. The environment begins to impact epigenetic processes *in utero* and continues to act, to greater and lesser degrees, across the lifespan. Despite the fact that epigenetic profile changes from fertilization onward, a clear change in the environment exists across the developmental time span we focus on: birth. Because critical changes with lasting consequences for the baby can occur *in utero,* we include this stage in our discussion, but with a clear distinction between the environment *in utero* and the postnatal environment.

#### *Prenatal Experiences*

The epigenome is referred to as "an archive of the prenatal environment" (Heijmans, Tobi, Lumey, & Slagboom, 2009). Fetal development represents a critical period for epigenetic programming for both the fetus and subsequent generations. Two normative epigenetic processes that occur soon after fertilization set the

stage for the massive reorganization that occurs to the epigenome during prenatal development and function as epigenetic resets to "ground state" of the organism after fertilization. First, germ cells, the cells that become the eggs and sperm that create the next generation (von Meyenn & Reik, 2015), are formed during fetal development. As these primordial germ cells are created, there is an almost total resetting (erasing) of epigenetic marks. The second reset to ground and stripping of epigenetic marks occurs for the pluripotent cells that will differentiate into all the cells of the developing organism (O'Neill, 2015). The profound implication of these epigenetic resets is that *three generations are impacted simultaneously*: the mother, her offspring, and the germ cells for the next generation. That said, these processes are maturational epigenetic resets. On top of this normative process, histone modification, methylation, and potentially ncRNA changes are critical processes during embryogenesis that define the initial germlines and drive the differentiation of the myriad cell types that will later become the fetus and newborn.

DNA methylation is the most studied epigenetic process as an outcome of prenatal experience (Cao-Lei, Laplante, & King, 2016). Initially after fertilization there is rapid demethythlation of the paternal genome, followed by demethylation of maternal marks in the developing zygote (Gluckman, Hanson, Buklijas, Low, & Beedle, 2009) and subsequently tissue-specific parental methylation marks in some locations are reestablished, so methylation patterns are undergoing a huge amount of reorganization in the earliest phases of development. These are often influenced by environmental experiences. In addition to these developmental changes, epigenetic programming of the fetus is also affected by maternal factors, including nutrition, toxin exposure (e.g., substance use, lead), and maternal psychosocial stressors. From this vantage point, the womb is the environment of the developing baby, which is defined by the mother and, indirectly or dyadically, her environment.

Whereas many epigenetic resets are normative processes, individual differences emerge across offspring due to the extent, location, and timing of these processes. Epigenetic adjustments can also adaptively shape fetal development to fit the environment, even if that environment is characterized by adversity. A number of different experiences reportedly affect methylation in the developing fetus. Fetal alcohol syndrome disorder (FASD) results in structural and neurocognitive difficulties across the lifespan, and teratogenic effects of alcohol exposure have an epigenetic root, including change in methylation patterns (Haycock, 2009). Ethanol administration to mice during gestation results in global hypomethylation (Garro, McBeth, Lima, & Lieber, 1991). This finding is not ubiquitous, however, and certain loci are predicted to be hypermethylated as a function of prenatal alcohol exposure (Kaminen-Ahola et al., 2010). Nor is alcohol the only toxin with defined epigenetic risk for the developing fetus. Both global and gene-specific individual differences in methylation have been observed in offspring of mothers who smoked during pregnancy (Breton et al., 2009). Toxin exposure has also been demonstrated to impact overall methylation patterns in both the fetus and the placenta (Hou, Zhang, Wang, & Baccarelli, 2011; Perera & Herbstman, 2011).

Maternal anxiety, stress, and depression have been linked not only to changes in risk of child psychopathology and regulation of the child's hypothalamic–pituitary–adrenal (HPA) axis but also changes in methylation of the glucocorticoid receptor (GR) (Mulligan, D'Errico, Stees, & Hughes, 2012; Oberlander et al., 2008; Stroud et al., 2016). The GR is a critical regulator of the HPA axis through its binding to cortisol, the putative "stress" hormone (see Thompson, Kiff, & McLaughlin, Chapter 5, this volume). Cortisol results from activation of a biochemical cascade within limbic regulatory regions in the brain that are propagated from the hypothalamus to the pituitary to the adrenal cortex (see Thompson et al., Chapter 5, this volume). This pathway, referred to as the HPA axis is implicated in a variety of health and behavioral disorders across the lifespan and is one of the most investigated physiological substrates of disease etiology. The glucocorticoid receptor itself is within the cell but must be transported into the nucleus of the cell before it can have effect. Proteins, such as *FKBP5,* have been identified that control the movement of the receptor within the cell. Methylation differences in both survivors of the Holocaust and their offspring have been reported in the regulatory regions of *FKBP5* (Yehuda et al., 2016), suggesting that epigenetic differences in genes may lead to biological differences (e.g., HPA axis reactivity) as a result of early experiences. It is apparent that many environmental factors likely interact to

contribute to widening individual differences in phenotypes across fetal development that are propagated through well-characterized biobehavioral pathways, for example, the HPA axis, and have epigenetic roots (i.e., methylation of the GR receptor or *FKBP5*).

Though not as well characterized as methylation, histones also appear to be modified by the prenatal environment. Normative fetal development depends on histone modifications *in utero,* including neural sexual differentiation (Tsai, Grant, & Rissman, 2009) and lineage-specific pluripotent cell differentiation (Azuara et al., 2006). Histone modification is a principal mechanistic change resulting from fetal alcohol exposure, which is consistent with findings in both mouse and human models that histone modification is a primary outcome of binge drinking later in life (Haycock, 2009; Park, Lim, & Shukla, 2005; Shukla et al., 2008; Wong, Mill, & Fernandes, 2011). Other environmental toxins, such as arsenic exposure *in utero,* also lead to changes in histone acetylation with health consequences (Cronican et al., 2013).

Whereas DNA methylation (and, to a certain extent, histone modification) represent slow and relatively intractable forms of epigenetic modification, changes in ncRNA likely represent epigenetic modification on a fast scale. For this reason, and because this facet of epigenetics is most recently characterized, less is known about the prenatal environment and ncRNA. There is evidence that miRNA plays a role in normative development of the embryo across embryogenesis and gametogenesis, including cardiac, neuronal, muscle, and lymphocyte development (Stefani & Slack, 2008). This evidence largely emerges from mouse models and has been identified as an important step toward refining the etiological basis of genetic disorders that may manifest through epigenetic "missing pieces."

In summary, the prenatal environment can exert profound changes upon epigenetic processes. These changes impact long-term developmental trajectories. Individual differences in epigenetic processes emerge early. Even prenatally, there is evidence of environmental effects across methylation, histones, and ncRNAs. The field of infant mental health has long recognized that dynamic interactions between the mother and her developing infant during the prenatal period are linked to later socioemotional development. These effects are likely mechanistically mediated by epigenetic changes driven by the confluence of psychosocial, nutritional, and environmental experiences. These experiences can imprint in the developing fetus, creating epigenetic patterns that influence the developmental trajectory across the lifespan.

### *Postnatal Experiences: The Parent–Child Relationship*

Building on the prenatal period, there is evidence that the postnatal environment, and particularly the early parent–child relationship, influence epigenetic processes in the infant. Changes in methylation and histone modification have also been observed in this developmental window. As with the prenatal research, the best characterized epigenetic aspect in relation to postnatal experiences is in association with DNA methylation.

The first study that demonstrated epigenetic changes associated with maternal care was conducted by Meaney and colleagues (Weaver et al., 2004) This seminal set of experiments, performed in rats, demonstrated differential methylation of the hippocampal GR gene promoter as a function of naturally occurring variation in maternal behavior. Mapping differences in methylation as a function of maternal behavior established a correlation between maternal behavior and methylation. This was followed by an adoption study in which pups of high- and low-quality maternal behavior (i.e., licking and grooming) dams were cross-fostered, and resulted in a pattern of methylation consistent with the rearing mother. Inhibition of the methylation pathway blocked the positive effects of cross-fostering, demonstrating a causative epigenetic pathway (Weaver et al., 2004). The idea that *naturally occurring variability* in the quality of maternal care in the first days of life *caused* persistent epigenetic differences that were subsequently predictive of adult phenotypes was a paradigm shift.

Later preclinical studies have examined similar pathways and demonstrated multi generational effects of maternal maltreatment. As another example of postnatal environments exerting an epigenetic impact, rats exposed to abusive behavior in the first week of life show altered methylation of brain-derived neurotropic factor (BDNF) and attenuated gene expression (Roth, Lubin, Funk, & Sweatt, 2009). BDNF methylation is partially rescued through cross-fostering, suggesting that variability in maternal care influences diverse pathways and that recovery with later environmental changes

is not uniform across these pathways (Roth et al., 2009; Roth & Sweatt, 2011).

Attempts to replicate these findings in humans are increasing and mostly consistent. One compelling piece of evidence comes from postmortem tissue of suicide victims and controls. DNA methylation of the GR promoter region was equivalent among controls and suicide victims without a history of abuse. However, those with a history of abuse had both lower GR expression and increased DNA methylation in the regulatory region of the gene in brain tissue. These data suggest that childhood abuse altered the HPA axis epigenetically, and that these effects persisted across their lives (McGowan et al., 2009). The inability to use controlled trials and limited access to central tissues (e.g., brain samples) for epigenetic studies in humans represent barriers to direct replication from the rodent literature.

Epigenetic effects of postnatal environmental toxin exposure and food availability also exist. A protein-restricted diet in rats has been linked to methylation patterns that limit GR expression (Burdge, Hanson, Slater-Jefferies, & Lillycrop, 2007). Arsenic is a common toxin found in drinking water, and millions of people are exposed to arsenic each year in developing countries (Vahter, 2007). Arsenic has been linked changes in gene expression in both newborns (Xie et al., 2007) and adults (Zhou, Sun, Ellen, Chen, & Costa, 2008). Cadmium, a component of cigarettes, has also been linked to both global and site-specific methylation changes (Huang, Zhang, Qi, Chen, & Ji, 2008; Kippler et al., 2013), and nickel has been linked to global hypomethylation (Lee et al., 1995). Epigenetic modification has been put forward as a critical mechanism characterizing risk associated with toxin exposure, above and beyond the threat posed by toxin-induced mutations to the genetic code (Ray, Yosim, & Fry, 2014); in the future, characterizing both the timing and extent of these effects will likely clarify the mechanism by which early toxin exposure impacts health and behavior across the lifespan.

Histone modification has been documented across the postnatal window as well; in fact, the effect of the maternal–child dyad on individual differences in infant histone patterns is best characterized by postnatal effects (Champagne, 2010). Histone modifications that facilitate GR expression have been observed with a protein-restricted diet, in addition to methylation patterns that inhibit GR expression, furthering the idea that these epigenetic processes operate in a discrete and functionally balanced, coordinated capacity (Burdge et al., 2007). Histone modification extends to the postnatal mother–child relationship, again suggesting that there is a complex dyadic environment that shapes the infant's development. For example, increased acetylation of glutamic acid decarboxylase 1 (GAD1) observed in rat pups varies with maternal behavior (Zhang et al., 2010). Interestingly, licking and grooming predicts increased methylation of the GAD promoter as well, again highlighting the coordinated nature of epigenetic processes as a result of predictive stimuli.

In summary, a robust and compelling animal literature links epigenetic changes to postnatal experience. While these studies have not been fully replicated in humans, this is likely due more to the methodological difficulty and tissue requirements necessary for such studies than to the lack of actual effects.

### *Epigenetic Differences Matter for Mental and Physical Health*

We have described several core epigenetic processes and provided select examples for how individual differences in epigenetic processes emerge as a function of environmental experiences beginning prenatally and continuing across early development. Our review suggests that environmental factors may lead to increases or decreases in gene expression. This helps us understand how genotypes differentiate into variable phenotypes. Epigenetic processes illustrate the profound depth of environmental experiences that modulate the genome. Environmental experiences range from toxins to the subtleties of the parent–child relationship. Furthermore, critical developmental periods do not just begin in infancy, but probably extend to the moment of fertilization. Yet the implications of epigenetics extend further. An emerging literature suggests that epigenetics matters for mental and physical health and that individual differences in the epigenetic code may translate into lasting phenotypic changes.

Before making wide-ranging claims about the innovation of epigenetics, however, it is important to keep in mind that these pathways reflect the complexity of the bidirectional interplay between the environment and biology. Through linking environment, epigenetic change, and mental health, we can gain novel mechanistic insight into intervention and treatment plan-

ning, but to date, few studies have been able to integrate these processes, and those that have are limited to preclinical animal studies. Building testable models that include relevant genetic, environmental, epigenetic, and outcome variables is a task that strains the capacity of modern methodology, but it is a critical next step.

Some work has examined the relationship between individual differences in epigenetic markers and health and behavioral outcomes. In rodents, for example, methylation of mu-opioid receptor (*OPRM1*) DNA has been linked to adolescent rat pup licking and grooming behavior (Hao, Huang, Nielsen, & Kosten, 2011). Kinnally and colleagues (2010) have observed that differences in CpG methylation of the serotonin transporter exacerbated the effects of early life stress (rearing environment) on behavioral stress reactivity (maternal separation) in infant rhesus macaques; this effect was more powerful than the impact of genotype in their investigation. In maternally deprived infants, macaques with higher methylation, regardless of genotype, exhibited the most activity during stressful separation.

In humans, the search for epigenetic predictors of health and behavior in infancy is somewhat more elusive. In the behavioral realm, most of the work with infants links epigenetic between-individual differences to changes in stress response trajectory within individuals, as mentioned earlier.

The HPA axis is probably the most extensively characterized pathway linking early negative environments and mental health risk through epigenetics, but this requires synthesizing the literature linking environment to HPA-relevant epigenomic changes and the literature linking the HPA axis to health and behavior. Also, elevated BDNF methylation status predicts increased ventromedial prefrontal cortex and anterior cingulate cortex activity in adults who received lower-quality maternal care, suggesting an environment–epigenome–neural activity link in humans (Moser et al., 2015). Many investigations have been conducted later in life examining the relationship between epigenetic markers and schizophrenia, posttraumatic stress disorder, suicide, mood disorders, and other behavioral outcomes, and an emerging literature suggests strong reciprocal relationships between these disorders and epigenetic markers (Dalton, Kolshus, & McLoughlin, 2014; Labonte, Azoulay, Yerko, Turecki, & Brunet, 2014; Lee & Sawa, 2014; Rampp, Binder, & Provençal, 2014; Roth, Matt, Chen, & Blaze, 2014).

Still, the relative impact during early childhood remains understudied, despite extensive evidence implicating this as a critical window of epigenetic change. This makes sense given the difficulty of collecting longitudinal, robust human epigenetic data from infancy into adolescence and beyond, which is required for examining these relationships. Investigations of this nature will be critical going forward and perhaps addressed by the new Environmental influences on Child Health Outcomes (ECHO) program spearheaded by the National Institute of Child Health and Human Development (NICHD; *www.nih.gov/echo*).

Relatedly, it has been proposed that epigenetic effects of early social experience inform social behavior later in life. Social interactions during the neonatal period potentially organize the subsequent expression of behavior by altering sensitivity to neuropeptides and steroids such as oxytocin, vasopressin, and estrogen (Cushing & Kramer, 2005) likely through epigenetic alterations to genes critical to the creation, release, and cellular and metabolic activity of these neuropeptides. In studies of abuse and neglect, there is evidence of epigenetic changes in a broader number of biological pathways including a host of neurotransmitters (e.g., dopamine, serotonin, gamma-aminobutyric acid, glutamate) and other neuroepeptides such as vasopressin, oxytocin, estrogen, and corticotropin-releasing hormone (Bird & Lawrence, 2009; Champagne, Diorio, Sharma, & Meaney, 2001; Champagne, Francis, Mar, & Meaney, 2003; Curley, Jensen, Mashoodh, & Champagne, 2011; Insel, 1989; Korosi & Baram, 2008; Lukas, Bredewold, Neumann, & Veenema, 2010; Lupien, McEwen, Gunnar, & Heim, 2009; Ognibene et al., 2008; Veenema, Blume, Niederle, Buwalda, & Neumann, 2006; Zhang, Chretien, Meaney, & Gratton, 2005). One interesting extension of the maternally driven infant experience is that, as the child grows, his or her social experience expands. It has been postulated that the postweaning social environment represents a time of "epigenetic reversibility" to early postpartum experience, such as when high-quality child care may compensate for intrafamilial risk factors (see Trigg & Keyes, Chapter 37, this volume).

Some attempt has been made to link the relationship between environment, epigenetics, and health and behavioral outcomes of relevance to

infant mental health specialists. Lahiri, Maloney, and Zawia (2009) have proposed a latent early-life-associated regulation model for the etiology of neurobiological diseases, including autism, schizophrenia, bipolar disorder, Parkinson's, and dementias. This model suggests that epigenetic perturbations experienced early in life can be organized through early experiences but remain dormant until gross expression changes manifest as a function of later development. This model has a precedent: The organizational–activational model of sex differentiation operates on a similar platform (Arnold, 2009), as does the concept of "sleeper effects," in which deficits emerge later in life as a result of missing early-life stimuli (Maurer, Mondloch, & Lewis, 2007). However, the broad-reaching and etiologically diverse outcomes associated with this framework remain speculative until more data can be gathered.

As with similar models (see Barker, 2004; Del Giudice, Ellis, & Shirtcliff, 2011), the latent early-life-associated regulation model suggests that (1) the environment can change epigenetic marks; (2) prenatal and infancy periods are particularly susceptible to environmental perturbations; (3) these perturbations result in individual differences in epigenetic signatures; and (4) these signatures likely have lasting consequences in health and behavior. Common disorders thought to have roots in infancy are predicted to result from coordinated, environmentally mediated epigenetic regulatory processes layered on top of genetically defined networks of risk signatures encoded in the genome. This gene × epigene × environment equilibrium is shifting continuously across development, with different processes having different time signatures. The complexity inherent in this conceptualization is a case for great optimism because it places the environment center stage in the infant experience—and this is good news because the environment is putatively the easiest target for prevention and intervention efforts.

## Conclusions

Genetics details the sequence of nucleotides in a person, but genes are not destiny. These nucleotides form the template for transcription and subsequent protein coding in cells, but it is the epigenetic layer that operates to influence how genes are expressed. Epigenetic change sits at the fulcrum of several spheres (e.g., environmental, biological, social factors), and as such, it is exciting to consider how these spheres operate simultaneously as a function of multiple interdependent relationships. Traditional theoretical models of within- and between-individual change prioritize the infant window as an origin point for later health and disease, and epigenetics extends this vantage point through mechanistic pathways.

Biological pathways may represent an important metric by which to measure treatment efficacy. Hearkening back to Waddington (1956, 2012), we suggest the idea that the organism develops as an integrated whole, and biological and behavioral outcomes will converge as the organism's development adapts to the environment. Failure to correct the underlying biological "scars" may lead to undetected elevated risk that over time manifests as behavioral problems. Epigenetics illustrates that the environment not only matters at the level of behavior and socioemotional functioning but also leaves an imprint under the skin.

This emerging perspective has five important implications for clinicians. First, epigenetics is best conceptualized as an ongoing, reciprocal relationship between environment and biology, as opposed to a linear relationship of cause and consequence. This developmental perspective introduces more variability into the developing human and minimizes the role of discrete pathological states in favor of a network of fluctuating processes.

Second, even small disruptions in the formative time frame of infancy can, unfortunately, have profound impacts across the life course if left uninterrupted. Epigenetic changes operate mechanistically on the root code of human beings. Decreasing flexibility was emphasized by Waddington's (1956, 2012) notion of experiential canalization, which increasingly narrows over time. Alternatively, epigenetics also allows ontogenetic change (albeit slowly) to unfold, rather than allowing individuals to be constrained by their phylogenetic heritage. The nature of a flexible, malleable system of regulation of gene expression is that environmental impacts can have outsized effects over compounded time as epigenetic flexibility narrows.

Third, developmental malleability operates as both a risk factor and an opportunity. Even though the empirical focus of epigenetic investigation to date has been on negative experiences, the malleability of epigenetic pathways also represents an opportunity through which

prevention and intervention efforts may result in profound positive changes.

Fourth, epigenetics cannot be considered a single phenomenon, but instead is best thought of as a set of interactive processes that achieve a specific downstream goal of altering gene expression. This complexity illustrates that, fundamentally, epigenetics is an adaptive process, wherein multiple regulatory and counterregulatory processes operate in a dynamic that can only be understood in a sense of the context to which the individual is adapting.

The fifth implication is perhaps most relevant, yet controversial for infant mental health experts. Because epigenetic processes are linked but discrete, environmental change can propagate across these epigenetic processes. Methylation, histone modification, and ncRNA regulation work in a coordinated fashion and therefore changes that shift equilibrium points in any one of these systems will likely be felt across all of them. This suggests that intervention efforts at the level of the DNA or epigenome are facing an uphill battle, in which other cellular events are likely to work against subtle epigenetic effects. Instead, a cascade of cellular events is expected to unfold in a coordinated manner, which allows the infant to adapt in response to these earliest environmental interventions. This has important yet controversial implications. As agents of environmental change, clinicians must carefully think through the ramifications of which level of action is best targeted, be it the infant, the dyadic relationship, or deeper molecular phenotypes. In the end, it is likely that rather than being discrete levels, these all work together across development.

Evidence that interventions manifest on an epigenetic level is sparse and very difficult to collect, though it exists (Naumova et al., 2012). A more robust literature exists, linking interventions to biomarkers such as cortisol that are regulated by the epigenome, suggesting that this level is critical. One is left to contend with the question of whether interventions are just "fixing the surface" or are, indeed, "getting under the skin." Our discussion suggests that deeper embedding really is possible. Behavioral intervention can plausibly induce biochemical and molecular changes, though to date this remains understudied.

In conclusion, infancy is a period of rapid change whose importance cannot be overstated on the level of the epigenome. It is intuitive for infant mental health experts to consider that changing an environment changes a child, but this likely extends deep into the regulatory processes within each cell. Understanding those biological regulatory processes, at that deep cellular level, does not reduce an infant to component elements. Instead, it allows us to see the infant as an amalgamation coordinated across multiple levels, from nucleotide to neighborhood, with all the requisite complexity that entails.

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