Epigenetics and Human Health

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Environmental Epigenomics in Health and Disease

Epigenetics and Disease Origins

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Watercolor painting entitled *Origins* © by Collin Murphy, Portland, Oregon artist (http://collinmurphyart.com), shows that identical twins can vary in their susceptibility to adult diseases because even though their genomes are essentially the same, their epigenomes often vary markedly due to different environmental exposures while in the womb.

Preface

In the spring of 1998 my co-editor, colleague and friend, Randy Jirtle, approached me at a scientific meeting in San Diego, CA to discuss something that clearly had him excited. Initiating our discussion, Randy displayed the unbridled enthusiasm that is so characteristic of him. He advised me that he had spoken with my boss about organizing an international scientific meeting that would merge an evolving field with toxicology. That field was genomic imprinting and on that afternoon as we sat, he was effusive in his description of monoallelic expression. While this was not my first time hearing about genomic imprinting or the field of epigenetics that imprinting is a component of, I realized I was becoming fascinated with the opportunity to work with Randy and make an impact on merging aspects of toxicology and epigenetics.

In 1998, we had no way of knowing where that initial conversation would lead us, but we began crafting a vision to merge toxicology with epigenetics, as it was clear that the environment plays a pivotal role with epigenetic processes. It is now reasonable to argue that this vision is being shared globally, after organizing three extremely well attended international scientific meetings focusing on the interactions between environmental agents, epigenetics, and disease susceptibility.

What has in fact transpired with regard to developing the interface between epigenetics and environmental exposures over the last 15 years has been nothing short of remarkable. The advent of high throughput technologies such as genome-wide bisulfite sequencing, along with ChIP-Seq, RNA-Seq and technologies to map chromatin accessibility resulted in the generation of terabytes of data. Novel computational tools have been and are continually being developed to both store data and analyze it, as well. Investments in large multi-institutional consortia such as the NIH Roadmap Epigenome Mapping Consortium (REMC), the NHGRI supported ENCODE program, and the International Human Epigenome Consortium (IHEC) have spurred the development of these technologies and analytic tools.

There are compelling human epidemiological and animal experimental data that indicate the risk of developing adult-onset complex diseases and neurological disorders is influenced by persistent epigenetic adaptations in response to prenatal and early postnatal exposures to environmental factors. The epigenetic programs

are established as stem cells differentiate during embryogenesis, and are faithfully reproduced during mitosis. Moreover, they can also be maintained during meiosis. The plasticity of the epigenome allows the genome to express specific gene programs in a cell specific pattern that is spatially and temporally regulated, resulting in phenotypes. The capacity of the epigenome to interpret both internal and external stimuli and alter expression programs is a critical component in normal development, aging, and disease pathogenesis. In the past decade, our field has witnessed an explosion of unprecedented research on and support for epigenetics, epigenomics, and their interface with human health and disease. This research is in large measure an effort to generate a more precise understanding of how DNA and gene expression are regulated by DNA sequence, functional DNA elements, chromatin states, epigenomic signatures, and epigenetic processes.

It is becoming increasingly apparent that exposure to environmental toxicants can be associated with epigenetic changes, such as altered patterns of DNA methylation. These changes can affect gene expression patterns, and likely contribute to disease or other phenotypes associated with exposure. DNA methylation is thought to be one of the last steps of epigenetic gene regulation – a read-out of chromatin states established by other proteins. In order to understand the mechanism by which toxicants impact gene expression, we must examine how exposures perturb the proteins and processes upstream of DNA methylation and other epigenetic marks.

Epigenetic modifications, such as DNA methylation or post-translational modifications to histone tails, modifies the DNA and/or the way it is packaged into chromatin, making certain genes either more or less accessible to trans-acting elements, such as transcription factors. These epigenetic marks, however, represent limited facets in this complex process. Other proteins or protein complexes act as 'readers', 'writers' and 'erasers' of the epigenetic code, depositing or removing epigenetic marks or binding to them and recruiting other proteins. In addition, other factors such as non-coding RNAs, chromatin remodeling complexes, inter- and intra-chromosomal interactions and functional genomic elements play important roles in this process. Thus, to understand the mechanisms involved in the environmental control of gene regulation and the central role of epigenetics in the process, it is critical to understand all of the interacting pathways.

Exposure to environmental toxicants has been associated with changes in gene expression and DNA methylation profiles, which together likely contribute to disease or other phenotypes associated with exposure. The chapters in these volumes address a wide range of environmental exposures, such as airborne particulates, cocaine, radiation, tobacco smoke, and xenoestrogens. The health outcomes associated with these exposures include autoimmune disorders, neurodevelopmental disorders, and cancer. Importantly, dietary supplements and drugs can modify the epigenetic effects induced by these agents, thereby reducing their toxicological impact.

In the two volumes of this book, a number of leading investigators in the field of epigenetics discuss patterns of epigenomic modifications in normal cells, and how environmentally-induced changes in them are associated with disease pathogenesis.

The authors comprehensively review epigenetic processes that occur in human embryonic stem cells, as well as in differentiating cells and organs such as the brain, discussing autism, schizophrenia, and even sexual dimorphism in the developing brains of males and females. Particular emphasis is placed on the consequences of environmental exposures during development on epigenetic reprogramming that influences adult disease pathogenesis.

The overall purpose of this book is to give readers an overview of how environmental exposures can influence the development of disease by disrupting epigenetic processes and reprogramming. When Randy approached me in 1998 at the scientific meeting in San Diego, I had no idea what we would accomplish together in moving this field forward. He has been able to produce many significant contributions to the field directly from his laboratory research. Moreover, he has trained a cadre of young investigators who will continue to make an impact in enhancing our understanding of how the environment can alter epigenetic processes and influence the development of human disease. I, on the other hand, have been privileged to be among the extramural scientists at the National Institutes of Health (NIH) who develop research programs that support cutting edge science in moving this field forward.

Since that initial conversation, Randy and I have collaborated on a number of epigenetic projects. This book represents our latest collaboration to bring this field of environmental epigenomics to a growing audience. It is my desire that the readers learn as much, and have as much fun reading the chapters that constitute both volumes of this book as I did.

Frederick L. Tyson, Ph.D.

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Part I
Fetal Origins of Adult Disease Susceptibility

Chapter 1
Epigenetics: How Genes and Environment Interact

Randy L. Jirtle

Abstract Two epigenetically regulated targets involved in the developmental origins of disease susceptibility are genes that are imprinted and those with metastable epialleles. Genes with metastable epialleles have highly variable expressions that result from random allelic changes in the epigenome. The isogenic agouti viable yellow (A^{vy}) mouse harbors a metastable *Agouti* gene. Maternal exposure of the A^{vy} mouse during pregnancy with nutritional supplements (e.g., methyl donors and genistein), toxicological agents (e.g., bisphenol A), and physical agents (e.g., ionizing radiation) changes the coat color and adult disease incidence in the offspring by altering DNA methylation at the A^{vy} locus. These studies clearly demonstrate that the developmental origins of disease risk can result from alterations in the epigenome. Genomic imprinting is an epigenetic form of gene silencing that results in monoallelic, parent-of-origin-dependent gene expression. It evolved in mammals with the advent of placentation and viviparity approximately 150 million years ago. Since only a single mutation or epigenetic event is required to alter imprinted gene function, these genes are implicated in a number of complex diseases and neurological conditions. Imprinting is controlled by parental-specific epigenetic marks that are established during gametogenesis and in early embryonic development. We refer to the complete set of these *cis*-acting epigenetic regulatory elements as the imprintome, a distinct and specially tasked subset of the epigenome. Significant species variation in the repertoire of imprinted genes and their epigenetic regulation mean that only humans can be used to define our imprintome.

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Keywords Agouti mouse • Developmental origins of health and disease (DOHaD) • Epigenetics • Evolution • Genomic imprinting • Imprintome • Metastable epialleles

Abbreviations

5hmC	5-Hydroxymethylcytosine
5mC	5-Methylcytosine
AO	Antioxidants
Air	Antisense Igf2r RNA
A^{vy}	Agouti viable yellow
$Axin^{Fu}$	Axin fused
BPA	Bisphenol A
$CABP^{IAP}$	CDK5 activator-binding protein-IAP
CNV	Copy-number variation
ChIP	Chromatin immunoprecipitation
CTCF	CCCTC-binding factor (zinc-finger protein)
CTCFL (BORIS)	CCCTC-binding factor (zinc-finger protein) like
DLGAP2	Discs large (Drosophila) homolog-associated protein 2
DNMT1	DNA (cytosine-5-)-methyltransferase 1
DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha
DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta
DNMT3L	DNA (cytosine-5-)-methyltransferase 3-like
DMR	Differentially methylated region
DOHaD	Developmental origins of health and disease
EMB	Embryo
FAM50B	Family with sequence similarity 50 member B
FWA	Homeobox-leucine zipper protein HDG6
GDMR	Gametic differentially methylated region
GWAS	Genome-wide association studies
H19	Imprinted maternally expressed transcript (non-protein coding)
HDAC	Histone deacetylase
HOTS	H19 opposite tumor suppressor
IAP	Intracisternal A particle
IGF2	Insulin-like growth factor 2
IGF2R	Insulin-like growth factor 2 receptor
ICR	Imprint control region
KCNK9	Potassium channel subfamily K, member 9
LDIR	Low-dose ionizing radiation
LOI	Loss of imprinting
MAGI2	Membrane-associated guanylate kinase WW and PDZ domain containing 2
MEA	Histone-lysine N-methyltransferase MEDEA

MBD	Methyl-CpG-binding protein
MC4R	Melanocortin 4 receptor
MeCP2	Methyl-CpG-binding protein 2
MeDIP-chip	Methylated DNA immunoprecipitation microarray
MeDIP-seq	Methylated DNA immunoprecipitation-sequence
MET1	DNA (cytosine-5)-methyltransferase 1
MethylCap-seq	Methyl-DNA-binding domain capture sequence
MYA	Million years ago
NGS	Next-generation sequencing
NTM	Neurotrimin
NcRNA	Noncoding RNA
PGC	Primordial germ cell
ROS	Reactive oxygen species
SMRT	Single-molecule real-time sequencing
TB	Trophoblasts
YS	Yolk sac
ZNF	Zinc finger

1.1 Introduction

The word "environment" means different things to people. For sociologists and psychologists, it conjures up visions of social group interactions, family dynamics, and maternal nurturing. Nutritionists envision food pyramids and dietary supplements, while toxicologists focus on water, soil, and air pollutants. This chapter provides evidence that these vastly different environments all interact with the genome, thereby altering gene expression and disease risk by modifying the epigenome.

A colleague of mine took a small vial out of his pocket during a scientific meeting and placed it on the table in front of him. When the meeting drew to a close, he picked up the vial and said, "This is a vial of DNA, and in the past two days, it hasn't done a damn thing!" Like a computer, the genes in our cells are impotent without their software, telling them when, where, and how to work. These programs are collectively referred to as the epigenome. Thus, a cell is comparable to a programmable computer. This is why even though the genome is essentially identical in all cells, there are over 250 different cell types in our body. Every cell type is chemically programmed during development to perform a different function – a function that is maintained throughout its life. Thus, not only does the DNA need to be duplicated with high fidelity during somatic cell division, but the epigenome also has to be faithfully replicated. The epigenome,

however, is particularly vulnerable to environmentally induced perturbations, especially when it is initially established pre- and postnatally.

One of the most important questions in biology is why do individuals vary in their susceptibility to diseases? To address this question, genome-wide association studies (GWAS) have focused on the identification of DNA alterations that are linked with disease phenotypes. This genome-centric approach has been successful in detecting mutations that are associated with the pathogenesis of a number of chronic conditions like diabetes and schizophrenia (Cirulli and Goldstein 2010; Gibson 2011). Nevertheless, the identified genetic variants often explain only a small portion of their heritability (Maher 2008).

This leads to a more perplexing question. Why do monozygotic twins also frequently vary in their susceptibility to diseases if the only thing important in developing this discordance is genetic variation (Bell and Spector 2011; Ollikainen and Craig 2011; Zwijnenburg et al. 2010)? One answer to this question is that although monozygotic twins are basically genetically identical, their epigenomes most likely are not the same. One potential cause for variation in epigenetic programming is that even though identical twins share the same womb, they do not necessarily receive identical maternal blood flow. Thus, the level of nutrition and even exposure to toxicants during early development most likely varies between the two developing fetuses. As discussed below, even small variations in such environmental exposures during pregnancy can have profound effects on the epigenome and subsequent disease susceptibility in adulthood (Bernal et al. 2013; Dolinoy et al. 2006, 2007; Waterland and Jirtle 2003).

1.2 Definition of Epigenetics

Epigenetics simply means “above the genetics” as epicenter means “above the center.” This field of research is presently growing exponentially, with a doubling in the number of papers published on this subject every 2–3 years (Jirtle 2009).

The developmental biologist Conrad Waddington (Waddington 1940) first defined epigenetics in the 1940s as “the interactions of genes with their environment which bring the phenotype into being.” The molecular mechanisms responsible for these interactions include covalent chemical DNA modifications, covalent modifications of histone tails (e.g., acetylation, methylation, phosphorylation, and ubiquitination), the expression of noncoding RNA (ncRNA), packaging of DNA around nucleosomes, and higher-order chromatin folding and attachment to the nuclear matrix. Thus, epigenetics is presently described as the study of changes in gene expression that occur not by changing the DNA sequence, but by modifying DNA methylation and remodeling chromatin. Moreover, epigenetic modifications are not only transmitted with high fidelity during somatic cell division, but they can also be inherited transgenerationally, thereby effecting the health of future generations (Anway et al. 2005; Morgan et al. 1999; Pembrey et al. 2006).

Epigenetically labile genes particularly vulnerable to environmentally induced epigenetic dysregulation include those that are imprinted or have metastable epialleles.

1.3 Epigenetically Labile Genes

Traditional research on the combined effects of the environment and genetics on individual variation in disease risk examined the relationship between disease susceptibility, environmental exposures, and germline mutations in the coding and promoter regions of genes. Such research efforts have highlighted the importance of genotype in human diseases. It is now clear, however, that a full understanding of environmental interactions with the genome will require epigenetic mechanisms to also be taken into account.

Human epidemiological and animal experimental data indicate that the risk of developing adult-onset diseases, such as asthma, diabetes, obesity, and cancer, is influenced by persistent adaptations to prenatal and early postnatal exposure to environmental factors (Jirtle and Skinner 2007; Yajnik 2004; Barker 2007; Gluckman 2012). The results of these studies support the hypothesis referred to as the developmental origins of health and disease (DOHaD). This theory posits the intriguing idea that the evolution of developmental plasticity, which enables an organism to adapt to environmental signals during early life, can also increase the risk of developing chronic diseases when there is a mismatch between the perceived environment and that which is actually encountered in adulthood. It is now known that developmental plasticity is evident when environmental exposure produces a broad range of adult phenotypes from a single genotype by epigenetically altering gene expression (Jirtle and Skinner 2007; Rakyan et al. 2002; Vasicek et al. 1997; Waterland and Jirtle 2003; Wolff et al. 1998).

1.3.1 Metastable Epialleles

The highly variable expression of genes with metastable epialleles results from stochastic allelic changes in the epigenome rather than mutations in the genome. The most actively investigated metastable genes are the murine *Agouti viable yellow* (A^{vy}) gene (Duhl et al. 1994), the *Axin-fused* ($Axin^{Fu}$) gene (Vasicek et al. 1997), and the *CDK5 activator-binding protein-IAP* ($Cabp^{IAP}$) gene (Druker et al. 2004), although additional metastable epialleles have recently been identified in both the mouse (Weinhouse et al. 2011) and human (Waterland et al. 2010).

The A^{vy} mouse harbors a metastable *Agouti* gene because of the insertion of a retroviral intracisternal A particle (IAP) approximately 100 kb upstream of the gene (Fig. 1.1a). The degree of IAP methylation at the cryptic promoter region in the proximal end of the IAP varies dramatically among individual isogenic A^{vy} mice,

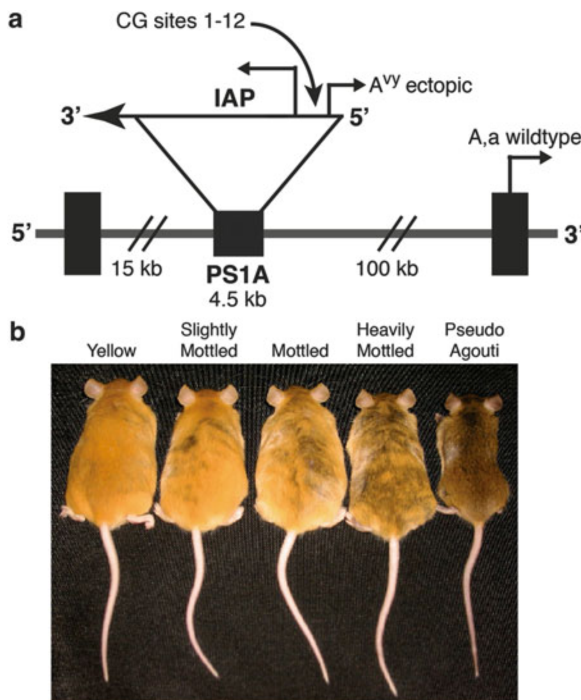

Fig. 1.1 A^{vy} locus and resulting mouse coat colors. (**a**) The *Agouti* gene encodes for a paracrine-signaling molecule that produces either black eumelanin [*a*] or yellow pheomelanin [*A*] from the wild-type promoter (arrowhead labeled *A*,*a* wild type). Normally, *Agouti* expression during follicle development results in *brown* (*agouti*) wild-type animals. The A^{vy} allele resulted from a spontaneous contraoriented insertion of an intracisternal A particle (*IAP*) into pseudoexon *PS1A* upstream of the wild-type promoter. This insertion carries a cryptic promoter (arrowhead labeled A^{vy} ectopic) controlled by the methylation of upstream CpG sites (Duhl et al. 1994; Jirtle and Skinner 2007). (**b**) The level of CpG methylation at the A^{vy} locus results in the formation of distinct coat-color phenotypes. Hypermethylation of the cryptic IAP promoter results in *brown*, pseudoagouti offspring, hypomethylation results in *yellow offspring*, and mottled mice are epigenetic *mosaic offspring*; black a/a offspring are not shown. (Redrawn from Dolinoy et al. (2006))

causing a wide distribution in coat color, ranging from brown (i.e., methylated) to yellow (i.e., unmethylated); mottled A^{vy} mice are epigenetically mosaic (Fig. 1.1b) (Miltenberger et al. 1997; Morgan et al. 1999). Hypomethylation of this alternative promoter causes inappropriate *Agouti* gene expression throughout the A^{vy} mouse. This not only leads to a yellow coat color, but it also antagonizes the melanocortin 4 receptor (MC4R) in the hypothalamus, causing the animals to overeat, thereby becoming obese, and subsequently developing diabetes and cancer at a high frequency. In contrast, the incidence of these diseases is markedly reduced in pseudoagouti (brown) offspring that develop when this promoter is hypermethylated, and the *Agouti* gene is developmentally expressed only in hair follicles (Jirtle and Skinner 2007; Williams et al. 2000; Yen et al. 1994). This makes

the A^{vy} mouse an excellent and exquisitely sensitive biosensor for identifying environmental exposures early in development that alter adult disease susceptibility by modifying the epigenome rather than by mutating the genome.

1.3.1.1 Nutritional Supplements

We initially used the A^{vy} mouse to investigate if maternal nutrition could change the coat-color distribution of the offspring by altering the epigenome (Fig. 1.2) (Waterland and Jirtle 2003). This study was the first to show that maternal dietary supplementation during pregnancy with methyl donors (i.e., folic acid, vitamin B_{12}, choline, and betaine) significantly increases the incidence of pseudoagouti offspring by increasing DNA methylation at the *Agouti* locus.

We also demonstrated that genistein, the weak phytoestrogenic compound present in soya products, similarly alters the coat-color distribution and decreases the incidence of obesity by increasing DNA methylation (Dolinoy et al. 2006). This finding is particularly interesting because genistein is not a methyl donor. Nevertheless, genistein at a level comparable to that found in an Asian diet also results in DNA hypermethylation. The intracellular signaling mechanism by which DNA methylation at the A^{vy} locus is enhanced by genistein is presently unknown; however, it may involve an alteration in the redox state of the cell, as observed following ionizing radiation exposure (Bernal et al. 2013). This intriguing possibility needs to be further investigated.

These initial studies demonstrated that changes in the nutritional environment during gestation result in alterations in offspring phenotype and disease risk not by mutations in the genome, but rather by modifications in the epigenome. They were transformational to the DOHaD research field because for the first time a memory system was identified by which environmental exposures during early development could affect disease risk years later in adulthood. Moreover, our findings ushered in the era of environmental epigenomics by demonstrating to scientists in a multitude of disciplines that their investigations also fit under the "big tent" of epigenetic research.

1.3.1.2 Bisphenol A

The effects on the fetal epigenome of maternal exposure to the endocrine disruptor and suspect carcinogen bisphenol A (BPA) (Soto and Sonnenschein 2010) were also determined with the use of A^{vy} mouse model (Dolinoy et al. 2007). BPA is a high-production volume chemical used in the manufacture of polycarbonate plastic and epoxy resins. It is present in many commonly used products including food and beverage containers, baby bottles, and dental composites. The detection of BPA in a majority of humans attests to its widespread use and our exposure to this chemical (Calafat et al. 2005).

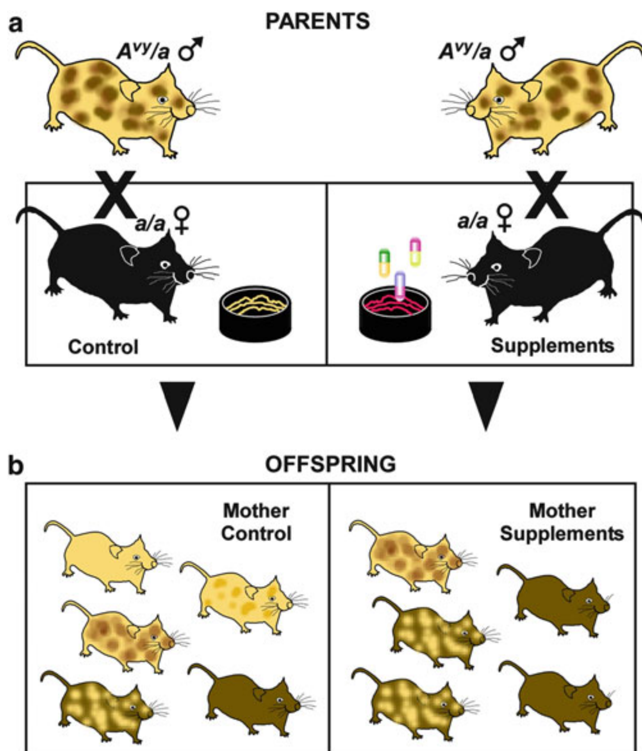

Fig. 1.2 Effect of maternal dietary supplementation on the phenotype and epigenotype of A^{vy}/a offspring. (**a**) Female mice are exposed to a control diet or a supplemented diet. (**b**) In this example, mothers whose diet is supplemented produce a higher proportion of offspring that are pseudoagouti and hypermethylated at the A^{vy} locus (Redrawn from Bernal and Jirtle (2010))

Maternal exposure to BPA shifted the coat-color distribution of A^{vy} mouse offspring toward yellow by decreasing DNA methylation in the retrotransposon upstream of the *Agouti* gene at the pluripotent stem cell stage of development (Dolinoy et al. 2007). Although the epigenetic response to BPA in the A^{vy} mouse was subsequently found to be dose dependent (Anderson et al. 2012), the dose used in the initial BPA epigenetic study results in plasma levels comparable to that found in humans. The hypomethylating effect of BPA is not confined to the *Agouti* gene since DNA methylation at the $Cabp^{IAP}$ locus is also decreased. These findings provide compelling evidence that the epigenetic effects of xenobiotic chemicals like BPA need to be included in the risk assessment process.

Importantly, maternal dietary supplementation with either methyl donors or genistein counteracts the DNA hypomethylating effect of BPA. Thus, early developmental exposure to BPA can change offspring phenotype and increase disease susceptibility by stably altering the epigenome, an effect that can be blocked by maternal dietary supplements. Two millennia after Hippocrates stated, “Let food be thy medicine, and medicine be thy food,” it is apparent that at least in diseases that

result from a dysregulated epigenome, *food is medicine*. In the twenty-first century, it will be critical to optimize the preventative use of nutritional supplements to counteract deleterious environmental influences on the epigenome.

1.3.1.3 Low-Dose Ionizing Radiation

The A^{vy} mouse model has shown that early nutritional (Dolinoy et al. 2006; Morgan et al. 2008; Waterland and Jirtle 2003) and chemical (Dolinoy et al. 2007; Kaminen-Ahola et al. 2010) exposures induce persistent epigenetic changes at the A^{vy} locus (Fig. 1.3). Recently, we have also demonstrated that a physical agent, low-dose ionizing radiation (LDIR), significantly increases DNA methylation at the A^{vy} locus and alters the coat-color distribution of the offspring in a sex- and dose-dependent manner (Fig. 1.4b) (Bernal et al. 2013).

Moreover, maternal antioxidant (AO) supplementation mitigates the increase in DNA methylation and the concomitant shift in coat color to pseudoagouti observed in irradiated male offspring (Fig. 1.4c). These findings support the postulate that LDIR increases DNA methylation at the A^{vy} locus in part through the generation of reactive oxygen species (ROS). Thus, the cellular redox state in pluripotent stem cells in response to LDIR plays an important role in determining the ultimate methylation status at the A^{vy} locus. Since pseudoagouti A^{vy} mice also have a reduced incidence of cancer, diabetes, and obesity (Jirtle and Skinner 2007; Williams et al. 2000; Yen et al. 1994), low doses of ionizing radiation are positively adaptive in this epigenetic isogenic mouse model system.

The risk to humans from low doses of ionizing radiation is presently predicted from high-dose epidemiological data using the LNT risk assessment model (Fig. 1.5) (Pearce et al. 2012; Sanders 2009). This dose–response model assumes that all doses of radiation are harmful. Nevertheless, there is a large body of scientific evidence that shows low doses of ionizing radiation are positively adaptive (Cameron 2005; Calabrese 2009; Sanders 2009).

The mechanism(s) by which LDIR induce adaptive biological responses have remained enigmatic (Vaiserman 2011). We now show, for the first time, that X-ray doses used diagnostically increase the incidence of pseudoagouti mice and decrease chronic disease incidence in A^{vy} mice by enhancing DNA methylation. These results do not support the LNT risk assessment model, but rather are consistent with the hormesis model of risk assessment (Fig. 1.5). Our findings not only have significant implications concerning the mechanism of hormesis, but they also emphasize the potential importance of this phenomenon in determining human risk at low radiation doses. Since the epigenome varies markedly between species, the effect of LDIR on the human epigenome needs to be defined. Epidemiological data alone will no longer suffice to assess our risk to clinically relevant doses of X-rays.

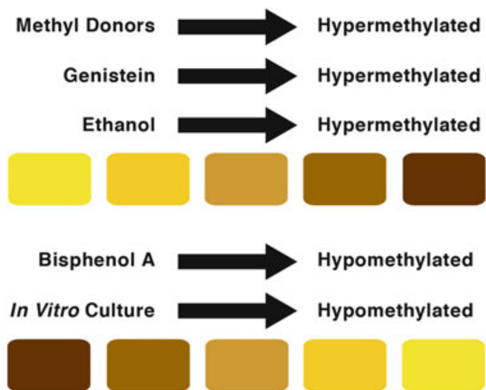

Fig. 1.3 The A^{vy} mouse as a biosensor of environmentally induced alterations in the epigenome. This animal model has shown that early developmental exposures to methyl donors (Waterland and Jirtle 2003), genistein (Dolinoy et al. 2006), ethanol (Kaminen-Ahola et al. 2010), bisphenol A (BPA) (Dolinoy et al. 2007), and in vitro culturing (Morgan et al. 2008) cause coat-color changes in the offspring by hypermethylating or hypomethylating an IAP inserted into the *Agouti* locus (see Fig. 1.1a). Boxes; Coat color

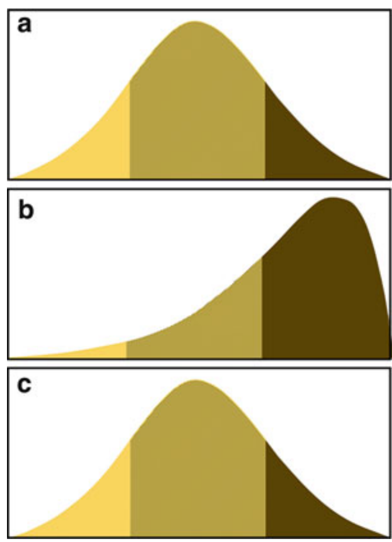

Fig. 1.4 Coat-color distribution of A^{vy} offspring that were (**a**) sham irradiated, (**b**) exposed in utero to low-dose ionizing radiation (*LDIR*), and (**c**) exposed in utero to LDIR + antioxidants. *Yellow*, yellow A^{vy} mice; *yellow* and *brown*, mottled A^{vy} mice; *brown*, pseudoagouti A^{vy} mice

1.3.2 Imprinted Genes

Animal breeding three millennia ago showed that crossing a female horse with a male donkey produced a mule. Contrary to Mendelian predictions, the reciprocal cross yields a phenotypically different offspring called a hinny (Hunter 2007).

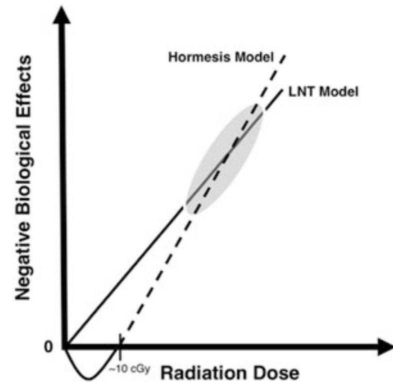

Fig. 1.5 Dose–response models for radiation risk assessment. The LNT model assumes that every dose of radiation increases the risk of adverse effects. The hormesis model is a biphasic dose–response curve where low doses result in a positive adaptive response and is supported by our LDIR epigenetic study (Bernal et al. 2013). Gray ellipse depicts where most radiation dose response data has been obtained

The first experimental evidence that the parental genomes of mammals are not functionally equivalent came from elegant mouse nuclear transplantation studies in the mid 1980s (Fig. 1.6) (Barton et al. 1984; McGrath and Solter 1984; Surani et al. 1984). They demonstrated that the abortive pregnancies resulting from diploid androgenotes derived from two male pronuclei are markedly different from those that developed from diploid gynogenotes derived from two female pronuclei. These studies suggested the presence of genes critical to normal development that are expressed in a parent-of-origin-dependent manner.

In 1991, murine *Igf2r* (Barlow et al. 1991) and *Igf2* (DeChiara et al. 1991) were the first genes shown to be imprinted and expressed only from the maternal and paternal allele, respectively (Fig. 1.7). The following year *H19* was demonstrated to be imprinted in humans (Fig. 1.7b) (Zhang and Tycko 1992). Presently, there are approximately 100 genes known to be imprinted in mice and 70 in humans (http://www.geneimprint.com). This is consistent with our predictions, using machine learning algorithms, that the repertoire of imprinted genes is species dependent and that there are fewer genes imprinted in the human than in the mouse (Luedi et al. 2005, 2007).

Some imprinted genes in Eutherians (e.g., human and mouse) are likewise imprinted in Metatherians (e.g., opossum) (Das et al. 2009, 2012). In contrast, genes tested for imprint status in Prototherians (e.g., echidna and platypus) and Aves (e.g., chicken) are biallelically expressed (Killian et al. 2001; Nolan et al. 2001; Renfree et al. 2009). These findings are consistent with genomic imprinting originating approximately 150 million years ago during the Jurassic/Cretaceous period in a common ancestor of the two Therian infraclasses, Metatherians and Eutherians (Fig. 1.8) (Killian et al. 2000, 2001; Nolan et al. 2001; O'Neill et al. 2000). Interestingly, the phenomenon of genomic imprinting also evolved independently in some insects and flowering plants (Khosla et al. 2006; Scott and Spielman 2006). The functional haploidy resulting from a gene being imprinted enables a single genetic mutation or epigenetic modification to alter the function of an imprinted gene, making imprinted genes unique disease susceptibility loci.

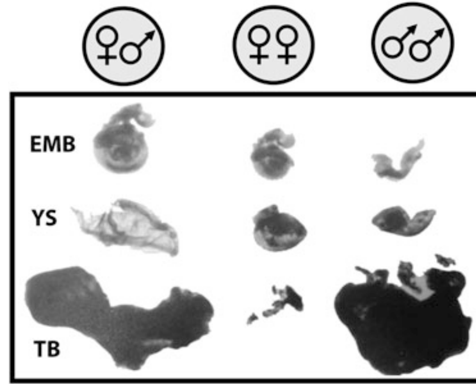

Fig. 1.6 Maternal and paternal genomes not functionally equivalent. Mouse nuclear transplantation studies show abnormal development of the embryo (*EMB*) and trophoblasts (*TB*) in diploid gynogenotes derived from two female pronuclei and diploid androgenotes derived from two male pronuclei. Normal mouse development occurs when one pronucleus is female and the other is male (Redrawn from Barton et al. (1984) and Surani et al. (1984)). Yolk sac (*YS*)

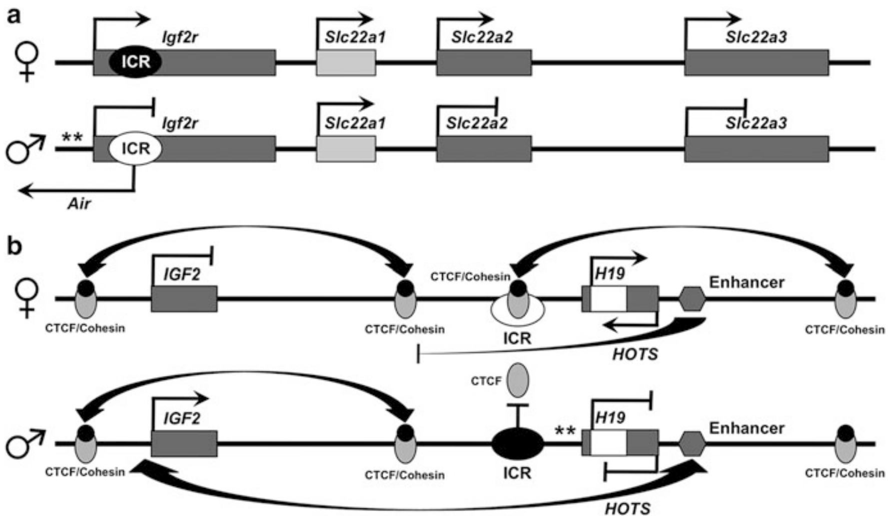

Fig. 1.7 Mechanisms of monoallelic gene expression controlled by parent-of-origin gametic and somatic differentially methylated regions (*DMRs*). (**a**) Noncoding RNA imprinting model. The inherited gametic DMR in intron 2 of the mouse *Igf2r* is the imprint control region (ICR) that regulates expression of *Air*. This antisense ncRNA inhibits the expression of the paternal allele of *Igf2r* by establishing a somatic DMR in the promoter region (**) (Barlow 2011). (**b**) Chromatin boundary imprinting model. Parent-of-origin-specific DNA methylation prevents CTCF binding and cohesion-mediated chromatin remodeling of the human *IGF2–H19–HOTS* imprinted domain. Differently arranged chromatin loops allow or prevent promoter–enhancer interaction, resulting in paternal expression of *IGF2* and maternal expression of *H19* and *HOTS* (redrawn from Skaar et al. (2012)). *White oval*, unmethylated ICR; *dark oval*, methylated ICR; *light gray oval*, CTCF; *black circle*, cohesin; methylated sDMR (**)

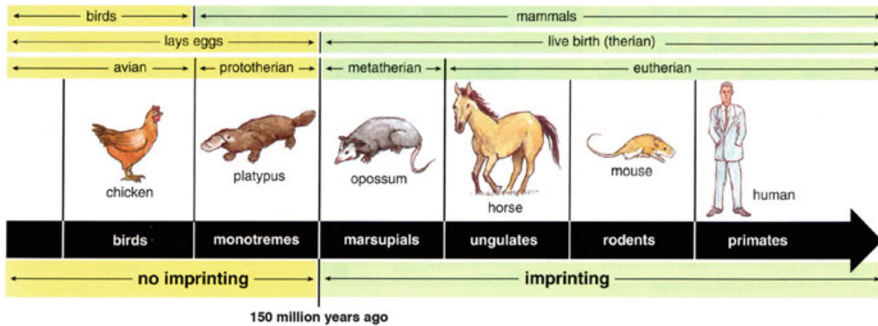

Fig. 1.8 Genomic imprinting evolved in mammals about 150 MYA with the genesis of placentation and viviparity. Thus, Metatherians and Eutherians have imprinted genes, but there is no evidence of imprinting in the egg-laying Prototherians or Avians (Jirtle and Weidman 2007)

1.3.2.1 Imprinting Evolution Theories

The evolutionary biologist Theodosius Dobzhansky (Dobzhansky 1973) wrote, "Nothing in biology makes sense except in the light of evolution." Nevertheless, scientists have struggled to formulate a theory to explain the adaptive evolutionary advantage of imprinted genes, since its evolution eliminates the protection that diploidy affords against the deleterious effects of recessive mutations.

Many theories are proposed to explain the evolution of genomic imprinting (Das et al. 2009), but the most widely debated and discussed model is the parental kinship or conflict hypothesis (Haig and Graham 1991; Moore and Haig 1991). This theory maintains that genomic imprinting developed in response to viviparity and polygamy and speculates that fitness effects during placental development were the principal factors that shaped its evolution. For the first time in evolutionary history, the placenta exists as an interface for both the paternal and maternal genomes to exert their influence on resource allocation within the intrauterine environment. Postnatal nurturing behavior, like lactation, further extends this interaction between the mother and offspring, indicating that imprinted genes also play an important role in brain development and behavioral formation (Curley 2011; Keverne 2011). This led to the theory that skewed expression of imprinted genes toward the paternally and maternally imprinted genes results in the behavioral conditions of autism and schizophrenia, respectively (Badcock and Crespi 2008; Badcock 2011).

According to the conflict theory, genes expressed from the paternal allele favor increased maternal investment to enhance an offspring's own fitness at a cost to all others. In contrast, genes expressed from the maternal allele maximize reproductive fitness of the mother ensuring availability of resources for all of her current and future progeny. This postulate explains successfully the parental allelic expression bias of a number of imprinted genes, including the paternal expression of *Igf2, a* growth enhancer, and the maternal expression of *Igf2r, a* growth inhibitor (Haig and Graham 1991; Haig 2004). Supporting evidence for this postulate also comes from

the discovery of imprinted genes in Eutherian mammals and marsupials, while none have been identified in the egg-laying monotremes or birds (Fig. 1.8) (Killian et al. 2000, 2001; Nolan et al. 2001; O'Neill et al. 2000).

1.3.2.2 Imprinting Mechanisms

Genomic imprinting appears to have evolved independently at least three times. In the mealybug species, imprinting is manifested as heterochromatization of the entire paternal genome in males selectively (Khosla et al. 2006). *MEA* and *FWA* in Arabidopsis are imprinted selectively in the nutrient-providing endosperm, and the mechanism involves demethylation activation by the maintenance methyltransferase, *MET1* (Scott and Spielman 2006).

The imprinting mechanism in vertebrates is more complex. The imprint regulatory marks necessarily undergo a cycle involving their establishment in the primordial germ cells (PGCs) of one generation, maintenance during somatic cell divisions throughout life in the resulting individual, and erasure and reestablishment in the germ cells during embryogenesis to reflect the sex of the individual in which they reside (Jirtle and Skinner 2007; Murphy and Jirtle 2003; Barlow 2011). DNA methylation is a candidate for this inherited marking system, since it can be modulated with the help of the de novo methyltransferases DNMT3A, DNMT3B, and DNMT3L in PGCs and then maintained throughout life with the aid of DNMT1 (Hermann et al. 2004; Jia et al. 2007; Turek-Plewa and Jagodzinski 2005). Proteins like CTCF, coupled with its testis-specific counterpart CTCFL (BORIS), ncRNAs, and methyl-CpG-binding proteins (MBDs) which recruit histone deacetylases (HDACs), are all involved in imprinting regulation, emphasizing the complexity of the imprinting process (Klenova et al. 2002; Lewis and Reik 2006; Barlow 2011).

Imprinting control regions (ICRs) are present in imprinted gene clusters as discrete *cis*-acting DNA elements that carry a heritable epigenetic mark that distinguishes the two parental alleles (Barlow 2011). The inherited gametic imprint mark in Eutherians contains DNA methylation at specific CpG sites, resulting in both a methylated and unmethylated allele in somatic cells or a gametic differentially methylated region (gDMR). For these ICRs, the inherited methylation marks can be paternally derived from the sperm, as in the gDMR or ICR located upstream of *H19* in the *IGF2*/*H19* cluster (Fig. 1.7b), or maternal in origin like the gDMR or ICR in intron 2 of murine *Igf2r* (Fig. 1.7a) (Reik and Walter 2001; Barlow 2011). CpG-rich sequences can also acquire gDMR-dependent, parental-specific DNA methylation marks soon after fertilization in the diploid somatic cells (sDMR), such as the sDMR in the promoter of *Igf2r* (Fig. 1.7a). Since these imprint regulatory marks are not always "read," monoallelic expression can be cell type, developmental stage, and sex of the individual dependent, resulting in significant expression variation (Gregg et al. 2010; Prickett and Oakey 2012). It has been proposed that this epigenetically mediated variation in imprinted gene expression has played an important role in mammalian speciation (Vrana et al. 1998), which

could explain why the repertoires of imprinted genes vary between mammalian species (Luedi et al. 2007) (http://www.geneimprint.com).

The parent-of-origin-dependent expression of imprinted genes is mediated by at least two different imprinting mechanisms. Briefly, the chromatin boundary imprinting model holds that allele-specific modifications at the ICR affect binding of insulator proteins (e.g., zinc-finger DNA-binding protein CTCF), thereby mediating gene silencing. Such is the case for the *IGF2*/*H19* imprinted domain (Fig. 1.7b) (Schoenherr et al. 2003). CTCF binds to the maternal unmethylated allele of the ICR, shielding *IGF2* from downstream *IGF2*/*H19* shared enhancers; this results in maternal expression of *H19* and *HOTS*. In contrast, CTCF is unable to bind to the methylated paternal allele of the ICR, allowing enhancer interaction with the *IGF2* promoter and paternal *IGF2* expression.

In the noncoding RNA imprinting model, production of antisense ncRNA is critical for the establishment of imprinting. Expression after fertilization of the *antisense Igf2r RNA (Air)* from the paternal allele of the *Igf2r* ICR silences the father's copy of the gene by the formation of promoter DNA methylation. The paternal alleles of two additional genes, *Slc22a2* and *Slc22a3,* are also silenced (Fig. 1.7a) (Barlow 2011). DNA methylation on the maternal allele of the ICR inhibits the production of *Air* from the mother's copy of the gene. Thus, the promoter on the maternal allele remains unmethylated permitting expression of *Igf2r, Slc22a2*, and *Slc22a3* only from the allele inherited from the mother. *IGF2R Air* is not produced in the human, at least in the normal tissues investigated, despite the presence of an ICR in intron 2 (Yotova et al. 2008). As a consequence, this gene is biallelically expressed in humans, except for sporadic monoallelic expression observed in the placenta and some Wilms' tumors (Monk et al. 2006; Yotova et al. 2008). Even more surprising is that *Air* has not been found in other mammals that show imprinted *IGF2R* expression, such as the dog (O'Sullivan et al. et al. 2007) and opossum (Weidman et al. 2006). This indicates that epigenetic modifications other than DNA methylation are involved in controlling imprinting at the *IGF2R* locus in these mammalian species. Further investigation into the role of histones and ncRNAs in imprinted gene regulation in Therians will undoubtedly help resolve these issues (Kacem and Feil 2009; Girardot et al. 2012).

1.3.2.3 Imprinting and Disease Susceptibility

George Orwell wrote in *Animal Farm*, "All animals are equal, but some animals are more equal than others." The same is true about genes when it comes to disease susceptibility, and those that are more equal are monoallelically expressed. The parental allele that is expressed can be chosen either randomly (Tycko 2010) or in a parental-specific manner, as observed in genomically imprinted genes (Jirtle and Weidman 2007; Barlow 2011). Genomic imprinting may be evolutionarily adaptive because of its apparent involvement in brain development (Badcock and Crespi 2006; Keverne 2001) and potential ability to accelerate mammalian speciation (Hunter 2007; Vrana et al. 1998). Nevertheless, the presence of functionally haploid

imprinted genes in the human genome can be disastrous to the health of a given individual.

A number of developmental disorders in humans, such as Beckwith–Wiedemann and Silver–Russell syndromes, result not only from genomic mutations but also from epigenetic dysregulation of imprinted genes (Ishida and Moore 2012). Interestingly, Silver–Russell syndrome, a congenital disease characterized by growth retardation, is the first human disorder shown to result from epigenetically mediated imprinting defects affecting two different chromosomes (Eggermann et al. 2008). Ten percent of patients present with maternal UPD of chromosome 7, while 40–65 % show hypomethylation at the ICR upstream of *H19* at chromosome location 11p15.5 (Fig. 1.7b) (Ishida and Moore 2012). Silver–Russell syndrome patients with *H19* ICR hypomethylation additionally exhibit altered DNA methylation at other imprinted loci, suggesting defects in the ability to establish and/or maintain imprinting postfertilization (Azzi et al. 2009). In contrast, hypermethylation of the *H19* ICR, with concomitant biallelic expression of *IGF2,* is associated with an overgrowth disorder, Beckwith–Wiedemann syndrome (Riccio et al. 2009). Consequently, these two developmental syndromes are clinically and epigenetically opposite diseases.

Genome-wide association studies (GWAS) and copy-number variation (CNV) studies have also identified genomic regions linked to complex disorders such as autism, bipolar disorder, schizophrenia, and Tourette's syndrome with a parent-of-origin inheritance preference, indicating the involvement of imprinted genes in their etiology (Morison et al. 2005). Two recently identified imprinted genes implicated in the development of autism and schizophrenia are *DLGAP2,* a membrane-associated guanylate kinases localized at postsynaptic density in neuronal cells (Luedi et al. 2007; Pinto et al. 2010), and *MAGI2,* a multi-PDZ domain scaffolding protein that interacts with several different ligands in brain, respectively (Barbaux et al. 2012; Karlsson et al. 2012; Koide et al. 2012; Luedi et al. 2007; Walsh et al. 2008). The potential involvement of imprinted genes in autism and schizophrenia are consistent with the imprinted brain theory proposed by Badcock and Crespi (Badcock and Crespi 2008; Badcock 2011).

Exposure to famine conditions while in utero increases the risk of developing cardiovascular disease, obesity, and diabetes (McMillen and Robinson 2005); it also doubles the incidence of schizophrenia (St Clair et al. 2005; Susser et al. 1996). Thus, a major reduction in nutrition during pregnancy markedly increases psychosis formation. In contrast, the calorie-rich Western diet is predicted to increase the prevalence of autism with a concomitant decrease in the incidence of schizophrenia (Badcock 2011), an intriguing situation presently observed in Western countries (Woogh 2001; Blaxill 2004). A better understanding of the mechanisms by which the environment affects the genesis of neurological disorders will clearly necessitate elucidating the role of imprinted gene expression in normal human neurological and behavioral development.

Determining the role of imprinted gene regulation in normal and abnormal behavior development has significant limitations, however, since brain-specific expression profiles cannot be measured in living individuals. This means relevant

correlations cannot readily be made between disease state and abnormal imprinted gene expression in brain tissue. Nevertheless, given the consistency of the parentally established epigenetic marks present in imprint control elements, inherited epigenetic abnormalities should be detectable in peripheral tissues that are amenable to analysis. Therefore, even in the absence of expression data, screening affected individuals for epigenetic disruptions in imprint regulatory elements should prove to be highly informative for ascertaining the role of genomic imprinting in psychiatric conditions.

Because imprinted genes are so integrally involved in regulating early growth and development, their deregulation in somatic cells also increases cancer risk (Feinberg et al. 2006; Uribe-Lewis et al. 2011). For imprinted tumor suppressor genes, only a single mutational or epigenetic event is required for complete gene inactivation because one allele is already nonfunctional due to imprinting. Consequently, the silenced allele of an imprinted gene has been equated to the first hit, as defined by Knudson (Knudson 2001) in his two-step model for carcinogenesis. Imprinted oncogenes can also be inappropriately overexpressed in somatic cells through loss of imprinting (LOI) in which the normally silent allele becomes transcriptionally active (Jelinic and Shaw 2007). These findings indicate that imprinted genes are also potential human cancer susceptibility loci, and approximately 30 % of imprinted genes are now known to be involved in its genesis (Skaar et al. 2012). Thus, to improve our understanding of the etiology of human diseases and neurological disorders, the complete repertoire of imprinted genes and their regulatory elements, the imprintome, must be defined. It is also important to determine the imprintomes for animals, such as cows and pigs, since reproductive fecundity, muscle mass, and fat deposition are in part dependent upon imprinted gene expression (Coster et al. 2012; Magee et al. 2010; Van Laere et al. 2003; Wylie et al. 2000).

1.3.2.4 The Imprintome

The concept of the "imprintome" first appeared in print in 2009 (Jirtle 2009) and subsequently has been used with varying definitions (Cooper and Constancia 2010; Monk 2010). As the term was first coined, the human imprintome is "the environmentally labile *cis*-acting imprint regulatory elements in the human genome." Since genomic imprinting is a direct consequence of epigenetic regulation, the imprintome should be viewed as part of the epigenome, rather than the genome or transcriptome.

The importance of understanding the imprintome comes from the recognition that the advent of imprinting represents a significant vertical progression in mammalian evolution (Fig. 1.8). The imprintome is critical for development and growth, and our understanding of many complex human diseases is improving significantly as the involvement of imprinted genes and imprint regulation is increasingly being established. It is also important to distinguish between parent-of-origin-dependent monoallelic gene expression resulting from the imprintome, and other genes that

are monoallelically expressed, but not in a parental-dependent manner (Tycko 2010). Random parental monoallelic expression appears to function as a dosage control mechanism (Gimelbrant et al. 2007) and employs epigenetic regulatory mechanisms that differ from those utilized by genomically imprinted genes (Luedi et al. 2007). The imprintome is also distinguished from the remainder of the epigenome by its consistency and fidelity. It always exhibits parent-of-origin-specific epigenetic marks with very little spatial, temporal, or interindividual variability.

Understanding the human imprintome has become a greater necessity because of the demonstration of species-specific differences in imprinting establishment and regulation. Evidence that mouse and human imprinted gene repertoires show only about 30 % overlap (Luedi et al. 2005, 2007) should be taken as a caveat for modeling any human system or complex disease that includes imprinted genes. Given the plasticity of the epigenome, and by extension, the imprintome, it can be inferred that epigenetic divergence between species is more rapid and extensive than genetic divergence. If epigenetic changes, specifically imprint regulatory changes, are a driving force in mammalian evolution and species divergence (Proudhon et al. 2012), then the relevance of any model organism's imprintome in understanding the human imprintome is diminished.

An even closer species comparison emphasizes this point. The divergence of rat and mouse from a common ancestor occurred in the same approximate time frame as the divergence of human and macaque, approximately 20 MYA (Springer et al. 2003). Nevertheless, the genetic divergence between mouse and rat is nearly double of that between human and macaque (Gibbs et al. 2004), but mouse and rat have similar encephalization quotients (ratio of actual brain size to expected brain size based on body size), while the human encephalization is at least six times that of macaque (Williams 2002). Given the comparative genetic stability between human and monkey, it has been postulated that epigenetics provides the adaptable variability responsible for this sort of difference between species (Keverne 2011). Thus, to more thoroughly understand the pathogenesis of neurological disorders, the human imprintome must be defined.

Human Imprintome Identification

Presently, only 22 DMRs associated with known human imprinted genes have been identified (Skaar et al. 2012); this represents less than half of the experimentally identified imprinted genes (http://www.geneimprint.com). The discovery of most of these DMRs was by screening known imprinted genes for differential methylation by dye-terminator sequencing following bisulfite conversion (Tremblay et al. 1997). Sequencing by this method is capable of qualitatively identifying stretches of CpGs with intermediate methylation of genomic DNA. Confirmation of these regions is usually accomplished by the sequencing of clones to definitively identify parental allele-specific stretches of hyper- and hypomethylation (Tremblay et al. 1997). Newer methods, including pyrosequencing and mass spectrometry-based

methylation analysis using a Sequenom MassArray system (Sequenom, San Diego, CA), are also capable of quantitative methylation analysis. Nevertheless, they are unable to determine contiguous allele-specific methylation, so cloning is still necessary to definitively identify an imprint regulatory DMR.

The latest methods for high-throughput, parallel, next-generation sequencing (NGS) are allele specific, generate discrete sequences from single DNA strands, and are also quantitative. Applications for targeted sequencing of methylated regions include sequencing of methylated DNA captured by either immunoprecipitation with 5mC-specific antibodies (MeDIP-seq) (Down et al. 2008) or affinity purification by the MDB domain of MeCP2 (MethylCap-seq) (Brinkman et al. 2010). Similar methods could also be used to map 5-hydroxymethylcytosine (5hmC) with the appropriate antibodies. These methods provide powerful whole-genome coverage, but the length of each sequence read is much shorter than other methods, reducing the number of consecutive CpG sites analyzed. Another limitation is that capture efficiency depends on CpG density, so that regions with higher CpG content are overrepresented. Thus, imprintome elements with low CpG content will not be as effectively captured and sequenced and may be missed without a great depth of sequencing.

Direct whole-genome sequencing on bisulfite-converted DNA by NGS is also possible and would avoid any pulldown biases, but trade one drawback for another. With the bisulfite conversion of all cytosines not in CpG pairs, and many that are, there are only three bases in most sequence outputs. Thus, the resulting sequences can be ambiguous for genome alignment, particularly with the short reads of NGS, making analysis of de novo sequencing results challenging. Finally, bisulfite conversion and sequencing cannot distinguish between 5hmC and 5mC, a difference that may be highly significant for regulation.

The most recently developed sequencing methods, such as Pacific Biosciences (Menlo Park, CA) single-molecule real-time (SMRT) sequencing, sequence single molecules in real time. Nanopore sequencing, such as the GridION system of Oxford Nanopore Technologies (Oxford, UK), also has the same potential. Of greatest significance, these methods have the potential to directly identify methylated and possibly also hydroxymethylated bases in single-strand sequencing, not with bisulfite conversion, but with the use of variation in DNA polymerase kinetics (Flusberg et al. 2010) or altered electrical current across a nanopore (Wallace et al. 2010). These high-throughput methods hold the promise of unambiguous and unbiased sequencing that will be capable of identifying differential methylation.

Until NGS whole-genome methylation sequencing is validated and standardized, the most cost-effective method for comparative methylation analysis with complete genomic coverage is MeDIP-chip, using affinity pulldowns as described previously, but with analysis on whole-genome tiling arrays. Methylated DNA collected by MeDIP is quantitated by hybridization to genome arrays, providing relative methylation levels for screening purposes. Imprintome DMRs are consistent across tissues, time, and individuals, so false positives can be reduced by using diverse samples to eliminate variability. While the completeness of

genome-wide detection by NGS depends on the depth and redundancy of sequencing, the entire genome is interrogated equally when arrays are scanned. The resolution of this method is limited to array probe length, but more significantly is the already described challenge of distinguishing DMRs, with their typically low CpG density, from non-imprintome methylation. This requires multiple samples to provide sufficient power to distinguish between hypermethylation and differential methylation.

To eliminate variability from non-imprintome methylation, methylation mapping of gametic cells should be helpful since this approach detects only the original parentally established marks. A major drawback of this approach is that while sperm DNA is plentiful, human oocyte DNA is difficult to obtain in sufficient quantity for this type of assay. Analysis of sperm DNA alone has two limitations. Firstly, it contains DNA methylation other than the DMR-establishing marks. Secondly, the majority of known DMRs are established by maternal methylation, so sperm analysis would likely miss most novel DMRs. The ideal screen would use oocyte DNA to subtract common methylation and thereby identify paternal- and maternal-specific DNA methylation marks. Unfortunately, though the use of gametic DNA will be useful in defining gDMRs, it will not help define sDMRs.

Genome-wide interrogation of DNA methylation is a more straightforward process than the determination of histone modification status. Nevertheless, whole-genome arrays and NGS have the potential to screen for chromatin structure or histone modifications indicative of imprinting. Chromatin immunoprecipitation (ChIP) with antibodies specific to histone modifications, followed by either microarray analysis (ChIP-chip) or sequencing (ChIP-seq), can identify differentially marked regions, comparable to the methylation screens described.

Informatic approaches have also proven useful in the ascertainment of imprinted genes and imprintome elements. The list of potential imprinted human genes from the previously mentioned prediction study (Luedi et al. 2007) has resulted in the experimental confirmation of *DLGAP2*, *FAM50B, KCNK9, MAGI2,* and *NTM* as imprinted (Barbaux et al. 2012; Luedi et al. 2007); a DMR for *FAM50B* linked to monoallelic expression has also been identified (Zhang et al. 2011). Continuation of this approach using the convergence of informatic predictions, methylation analysis, and histone modification studies could have a great deal of power to specifically identify imprintome elements.

Unless an imprint regulatory element is in the promoter or resides within a gene, our knowing the imprintome does not identify the genes that are imprinted. Establishing imprint status requires determining if the expressions of genes in the genomic region of an identified DMR are parental allele dependent. Obtaining experimental confirmation of this unique expression pattern for imprinted genes can be a daunting task, particularly in humans, since monoallelic expression of imprinted genes can vary by cell type, developmental stage, and the sex of the individual (Gregg et al. 2010; Prickett and Oakey 2012). Interestingly, rare microdeletions and amplifications (i.e., CNVs) that alter disease phenotype in a parent-of-origin-dependent manner should be helpful in determining the imprinted genes regulated by the DMRs defined in the imprintome. In effect, such CNVs can

act as spotlights to focus our attention on those genes that are monoallelically expressed.

Ultimately, not only will the complete repertoire of imprinted genes be identified in the human but also in other mammalian species. By performing phylogenetic comparisons of the full complements of imprinted genes and their imprintomes, we will be able to determine the role they play in mammalian evolution and disease susceptibility.

1.4 Conclusions

Every time we look into a mirror or step on a scale, we know we are what we eat; however, we now know that we may also be what our mother was exposed to while we were in her womb (Jirtle and Skinner 2007; Waterland and Jirtle 2003). Furthermore, since epigenetic marks are not always erased in the egg and the sperm (Anway et al. 2005; Daxinger and Whitelaw 2012; Jirtle and Skinner 2007; Pembrey et al. 2006), we may even be what our grandparents and great grandparents were exposed to during their lives. Thus, recent epigenetic investigations have brought a novel biological perspective to the Biblical warning that the sins of the fathers are visited upon the children unto the third or fourth generation (Exodus 20:5). It is now evident that the ability to optimally diagnose, prevent, and treat diseases will remain elusive until it is more fully appreciated that human health and disease are not dependent solely upon genetic variations, but rather upon complex interactions between alterations in the rigid genome and the plastic epigenome.

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References

Anderson OS, Nahar MS, Faulk C, Jones TR, Liao C, Kannan K, Weinhouse C, Rozek LS, Dolinoy DC (2012) Epigenetic responses following maternal dietary exposure to physiologically relevant levels of bisphenol A. Environ Mol Mutagen 53:334–342

Anway MD, Cupp AS, Uzumcu M, Skinner MK (2005) Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308:1466–1469

Azzi S, Rossignol S, Steunou V, Sas T, Thibaud N, Danton F, Le Jule M, Heinrichs C, Cabrol S, Gicquel C, Le Bouc Y, Netchine I (2009) Multilocus methylation analysis in a large cohort of 11p15-related foetal growth disorders (Russell Silver and Beckwith Wiedemann syndromes) reveals simultaneous loss of methylation at paternal and maternal imprinted loci. Hum Mol Genet 18:4724–4733

Badcock C (2011) The imprinted brain: How genes set the balance between autism and psychosis. Epigenomics 3:345–359

Badcock C, Crespi B (2006) Imbalanced genomic imprinting in brain development: an evolutionary basis for the aetiology of autism. J Evol Biol 19:1007–1032
Badcock C, Crespi B (2008) Battle of the sexes may set the brain. Nature 454:1054–1055
Barbaux S, Gascoin-Lachambre G, Buffat C, Monnier P, Mondon F, Tonanny MB, Pinard A, Auer J, Bessieres B, Barlier A, Jacques S, Simeoni U, Dandolo L, Letourneur F, Jammes H, Vaiman D (2012) A genome-wide approach reveals novel imprinted genes expressed in the human placenta. Epigenetics 7:1079–1090
Barker DJ (2007) The origins of the developmental origins theory. J Intern Med 261:412–417
Barlow DP (2011) Genomic imprinting: a mammalian epigenetic discovery model. Annu Rev Genet 45:379–403
Barlow DP, Stoger R, Herrmann BG, Saito K, Schweifer N (1991) The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. Nature 349:84–87
Barton SC, Surani MA, Norris ML (1984) Role of paternal and maternal genomes in mouse development. Nature 311:374–376
Bell JT, Spector TD (2011) A twin approach to unraveling epigenetics. Trends Genet 27:116–125
Bernal AJ, Jirtle RL (2010) Epigenomic disruption: the effects of early developmental exposures. Birth Defects Res A Clin Mol Teratol 88:938–944
Bernal AJ, Dolinoy DC, Huang D, Skaar DA, Weinhouse C, Jirtle RL (2013) Adaptive radiation-induced epigenetic alterations mitigated by antioxidants. FASEB J 27:665–671
Blaxill MF (2004) What's going on? The question of time trends in autism. Public Health Rep 119:536–551
Brinkman AB, Simmer F, Ma K, Kaan A, Zhu J, Stunnenberg HG (2010) Whole-genome DNA methylation profiling using MethylCap-seq. Methods 52:232–236
Calabrese EJ (2009) The road to linearity: why linearity at low doses became the basis for carcinogen risk assessment. Arch Toxicol 83:203–225
Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Ekong J, Needham LL (2005) Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. Environ Health Perspect 113:391–395
Cameron JR (2005) Moderate dose rate ionizing radiation increases longevity. Br J Radiol 78:11–13
Cirulli ET, Goldstein DB (2010) Uncovering the roles of rare variants in common disease through whole-genome sequencing. Nat Rev Genet 11:415–425
Cooper WN, Constancia M (2010) How genome-wide approaches can be used to unravel the remaining secrets of the imprintome. Brief Funct Genomics 9:315–328
Coster A, Madsen O, Heuven HC, Dibbits B, Groenen MA, van Arendonk JA, Bovenhuis H (2012) The imprinted gene DIO3 is a candidate gene for litter size in pigs. PLoS One 7:e31825
Curley JP (2011) Is there a genomically imprinted social brain? Bioessays 33:662–668
Das R, Hampton DD, Jirtle RL (2009) Imprinting evolution and human health. Mamm Genome 20:563–572
Das R, Anderson N, Koran MI, Weidman JR, Mikkelsen TS, Kamal M, Murphy SK, Linblad-Toh K, Greally JM, Jirtle RL (2012) Convergent and divergent evolution of genomic imprinting in the marsupial *Monodelphis domestica*. BMC Genomics 13:394
Daxinger L, Whitelaw E (2012) Understanding transgenerational epigenetic inheritance via the gametes in mammals. Nat Rev Genet 13:153–162
DeChiara TM, Robertson EJ, Efstratiadis A (1991) Parental imprinting of the mouse insulin-like growth factor II gene. Cell 64:849–859
Dobzhansky T (1973) Nothing in biology makes sense except in the light of evolution. Am Biol Teach 35:125–129
Dolinoy DC, Weidman JR, Waterland RA, Jirtle RL (2006) Maternal genistein alters coat color and protects A^{vy} mouse offspring from obesity by modifying the fetal epigenome. Environ Health Perspect 114:567–572

Dolinoy DC, Huang D, Jirtle RL (2007) Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci USA 104:13056–13061

Down TA, Rakyan VK, Turner DJ, Flicek P, Li H, Kulesha E, Graf S, Johnson N, Herrero J, Tomazou EM, Thorne NP, Backdahl L, Herberth M, Howe KL, Jackson DK, Miretti MM, Marioni JC, Birney E, Hubbard TJ, Durbin R, Tavare S, Beck S (2008) A Bayesian deconvolution strategy for immunoprecipitation-based DNA methylome analysis. Nat Biotechnol 26:779–785

Druker R, Bruxner TJ, Lehrbach NJ, Whitelaw E (2004) Complex patterns of transcription at the insertion site of a retrotransposon in the mouse. Nucleic Acids Res 32:5800–5808

Duhl DM, Vrieling H, Miller KA, Wolff GL, Barsh GS (1994) Neomorphic agouti mutations in obese yellow mice. Nat Genet 8:59–65

Eggermann T, Eggermann K, Schonherr N (2008) Growth retardation versus overgrowth: silver-Russell syndrome is genetically opposite to Beckwith-Wiedemann syndrome. Trends Genet 24:195–204

Feinberg AP, Ohlsson R, Henikoff S (2006) The epigenetic progenitor origin of human cancer. Nat Rev Genet 7:21–33

Flusberg BA, Webster DR, Lee JH, Travers KJ, Olivares EC, Clark TA, Korlach J, Turner SW (2010) Direct detection of DNA methylation during single-molecule, real-time sequencing. Nat Methods 7:461–465

Gibbs RA, Weinstock GM, Metzker ML, Muzny DM, Sodergren EJ, Scherer S, Scott G, Steffen D, Worley KC, Burch PE, Okwuonu G, Hines S, Lewis L, DeRamo C, Delgado O, Dugan-Rocha S, Miner G, Morgan M, Hawes A, Gill R, Celera Holt RA, Adams MD, Amanatides PG, Baden-Tillson H, Barnstead M, Chin S, Evans CA, Ferriera S, Fosler C, Glodek A, Gu Z, Jennings D, Kraft CL, Nguyen T, Pfannkoch CM, Sitter C, Sutton GG, Venter JC, Woodage T, Smith D, Lee HM, Gustafson E, Cahill P, Kana A, Doucette-Stamm L, Weinstock K, Fechtel K, Weiss RB, Dunn DM, Green ED, Blakesley RW, Bouffard GG, De Jong PJ, Osoegawa K, Zhu B, Marra M, Schein J, Bosdet I, Fjell C, Jones S, Krzywinski M, Mathewson C, Siddiqui A, Wye N, McPherson J, Zhao S, Fraser CM, Shetty J, Shatsman S, Geer K, Chen Y, Abramzon S, Nierman WC, Havlak PH, Chen R, Durbin KJ, Egan A, Ren Y, Song XZ, Li B, Liu Y, Qin X, Cawley S, Worley KC, Cooney AJ, D'Souza LM, Martin K, Wu JQ, Gonzalez-Garay ML, Jackson AR, Kalafus KJ, McLeod MP, Milosavljevic A, Virk D, Volkov A, Wheeler DA, Zhang Z, Bailey JA, Eichler EE, Tuzun E, Birney E, Mongin E, Ureta-Vidal A, Woodwark C, Zdobnov E, Bork P, Suyama M, Torrents D, Alexandersson M, Trask BJ, Young JM, Huang H, Wang H, Xing H, Daniels S, Gietzen D, Schmidt J, Stevens K, Vitt U, Wingrove J, Camara F, Mar Alba M, Abril JF, Guigo R, Smit A, Dubchak I, Rubin EM, Couronne O, Poliakov A, Hubner N, Ganten D, Goesele C, Hummel O, Kreitler T, Lee YA, Monti J, Schulz H, Zimdahl H, Himmelbauer H, Lehrach H, Jacob HJ, Bromberg S, Gullings-Handley J, Jensen-Seaman MI, Kwitek AE, Lazar J, Pasko D, Tonellato PJ, Twigger S, Ponting CP, Duarte JM, Rice S, Goodstadt L, Beatson SA, Emes RD, Winter EE, Webber C, Brandt P, Nyakatura G, Adetobi M, Chiaromonte F, Elnitski L, Eswara P, Hardison RC, Hou M, Kolbe D, Makova K, Miller W, Nekrutenko A, Riemer C, Schwartz S, Taylor J, Yang S, Zhang Y, Lindpaintner K, Andrews TD, Caccamo M, Clamp M, Clarke L, Curwen V, Durbin R, Eyras E, Searle SM, Cooper GM, Batzoglou S, Brudno M, Sidow A, Stone EA, Venter JC, Payseur BA, Bourque G, Lopez-Otin C, Puente XS, Chakrabarti K, Chatterji S, Dewey C, Pachter L, Bray N, Yap VB, Caspi A, Tesler G, Pevzner PA, Haussler D, Roskin KM, Baertsch R, Clawson H, Furey TS, Hinrichs AS, Karolchik D, Kent WJ, Rosenbloom KR, Trumbower H, Weirauch M, Cooper DN, Stenson PD, Ma B, Brent M, Arumugam M, Shteynberg D, Copley RR, Taylor MS, Riethman H, Mudunuri U, Peterson J, Guyer M, Felsenfeld A, Old S, Mockrin S, Collins F (2004) Genome sequence of the Brown Norway rat yields insights into mammalian evolution. Nature 428:493–521

Gibson G (2011) Rare and common variants: twenty arguments. Nat Rev Genet 13:135–145

Gimelbrant A, Hutchinson JN, Thompson BR, Chess A (2007) Widespread monoallelic expression on human autosomes. Science 318:1136–1140
Girardot M, Cavaillé J, Feil R (2012) Small regulatory RNAs controlled by genomic imprinting and their contribution to human disease. Epigenetics 7:1341–1348
Gluckman PD (2012) Epigenetics and metabolism in 2011: epigenetics, the life-course and metabolic disease. Nat Rev Endocrinol 8:74–76
Gregg C, Zhang J, Butler JE, Haig D, Dulac C (2010) Sex-specific parent-of-origin allelic expression in the mouse brain. Science 329:682–685
Haig D (2004) Genomic imprinting and kinship: how good is the evidence? Annu Rev Genet 38:553–585
Haig D, Graham C (1991) Genomic imprinting and the strange case of the insulin-like growth factor II receptor. Cell 64:1045–1046
Hermann A, Gowher H, Jeltsch A (2004) Biochemistry and biology of mammalian DNA methyltransferases. Cell Mol Life Sci 61:2571–2587
Hunter P (2007) The silence of genes. Is genomic imprinting the software of evolution or just a battleground for gender conflict? EMBO Rep 8:441–443
Ishida M, Moore GE (2012) The role of imprinted genes in humans. Mol Aspects Med [Epub ahead of print]
Jelinic P, Shaw P (2007) Loss of imprinting and cancer. J Pathol 211:261–268
Jia D, Jurkowska RZ, Zhang X, Jeltsch A, Cheng X (2007) Structure of Dnmt3a bound to Dnmt3L suggests a model for de novo DNA methylation. Nature 449:248–251
Jirtle RL (2009) Epigenome: the program for human health and disease. Epigenomics 1:13–16
Jirtle RL, Skinner MK (2007) Environmental epigenomics and disease susceptibility. Nat Rev Genet 8:253–262
Jirtle RL, Weidman JR (2007) Imprinted and more equal. Am Sci 95:143–149
Kacem S, Feil R (2009) Chromatin mechanisms in genomic imprinting. Mamm Genome 20:544–556
Kaminen-Ahola N, Ahola A, Maga M, Mallitt KA, Fahey P, Cox TC, Whitelaw E, Chong S (2010) Maternal ethanol consumption alters the epigenotype and the phenotype of offspring in a mouse model. PLoS Genet 6:e1000811
Karlsson R, Graae L, Lekman M, Wang D, Favis R, Axelsson T, Galter D, Belin AC, Paddock S (2012) MAGI1 copy number variation in bipolar affective disorder and schizophrenia. Biol Psychiatry 71:922–930
Keverne EB (2001) Genomic imprinting, maternal care, and brain evolution. Horm Behav 40:146–155
Keverne EB (2011) Epigenetics and brain evolution. Epigenomics 3:183–191
Khosla S, Mendiratta G, Brahmachari V (2006) Genomic imprinting in the mealybugs. Cytogenet Genome Res 113:41–52
Killian JK, Byrd JC, Jirtle JV, Munday BL, Stoskopf MK, MacDonald RG, Jirtle RL (2000) M6p/igf2r imprinting evolution in mammals. Mol Cell 5:707–716
Killian JK, Nolan CM, Stewart N, Munday BL, Andersen NA, Nicol S, Jirtle RL (2001) Monotreme IGF2 expression and ancestral origin of genomic imprinting. J Exp Zool 291:205–212
Klenova EM, Morse HC 3, Ohlsson R, Lobanenkov VV (2002) The novel BORIS + CTCF gene family is uniquely involved in the epigenetics of normal biology and cancer. Semin Cancer Biol 12:399–414
Knudson AG (2001) Two genetic hits (more or less) to cancer. Nat Rev Cancer 1:157–162
Koide T, Banno M, Aleksic B, Yamashita S, Kikuchi T, Kohmura K, Adachi Y, Kawano N, Kushima I, Nakamura Y, Okada T, Ikeda M, Ohi K, Yasuda Y, Hashimoto R, Inada T, Ujike H, Iidaka T, Suzuki M, Takeda M, Iwata N, Ozaki N (2012) Common variants in MAGI2 gene are associated with increased risk for cognitive impairment in schizophrenic patients. PLoS One 7: e36836
Lewis A, Reik W (2006) How imprinting centres work. Cytogenet Genome Res 113:81–89

Luedi PP, Hartemink AJ, Jirtle RL (2005) Genome-wide prediction of imprinted murine genes. Genome Res 15:875–884

Luedi PP, Dietrich FS, Weidman JR, Bosko JM, Jirtle RL, Hartemink AJ (2007) Computational and experimental identification of novel human imprinted genes. Genome Res 17:1723–1730

Magee DA, Sikora KM, Berkowicz EW, Berry DP, Howard DJ, Mullen MP, Evans RD, Spillane C, MacHugh DE (2010) DNA sequence polymorphisms in a panel of eight candidate bovine imprinted genes and their association with performance traits in Irish Holstein-Friesian cattle. BMC Genet 11:93

Maher B (2008) Personal genomes: the case of the missing heritability. Nature 456:18–21

McGrath J, Solter D (1984) Completion of mouse embryogenesis requires both the maternal and paternal genomes. Cell 37:179–183

McMillen IC, Robinson JS (2005) Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol Rev 85:571–633.

Miltenberger RJ, Mynatt RL, Wilkinson JE, Woychik RP (1997) The role of the agouti gene in the Yellow Obese Syndrome. J Nutr 127:1902S–1907S

Monk D (2010) Deciphering the cancer imprintome. Brief Funct Genomics 9:329–339

Monk D, Arnaud P, Apostolidou S, Hills FA, Kelsey G, Stanier P, Feil R, Moore GE (2006) Limited evolutionary conservation of imprinting in the human placenta. Proc Natl Acad Sci USA 103:6623–6628

Moore T, Haig D (1991) Genomic imprinting in mammalian development: a parental tug-of-war. Trends Genet 7:45–49

Morgan HD, Sutherland HGE, Martin DIK, Whitelaw E (1999) Epigenetic inheritance at the agouti locus in the mouse. Nat Genet 23:314–318

Morgan HD, Jin XL, Li A, Whitelaw E, O'Neill C (2008) The culture of zygotes to the blastocyst stage changes the postnatal expression of an epigenetically labile allele, agouti viable yellow, in mice. Biol Reprod 79:618–623

Morison IM, Ramsay JP, Spencer HG (2005) A census of mammalian imprinting. Trends Genet 21:457–465

Murphy SK, Jirtle RL (2003) Imprinting evolution and the price of silence. Bioessays 25:577–588

Nolan CM, Killian JK, Petitte JN, Jirtle RL (2001) Imprint status of M6P/IGF2R and IGF2 in chickens. Dev Genes Evol 211:179–183

Ollikainen M, Craig JM (2011) Epigenetic discordance at imprinting control regions in twins. Epigenomics 3:295–306

O'Neill MJ, Ingram RS, Vrana PB, Tilghman SM (2000) Allelic expression of IGF2 in marsupials and birds. Dev Genes Evol 210:18–20

O'Sullivan FM, Murphy SK, Simel LR, McCann A, Callanan JJ, Nolan CM (2007) Imprinted expression of the canine IGF2R, in the absence of an anti-sense transcript or promoter methylation. Evol Dev 9:579–589

Pearce MS, Salotti JA, Little MP, McHugh K, Lee C, Kim KP, Howe NL, Ronckers CM, Rajaraman P, Sir Craft AW, Parker L, de Gonzalez AB (2012) Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a retrospective cohort study. Lancet 380:499–505

Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjostrom M, Golding J (2006) Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet 14:159–166

Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R, Conroy J, Magalhaes TR, Correia C, Abrahams BS, Almeida J, Bacchelli E, Bader GD, Bailey AJ, Baird G, Battaglia A, Berney T, Bolshakova N, Bolte S, Bolton PF, Bourgeron T, Brennan S, Brian J, Bryson SE, Carson AR, Casallo G, Casey J, Chung BH, Cochrane L, Corsello C, Crawford EL, Crossett A, Cytrynbaum C, Dawson G, de Jonge M, Delorme R, Drmic I, Duketis E, Duque F, Estes A, Farrar P, Fernandez BA, Folstein SE, Fombonne E, Freitag CM, Gilbert J, Gillberg C, Glessner JT, Goldberg J, Green A, Green J, Guter SJ, Hakonarson H, Heron EA, Hill M, Holt R, Howe JL, Hughes G, Hus V, Igliozzi R, Kim C, Klauck SM, Kolevzon A, Korvatska O, Kustanovich V, Lajonchere CM, Lamb JA, Laskawiec M, Leboyer M, Le Couteur A, Leventhal BL, Lionel

AC, Liu XQ, Lord C, Lotspeich L, Lund SC, Maestrini E, Mahoney W, Mantoulan C, Marshall CR, McConachie H, McDougle CJ, McGrath J, McMahon WM, Merikangas A, Migita O, Minshew NJ, Mirza GK, Munson J, Nelson SF, Noakes C, Noor A, Nygren G, Oliveira G, Papanikolaou K, Parr JR, Parrini B, Paton T, Pickles A, Pilorge M, Piven J, Ponting CP, Posey DJ, Poustka A, Poustka F, Prasad A, Ragoussis J, Renshaw K, Rickaby J, Roberts W, Roeder K, Roge B, Rutter ML, Bierut LJ, Rice JP, Salt J, Sansom K, Sato D, Segurado R, Sequeira AF, Senman L, Shah N, Sheffield VC, Soorya L, Sousa I, Stein O, Sykes N, Stoppioni V, Strawbridge C, Tancredi R, Tansey K, Thiruvahindrapduram B, Thompson AP, Thomson S, Tryfon A, Tsiantis J, Van Engeland H, Vincent JB, Volkmar F, Wallace S, Wang K, Wang Z, Wassink TH, Webber C, Weksberg R, Wing K, Wittemeyer K, Wood S, Wu J, Yaspan BL, Zurawiecki D, Zwaigenbaum L, Buxbaum JD, Cantor RM, Cook EH, Coon H, Cuccaro ML, Devlin B, Ennis S, Gallagher L, Geschwind DH, Gill M, Haines JL, Hallmayer J, Miller J, Monaco AP, Nurnberger JIJ, Paterson AD, Pericak-Vance MA, Schellenberg GD, Szatmari P, Vicente AM, Vieland VJ, Wijsman EM, Scherer SW, Sutcliffe JS, Betancur C (2010) Functional impact of global rare copy number variation in autism spectrum disorders. Nature 466:368–372

Prickett AR, Oakey RJ (2012) A survey of tissue-specific genomic imprinting in mammals. Mol Genet Genomics 287:621–630

Proudhon C, Duffie R, Ajjan S, Cowley M, Iranzo J, Carbajosa G, Saadeh H, Holland ML, Oakey RJ, Rakyan VK, Schulz R, Bourc'his D (2012) Protection against de novo methylation is instrumental in maintaining parent-of-origin methylation inherited from the gametes. Mol Cell 47:909–920

Rakyan VK, Blewitt ME, Druker R, Preis JI, Whitelaw E (2002) Metastable epialleles in mammals. Trends Genet 18:348–351

Reik W, Walter J (2001) Genomic imprinting: parental influence on the genome. Nat Rev Genet 2:21–32

Renfree MB, Hore TA, Shaw G, Marshall Graves JA, Pask AJ (2009) Evolution of genomic imprinting: insights from marsupials and monotremes. Annu Rev Genomics Hum Genet 10:241–262

Riccio A, Sparago A, Verde G, De Crescenzo A, Citro V, Cubellis MV, Ferrero GB, Silengo MC, Russo S, Larizza L, Cerrato F (2009) Inherited and sporadic epimutations at the IGF2-H19 locus in Beckwith-Wiedemann syndrome and Wilms' tumor. Endocr Dev 14:1–9

Sanders C (2009) Radiation hormesis and the linear-no-threshold assumption. Springer, New York

Schoenherr CJ, Levorse JM, Tilghman SM (2003) CTCF maintains differential methylation at the Igf2/H19 locus. Nat Genet 33:66–69

Scott RJ, Spielman M (2006) Genomic imprinting in plants and mammals: how life history constrains convergence. Cytogenet Genome Res 113:53–67

Skaar DA, Li Y, Bernal AJ, Hoyo C, Murphy SK, Jirtle RL (2012) The human imprintome: regulatory mechanisms, methods of ascertainment, and roles in disease susceptibility. ILAR J 53:341–358

Soto AM, Sonnenschein C (2010) Environmental causes of cancer: endocrine disruptors as carcinogens. Nat Rev Endocrinol 6:363–370

Springer MS, Murphy WJ, Eizirik E, O'Brien SJ (2003) Placental mammal diversification and the Cretaceous-Tertiary boundary. Proc Natl Acad Sci U S A 100:1056–1061

St Clair D, Xu M, Wang P, Yu Y, Fang Y, Zhang F, Zheng X, Gu N, Feng G, Sham P, He L (2005) Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959–1961. JAMA 294:557–562

Surani MA, Barton SC, Norris ML (1984) Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. Nature 308:548–550

Susser E, Neugebauer R, Hoek HW, Brown AS, Lin S, Labovitz D, Gorman JM (1996) Schizophrenia after prenatal famine. Further evidence. Arch Gen Psychiatry 53:25–31

Tremblay KD, Duran KL, Bartolomei MS (1997) A 5′ 2-kilobase-pair region of the imprinted mouse H19 gene exhibits exclusive paternal methylation throughout development. Mol Cell Biol 17:4322–4329
Turek-Plewa J, Jagodzinski PP (2005) The role of mammalian DNA methyltransferases in the regulation of gene expression. Cell Mol Biol Lett 10:631–647
Tycko B (2010) Allele-specific DNA methylation: beyond imprinting. Hum Mol Genet 19: R210–R220
Uribe-Lewis S, Woodfine K, Stojic L, Murrell A (2011) Molecular mechanisms of genomic imprinting and clinical implications for cancer. Expert Rev Mol Med 13:e2
Vaiserman AM (2011) Hormesis and epigenetics: is there a link? Ageing Res Rev 10:413–421
Van Laere AS, Nguyen M, Braunschweig M, Nezer C, Collette C, Moreau L, Archibald AL, Haley CS, Buys N, Tally M, Andersson G, Georges M, Andersson L (2003) A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature 425:832–836
Vasicek TJ, Zeng L, Guan XJ, Zhang T, Costantini F, Tilghman SM (1997) Two dominant mutations in the mouse fused gene are the result of transposon insertions. Genetics 147:777–786
Vrana PB, Guan XJ, Ingram RS, Tilghman SM (1998) Genomic imprinting is disrupted in interspecific Peromyscus hybrids. Nat Genet 20:362–365
Waddington CH (1940) Organisers and genes. Cambridge University Press, Cambridge, UK
Wallace EV, Stoddart D, Heron AJ, Mikhailova E, Maglia G, Donohoe TJ, Bayley H (2010) Identification of epigenetic DNA modifications with a protein nanopore. Chem Commun (Camb) 46:8195–8197
Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM, Nord AS, Kusenda M, Malhotra D, Bhandari A, Stray SM, Rippey CF, Roccanova P, Makarov V, Lakshmi B, Findling RL, Sikich L, Stromberg T, Merriman B, Gogtay N, Butler P, Eckstrand K, Noory L, Gochman P, Long R, Chen Z, Davis S, Baker C, Eichler EE, Meltzer PS, Nelson SF, Singleton AB, Lee MK, Rapoport JL, King MC, Sebat J (2008) Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. Science 320:539–543
Waterland RA, Jirtle RL (2003) Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 23:5293–5300
Waterland RA, Kellermayer R, Laritsky E, Rayco-Solon P, Harris RA, Travisano M, Zhang W, Torskaya MS, Zhang J, Shen L, Manary MJ, Prentice AM (2010) Season of conception in rural gambia affects DNA methylation at putative human metastable epialleles. PLoS Genet 6: e1001252
Weidman JR, Dolinoy DC, Maloney KA, Cheng JF, Jirtle RL (2006) Imprinting of opossum Igf2r in the absence of differential methylation and air. Epigenetics 1:49–54.
Weinhouse C, Anderson OS, Jones TR, Kim J, Liberman SA, Nahar MS, Rozek LS, Jirtle RL, Dolinoy DC (2011) An expression microarray approach for the identification of metastable epialleles in the mouse genome. Epigenetics 6:1105–1113
Williams MF (2002) Primate encephalization and intelligence. Med Hypotheses 58:284–290
Williams G, Harrold JA, Cutler DJ (2000) The hypothalamus and the regulation of energy homeostasis: lifting the lid on a black box. Proc Nutr Soc 59:385–396
Wolff GL, Kodell RL, Moore SR, Cooney CA (1998) Maternal epigenetics and methyl supplements affect agouti gene expression in A^{vy}/a mice. FASEB J 12:949–957
Woogh C (2001) Is schizophrenia on the decline in Canada? Can J Psychiatry 46:61–67
Wylie AA, Murphy SK, Orton TC, Jirtle RL (2000) Novel imprinted DLK1/GTL2 domain on human chromosome 14 contains motifs that mimic those implicated in IGF2/H19 regulation. Genome Res 10:1711–1718
Yajnik CS (2004) Early life origins of insulin resistance and type 2 diabetes in India and other Asian countries. J Nutr 134:205–210
Yen TT, Gill AM, Frigeri LG, Barsh GS, Wolff GL (1994) Obesity, diabetes, and neoplasia in yellow A(vy)/- mice: ectopic expression of the agouti gene. FASEB J 8:479–488

Yotova IY, Vlatkovic IM, Pauler FM, Warczok KE, Ambros PF, Oshimura M, Theussl HC, Gessler M, Wagner EF, Barlow DP (2008) Identification of the human homolog of the imprinted mouse Air non-coding RNA. Genomics 92:464–473

Zhang Y, Tycko B (1992) Monoallelic expression of the human H19 gene. Nat Genet 1:40–44

Zhang A, Skaar DA, Li Y, Huang D, Price TM, Murphy SK, Jirtle RL (2011) Novel retrotransposed imprinted locus identified at human 6p25. Nucleic Acids Res 39:5388–5400

Zwijnenburg PJ, Meijers-Heijboer H, Boomsma DI (2010) Identical but not the same: the value of discordant monozygotic twins in genetic research. Am J Med Genet B Neuropsychiatr Genet 153B:1134–1149

Chapter 2
Developmental Epigenomics and Metabolic Disease

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Abstract Organisms have evolved the processes of developmental plasticity to adjust their developmental trajectory in response to early life exposure to environmental cues. The potentially adaptive nature of such anticipatory responses is aimed at promoting Darwinian fitness; however, a mismatch between the induced phenotype and the later life environment increases the risk of disease, as evolutionary processes act to maximize reproductive success and not to maintain health and longevity. We focus on the developmental origins of later life metabolic disease in humans particularly with respect to early life under- and overnutrition and review the experimental, epidemiological, and clinical evidence implicating epigenetic mechanisms in the modulation of disease risk. There is growing evidence that epigenetic marks—and hence possibly disease risk—may be transgenerationally transmitted via non-genomic pathways of inheritance. This has implications both for the perpetuation of disease risk across generations and for the potential impact on evolution should epigenetic marks become genomically fixed. We also discuss the potential of developmental epigenomics in identifying prognostic biomarkers and therapeutic targets and raise several technical and methodological caveats to be borne in mind when undertaking and interpreting epigenomic research.

Keywords Childhood abuse • Development • Developmental origins of health and disease • Developmental plasticity • Diabetes • Environmental toxins • Epigenetics •

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Epigenomics • Evolution • Fitness • Health • Intervention • Maternal care • Maternal nutrition • Metabolic disease • Mismatch • Obesity • Overnutrition • Phenotypic inertia • Predictive adaptive response • Stress • Trade-off • Undernutrition

Abbreviations

DOHaD	Developmental origins of health and disease
HPA	Hypothalamic-pituitary-adrenal
IAR	Immediate adaptive response
PAR	Predictive adaptive response

2.1 Introduction

Gene-environment interactions have traditionally been used to explain phenotypic variation in humans, such as differential susceptibility to disease. However, such a dichotomous perspective is increasingly becoming outmoded in light of the accumulating data pointing to the role of early life development in disease causation. In this chapter we discuss how the processes of developmental plasticity—whereby phenotypic development is influenced by developmental experiences—affect an individual's interaction with its environment at maturity and, in turn, its risk of metabolic disease. Developmental plasticity is now well recognized as being underpinned by epigenetic mechanisms, which are phylogenetically old processes that regulate gene expression in response to environmental cues through covalent modification of DNA and its associated molecules. We also discuss how the emerging field of developmental epigenomics may be exploited to identify prognostic biomarkers and indicate targets for therapy and the attendant methodological pitfalls of which to be mindful.

2.2 Developmental Plasticity

Far from passively adhering to a rigid genetic blueprint during early development, the developing organism is receptive to external cues and responds by adjusting its phenotypic development, thus resulting in several different possible phenotypes arising from a single genotype. It achieves this via the processes of developmental plasticity, which are ecologically prevalent and seen across many taxa (Bateson et al. 2004). Examples include the female honeybee which develops into either a queen or a worker according to how the early larva is fed; the water flea which

forms a defensive helmet and tail spike when its mother is exposed to chemicals released by predators, allowing it to cope with a potentially dangerous postnatal environment; the larval desert locust which receives parental chemical signals that act as a proxy for population density and in response develops into either a nocturnal, solitary morph or a diurnal, gregarious morph; and the spade-foot tadpole which undergoes earlier metamorphosis from tadpole to terrestrial form to promote immediate survival when habitat desiccation is imminent. In this review we shall focus on the potential role of the processes of developmental plasticity in humans and consider how these may contribute to the risk of metabolic disease in later life.

Developmental plasticity clearly plays an essential role in coping with changing environments on a time-scale intermediate between coping via homeostasis and responding through phenotype selection. It has evolved to enable an organism to modulate its phenotypic development and life course in potentially adaptive ways (Bateson et al. 2004; Bateson and Gluckman 2011), that is, increasing Darwinian fitness to allow gene flow to the next generation. With respect to cues such as nutritional state, it is likely that there is some level of phenotypic inertia in the formation of responses to environmental cues: taking into account a range of environmental conditions over a longer time scale may enable a more accurate picture of the future environment to be obtained (Kuzawa and Thayer 2011).

The potential reaction norm of induced phenotypes provided by developmental plasticity is presumably limited by the range of environmental exposures previously experienced by the lineage. A further constraint emerges from the likely developmental and energetic costs involved in maintaining the capacity for plasticity. Hence, these processes tend to be limited to windows during early development (Bateson and Gluckman 2011). The mechanisms underlying developmental plasticity include changes in epigenetic marks that modulate the expression of specific genes under specific contexts (Waterland and Jirtle 2003; Gluckman et al. 2011). There are also emerging hints that epigenetic changes may be transgenerationally transmitted through non-genomic mechanisms of inheritance (Low et al. 2011).

2.2.1 Environmental Interactions During Development

Developmental plasticity is one of several responses that organisms employ during development in response to environmental exposures. The type of response elicited is dependent on where on the spectrum of severity and evolutionary novelty the environmental exposures lie. Extreme environmental challenges that overwhelm the adaptive capacity, or which are entirely novel and thus are not countered by evolved defenses, can result in irreversible disruption of the normal developmental program. Limb malformation in children whose mothers were treated with thalidomide during pregnancy is an example of such a teratogenic response. Challenges that are less severe, and particularly those that occur within the normative

ecological context, generally induce a potentially adaptive plastic response (Gluckman et al. 2009).

The phase within the life course when the phenotypic consequences of these responses become apparent allows an additional level of classification into immediate adaptive responses (IARs) or predictive adaptive responses (PARs), although there is no sharp delineation between the two and both can be induced by the same cue (Gluckman et al. 2005b). IARs are made when survival is immediately threatened and a prompt response is needed, but this is usually followed by longer-term trade-offs. For example, the fetus challenged by placental dysfunction may be born prematurely and growth retarded so as to improve its immediate chances of survival, but this incurs an unavoidable trade-off of greater morbidity and mortality postnatally (Gluckman et al. 2005b).

Cues of a more subtle nature may evoke PARs which do not lead to an overt phenotype at birth, but nevertheless bestow potentially adaptive advantages later in life. However, what has often been misunderstood is that these responses are most likely to be fitness enhancing in childhood through to the early reproductive years (Godfrey et al. 2010), as human fitness is largely determined by childhood and adolescent survival (Jones 2009). There may be a loss of potential advantage under some circumstances from the induction of such responses in mismatched environments later in life, but this is inconsequential in evolutionary terms. By adapting the phenotype better to the predicted ecological (e.g., nutrition and stress) environment in childhood and adolescence, fitness is likely to be enhanced. Such a model is supported by the observation that puberty is accelerated in girls of lower birth size (Sloboda et al. 2007).

Anticipatory responses are a widespread phenomenon, and the PAR model is supported by much experimental and clinical evidence. The coat characteristics of the meadow vole are particularly illustrative—using day length cues as transduced by maternal melatonin, voles are born with a thin coat during spring in anticipation of a hot summer or a thick coat in autumn which only confers an advantage later in the winter season (Lee and Zucker 1988). The ability to use prenatal cues to predict the extremes of temperatures encountered in adulthood therefore promotes survival and reproduction. Fetally undernourished rats fed a normal postnatal diet become obese, hypertensive, and insulin and leptin resistant and develop behavioral modifications reflected in increased food consumption and decreased physical activity (Vickers et al. 2000). This set of features comprises an integrated phenotype that had been tailored to cope with the nutritional scarcity anticipated to prevail postnatally. A high-fat diet amplifies these features, but administration of the adipokine leptin to neonatal rats can prevent development of all aspects of metabolic dysfunction (Vickers et al. 2005). In humans, retrospective analysis of a cohort of adults who were gravely undernourished in childhood showed that those who were smaller at birth developed marasmus, a more benign form of malnutrition, compared to those larger at birth who developed kwashiorkor, a form with a poorer survival prognosis (Forrester et al. 2012). They also had persistent changes in metabolic physiology. This suggests that potential marasmics were able to make

PARs enabling the development of a metabolic physiology that is more compatible with a low-nutrient environment.

2.2.2 The Role of Epigenetics in Developmental Plasticity

A rapidly accumulating body of literature indicates that epigenetically mediated modulation of the expression of specific genes and pathways plays a crucial role in the processes of developmental plasticity. This understanding has been considerably aided by next-generation technological advances in sequencing analysis over the past few years (Ku et al. 2011). Epigenetic mechanisms affecting gene dosage underpin several developmental processes including cell differentiation, genomic imprinting, and X-chromosome inactivation in female mammals. It would appear that the same biological processes have been co-opted for a range of core functions. While these other uses of epigenetic processes are generally stable, developmental plasticity involves contingent changes in gene regulation that may be transient or persist across the life course.

The honeybee referred to above provides a good example of how epigenetic processes mediate developmental plasticity. Although early life nutrition in terms of duration of access to royal jelly normally determines whether larvae become queens or workers, experimentally induced silencing of *Dnmt3*, which encodes the DNA methyltransferase enzyme responsible for establishing new methylation marks, causes most larvae to develop into queens with the characteristic enlarged ovaries (Kucharski et al. 2008).

2.2.3 Epigenetics and Evolution

Epigenetic processes, such as DNA methylation and small RNA activity, are thought to have evolved as defense mechanisms to protect the genome against transposable elements—potentially mutagenic mobile DNA segments that move from one location of the genome to another (Slotkin and Martienssen 2007). Epigenetic silencing may have evolved to counter viral infections or to silence the second genome during cell fusion in bacteria. Alternatively, DNA methylation may also have enabled gene dosage rebalance after gene duplication (Chang and Liao 2012). Others have suggested that the epigenetic function of changes in histone structure may have arisen as an incidental outcome from their primary role as regulators of gene expression (Felsenfeld and Groudine 2003). Mattick has also proposed that noncoding RNAs may have evolved as a means of regulating the complex processes of plasticity underpinning cognition and brain function, perhaps in part through their ability to be differentially edited depending on the context (Mattick 2011).

2.2.3.1 Potential Role of Epigenetic Change in Evolutionary Processes

Could epigenetic processes play a role in guiding genetic evolution? West-Eberhard (2003) has suggested that changes in phenotype induced by the environment can lead to adaptive evolution and the origin of evolutionary novelties. Thus, rather than being the originator of evolutionary change, genes may instead serve as "followers" in maintaining developmentally induced phenotypic changes. Indeed, a growing body of evidence points towards the transgenerational transmission of developmentally induced, epigenetically regulated phenotypes (Gluckman et al. 2007a), but the discussion to date on whether heritable induced phenotypes could drive evolutionary change has been limited by insufficient empirical evidence for a mechanism.

A key question is whether changes in epigenetic marks prompted by early life exposures could become incorporated into the genome. Theoretically, this may be achieved through several routes. Firstly, recurrence of the inducing cues over multiple generations may stabilize the phenotype until genomic fixation occurs via genetic accommodation, the processes of which are still poorly defined. Secondly, the propensity of methylated or hydroxymethylated cytosine to spontaneously hypermutate into thymine or uracil, respectively (Pfeifer 2006), causing nonrandom changes to the DNA sequence, may generate a range of phenotypes that undergo natural selection and become genomically fixed. Finally, increasing data from animal studies examining male germline inheritance have suggested that epigenetic marks may pass meiosis by way of microRNAs (Wagner et al. 2008) or of methylation and/or histone marks in sperm (Ng et al. 2010; Morgan and Bale 2011). This supports the plausibility of selection acting on the phenotype induced by a particular epigenetic mark; phenotypes that enhance fitness could conceivably then become genomically fixed and drive evolutionary change.

2.3 Developmental Plasticity, Health, and Disease

2.3.1 Developmental and Evolutionary Pathways to Disease

We proposed that PARs act to confer longer-term adaptive advantages that enhance an organism's fitness (Gluckman et al. 2005a). However, the efficacy of this strategy is dependent on the accuracy of early life cues received. If the cues are not faithfully transmitted or are not reflective of the actual environment, the fetus will consequently make erroneous predictions and adopt a developmental trajectory that is unsuited to its mature environment, thereby elevating risk of disease in later life. This scenario is known as a developmental mismatch (Gluckman and Hanson 2006). For example, if the fetus receives inadequate nutrition, whether due to placental dysfunction, poor maternal diet, or maternal constraint (maternally imposed physiological limitations on fetal growth to reduce the risk of obstructed

labor), an energy-poor environment is predicted, and an energy-conserving metabolic physiology is developed—even though the environment may be nutritionally abundant in reality. It then becomes mismatched to a postnatal obesogenic environment, potentially leading to metabolic disease. The conditions under which predictive responses are evolutionarily protected have been defined—selective advantage is possible even if the prediction is not always accurate (Jablonka et al. 1995).

The consequences of mismatch need not be symmetrical: there is a greater fitness disadvantage for a human in predicting a nutritionally rich environment and being born into a nutritionally poor environment than vice versa. Both survival and fertility are more likely to be compromised in the former, and the Paleolithic human was most likely to be exposed to less nutritionally rich environments. The presence of maternal constraint further biases the prediction. The key issue is when in the life course the disadvantage appears. In general the impact of a nutritionally rich environment does not affect health until after peak fertility, at least in females. As evolution is driven by fitness, and not by health or longevity per se, such late-onset disadvantage is generally of no evolutionary consequence.

Evidence of developmental mismatch in humans abounds and will be discussed in detail below. Migrants or refugees who were born in conditions of poor nutrition and have moved to countries with a more energy-dense, fat- and sugar-laden diet are at greater risk of diabetes and cardiovascular disease (Jeemon et al. 2009). Similar health problems are also increasingly seen in countries undergoing rapid economic growth and hence nutritional transition to a diet more comparable in composition and quantity to the "Western" diet, such as in India and China.

Two points should be noted regarding the nature of mismatch. Firstly, not every individual who is mismatched to their environment will inexorably develop disease. Other factors such as the individual's specific developmental history, later life environment, and their genome may also play a role. Mismatch simply increases the risk of developing disease in later life. Secondly, because developmental plasticity operates under normal as well as under adverse conditions (Gluckman et al. 2011), the induction of variable risk in a mismatched environment is not limited to extreme scenarios. For instance, risk of death from heart disease is higher among those of low birth weight, but the effect is graded across the normal range of birth weights, indicating that plastic responses are occurring even in uneventful pregnancies (Godfrey 2006). Similar graded effects have also been observed for risk of diabetes, obesity, and hypertension. Since no obvious impact is seen in the fetus or mother, and such effects can be induced by normal physiological cues (i.e., maternal nutritional status throughout the offspring's early development), "normal" birth weight may well be obscuring subtle developmental effects that nonetheless increase risk of later life disease. It is important to note that the "normal" range in terms of nutrition may include over- as well as undernutrition, either of which can be viewed as unbalanced nutrition leading to potential mismatch in the offspring.

2.3.2 Developmental Origins of Health and Disease

The involvement of a developmental component in determining disease risk in later life—a phenomenon dubbed the Developmental Origins of Health and Disease (DOHaD)—is a relatively new field of research. While there was a series of earlier clinical and experimental papers (reviewed in Gluckman et al. (2010)), it only received more focused attention after the publication of several highly influential papers from David Barker's group. They showed that reduced fetal growth, as measured by low birth weight, was associated with greater risk of cardiovascular-related mortality in later life (Barker et al. 1989). These findings proved provocative as they challenged preconceived assumptions that adverse cues experienced during gestation would only become manifest during the immediate postnatal period and not produce effects after a lag of several decades. However, further research has since verified this phenomenon by demonstrating similar associations for other metabolic disorders including insulin resistance, obesity, hypertension, diabetes, and osteoporosis (Godfrey 2006) coupled with a plethora of studies in a number of animal species. Today there is an increasing appreciation of the importance of DOHaD research in understanding contemporary human morbidity and mortality.

2.3.3 Developmental Origins of Obesity and Related Metabolic Diseases

An extensive body of experimental studies in animals involving maternal dietary and behavioral or hormonal manipulation from pregnancy to weaning has revealed how offspring can be "primed" to develop characteristics akin to that of human cardiometabolic disease in later life. Two widely used rat models involve subjecting pregnant dams to a hypocaloric or protein-restricted diet during pregnancy and/or lactation (Vickers et al. 2000; Lillycrop et al. 2009). Offspring later develop obesity, hypertension, impairments in glucose homeostasis and lipid metabolism, and dietary fat preference. These effects are exacerbated when offspring are fed a high-fat postnatal diet, which simulates a situation of greater mismatch. Recent work in rodents has suggested that such developmental induction can also be effected by paternal factors (Carone et al. 2010; Ng et al. 2010; Morgan and Bale 2011).

In humans, the prolonged study periods required for longitudinal analysis have limited experimental and clinical studies. Therefore, retrospective analysis of so-called natural experiments, such as famines and pandemics which have exposed population groups to a putative inducer of developmental programming, has yielded the most useful data (Vaiserman 2011). A cohort of Dutch individuals whose mothers were subjected to an acute period of famine during pregnancy as a result of Nazi-imposed food restrictions have served as a valuable resource.

Compared to their unexposed siblings, these people as adults tend to have higher plasma lipid levels and higher rates of obesity and coronary artery disease (Heijmans et al. 2009). Comparable observations have also been made in Nigerian individuals exposed to conditions of famine during early life, who were then at higher risk of hypertension and impaired glucose tolerance in adulthood (Hult et al. 2010).

Prenatal undernutrition is not the only developmental route by which predisposition to metabolic disease could occur. Early life overnutrition, maternal adiposity, gestational diabetes, and excessive weight gain during pregnancy are also risk factors for a similar outcome (Boney et al. 2005). The downstream metabolic effects of early life overnutrition have been corroborated by many animal studies. For example in mice, offspring of mothers fed a high-fat diet from weaning until lactation developed hypertension, fatty liver, and lipid metabolism dysregulation in adulthood (Elahi et al. 2009). In rats, a maternal high-fat diet induced hepatic fat deposition and insulin insensitivity in offspring at adulthood (Bruce et al. 2009; Dunn and Bale 2009).

The apparent paradox of diametrically different exposures giving rise to similar health outcomes in later life suggests that multiple developmental pathways are at play. Our understanding of the biological basis of how early life overnutrition is linked to adverse health outcomes is rudimentary. There are suggestions that disruption of mitochondrial function, oxidative stress, and upregulated placental transport could contribute (Bruce and Hanson 2010); however, there is now also growing evidence that epigenetic factors contribute to both pathways, as discussed later.

Although gestational diabetes has adverse consequences on offspring health, evolutionary explanations could account for the lack of negative selection against it: as mild fetal hyperinsulinemia is growth promoting, the placenta may have evolved to buffer against fetal undernutrition rather than overnutrition; furthermore obesogenic environments would have been highly unlikely during the course of our evolutionary history, and it is only with the current ubiquity of nutritional overload that an evolutionary mismatch has arisen (Ma et al. submitted).

The trends towards poorer dietary quality and increasing rates of maternal obesity and gestational diabetes in many developing and developed countries are raising concerns about the health impact on the next generation and in particular the perpetuation of a vicious cycle of "diabesity" (Poston 2011). Paternal influences at the time of conception may also play a role: daughters of male rats chronically fed a high-fat diet have impaired pancreatic function, suggesting that the generational effects must have been caused by altered sperm development or molecular programming in the father's germline (Ng et al. 2010). Food preferences may be shaped by the nature of maternal diets and the foods onto which children are weaned (Beauchamp and Mennella 2009). Infant diet can also modulate disease risk. For example, an early transition from breast milk to formula feeding increases the risk of being overweight in adolescence (Gillman et al. 2001).

2.3.3.1 Epigenetics: Experimental Evidence

The initial resistance towards the idea that disease with a later life onset could have developmental origins was partially due to the lack of evidence for a tenable biological mechanism that could account for the epidemiological observations. However, a wealth of animal data have now identified modifications of specific epigenetic marks in association with alterations in gene expression and disease susceptibility. In the rat undernutrition model described above, increased methylation at the promoter region of *Ppara*, which encodes a major regulator of lipid metabolism, was seen in conjunction with decreased gene expression in hepatocytes (Gluckman et al. 2007b). Additionally, both epigenetic and phenotypic changes in these rats could be reversed if leptin was administered at the neonatal stage (Vickers et al. 2005; Gluckman et al. 2007b). This suggests that the presence of an adipose-derived hormone may act as a cue to alter fetally made predictions of a scarce nutritional environment and thus adopt a different developmental trajectory to adapt accordingly. A similar approach found hypomethylation of hypothalamic *Pomc*, encoding an anorexigenic peptide, in association with the rescuing effects of orally administered leptin (Palou et al. 2011). The degree of nutritional deficit need not be severe for epigenetic effects to be seen. In baboons, moderate undernutrition that did not affect birth weight—that is, no overt phenotypic change was induced—nevertheless led to molecular changes as reflected by hypomethylation at the *PCK1* promoter, the gene product of which is crucial for normal gluconeogenesis (Nijland et al. 2010).

In the maternal protein restriction rat model, decreased methylation at the *Ppara* and *GR* promoters was observed together with corresponding increases in gene expression (Lillycrop et al. 2005, 2007, 2008); histone modifications biased towards *GR* transcription were also detected. In a similar mouse model, offspring showed hypermethylation at the *Lxra* promoter and reduced expression of its gene product, a key protein in lipid metabolism (van Straten et al. 2009). Lower levels of leptin gene transcription and protein expression were found in adipose tissue of offspring of protein-starved mice. This was also associated with demethylation of specific CpGs at the leptin gene promoter (Jousse et al. 2011). Of potential functional relevance to hypertension, hypomethylation at the promoter of mouse brain *Ace-1*, coding for a component of the renin-angiotensin system, has been observed alongside upregulation of microRNAs that regulate its translation (Goyal et al. 2010).

Placental insufficiency can be experimentally simulated by uterine ligation during pregnancy. Offspring of these rats eventually become diabetic. Molecular investigations demonstrate that this outcome is underpinned by multiple epigenetic changes. These included histone deacetylation and reconfiguration of histone methylation and, in parallel, gradual *Pdx1* promoter hypermethylation leading to underexpression of a transcription factor necessary for pancreatic development (Park et al. 2008). Neonatal treatment of offspring with exendin-4, a glucagon-like

peptide-1 analogue, could normalize the histone modifications and *Pdx1* transcription, preventing the onset of diabetes (Pinney et al. 2011).

There is increasing evidence for the epigenetic basis by which early life overnutrition gives rise to metabolic dysfunction in adulthood. In a study of knockout rats that lack apolipoprotein E and are hypercholesterolemic, it was shown that offspring fed a high-cholesterol diet had as adults differential histone marks in the vasculature compared to counterparts from wild-type mothers fed an identical diet (Alkemade et al. 2010). Changes appear to manifest early in postnatal life in the rat model of maternal high-fat nutrition since hypomethylation of *Cdkn1a,* which encodes for the hepatic cell cycle inhibitor, was observed at postnatal day two (Dudley et al. 2011). Various changes in histone marks have been detected for hepatic *Pck1* (Strakovsky et al. 2011), which codes for a key regulator of plasma glucose homeostasis. A neurobehavioral link has been suggested wherein mice born to maternal high-fat diet dams showed both global and gene promoter-specific hypomethylation in the brain, including that of opioid- and dopamine-related genes (Vucetic et al. 2011). The altered epigenetic regulation within the mesocorticolimbic reward circuitry may likewise account for dietary preferences biased towards sucrose and fat.

In a nonhuman primate model, Japanese macaques fetuses, which were exposed to a high-fat diet during gestation, showed symptoms of nonalcoholic fatty liver disease. Molecular investigations revealed hyperacetylation of the histone mark H3K14 in hepatocytes and decreased expression of the histone deacetylase HDAC1 (Aagaard-Tillery et al. 2008).

2.3.3.2 Epigenetics: Clinical Evidence

The obvious constraints of using humans as experimental subjects in investigations of the developmental induction of later life disease have limited the availability of high-quality molecular evidence. Adult offspring born during the Dutch Hunger Winter famine cohort were examined for their epigenetic state of several genes known to be linked to growth and metabolic disease. A birth weight-independent decrease in DNA methylation was seen at the promoter of *IGF2*, an imprinted gene that promotes cell proliferation, when compared to that in unexposed siblings (Heijmans et al. 2008). While the effect size was very small, these data suggest that an epigenetic change can persist for at least six decades after transient exposure to the inducing cue. Interestingly, *IGF2* methylation is not affected in preterm babies born small for gestational age, suggesting some caution in interpretation (Tobi et al. 2011).

Molecular support for the concept that maternal preconceptional body composition influences offspring metabolic profile has been provided by a study which found that prepregnancy BMI was moderately correlated with methylation of umbilical cord *PPARGC1A*, the gene product of which regulates energy metabolism (Gemma et al. 2009). Since none of the mothers was diabetic, it can be inferred that developmental influences may still be exerted in unexceptional pregnancies.

Perhaps the strongest data come from a recent clinical study following children born to normal pregnancies from birth to up to 9 years, to determine potential associations between early life epigenetic marks and possible prodromal markers of metabolic disease. It was found in two independent cohorts and in two independent replicates within one cohort that in DNA extracted from the umbilical cord at term, the methylation level at a specific site in the *RXRA* promoter correlated with childhood body fat (Godfrey et al. 2011). RXRA is a transcription factor involved in fat metabolism and insulin sensitivity, providing physiological relevance. Maternal carbohydrate consumption in early pregnancy has previously been linked to neonatal adiposity, and in this study higher *RXRA* promoter methylation was indeed linked to low-carbohydrate intake. That the association was detected in healthy subjects and not dependent on birth weight underscores the normative nature of developmentally plasticity. As a substantial proportion of the variance in adiposity could be accounted for by the epigenetic state, epigenotypic measures may lead to the ability to quantify the importance of the developmental contribution.

2.3.4 Epigenetic Contributions to the Developmental Origins of Other Disorders

2.3.4.1 Early Life Stress

Early life stresses in humans can lead to dysfunctions in neurodevelopment and behavior (Champagne 2010). Furthermore, maternal rat behaviors including tactile stimulation, such as licking and grooming of pups, influence their later life behavioral and hypothalamic-pituitary-adrenal (HPA) responses to stress. Higher levels of maternal care lead to lower methylation levels at the hippocampal *GR* promoter and lower expression of GR, which plays a role in the regulation of HPA activity (Weaver et al. 2004). These rats are better able to cope with stressors and grow up to demonstrate a similar level of care towards their offspring, inducing the same epigenetic changes in them. Known as niche re-creation, this is one pathway by which developmentally induced epigenetic changes can be passed onto subsequent generations. Maternal separation, another inducer of early life stress in mice, caused hypomethylation at the enhancer region of brain *Avp*, which codes for the neuropeptide arginine vasopressin linked to mood and cognitive behaviors (Murgatroyd et al. 2009). Alterations were particularly apparent at CpGs located in the MeCP2 binding site. The epigenetic changes, together with the induced endocrine and behavioral phenotypes, persisted for at least 1 year.

In recent years, a number of human studies have been published that draw some parallels with the animal work. Hypermethylation at the promoter of hippocampal *GR,* and a reduction in its expression, was found in individuals who had suffered childhood abuse compared with those who did not experience such adversity (McGowan et al. 2009). Furthermore, newborn children whose mothers

experienced depression in late pregnancy had hypermethylated cord blood *GR* and at 3 months of age showed heightened HPA stress responses as determined by salivary cortisol after stress exposure (Oberlander et al. 2008). This study did not examine if the epigenetic changes endured past the neonatal period. Recently, a positive association was found between methylation levels in blood samples from adolescent children and maternal experience of violence from an intimate partner during pregnancy (Radtke et al. 2011), which highlights the durability of epigenetic marks established in utero. Finally, other work focusing on the impact of childhood abuse found an influence on methylation at the promoter region of *5HTT*, encoding the serotonin transporter; degree of methylation also correlated with symptoms of antisocial personality disorder (Beach et al. 2011).

2.3.4.2 Environmental Toxins

It is now well established that prenatal exposure to environmental toxins affects global and gene-specific DNA methylation. Children whose mothers were tobacco smokers during pregnancy showed global hypomethylation in cord blood (Guerrero-Preston et al. 2010). Moreover, these changes appear to be durable, as suggested by a preliminary report showing lower genomic methylation of *Sat2* and *Alu* in adults who had been prenatally exposed to smoke (Flom et al. 2011). Traffic-related airborne polycyclic aromatic hydrocarbon exposure is also linked to increased *ACSL3* methylation in cord blood, concomitant with lowered gene expression in fetal placental tissue (Perera et al. 2009). It is of concern that the degree of methylation is associated with incidence of childhood asthma, although it is unclear if the physiological functions of ACSL, an enzyme involved in fatty acid metabolism, directly affect asthma.

2.4 Developmental Epigenomics

2.4.1 Problems and Pitfalls

The literature reviewed above points to a role for epigenetics in developmental processes of functional significance in humans; however, these studies are at an early stage and several caveats are needed. Experimental studies generally involve a single large manipulation, making it potentially difficult to translate the findings to humans. The field of epigenomic epidemiology likewise needs to benefit from the experience of genomic research and become more aware of the importance of experimental replication. To date it appears that only the study by Godfrey et al. (2011) has validated a finding by replication both within and between cohorts.

Beyond these cautionary points, the issues of which epigenetic marks to study are pertinent. In general metabolic disease and obesity involve the gradual

appearance of pathophysiology over many years. As the physiology of metabolic homeostasis is complex and only subtle physiological change is needed for symptoms to appear over time, epigenetic changes of an on/off nature would not be expected to occur as they do in cell differentiation. Rather, one would anticipate context-dependent changes in regulation of sensitive components within complex networks. Thus, we may be looking for epigenetic marks distal to the proximal promoter which may have no effect on expression changes, except in particular contexts. The search strategy used by Godfrey et al. (2011) was an attempt to be unbiased. A microarray discovery approach was used to define genomic regions where DNA methylation showed high variance within a population; then phenotypic correlates were searched for. Since then the potential for better and more complete scanning by whole genome approaches has grown, but the challenges are no less diminished. Ultimately, whatever epigenetic marks are identified, their functional importance will need to be validated by in vitro experiments since the presence or absence of expression changes under basal conditions may not be informative given the context-specific nature of the change.

A further problem concerns which tissue to study and the need to consider potential changes in the number of cells of any type within the tissue since this may be the developmental change of interest, rather than simply being a confounder. A further problem will be how best to analyze the data since its complexity will be orders of magnitude higher than that associated with genomic analyses. Furthermore, we have recently found that many epigenetic measures do not follow normative distributions and require more complex forms of analysis (Sheppard et al. submitted for publication). Such concerns mean that caution must be exercised, particularly when subtle changes in methylation are reported, as seen in the study of *IGF2* in the Dutch Hunger Winter study (Heijmans et al. 2008).

There inherently seems to be greater confidence in data when the magnitude of the epigenetic change is large, and the phenotypic change is strongly associated in a graded manner with the change in the epigenetic mark (Godfrey et al. 2011). Nevertheless, some epigenetic changes may occur in stepwise fashion or follow U-shaped relationships to the phenotype or cue. This might either reflect a canalization process or show that the epigenetic change measured is distal to the induction pathway and of secondary importance.

Beyond the birth period when perinatal tissues can be collected, the range of sample types from which DNA can be extracted is limited. This creates another potential complication. We have found that epigenetic marks in umbilical tissue do not necessarily correlate with those from buccal smear samples, and validation techniques may have to be age and tissue specific. Unless these issues are addressed, we will soon have an obfuscating, confused literature where the technical issues could obscure important biological phenomena.

2.4.2 The Potential of Developmental Epigenetics

Notwithstanding these difficulties, the application of developmental epigenetics has enormous potential in more clearly defining the etiology of metabolic diseases. The reality is that we have little knowledge of the optimal nutritional management of mother and infant to reduce long-term risk of metabolic diseases in contemporary environments. To date, we have mainly used crude measures of birth outcomes and infant growth. It seems feasible to use epigenetic marks to profile infants in relationship to their nutritional exposures and use such measures to better address the issues of preconceptional, gestational, lactational, and infant feeding. Interventional studies which have long-term disease outcomes as their endpoints are clearly impractical, and epigenetic measures may become useful biomarkers of desirable changes in the developmental trajectory.

Birth weight provides a very limited assessment of the neonatal state and intrauterine history, as evidenced by many children who are neither very large nor small at birth having altered development. We are currently evaluating the potential of epigenetic measures made at birth to interrogate the developmental history further. This may be useful in stratifying subjects for intervention, potentially at any stage in the life course.

There remains much uncertainty regarding the relative importance of developmental as opposed to genetic and lifestyle factors in the etiology of noncommunicable disease. The data of Godfrey et al. (2011) indicate a greater developmental component than was generally thought. Nevertheless, much more data, together with various approaches to validation across different populations, will be needed to reach firm conclusions.

As epigenetic biology evolves, the potential for it to identify new therapeutic targets will become clearer. Epigenetic manipulation has already found utility in cancer therapy. If therapeutic strategies are also discovered that allow for the specific targeting of epigenetic changes involved in the genesis of metabolic diseases, the ability to improve human health will be greatly enhanced.

2.5 Final Comments

An in-depth understanding of the developmental origins of obesity, diabetes, and other related metabolic diseases will have important implications for intervention strategies to deal with the epidemic of noncommunicable disease. There is low adherence in the UK to a prudent diet among women of reproductive age, even if pregnancy was planned (Inskip et al. 2009). There is also little appreciation of the importance of nutritional influences in early infancy on lifelong health among first-time mothers (Gage et al. 2011). This suggests that effective nutritional and health education programs targeted at young women could be of immense value in mitigating the soaring rates of obesity and related metabolic diseases. Epigenetic

measurements could well assist in the development of optimal nutritional strategies for health improvement both in the mother and infant, to stratify at-risk populations and to monitor interventions. As global approaches to interrogate the epigenome become more available, novel epigenetic targets for therapeutic intervention will continue to be identified.

References

Aagaard-Tillery KM, Grove K, Bishop J, Ke X, Fu Q, McKnight R, Lane RH (2008) Developmental origins of disease and determinants of chromatin structure: maternal diet modifies the primate fetal epigenome. J Mol Endocrinol 41:91–102

Alkemade FE, van Vliet P, Henneman P, van Dijk KW, Hierck BP, van Munsteren JC, Scheerman JA, Goeman JJ, Havekes LM, Gittenberger-de Groot AC, van den Elsen PJ, DeRuiter MC (2010) Prenatal exposure to apoE deficiency and postnatal hypercholesterolemia are associated with altered cell-specific lysine methyltransferase and histone methylation patterns in the vasculature. Am J Pathol 176:542–548

Barker DJP, Osmond C, Golding J, Kuh D, Wadsworth ME (1989) Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ 298:564–567

Bateson P, Gluckman P (2011) Plasticity, robustness, development and evolution. Cambridge University Press, Cambridge

Bateson P, Barker D, Clutton-Brock T, Deb D, D'Udine B, Foley RA, Gluckman P, Godfrey K, Kirkwood T, Mirazon Lahr M, McNamara J, Metcalfe NB, Monaghan P, Spencer HG, Sultan SE (2004) Developmental plasticity and human health. Nature 430:419–421

Beach SRH, Brody GH, Todorov AA, Gunter TD, Philibert RA (2011) Methylation at *5HTT* mediates the impact of child sex abuse on women's antisocial behavior: an examination of the Iowa adoptee sample. Psychosom Med 73:83–87

Beauchamp GK, Mennella JA (2009) Early flavor learning and its impact on later feeding behavior. J Pediatr Gastroenterol Nutr 48:S25–S30

Boney CM, Verma A, Tucker R, Vohr BR (2005) Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. Pediatrics 115:290–296

Bruce KD, Hanson MA (2010) The developmental origins, mechanisms, and implications of metabolic syndrome. J Nutr 140:648–652

Bruce KD, Felino RC, Marco A, Junlong Z, Priya LE, Graham CB, Adrian CB, Geraldine FC, Lucilla P, Mark AH, Josie MM, Christopher DB (2009) Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. Hepatology 50:1796–1808

Carone BR, Fauquier L, Habib N, Shea JM, Hart CE, Li R, Bock C, Li C, Gu H, Zamore PD, Meissner A, Weng Z, Hofmann HA, Friedman N, Rando OJ (2010) Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. Cell 143:1084–1096

Champagne FA (2010) Early adversity and developmental outcomes. Perspect Psychol Sci 5:564–574

Chang AY-F, Liao B-Y (2012) DNA methylation rebalances gene dosage after mammalian gene duplications. Mol Biol Evol 29:133–144

Jousse C, Parry L, Lambert-Langlais S, Maurin A-C, Averous J, Bruhat A, Carraro VR, Tost J, Letteron P, Chen P, Jockers R, Launay J-M, Mallet J, Fafournoux P (2011) Perinatal undernutrition affects the methylation and expression of the leptin gene in adults: implication for the understanding of metabolic syndrome. FASEB J 25:3271–3278

Dudley KJ, Sloboda DM, Connor KL, Beltrand J, Vickers MH (2011) Offspring of mothers fed a high fat diet display hepatic cell cycle inhibition and associated changes in gene expression and DNA methylation. PLoS One 6:e21662

Dunn GA, Bale TL (2009) Maternal high-fat diet promotes body length increases and insulin insensitivity in second-generation mice. Endocrinology 150:4999–5009

Elahi MM, Cagampang FR, Mukhter D, Anthony FW, Ohri SK, Hanson MA (2009) Long-term maternal high-fat feeding from weaning through pregnancy and lactation predisposes offspring to hypertension, raised plasma lipids and fatty liver in mice. Br J Nutr 102:514–519

Felsenfeld G, Groudine M (2003) Controlling the double helix. Nature 421:448–453

Flom J, Ferris J, Gonzalez K, Santella R, Terry MB (2011) Prenatal tobacco smoke exposure and genomewide methylation in adulthood. Cancer Epidemiol Biomarkers Prev 20:720

Forrester TE, Badaloo AV, Boyne MS, Osmond C, Thompson D, Green C, Taylor-Bryan C, Barnett A, Soares-Wynter S, Hanson MA, Beedle AS, Gluckman PD (2012) Prenatal factors contribute to the emergence of kwashiorkor or marasmus in severe undernutrition: evidence for the predictive adaptation model. PLoS One 7:e35907

Gage H, Raats M, Williams P, Egan B, Jakobik V, Laitinen K, Martin-Bautista E, Schmid M, von Rosen-von HJ, Campoy C, Decsi T, Morgan J, Koletzko B (2011) Developmental origins of health and disease: the views of first-time mothers in 5 European countries on the importance of nutritional influences in the first year of life. Am J Clin Nutr 94:2018S–2024S

Gemma C, Sookoian S, Alvarinas J, Garcia SI, Quintana L, Kanevsky D, Gonzalez CD, Pirola CJ (2009) Maternal pregestational BMI is associated with methylation of the *PPARGC1A p*romoter in newborns. Obesity 17:1032–1039

Gillman MW, Rifas-Shiman SL, Camargo CA, Berkey CS, Frazier AL, Rockett HRH, Field AE, Colditz GA (2001) Risk of overweight among adolescents who were breastfed as infants. JAMA 285:2461–2467

Gluckman P, Hanson M (2006) Mismatch: why our world no longer fits our bodies. Oxford University Press, Oxford

Gluckman PD, Hanson MA, Spencer HG (2005a) Predictive adaptive responses and human evolution. Trends Ecol Evol 20:527–533

Gluckman PD, Hanson MA, Spencer HG, Bateson P (2005b) Environmental influences during development and their later consequences for health and disease: implications for the interpretation of empirical studies. Proc R Soc B 272:671–677

Gluckman PD, Hanson MA, Beedle AS (2007a) Non-genomic transgenerational inheritance of disease risk. Bioessays 29:145–154

Gluckman PD, Lillycrop KA, Vickers MH, Pleasants AB, Phillips ES, Beedle AS, Burdge GC, Hanson MA (2007b) Metabolic plasticity during mammalian development is directionally dependent on early nutritional status. Proc Natl Acad Sci U S A 104:12796–12800

Gluckman PD, Hanson MA, Bateson P, Beedle AS, Law CM, Bhutta ZA, Anokhin KV, Bougneres P, Chandak GR, Dasgupta P, Smith GD, Ellison PT, Forrester TE, Gilbert SF, Jablonka E, Kaplan H, Prentice AM, Simpson SJ, Uauy R, West-Eberhard MJ (2009) Towards a new developmental synthesis: adaptive developmental plasticity and human disease. Lancet 373:1654–1657

Gluckman PD, Hanson MA, Buklijas T (2010) A conceptual framework for the developmental origins of health and disease. J Dev Orig Health Dis 1:6–18

Gluckman PD, Hanson MA, Low FM (2011) The role of developmental plasticity and epigenetics in human health. Birth Defects Res C Embryo Today 93:12–18

Godfrey K (2006) The 'developmental origins' hypothesis: epidemiology. In: Gluckman PD, Hanson MA (eds) Developmental origins of health and disease. Cambridge University Press, Cambridge, pp 6–32

Godfrey KM, Gluckman PD, Hanson MA (2010) Developmental origins of metabolic disease: life course and intergenerational perspectives. Trends Endocrinol Metab 21:199–205

Godfrey KM, Sheppard A, Gluckman PD, Lillycrop KA, Burdge GC, McLean C, Rodford J, Slater-Jefferies JL, Garratt E, Crozier SR, Emerald BS, Gale CR, Inskip HM, Cooper C,

Hanson MA (2011) Epigenetic gene promoter methylation at birth is associated with child's later adiposity. Diabetes 60:1528–1534

Goyal R, Goyal D, Leitzke A, Gheorghe CP, Longo LD (2010) Brain renin-angiotensin system: fetal epigenetic programming by maternal protein restriction during pregnancy. Reprod Sci 17:227–238

Guerrero-Preston R, Goldman LR, Brebi-Mieville P, Ili-Gangas C, LeBron C, Hernández-Arroyo M, Witter FR, Apelberg BJ, Roystacher M, Jaffe A, Halden RU, Sidransky D (2010) Global DNA hypomethylation is associated with in utero exposure to cotinine and perfluorinated alkyl compounds. Epigenetics 5:539–546

Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE, Lumey LH (2008) Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A 105:17046–17049

Heijmans BT, Tobi EW, Lumey LH, Slagboom PE (2009) The epigenome: archive of the prenatal environment. Epigenetics 4:1–6

Hult M, Tornhammar P, Ueda P, Chima C, Edstedt Bonamy A-K, Ozumba B, Norman M (2010) Hypertension, diabetes and overweight: looming legacies of the Biafran famine. PLoS One 5: e13582

Inskip HM, Crozier SR, Godfrey KM, Borland SE, Cooper C, Robinson SM, Southampton Women's Survey Study Group (2009) Women's compliance with nutrition and lifestyle recommendations before pregnancy: general population cohort study. BMJ 338:b481

Jablonka E, Oborny B, Molnar I, Kisdi E, Hofbauer J, Czaran T (1995) The adaptive advantage of phenotypic memory in changing environments. Philos Trans R Soc B Biol Sci 350:133–141

Jeemon P, Neogi S, Bhatnagar D, Cruickshank KJ, Prabhakaran D (2009) The impact of migration on cardiovascular disease and its risk factors among people of Indian origin. Curr Sci 97:378–384

Jones JH (2009) The force of selection on the human life cycle. Evol Hum Behav 30:305–314

Ku CS, Naidoo N, Wu M, Soong R (2011) Studying the epigenome using next generation sequencing. J Med Genet 48:721–730

Kucharski R, Maleszka J, Foret S, Maleszka R (2008) Nutritional control of reproductive status in honeybees via DNA methylation. Science 319:1827–1830

Kuzawa CW, Thayer ZM (2011) Timescales of human adaptation: the role of epigenetic processes. Epigenomics 3:221–234

Lee TM, Zucker I (1988) Vole infant development is influenced perinatally by maternal photoperiodic history. Am J Physiol 255:R831–R838

Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC (2005) Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. J Nutr 135:1382–1386

Lillycrop KA, Slater-Jefferies JL, Hanson MA, Godfrey KM, Jackson AA, Burdge GC (2007) Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. Br J Nutr 97:1064–1073

Lillycrop KA, Phillips ES, Torrens C, Hanson MA, Jackson AA, Burdge GC (2008) Feeding pregnant rats a protein-restricted diet persistently alters the methylation of specific cytosines in the hepatic PPARa promoter of the offspring. Br J Nutr 100:278–282

Lillycrop KA, Hanson MA, Burdge GC (2009) Epigenetics and the influence of maternal diet. In: Newnham JP, Ross MG (eds) Early life origins of human health and disease. Karger, Basel, pp 11–20

Low FM, Gluckman PD, Hanson MA (2011) Developmental plasticity and epigenetic mechanisms underpinning metabolic and cardiovascular diseases. Epigenomics 3:279–294

Mattick JS (2011) The central role of RNA in human development and cognition. FEBS Lett 585:1600–1616

McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonté B, Szyf M, Turecki G, Meaney MJ (2009) Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat Neurosci 12:342–348

Morgan CP, Bale TL (2011) Early prenatal stress epigenetically programs dysmasculinization in second-generation offspring via the paternal lineage. J Neurosci 31:11748–11755

Murgatroyd C, Patchev AV, Wu Y, Micale V, Bockmuhl Y, Fischer D, Holsboer F, Wotjak CT, Almeida OFX, Spengler D (2009) Dynamic DNA methylation programs persistent adverse effects of early-life stress. Nat Neurosci 12:1559–1566

Ng S-F, Lin RCY, Laybutt DR, Barres R, Owens JA, Morris MJ (2010) Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. Nature 467:963–966

Nijland MJ, Mitsuya K, Li C, Ford S, McDonald TJ, Nathanielsz PW, Cox LA (2010) Epigenetic modification of fetal baboon hepatic phosphoenolpyruvate carboxykinase following exposure to moderately reduced nutrient availability. J Physiol 588:1349–1359

Oberlander TF, Weinberg J, Papsdorf M, Grunau R, Misri S, Devlin AM (2008) Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (*NR3C1*) and infant cortisol stress responses. Epigenetics 3:97–106

Palou M, Picó C, McKay JA, Sánchez J, Priego T, Mathers JC, Palou A (2011) Protective effects of leptin during the suckling period against later obesity may be associated with changes in promoter methylation of the hypothalamic pro-opiomelanocortin gene. Br J Nutr 106:769–778

Park JH, Stoffers DA, Nicholls RD, Simmons RA (2008) Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of *Pdx1*. J Clin Invest 118:2316–2324

Perera F, Tang W-Y, Herbstman J, Tang D, Levin L, Miller R, Ho S-M (2009) Relation of DNA methylation of 5′-CpG island of *ACSL3* to transplacental exposure to airborne polycyclic aromatic hydrocarbons and childhood asthma. PLoS One 4:e4488

Pfeifer GP (2006) Mutagenesis at methylated CpG sequences. Curr Top Microbiol Immunol 301:259–281

Pinney S, Jaeckle Santos L, Han Y, Stoffers D, Simmons R (2011) Exendin-4 increases histone acetylase activity and reverses epigenetic modifications that silence *Pdx1* in the intrauterine growth retarded rat. Diabetologia 54:2606–2614

Poston L (2011) Intergenerational transmission of insulin resistance and type 2 diabetes. Prog Biophys Mol Biol 106:315–322

Radtke KM, Ruf M, Gunter HM, Dohrmann K, Schauer M, Meyer A, Elbert T (2011) Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor. Transl Psychiatry 1:e21

Sloboda DM, Hart R, Doherty DA, Pennell CE, Hickey M (2007) Age at menarche: influences of prenatal and postnatal growth. J Clin Endocrinol Metab 92:46–50

Slotkin RK, Martienssen R (2007) Transposable elements and the epigenetic regulation of the genome. Nat Rev Genet 8:272–285

Strakovsky RS, Zhang X, Zhou D, Pan Y-X (2011) Gestational high fat diet programs hepatic phosphoenolpyruvate carboxykinase gene expression and histone modification in neonatal offspring rats. J Physiol 589:2707–2717

Tobi EW, Heijmans BT, Kremer D, Putter H, Delemarre-van de Waal HA, Finken MJJ, Wit JM, Slagboom PE (2011) DNA methylation of *IGF2*, *GNASAS*, *INSIGF* and *LEP* and being born small for gestational age. Epigenetics 6:171–176

Vaiserman A (2011) Early-life origin of adult disease: evidence from natural experiments. Exp Gerontol 46:189–192

van Straten EME, Bloks VW, Huijkman NCA, Baller JFW, van Meer H, Lutjohann D, Kuipers F, Plosch T (2009) The Liver X-Receptor (LXR) gene promoter is hypermethylated in a mouse model of prenatal protein restriction. Am J Physiol Regul Integr Comp Physiol 298: R275–R282

Vickers MH, Breier BH, Cutfield WS, Hofman PL, Gluckman PD (2000) Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. Am J Physiol 279:E83–E87

Vickers MH, Gluckman PD, Coveny AH, Hofman PL, Cutfield WS, Gertler A, Breier BH, Harris M (2005) Neonatal leptin treatment reverses developmental programming. Endocrinology 146:4211–4216

Vucetic Z, Kimmel J, Reyes TM (2011) Chronic high-fat diet drives postnatal epigenetic regulation of μ-opioid receptor in the brain. Neuropsychopharmacology 36:1199–1206

Wagner KD, Wagner N, Ghanbarian H, Grandjean V, Gounon P, Cuzin F, Rassoulzadegan M (2008) RNA induction and inheritance of epigenetic cardiac hypertrophy in the mouse. Dev Cell 14:962–969

Waterland RA, Jirtle RL (2003) Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 23:5293–5300

Weaver ICG, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ (2004) Epigenetic programming by maternal behavior. Nat Neurosci 7:847–854

West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press, New York

Chapter 3
Sculpting Our Future: Environmental Nudging of the Imprintome

Susan K. Murphy and Cathrine Hoyo

Abstract Over the past several decades, there has been a dramatic increase in awareness of the importance of epigenetics in the regulation of gene expression and how this relates to disease. The initial focus on cancer as a potential outcome of epigenetic alterations has grown to include the role of epigenetics in neurodevelopmental disorders, obesity, diabetes, memory, and even deviation in complex human social interactions. Prominent among the genes implicated in all of these conditions are those subject to genomic imprinting. These genes are regulated by epigenetic mechanisms, including DNA methylation that is established during early development and results in parent of origin dependent expression. Advances in the ability to accurately measure DNA methylation using high throughput techniques have now paved the way for study of this epigenetic modification in epidemiologic studies. In this chapter, we will examine the relationship of the early origins hypothesis to imprinted genes and how emerging studies strongly suggest that early origins may in part have its roots in epigenetic changes at these imprinted regulatory regions during early life.

Keywords Cigarette smoking • DNA methylation • Early origins • Epigenetic reprogramming • Folic acid • Imprintome

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Abbreviations

BWS	Beckwith Wiedemann Syndrome
CDKN1C	Cyclin-Dependent Kinase Inhibitor 1C
CTCF	CCCTC Binding Factor
DLK1	Delta Like 1 Homolog (Drosophila)
DMR	Differentially Methylated Region
DNMT	DNA Methyltransferase
GNAS	Guanine Nucleotide Binding Protein (G protein) Alpha Stimulating Activity Polypeptide
GRB10	Growth Factor Receptor-Bound Protein 10
H19	Maternally Expressed H19
HYMAI	Hydatidiform Mole Associated and Imprinted (Non-Protein Coding)
IGF1R	Insulin-like Growth Factor Type I Receptor
IGF2	Insulin-like Growth Factor II
KCNQ1OT1 KCNQ1	Opposite Strand/Antisense Transcript (Non-Protein Coding)
MEG3	Maternally Expressed Gene 3 (Non-Protein Coding)
MEST	Mesoderm Specific Transcript Homolog (Mouse)
NDN	Necdin
NEST	Newborn Epigenetics STudy
NNAT	Neuronatin
PLAGL1	Pleomorphic Adenoma Gene-Like 1
PEG3	Paternally Expressed Gene 3
SGCE	Epsilon Sarcoglycan
SLC38A4	Solute Carrier Family 38 Member 4

3.1 Introduction

As early as the 1970s, Ravelli et al. (1976), Forsdahl (1977), and later Barker (1988) put forth the hypothesis that the adult risk of diseases in humans like bronchitis, hypertension, stroke, and cardiovascular disease is related to the environmental conditions experienced during gestation and early childhood. Despite the evidence connecting early development with later disease, causal mechanisms have remained elusive. Over the last decade, however, epigenetics has come into the fore as a potential explanation for the influence of the early environment on outcomes with repeated demonstration of the importance of epigenetic mechanisms to gene regulation. Environmental epigenetics is now a major focus of many labs around the world, including our own.

Epigenetics refers to the mechanisms that bring about a change in phenotype without depending on a change in nucleotide sequence. There are multiple forms of epigenetic regulation, including noncoding RNAs, posttranslational modifications of histone proteins, and DNA methylation. DNA methylation occurs predominantly at 5′-CG-3′ dinucleotide pairs. The prevalence of CG dinucleotides is reduced throughout the genome when compared to the other 15 possible dinucleotide combinations (Bird 1987). This observation has been attributed to the activity of endogenous deaminase enzymes, which target methylated cytosines (Duncan and Miller 1980), and may have led to the depletion of CG dinucleotide pairs in the genome over time. About 20 % of CG dinucleotides are grouped into high CG-density regions referred to as CG islands, with criteria including length greater than 500 bp, GC content >55 %, and observed CG to expected CG ratio >0.65 (Takai and Jones 2002). CG islands are often located at the promoter regions of genes and the CG dinucleotides contained within these islands are most often unmethylated, with exceptions including CG islands associated with genes subject to X chromosome inactivation in females and genes whose expression is subject to genomic imprinting.

3.2 Genomic Imprinting

Genomic imprinting is an intriguing phenomenon that results in transcription from only one of the two parentally derived chromosomes in a manner that is entirely dependent on the parent of origin's sex (Monk 1988). There are approximately 65 confirmed imprinted genes in humans (Table 3.1), although another ~150 have been computationally predicted to exist (Luedi et al. 2007). Much debate has ensued over the reasons for why genomic imprinting initially arose and why it has been retained over time (Reik and Walter 2001; Sleutels and Barlow 2002). There is evidence to support that imprinting arose as a consequence of the drive to epigenetically silence repetitive elements in the genome (Pask et al. 2009; Warren et al. 2008), and that neighboring genes acquired their imprinted status as a consequence of a bystander effect and subsequent selection. A corollary to this idea is that it implies distinct abilities in the gametes to differentially silence particular regions of the genome, an implication supported by the observation that imprint marks are erased and then reestablished in the gametes such that they are specific to the sex of the individual now carrying the developing germ cells. Others have proposed that imprinting arose in early mammals as a result of conflicts over genetic investment and overall competitive fitness of the offspring (Moore and Haig 1991). In this scenario, imprinting might provide a growth advantage to offspring of males through manipulation of maternal resources (e.g., placental nourishment) and preferential investment in nurturing (e.g., nursing, provision of warmth, licking and grooming) in the context of concurrent paternities. In contrast, females desire equal fitness for all offspring they gestate. Thus, females developed the ability to selectively silence transmitted genes that promote growth and increase the demand for maternal

Table 3.1 Known imprinted genes in humans

Gene	Location	Expressed allele	Gene	Location	Expressed allele
TP73	1p36.32	Maternal	*PHLDA2*	11p15.5	Maternal
DIRAS3	1p31.3	Paternal	*OSBPL5*	11p15.4	Maternal
LRRTM1	2p12	Paternal	*SMPD1*	11p15.4	Maternal
NAP1L5	4q22.1	Paternal	*WT1*	11p13	Paternal
PLAGL1	6q24.2	Paternal	*RBP5*	12p13.31	Maternal
HYMAI	6q24.2	Paternal	*DLK1*	14q32.2	Paternal
SLC22A2	6q25.3	Maternal	*MEG3*	14q32.2	Maternal
SLC22A3	6q25.3	Maternal	*PWCR1*	15q11.2	Paternal
DDC	7p12.2	Isoform dependent	*NDN*	15q11.2	Paternal
GRB10	7p12.1	Isoform dependent	*SNURF*	15q11.2	Paternal
TFPI2	7q21.3	Maternal	*SNORD107*	15q11.2	Paternal
SGCE	7q21.3	Paternal	*SNORD64*	15q11.2	Paternal
PEG10	7q21.3	Paternal	*SNORD108*	15q11.2	Paternal
PPP1R9A	7q21.3	Maternal	*SNORD109B*	15q11.2	Paternal
DLX5	7q21.3	Maternal	*MKRN3*	15q11.2	Paternal
CPA4	7q32.2	Maternal	*MAGEL2*	15q11.2	Paternal
MEST	7q32.2	Paternal	*SNRPN*	15q11.2	Paternal
MESTIT1	7q32.2	Paternal	*SNORD109A*	15q11.2	Paternal
KLF14	7q32.3	Maternal	*SNORD115*	15q11.2	Paternal
COPG2IT1	7q32	Paternal	*SNORD115-48*	15q11.2	Paternal
DLGAP2	8p23	Paternal	*UBE3A*	15q11.2	Maternal
KCNK9	8q24.3	Maternal	*ATP10A*	15q11.2	Maternal
INPP5F V2	10q26.11	Paternal	*ANKRD11*	16q24.3	Maternal
KCNQ1OT1	11p15.5	Paternal	*TCEB3C*	18q21.1	Maternal
H19	11p15.5	Maternal	*ZIM2*	19q13.43	Paternal
IGF2	11p15.5	Paternal	*PEG3*	19q13.43	Paternal
IGF2AS	11p15.5	Paternal	*ZNF264*	19q13.4	Maternal
INS	11p15.5	Paternal	*BLCAP*	20q11.23	Isoform dependent
KCNQ1	11p15.5	Maternal	*NNAT*	20q11.23	Paternal
KCNQ1DN	11p15.4	Maternal	*L3MBTL1*	20q13.12	Paternal
CDKN1C	11p15.5	Maternal	*GNASAS*	20q13.32	Paternal
SLC22A18	11p15.5	Maternal	*GNAS*	20q13.32	Isoform dependent

nurturing and resources. In contrast, males developed the ability to selectively repress expression of transmitted genes that function to keep growth in check and genes that place limitations on maternal resource extraction.

While these are intriguing hypotheses and are not mutually exclusive, the true reasons behind the evolution of imprinting remain an enigma. Nevertheless, imprinted genes represent a substantial group of genes that are expressed and regulated very differently from the remainder of the genome, in a manner that

allows for only one functional copy. Epigenetics is integral to this regulation and as such may be the imprinting "Achilles' heel" in terms of preventing chaos resulting from disruption of normal imprinting. In this chapter, we will discuss the implications for the imposition of monoallelic expression on this group of genes, the potential vulnerabilities that might trigger detrimental shifts in expression over the course of our lifetimes, and how this might link early life environmental adaptations to later life outcomes.

3.3 The Imprintome

The imprintome comprises all of the epigenetic features required to bring about imprinted gene expression. It is distinct from the process of transcription but is involved in establishing imprint status as well as eliciting and coordinating the transcriptional activity from this group of genes. Thus, the imprintome minimally consists of the patterns of DNA methylation established in the gametes and early embryo along with the requisite histone modifications and may also involve the action of noncoding RNAs that contribute to establishment and maintenance of imprinted expression.

The existence of the imprintome means that the group of genes regulated by this mechanism is present in a permanent state of half-functionality. Unlike genes subject to random monoallelic expression by which one of the two copies is inactivated but the choice of copy is random (Zakharova et al. 2009), the imprintome necessarily imposes inactivation on the same parental copy in all cells. The molecular mechanisms required to dictate this pattern of regulation have been maintained for millions of years (Killian et al. 2000), supporting an important driving force for this complicated and resource-usurping ability. The risks this imposes to health and development in light of the relative ease of overriding the imprintome with a single genetic or epigenetic hit further underscores a strong evolutionary drive to keep this mechanism in place.

3.4 The Early Origins of Disease

Abundant anecdotal evidence from well-documented population health records has repeatedly shown that there is increased risk of long-term health effects for individuals who experienced nutritional deprivation during gestation. During the Dutch Hunger Winter of 1944–1945, an embargo imposed toward the end of World War II resulted in food rationing such that the population of about 4.5 million people living in the part of the Netherlands occupied by Germany was restricted to consuming ~600 kcal per day. This included many pregnant women, deprived of adequate nutrition required to support a healthy pregnancy. Individuals born to mothers who became pregnant during the time of the famine show an increased risk

of diabetes, obesity, and cardiovascular disease, while those born to mothers who were in the second trimester of pregnancy at the time the famine have an increased risk of developing schizophrenia (Roseboom et al. 2006; Susser et al. 1996).

The early origins hypothesis postulates that metabolic adaptation to these conditions resulted in what has been referred to as "thrifty phenotype" (Hales and Barker 2001). In the case of nutrient deprivation during pregnancy, an expression profile is instituted that is hyperefficient at caloric use and storage, which allows the developing fetus to survive. Once a particular metabolic gene expression profile is established, it becomes fixed and destined to govern that individual's metabolism throughout the course of their life, regardless of conditions experienced later. Thus, a thrifty phenotype brought about by exposure to limited nutrition during early pregnancy leads to a metabolic profile in postnatal life and adulthood that is extremely efficient at caloric utilization and storage, and this may lead to development of obesity due to the tendency to utilize minimal calories for basal metabolic functions while maximally retaining unused calories, stored as fat. In contrast, adequate nutrition during pregnancy creates a more balanced metabolic profile that is not programmed toward establishment of caloric reserves but instead more efficiently balances caloric intake with expenditure. Thus, the propensity toward a particular metabolic profile and diabetes as well as obesity in postnatal and adult life is dependent on the conditions encountered during pregnancy. Epigenetic mechanisms are thought to explain the establishment of these disparate and persistent metabolic profiles (Wadhwa et al. 2009).

3.5 Imprinted Genes and Early Origins

While there are undoubtedly many genes that are involved in the establishment of individual metabolic profiles, many imprinted genes have demonstrated roles in glucose utilization and adipose tissue formation. Thus, this group of genes, being epigenetically regulated, may link the early nutritional environment with later propensity toward metabolic disorder, diabetes and obesity, along with the increased risk of developing obesity-associated cardiovascular disease and cancer. Insulin-like Growth Factor II (IGF2) protein is produced from mRNAs originating from paternally derived chromosome 11 and functions as a growth mitogen by signaling through the Insulin and IGF1R receptors. Delta-like 1 homolog (Drosophila) (DLK1) is also paternally expressed from chromosome 14 and functions as both a growth factor and an inhibitor of adipocyte differentiation. Polymorphisms in both *IGF2* and *DLK1* have been associated with obesity (Chacon et al. 2008; O'Connell et al. 2011).

Paternally expressed *NNAT* is an acute diet-responsive gene that is expressed in white adipose tissue and in the hypothalamus (Li et al. 2010), is regulated by leptin and associated with obesity (Vrang et al. 2010) and implicated in pancreatic beta cell function and type 2 diabetes (Joe et al. 2008). Paternally expressed *MEST* is highly expressed in adipose tissue and has been causally linked to its expansion

(Koza et al. 2006; Kozak et al. 2010). Mutation of *Peg3*, also paternally expressed, leads to inability to regulate body temperature, increased leptin levels and obesity in mice (Curley et al. 2005). *PLAGL1* and *HYMA1* are both paternally expressed and the only genes localized within the region of chromosome 6 causing transient neonatal diabetes (Docherty et al. 2010).

Imprinted genes play fundamental roles in biological processes beyond those involved in metabolism. They are also involved in neurological function, including learning (Drake et al. 2011), the consolidation and enhancement of memory (Chen et al. 2011), extinction of fear memories (Agis-Balboa et al. 2011), and function in social and nurturing behaviors (Champagne et al. 2009; Lefebvre et al. 1998; Li et al. 1999). As such, imprint deregulation contributes to numerous neurological disorders, including those attributed to overt defects in imprinted domains like those on chromosome 15 associated with Prader-Willi and Angelman syndromes, as well as those associated with uniparental disomies of chromosomes 7, 11, and 14 (Murphy and Jirtle 2003; Wilkins and Ubeda 2011). Imprinted genes have also been implicated in autism, a neurodevelopmental disorder that disproportionately affects males (Fradin et al. 2010; Guffanti et al. 2011; Kato et al. 2008; Nurmi et al. 2003).

3.6 Windows of Vulnerability During Reprogramming of the Imprintome

The imprinted status of a gene is reset in each generation. This process takes place in the gametes, the only cellular compartment in which the parentally derived chromosomes can be acted upon in isolation. During gametogenesis, the methylation imprint marks are completely erased. This effectively eliminates the "memory" of the parental origin of each of the chromosomes such that new imprint marks can be established that are specific to the sex of the developing embryo in which the chromosomes are then housed. DNA methylation is thought to represent the fundamental imprint mark that establishes these domains as being imprinted, but histone modifications may also be involved. During spermatogenesis, histone proteins are replaced with protamines (Miller et al. 2010; Ooi and Henikoff 2007), a characteristic previously thought to negate the ability of histones to transmit epigenetic information during the process of gametogenesis. However, it is now known that some histones are retained during protamine replacement (Hammoud et al. 2009), suggesting that modifications that are present on these retained histone proteins may help to mark the relevant chromosomal regions as those requiring the methylation marks that specify imprinting.

In humans, gametogenesis begins during embryonic development and marks one of two developmental time points during which extensive epigenetic remodeling occurs. The other occurs just after fertilization, when the parentally derived chromosomes unite in the zygote and undergo methylation erasure, with the exception of methylation present at imprinted loci that was established in the

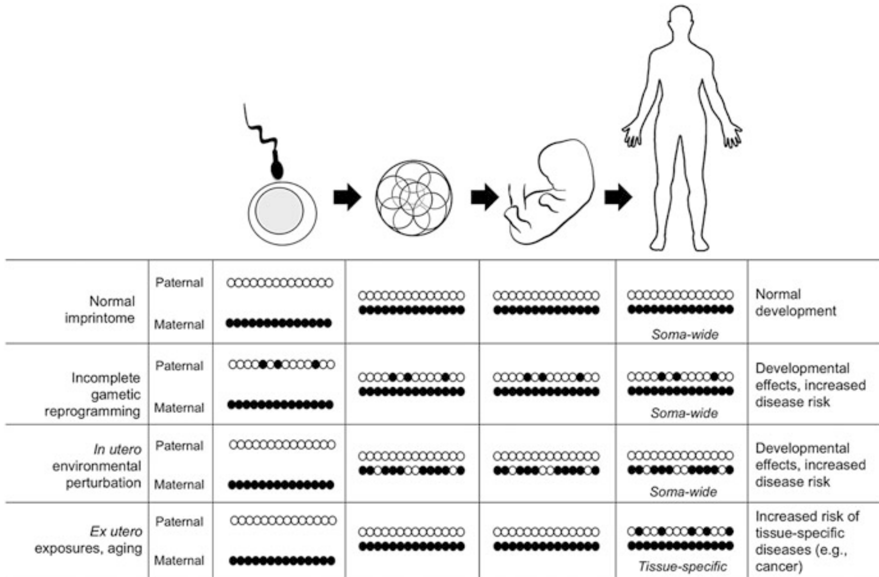

Fig. 3.1 Implications for nudging the imprintome at critical developmental windows of vulnerability. Shown is a hypothetical imprinted DMR that normally carries a maternal methylation mark, indicated by the *filled circles*. Normally, this methylation is established in the oocyte. During spermatogenesis, an unmethylated state is established for the same sequence. After fertilization, this methylation pattern is carried forward throughout the lifespan of the individual in all somatic cells. The imprintome can be nudged in various ways, including the effects of incomplete reprogramming of methylation profiles in the gametes (shown as incomplete erasure during spermatogenesis) or through environmental perturbations (shown as loss of methylation on the paternally derived allele). If these effects occur prior to gastrulation, they are carried forward soma-wide throughout life. Imprintome nudging can also occur as a result of environmental exposures encountered *ex utero* (shown as gain of methylation on the maternal allele), or as a result of the aging process, or both. In these cases, the alterations may be tissue-specific. None of these effects are mutually exclusive. Cumulative effects of imprintome nudging may shift expression levels and impair proper function of imprinted genes and could potentially induce a loss of fidelity of the imprinting mechanism, leading to aberrant activation of expression of the normally silent parental allele or conversely, to the aberrant silencing of the normally active allele

gametes. It is perhaps during these periods of development, when the epigenome undergoes physical depletion followed by renewal and is in a highly dynamic state of flux, that any endogenous or exogenous influences may be most likely to induce persistent changes in methylation (Fig. 3.1).

3.7 Environmental Influences *In Utero* Shift Methylation in the Imprintome

The Newborn Epigenetics STudy (NEST) was initiated in North Carolina to begin to address how the *in utero* environment impacts epigenetic profiles in newborns and how this relates to development and disease. The first genes undergoing analysis within NEST are imprinted genes, using bisulfite pyrosequencing to quantify DNA methylation at the differentially methylated regulatory regions that regulate imprinted expression. While data generation and analyses are ongoing, as described below, we first examined two differentially methylated regions associated with the *IGF2*/*H19* imprinted domain at human chromosome 11p15.5. Paternally expressed *IGF2* encodes an endocrine and autocrine/paracrine acting factor important in directing growth during prenatal development (LeRoith and Lowe 2005). Maternally expressed *H19* encodes a long noncoding RNA that has been implicated in the *trans* regulation of *IGF2* (Wilkin et al. 2000), as a tumor suppressive RNA (Yoshimizu et al. 2008), an oncofetal RNA (Ariel et al. 1997), and most recently as producing a protein from an antisense transcript with tumor suppressive activity (Onyango and Feinberg 2011).

Regulation of the *IGF2*/*H19* imprinted domain is relatively well established in comparison to what is known about other imprinted domains. There are two DMRs that have been previously shown to contribute to the establishment and maintenance of *IGF2* imprinting, including the *H19* DMR, located proximal to the promoter region of *H19*, approximately 140 kb downstream from *IGF2*, and the *IGF2* DMR located in an intragenic region upstream of the three closely positioned *IGF2* promoters that are subject to genomic imprinting. Both regions have previously been shown to exhibit altered methylation that is associated with skewed expression and/or imprinting, with phenotypic outcomes of perturbations in growth, obesity, diabetes, neurological deficits, and multiple types of cancer (Chao and D'Amore 2008). Of particular intrigue, periconceptional exposure to famine conditions is associated with shifts in methylation at the *IGF2* DMR as measured six decades postexposure (Heijmans et al. 2008), indicative of the responsiveness of imprinted gene DMRs to environmental influences as well as the persistence of such alterations.

3.7.1 The Imprintome and Folic Acid

DNA methylation reactions are enzymatically catalyzed by DNA methyltransferase enzymes, which catalyze the transfer of a methyl group from the universal methyl group donor, S-adenosylmethionine, to the five-carbon position of cytosines located 5' to guanines in the DNA sequence. Generation of S-adenosylmethionine occurs through the one-carbon metabolism pathway, which is supplied with carbon units through dietary sources, including foods rich in folate, methionine, choline, and betaine. Folic acid, the synthetic form of folate, can also be consumed as a dietary

supplement and is included in many multivitamin and prenatal vitamin supplements. Fortification of milled grain products, including breads and cereals, began in the United States in 1998 upon recognition that 400 micrograms of daily folic acid taken by women prior to and throughout early pregnancy reduces the risk of neural tube defects (Pitkin 2007). Providing the strongest impetus for fortification was the fact that approximately half of pregnancies in the United States are unplanned. In these instances, folic acid supplementation would typically not occur until after knowledge of pregnancy, beyond the window of potential benefit. The consequences of dietary fortification of the general population are not fully understood (Gibson et al. 2011; Kim 2007; Sauer et al. 2009; Solomons 2007) nor are the consequences for children conceived to women who began taking folic acid supplements prior to becoming pregnant (via recommendations for prenatal vitamin consumption) *along with* foods fortified with folic acid and those containing folate.

Animal studies have clearly demonstrated that maternal supplementation with one carbon donor nutrients has a direct and persistent effect on methylation in the offspring that also leads to phenotypic changes and increased risk of obesity and cancer (Dolinoy et al. 2006; Waterland and Jirtle 2003). We therefore wished to determine if variable levels of folic acid intake during pregnancy are associated with differences in methylation at the regulatory elements of imprinted loci and focused on the two DMRs associated with the *IGF2*/*H19* imprinted domain. There were no significant differences in methylation at the *IGF2* DMR, consistent with the lack of association reported for folate levels and methylation of the proximal P2 or P3 promoters of *IGF2* (Ba et al. 2011). Methylation levels at the *H19* DMR were 3–5 % lower in infants born to women who took folic acid before and during pregnancy versus infants born to women who did not take supplements, respectively, and this effect was most prominent in male infants (Hoyo et al. 2011). The average level of methylation detected at this DMR was ~61 % in the entire cohort, indicating that there is some methylation present on the normally unmethylated maternal chromosome. These results suggest that at least for this particular region, the effect of folic acid intake during pregnancy may be beneficial in that it contributes toward a more normal (i.e., closer to 50 %) methylation profile.

3.7.2 The Imprintome and Smoking

Harmful effects from *in utero* exposure to cigarette smoking include increased risk of birth defects, premature birth as well as being small for gestational age and low birth weight, yet 13 % of women smoke during their pregnancies (Dietz et al. 2011). Attitudes regarding smoking during pregnancy have dramatically changed versus those dominant a half-century ago, when smoking during pregnancy was much more common, reported in one study to be 62 % of pregnant women in the 1960s (Paradis et al. 2011), and at the time not clearly associated with detrimental outcomes (Stewart 1967). We examined the relationship between methylation of the *IGF2*/*H19* DMRs and *in utero* exposure to cigarette smoking. We quantified

methylation of these two regions using umbilical cord blood, which is naïve to direct exposures to the external environment and thus should reflect *in utero* exposures. Here the effects of exposure were specific to the *IGF2* DMR. There were no observable differences in methylation at the *H19* DMR. Infants born to mothers who smoked throughout pregnancy showed approximately 5 % higher methylation at the *IGF2* DMR than did infants born to never smoking mothers. Infants born to mothers who quit smoking during pregnancy also exhibited methylation levels comparable to the infants born to never smoking mothers. Similar to our findings with folic acid intake, the methylation shifts were specific to male infants (Murphy et al. 2011). Others have reported a 1.2 % decrease in methylation of the *IGF2* DMR in response to maternal smoking during pregnancy that approached significance ($p = 0.054$), although stratification by sex showed no apparent differences (Tobi et al. 2011). In this study, peripheral blood from individuals at age 19 was used for analysis, suggesting that the methylation effects from exposure to cigarette smoke during gestation persist for at least two decades.

3.7.3 The Imprintome and Antidepressant Use

We analyzed the NEST cohort with respect to maternal depression and use of antidepressant medication during pregnancy, and their potential influence on methylation profiles at the *IGF2* and *H19* DMRs. While there was no evident association between depression and methylation profiles at these DMRs, there was an approximate 7 % increase in methylation at the *H19* DMR in infants born to mothers who used antidepressant medications during pregnancy, an effect observed in African Americans but not in Caucasians (Soubry et al. 2011).

3.8 Breast Feeding May Counter Negative Environmental Effects on the Imprintome

As described above, multiple imprinted genes play a role in the ability to utilize and store energy and from studies in adults, they have been shown to play a role in obesity. In NEST, we analyzed how growth trajectories for infants during the first year of life are related to methylation at the *IGF2* and *H19* DMRs and how this relates to feeding patterns of the infants, particularly breast feeding. Infants classified as overweight or obese at 1 year of age had higher levels of methylation at the *H19* DMR, while the *IGF2* DMR showed no significant differences. Intriguingly, *H19* DMR hypermethylation was not present in obese infants who breastfed at any point during the first year of life (Perkins et al. 2012).

3.9 Childhood Exposure to Lead Induces Imprintome Methylation Alterations

Low-level lead exposure is known to impair cognitive abilities in children (Bellinger et al. 1992; Canfield et al. 2003; Lanphear et al. 2005; Mazumdar et al. 2011). The use of lead-based paint was banned in 1978 to minimize exposure of children to this heavy metal. Despite these efforts, lead exposure is still of concern since housing built before 1978 may still contain lead-based paint which becomes a problem as it deteriorates. The mechanisms by which lead exposure induces harmful effects may in part be explained by effects on the epigenome (Pilsner et al. 2009; Wright et al. 2010). Quantitative analysis of multiple imprinted gene DMRs in peripheral blood from individuals followed as part of the Cincinnati Lead Study (Dietrich et al. 2001) showed significant hypomethylation of *PLAGL1* associated with early childhood lead exposure; an association that appeared to be more pronounced in males than females (our unpublished data). These methylation changes were also associated with criminal behavior in adult life. Prior analysis of this cohort has shown that an increased level of lead in the peripheral blood during childhood is associated with gray matter atrophy (Brubaker et al. 2010; Cecil et al. 2008) and later delinquent and antisocial behaviors (Wright et al. 2008) as well as poorer performance on standardized measures of intelligence (Hornung et al. 2009). It is not yet clear how deregulation of *PLAGL1* or other imprinted genes might contribute to these findings.

3.10 Implications of Nudging the Imprintome

There is now mounting evidence that there are indeed epigenetic consequences resulting from environmental exposures and that the targets of these exposures include the imprintome. As opposed to the relatively large shifts in methylation that are frequently reported in human pathological conditions, the effect sizes associated with environmental exposures discussed here have thus far been small. This is not necessarily surprising, since larger changes are indeed associated with overt pathology. While research still seeks to generate knowledge on the types of environmental influences that change methylation and which loci are affected by these changes, it will also be of fundamental importance to understand the consequence of those changes. To this end, use of integrated technologies, such as genome-wide methylation profiling along with gene expression, microRNA, and proteomics data, will be essential to decipher such outcomes and to help understand how these changes might be prevented or ameliorated.

Using gene expression microarray data generated from umbilical cord blood of a subset of the infants analyzed in our NEST cohort, we found that a 1 % decrease in methylation at the *IGF2* DMR was associated with a near doubling of *IGF2* transcript levels. At the *H19* DMR, every 1 % decrease in methylation was

associated with a 2.2-fold increase in *IGF2* transcript levels. The increased methylation at the *IGF2* DMR in male infants born to smoking mothers was found to mediate 20 % of the reduced birth weight in these children (Murphy et al. 2011). This finding is consistent with the important role of *IGF2* in promoting growth during prenatal development, since increased methylation in the infants born to smokers was associated with lower *IGF2* transcript levels and also with risk of low birth weight. As described above, low birth weight has been linked to risk of numerous adult-onset chronic diseases and conditions (Barker 2004).

Decoding these relationships in human studies is difficult due to the complexity of exposures, the genetic heterogeneity in the human population and our lack of understanding of how genetic variants contribute to epigenetic vulnerability and efficiency of reprogramming, combined with the restricted tissue types available for study from living humans. Nonetheless, it will be important to determine the downstream consequences of environmentally induced nudging of the imprintome.

3.10.1 Perturbing Imprintome Function May Disrupt an Integrated Gene Network

Accumulating evidence suggests that many imprinted genes function together in a coordinately regulated network. The first demonstration of coordinate regulation came from studies of BWS, a congenital overgrowth disorder caused by defects in imprinting on chromosome 15 with increased risk of Wilms' tumor or hepatoblastoma (Enklaar et al. 2006). In this study, Arima and colleagues (2005) examined the paternally expressed gene *PLAGL1* (aka *ZAC*) on chromosome 6, implicated in transient neonatal diabetes when overexpressed (Ma et al. 2004), and the maternally expressed gene *CDKN1C* (aka $p57^{KIP2}$) that is causally associated with BWS and located on chromosome 15. They observed that in mice, *Plagl1* and *Cdkn1c* share strikingly similar spatial patterns of gene expression. They also found that human PLAGL1, a zinc finger protein, binds in a methylation-sensitive manner to the nearby imprinted *KCNQ1OT1* (*LIT1*) promoter CpG island that regulates expression of *CDKN1C in cis* to activate transcription. They reported loss of methylation of the *KCNQ1OT1* promoter in several patients with transient neonatal diabetes and altered expression and methylation of *PLAGL1*, suggesting that interaction between these two imprinted domains may contribute to this condition and that these findings may portend a more global methylation defect.

Subsequently it was shown by Varrault and colleagues (2006) that *Plagl1* null mice die perinatally, exhibit growth restriction and abnormal bone formation. Microarray analysis showed that *Plagl1* knockout disrupts a network of coordinately regulated genes that contains an unusually large number that are imprinted, including *Igf2*, *H19*, *Cdkn1c*, *Dlk1*, *Meg3*, *Sgce*, *Peg3*, *Ndn*, *Mest*, *Gnas*, and *Grb10*. Experiments in vitro showed induction of *Igf2*, *Cdkn1c*, *H19*, *Dlk1*, and *Mest* when Plagl1 was overexpressed. In contrast, liver tissue from *Plagl1* null mice

showed repression of *Igf2*, *Cdkn1c*, *H19*, and *Dlk1*. Plagl1 was found to bind to the endodermal enhancers that drive paternal expression of *Igf2* and maternal expression of *H19* (located downstream of *H19*), trans-activating the promoters of both genes. Further support for a coordinated network of imprinted genes that function to help guide appropriate patterns of growth came from the finding that imprinted *Igf2*, *H19*, *Plagl1*, *Mest*, *Peg3*, *Dlk1*, *Meg3*, *Grb10*, *Ndn*, *Cdkn1c*, and *SLC38a4* all normally exhibit coordinate transcriptional downregulation concomitant with growth deceleration as adult body size is reached (Lui et al. 2008).

Using a different approach called circular chromosomal conformation capture (4C), Zhao et al. (2006) revealed additional evidence of an imprinted gene network from analysis of chromosomal domains that interact in trans with the maternally derived *H19* imprint control region (ICR). Remarkably, 114 other regions of the genome were found to interact with the *H19* ICR, including domains associated with 15 imprinted genes. These interactions differed in undifferentiated embryonic stem cells versus embryoid bodies, suggesting variable functions of these relationships based on cell type. Up to four distinct chromosomal regions were interacting with the *H19* ICR at any one time, which may indicate a highly active process, and support that an altered epigenetic state at the *H19* ICR has the potential to hinder multiple interchromosomal interactions and consequent downstream effects.

Berg et al. also found a near tenfold overrepresentation of multiple imprinted genes among those genes comprising a stem cell signature in long-term murine hematopoietic, muscle, and epidermal stem/progenitor cells (Berg et al. 2011). The level of expression of these imprinted genes was repressed upon differentiation. These results indicate that, in addition to the requirement for coordinate regulation of these genes to orchestrate growth and development, these genes also have a fundamental role in maintaining somatic tissues throughout life.

If imprinted genes are subject to multi-locus coordinate transcriptional regulation in humans, then disrupted expression through epigenetic or genetic alterations of the upstream regulators of such a network could have widespread ramifications for the functioning of the entire network, with phenotypic consequences depending on the severity of the defect. Major disruptions may go unrecognized, given the likelihood of embryonic or fetal lethality of such events. Indeed, altered methylation and expression of multiple imprinted genes has been observed in conceptal tissues from spontaneous miscarriages (Doria et al. 2010; Pliushch et al. 2010), with a disproportionate number of males showing this effect (Pliushch et al. 2010). More minor alterations likely raise the risk of disorders associated with imprinted genes, including (neuro)developmental disorders and cancer.

Both paternally expressed *PLAGL1* (Varrault et al. 2006) and maternally expressed *H19* (Gabory et al. 2009; Sandhu et al. 2009) have been implicated as regulatory hubs of a transcriptional imprinting network. As such, disruption of expression of these genes, or in the case of the *H19* DMR, altered binding of the CTCF protein, may lead to extensive effects on either the establishment of imprinted expression or the coordination of expression levels among the imprinted and non-imprinted genes that are connected within this network.

Epigenetic deregulation at these loci does not necessarily result solely from environmental exposures. It is also conceivable that altered methylation that disrupts network function could arise from lack of fidelity in epigenetic reprogramming in the gametes (Fig. 3.1). If erasure of the imprint marks from the prior generation and/or establishment of the new imprint marks in the developing gametes is incomplete, this skewed imprintome will be transmitted faithfully to the next generation. Such defects could arise, for example, due to declining function of the reprogramming machinery as a result of the aging process, dietary deficiencies, or genetic variants that affect an inability to undergo complete reprogramming of epigenetic marks in the gametes, or from a combination of these effects.

3.10.2 Long-Term Implications of Nudging Imprintome Function

The ability to maintain health depends on appropriate expression and functioning of the genes throughout the genome, not just those that are imprinted. However, imprinted genes appear to comprise a special subgroup whose temporal and spatial patterns of expression are particularly important to maintenance of neurological and physiological health. The deviations in imprinted gene regulation that result from diet or environmental exposure, or even from inefficiencies with epigenetic reprogramming, can have indirect or direct adverse effects on development with long term consequences not only to the individual but also to society in the increased expense associated with providing intervention, health care, and addressing special needs related to alterations in imprintome function.

3.10.2.1 Costs to the Individual

It may be easy to dismiss small shifts in the imprintome result from environmental exposures. But are they inconsequential? We certainly do not yet have a grasp of the breadth of imprintome changes that occur in response to environmental perturbations, the relevant targets for each exposure, nor do we understand the thresholds of methylation changes that are tolerable (i.e., that do not induce a measurable change in phenotype). Nevertheless, we do know that there are changes in imprintome methylation that can directly mediate profound neurological and/or developmental disorders, and we are finding that more subtle alterations are linked to phenotypic changes that include shifts in the expression of growth regulatory genes. Increased risk of metabolic syndrome and diabetes from adaptive epigenetic programming *in utero* may have a tremendous impact on health-care costs. Individuals with obesity, hypertension, and diabetes are at increased risk of stroke and cardiovascular disease, sequelae that may well have been preventable through careful attention not only to avoiding relevant exposures but also through the quality and quantity of nutritional intake during pregnancy. Thus, costs to the individual resulting from imprintome nudging can include alterations in normal

patterns of growth, developmental deficits, propensity to develop obesity, cardiovascular disease, and increased risk of stroke, diabetes, and cancer.

3.10.2.2 Costs to Society

Imprinted genes and the imprintome have essential functions as growth continues during gestation and childhood. Particular imprinted genes may also have critical functions in preserving the function of somatic stem cells and thus of maintaining homeostasis of adult tissues (Berg et al. 2011; Ferron et al. 2011). As described above, scientific inquiry has shown irrefutable evidence of the importance of the imprintome to maintaining health, and conversely the disease that can result when perturbed. Thus, the potential societal costs to imprintome nudging should not be overlooked. To our knowledge, no one has performed cost projection analyses that assess the expense associated with the increased need for interventional and educational services and health care related to imprintome deregulation.

Low birth weight, as we have found is mediated in part through alterations associated with maternal smoking-induced methylation changes at *IGF2* (Murphy et al. 2011), results in increased risk of smoking-related morbidities and pregnancy complications that often require additional care. In a study examining health-care costs associated with births in Iowa, low birth weight attributable to smoking during pregnancy was estimated to add an excess $2,085,907 annually (2007 dollars) to health-care costs during the neonatal period alone (Udeh and Losch 2008). Conversely, childhood obesity is estimated to add $14.1 billion dollars annually to normal health-care costs for children in the United States (Trasande and Chatterjee 2009). That a number of imprinted genes are involved in energy management, and even in the differentiation of adipocytes, suggests that imprintome deregulation could substantially contribute to these additional costs.

Aside from the influence of the imprintome on cost of health care and general well-being, additional societal costs result from effects on neurological function and cognitive abilities. For example, low birth weight associated with smoking during pregnancy is linked to decreased cognitive abilities in the child (Grantham-McGregor 1998) as well as increased risk of attention deficit disorder (Nomura et al. 2010; Sciberras et al. 2011). In addition to the costs associated with providing services in the schools to assist these children, the reduction in productivity, reduced wage earning potential, and overall reduction in societal contributions must also be taken into account.

3.11 Can Imprintome Deregulation Be Treated or Prevented?

There are certainly non-preventable causes of imprintome deregulation, such as errors in chromosome segregation and copy number alterations that affect imprinted domains. However, environmentally induced shifts in the imprintome

and the associated morbidities may indeed be preventable, especially as we begin to identify and understand the specific types of exposures that should be avoided. One of the most exciting aspects related to epigenetic modifications is that they are potentially reversible. Animal models as well as studies of human disease show that this may be possible through dietary or pharmaceutical interventions. Reversal of altered methylation has been shown to occur in mice with the use of methyl group donor nutrients as well as with genistein, a phytoestrogen present in soy products (Dolinoy et al. 2007). Use of DNA methyltransferase inhibitors has also shown efficacy in reversing aberrantly hypermethylated genes in the treatment of cancer (Issa 2007).

While methylation can be modulated through these types of interventions, the problem with all of the current approaches is that they are nonspecific. There is currently no mechanism that allows for effectively targeting specific loci to alter DNA methylation. This may present a substantial hurdle since there are regions of the genome that should normally be methylated (e.g., transposable elements) and using these approaches may inadvertently lead to hypomethylation of these regions and create other unintended problems. In addition, the stability of the imprintome methylation profiles and the notion that the imprintome may only be vulnerable to shifts in methylation during certain developmental windows may make correcting epigenetic defects within the imprintome particularly challenging. It is thus crucial to continue investigation into the mechanisms responsible for the writing and reading of the imprintome as well as where vulnerabilities exist so that we may identify the opportunities, targets, and artillery for beneficial interventions.

3.12 Conclusions

The imprintome is a unique and powerful regulatory mechanism that is capable of dictating much about not only our individual health, but also appears to have the potential, when disrupted, to impact society through detrimental effects at considerable cost to the individual, health-care system, and society as a whole. Insuring the appropriate reprogramming of the imprintome in the gametes and safeguarding it from potentially detrimental environmental influences during development should be a top priority if we hope to circumvent these problems. However, before interventions can be implemented, a systematic examination of environmental factors that are associated with epigenetic perturbation will be required, the windows of vulnerability defined, and the resultant phenotypes elucidated. Such observations should be mechanistically evaluated in both in vitro and in vivo studies.

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References

Agis-Balboa RC, Arcos-Diaz D, Wittnam J, Govindarajan N, Blom K, Burkhardt S, Haladyniak U, Agbemenyah HY, Zovoilis A, Salinas-Riester G, Opitz L, Sananbenesi F, Fischer A (2011) A hippocampal insulin-growth factor 2 pathway regulates the extinction of fear memories. EMBO J 30:4071–4083

Ariel I, Ayesh S, Perlman EJ, Pizov G, Tanos V, Schneider T, Erdmann VA, Podeh D, Komitowski D, Quasem AS, de Groot N, Hochberg A (1997) The product of the imprinted H19 gene is an oncofetal RNA. Mol Pathol 50:34–44

Arima T, Kamikihara T, Hayashida T, Kato K, Inoue T, Shirayoshi Y, Oshimura M, Soejima H, Mukai T, Wake N (2005) Zac, lit1 (kcnq1ot1) and p57kip2 (cdkn1c) are in an imprinted gene network that may play a role in Beckwith-Wiedemann syndrome. Nucleic Acids Res 33:2650–2660

Ba Y, Yu H, Liu F, Geng X, Zhu C, Zhu Q, Zheng T, Ma S, Wang G, Li Z, Zhang Y (2011) Relationship of folate, vitamin b(12) and methylation of insulin-like growth factor-ii in maternal and cord blood. Eur J Clin Nutr 65:480–485

Barker DJ (1988) Childhood causes of adult diseases. Arch Dis Child 63:867–869

Barker DJ (2004) The developmental origins of adult disease. J Am Coll Nutr 23:588S–595S

Bellinger DC, Stiles KM, Needleman HL (1992) Low-level lead exposure, intelligence and academic achievement: a long-term follow-up study. Pediatrics 90:855–861

Berg JS, Lin KK, Sonnet C, Boles NC, Weksberg DC, Nguyen H, Holt LJ, Rickwood D, Daly RJ, Goodell MA (2011) Imprinted genes that regulate early mammalian growth are coexpressed in somatic stem cells. PLoS One 6:e26410

Bird A (1987) Cpg islands as gene markers in the vertebrate nucleus. Trends Genet 3:342–347

Brubaker CJ, Dietrich KN, Lanphear BP, Cecil KM (2010) The influence of age of lead exposure on adult gray matter volume. Neurotoxicology 31:259–266

Canfield RL, Henderson CR Jr, Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP (2003) Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. N Engl J Med 348:1517–1526

Cecil KM, Brubaker CJ, Adler CM, Dietrich KN, Altaye M, Egelhoff JC, Wessel S, Elangovan I, Hornung R, Jarvis K, Lanphear BP (2008) Decreased brain volume in adults with childhood lead exposure. PLoS Med 5:e112

Chacon MR, Miranda M, Jensen CH, Fernandez-Real JM, Vilarrasa N, Gutierrez C, Naf S, Gomez JM, Vendrell J (2008) Human serum levels of fetal antigen 1 (fa1/dlk1) increase with obesity, are negatively associated with insulin sensitivity and modulate inflammation in vitro. Int J Obes 32:1122–1129

Champagne FA, Curley JP, Swaney WT, Hasen NS, Keverne EB (2009) Paternal influence on female behavior: the role of peg3 in exploration, olfaction, and neuroendocrine regulation of maternal behavior of female mice. Behav Neurosci 123:469–480

Chao W, D'Amore PA (2008) Igf2: epigenetic regulation and role in development and disease. Cytokine Growth Factor Rev 19:111–120

Chen DY, Stern SA, Garcia-Osta A, Saunier-Rebori B, Pollonini G, Bambah-Mukku D, Blitzer RD, Alberini CM (2011) A critical role for IGF-II in memory consolidation and enhancement. Nature 469:491–497

Curley JP, Pinnock SB, Dickson SL, Thresher R, Miyoshi N, Surani MA, Keverne EB (2005) Increased body fat in mice with a targeted mutation of the paternally expressed imprinted gene peg3. FASEB J 19:1302–1304

Dietrich KN, Ris MD, Succop PA, Berger OG, Bornschein RL (2001) Early exposure to lead and juvenile delinquency. Neurotoxicol Teratol 23:511–518

Dietz PM, Homa D, England LJ, Burley K, Tong VT, Dube SR, Bernert JT (2011) Estimates of nondisclosure of cigarette smoking among pregnant and nonpregnant women of reproductive age in the United States. Am J Epidemiol 173:355–359

Docherty LE, Poole RL, Mattocks CJ, Lehmann A, Temple IK, Mackay DJ (2010) Further refinement of the critical minimal genetic region for the imprinting disorder 6q24 transient neonatal diabetes. Diabetologia 53:2347–2351

Dolinoy DC, Weidman JR, Waterland RA, Jirtle RL (2006) Maternal genistein alters coat color and protects A^{vy} mouse offspring from obesity by modifying the fetal epigenome. Environ Health Perspect 114:567–572

Dolinoy DC, Huang D, Jirtle RL (2007) Maternal nutrient supplementation counteracts bisphenol a-induced DNA hypomethylation in early development. Proc Natl Acad Sci USA 104:13056–13061

Doria S, Sousa M, Fernandes S, Ramalho C, Brandao O, Matias A, Barros A, Carvalho F (2010) Gene expression pattern of IGF2, PHLDA2, PEG10 and CDKN1C imprinted genes in spontaneous miscarriages or fetal deaths. Epigenetics 5:444–450

Drake NM, DeVito LM, Cleland TA, Soloway PD (2011) Imprinted rasgrf1 expression in neonatal mice affects olfactory learning and memory. Genes Brain Behav 10:392–403

Duncan BK, Miller JH (1980) Mutagenic deamination of cytosine residues in DNA. Nature 287:560–561

Enklaar T, Zabel BU, Prawitt D (2006) Beckwith-Wiedemann syndrome: multiple molecular mechanisms. Expert Rev Mol Med 8:1–19

Ferron SR, Charalambous M, Radford E, McEwen K, Wildner H, Hind E, Morante-Redolat JM, Laborda J, Guillemot F, Bauer SR, Farinas I, Ferguson-Smith AC (2011) Postnatal loss of dlk1 imprinting in stem cells and niche astrocytes regulates neurogenesis. Nature 475:381–385

Forsdahl A (1977) Are poor living conditions in childhood and adolescence an important risk factor for arteriosclerotic heart disease? Br J Prev Soc Med 31:91–95

Fradin D, Cheslack-Postava K, Ladd-Acosta C, Newschaffer C, Chakravarti A, Arking DE, Feinberg A, Fallin MD (2010) Parent-of-origin effects in autism identified through genome-wide linkage analysis of 16,000 SNPS. PLoS One 5:e12513

Gabory A, Ripoche MA, Le Digarcher A, Watrin F, Ziyyat A, Forne T, Jammes H, Ainscough JF, Surani MA, Journot L, Dandolo L (2009) H19 acts as a *trans* regulator of the imprinted gene network controlling growth in mice. Development 136:3413–3421

Gibson TM, Weinstein SJ, Pfeiffer RM, Hollenbeck AR, Subar AF, Schatzkin A, Mayne ST, Stolzenberg-Solomon R (2011) Pre- and postfortification intake of folate and risk of colorectal cancer in a large prospective cohort study in the United States. Am J Clin Nutr 94:1053–1062

Grantham-McGregor SM (1998) Small for gestational age, term babies, in the first six years of life. Eur J Clin Nutr 52(Suppl 1):S59–S64

Guffanti G, Strik Lievers L, Bonati MT, Marchi M, Geronazzo L, Nardocci N, Estienne M, Larizza L, Macciardi F, Russo S (2011) Role of UBE3A and ATP10A genes in autism susceptibility region 15q11-q13 in an Italian population: a positive replication for UBE3A. Psychiatry Res 185:33–38

Hales CN, Barker DJ (2001) The thrifty phenotype hypothesis. Br Med Bull 60:5–20

Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT, Cairns BR (2009) Distinctive chromatin in human sperm packages genes for embryo development. Nature 460:473–478

Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE, Lumey LH (2008) Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci USA 105:17046–17049

Hornung RW, Lanphear BP, Dietrich KN (2009) Age of greatest susceptibility to childhood lead exposure: a new statistical approach. Environ Health Perspect 117:1309–1312

Hoyo C, Murtha AP, Schildkraut JM, Jirtle RL, Demark-Wahnefried W, Forman MR, Iversen ES, Kurtzberg J, Overcash F, Huang Z, Murphy SK (2011) Methylation variation at IGF2 differentially methylated regions and maternal folic acid use before and during pregnancy. Epigenetics 6:928–936

Issa JP (2007) DNA methylation as a therapeutic target in cancer. Clin Cancer Res 13:1634–1637

Joe MK, Lee HJ, Suh YH, Han KL, Lim JH, Song J, Seong JK, Jung MH (2008) Crucial roles of neuronatin in insulin secretion and high glucose-induced apoptosis in pancreatic beta-cells. Cell Signal 20:907–915

Kato C, Tochigi M, Ohashi J, Koishi S, Kawakubo Y, Yamamoto K, Matsumoto H, Hashimoto O, Kim SY, Watanabe K, Kano Y, Nanba E, Kato N, Sasaki T (2008) Association study of the 15q11-q13 maternal expression domain in Japanese autistic patients. Am J Med Genet B Neuropsychiatr Genet 147B:1008–1012

Killian JK, Byrd JC, Jirtle JV, Munday BL, Stoskopf MK, MacDonald RG, Jirtle RL (2000) M6P/IGF2R imprinting evolution in mammals. Mol Cell 5:707–716

Kim YI (2007) Folic acid fortification and supplementation–good for some but not so good for others. Nutr Rev 65:504–511

Koza RA, Nikonova L, Hogan J, Rim JS, Mendoza T, Faulk C, Skaf J, Kozak LP (2006) Changes in gene expression foreshadow diet-induced obesity in genetically identical mice. PLoS Genet 2:e81

Kozak LP, Newman S, Chao PM, Mendoza T, Koza RA (2010) The early nutritional environment of mice determines the capacity for adipose tissue expansion by modulating genes of caveolae structure. PLoS One 5:e11015

Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, Canfield RL, Dietrich KN, Bornschein R, Greene T, Rothenberg SJ, Needleman HL, Schnaas L, Wasserman G, Graziano J, Roberts R (2005) Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect 113:894–899

Lefebvre L, Viville S, Barton SC, Ishino F, Keverne EB, Surani MA (1998) Abnormal maternal behaviour and growth retardation associated with loss of the imprinted gene Mest. Nat Genet 20:163–169

LeRoith D, Lowe WLJ (2005) Growth factors. In: Melmed S, Conn MP (eds) Endocrinology: basic and clinical principles. Humana Press, Totowa, pp 85–91

Li L, Keverne EB, Aparicio SA, Ishino F, Barton SC, Surani MA (1999) Regulation of maternal behavior and offspring growth by paternally expressed Peg3. Science 284:330–333

Li X, Thomason PA, Withers DJ, Scott J (2010) Bio-informatics analysis of a gene co-expression module in adipose tissue containing the diet-responsive gene NNAT. BMC Syst Biol 4:175

Luedi PP, Dietrich FS, Weidman JR, Bosko JM, Jirtle RL, Hartemink AJ (2007) Computational and experimental identification of novel human imprinted genes. Genome Res 17:1723–1730

Lui JC, Finkielstain GP, Barnes KM, Baron J (2008) An imprinted gene network that controls mammalian somatic growth is down-regulated during postnatal growth deceleration in multiple organs. Am J Physiol Regul Integr Comp Physiol 295:R189–R196

Ma D, Shield JP, Dean W, Leclerc I, Knauf C, Burcelin RR, Rutter GA, Kelsey G (2004) Impaired glucose homeostasis in transgenic mice expressing the human transient neonatal diabetes mellitus locus, TNDM. J Clin Invest 114:339–348

Mazumdar M, Bellinger DC, Gregas M, Abanilla K, Bacic J, Needleman HL (2011) Low-level environmental lead exposure in childhood and adult intellectual function: a follow-up study. Environ Health 10:24

Miller D, Brinkworth M, Iles D (2010) Paternal DNA packaging in spermatozoa: more than the sum of its parts? DNA, histones, protamines and epigenetics. Reproduction 139:287–301

Monk M (1988) Genomic imprinting. Genes Dev 2:921–925

Moore T, Haig D (1991) Genomic imprinting in mammalian development: a parental tug-of-war. Trends Genet 7:45–49

Murphy SK, Jirtle RL (2003) Imprinting evolution and the price of silence. Bioessays 25:577–588

Murphy SK, Adigun A, Huang Z, Overcash F, Wang F, Jirtle RL, Schilder JM, Murtha AP, Iversen ES, Hoyo C (2011) Gender-specific methylation differences in relation to prenatal exposure to cigarette smoke. Gene 494(1):36–43

Nomura Y, Marks DJ, Halperin JM (2010) Prenatal exposure to maternal and paternal smoking on attention deficit hyperactivity disorders symptoms and diagnosis in offspring. J Nerv Ment Dis 198:672–678

Nurmi EL, Amin T, Olson LM, Jacobs MM, McCauley JL, Lam AY, Organ EL, Folstein SE, Haines JL, Sutcliffe JS (2003) Dense linkage disequilibrium mapping in the 15q11-q13 maternal expression domain yields evidence for association in autism. Mol Psychiatry 8:624–634

O'Connell J, Lynch L, Hogan A, Cawood TJ, O'Shea D (2011) Preadipocyte factor-1 is associated with metabolic profile in severe obesity. J Clin Endocrinol Metab 96:E680–E684

Onyango P, Feinberg AP (2011) A nucleolar protein, H19 opposite tumor suppressor (HOTS), is a tumor growth inhibitor encoded by a human imprinted H19 antisense transcript. Proc Natl Acad Sci USA 108:16759–16764

Ooi SL, Henikoff S (2007) Germline histone dynamics and epigenetics. Curr Opin Cell Biol 19:257–265

Paradis AD, Fitzmaurice GM, Koenen KC, Buka SL (2011) Maternal smoking during pregnancy and criminal offending among adult offspring. J Epidemiol Community Health 65:1145–1150

Pask AJ, Papenfuss AT, Ager EI, McColl KA, Speed TP, Renfree MB (2009) Analysis of the platypus genome suggests a transposon origin for mammalian imprinting. Genome Biol 10:R1

Perkins E, Murphy SK, Murtha AP, Schildkraut J, Jirtle RL, Demark-Wahnefried W, Forman MR, Kurtzberg J, Overcash F, Huang Z, Hoyo C (2012) Insulin-Like Growth Factor 2/H19 methylation at birth and risk of overweight and obesity in children. J Pediatr 161:31–39

Pilsner JR, Hu H, Ettinger A, Sanchez BN, Wright RO, Cantonwine D, Lazarus A, Lamadrid-Figueroa H, Mercado-Garcia A, Tellez-Rojo MM, Hernandez-Avila M (2009) Influence of prenatal lead exposure on genomic methylation of cord blood DNA. Environ Health Perspect 117:1466–1471

Pitkin RM (2007) Folate and neural tube defects. Am J Clin Nutr 85:285S–288S

Pliushch G, Schneider E, Weise D, El Hajj N, Tresch A, Seidmann L, Coerdt W, Muller AM, Zechner U, Haaf T (2010) Extreme methylation values of imprinted genes in human abortions and stillbirths. Am J Pathol 176:1084–1090

Ravelli GP, Stein ZA, Susser MW (1976) Obesity in young men after famine exposure *in utero* and early infancy. N Engl J Med 295:349–353

Reik W, Walter J (2001) Genomic imprinting: parental influence on the genome. Nat Rev Genet 2:21–32

Roseboom T, de Rooij S, Painter R (2006) The Dutch famine and its long-term consequences for adult health. Early Hum Dev 82:485–491

Sandhu KS, Shi C, Sjolinder M, Zhao Z, Gondor A, Liu L, Tiwari VK, Guibert S, Emilsson L, Imreh MP, Ohlsson R (2009) Nonallelic transvection of multiple imprinted loci is organized by the H19 imprinting control region during germline development. Genes Dev 23:2598–2603

Sauer J, Mason JB, Choi SW (2009) Too much folate: a risk factor for cancer and cardiovascular disease? Curr Opin Clin Nutr Metab Care 12:30–36

Sciberras E, Ukoumunne OC, Efron D (2011) Predictors of parent-reported attention-deficit/hyperactivity disorder in children aged 6–7 years: a national longitudinal study. J Abnorm Child Psychol 39:1025–1034

Sleutels F, Barlow DP (2002) The origins of genomic imprinting in mammals. Adv Genet 46:119–163

Solomons NW (2007) Food fortification with folic acid: has the other shoe dropped? Nutr Rev 65:512–515

Soubry A, Murphy SK, Huang Z, Murtha A, Schildkraut JM, Jirtle RL, Wang F, Kurtzberg J, Demark-Wahnefried W, Forman MR, Hoyo C (2011) The effects of depression and use of antidepressive medicines during pregnancy on the methylation status of the IGF2 imprint control regions in the offspring. Clin Epigenetics 3:2

Stewart WH (1967) In: Health EaW (ed) The health consequences of smoking: a public health service review. United States, Public Health Service, Office of the Surgeon General, Washington, DC

Susser E, Neugebauer R, Hoek HW, Brown AS, Lin S, Labovitz D, Gorman JM (1996) Schizophrenia after prenatal famine. Further evidence. Arch Gen Psychiatry 53:25–31

Takai D, Jones PA (2002) Comprehensive analysis of CPG islands in human chromosomes 21 and 22. Proc Natl Acad Sci USA 99:3740–3745

Tobi EW, Heijmans BT, Kremer D, Putter H, Delemarre-van de Waal HA, Finken MJ, Wit JM, Slagboom PE (2011) DNA methylation of igf2, gnasas, insigf and lep and being born small for gestational age. Epigenetics 6:171–176

Trasande L, Chatterjee S (2009) The impact of obesity on health service utilization and costs in childhood. Obesity 17:1749–1754

Udeh BL, Losch ME (2008) Tobacco & low birth weight cost analysis: CSBR white paper #2. Iowa Department of Public Health, Des Moines, pp 1–37

Varrault A, Gueydan C, Delalbre A, Bellmann A, Houssami S, Aknin C, Severac D, Chotard L, Kahli M, Le Digarcher A, Pavlidis P, Journot L (2006) Zac1 regulates an imprinted gene network critically involved in the control of embryonic growth. Dev Cell 11:711–722

Vrang N, Meyre D, Froguel P, Jelsing J, Tang-Christensen M, Vatin V, Mikkelsen JD, Thirstrup K, Larsen LK, Cullberg KB, Fahrenkrug J, Jacobson P, Sjostrom L, Carlsson LM, Liu Y, Liu X, Deng HW, Larsen PJ (2010) The imprinted gene neuronatin is regulated by metabolic status and associated with obesity. Obesity 18:1289–1296

Wadhwa PD, Buss C, Entringer S, Swanson JM (2009) Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. Semin Reprod Med 27:358–368

Warren WC, Hillier LW, Marshall Graves JA, Birney E, Ponting CP, Grutzner F, Belov K, Miller W, Clarke L, Chinwalla AT, Yang SP, Heger A, Locke DP, Miethke P, Waters PD, Veyrunes F, Fulton L, Fulton B, Graves T, Wallis J, Puente XS, Lopez-Otin C, Ordonez GR, Eichler EE, Chen L, Cheng Z, Deakin JE, Alsop A, Thompson K, Kirby P, Papenfuss AT, Wakefield MJ, Olender T, Lancet D, Huttley GA, Smit AF, Pask A, Temple-Smith P, Batzer MA, Walker JA, Konkel MK, Harris RS, Whittington CM, Wong ES, Gemmell NJ, Buschiazzo E, Vargas Jentzsch IM, Merkel A, Schmitz J, Zemann A, Churakov G, Kriegs JO, Brosius J, Murchison EP, Sachidanandam R, Smith C, Hannon GJ, Tsend-Ayush E, McMillan D, Attenborough R, Rens W, Ferguson-Smith M, Lefevre CM, Sharp JA, Nicholas KR, Ray DA, Kube M, Reinhardt R, Pringle TH, Taylor J, Jones RC, Nixon B, Dacheux JL, Niwa H, Sekita Y, Huang X, Stark A, Kheradpour P, Kellis M, Flicek P, Chen Y, Webber C, Hardison R, Nelson J, Hallsworth-Pepin K, Delehaunty K, Markovic C, Minx P, Feng Y, Kremitzki C, Mitreva M, Glasscock J, Wylie T, Wohldmann P, Thiru P, Nhan MN, Pohl CS, Smith SM, Hou S, Nefedov M, de Jong PJ, Renfree MB, Mardis ER, Wilson RK (2008) Genome analysis of the platypus reveals unique signatures of evolution. Nature 453:175–183

Waterland RA, Jirtle RL (2003) Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 23:5293–5300

Wilkin F, Paquette J, Ledru E, Hamelin C, Pollak M, Deal CL, Mamelin C (2000) H19 sense and antisense transgenes modify insulin-like growth factor-II mRNA levels. Eur J Biochem 267:4020–4027

Wilkins JF, Ubeda F (2011) Diseases associated with genomic imprinting. Prog Mol Biol Transl Sci 101:401–445

Wright JP, Dietrich KN, Ris MD, Hornung RW, Wessel SD, Lanphear BP, Ho M, Rae MN (2008) Association of prenatal and childhood blood lead concentrations with criminal arrests in early adulthood. PLoS Med 5:e101

Wright RO, Schwartz J, Wright RJ, Bollati V, Tarantini L, Park SK, Hu H, Sparrow D, Vokonas P, Baccarelli A (2010) Biomarkers of lead exposure and DNA methylation within retrotransposons. Environ Health Perspect 118:790–795

Yoshimizu T, Miroglio A, Ripoche MA, Gabory A, Vernucci M, Riccio A, Colnot S, Godard C, Terris B, Jammes H, Dandolo L (2008) The h19 locus acts in vivo as a tumor suppressor. Proc Natl Acad Sci USA 105:12417–12422

Zakharova IS, Shevchenko AI, Zakian SM (2009) Monoallelic gene expression in mammals. Chromosoma 118:279–290

Zhao Z, Tavoosidana G, Sjolinder M, Gondor A, Mariano P, Wang S, Kanduri C, Lezcano M, Sandhu KS, Singh U, Pant V, Tiwari V, Kurukuti S, Ohlsson R (2006) Circular chromosome conformation capture (4c) uncovers extensive networks of epigenetically regulated intra- and interchromosomal interactions. Nat Genet 38:1341–1347

Part II
Epigenetics and Environmental Exposures

Chapter 4
Complex Phenotypes: Epigenetic Manifestation of Environmental Exposures

Christopher Faulk and Dana C. Dolinoy

Abstract Environmental influences in early development alter the epigenome and lead to complex phenotypes and disease susceptibility throughout the life course. Five primary factors, including nutrition, behavior, stress, toxins, and stochasticity, act to influence the epigenome during this critical period. To illustrate how changes in early environment can dramatically affect the epigenome, we provide examples from diverse members of the animal kingdom, spanning insects to human. Specific to mammalian early embryogenesis, DNA methylation, and other epigenetic marks are reset at two specific times in distinct cell lineages leading to epigenetic programming of gametic and somatic cells. These two waves of genomic demethylation and reestablishment of methylation frame the sensitive times for early environmental influences. Evaluating the complex effects of environmental exposures on the developing epigenome requires novel and comprehensive approaches. In this chapter we outline a strategy for the evaluation of environmentally induced epigenetic effects across animal models and human samples, highlighting the necessity for careful assessment of dose and resulting phenotypic changes across the life course. Herein we review the history, environmental factors, critical time points, and vulnerable genomic structures of epigenome–environment interactions. We also provide a framework to further explore epigenomic changes and translate this knowledge from mouse to man.

Keywords Epigenetics • Development • Environment • DNA methylation • Plasticity • Environmental epigenomics

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Abbreviations

BPA	Bisphenol A
CDK	Cyclin-dependent kinase
DMR	Differentially methylated region
DOHaD	Developmental origins of health and disease
GR	Glucocorticoid receptor
IAP	Intracisternal A-particle
LTR	Long terminal repeat
PGC	Primordial germ cells
PRC2	Polycomb repressive complex 2
RAMs	Regions of altered methylation
tRNA	Transfer RNA

4.1 Introduction

The developmental environment is emerging as an influential predictor of subsequent phenotypes and disease risk in later life. The "developmental origins of health and disease" (DOHaD) hypothesis posits that gene–environment interactions during early life result in long-lasting effects and points to epigenetic inheritance as a prime mechanism (Barker et al. 2002). Epigenetics is the study of changes in gene expression that are heritable from cell to cell, hence through cell lineage development, or in rare cases, transgenerationally from parent to offspring to grand-offspring (Youngson and Whitelaw 2008). Increasingly, we are recognizing that early environmental influences on the epigenome are diverse and include dietary (total caloric intake, specific nutrient level, phytochemicals), physical (behavior, temperature, species density, stress), chemical (toxins, endocrine disruptors, pharmaceuticals), or unknown (stochastic, random) effects. Thus, the convergence of environmental toxicology and epigenetic gene regulation is particularly important during the earliest stages of development when epigenetic modifications, such as DNA methylation, are the most sensitive to perturbation.

In this chapter, we introduce the reader to complex phenotypes emerging from environmental perturbations on the developmental epigenome. Firstly, we discuss five relevant environmental influences on epigenetic modifications in development. Here, we showcase examples in animals from insect to human where the environment influences the epigenome through early developmental exposures. Secondly, we review the timing of epigenomic reprogramming, focusing on the post-fertilization and germ cell differentiation stages in male and female offspring. Thirdly, we elucidate the major genome structures and mechanistic targets most vulnerable to environmental perturbations. Finally, we introduce strategies for evaluating

environmental effects on the developing epigenome, and for detecting and verifying epigenetic modifications using combined approaches in animal models and human clinical and epidemiological populations.

4.2 Developmental Influences on the Epigenome

The importance of the early environment in modifying developmental trajectory is not new. For example, in 1809 Jean-Baptiste Lamarck proposed that the increased use of a body part would cause a heritable increase in the size of that body part (Lamarck 1809). Mechanistically, Baptiste hypothesized that organisms have a "tendency to progression" in which offspring inherit traits acquired by the habits of their parents (Gould 2002). Likewise in the early twentieth century, early environmental manipulations were attempted by now-discredited Soviet biologist Trofim Lysenko in his claims that crop yields could be adapted to cold climates by exposing seeds to cold temperatures (Soyfer 2001). This culminated in Lysenko's experimental feeding of special diets to gestating cattle hybrids to produce offspring with greater milk productivity (News of Science 1957). Such misconceptions surrounding the environmental influence on developmental trajectory, therefore, persisted for decades despite early refutations such as August Weismann's experiment in cutting the tails off of rats over five generations while never observing the birth of a tailless rat (Weismann 1891). Epigenetics was revived as a modern science by Conrad Waddington in 1947 and by the end of the twentieth century had adopted its current definition (Jablonka and Lamb 2002).

There are currently a variety of recognized developmental influences resulting in lifelong phenotypic change, mediated by epigenetic gene regulation. The five early developmental influences described here, nutrition, behavior, stress, toxins, and stochasticity interact to influence methylation and other epigenetic marks that in turn affect life-stage phenotype and disease (Fig. 4.1). As indicated by the wide number of animal species discussed (from insects to humans), it is likely that the capacity for epigenetic plasticity is evolutionarily selected. Therefore, it is likely that many more instances of environmental epigenetic influences remain to be elucidated (Jablonka and Raz 2009). Of important note, however, is that not all animals use DNA methylation to repress gene function; for example, the model organisms, fruit fly (*Drosophila melanogaster*) and roundworm (*Caenorhabditis elegans*). To ultimately succeed in identifying environmental factors that affect the epigenome and lead to complex phenotype and disease, researchers must integrate the layers of epigenetic changes in response to mixtures of environmental exposures, paying attention to the times of sensitivity and the model system of evaluation.

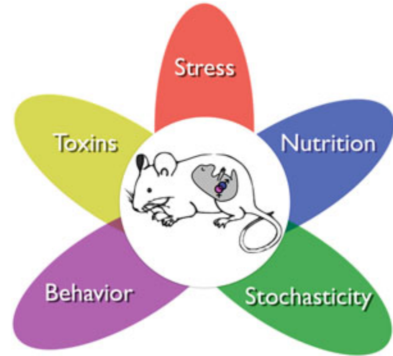

Fig. 4.1 Environmental factors working individually and in concert. Five environmental influences that affect the developing embryo and its primordial germ cells (represented by the *pink* and *blue dots*). Each of these factors can act through a variety of mechanisms, and result in an array of changes in epigenetic marks

4.2.1 Nutrition

Nutrition in early life is never guaranteed or consistent. In bees (*Apis mellifera*), early life nutritionally induced changes are the underlying mediators of queen and worker honeybee differentiation. Bee larva fed royal jelly, a diet specially enhanced with royalactin proteins, shifts development to the queen phenotype, and shows similar effects in the fruit fly (*Drosophila melanogaster*) (Kamakura 2011). DNA methylation is a primary mechanism by which royal jelly acts on the genome through the diet (Kucharski et al. 2008). For example, methylation of the promoter region of *dynactin p62* is decreased in worker bee heads as compared to bodies and averages 10 % lower methylation in the queen bee's complementary tissues, an effect that has been experimentally induced by siRNA-mediated silencing of *Dnmt3*. Additionally, in utero supplementation of animals with methyl-donor rich diets permanently shifts the coat color pattern of mice carrying the *Agouti* viable yellow allele (Waterland and Jirtle 2003) and increases methylation and suppresses transcription of the *Runx3* gene in lung tissue (Hollingsworth et al. 2008). Similarly, dietary supplementation with genistein, the major phytoestrogen in soy, interacts with the methyl-donor pathway to similarly shift the coat color distribution of *Agouti* viable yellow mice, which have emerged as a model biosensor for in utero effects on the fetal epigenome (Dolinoy et al. 2006).

Whole genome hypomethylation is also seen in animals receiving folate deficient diets during gestation and lactation (McKay et al. 2010). For example, in mice, an early postnatal methyl-donor deficient diet reduced methylation at the imprinted gene, *Igf2* (Waterland et al. 2006b). In fact, in utero malnutrition in rodents not only directly affects the expression and methylation of several genes, such as glucocorticoid receptor, *Nr3c1*, and peroxisome proliferator-activated receptor alpha, *Pparα*, but also the neonatal response to leptin. Moreover, these epigenetic effects persist later in life and affect the ultimate adult phenotype, adiposity (Gluckman et al. 2007). Humans are also affected by early life nutritional status as shown in the DNA methylation changes at the *IGF2* locus in whole blood from individuals subject to the Dutch hunger winter (Heijmans et al. 2008). Human

longevity also appears correlated to food abundance available to our grandparents during their prepubertal growth, an effect hypothesized to be epigenetic in origin, although the direct epigenetic mechanism remains unknown (Kaati et al. 2007). Candidate gene studies followed by whole epigenome analysis will become important to environmental epigenomics especially in the translation from mouse to human research.

4.2.2 Behavior and Stress

Behavior- and stress-induced changes are likewise widespread from insects to mammals. The desert locust (*Schistocerca gregaria*) produces more offspring of the gregarious swarming phenotype when bred in crowded conditions (Maeno and Tanaka 2010). Similarly, rats show persistent DNA methylation changes of the glucocorticoid receptor and many other loci in the hippocampus due to high versus low levels of maternal care in the first week of life (McGowan et al. 2011). Falling under both behavior and stress, humans abused in early life also show increased DNA methylation at the *NR3C1* glucocorticoid receptor promoter in the hippocampus (McGowan et al. 2009).

Stress induces epigenetically controlled phenotypic changes in many animals. Insects show widely varying genomic methylation levels between species and changes within a species during development, suggesting a role for methylation in gene–environment interactions (Kronforst et al. 2008). The pea aphid (*Acyrthosiphon pisum*) has a functional DNA methylation system, and under stress from crowded conditions or predators will produce more winged offspring (Weisser et al. 1999). The crowded mothers express more DNMT enzyme, and the winged offspring show increased methylation at the *ApJHBP* locus (Walsh et al. 2010). This illustrates the importance of verifying concordant changes in message level and translation with DNA methylation. Emerging evidence also suggests that regulation of epigenetic marks is associated with stress and environmental response genes in the pacific oyster (*Crassostrea gigas*) and in the basal chordate *Ciona intestinalis* (Gavery and Roberts 2010; Sasaki and Satoh 2007). Furthermore, after being in close proximity to cats, rats exhibit symptoms of post-traumatic stress syndrome concomitant with increases of methylation in the *Bdnf* gene in the hippocampus (Roth et al. 2011). Interestingly, increased methylation of this same gene is also seen in human suicide victims (Keller et al. 2010).

In mice, stress in early life results in increased adult brain expression of arginine vasopressin (AVP) protein correlating with increased methylation of the *Avp* gene in neurons (Murgatroyd et al. 2009). Stress from maternal separation in mice results in depressive behavior coupled with both increased and decreased DNA methylation in a number of genes (Franklin et al. 2010). Interestingly, these mice can transmit the DNA methylation pattern and phenotype transgenerationally through

the male line (Franklin et al. 2010). Early life stress in humans is also linked with gene expression changes for a polymorphic form of serotonin receptor (Caspi et al. 2003).

4.2.3 Chemical Toxicants

Chemical toxins are widely dispersed and have been shown to impact epigenetic processes and marks following early life exposures. Water fleas (*Daphnia magna*) show decreased global DNA methylation when reared in the presence of vinclozolin, a fungicide and endocrine disruptor (Vandegehuchte et al. 2010). Moving from insects to mammals, female rats exposed to vinclozolin during gestation produce male offspring with methylation changes in numerous genes in their sperm. In the female offspring there is a greater incidence of tumor formation and pregnancy abnormalities, which in some cases persists transgenerationally through the germ line (Anway et al. 2005; Guerrero-Bosagna et al. 2010; Nilsson et al. 2008). A whole epigenome approach will offer a more complete understanding of these changes. Because F1 and F2 germ cells are present in the exposed gestating female, they are exposed to vinclozolin during early development. Thus, the effects on the epigenome can be inherited transgenerationally (Fig. 4.2).

While a complete review of the full suite of chemicals affecting the developing epigenome is beyond the scope of this chapter, a number of classes of chemicals beyond pesticides have also been elucidated to act on the epigenome. For example, bisphenol A (BPA), a widely studied endocrine disruptor, is ubiquitous in our environment and is repeatedly shown to affect DNA methylation in multiple rodent tissues, such as liver and brain (Dolinoy et al. 2010; Yaoi et al. 2008). In primates, early exposure to the metal lead (Pb) results in decreased DNA methyltransferase activity in the brain even 23 years later, again underscoring the importance of correlating message level to methylation (Wu et al. 2008). Given the emerging weight of evidence linking developmental toxicant exposures to later disease states in animal models via methylation, a clear path from dosage experiments in model organisms to candidate genes in human studies is particularly crucial.

4.2.4 Stochasticity

Lastly, stochastically or randomly placed methylation marks laid down in early development have been observed at several loci. The model biosensor, the viable yellow (A^{vy}) mouse, varies from brown, pseudoagouti, to yellow fur coloration due to randomly established levels of DNA methylation at a recently inserted intracisternal A-particle (IAP) element within the 5′ end of the *Agouti* gene. DNA methylation can vary by over 80 % at several CpG sites within this IAP between animals (Dolinoy et al. 2010). Similarly the $Cabp^{IAP}$ locus in C57BL/6 mice also contains a contraoriented IAP element in intron 6, capable of stochastic DNA

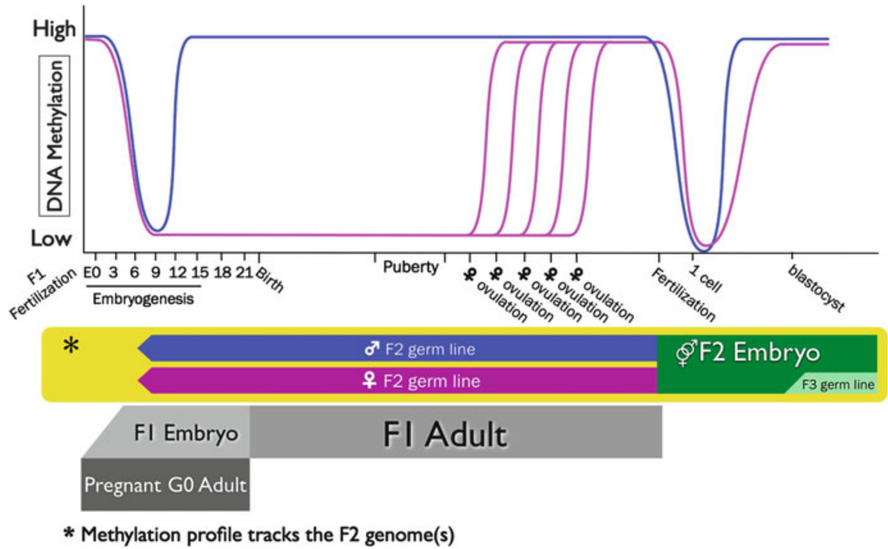

Fig. 4.2 Methylation reprogramming in a single genome occurs in two waves. Global demethylation events from the perspective of the mouse F2 genome from germ cells to newly conceived F2 embryo. Within the pregnant G0 mouse, the F1 embryo generates a group of cells destined to become its gametes, which will form the F2 generation. These primordial germ cells (*PGC*) begin to migrate to the genital ridge around embryonic day 7.25 in the mouse. During this time they become demethylated in preparation to adopt the somatic methylation pattern, and for imprinted genes, the gender-specific methylation pattern to match the genotype of the individual in which they reside. In males, methylation is reestablished by E14. In females the PGC remain largely unmethylated until maturation in the F1 adult during each estrous cycle. During fertilization, the F2 gametes combine and undergo the second, more complete, wave of demethylation in preparation to establish somatic methylation patterns (with the exception of the F3 PGCs). Any environmental influences on the pregnant G0 adult can affect the development and adult disease susceptibility of both the F1 and F2 generations as their somatic and germline methylation patterns are being established, respectively

methylation (Druker 2004). The Axin-fused ($Axin^{Fu}$) mouse has a dramatic kinky tail phenotype caused by stochastic DNA methylation of another intronic IAP element (Vasicek et al. 1997). Thus, IAP elements are important proxies for genome-wide methylation determination as well as for understanding methylation resetting during development, as will be discussed in more detail in Sect. 4.4.1.

These alleles are termed “metastable epialleles,” as they are variably expressed in genetically identical organisms due to epigenetic modifications that are established during early development (Rakyan et al. 2002). The epigenetic marks are variable between individuals but consistent and stable in their patterns within a mouse throughout its life, implying that the level of methylation is set early in development and stable for life (Weinhouse et al. 2011). The distribution of variable expressivity is shifted at these metastable epialleles following maternal exposure to nutritional and environmental factors (Cooney et al. 2002; Dolinoy et al. 2006, 2007; Kaminen-Ahola et al. 2010; Waterland and Jirtle 2003; Waterland

et al. 2006b). In humans, we see variation in methylation in monozygotic twins where spinal deformation was associated with increased *AXIN1* methylation in one twin with lack of methylation and associated physical anomaly in the other one (Oates et al. 2006). It is likely that the underlying stochastic distribution of methylation at metastable epialleles may be affected by as yet uncharacterized environmental factors.

4.3 Time Points of DNA Methylation Lability

DNA methylation is primarily a stable repressive mark; however, its regulation is more dynamic than previously believed and can be actively removed at specific loci and genome-wide at several stages during development (Wu and Zhang 2010). In the context of early environmentally modifiable epigenetic marks, it is important to determine the windows of greatest susceptibility. The epigenome is most vulnerable to environmental factors during embryogenesis because the DNA synthetic rate is high, and the elaborate DNA methylation patterning required for normal tissue development is established. During adulthood, somatic tissues vary widely in cellular turnover rate and environmental exposures (see Sect. 4.3.3). The mammalian genome undergoes two waves of global DNA demethylation followed by de novo methylation, as illustrated in Fig. 4.2 using the mouse as a representative mammalian animal model (Bernal and Jirtle 2010). In mammals, the mother, G0, hosts the development of the F1 offspring from zygote stage to birth. During the development of the F1 offspring, a separate lineage of cells within the F1, called the primordial germ cells (PGCs), migrate and differentiate into gamete precursor cells that will eventually become the F2 generation. By convention, the first wave of methylation resetting refers to the reprogramming of the epigenome within these PGCs, and the second wave refers to the reprogramming that happens shortly after zygote formation. Exposure of a pregnant mother can affect methylation status of both the first wave (in the F2 PGCs) and the second wave (in the post-fertilization F1 pluripotent somatic cells).

4.3.1 Germ Cell Methylation Reprogramming

In mice, the first wave of reprogramming occurs in primordial germ cells (PGCs) during and after their migration to the genital ridge (Yamazaki et al. 2003). This demethylation is complete in the mouse before birth and allows resetting of imprinted genes in the PGCs to match the sex of the host in which they now find themselves (Lees-Murdock and Walsh 2008; Sasaki and Matsui 2008). A number of repetitive elements, including IAPs, are also protected from demethylation to varying extents during this reprogramming wave in developing oocytes (Hajkova

et al. 2002). These genetic loci are more susceptible to loss of heterozygosity and increased methylation instability, respectively (Dolinoy and Jirtle 2008).

In male mouse fetuses, PGCs differentiate into prospermatogonia, enter mitotic arrest, and reestablish methylation before birth (Kota and Feil 2010; Reik et al. 2001). Consequently, this window is especially important for disruptions to loci that escape demethylation as well as resetting of global methylation in male offspring with any effects likely to be seen in the F2 generation (Guerrero-Bosagna et al. 2010; Hanel and Wevrick 2001). Nonmammalian animals, in general, do not have imprinted genes (Kaneda et al. 2004). Developmental exposures may affect the growth of the offspring by inheritance without necessarily having a lasting impact on the parent.

After sexual maturation in all male mammals, the prospermatogonia complete meiosis and differentiate into mature sperm, and during this process, the chromosomes are almost entirely stripped of histones and repackaged with highly basic protamines. Because the protamines do not contain any modifiable tails, any epigenetic information carried on histones is unable to be passed through the male germ line (Balhorn 2007). A small number of histones are retained in mammalian sperm; however, it is unknown whether they play a role in passing on any epigenetic information to the resulting zygote (Gaucher et al. 2010; Kota and Feil 2010).

In contrast to males, F1 female mammalian PGCs complete meiosis I while still in the developing embryo, followed by cell arrest until puberty (Kota and Feil 2010). Thus, in human females, for example, the oocytes remain in a haploid demethylated state for years. Therefore, the window of possible disruption to the establishment of methylation patterns in oocytes is much longer and repeatedly occurs during the maturation of each egg throughout fertility (Fig. 4.2) (Sasaki and Matsui 2008).

4.3.2 Zygotic Methylation Reprogramming

The second wave of global demethylation occurs shortly after fertilization and before implantation. The male pronucleus is stripped of the protamines, while the DNA is actively demethylated and repackaged with newly synthesized histones in the zygote (Nonchev and Tsanev 1990; Santos et al. 2002). The female complement of chromosomes becomes demethylated via a passive mechanism during replication (Hemberger et al. 2009). Not every gene is demethylated since the oocyte contains egg-specific isoforms of DNMT1, and the early embryo synthesizes its own somatic DNMT1 isoform. The presence of these maintenance methyltransferases is required to ensure the preservation of gametically derived differential methylation for imprinted genes, particularly during global demethylation of most other regions of the genome (Cirio et al. 2008). Application of whole epigenome methods in model organisms will complete the list of candidate targets for environmental sensitivity.

The zygotic round of demethylation is less comprehensive than the reprogramming in PGCs, with imprinting control regions retaining differential DNA methylation depending on their parent-of-origin and some classes of repetitive elements also retaining their methylation (Lees-Murdock and Walsh 2008). This wave of methylation cycling sets the pattern for all somatic cells in the resulting embryo and adult except for the PGCs, which will form the gametes for the next generation. The embryo (F1) would be most vulnerable during this window of time to environmental influences that disrupt the reestablishment of DNA methylation. Thus, any epigenetic disruptions early in development would affect tissues in all three germ layers (Weinhouse et al. 2011).

4.3.3 Somatic Methylation Lability

The major global demethylation event that occurs in the somatic cells of adults is associated with aging and disease states (Calvanese et al. 2009). As mammals age, they undergo gradual genome-wide DNA hypomethylation concomitant with hypermethylation at normally unmethylated CpG islands (Murgatroyd et al. 2010). Additionally, adult tissues undergo widely varying life spans and cellular turnover rates. Neurons can last a lifetime, while gut epithelial cells live only a few days (Creamer et al. 1961). Cell types have differing exposure profiles as well, with some exposed more directly to toxins and nutrients like the gut and intestinal tract. Other tissues are exposed primarily to chemical metabolites, and some, like liver, are exposed to both. Location, length of exposure, and cellular life span all play a role in the dynamics of DNA methylation change for a given organ over time. Since cancer is a heterogeneous disease, it displays inconsistent methylation profiles, but in general, the genome is widely hypomethylated as compared to normal tissue with the notable exception of hypermethylation of tumor suppressor gene promoter regions (Feinberg 2007).

4.4 Vulnerable Genomic Structures

The genome is not homogenous in content, expression, or epigenetic marks; some loci are more likely to resist reprogramming or to be environmentally sensitive. As discussed in Sects. 4.2 and 4.3, repetitive elements and imprinted genes can be incompletely reprogrammed or unusually labile, respectively. Below we examine these types of loci in greater detail.

4.4.1 Repetitive Elements

Human and mouse genomes are comprised of 46 % and 39 % repetitive elements, respectively. These elements fall under several different types based on their methods of duplication including cut-and-paste, copy-and-paste, tandem duplication, and gene conversion (Cordaux and Batzer 2009). From an evolutionary perspective, selection acts to limit the spread of these potentially genome destabilizing elements. Epigenetics, particularly DNA methylation, plays a major role in their suppression. As an example, the long terminal repeat (LTR) class of elements is widely active in mice with several thousand copies accumulating and still being quite active in reshaping the genome today (Qin et al. 2010). These elements are generally silenced to a high degree in normal somatic cells; however, they can act as gene promoters in both mouse and human (Bernal and Jirtle 2010; Cohen et al. 2009). Additionally, the same elements can exhibit differing species-specific methylation patterns (Carbone et al. 2009). Elements of different classes regain methylation at different times as well. For examples, some LTR-class IAPs can remain unmethylated in the female oocyte until maturation, whereas LINE-1 elements are remethylated much sooner, around the time of birth (Lees-Murdock and Walsh 2008). Sex-specific differences are also evident in these same elements, with both classes becoming methylated in male germ cells immediately following the global demethylation event in migrating primordial germ cells. Clearly, the genomic identity of a region plays a large role in the timing in the laying down of epigenetic marks.

4.4.2 Imprinting

Epigenetic systems in mammals may have developed as a consequence of totipotency and the need to activate genes in only certain cell types despite the fact that all cells share the same genetic components (Jablonka and Lamb 2002). One of the most extensively studied epigenetic phenomena in mammals is genomic imprinting, in which one parental allele is epigenetically altered resulting in parent-of-origin modification of gene transcription (Murphy and Jirtle 2003; Reik and Walter 2001). Imprinted genes were first hypothesized following nuclear transplantation studies conducted by Surani and colleagues in the 1980s in which diploid androgenotes derived from two male pronuclei and diploid gynogenotes derived from two female pronuclei developed improperly (Barton et al. 1984; Surani et al. 1984). It was not until 1991, however, that the first imprinted genes were identified. Since the demonstration that *insulin-like growth factor 2* (*Igf2*), a potent growth factor (DeChiara et al. 1991), and *insulin-like growth factor 2 receptor* (*Igf2r*) (Barlow et al. 1991) are imprinted, approximately 80 imprinted genes have been identified in mice and humans, with 29, or about one third being imprinted in both species (Morison et al. 2005).

Since imprinted genes are functionally haploid, the health consequences of genomic imprinting are potentially disastrous. Monoallelic expression eliminates the protection that diploidy normally affords against deleterious effects of recessive mutations. The most widely accepted theory of imprinting evolution, "the conflict hypothesis," posits that imprinting arose because of a genetic tug-of-war between the parents to control the amount of nutrients extracted from the mother by her offspring (Haig and Graham 1991; Wilkins and Haig 2003). Work in the early 2000s showed that imprinting evolved approximately 180 million years ago following the divergence of Prototherian (i.e., monotremes) from Therian (i.e., marsupials and eutherians) mammals (Killian et al. 2000; Murphy and Jirtle 2003). Thus, genomic imprinting arose in mammals with the evolution of the placenta and the advent of viviparity.

Imprinted genes and their associated regulatory components may be particularly sensitive to developmental environmental perturbations on the epigenome. In fact, as discussed above, individuals conceived during the Dutch hunger winter at the end of World War II, were shown 60 years later to have altered DNA methylation at the *IGF2* locus (Heijmans et al. 2008). Imprinted genes are associated with differentially methylated regions, termed DMRs (Ferguson-Smith 2011). Since DMRs, unlike most regions of the genome, must retain methylation on a single copy on one chromosome with the sister chromosomal locus hypomethylated, they are naturally sensitive to methylation disruption (Bernal and Jirtle 2010). The classic example of complementary genes being imprinted is the maternally expressed *Igf2r* and paternally expressed *Igf2* genes in mice. Although these regions are believed to have evolved rapidly, the *Igf2r* DMR is maintained in humans despite the now biallelic expression of the gene (Kalscheuer et al. 1993). Moreover, the imprinting status is often, but not always, preserved between species (Ferguson-Smith 2011; Weidman et al. 2004). The monoallelic expression of imprinted genes can also be specific to a tissue or developmental time point. Consequently, developmental dysregulation by toxins or other environmental exposures can vary in tissue and time-dependent manner further complicating risk assessment.

4.5 Mechanistic Targets of Environmental Exposure

Mounting evidence suggests that environmental pressures can exert effects on multiple levels of gene regulation. The weight of evidence is currently focusing on gene transcription and chromatin structure controlling access to transcriptional machinery.

4.5.1 Gene Transcription

The original function of DNA methylation is likely to be host defense against rogue transposable elements but has since been co-opted to also serve a gene regulatory function (Zemach and Zilberman 2010). Gene transcription can be suppressed by the DNA methylation of CpG islands in promoters as well as histone modification and nucleosome placement. For example, the methyl group of the 5-methyl cytosine extends into the major groove of DNA, inhibiting transcription by interfering with transcription factor binding proteins. In addition, DNMTs and methylated DNA interact with higher-order chromatin proteins to affect histone modifications and further compact chromatin. Thus, DNA methylation and histone modifications act synergistically to maintain silencing and inhibit access to transcription factors.

As introduced previously, the insertion of a metastable IAP in the *Agouti* gene in the A^{vy} mouse strain provides a locus for variable DNA methylation to affect transcription. The $Cabp^{IAP}$ and $Axin^{Fu}$ loci also show a correlation of increased methylation and decreased expression affected by environmental exposures. These loci provide a basis for the investigation of genome-wide changes due to methylation, and a number of studies find novel metastable epialleles with variable methylation (Luedi et al. 2005; Waterland et al. 2010). Repetitive element insertions can also be the cause of several human diseases (Callinan and Batzer 2006). Although they can disrupt gene transcription, they can also be silenced by surveillance mechanisms, as we see in the case of the A^{vy} mouse IAP element. Environmentally induced derepression of these repetitive elements is an active area of research.

4.5.2 Chromatin Remodeling Complexes

Chromatin modifying complexes act throughout the genome changing the higher-order chromatin structure to inhibit or increase access to transcriptional machinery. The methyltransferase *EZH2* catalyzes H3K27 trimethlyation as part of the PRC2, and it can also be phosphorylated by cyclin-dependent kinases (CDK) (Hansen et al. 2008). After phosphorylation it loses the ability to bind to noncoding RNAs and is thus unable to place the H3K27me3 mark in a sequence-specific manner (Zeng et al. 2011). Importantly, this mark is propagated from cell to cell, preserving the epigenetic silencing of marked regions. Any environmental influences that alter the activity of *EZH2*, or its upstream CDK enzymes, can thereby have widespread developmental effects lasting into adulthood. Placental mammals also have *REX1*, a DNA-binding transcription factor thought to target chromatin remodeling complexes (Kim et al. 2007). In mouse *Rex1* null mutants, DNA methylation profiles at the DMRs of several imprinted loci are disrupted in adult tissues despite the fact that *Rex1* is only expressed very early in development (Kim et al. 2011).

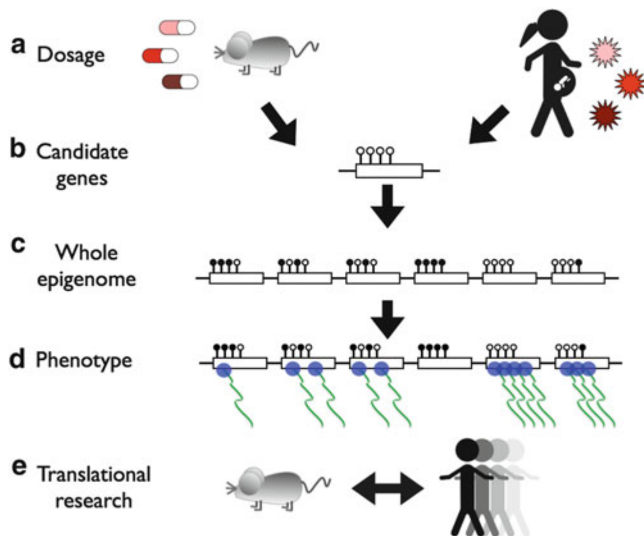

Fig. 4.3 Comprehensive strategy for the analysis of environmental disruption on the developing epigenome from mice to men. (**a**) Multiple dosages in animal model studies better reflect relevant human exposure ranges allowing for the detection of non-monotonic epigenomic effects, often characteristic of endocrine active compounds such as bisphenol A (BPA). (**b**) Candidate gene assays are quick, reproducible, and relatively inexpensive and are able to be used for epidemiological research. Conversely, this approach alone is limited in the ability to identify new targets of environmentally influenced epigenetic changes. (**c**) Epigenome-wide studies, enabled by advances in technology, provide a comprehensive overview of DNA methylation changes over the entire genome, enabling the discovery of new regions of metastability. (**d**) Phenotype can be measured quantitatively by message level and correlated to genomic DNA methylation. An environmentally induced change in an epigenetic mark does not necessarily reflect a corresponding change in message, such as mRNA levels. (**e**) Translational research from animal models to human clinical samples to human populations is necessary to locate and quantify environmentally induced changes (e.g. BPA) on the epigenome

4.6 Mapping Environmental Influences on the Developing Epigenome: A Representative Approach from Mice to Men

Until recently, most attempts to elucidate the effects on the epigenome following nutritional and environmental exposure were either candidate gene driven or based on epigenetic techniques with limited genome coverage/sensitivity, restricted in dose–response assessment, or confined to animal models. Using bisphenol A (BPA) as a representative early environmental exposure, we have developed a comprehensive strategy for evaluating effects on the developing epigenome (Fig. 4.3). This approach combines multi-dose studies in animal models with careful environmental characterization of human clinical samples and extended analysis of epidemiological-characterized human population samples. Integral to this strategy and important for identifying biomarkers for disease risk and progression is the requirement that

environmentally induced changes on the developmental epigenome be correlated with subsequent gene expression and phenotypic effects across the life course.

BPA is a high-production volume monomer used in the manufacture of polycarbonate plastic and epoxy resins. It is present in many commonly used products including food and beverage containers, baby bottles, dental composites, and thermal receipt paper. Furthermore, BPA is associated with epigenetic alterations following developmental exposure (Dolinoy et al. 2007; Ho et al. 2006; Yaoi et al. 2008). In the rat model, Ho et al. observed multiple changes in gene-specific DNA methylation patterns in the adult male prostate, including hypomethylation of the phosphodiesterase type 4 variant 4 (*Pde4d4*) gene following neonatal exposure to both estradiol and low-level BPA (10 μg/kg of body weight BPA). Decreased *Pde4d4* methylation is associated with a marked increase in prostate cancer risk. Using the viable yellow *Agouti* (A^{vy}) mouse model, we showed that maternal dietary exposure to moderate levels of BPA (50 mg BPA/kg diet) results in decreased DNA methylation at the A^{vy}, and $Cabp^{IAP}$ metastable epialleles (Dolinoy et al. 2007). Finally, using restriction-enzyme based methylation technology, Yaoi and colleagues reported both hyper- and hypomethylation at *NotI* loci in murine offspring forebrain following gestational exposure to 20 μg/kg of body weight BPA (Yaoi et al. 2008). These recent attempts, including our own, were limited to (1) a candidate gene driven approach, (2) restricted dose–response assessment, (3) less than comprehensive timing and expression effects, and finally (4) animal models.

Capitalizing on advances in unbiased epigenomic and high-throughput quantitative DNA methylation technologies, we have developed a comprehensive approach to identify the constellation of genomic loci with altered epigenetic status following dose-dependent in utero BPA exposure. Using a “tiered focusing approach,” our strategy proceeds from unbiased broad DNA methylation analysis using methylation-based next generation sequencing technology to in-depth quantitative site-specific CpG methylation determination using the Sequenom MassARRAY and QIAgen pyrosequencing platforms. Innovative to this design, we employ this approach across both mice and humans in order to identify species specificity in lability from early exposure to environmental agents. Additional toxicologically relevant animal models, including rats and sheep are also being considered for this approach, resulting in a comparative epigenomic analysis across mammalian species. Using bioinformatics and biostatistical methods, we compare the regions of altered methylation (RAMs) following BPA exposure, and the cellular pathways in which the genes with nearby RAMs function.

Human BPA exposure, including time-dependent intra-individual variation, is an extremely active area of research as well as being controversial. Examination of 2,517 individuals ($\geq$6 years) from 2003 to 2004 NHANES survey showed urinary BPA concentrations ranging from 0.4 to 149 ug/L (mean 2.6 ug/L) (Calafat et al. 2008). Levels were lower in Mexican Americans compared to non-Hispanic blacks and whites and higher in women and children as well as individuals of low socioeconomic status (Calafat et al. 2008). A study of circulating blood BPA levels in pregnant women in Michigan indicated exposure levels between 0.5 and 22.3 ug/L (mean 5.9 ug/L) (Padmanabhan et al. 2008). These trends indicate that in utero

development and infancy may be particularly vulnerable time periods for exposure to BPA. Collaborating with analytical chemists, we are evaluating BPA concentrations in human placental and fetal tissue and employing epigenome-wide techniques to correlate human in utero BPA exposure levels with distinct methylation profiles. These samples represent a unique opportunity to not only measure BPA levels in tissue matrices but to also conduct epigenome-wide and transcriptomic analysis, an endeavor that requires relatively large amounts of DNA and RNA. Moving from human clinical samples to human population studies with well-characterized early environmental exposure data, as well as life-course demographic and nutrition data, is a crucial next step in this pipeline.

The strategy described herein utilizes BPA as a representative environmental toxicant that is easily applied to other environmental factors of interest. The elucidation of epigenomic loci dysregulated in a dose-dependent manner in both animal and human genomes will ultimately strengthen human health risk assessment and shape diagnostic and therapeutic strategies for disease. As mentioned above, the mouse is a tractable and popular model for human diseases; however, animal models for toxicology studies may not be the best choice for modeling the potential impact on the human genome if the repertoire of epigenetically labile genes is markedly species dependent. For example, recently Jirtle and colleagues employed machine-learning algorithms to identify epigenetically regulated imprinted genes throughout the genome. This approach uncovered 600 novel imprinted candidate genes in the mouse and 156 in the human (Luedi et al. 2005, 2007). Interestingly, humans are predicted to have not only fewer imprinted genes than mice, but also a markedly different repertoire. The divergence of imprinted genes, and potentially other epigenetically regulated loci such as metastable epialleles, between mouse and human could have serious consequences for the reliance on rodents as models of human disease. Further, the use of whole genome and unbiased deep sequencing approaches allows for the identification of epigenetic biomarkers for exposure that will be useful in enabling clinicians to identify at-risk individuals prior to disease onset.

4.7 Moving Forward

It is increasingly recognized that environmental exposure to chemical, nutritional, and behavioral factors alters gene expression and affects health and disease, by not only mutating promoter and coding regions of genes but also by altering gene expression through the modification of the epigenome. The investigation of early environmental effects can inform the fields of toxicology and environmental epidemiology by elucidating the mechanisms underlying developmental exposure and adult disease. Of the five environmental factors we highlighted as affecting development, nutrition is the best studied. Given the ubiquity of environmental toxins in our environment, a comprehensive plan is needed to assess their effects. Dosage levels, corresponding mRNA message levels, protein translation, and resulting

phenotypic consequences, ideally must all be determined in model organisms. Candidate gene approaches will be enhanced by concomitant whole epigenome technologies. Epidemiological studies must then translate these results from mouse to man.

In order to integrate epigenetic research into risk assessment or clinical practice, we must first understand the most sensitive time points in the resetting of epigenetic marks. These vulnerable periods are likely distinct for males and females as well as for offspring and grand-offspring and may require specialized preventive and/or corrective actions. Additionally, the molecular mechanisms linking these sensitive time points to the adult presentation of disease need to be fully characterized. Ultimately scientists must integrate the layers of epigenetic changes with the times of sensitivity to generate the best prescriptions for human health. Since epigenetic profiles, unlike genetic mutations, are potentially reversible, approaches for prevention and treatment, such as nutritional supplementation and/or pharmaceutical therapies, may have significant impact on human health and disease trajectories.

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References

Anway MD, Cupp AS, Uzumcu M, Skinner MK (2005) Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308:1466–1469

Balhorn R (2007) The protamine family of sperm nuclear proteins. Genome Biol 8:227

Barker DJ, Eriksson JG, Forsen T, Osmond C (2002) Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol 31:1235–1239

Barlow DP, Stoger R, Herrmann BG, Saito K, Schweifer N (1991) The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. Nature 349:84–87

Barton S, Surani M, Norris M (1984) Role of paternal and maternal genomes in mouse development. Nature 311:374–376

Bernal AJ, Jirtle RL (2010) Epigenomic disruption: the effects of early developmental exposures. Birth Defects Res A Clin Mol Teratol 88:938–944

Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL (2008) Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. Environ Health Perspect 116:39–44

Callinan PA, Batzer MA (2006) Retrotransposable elements and human disease. Genome Dyn 1:104–115

Calvanese V, Lara E, Kahn A, Fraga MF (2009) The role of epigenetics in aging and age-related diseases. Ageing Res Rev 8:268–276

Carbone L, Harris RA, Vessere GM, Mootnick AR, Humphray S, Rogers J, Kim SK, Wall JD, Martin D, Jurka J, Milosavljevic A, de Jong PJ (2009) Evolutionary breakpoints in the gibbon suggest association between cytosine methylation and karyotype evolution. PLoS Genet 5: e1000538

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301:386–389

Cirio MC, Ratnam S, Ding F, Reinhart B, Navara C, Chaillet JR (2008) Preimplantation expression of the somatic form of Dnmt1 suggests a role in the inheritance of genomic imprints. BMC Dev Biol 8:9

Cohen CJ, Lock WM, Mager DL (2009) Endogenous retroviral LTRs as promoters for human genes: a critical assessment. Gene 448:105–114

Cooney CA, Dave AA, Wolff GL (2002) Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. J Nutr 132:2393–2400

Cordaux R, Batzer MA (2009) The impact of retrotransposons on human genome evolution. Nat Rev Genet 10:691–703

Creamer B, Shorter RG, Bamforth J (1961) The turnover and shedding of epithelial cells. I. The turnover in the gastro-intestinal tract. Gut 2:110–118

DeChiara TM, Robertson EJ, Efstratiadis A (1991) Parental imprinting of the mouse insulin-like growth factor ii gene. Cell 64:849–859

Dolinoy DC, Jirtle RL (2008) Environmental epigenomics in human health and disease. Environ Mol Mutagen 49:4–8

Dolinoy DC, Weidman JR, Waterland RA, Jirtle RL (2006) Maternal genistein alters coat color and protects A^{vy} mouse offspring from obesity by modifying the fetal epigenome. Environ Health Perspect 114:567–572

Dolinoy DC, Huang D, Jirtle RL (2007) Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci USA 104:13056–13061

Dolinoy DC, Weinhouse C, Jones TR, Rozek LS, Jirtle RL (2010) Variable histone modifications at the A (vy) metastable epiallele. Epigenetics 5:637–644

Druker R (2004) Complex patterns of transcription at the insertion site of a retrotransposon in the mouse. Nucleic Acids Res 32:5800–5808

Feinberg AP (2007) Phenotypic plasticity and the epigenetics of human disease. Nature 447:433–440

Ferguson-Smith AC (2011) Genomic imprinting: the emergence of an epigenetic paradigm. Nat Rev Genet 12:565–575

Franklin TB, Russig H, Weiss IC, Graff J, Linder N, Michalon A, Vizi S, Mansuy IM (2010) Epigenetic transmission of the impact of early stress across generations. Biol Psychiatry 68:408–415

Gaucher J, Reynoird N, Montellier E, Boussouar F, Rousseaux S, Khochbin S (2010) From meiosis to postmeiotic events: the secrets of histone disappearance. FEBS J 277:599–604

Gavery MR, Roberts SB (2010) DNA methylation patterns provide insight into epigenetic regulation in the Pacific oyster (*Crassostrea gigas*). BMC Genomics 11:483

Gluckman PD, Lillycrop KA, Vickers MH, Pleasants AB, Phillips ES, Beedle AS, Burdge GC, Hanson MA (2007) Metabolic plasticity during mammalian development is directionally dependent on early nutritional status. Proc Natl Acad Sci USA 104:12796–12800

Gould SJ (2002) The structure of evolutionary theory. Belknap Press of Harvard University Press, Cambridge, MA

Guerrero-Bosagna C, Settles M, Lucker B, Skinner MK (2010) Epigenetic transgenerational actions of vinclozolin on promoter regions of the sperm epigenome. PLoS One 5:e13100

Haig D, Graham C (1991) Genomic imprinting and the strange case of the insulin-like growth factor II receptor. Cell 64:1045–1046

Hajkova P, Erhardt S, Lane N, Haaf T, El-Maarri O, Reik W, Walter J, Surani MA (2002) Epigenetic reprogramming in mouse primordial germ cells. Mech Dev 117:15–23

Hanel ML, Wevrick R (2001) Establishment and maintenance of DNA methylation patterns in mouse Ndn: implications for maintenance of imprinting in target genes of the imprinting center. Mol Cell Biol 21:2384–2392

Hansen KH, Bracken AP, Pasini D, Dietrich N, Gehani SS, Monrad A, Rappsilber J, Lerdrup M, Helin K (2008) A model for transmission of the H3K27me3 epigenetic mark. Nat Cell Biol 10:1291–1300

Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE, Lumey LH (2008) Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci USA 105:17046–17049

Hemberger M, Dean W, Reik W (2009) Epigenetic dynamics of stem cells and cell lineage commitment: digging Waddington's canal. Nat Rev Mol Cell Biol 10:526–537

Ho SM, Tang WY, Belmonte de Frausto J, Prins GS (2006) Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Res 66:5624–5632

Hollingsworth JW, Maruoka S, Boon K, Garantziotis S, Li Z, Tomfohr J, Bailey N, Potts EN, Whitehead G, Brass DM, Schwartz DA (2008) In utero supplementation with methyl donors enhances allergic airway disease in mice. J Clin Invest 118:3462–3469

Jablonka E, Lamb MJ (2002) The changing concept of epigenetics. Ann N Y Acad Sci 981:82–96

Jablonka E, Raz G (2009) Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. Q Rev Biol 84:131–176

Kaati G, Bygren LO, Pembrey M, Sjöström M (2007) Transgenerational response to nutrition, early life circumstances and longevity. Eur J Hum Genet 15:784–790

Kalscheuer VM, Mariman EC, Schepens MT, Rehder H, Ropers HH (1993) The insulin-like growth factor type-2 receptor gene is imprinted in the mouse but not in humans. Nat Genet 5:74–78

Kamakura M (2011) Royalactin induces queen differentiation in honeybees. Nature 473 (7348):478–483

Kaminen-Ahola N, Ahola A, Maga M, Mallitt K-A, Fahey P, Cox T, Whitelaw E, Chong S (2010) Maternal Ethanol Consumption Alters the Epigenotype and the Phenotype of Offspring in a Mouse Model. PLoS Genet 6:e1000811

Kaneda M, Okano M, Hata K, Sado T, Tsujimoto N, Li E, Sasaki H (2004) Essential role for de novo DNA methyltransferase *Dnmt3*a in paternal and maternal imprinting. Nature 429:900–903

Keller S, Sarchiapone M, Zarrilli F, Videtic A, Ferraro A, Carli V, Sacchetti S, Lembo F, Angiolillo A, Jovanovic N, Pisanti F, Tomaiuolo R, Monticelli A, Balazic J, Roy A, Marusic A, Cocozza S, Fusco A, Bruni CB, Castaldo G, Chiariotti L (2010) Increased *BDNF* promoter methylation in the Wernicke area of suicide subjects. Arch Gen Psychiatry 67:258–267

Killian J, Byrd J, Jirtle J, Munday B, Stoskopf M, MacDonald R, Jirtle R (2000) M6P/IGF2R imprinting evolution in mammals. Mol Cell 5:707–716

Kim JD, Faulk C, Kim J (2007) Retroposition and evolution of the DNA-binding motifs of YY1, YY2 and REX1. Nucleic Acids Res 35:3442–3452

Kim JD, Kim H, Ekram MB, Yu S, Faulk C, Kim J (2011) Rex1/Zfp42 as an epigenetic regulator for genomic imprinting. Hum Mol Genet 20:1353–1362

Kota SK, Feil R (2010) Epigenetic transitions in germ cell development and meiosis. Dev Cell 19:675–686

Kronforst MR, Gilley DC, Strassmann JE, Queller DC (2008) DNA methylation is widespread across social hymenoptera. Curr biol CB 18:R287–R288

Kucharski R, Maleszka J, Foret S, Maleszka R (2008) Nutritional control of reproductive status in honeybees via DNA methylation. Science 319:1827–1830

Lamarck J-B (1809) Philosophie Zoologique ou exposition des considérations relatives à l'histoire naturelle des animaux. Dentu et L'Auteur, Paris

Lees-Murdock DJ, Walsh CP (2008) DNA methylation reprogramming in the germ line. Epigenetics 3:5–13

Luedi P, Hartemink A, Jirtle R (2005) Genome-wide prediction of imprinted murine genes. Genome Res 15:875–884

Luedi PP, Dietrich FS, Weidman JR, Bosko JM, Jirtle RL, Hartemink AJ (2007) Computational and experimental identification of novel human imprinted genes. Genome Res 17:1723–1730
Maeno K, Tanaka S (2010) Epigenetic transmission of phase in the desert locust, *Schistocerca gregaria*: determining the stage sensitive to crowding for the maternal determination of progeny characteristics. J Insect Physiol 56:1883–1888
McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonté B, Szyf M, Turecki G, Meaney MJ (2009) Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat Neurosci 12:342–348
McGowan PO, Suderman M, Sasaki A, Huang TCT, Hallett M, Meaney MJ, Szyf M (2011) Broad epigenetic signature of maternal care in the brain of adult rats. PLoS One 6:e14739
McKay JA, Waltham KJ, Williams EA, Mathers JC (2010) Folate depletion during pregnancy and lactation reduces genomic DNA methylation in murine adult offspring. Genes Nutr 6 (2):189–196
Morison IM, Ramsay JP, Spencer HG (2005) A census of mammalian imprinting. Trends Genet 21:457–465
Murgatroyd C, Patchev AV, Wu Y, Micale V, Bockmuhl Y, Fischer D, Holsboer F, Wotjak CT, Almeida OF, Spengler D (2009) Dynamic DNA methylation programs persistent adverse effects of early-life stress. Nat Neurosci 12:1559–1566
Murgatroyd C, Wu Y, Bockmühl Y, Spengler D (2010) The Janus face of DNA methylation in aging. Aging 2:107–110
Murphy SK, Jirtle RL (2003) Imprinting evolution and the price of silence. Bioessays 25:577–588
News of Science (1957) Science 126:157–161
Nilsson EE, Anway MD, Stanfield J, Skinner MK (2008) Transgenerational epigenetic effects of the endocrine disruptor vinclozolin on pregnancies and female adult onset disease. Reproduction 135:713–721
Nonchev S, Tsanev R (1990) Protamine-histone replacement and DNA replication in the male mouse pronucleus. Mol Reprod Dev 25:72–76
Oates NA, van Vliet J, Duffy DL, Kroes HY, Martin NG, Boomsma DI, Campbell M, Coulthard MG, Whitelaw E, Chong S (2006) Increased DNA methylation at the *AXIN1* gene in a monozygotic twin from a pair discordant for a caudal duplication anomaly. Am J Hum Genet 79:155–162
Padmanabhan V, Siefert K, Ransom S, Johnson T, Pinkerton J, Anderson L, Tao L, Kannan K (2008) Maternal bisphenol-A levels at delivery: a looming problem? J Perinatol 28:258–263
Qin C, Wang Z, Shang J, Bekkari K, Liu R, Pacchione S, McNulty KA, Ng A, Barnum JE, Storer RD (2010) Intracisternal A particle genes: distribution in the mouse genome, active subtypes, and potential roles as species-specific mediators of susceptibility to cancer. Mol Carcinog 49:54–67
Rakyan VK, Blewitt ME, Druker R, Preis JI, Whitelaw E (2002) Metastable epialleles in mammals. Trends Genet 18:348–351
Reik W, Walter J (2001) Genomic imprinting: parental influence on the genome. Nat Rev Genet 2:21–32
Reik W, Dean W, Walter J (2001) Epigenetic reprogramming in mammalian development. Science 293:1089–1093
Roth TL, Zoladz PR, Sweatt JD, Diamond DM (2011) Epigenetic modification of hippocampal Bdnf DNA in adult rats in an animal model of post-traumatic stress disorder. J Psychiatr Res 45 (7):919–926
Santos F, Hendrich B, Reik W, Dean W (2002) Dynamic reprogramming of DNA methylation in the early mouse embryo. Dev Biol 241:172–182
Sasaki H, Matsui Y (2008) Epigenetic events in mammalian germ-cell development: reprogramming and beyond. Nat Rev Genet 9:129–140
Sasaki A, Satoh N (2007) Effects of 5-aza-2′-deoxycytidine on the gene expression profile during embryogenesis of the Ascidian *Ciona intestinalis*: a microarray analysis. Zoolog Sci 24:648–655

Soyfer VN (2001) The consequences of political dictatorship for Russian science. Nat Rev Genet 2:723–729

Surani MA, Barton SC, Norris ML (1984) Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. Nature 308:548–550

Vandegehuchte MB, Lemière F, Vanhaecke L, Vanden Berghe W, Janssen CR (2010) Direct and transgenerational impact on *Daphnia magna* of chemicals with a known effect on DNA methylation. Comp Biochem Physiol C Toxicol Pharmacol 151:278–285

Vasicek TJ, Zeng L, Guan XJ, Zhang T, Costantini F, Tilghman SM (1997) Two dominant mutations in the mouse fused gene are the result of transposon insertions. Genetics 147:777–786

Walsh TK, Brisson JA, Robertson HM, Gordon K, Jaubert-Possamai S, Tagu D, Edwards OR (2010) A functional DNA methylation system in the pea aphid, *Acyrthosiphon pisum*. Insect Mol Biol 19(Suppl 2):215–228

Waterland R, Jirtle R (2003) Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 23:5293–5300

Waterland RA, Dolinoy DC, Lin JR, Smith CA, Shi X, Tahiliani KG (2006a) Maternal methyl supplements increase offspring DNA methylation at Axin fused. Genesis 44:401–406

Waterland RA, Lin J-R, Smith CA, Jirtle RL (2006b) Post-weaning diet affects genomic imprinting at the insulin-like growth factor 2 (Igf2) locus. Hum Mol Genet 15:705–716

Waterland RA, Kellermayer R, Laritsky E, Rayco-Solon P, Harris RA, Travisano M, Zhang W, Torskaya MS, Zhang J, Shen L, Manary MJ, Prentice AM (2010) Season of conception in rural gambia affects DNA methylation at putative human metastable epialleles. PLoS Genet 6: e1001252

Weidman JR, Murphy SK, Nolan CM, Dietrich FS, Jirtle RL (2004) Phylogenetic footprint analysis of IGF2 in extant mammals. Genome Res 14:1726–1732

Weinhouse C, Anderson OS, Jones TR, Kim J, Liberman SA, Nahar MS, Rozek LS, Jirtle RL, Dolinoy DC (2011) An expression microarray approach for the identification of metastable epialleles in the mouse genome. Epigenetics 6:1105–1113

Weismann A (1891) Essays upon heredity. Clarendon, Oxford

Weisser WW, Braendle C, Minoretti N (1999) Predator-induced morphological shift in the pea aphid. Proc R Soc Lond B Biol Sci 266:1175–1181

Wilkins JF, Haig D (2003) What good is genomic imprinting: the function of parent-specific gene expression. Nat Rev Genet 4:359–368

Wu SC, Zhang Y (2010) Active DNA demethylation: many roads lead to Rome. Nat Rev Mol Cell Biol 11:607–620

Wu J, Basha MR, Brock B, Cox DP, Cardozo-Pelaez F, McPherson CA, Harry J, Rice DC, Maloney B, Chen D, Lahiri DK, Zawia NH (2008) Alzheimer's disease (AD)-like pathology in aged monkeys after infantile exposure to environmental metal lead (Pb): evidence for a developmental origin and environmental link for AD. J Neurosci 28:3–9

Yamazaki Y, Mann MRW, Lee SS, Marh J, McCarrey JR, Yanagimachi R, Bartolomei MS (2003) Reprogramming of primordial germ cells begins before migration into the genital ridge, making these cells inadequate donors for reproductive cloning. Proc Natl Acad Sci USA 100:12207–12212

Yaoi T, Itoh K, Nakamura K, Ogi H, Fujiwara Y, Fushiki S (2008) Genome-wide analysis of epigenomic alterations in fetal mouse forebrain after exposure to low doses of bisphenol A. Biochem Biophys Res Commun 376:563–567

Youngson NA, Whitelaw E (2008) Transgenerational epigenetic effects. Annu Rev Genomics Hum Genet 9:233–257

Zemach A, Zilberman D (2010) Evolution of eukaryotic DNA methylation and the pursuit of safer sex. Curr Biol 20:R780–R785

Zeng X, Chen S, Huang H (2011) Phosphorylation of EZH2 by CDK1 and CDK2: a possible regulatory mechanism of transmission of the H3K27me3 epigenetic mark through cell divisions. Cell Cycle 10:579–583

Chapter 5
Epigenetic Effects of Ionizing Radiation

Olga Kovalchuk

Abstract Over the past decade, research efforts have focused on elucidating the cellular and molecular mechanisms of ionizing radiation (IR)-induced effects in eukaryotic and, most importantly, mammalian cells. The primary sources of radiation exposure stem from diagnostic tests, therapeutic treatments, occupational exposures, nuclear tests, nuclear accidents, as well as the growing production of radioactive waste. It is now well accepted that the effects of IR exposure can be noticed far beyond the borders of the directly irradiated tissue. IR can affect neighboring cells, giving rise to a bystander effect. IR effects can also span several generations and influence the progeny of exposed parents, leading to transgenerational effects. Bystander and transgenerational IR effects are linked to the phenomenon of the IR-induced genome instability that manifests itself as chromosome aberrations, gene mutations, late cell death, and aneuploidy. While the occurrence of these phenomena is well documented, the mechanisms that lead to their development are still being defined. Mounting evidence suggests that IR-induced genome instability and bystander and transgenerational effects may be epigenetically mediated. The epigenetic alterations include DNA methylation, histone modification, and RNA-associated silencing. Recent studies show that IR exposure alters epigenetic parameters not only in the directly exposed tissues but also in the distant bystander tissues. Furthermore, transgenerational radiation effects are proposed to be of an epigenetic nature. In this chapter, I will discuss the role of the epigenetics in IR-induced direct responses, as well as in bystander and transgenerational effects.

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Abbreviations

3D	Three-dimensional
ATR	Ataxia telangiectasia and Rad3 related
BORIS	Brother of the regulator of imprinted sites
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
ESTR	Expanded simple tandem repeat
DSBs	Double-strand breaks
IR	Ionizing radiation
LSH	Lymphoid-specific helicase
MBD	Methyl CpG-binding domain
mRNA	Messenger RNA
miRNA	MicroRNA
piRNA	Piwi-interacting RNA
RB	Retinoblastoma
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
UTR	Untranslated region

5.1 Introduction

All living organisms are exposed daily to radiation. In addition to diagnostic and therapeutic medical exposures, we are exposed chronically to background radiation from cosmic rays, radioactive waste, radon decay, nuclear tests, and accidents at the Chernobyl, Fukushima, and other nuclear power plants. On the one hand, IR is a well-known cancer-inducing agent. The carcinogenic potential of IR was recognized very soon after its discovery, when the first radiation-induced tumor was reported in 1902 (Chauveinc et al. 1998; Little 2000, 2003; Kovalchuk and Baulch 2008). On the other hand, IR is one of the primary clinical methods used for detecting and treating human malignancies, which makes understanding the underlying mechanisms of IR-induced biological effects imperative.

5.2 Direct Effects of Radiation Exposure: Radiation-Induced Cancers

The risk of radiation therapy-related deleterious effects, including secondary cancers, is a growing problem (Boice et al. 1992; Leone et al. 1999; Brenner et al. 2000, 2005; Copelan et al. 2004; Hall 2006). Furthermore, cancer risk may also stem from exposure to low doses of IR during diagnostic procedures such as computed tomography (Hildreth et al. 1989; Preston-Martin et al. 1989; Infante-Rivard et al. 2000; Mori et al. 2001; Hall 2002; Shu et al. 2002; Brenner and Hall 2004; Kleinerman 2006; Liu et al. 2006).

Elevated cancer rates have been reported in atomic bomb survivors, occupationally exposed individuals, and populations exposed to IR as a result of nuclear tests and nuclear power plant accidents. Analysis of the Hiroshima and Nagasaki A-bomb survivors cohort revealed elevated rates of leukemia (Folley et al. 1952; Watanabe et al. 1972; Wakabayashi et al. 1983), breast cancer (Wakabayashi et al. 1983; Carmichael et al. 2003), thyroid carcinoma (Watanabe et al. 1972; Wakabayashi et al. 1983), and stomach and lung cancers (Wakabayashi et al. 1983).

Significantly increased rates of leukemia were reported in residents of the areas adjacent to the Mayak nuclear facility in the southern Urals region of Russia (Kossenko 1996; Shilnikova et al. 2003). Increased mutation and cancer rates were also seen in the population of the Semipalatinsk nuclear test site in Kazakhstan (Salomaa et al. 2002; Tanaka et al. 2006).

In April of 1986, the explosion of the 4th reactor at the of Chernobyl Nuclear Power Plant led to an enormous release of radioactive materials into the atmosphere and the formation of complex patterns of radioactive contamination over vast territories of Europe. In Chernobyl, the most significant human exposure was to radioactive iodine-131. This exposure resulted in a subsequent rise in the number of thyroid carcinomas (Bogdanova et al. 2006; Likhtarov et al. 2006; Williams and Baverstock 2006; Williams 2006). IR exposure from the Chernobyl accident also resulted in elevated levels of other cancers such as leukemia and lymphoma (Gluzman et al. 2005; Balonov 2007), breast cancer (Pukkala et al. 2006; Prysyazhnyuk et al. 2007), bladder cancer (Morimura et al. 2004), and renal-cell carcinomas (Romanenko et al. 2000). The rates of the IR-induced solid cancers continue to rise even now, more than 25 years after the accident (Baverstock 2000; Baverstock and Williams 2006, 2007; Williams and Baverstock 2006). Another devastating nuclear accident happened in 2012 in Fukushima, and its health effects still remain to be explored. Based on the similarities between the Chernobyl and Fukushima accidents, it is predicted that the reactor accident in Japan will also lead to an increased cancer incidence in the exposed populations.

Over the past decade, intense research efforts have been made to elucidate the cellular and molecular mechanisms of IR-induced carcinogenesis in eukaryotes and, most importantly, in humans. IR causes a variety of responses in exposed cells. It can cause changes in gene expression, disruption of mitochondrial function, cell cycle arrest, and apoptotic cell death (Khodarev et al. 2001; Amundson et al. 2003;

Amundson and Fornace 2003; Criswell et al. 2003; Fei and El-Deiry 2003; Iliakis et al. 2003; Powell and Kachnic 2003; Andreev et al. 2006; Jeggo and Lobrich 2006; Rodemann and Blaese 2007; Valerie et al. 2007). Moreover, IR is a potent DNA damaging agent capable of producing DNA damage such as cross-linking, nucleotide base damage, and single- and double-strand breaks (Little 2000; Huang et al. 2003). The accumulation of DNA damage caused by IR in conjunction with disrupted cellular regulation processes can lead to cancer (Little 2000).

5.3 Indirect Effects of Radiation Exposure: Genome Instability and Bystander Effects

Historically, the central dogma of radiation biology stated that the effects of IR were restricted to the directly hit cells. This paradigm has been challenged by numerous observations in which cells that were not directly traversed by the IR exhibited responses similar to those of the directly irradiated cells. These responses were demonstrated in the cells that were descendents of the directly irradiated cells and were postulated to occur because of a phenomenon called *radiation-induced genome instability* (Morgan 2003a, b; Nagar et al. 2003; Morgan and Sowa 2005, 2007, 2009). Genomic instability is characterized by an increased acquisition of alterations in the genome. It manifests itself as an induction of chromosomal aberrations, aneuploidy, micronuclei, gene mutations and amplifications, microsatellite instability, and cell death (Huang et al. 2003; Morgan 2003a, b; Nagar et al. 2003; Suzuki et al. 2003). IR-induced genomic instability occurs in the irradiated cell at extended times after irradiation and even in the progeny of the irradiated cell generations after exposure (Morgan et al. 1996; Wright 1998; Little 2000). There are many signaling pathways involved in the initiation and perpetuation of genomic instability (Kaplan et al. 1997). Furthermore, the relative contribution of the different pathways depends upon the genetic background of the irradiated cell or organism (Paquette and Little 1994). It has long been proposed that genomic instability also plays a significant role in tumorigenesis (Goldberg 2003; Little 2003).

Radiation responses also occur in the naïve bystander cells that were in contact with irradiated cells or received signals from the directly irradiated cells. These *bystander effects* challenge the classic radiation biology dogma (Morgan et al. 2002; Mothersill and Seymour 2003, 2004, 2006; Morgan and Sowa 2005). IR-induced bystander effects encompass a number of different endpoints. Some, but not all, of these are detrimental to the cell. Similar to genomic instability, bystander effects are measured by the induction of gross genome rearrangements, chromosome aberrations, sister chromatid exchanges, deletions, duplications, mutations and amplifications, and cell death (Zhou et al. 2000; Lorimore et al. 2001, 2005; Zhou et al. 2002a, b; Klokov et al. 2004; Hamada et al. 2007; Han et al. 2007). Interestingly, bystander effects are also observed following cytoplasmic irradiation,

demonstrating that the target for genetic events is not just the nucleus (Folkard et al. 1997; Wu et al. 1999; Randers-Pehrson et al. 2001). They were seen in cells that were not traversed by radiation but were in the same environment as the irradiated cell. These bystanders received signals from irradiated cells that generated a response in the bystander cells. Evidence suggests that bystander effects are communicated between cells by means of either gap junctions or transmission of soluble factors between the irradiated cell and the nonirradiated cell through cell culture medium. Among the candidate soluble factors are reactive oxygen species and cytokines (Morgan 2003a, b; Nagar et al. 2003; Kovalchuk and Baulch 2008). Responses seen in nonirradiated bystander cells include cell death, neoplastic transformation, and genomic instability (Zhou et al. 2000, 2002a, b; Lorimore et al. 2001, 2005; Suzuki et al. 2003; Azzam and Little 2004; Gaugler et al. 2007; Han et al. 2007; Maguire et al. 2007). Bystander effects are also observed in three-dimensional tissue models, including spheroids (Persaud et al. 2005, 2007), and reconstructed human tissue models (Belyakov et al. 2005; Sedelnikova et al. 2007; Kovalchuk et al. 2010). Thus, bystander effects are a ubiquitous consequence of radiation exposure (Mothersill and Seymour 2004).

Bystander effects also manifest themselves in the whole-organism context; however, conclusive evidence in vivo is relatively scarce (Goldberg and Lehnert 2002; Hall 2003; Koturbash et al. 2007; Mothersill et al. 2007; Kovalchuk and Baulch 2008). Radiation exposure leads to clastogenic activity in the plasma of the patients receiving high-dose radiotherapy and in individuals accidentally exposed to radiation (Goh and Sumner 1968; Pant and Kamada 1977; Emerit et al. 1994, 1995; Marozik et al. 2007; Kovalchuk and Baulch 2008; Ilnytskyy and Kovalchuk 2011). Moreover, these factors are capable of inducing chromosome damage in the cultured cells (Goh and Sumner 1968; Hollowell and Littlefield 1968; Pant and Kamada 1977; Emerit et al. 1994, 1995; Marozik et al. 2007).

Bystander effects also occur within an exposed organ. When the lung base is irradiated, significant molecular and cellular damage occurs in the shielded lung apex (Khan et al. 1998, 2003). The same group also showed that exposure of one lung, either right or left, results in a marked increase of micronuclei in the unexposed, shielded lung (Khan et al. 1998, 2003). Similar within-the-organ bystander effects are observed during partial liver irradiation (Brooks et al. 1974; Brooks 2004). The existence of the somatic bystander effects has been confirmed through the use of rodent skin and spleen models in which one part of the animal body is exposed to IR, while the other part is protected by a medical grade shield (Koturbash et al. 2006b, 2007, 2008a, b; Ilnytskyy et al. 2009; Ilnytskyy and Kovalchuk 2011). These studies show that X-ray exposure of one side of the animal causes profound changes in the unexposed bystander portion of the body.

5.4 Transgenerational Effects of Radiation Exposure

The effects of radiation exposure can also span several generations in animals and humans. Evidence to support the hypothesis for germline heritable effects in humans exposed to ionizing radiation is subject to much debate. In contrast, the data from animal models clearly demonstrate that the biological effects of parental radiation exposure are transmitted to the progeny through the germline of the irradiated parent (Morgan 2003a). Early studies of these *transgenerational effects* used various tests such as the specific locus, dominant lethal, and heritable translocation assays (Generoso et al. 1980; Russell and Kelly 1982; Green et al. 1987; Russell et al. 1998). Other studies focused on heritable alterations in cancer incidence and teratogenesis following parental preconception irradiation (Nomura 1988; Tomatis 1994; Mohr et al. 1999; Nomura 2003; Nomura et al. 2004; Dasenbrock et al. 2005). It is now also apparent that the risks to the progeny of irradiated parents include transgenerational genomic instability (Tomatis 1994; Carls and Schiestl 1999; Mohr et al. 1999; Barber et al. 2002; Dubrova 2003a, b; Niwa 2003; Nomura 2003; Nomura et al. 2004; Dasenbrock et al. 2005; Barber and Dubrova 2006; Kovalchuk and Baulch 2008; Ilnytskyy and Kovalchuk 2011).

While the exact molecular mechanisms of transgenerational IR-induced genome instability have yet to be adequately defined, evidence points to an epigenetic basis for this phenomena (Morgan 2003c; Jirtle and Skinner 2007; Kovalchuk and Baulch 2008; Ilnytskyy and Kovalchuk 2011). Specifically, several independent studies demonstrate that the IR-induced cellular reprogramming persists for multiple generations. Wiley and colleagues used a mouse preimplantation embryo chimera assay to demonstrate heritable effects of paternal irradiation on embryonic cell proliferation that persisted for two generations with no decrease in the incidence or severity of the effect between generations (Wiley et al. 1997; Baulch et al. 2001; Vance et al. 2002; Baulch and Raabe 2005).

The mechanism(s) that underlies the observed persistent transgenerational gene expression phenotype is termed *epigenomic instability* (Baulch and Raabe 2005). Studies using the neutral pH sperm comet assay show effects on the DNA electrophoretic mobility of sperm from irradiated male mice 7 weeks postexposure (Anderson and Brinkworth 2007; Baulch et al. 2007; Kovalchuk and Baulch 2008). This same assay also demonstrated DNA damage and changes in chromatin conformation in the unexposed offspring of irradiated male mice (Anderson and Brinkworth 2007; Baulch et al. 2007).

Dubrova and colleagues pioneered the analysis of transgeneration instability of repeat sequences in the genome (Dubrova et al. 1993). Initially, these repeat sequences were referred to as minisatellites but later were called expanded simple tandem repeat (ESTR) loci. ESTR analysis was used to demonstrate the induction of heritable genomic instability in the progeny of irradiated mice (Dubrova et al. 1993; Barber et al. 2002; Yauk et al. 2002) and in human populations, including individuals initially exposed in the Chernobyl accident, the Chernobyl cleanup

workers, and people living around the Semipalatinsk nuclear test site (Dubrova et al. 1996, 1997, 2002).

A great deal of data attests to the existence and manifestation of genomic instability and the bystander effect in cultured cells, 3D models of tissues, organs, and organisms. Unfortunately, the mechanisms responsible for the bystander effect in these model systems remain relatively unexplored. The high frequency of induction and the transgenerational persistence of the bystander responses, however, suggest an epigenetic genesis of this phenomenon (Lorimore et al. 2003; Morgan 2003a, b; Nagar et al. 2003; Kaup et al. 2006; Kovalchuk and Baulch 2008; Ilnytskyy and Kovalchuk 2011).

5.5 Epigenetic Changes and Their Roles in the Cell

Epigenetic changes are mitotically stable and potentially meiotically heritable alterations in gene expression. They include DNA methylation, histone modification, and RNA-associated silencing (Jaenisch and Bird 2003). Cytosine DNA methylation was the first epigenetic alteration identified and is the most widely studied of the known epigenetic mechanisms. It is crucial for normal development, cell proliferation, and the proper maintenance of genome stability in an organism (Robertson and Wolffe 2000; Scarano et al. 2005; Klose and Bird 2006; Shames et al. 2007). In mammals, DNA methylation occurs mostly in the context of CG dinucleotides that are methylated normally from 60 % to 80 % (Weber and Schubeler 2007). The process of DNA methylation is mediated by the DNA methyltransferase enzymes, DNMT1, DNMT3a, and DNMT3b (Robertson 2001; Rountree et al. 2001; Goll and Bestor 2005; Weber et al. 2005, 2007; Wilson et al. 2006). DNMT1 is the major enzyme involved in the maintenance of the pattern of DNA methylation after DNA replication (Liang et al. 2002). DNMT3A and DNMT3B are de novo methyltransferases targeting unmethylated and hemimethylated sites (Okano et al. 1999; Goll and Bestor 2005; Weber and Schubeler 2007). The association of DNA methylation with transcriptional repression is mediated by the MBD (methyl CpG-binding domain) proteins (MeCP2, MBD1, MBD2, and MBD3) (Klose and Bird 2006). These proteins selectively interact with methylated DNA (Robertson and Wolffe 2000; Robertson 2002; Hendrich and Tweedie 2003; Jaenisch and Bird 2003; Klose and Bird 2006). The regulatory potential of DNA methylation manifests in promoter regions that control the expression of adjacent genes. Hypermethylated promoters lead to an “off” state of expression, while those less methylated are deemed as “on” (Jaenisch and Bird 2003). Moreover, methylated cytosines themselves can physically prevent the proper binding of transcription factors to promoter regions (Weber and Schubeler 2007).

DNA methylation changes are closely connected with alterations in the other components of chromatin structure, primarily histone modifications. In this instance, however, there are in excess of 50 posttranslational modifications that

may occur at key amino acid residues within histones of the nucleosome. Histone modifications encompassing acetylation, methylation, phosphorylation, ubiquitination, and sumoylation are important in maintaining transcriptional regulation and genome stability (Jenuwein and Allis 2001; Weidman et al. 2007).

For example, acetylation of several lysine residues within the N-terminal tail of H3 and H4 gives rise to an open or "on" chromatin state typically associated with DNA hypomethylation and expressed genes, while deacetylation tends to coincide with hypermethylated DNA loci that are not expressed (Munshi et al. 2009). Histone methylation can lead to different transcriptional consequences based on the residue affected (Jenuwein and Allis 2001; Cheung and Lau 2005). Moreover, histone residues can be mono-, di-, and trimethylated, leading to an enormous complexity in the mostly uncharted histone code (Cheung and Lau 2005; Saha et al. 2006; Weidman et al. 2007; He et al. 2008).

In addition, epigenetic control can be mediated by the recently discovered small regulatory RNAs. Among those of special interest are the microRNAs (miRNAs) that can inhibit the translation of a variety of proteins. MicroRNAs are abundant, small, single-stranded noncoding RNAs that are potent regulators of gene expression (Hwang and Mendell 2006; Sevignani et al. 2006). To control the translation of their target mRNAs, miRNAs associate with the RNA-induced silencing complex (RISC) proteins and bind to the 3′UTR of their cognate mRNAs. Thus, they serve as translational suppressors that regulate protein synthesis (Hutvagner and Zamore 2002a, b). Regulatory microRNAs impact a wide variety of cellular processes, such as cellular differentiation, proliferation, apoptosis, genome stability, and even predisposition to cancer (Chang and Mendell 2007; Fabbri et al. 2007b).

5.6 Epigenetic Changes in the Directly Exposed Tissue

Direct radiation exposure strongly influences epigenetic effectors. DNA damaging agents including ionizing radiation have been reported to affect DNA methylation patterns (Kalinich et al. 1989; Tawa et al. 1998; Minamoto et al. 1999; Kovalchuk et al. 2004). Acute exposures to low LET X-rays or γ-rays were noted to result in global hypomethylation (Kalinich et al. 1989; Tawa et al. 1998). It was recently shown that the IR exposure leads to the profound dose-dependent and sex- and tissue-specific global DNA hypomethylation (Pogribny et al. 2004; Raiche et al. 2004; Koturbash et al. 2005; Loree et al. 2006). The IR exposure also affects methylation of the promoter of the p16 tumor suppressor in a sex- and tissue-specific manner (Kovalchuk et al. 2004). The DNA hypomethylation observed after irradiation was related to DNA repair (Pogribny et al. 2004). It also correlated with the radiation-induced alterations in the expression of DNA methyltransferases, especially de novo methyltransferases, DNMT3a and DNMT3b (Raiche et al. 2004; Pogribny et al. 2005). Most importantly, the radiation-induced global genome DNA hypomethylation appears to be linked to genome instability in the exposed tissue (Pogribny et al. 2004, 2005; Raiche et al. 2004; Loree et al. 2006).

Although much attention has been given to the IR-induced changes in DNA methylation, histones have been largely overlooked. Among the histone modifications that change upon radiation exposure, phosphorylation of histone H2AX has been studied most intensively. Histone H2AX, a variant of histone H2A, is rapidly phosphorylated at Ser139 upon the induction of DNA strand breaks by irradiation, and it can be visualized effectively within repair foci using phospho-specific antibodies (Sedelnikova et al. 2003; Pogribny et al. 2005). Animal studies demonstrate that changes also in histone methylation, specifically with the loss of histone H4 lysine trimethylation, also correlate with IR-induced global loss of DNA methylation (Pogribny et al. 2005). Furthermore, histone protein modification may influence the radiation sensitivity of the tissues (Lee et al. 2012).

IR also results in significant changes to the microRNAome. Recent research efforts have led to the identification of over 150 potential IR-responsive miRNAs (Weidhaas et al. 2007; Maes et al. 2008; Cha et al. 2009a, b; Simone et al. 2009; Wagner-Ecker et al. 2010; Templin et al. 2011). MicroRNAome changes are present in exposed animal tissues, as well as in human cells and 3D models of human tissue. These changes are detected as early as several hours after exposure to IR (Ilnytskyy et al. 2008; Koturbash et al. 2008c; Ilnytskyy et al. 2009) and persist for days, weeks (Koturbash et al. 2008c; Tamminga et al. 2008a; Ilnytskyy et al. 2009), and even months (Koturbash et al. 2007). Analysis of miRNA profiles in different tissues after IR exposure delineates tissue-dependent and sex-specific changes in miRNA regulation (Koturbash et al. 2007, 2008c; Ilnytskyy et al. 2008; Tamminga et al. 2008a).

Interestingly, very profound effects of IR exposure on miRNAome were seen in IR-sensitive hematopoietic tissues, spleen, and thymus in a sex-specific manner (Ilnytskyy et al. 2008). Among the regulated miRNAs, the most prominent changes were seen in the expression of miR-34a and miR-7. These miRNAs may be involved in important protective mechanisms counteracting radiation cytotoxicity (Ji et al. 2008; Tahara et al. 2010). IR exposure leads to a significant increase in the expression of the tumor-suppressor miRNA, miR-34a, paralleled by a decrease in the expression of its target oncogenes, *NOTCH1*, *MYC*, *E2F3*, and *CYCLIN D1*. MiR-7 targets the lymphoid-specific helicase LSH, a pivotal regulator of DNA methylation and genome stability. While miR-7 is significantly downregulated in response to radiation exposure, LSH is upregulated. Taken together, these miRNAome changes may constitute a cellular protective effect and an attempt of the cell to counteract radiation-induced DNA hypomethylation (Ilnytskyy et al. 2008).

Analysis of miRNA expression in the testes of whole-body irradiated mice reveals that a large number of miRNAs are significantly changed following IR exposure; the majority of which are upregulated (Tamminga et al. 2008a). MiR-709 is highly expressed in control testes, but its expression is significantly increased following irradiation. This miRNA is controlled through the DNA damage response ATR/Rfx1 pathway that controls the expression of Brother of the Regulator of Imprinted Sites (BORIS). This is a testes-specific gene that directs epigenetic reprogramming and DNA methylation during male germ cell differentiation.

IR-induced changes in miR-709 levels are associated with altered levels of BORIS and DNA methylation in exposed murine testes. Studies indicate that an increase in miR-709 expression in irradiated testes may elicit a protective effect through decreasing the cellular level of BORIS, thereby preventing aberrant erasure of DNA methylation (Tamminga et al. 2008a).

The effect of X-ray irradiation on microRNA expression in the hippocampus, frontal cortex, and cerebellum of male and female mice also revealed a sex- and brain region-specific expression pattern of miRNAs (Ilnytskyy et al. 2009; Koturbash et al. 2010a). The most pronounced changes in response to IR were observed in the frontal cortex. In total, 38 miRNAs were found to be deregulated in females and 13 in males. Most interestingly, miRNAs of the miR-29 family, miR-29a and miR-29c, are exclusively downregulated in the frontal cortex tissue of exposed female mice. This family of miRNAs is involved in several very important processes, including the establishment of DNA methylation patterns by regulating the expression of the de novo DNA methyltransferases, DNMT3a and DNMT3b (Fabbri et al. 2007a). Protein analysis reveals increased levels of DNMT3a in the female frontal cortex of female mice. This correlation suggests that increased DNMT3a levels may result from IR-induced downregulation of the miR-29 family of miRNA in the frontal cortex of female mice. Therefore, the miR-29-mediated increase of DNMT3a in the female frontal cortex may be a protective mechanism involved in restoring and stabilizing the levels of global genomic methylation after IR exposure.

Overall, IR-induced microRNAome alterations are detectable as early as several hours after exposure (Ilnytskyy et al. 2008, 2009; Koturbash et al. 2008c), and they can persists for months (Koturbash et al. 2007). MiRNA changes may have a protective effect after IR exposure, yet more studies are needed to determine the roles of miRNAs in whole-body IR responses. The exact roles of miRNAs in IR-induced carcinogenesis also need to be delineated (Koturbash et al. 2010b).

5.7 Epigenetic Determinants of the Indirect Radiation Effects: Bystander Effect

Although a significant body of evidence points toward the epigenetic nature of the IR-induced bystander and transgenerational effects, until recently, very few studies have investigated the epigenetic changes involved in these indirect radiation responses. The pioneering work of Kaup and colleagues showed that DNA methylation is important for the maintenance of the IR-induced bystander effect in cultured cells. Using cultured human keratinocytes, they demonstrated that the dysregulation of DNA methylation in naïve cells exposed to the medium from the irradiated cells persists for 20 passages. Over a similar period of culture and under the similar culture conditions, these cells have also exhibited increased and

persistent levels of chromosome and chromatid aberrations, reproductive cell death, apoptosis, and other signs of genome instability (Kaup et al. 2006).

Epigenetic changes have been shown to be important in both tissue- and organism-based bystander effect models. The study by Sedelnikova and colleagues examined bystander effects in two reconstructed human 3D tissue models and in airway tissue and full-thickness skin (Sedelnikova et al. 2007). Following the microbeam irradiation of cells located in a thin plane through these biological samples, a variety of biological endpoints were analyzed in distal bystander cells (i.e., up to 2.5 mm away from the irradiated cell plane) as a function of postexposure time (i.e., 0 h to 7 days). In this study, a significant increase in the levels of phosphorylated H2AX was found in bystander tissues, a histone for which bystander effects were previously demonstrated in culture (Sokolov et al. 2005; Smilenov et al. 2006; Burdak-Rothkamm et al. 2007; Yang et al. 2007). Furthermore, extensive long-term increases in apoptosis and micronucleus formation, as well as the loss of nuclear DNA methylation, persistent growth arrest, and the increasing number of senescent cells were observed. Of special interest is the loss of DNA methylation in bystander cells. DNA methylation is an important epigenetic phenomenon involved in the regulation of gene expression and genome stability. Since changes in DNA methylation are linked to other epigenetic effectors, the observed alteration of DNA methylation in bystander cells may be indicative of an epigenetic basis of the bystander effect in a 3D model of human tissue (Sedelnikova et al. 2007).

Further analysis of bystander effects in a 3D model of human tissue revealed that IR leads to a deregulation of miRNA expression in bystander tissues (Fig. 5.1). Indeed, the major bystander end points, including apoptosis, cell cycle deregulation, and DNA hypomethylation, are mediated by altered expression of miRNAs. Specifically, c-MYC-mediated upregulation of the miR-17 family seen in bystander tissues was associated with decreased levels of E2F1 and RB1, indicating a switch to a proliferative state, while priming bystander cells for imminent death signals. Upregulation of the miR-29 family of microRNAs resulted in decreased levels of their targets DNMT3a and MCL1 proteins, thus influencing DNA methylation and apoptosis in bystander tissues. Altered expression of miR-16 also leads to changes in expression of BCL2, indicating modulation of apoptosis in bystander cells. These data clearly suggest that miRNAs play a profound role in the manifestation of IR-induced bystander effect endpoints (Fig. 5.2) (Kovalchuk et al. 2010; Dickey et al. 2011b).

Based on their size, stability, and potent regulatory roles in the cells, microRNAs appear to serve as a primary signaling mechanism for bystander effect propagation. Yet, no major differences in the levels of IR-induced γ-H2AX foci were found in matched human colon carcinoma cell lines with wild-type or depleted levels of mature miRNAs. Thus, even though miRNAs play a role in bystander effect manifestation, they appear not to be the primary bystander signaling molecules in the formation of bystander effect-induced DSBs (Dickey et al. 2011a, b).

A very significant insight into the role of epigenetic changes in bystander effects comes from the animal-based studies, where IR exposure induces DNA damage and

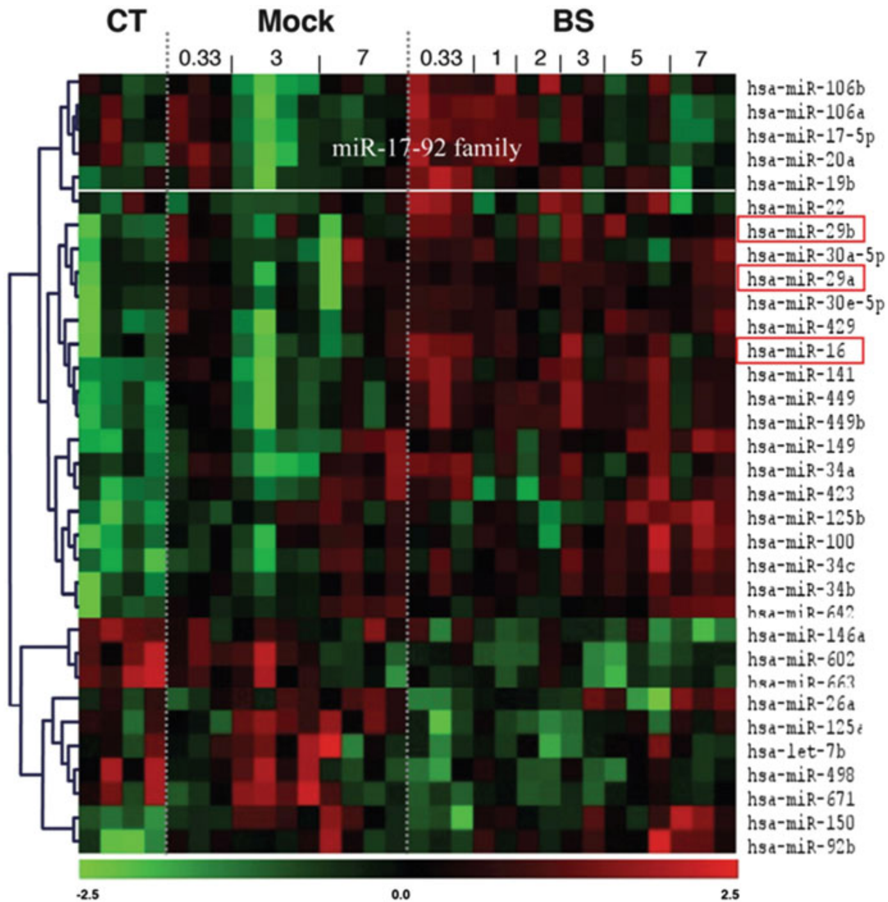

Fig. 5.1 Heat map of ANOVA ($p<0.05$) gene expression analysis following radiation treatments. *Vertical columns* denote days after irradiation for the mock treatment (*Mock*) and the bystander tissues (*BS*), unirradiated control samples (*CT*). The *white box* includes co-regulated members of the miR-17-92 family. The *red boxes* highlight important miRNAs discussed in the article. Each column represents an independent biological replicate. *Red denotes* high gene expression, whereas *green* depicts low gene expression (Adapted with permission from Kovalchuk et al. (2010))

modulates the epigenetic effectors in distant bystander tissues. The Kovalchuk and Engelward laboratories pioneered studies on the role of epigenetic changes in IR-induced bystander effects in vivo. To analyze in vivo bystander effects, they developed a mouse model whereby half of an animal body was exposed to radiation, while the other half was protected by a medical grade shield (Koturbash et al. 2006b) (Fig. 5.3). Alternatively, the animal head can be exposed while animal body is shielded by lead. This model system is used to monitor the induction and repair of DNA strand breaks in the cutaneous tissue. In addition to this well-established endpoint, epigenetic mechanisms (i.e., DNA methylation and alterations in DNA

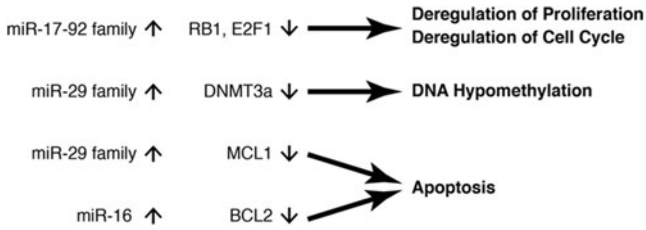

Fig. 5.2 MiRNAs involved in the regulation of bystander effects through the targeting of critical protein regulators (Adapted with permission from Kovalchuk et al. (2010))

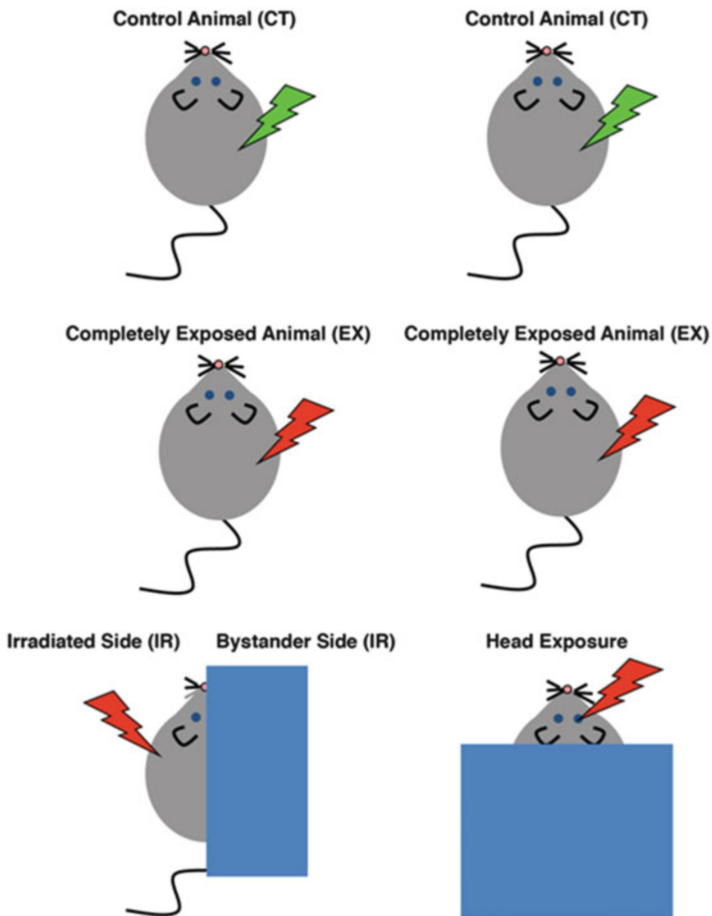

Fig. 5.3 Rodent models to study IR-induced bystander effects. Radiation shield, *blue rectangle*; *green lightning bolt*, sham irradiated; *red lightning bolt*, irradiated

methyltransferases and methyl-binding proteins) were investigated in the generation and/or maintenance of an IR-induced bystander effect in cutaneous tissue. They showed that radiation exposure of half the body leads to elevated levels of DNA strand breaks and altered levels of key proteins that modulate methylation patterns and silencing in the unirradiated bystander half of the body. These are some of the first findings to clearly demonstrate that the epigenetic alterations are involved in the bystander effects occurring in vivo (Koturbash et al. 2006b).

To be relevant for carcinogenesis, the epigenetic manifestations of bystander effects should accumulate and/or persist over a long period of time. To investigate the possibility that localized X-ray irradiation induces persistent epigenetically modulated bystander effects in distant tissues, Koturbash and colleagues monitored the occurrence of epigenetic changes (i.e., DNA methylation, histone methylation, and miRNA expression) in spleen tissue 7 months after localized cranial irradiation. This analysis revealed that localized cranial exposure causes decreased levels of global DNA methylation. It also alters the levels of key proteins that modulate methylation patterns and silencing (i.e., de novo methyltransferase DNMT3a and methyl-binding protein MeCP2) and contributes to the reactivation of the LINE1 retrotransposon in the bystander spleen. This study also provided the first evidence that downregulation of DNMT3a and MeCP2 is triggered and maintained by *miR-194* expression in the bystander rat spleen. The use of a microarray platform also showed that radiation exposure increased MiR-194 expression in bystander spleen, while miR-148a was changed in bystander skin (Ilnytskyy et al. 2009; Dickey et al. 2011b). These data clearly demonstrate that the bystander effect occurs in vivo in distant tissue, persists over a long period of time, and is epigenetically regulated (Koturbash et al. 2007; Dickey et al. 2011b).

5.8 Epigenetic Determinants of the Indirect Radiation Effects: Transgenerational Effects

A wealth of evidence has accumulated on the nature of transgenerational changes in the somatic tissues of the progeny of IR-exposed parents (Nomura 2003; Barber and Dubrova 2006). There is evidence that genome instability and elevated mutation rates in the progeny of exposed parents exposed to chemical agents occur because of alterations in the epigenome (Jirtle and Skinner 2007). Nevertheless, the molecular mechanisms leading to IR-induced transgenerational genome instability and carcinogenesis remain elusive.

The first direct evidence for epigenetic effector involvement in transgenerational responses came from the study by Koturbash and colleagues (Koturbash et al. 2006a). They utilized an in vivo mouse model to analyze the role of epigenetic parameters in transgenerational radiation effects. A significant loss of DNA methylation is observed in the thymus of offspring upon paternal and combined parental exposure. The DNA methylation changes are correlated with alterations in the

levels of DNA methyltransferases and methyl-binding proteins. Specifically, DNMT1 expression is dramatically decreased in the thymus tissue of the progeny of exposed males and the progeny with combined paternal and maternal exposure. The levels of DNMT3a and DNMT3b are also significantly downregulated in the progeny of exposed males and in the combined parental exposure group. The decrease in global cytosine DNA methylation and DNMT levels observed in the thymus of the progeny upon paternal and combined parental irradiation is also correlated with a significant decrease in the level of methyl-binding protein MeCP2. This protein selectively recognizes methylated DNA. It is important for methylation-mediated gene silencing and chromatin remodeling and is implicated in carcinogenesis (Jaenisch and Bird 2003; Koturbash et al. 2006a). Mammalian genomes heavily depend upon properly set patterns of methylated cytosines for their function. The global loss of DNA methylation and altered levels of DNMT1, DNMT3a, or DNMT3b can lead to the activation of transposable elements contributing to genome instability (Jirtle and Skinner 2007). Therefore, it is suggested that the global loss of DNA methylation observed in the progeny of irradiated parents may influence retrotransposons and satellite DNA, thus underlying transgenerational genome instability. Importantly, this postulate helps explain satellite DNA instability in the progeny of exposed parents. Parental irradiation also results in significantly increased levels of phosphorylated histone H2AX in the thymus of progeny of exposed males and both exposed parents. The observed accumulation of phosphorylated H2AX is an important epigenetic alteration, and it may be an early sign of predisposition to carcinogenesis (Sedelnikova and Bonner 2006; Bonner et al. 2008).

Histone modifications (e.g., phosphorylation of histone H2AX) also play an important role in the transgenerational radiation-induced changes. Barber and colleagues investigated the inheritance of transgenerational mutation rates and DNA damage in the germline and somatic tissues of the first generation offspring of the irradiated inbred mice from two different mouse strains (Barber et al. 2006). They observed marked elevation in transgenerational mutation rates. This finding was attributed to persistently elevated levels of phosphorylated histone, H2AX. This underscores the importance of H2AX in the indirect effects of radiation and IR-induced genome instability. Furthermore, this study suggests the intriguing possibility that the persistence of elevated mutation rates in the tissues of the offspring may be due to epigenetic inheritance of instability signals through the male germline.

DNA methylation is also proposed to be a plausible candidate for an epigenetic signal that leads to transgenerational mutagenesis. Altered DNA methylation levels in sperm may profoundly affect the fertilized embryo (Aitken and De Iuliis 2007). Methylation can be transmitted over many cell divisions. Additionally, it can result in the long-term gene expression changes by affecting genes responsible for the genome stability maintenance. The recent study by Hatch and colleagues has reinforced the conclusion that DNA methylation plays an important role in the transgenerational changes in mutation and recombination rates in the progeny of exposed animals (Hatch et al. 2007).

The majority of current research efforts on transgenerational IR effects have focused on the role of whole-body exposure, yet such exposure is relatively rare. In contrast, localized body-part exposures occur very frequently during processes such as radiation diagnostics and therapy. Recent research shows that localized cranial exposure leads to DNA damage and epigenetic changes in the germline and also to transgenerational epigenetic changes in the progeny (Tamminga et al. 2008b). In this study, an in vivo rat model revealed that cranial paternal irradiation results in a profound accumulation of DNA damage and global loss of DNA methylation in the germline even though the gonads are shielded. These changes further lead to deleterious molecular effects in the unexposed progeny. Specifically, it results in a pronounced loss of DNA methylation in the bone marrow, thymus, and spleen of the offspring, with loss of DNA methylation being most pronounced in the bone marrow.

To fully understand the nature and mechanisms of transgenerational genome instability, it is important to establish what happens in the germline of exposed parents as well as in the progeny. Filkowski and colleagues recently investigated the mechanisms underlying the disruption of DNA methylation and miRNA expression status in the germline and progeny of exposed parents (Filkowski et al. 2010). They established that paternal irradiation leads to deregulation of microRNAome in the exposed germline. IR exposure caused upregulation of the miR-29 family in the exposed male germline and led to decreased expression of de novo methyltransferase, *DNMT3a*, and profound hypomethylation of transposable LINE1 and SINE B2 sequences. Therefore, radiation-induced hypomethylation in the male germline can be explained in part by the IR-induced alteration in *miR-29* expression.

Interestingly, DNA methylation and microRNAome changes observed in the male germline are likewise associated with molecular effects in the somatic thymus tissue of the progeny of exposed animals, including hypomethylation of LINE1 and SINE B2 and a significant decrease in the expression of LSH. LSH is an important regulatory protein reported to be crucial for maintaining DNA methylation and silencing of repetitive elements by cooperating with DNMTs.

Furthermore, paternal irradiation results in microRNAome changes in the progeny (Fig. 5.4) (Filkowski et al. 2010). Expression of miR-468 is significantly increased and significantly decreases the LSH level in the thymus of the progeny of exposed parents. This study further suggests that miR-468-mediated suppression of LSH causes aberrant methylation of LINE1 and SINE B2 in the thymus of the progeny of exposed parents. Thus, altered microRNAome levels, global DNA hypomethylation, and reactivation of retroelements all constitute deleterious effects that may significantly influence genome stability and therefore lead to carcinogenesis.

It is important to note that epigenetic changes in the progeny are linked to male parent exposure. The paternal genome is extremely sensitive to the exposure to genotoxic agents. Moreover, the mammalian genomes undergo marked methylation reprogramming after fertilization in order to establish correct parent-of-origin developmental programs (Jirtle and Skinner 2007). The epigenetic changes

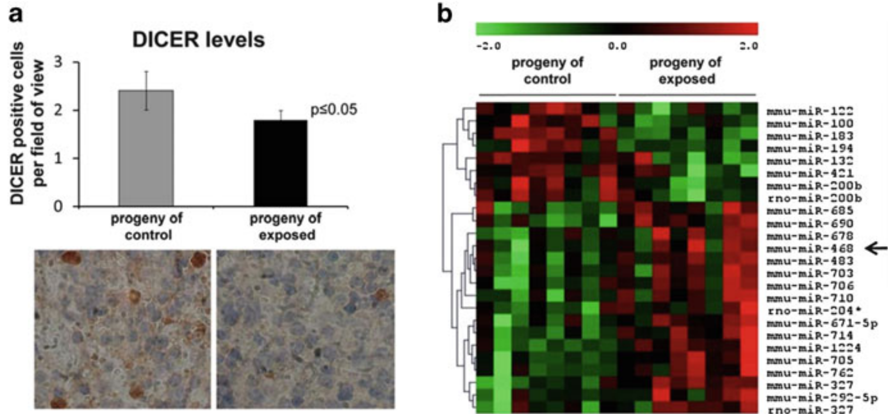

Fig. 5.4 Paternal radiation exposure and microRNAome deregulation in the thymus of unexposed progeny (**a**) Decreased levels of Dicer in the thymus tissue of the progeny of exposed animals as determined by immunohistochemistry. Levels of Dicer-positive cells per field of view are shown, mean values ± SEM, $n = 10$ fields per animals, 40 cells per field, *$p<0.05$, student's t-test. (**b**) Hierarchical clustering of differentially expressed *miRNA* genes in the thymus tissue of the progeny of control and IR-exposed mice. *Red* denotes high gene expression, whereas *green* depicts low gene expression (Adapted with permission from Filkowski et al. (2010))

observed in irradiated sperm may interfere with postfertilization epigenetic reprogramming, leading to subsequent detrimental changes in the developing embryo. Interestingly, some studies reported that transgenerational genome instability is more pronounced in mice than in humans. The recent data reported by Filkowski and colleagues may shed light on this apparent species difference in the magnitude of transgenerational effects (Filkowski et al. 2010). Their analysis of miRNA and genome databases shows that miR-468 is a mouse-specific miRNA. While the 3′-UTR of the human and mouse *LSH* genes are 98 % identical and the miR-468-binding site is preserved in both species, functional miR-468 does not exist in the human genome. The lack of miR-468 may explain in part why transgenerational genome instability is less pronounced in humans. It is clear that further in-depth studies are needed to understand the roles of miRNAs in germline and transgenerational effects.

In conclusion, the function of small regulatory RNAs in the genesis of transgenerational effects resulting from IR exposure needs to be further investigated. Small RNAs, and especially microRNAs, may constitute an epigenetic signal that can be inherited through the male germline and influence the chromatin packaging and gene expression in the fertilized egg. Another group of short RNAs are the 29- to 30-nucleotide-long RNAs that form complexes with Piwi proteins (piRNAs) (Aravin et al. 2007a, b; Brennecke et al. 2007; Chu and Rana 2007; O'Donnell and Boeke 2007; Yin and Lin 2007; Aravin and Hannon 2008; Assis and Kondrashov 2009; Reuter et al. 2009; Wang et al. 2009). This novel class of small RNA molecules, discovered in 2007, is expressed in mammalian germline.

PiRNA-Piwi complexes are linked to the silencing of retrotransposons and other germline genetic elements (O'Donnell and Boeke 2007; Aravin et al. 2008; Brennecke et al. 2008; Kuramochi-Miyagawa et al. 2008). Therefore, they may also be important regulators of transgenerational effects, but their role in transgenerational genome instability has yet to be defined (Ilnytskyy and Kovalchuk 2011).

5.9 Perspectives

Determining the role of epigenetic alterations in bystander effects, transgenerational effects, and radioadaptation is critically important since human exposure to radiation has significantly increased since the turn of the twentieth century because both environmental and medical exposures have increased. While radioadaptation at low radiation doses may be a positive effect, hypomethylation and increased genome instability observed at high doses are most likely deleterious outcomes. Thus, a better understanding of the molecular changes that occur in response to radiation should assist in improving radiation risk assessment and in the development of measures to ameliorate the deleterious effects of IR on the epigenome.

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References

Aitken J, De Iuliis GN (2007) Origins and consequences of DNA damage in male germ cells. Reprod Biomed Online 14:727–733

Amundson SA, Fornace AJ (2003) Monitoring human radiation exposure by gene expression profiling: possibilities and pitfalls. Health Phys 85:36–42

Amundson SA, Bittner M, Fornace AJ (2003) Functional genomics as a window on radiation stress signaling. Oncogene 22:5828–5833

Anderson D, Brinkworth MH (2007) Male-mediated developmental toxicity. RSC Publishing, Cambridge, UK

Andreev SG, Eidelman YA, Salnikov IV, Khvostunov IK (2006) Mechanistic modelling of genetic and epigenetic events in radiation carcinogenesis. Radiat Prot Dosim 122:335–339

Aravin AA, Hannon GJ (2008) Small RNA silencing pathways in germ and stem cells. Cold Spring Harb Symp Quant Biol 73:283–290

Aravin AA, Sachidanandam R, Girard A, Fejes-Toth K, Hannon GJ (2007a) Developmentally regulated piRNA clusters implicate MILI in transposon control. Science 316:744–747

Aravin AA, Hannon GJ, Brennecke J (2007b) The Piwi-piRNA pathway provides an adaptive defense in the transposon arms race. Science 318:761–764

Aravin AA, Sachidanandam R, Bourc'his D, Schaefer C, Pezic D, Toth KF, Bestor T, Hannon GJ (2008) A piRNA pathway primed by individual transposons is linked to de novo DNA methylation in mice. Mol Cell 31:785–799

Assis R, Kondrashov AS (2009) Rapid repetitive element-mediated expansion of piRNA clusters in mammalian evolution. Proc Natl Acad Sci USA 106:7079–7082

Azzam EI, Little JB (2004) The radiation-induced bystander effect: evidence and significance. Hum Exp Toxicol 23:61–65

Balonov MI (2007) The Chernobyl Forum: major findings and recommendations. J Environ Radioact 96:6–12

Barber RC, Dubrova YE (2006) The offspring of irradiated parents, are they stable? Mutat Res 598:50–60

Barber R, Plumb MA, Boulton E, Roux I, Dubrova YE (2002) Elevated mutation rates in the germ line of first- and second-generation offspring of irradiated male mice. Proc Natl Acad Sci USA 99:6877–6882

Barber RC, Hickenbotham P, Hatch T, Kelly D, Topchiy N, Almeida GM, Jones GD, Johnson GE, Parry JM, Rothkamm K, Dubrova YE (2006) Radiation-induced transgenerational alterations in genome stability and DNA damage. Oncogene 25:7336–7342

Baulch JE, Raabe OG (2005) Gamma irradiation of type B spermatogonia leads to heritable genomic instability in four generations of mice. Mutagenesis 20:337–343

Baulch JE, Raabe OG, Wiley LM (2001) Heritable effects of paternal irradiation in mice on signaling protein kinase activities in F3 offspring. Mutagenesis 16:17–23

Baulch JE, Li MW, Raabe OG (2007) Effect of ATM heterozygosity on heritable DNA damage in mice following paternal F0 germline irradiation. Mutat Res 616:34–45

Baverstock K (2000) Radiation-induced genomic instability: a paradigm-breaking phenomenon and its relevance to environmentally induced cancer. Mutat Res 454:89–109

Baverstock K, Williams D (2006) The Chernobyl accident 20 years on: an assessment of the health consequences and the international response. Environ Health Perspect 114:1312–1317

Baverstock K, Williams D (2007) The Chernobyl accident 20 years on: an assessment of the health consequences and the international response. Cien Saude Colet 12:689–698

Belyakov OV, Mitchell SA, Parikh D, Randers-Pehrson G, Marino SA, Amundson SA, Geard CR, Brenner DJ (2005) Biological effects in unirradiated human tissue induced by radiation damage up to 1 mm away. Proc Natl Acad Sci USA 102:14203–14208

Bogdanova TI, Zurnadzhy LY, Greenebaum E, McConnell RJ, Robbins J, Epstein OV, Olijnyk VA, Hatch M, Zablotska LB, Tronko MD (2006) A cohort study of thyroid cancer and other thyroid diseases after the Chernobyl accident: pathology analysis of thyroid cancer cases in Ukraine detected during the first screening (1998–2000). Cancer 107:2559–2566

Boice JD Jr, Harvey EB, Blettner M, Stovall M, Flannery JT (1992) Cancer in the contralateral breast after radiotherapy for breast-cancer. New Engl J Med 326:781–785

Bonner WM, Redon CE, Dickey JS, Nakamura AJ, Sedelnikova OA, Solier S, Pommier Y (2008) Gamma H2AX and cancer. Nat Rev Cancer 8:957–967

Brennecke J, Aravin AA, Stark A, Dus M, Kellis M, Sachidanandam R, Hannon GJ (2007) Discrete small RNA-generating loci as master regulators of transposon activity in drosophila. Cell 128:1089–1103

Brennecke J, Malone CD, Aravin AA, Sachidanandam R, Stark A, Hannon GJ (2008) An epigenetic role for maternally inherited piRNAs in transposon silencing. Science 322:1387–1392

Brenner DJ, Hall EJ (2004) Risk of cancer from diagnostic X-rays. Lancet 363:2192–2193

Brenner DJ, Curtis RE, Hall EJ, Ron E (2000) Second malignancies in prostate carcinoma patients after radiotherapy compared with surgery. Cancer 88:398–406

Brenner DJ, Hall EJ, Curtis RE, Ron E (2005) Prostate radiotherapy is associated with second cancers in many organs, not just the colorectum. Gastroenterology 129:773–774, author reply 774–775

Brooks AL (2004) Evidence for 'bystander effects' in vivo. Hum Exp Toxicol 23:67–70

Brooks AL, Retherford JC, McClellan RO (1974) Effect of 239PuO2 particle number and size on the frequency and distribution of chromosome aberrations in the liver of the Chinese hamster. Radiat Res 59:693–709

Burdak-Rothkamm S, Short SC, Folkard M, Rothkamm K, Prise KM (2007) ATR-dependent radiation-induced gamma H2AX foci in bystander primary human astrocytes and glioma cells. Oncogene 26:993–1002

Carls N, Schiestl RH (1999) Effect of ionizing radiation on transgenerational appearance of p(un) reversions in mice. Carcinogenesis 20:2351–2354

Carmichael A, Sami AS, Dixon JM (2003) Breast cancer risk among the survivors of atomic bomb and patients exposed to therapeutic ionising radiation. Eur J Surg Oncol 29:475–479

Cha HJ, Shin S, Yoo H, Lee EM, Bae S, Yang KH, Lee SJ, Park IC, Jin YW, An S (2009a) Identification of ionizing radiation-responsive microRNAs in the IM9 human B lymphoblastic cell line. Int J Oncol 34:1661–1668

Cha HJ, Shin S, Yoo H, Lee EM, Bae S, Yang KH, Lee SJ, Park IC, Jin YW, An S (2009b) Identification of specific microRNAs responding to low and high dose gamma-irradiation in the human lymphoblast line IM9. Oncol Rep 22:863–868

Chang TC, Mendell JT (2007) microRNAs in vertebrate physiology and human disease. Annu Rev Genomics Hum Genet 8:215–239

Chauveinc L, Giraud P, Dahnier S, Mounier N, Cosset JM (1998) Radiotherapy-induced solid tumors: review of the literature and risk assessment. Cancer Radiother 2:12–18

Cheung P, Lau P (2005) Epigenetic regulation by histone methylation and histone variants. Mol Endocrinol 19:563–573

Chu CY, Rana TM (2007) Small RNAs: regulators and guardians of the genome. J Cell Physiol 213:412–419

Copelan E, Hoshaw-Woodard S, Elder P, Penza S, Farag S, Marcucci G, Lin T, Ezzone S, Scholl MD, Bechtel T, Lemeshow S, Avalos B (2004) Therapy-related myelodysplasia and leukemia occur infrequently following VP-16 priming and autotransplantation without total body irradiation. Bone Marrow Transplant 34:85–87

Criswell T, Leskov K, Miyamoto S, Luo G, Boothman DA (2003) Transcription factors activated in mammalian cells after clinically relevant doses of ionizing radiation. Oncogene 22:5813–5827

Dasenbrock C, Tillmann T, Ernst H, Behnke W, Kellner R, Hagemann G, Kaever V, Kohler M, Rittinghausen S, Mohr U, Tomatis L (2005) Maternal effects and cancer risk in the progeny of mice exposed to X-rays before conception. Exp Toxicol Pathol 56:351–360

Dickey JS, Zemp FJ, Altamirano A, Sedelnikova OA, Bonner WM, Kovalchuk O (2011a) H2AX phosphorylation in response to DNA double-strand break formation during bystander signalling: effect of microrna knockdown. Radiat Prot Dosim 143:264–269

Dickey JS, Zemp FJ, Martin OA, Kovalchuk O (2011b) The role of miRNA in the direct and indirect effects of ionizing radiation. Radiat Environ Biophys 50:491–499

Dubrova YE (2003a) Germline mutation induction at mouse and human tandem repeat DNA loci. Adv Exp Med Biol 518:115–129

Dubrova YE (2003b) Radiation-induced transgenerational instability. Oncogene 22:7087–7093

Dubrova YE, Jeffreys AJ, Malashenko AM (1993) Mouse minisatellite mutations induced by ionizing radiation. Nat Genet 5:92–94

Dubrova YE, Nesterov VN, Krouchinsky NG, Ostapenko VA, Neumann R, Neil DL, Jeffreys AJ (1996) Human minisatellite mutation rate after the Chernobyl accident. Nature 380:683–686

Dubrova YE, Nesterov VN, Krouchinsky NG, Ostapenko VA, Vergnaud G, Giraudeau F, Buard J, Jeffreys AJ (1997) Further evidence for elevated human minisatellite mutation rate in Belarus eight years after the Chernobyl accident. Mutat Res 381:267–278

Dubrova YE, Grant G, Chumak AA, Stezhka VA, Karakasian AN (2002) Elevated minisatellite mutation rate in the post-Chernobyl families from Ukraine. Am J Hum Genet 71:801–809

Emerit I, Levy A, Cernjavski L, Arutyunyan R, Oganesyan N, Pogosian A, Mejlumian H, Sarkisian T, Gulkandanian M, Quastel M, Goldsmith J, Riklis E, Kordysh E, Poliak S, Merklin L (1994)

Transferable clastogenic activity in plasma from persons exposed as salvage personnel of the Chernobyl reactor. J Cancer Res Clin Oncol 120:558–561

Emerit I, Oganesian N, Sarkisian T, Arutyunyan R, Pogosian A, Asrian K, Levy A, Cernjavski L (1995) Clastogenic factors in the plasma of Chernobyl accident recovery workers – anticlastogenic effect of Ginkgo-biloba extract. Radiat Res 144:198–205

Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, Liu S, Alder H, Costinean S, Fernandez-Cymering C, Volinia S, Guler G, Morrison CD, Chan KK, Marcucci G, Calin GA, Huebner K, Croce CM (2007a) MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proc Natl Acad Sci USA 104:15805–15810

Fabbri M, Ivan M, Cimmino A, Negrini M, Calin GA (2007b) Regulatory mechanisms of microRNAs involvement in cancer. Expert Opin Biol Ther 7:1009–1019

Fei PW, El-Deiry WS (2003) P53 and radiation responses. Oncogene 22:5774–5783

Filkowski JN, Ilnytskyy Y, Tamminga J, Koturbash I, Golubov A, Bagnyukova T, Pogribny IP, Kovalchuk O (2010) Hypomethylation and genome instability in the germline of exposed parents and their progeny is associated with altered miRNA expression. Carcinogenesis 31:1110–1115

Folkard M, Vojnovic B, Prise KM, Bowey AG, Locke RJ, Schettino G, Michael BD (1997) A charged-particle microbeam: I. Development of an experimental system for targeting cells individually with counted particles. Int J Radiat Biol 72:375–385

Folley JH, Borges W, Yamawaki T (1952) Incidence of leukemia in survivors of the atomic bomb in Hiroshima and Nagasaki, Japan. Am J Med 13:311–321

Gaugler MH, Neunlist M, Bonnaud S, Aubert P, Benderitter M, Paris F (2007) Intestinal epithelial cell dysfunction is mediated by an endothelial-specific radiation-induced bystander effect. Radiat Res 167:185–193

Generoso WM, Bishop JB, Gosslee DG, Newell GW, Sheu CJ, von Halle E (1980) Heritable translocation test in mice. Mutat Res 76:191–215

Gluzman D, Imamura N, Sklyarenko L, Nadgornaya V, Zavelevich M, Machilo V (2005) Malignant diseases of hematopoietic and lymphoid tissues in Chernobyl clean-up workers. Hematol J 5:565–571

Goh K, Sumner H (1968) Breaks in normal human chromosomes: are they induced by a transferable substance in the plasma of persons exposed to total-body irradiation? Radiat Res 35:171–181

Goldberg Z (2003) Clinical implications of radiation-induced genomic instability. Oncogene 22:7011–7017

Goldberg Z, Lehnert BE (2002) Radiation-induced effects in unirradiated cells: a review and implications in cancer. Int J Oncol 21:337–349

Goll MG, Bestor TH (2005) Eukaryotic cytosine methyltransferases. Annu Rev Biochem 74:481–514

Green S, Lavappa KS, Manandhar M, Sheu CJ, Whorton E, Springer JA (1987) A guide for mutagenicity testing using the dominant lethal assay. Mutation Res 189:167–174

Hall EJ (2002) Lessons we have learned from our children: cancer risks from diagnostic radiology. Pediatr Radiol 32:700–706

Hall EJ (2003) The bystander effect. Health Phys 85:31–35

Hall EJ (2006) Intensity-modulated radiation therapy, protons, and the risk of second cancers. Int J Radiat Oncol Biol Phys 65:1–7

Hamada N, Matsumoto H, Hara T, Kobayashi Y (2007) Intercellular and intracellular signaling pathways mediating ionizing radiation-induced bystander effects. J Radiat Res 48:87–95

Han W, Wu L, Chen S, Bao L, Zhang L, Jiang E, Zhao Y, Xu A, Hei TK, Yu Z (2007) Constitutive nitric oxide acting as a possible intercellular signaling molecule in the initiation of radiation-induced DNA double strand breaks in non-irradiated bystander cells. Oncogene 26:2330–2339

Hatch T, Derijck AA, Black PD, van der Heijden GW, de Boer P, Dubrova YE (2007) Maternal effects of the scid mutation on radiation-induced transgenerational instability in mice. Oncogene 26:4720–4724

He S, Dunn KL, Espino PS, Drobic B, Li L, Yu J, Sun JM, Chen HY, Pritchard S, Davie JR (2008) Chromatin organization and nuclear microenvironments in cancer cells. J Cell Biochem 104:2004–2015

Hendrich B, Tweedie S (2003) The methyl-CpG binding domain and the evolving role of DNA methylation in animals. Trends Genet 19:269–277

Hildreth NG, Shore RE, Dvoretsky PM (1989) The Risk of breast-cancer after irradiation of the thymus in infancy. New Engl J Med 321:1281–1284

Hollowell JG Jr, Littlefield LG (1968) Chromosome damage induced by plasma of x-rayed patients – an indirect effect of x-ray. Proc Soc Exp Biol Med 129:240–244

Huang L, Snyder AR, Morgan WF (2003) Radiation-induced genomic instability and its implications for radiation carcinogenesis. Oncogene 22:5848–5854

Hutvagner G, Zamore PD (2002a) A microRNA in a multiple-turnover RNAi enzyme complex. Science 297:2056–2060

Hutvagner G, Zamore PD (2002b) RNAi: nature abhors a double-strand. Curr Opin Genet Dev 12:225–232

Hwang HW, Mendell JT (2006) MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer 94:776–780

Iliakis G, Wang Y, Guan J, Wang H (2003) DNA damage checkpoint control in cells exposed to ionizing radiation. Oncogene 22:5834–5847

Ilnytskyy Y, Kovalchuk O (2011) Non-targeted radiation effects-an epigenetic connection. Mutat Res 714:113–125

Ilnytskyy Y, Zemp FJ, Koturbash I, Kovalchuk O (2008) Altered microRNA expression patterns in irradiated hematopoietic tissues suggest a sex-specific protective mechanism. Biochem Biophys Res Commun 377:41–45

Ilnytskyy Y, Koturbash I, Kovalchuk O (2009) Radiation-induced bystander effects in vivo are epigenetically regulated in a tissue-specific manner. Environ Mol Mutagen 50:105–113

Infante-Rivard C, Mathonnet G, Sinnett D (2000) Risk of childhood leukemia associated with diagnostic irradiation and polymorphisms in DNA repair genes. Environ Health Persp 108:495–498

Jaenisch R, Bird A (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 33:245–254

Jeggo P, Lobrich M (2006) Radiation-induced DNA damage responses. Radiat Prot Dosim 122:124–127

Jenuwein T, Allis CD (2001) Translating the histone code. Science 293:1074–1080

Ji Q, Hao X, Meng Y, Zhang M, Desano J, Fan D, Xu L (2008) Restoration of tumor suppressor miR-34 inhibits human p53-mutant gastric cancer tumorspheres. BMC Cancer 8:266

Jirtle RL, Skinner MK (2007) Environmental epigenomics and disease susceptibility. Nat Rev Genet 8:253–262

Kalinich JF, Catravas GN, Snyder SL (1989) The effect of gamma radiation on DNA methylation. Radiat Res 117:185–197

Kaplan MI, Limoli CL, Morgan WF (1997) Perpetuating radiation-induced chromosomal instability. Radiat Oncol Investig 5:124–128

Kaup S, Grandjean V, Mukherjee R, Kapoor A, Keyes E, Seymour CB, Mothersill CE, Schofield PN (2006) Radiation-induced genomic instability is associated with DNA methylation changes in cultured human keratinocytes. Mutat Res-Fundam Mol Mech 597:87–97

Khan MA, Hill RP, Van Dyk J (1998) Partial volume rat lung irradiation: an evaluation of early DNA damage. Int J Radiat Oncol Biol Phys 40:467–476

Khan MA, Van Dyk J, Yeung IW, Hill RP (2003) Partial volume rat lung irradiation; assessment of early DNA damage in different lung regions and effect of radical scavengers. Radiother Oncol 66:95–102

Khodarev NN, Park JO, Yu J, Gupta N, Nodzenski E, Roizman B, Weichselbaum RR (2001) Dose-dependent and independent temporal patterns of gene responses to ionizing radiation in normal and tumor cells and tumor xenografts. Proc Natl Acad Sci USA 98:12665–12670

Kleinerman RA (2006) Cancer risks following diagnostic and therapeutic radiation exposure in children. Pediatr Radiol 36:121–125

Klokov D, Criswell T, Leskov KS, Araki S, Mayo L, Boothman DA (2004) IR-inducible clusterin gene expression: a protein with potential roles in ionizing radiation-induced adaptive responses, genomic instability, and bystander effects. Mutat Res-Fundam Mol Mech 568:97–110

Klose RJ, Bird AP (2006) Genomic DNA methylation: the mark and its mediators. Trends Biochem Sci 31:89–97

Kossenko MM (1996) Cancer mortality in the exposed population of the Techa River area. World Health Stat Q 49:17–21

Koturbash I, Pogribny I, Kovalchuk O (2005) Stable loss of global DNA methylation in the radiation-target tissue–a possible mechanism contributing to radiation carcinogenesis? Biochem Biophys Res Commun 337:526–533

Koturbash I, Baker M, Loree J, Kutanzi K, Hudson D, Pogribny I, Sedelnikova O, Bonner W, Kovalchuk O (2006a) Epigenetic dysregulation underlies radiation-induced transgenerational genome instability in vivo. Int J Radiat Oncol 66:327–330

Koturbash I, Rugo RE, Hendricks CA, Loree J, Thibault B, Kutanzi K, Pogribny I, Yanch JC, Engelward BP, Kovalchuk O (2006b) Irradiation induces DNA damage and modulates epigenetic effectors in distant bystander tissue in vivo. Oncogene 25:4267–4275

Koturbash I, Boyko A, Rodriguez-Juarez R, McDonald RJ, Tryndyak VP, Kovalchuk I, Pogribny IP, Kovalchuk O (2007) Role of epigenetic effectors in maintenance of the long-term persistent bystander effect in spleen in vivo. Carcinogenesis 28:1831–1838

Koturbash I, Loree J, Kutanzi K, Koganow C, Pogribny I, Kovalchuk O (2008a) In vivo bystander effect: cranial X-irradiation leads to elevated DNA damage, altered cellular proliferation and apoptosis, and increased p53 levels in shielded spleen. Int J Radiat Oncol 70:554–562

Koturbash I, Kutanzi K, Hendrickson K, Rodriguez-Juarez R, Kogosov D, Kovalchuk O (2008b) Radiation-induced bystander effects in vivo are sex specific. Mutat Res-Fundam Mol Mech 642:28–36

Koturbash I, Zemp FJ, Kutanzi K, Luzhna L, Loree J, Kolb B, Kovalchuk O (2008c) Sex-specific microRNAome deregulation in the shielded bystander spleen of cranially exposed mice. Cell Cycle 7:1658–1667

Koturbash I, Zemp F, Kolb B, Kovalchuk O (2010a) Sex-specific radiation-induced microRNAome responses in the hippocampus, cerebellum and frontal cortex in a mouse model. Mutat Res 22:114–118

Koturbash I, Zemp FJ, Pogribny I, Kovalchuk O (2010b) Small molecules with big effects: the role of the microRNAome in cancer and carcinogenesis. Mutat Res 722:94–105

Kovalchuk O, Baulch JE (2008) Epigenetic changes and nontargeted radiation effects–is there a link? Environ Mol Mutagen 49:16–25

Kovalchuk O, Burke P, Besplug J, Slovack M, Filkowski J, Pogribny I (2004) Methylation changes in muscle and liver tissues of male and female mice exposed to acute and chronic low-dose X-ray-irradiation. Mutat Res 548:75–84

Kovalchuk O, Zemp FJ, Filkowski JN, Altamirano AM, Dickey JS, Jenkins-Baker G, Marino SA, Brenner DJ, Bonner WM, Sedelnikova OA (2010) microRNAome changes in bystander three-dimensional human tissue models suggest priming of apoptotic pathways. Carcinogenesis 31:1882–1888

Kuramochi-Miyagawa S, Watanabe T, Gotoh K, Totoki Y, Toyoda A, Ikawa M, Asada N, Kojima K, Yamaguchi Y, Ijiri TW, Hata K, Li E, Matsuda Y, Kimura T, Okabe M, Sakaki Y, Sasaki H, Nakano T (2008) DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes. Genes Dev 22:908–917

Lee M, Urata SM, Aguilera JA, Perry CC, Milligan JR (2012) Modeling the influence of histone proteins on the sensitivity of DNA to ionizing radiation. Radiat Res 177:152–163
Leone G, Mele L, Pulsoni A, Equitani F, Pagano L (1999) The incidence of secondary leukemias. Haematologica 84:937–945
Liang G, Chan MF, Tomigahara Y, Tsai YC, Gonzales FA, Li E, Laird PW, Jones PA (2002) Cooperativity between DNA methyltransferases in the maintenance methylation of repetitive elements. Mol Cell Biol 22:480–491
Likhtarov I, Kovgan L, Vavilov S, Chepurny M, Ron E, Lubin J, Bouville A, Tronko N, Bogdanova T, Gulak L, Zablotska L, Howe G (2006) Post-Chernobyl thyroid cancers in Ukraine. Report 2: risk analysis. Radiat Res 166:375–386
Little JB (2000) Radiation carcinogenesis. Carcinogenesis 21:397–404
Little JB (2003) Genomic instability and radiation. J Radiol Prot 23:173–181
Liu Z, Mothersill CE, McNeill FE, Lyng FM, Byun SH, Seymour CB, Prestwich WV (2006) A dose threshold for a medium transfer bystander effect for a human skin cell line. Radiat Res 166:19–23
Loree JM, Koturbash I, Kutanzi K et al (2006) Molecular mechanisms of radiation-induced bystander effect in vivo in spleen. Environ Mol Mutagen 47:469
Lorimore SA, Coates PJ, Scobie GE, Milne G, Wright EG (2001) Inflammatory-type responses after exposure to ionizing radiation in vivo: a mechanism for radiation-induced bystander effects? Oncogene 20:7085–7095
Lorimore SA, Coates PJ, Wright EG (2003) Radiation-induced genomic instability and bystander effects: inter-related nontargeted effects of exposure to ionizing radiation. Oncogene 22:7058–7069
Lorimore SA, McIlrath JM, Coates PJ, Wright EG (2005) Chromosomal instability in unirradiated hemopoietic cells resulting from a delayed in vivo bystander effect of gamma radiation. Cancer Res 65:5668–5673
Maes OC, An J, Sarojini H, Wu H, Wang E (2008) Changes in microRNA expression patterns in human fibroblasts after low-LET radiation. J Cell Biochem 105:824–834
Maguire P, Mothersill C, McClean B, Seymour C, Lyng FM (2007) Modulation of radiation responses by pre-exposure to irradiated cell conditioned medium. Radiat Res 167:485–492
Marozik P, Mothersill C, Seymour CB, Mosse I, Melnov S (2007) Bystander effects induced by serum from survivors of the Chernobyl accident. Exp Hematol 35:55–63
Minamoto T, Mai M, Ronai Z (1999) Environmental factors as regulators and effectors of multistep carcinogenesis. Carcinogenesis 20:519–527
Mohr U, Dasenbrock C, Tillmann T, Kohler M, Kamino K, Hagemann G, Morawietz G, Campo E, Cazorla M, Fernandez P, Hernandez L, Cardesa A, Tomatis L (1999) Possible carcinogenic effects of X-rays in a transgenerational study with CBA mice. Carcinogenesis 20:325–332
Morgan WF (2003a) Is there a common mechanism underlying genomic instability, bystander effects and other nontargeted effects of exposure to ionizing radiation? Oncogene 22:7094–7099
Morgan WF (2003b) Non-targeted and delayed effects of exposure to ionizing radiation: I. radiation-induced genomic instability and bystander effects in vitro. Radiat Res 159:567–580
Morgan WF (2003c) Non-targeted and delayed effects of exposure to ionizing radiation: II. radiation-induced genomic instability and bystander effects in vivo, clastogenic factors and transgenerational effects. Radiat Res 159:581–596
Morgan WF, Sowa MB (2005) Effects of ionizing radiation in nonirradiated cells. Proc Natl Acad Sci USA 102:14127–14128
Morgan WF, Sowa MB (2007) Non-targeted bystander effects induced by ionizing radiation. Mutat Res-Fundam Mol Mech 616:159–164
Morgan WF, Sowa MB (2009) Non-targeted effects of ionizing radiation: implications for risk assessment and the radiation dose response profile. Health Phys 97:426–432
Morgan WF, Day JP, Kaplan MI, McGhee EM, Limoli CL (1996) Genomic instability induced by ionizing radiation. Radiat Res 146:247–258

Morgan WF, Hartmann A, Limoli CL, Nagar S, Ponnaiya B (2002) Bystander effects in radiation-induced genomic instability. Mutat Res-Fundam Mol Mech 504:91–100
Mori N, Matsumoto Y, Okumoto M, Suzuki N, Yamate J (2001) Variations in Prkdc encoding the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) and susceptibility to radiation-induced apoptosis and lymphomagenesis. Oncogene 20:3609–3619
Morimura K, Romanenko A, Min W, Salim EI, Kinoshita A, Wanibuchi H, Vozianov A, Fukushima S (2004) Possible distinct molecular carcinogenic pathways for bladder cancer in Ukraine, before and after the Chernobyl disaster. Oncol Rep 11:881–886
Mothersill C, Seymour C (2003) Radiation-induced bystander effects, carcinogenesis and models. Oncogene 22:7028–7033
Mothersill C, Seymour CB (2004) Radiation-induced bystander effects – implications for cancer. Nat Rev Cancer 4:158–164
Mothersill C, Seymour CB (2006) Radiation-induced bystander effects and the DNA paradigm: an “out of field” perspective. Mutat Res-Fundam Mol Mech 597:5–10
Mothersill C, Smith RW, Agnihotri N, Seymour CB (2007) Characterization of a radiation-induced stress response communicated in vivo between zebrafish. Environ Sci Technol 41:3382–3387
Munshi A, Shafi G, Aliya N, Jyothy A (2009) Histone modifications dictate specific biological readouts. J Genet Genomics 36:75–88
Nagar S, Smith LE, Morgan WF (2003) Characterization of a novel epigenetic effect of ionizing radiation: the death-inducing effect. Cancer Res 63:324–328
Niwa O (2003) Induced genomic instability in irradiated germ cells and in the offspring; reconciling discrepancies among the human and animal studies. Oncogene 22:7078–7086
Nomura T (1988) X-ray- and chemically induced germ-line mutation causing phenotypical anomalies in mice. Mutat Res 198:309–320
Nomura T (2003) Transgenerational carcinogenesis: induction and transmission of genetic alterations and mechanisms of carcinogenesis. Mutat Res 544:425–432
Nomura T, Nakajima H, Ryo H, Li LY, Fukudome Y, Adachi S, Gotoh H, Tanaka H (2004) Transgenerational transmission of radiation- and chemically induced tumors and congenital anomalies in mice: studies of their possible relationship to induced chromosomal and molecular changes. Cytogenet Genome Res 104:252–260
O’Donnell KA, Boeke JD (2007) Mighty Piwis defend the germline against genome intruders. Cell 129:37–44
Okano M, Bell DW, Haber DA, Li E (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99:247–257
Pant GS, Kamada N (1977) Chromosome aberrations in normal leukocytes induced by the plasma of exposed individuals. Hiroshima J Med Sci 26:149–154
Paquette B, Little JB (1994) In vivo enhancement of genomic instability in minisatellite sequences of mouse C3H/10T1/2 cells transformed in vitro by X-rays. Cancer Res 54:3173–3178
Persaud R, Zhou H, Baker SE, Hei TK, Hall EJ (2005) Assessment of low linear energy transfer radiation-induced bystander mutagenesis in a three-dimensional culture model. Cancer Res 65:9876–9882
Persaud R, Zhou H, Hei TK, Hall EJ (2007) Demonstration of a radiation-induced bystander effect for low dose low LET beta-particles. Radiat Environ Biophys 46:395–400
Pogribny I, Raiche J, Slovack M, Kovalchuk O (2004) Dose-dependence, sex- and tissue-specificity, and persistence of radiation-induced genomic DNA methylation changes. Biochem Biophys Res Commun 320:1253–1261
Pogribny I, Koturbash I, Tryndyak V, Hudson D, Stevenson SM, Sedelnikova O, Bonner W, Kovalchuk O (2005) Fractionated low-dose radiation exposure leads to accumulation of DNA damage and profound alterations in DNA and histone methylation in the murine thymus. Mol Cancer Res 3:553–561

Powell SN, Kachnic LA (2003) Roles of BRCA1 and BRCA2 in homologous recombination, DNA replication fidelity and the cellular response to ionizing radiation. Oncogene 22:5784–5791

Preston-Martin S, Thomas DC, Yu MC, Henderson BE (1989) Diagnostic radiography as a risk factor for chronic myeloid and monocytic leukemia (CML). Brit J Cancer 59:639–644

Prysyazhnyuk A, Gristchenko V, Fedorenko Z, Gulak L, Fuzik M, Slipenyuk K, Tirmarche M (2007) Twenty years after the Chernobyl accident: solid cancer incidence in various groups of the Ukrainian population. Radiat Environ Biophys 46:43–51

Pukkala E, Kesminiene A, Poliakov S, Ryzhov A, Drozdovitch V, Kovgan L, Kyyrönen P, Malakhova IV, Gulak L, Cardis E (2006) Breast cancer in Belarus and Ukraine after the Chernobyl accident. Int J Cancer 119:651–658

Raiche J, Rodriguez-Juarez R, Pogribny I, Kovalchuk O (2004) Sex- and tissue-specific expression of maintenance and de novo DNA methyltransferases upon low dose X-irradiation in mice. Biochem Biophys Res Commun 325:39–47

Randers-Pehrson G, Geard CR, Johnson G, Elliston CD, Brenner DJ (2001) The Columbia University single-ion microbeam. Radiat Res 156:210–214

Reuter M, Chuma S, Tanaka T, Franz T, Stark A, Pillai RS (2009) Loss of the Mili-interacting Tudor domain-containing protein-1 activates transposons and alters the Mili-associated small RNA profile. Nat Struct Mol Biol 16:639–646

Robertson KD (2001) DNA methylation, methyltransferases, and cancer. Oncogene 20:3139–3155

Robertson KD (2002) DNA methylation and chromatin – unraveling the tangled web. Oncogene 21:5361–5379

Robertson KD, Wolffe AP (2000) DNA methylation in health and disease. Nat Rev Genet 1:11–19

Rodemann HP, Blaese MA (2007) Responses of normal cells to ionizing radiation. Semin Radiat Oncol 17:81–88

Romanenko A, Morell-Quadreny L, Nepomnyaschy V, Vozianov A, Llombart-Bosch A (2000) Pathology and proliferative activity of renal-cell carcinomas (RCCS) and renal oncocytomas in patients with different radiation exposure after the Chernobyl accident in Ukraine. Int J Cancer 87:880–883

Rountree MR, Bachman KE, Herman JG, Baylin SB (2001) DNA methylation, chromatin inheritance, and cancer. Oncogene 20:3156–3165

Russell WL, Kelly EM (1982) Mutation frequencies in male mice and the estimation of genetic hazards of radiation in men. Proc Natl Acad Sci USA 79:542–544

Russell WL, Bangham JW, Russell LB (1998) Differential response of mouse male germ-cell stages to radiation-induced specific-locus and dominant mutations. Genetics 148:1567–1578

Saha A, Wittmeyer J, Cairns BR (2006) Chromatin remodelling: the industrial revolution of DNA around histones. Nat Rev Mol Cell Biol 7:437–447

Salomaa S, Lindholm C, Tankimanova MK, Mamyrbaeva ZZ, Koivistoinen A, Hultén M, Mustonen R, Dubrova YE, Bersimbaev RI (2002) Stable chromosome aberrations in the lymphocytes of a population living in the vicinity of the Semipalatinsk nuclear test site. Radiat Res 158:591–596

Scarano MI, Strazzullo M, Matarazzo MR, D'Esposito M (2005) DNA methylation 40 years later: its role in human health and disease. J Cell Physiol 204:21–35

Sedelnikova OA, Bonner WM (2006) Gamma H2AX in cancer cells. Cell Cycle 5:2909–2913

Sedelnikova OA, Pilch DR, Redon C, Bonner WM (2003) Histone H2AX in DNA damage and repair. Cancer Biol Ther 2:233–235

Sedelnikova OA, Nakamura A, Kovalchuk O, Koturbash I, Mitchell SA, Marino SA, Brenner DJ, Bonner WM (2007) DNA double-strand breaks form in bystander cells after microbeam irradiation of three-dimensional human tissue models. Cancer Res 67:4295–4302

Sevignani C, Calin GA, Siracusa LD, Croce CM (2006) Mammalian microRNAs: a small world for fine-tuning gene expression. Mamm Genome 17:189–202

Shames DS, Minna JD, Gazdar AF (2007) DNA methylation in health, disease, and cancer. Curr Mol Med 7:85–102

Shilnikova NS, Preston DL, Ron E, Gilbert ES, Vassilenko EK, Romanov SA, Kuznetsova IS, Sokolnikov ME, Okatenko PV, Kreslov VV, Koshurnikova NA (2003) Cancer mortality risk among workers at the Mayak nuclear complex. Radiat Res 159:787–798

Shu XO, Potter JD, Linet MS, Severson RK, Han D, Kersey JH, Neglia JP, Trigg ME, Robison LL (2002) Diagnostic X-rays and ultrasound exposure and risk of childhood acute lymphoblastic leukemia by immunophenotype. Cancer Epidemiol Biomark Prev 11:177–185

Simone NL, Soule BP, Ly D, Saleh AD, Savage JE, Degraff W, Cook J, Harris CC, Gius D, Mitchell JB (2009) Ionizing radiation-induced oxidative stress alters miRNA expression. PLoS One 4:e6377

Smilenov LB, Hall EJ, Bonner WM, Sedelnikova OA (2006) A microbeam study of DNA double-strand breaks in bystander primary human fibroblasts. Radiat Prot Dosimetry 122:256–259

Sokolov MV, Smilenov LB, Hall EJ, Panyutin IG, Bonner WM, Sedelnikova OA (2005) Ionizing radiation induces DNA double-strand breaks in bystander primary human fibroblasts. Oncogene 24:7257–7265

Suzuki K, Ojima M, Kodama S, Watanabe M (2003) Radiation-induced DNA damage and delayed induced genomic instability. Oncogene 22:6988–6993

Tahara E, Yasui W, Ito H, Harris CC (2010) Recent progress in carcinogenesis, progression and therapy of lung cancer: the 19th Hiroshima cancer seminar: the 3rd three universities' consortium international symposium, November 2009. Jpn J Clin Oncol 40:702–708

Tamminga J, Kathiria P, Koturbash I, Kovalchuk O (2008a) DNA damage-induced upregulation of miR-709 in the germline downregulates BORIS to counteract aberrant DNA hypomethylation. Cell Cycle 7:3731–3736

Tamminga J, Koturbash I, Baker M, Kutanzi K, Kathiria P, Pogribny IP, Sutherland RJ, Kovalchuk O (2008b) Paternal cranial irradiation induces distant bystander DNA damage in the germline and leads to epigenetic alterations in the offspring. Cell Cycle 7:1238–1245

Tanaka K, Iida S, Takeichi N, Chaizhunusova NJ, Gusev BI, Apsalikov KN, Inaba T, Hoshi M (2006) Unstable-type chromosome aberrations in lymphocytes from individuals living near Semipalatinsk nuclear test site. J Radiat Res (Tokyo) 47(Suppl A):A159–A164

Tawa R, Kimura Y, Komura J, Miyamura Y, Kurishita A, Sasaki MS, Sakurai H, Ono T (1998) Effects of X-ray irradiation on genomic DNA methylation levels in mouse tissues. J Radiat Res (Tokyo) 39:271–278

Templin T, Paul S, Amundson SA, Young EF, Barker CA, Wolden SL, Smilenov LB (2011) Radiation-induced micro-rna expression changes in peripheral blood cells of radiotherapy patients. Int J Radiat Oncol Biol Phys 80:549–557

Tomatis L (1994) Transgeneration carcinogenesis: a review of the experimental and epidemiological evidence. Jpn J Cancer Res 85:443–454

Valerie K, Yacoub A, Hagan MP, Curiel DT, Fisher PB, Grant S, Dent P (2007) Radiation-induced cell signaling: inside-out and outside-in. Mol Cancer Ther 6:789–801

Vance MM, Baulch JE, Raabe OG, Wiley LM, Overstreet JW (2002) Cellular reprogramming in the F3 mouse with paternal F0 radiation history. Int J Radiat Biol 78:513–526

Wagner-Ecker M, Schwager C, Wirkner U, Abdollahi A, Huber PE (2010) MicroRNA expression after ionizing radiation in human endothelial cells. Radiat Oncol 5:25

Wakabayashi T, Kato H, Ikeda T, Schull WJ (1983) Studies of the mortality of A-bomb survivors, report 7. Part III. incidence of cancer in 1959–1978, based on the tumor registry, Nagasaki. Radiat Res 93:112–146

Wang J, Saxe JP, Tanaka T, Chuma S, Lin H (2009) Mili interacts with tudor domain-containing protein 1 in regulating spermatogenesis. Curr Biol 19:640–644

Watanabe S, Shimosato Y, Okita T, Ezaki H, Shigemitsu T (1972) Leukemia and thyroid carcinoma found among A-bomb survivors in Hiroshima. Recent Results Cancer Res 39:57–83

Weber M, Schubeler D (2007) Genomic patterns of DNA methylation: targets and function of an epigenetic mark. Curr Opin Cell Biol 19:273–280

Weber M, Davies JJ, Wittig D, Oakeley EJ, Haase M, Lam WL, Schübeler D (2005) Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. Nat Genet 37:853–862

Weber M, Hellmann I, Stadler MB, Ramos L, Pääbo S, Rebhan M, Schübeler D (2007) Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. Nat Genet 39:457–466

Weidhaas JB, Babar I, Nallur SM, Trang P, Roush S, Boehm M, Gillespie E, Slack FJ (2007) MicroRNAs as potential agents to alter resistance to cytotoxic anticancer therapy. Cancer Res 67:11111–11116

Weidman JR, Dolinoy DC, Murphy SK, Jirtle RL (2007) Cancer susceptibility: epigenetic manifestation of environmental exposures. Cancer J 13:9–16

Wiley LM, Baulch JE, Raabe OG, Straume T (1997) Impaired cell proliferation in mice that persists across at least two generations after paternal irradiation. Radiat Res 148:145–151

Williams ED (2006) Chernobyl and thyroid cancer. J Surg Oncol 94:670–677

Williams D, Baverstock K (2006) Chernobyl and the future: too soon for a final diagnosis. Nature 440:993–994

Wilson IM, Davies JJ, Weber M, Brown CJ, Alvarez CE, MacAulay C, Schübeler D, Lam WL (2006) Epigenomics: mapping the methylome. Cell Cycle 5:155–158

Wright EG (1998) Radiation-induced genomic instability in haemopoietic cells. Int J Radiat Biol 74:681–687

Wu LJ, Randers-Pehrson G, Xu A, Waldren CA, Geard CR, Yu Z, Hei TK (1999) Targeted cytoplasmic irradiation with alpha particles induces mutations in mammalian cells. Proc Natl Acad Sci USA 96:4959–4964

Yang H, Anzenberg V, Held KD (2007) The time dependence of bystander responses induced by iron-ion radiation in normal human skin fibroblasts. Radiat Res 168:292–298

Yauk CL, Dubrova YE, Grant GR, Jeffreys AJ (2002) A novel single molecule analysis of spontaneous and radiation-induced mutation at a mouse tandem repeat locus. Mutat Res 500:147–156

Yin H, Lin H (2007) An epigenetic activation role of Piwi and a Piwi-associated piRNA in *Drosophila melanogaster*. Nature 450:304–308

Zhou H, Randers-Pehrson G, Waldren CA, Vannais D, Hall EJ, Hei TK (2000) Induction of a bystander mutagenic effect of alpha particles in mammalian cells. Proc Natl Acad Sci USA 97:2099–2104

Zhou H, Randers-Pehrson G, Suzuki M, Waldren CA, Hei TK (2002a) Genotoxic damage in non-irradiated cells: contribution from the bystander effect. Radiat Prot Dosim 99:227–232

Zhou H, Suzuki M, Geard CR, Hei TK (2002b) Effects of irradiated medium with or without cells on bystander cell responses. Mutat Res-Fundam Mol Mech 499:135–141

Chapter 6
The Intersection of Genetics and Epigenetics: Reactivation of Mammalian LINE-1 Retrotransposons by Environmental Injury

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Abstract Transposable elements such as LINE-1 (long interspersed nuclear element-1 or L1) are mobile genetic moieties within the genome. L1 retrotransposons comprise 21 % of the human genome by mass, and up to 100 are believed to remain retrotransposition competent within the human genome. During embryonic development, the genome undergoes reprogramming events defined by specific patterns of DNA methylation established de novo after implantation and preferentially targeted to repetitive sequences. Recent studies in the Ramos laboratory have shown that the ability of polycyclic aromatic hydrocarbon carcinogens, such as benzo(a)pyrene, to reactivate L1 transcription and retrotransposition in mammalian cells involves dysregulation of epigenetic programming mediated in part via mechanisms involving the aryl hydrocarbon receptor, a ligand-activated transcription factor and regulator of several other biological processes. The most detrimental effect of L1 on the genome is believed to be insertion into functional sequences that severely compromise gene function. Other studies have shown that L1 reactivation mediates changes in genetic programming of differentiation networks. Because L1 insertions can have a profound impact on primary genetic structure as well as epigenetic status of the host, they

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represent ideal molecular targets for development of novel epigenetic therapies targeting medical conditions that involve derangements of L1 activity.

Keywords Aryl hydrocarbon receptor (AHR) • Benzo(a)pyrene (BaP) • Bioinformatics • Chromatin modifications • CpG island • Disease phenotypes • DNA methylation • Environmental injury • Epigenetic programming • Epigenetic therapies • Genetic diversity • Genetic network • Genome • Genome evolution • Genomic imprinting • Genomic instability • Global hypomethylation • Global methylation • Heterochromatin • Histone • Histone deacetylase inhibitor • Histone tail covalent modification • L1 insertion • LINE-1 or L1 • Nucleosome • Personalized medicine • Polycyclic aromatic hydrocarbon • Transcription • Transcription factor • Transposable elements • Redox stress • Retrotransposition • Reverse transcriptase inhibitors • Xenobiotic

Abbreviations

3′ UTR	3′ Untranslated region
5′ UTR	5′ Untranslated region
α NP or ANF	α-Naphthoflavone
Ac	Activator
AHR	Aryl hydrocarbon receptor
AIDS	Acquired immune deficiency syndrome
AML	Acute myeloid leukemia
AP-1	Activator protein-1
APL	Acute promyelocytic leukemia
AR	Androgen receptor
ARE/EpRE	Antioxidant/electrophile response element
ARNT	Aryl hydrocarbon receptor nuclear translocator protein
ATM	Ataxia telangiectasia mutated
AZT	Azidothymidine
BaP	Benzo(a)pyrene
bHLH	Basic helix-loop-helix
c-myc	Myelocytomatosis viral oncogene homolog (avian)
C/EBP	CCAAT/enhancer-binding protein
CpG	5′ Cytosine-phospho-guanine
CREB	Cyclic AMP-responsive element binding protein 1
Ds	Dissociator
DNMT	DNA methyltransferase
ERα	Estrogen receptor α
ETO	Eight-twenty-one
GFP	Green fluorescent protein

H2BK	Histone H2B lysine residue
H3K	Histone H3 lysine residue
HAT	Histone acetylase
HATi	Histone acetylase inhibitor
HCG or Beta-HCG	Human chorionic gonadotropin
HDAC	Histone deacetylase
HDACi	Histone deacetylase inhibitor
HIV	Human immunodeficiency virus
HMEC	Human mammary epithelial cell
HMT	Histone methyltransferases
HP-1	Heterochromatin protein-1
Hsp90	90 kDa heat shock protein
IAP	Intracisternal A-particle
L1Md	L1 in *Mus domesticus*
L1Rn	L1 in *Rattus norvegicus*
LINE-1 or L1	Long interspersed nuclear element-1
LTR	Long terminal repeat
MBD	Methyl binding protein
MGST 1	Microsomal glutathione S-transferase 1
mVSMC	Murine vascular smooth muscle cells
NAC	N-Acetyl-L-cysteine
N-CoR	Nuclear receptor co-repressor 1
nnRTI	Non-nucleoside reverse transcriptase inhibitor
Nrf-2	Nuclear factor erythroid 2-related factor 2
nRTI	Nucleoside reverse transcriptase inhibitor
ORF	Open reading frame
PAH	Polycyclic aromatic hydrocarbon
PER	Period circadian protein
PCAF	p300/CBP-associated factor
PLZF	Promyelocytic leukemia zinc finger protein
PML	Promyelocytic leukemia
RAR	Retinoic acid receptor alpha
RB	Retinoblastoma
RP2	Retinitis pigmentosa
RT	Reverse transcriptase
RUNX	Runt-domain transcription factor
SGPL1	Sphingosine phosphate lyase 1
SIM	Single-minded protein
SINE	Short interspersed nuclear elements
siRNA	Small interfering RNA
SP-1	Specificity protein 1
SynBP	Syndecan binding protein

TCDD	2,3,7,8 Tetrachlorodibenzo-p-dioxin
TERT	Telomerase reverse transcriptase
WT1	Wilms' tumor suppressor
YY1	Yin yang 1

6.1 Introduction

In her pioneering work studying "mutable loci" in maize, Barbara McClintock identified "*Dissociator*" (*Ds*) and "*Activator*" (*Ac*) as loci that not only induced chromosome breaks but that also changed position (i.e., transposed) to effect gene expression (McClintock 1950). This novel concept of transposition was inconsistent with the long-held view of a static genome and, therefore, rejected for many years. The discovery of transposable elements at multiple levels of organization, coupled to the discovery of genomic plasticity, led to wider acceptance of McClintock's early hypotheses and a Nobel Prize for her in 1983. It is now understood that genomes have coevolved with their transposable elements, and moreover, transposons play a major role in genetic evolution and regulation of gene expression.

Transposable elements are genetic moieties that can change locations within the genome. There are two main classes of transposable elements: DNA transposons and retrotransposons (Babushok and Kazazian 2007). DNA transposons utilize encoded transposase and inverse terminal repeats for propagation, while retrotransposons propagate via RNA intermediates utilizing self-encoded reverse transcriptase activity (Reznikoff 2003). One of the most surprising outcomes of the analyses of the human genome was that more than 47 % of the sequence consisted of transposable elements (Kazazian and Moran 1998). Their relative contribution to the human genome includes long terminal repeat (LTR), 9 %; DNA transposons, 3 %; and long interspersed nuclear elements (LINEs or L1) and short interspersed nuclear elements (SINEs) which combined represent 35 % (Lander et al. 2001). In mice, transposable elements account for 37 % of the genome, with L1 representing up to 20 % (Deininger and Batzer 2002; Waterston et al. 2002).

L1 elements emerged around 120 million years ago (Smit et al. 1995; Lee et al. 2007) and represent the largest family of transposable elements among all studied mammalian genomes (Brouha et al. 2003; Lander et al. 2001; Smith 1976; Szak et al. 2002). Retrotranspositional explosion in ancestral primates occurred 40–50 million years ago (Ohshima et al. 2003). Although the causes that promoted this explosion remain unclear, changes in the environment are most likely involved. Species-specific L1 insertions in both the human and chimpanzee genomes indicate recent mobilization (Mills et al. 2006).

6.2 Genetics of LINE-1 Retrotransposon

Retrotransposons are present in all eukaryotic genomes examined so far (Kumar and Bennetzen 1999; Lovsin et al. 2001) and have contributed to genome structure and evolution. These genetic elements propagate via a "copy-and-paste" mechanism that involves reverse transcriptase and RNA intermediates. Autonomous retrotransposons encode the activities necessary for their mobility, while nonautonomous retrotransposons (e.g., *Alu* elements) do not encode any proteins and, therefore, require activities of autonomous retrotransposons for mobility (Ostertag and Kazazian 2001). Two classes of retrotransposons exist: LTR and non-LTR retrotransposons. LTR retrotransposons have direct terminal repeats that range in size from 100 bp to over 5 kb, e.g., Ty1 and Ty3 retrotransposons in yeast and retroviruses (Li et al. 2004). Non-LTR retrotransposons lack terminal repeats but have a 3′ UTR which contains a polyadenylation signal (Dombroski et al. 1991). Examples of non-LTR retrotransposons are the LINEs and SINEs. A simplified functional classification of transposable elements is illustrated in Fig. 6.1.

The most abundant non-LTR retrotransposon in the mammalian genome is L1. L1 retrotransposons comprise 21 % of the human genome by mass, and although most are inactive, 80–100 in human (Kazazian and Moran 1998) and 400 in mouse are believed to remain retrotransposition competent (Sassaman et al. 1997). A full-length mammalian L1 is approximately 6.0–7.5 kb in length and contains three major components: a 5′ untranslated region (5′ UTR), two open reading frames (ORFs), and a 3′ UTR with a poly A tail (Dombroski et al. 1991). ORF1 encodes a protein with RNA binding capacity (Yang et al. 2005), while ORF2 encodes the endonuclease and reverse transcriptase activities necessary for retrotransposition (Feng et al. 1996), along with a zinc finger domain of unknown function. The human L1 is transcribed from an internal promoter located within the 5′ UTR (Minakami et al. 1992). This promoter is approximately 907 bp long and contains a 371 bp CpG island within which several putative transcription factor binding sites including SP-1, AP-1, C/EBP, CREB, and c-myc have been identified (Kedar et al. 1991; Hann et al. 1994; Proffitt et al. 1995; Fabbro et al. 1999; Fawcett et al. 1999; Adhikary et al. 2005; Teneng et al. 2011). Figure 6.2 compares the structures of human and mouse L1.

L1 is believed to be the only autonomous, mobile element currently active in humans (Waterston et al. 2002). Most full-length L1s in the genome are 5′ truncated and, therefore, incapable of propagation. Other mechanisms of L1 inactivation include point mutations or inversion (Ostertag and Kazazian 2001). $L1^{\beta\text{-thal}}$ and $L1^{RP}$ are two examples of full-length, mobilization-competent L1 retrotransposons in humans (Roepman et al. 1996; Schwahn et al. 1998; Kimberland et al. 1999). $L1^{RP}$ is about 6 kb and 99.9 % identical to the consensus human L1 sequence, with its encoded proteins containing only three amino acid changes from the consensus (Kimberland et al. 1999). $L1^{RP}$ resides in intron 1 of the retinitis pigmentosa (RP2) gene of a patient with X-linked retinitis pigmentosa (Dombroski et al. 1991; Roepman et al. 1996; Schwahn et al. 1998). This condition is associated with loss

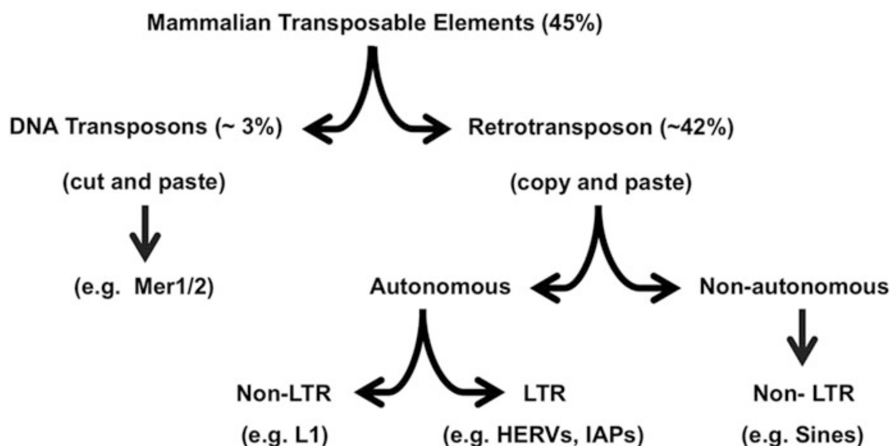

Fig. 6.1 Functional classification of transposable elements. Approximately 45 % of the human genome consists of transposable elements. Transposable elements are classified into DNA transposons and retrotransposon based on their mechanism of movement. DNA transposons can excise themselves from the genome, move as DNA, and insert themselves into new genomic sites. They use their inverted terminal repeats and homologous recombination to transpose, hence the so-called cut-and-paste mechanism. Retrotransposons copy themselves or other elements (e.g., SINES) and use their encoded reverse transcriptase activity to transpose to other locations, hence the name copy and paste. Functionally, retrotransposons are divided into autonomous and nonautonomous retrotransposons. Autonomous retrotransposons (e.g., LINE-1) have the complete machinery needed to transpose to new locations, while nonautonomous retrotransposons, such as SINES, borrow the retrotransposition machinery to transpose to new locations. Structurally, retrotransposons can be subdivided into two groups distinguished by the presence or absence of long terminal repeats (*LTRs*). Autonomous LTRs include HERVs (human endogenous retroviruses) and IAPs (intracisternal A-particles). L1s and SINES such as Alu and SVu are examples of non-LTR retrotransposon

of photoreceptor function and viability, resulting in tunnel vision, night blindness, and ultimately complete blindness (Farrar et al. 2002).

L1Md (L1 in *Mus domesticus*) is a major mouse retrotransposon family, represented by the consensus L1MdA element which is about 7.8 kb long. Like the human L1, mouse L1 consists of two ORFs, but the architecture of the mouse promoter (Fig. 6.2) is considerably different from its human counterpart in that it contains variable short repeats called monomers (205–210 bp). The relative strength of the mouse promoter is directly correlated to the number of monomers (Fanning 1983; Furano et al. 1988). An example is L1Md-A5, a mouse retrotransposon which contains 3 and 2/3 repeats that confer strong promoter activity and, like the human L1 element, contain putative carcinogen-responsive elements (Lu and Ramos 2003).

Given the preponderance of L1s in the human and mouse genomes, research in the Ramos laboratory over the past 20 years has focused on $L1^{RP}$ and *L1Md*, respectively, as human and mouse model systems of L1 biology. Elucidation of the mechanisms of regulation of L1 in mammalian cells is paramount given that

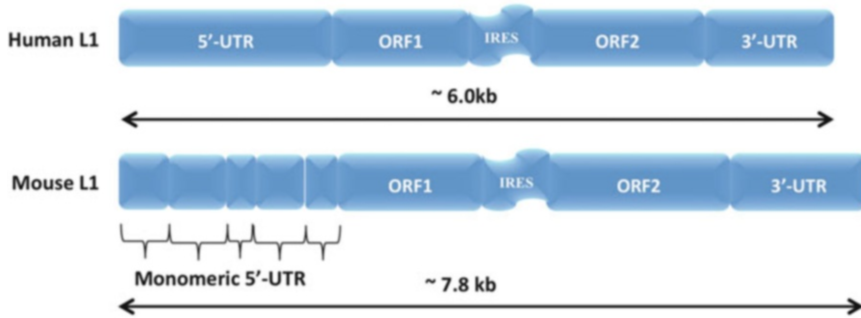

Fig. 6.2 Typical gene structures of human and mouse L1. L1 elements contain a 5′ UTR with an internal promoter and two open reading frames (*ORFs*). ORF2 encodes endonuclease and reverse transcriptase functions required for retrotransposition. The 3′ UTR contains a polyadenylation signal. The mouse L1Md promoter contains 200 bp repeats called monomers on the 5′ UTR region. A typical human L1 is 6.0 kb, while mouse L1s could be up to 7.7 kb in length depending on monomer composition

differences in L1 content and retrotransposition may contribute to variations in gene expression and mutagenic load in individuals. Recent studies have shown that the presence of L1 within a transcriptional unit can affect the structure of target DNA and the amount of mRNA produced (Han et al. 2004). Other biological consequences of L1 integration include insertional inactivation (Kazazian et al. 1988), deletions, gene rearrangements, nonallelic homologous recombination (Casavant et al. 1988), introduction of alternative splicing sites causing exon skipping, or activation of cryptic splice sites (Takahara et al. 1996; Musova et al. 2006). As such, retrotransposable elements likely represent the most important determinant of global genome evolution (Lander et al. 2001).

6.3 Transcriptional Regulation of LINE-1

The initial step in human L1 retrotransposition requires transcription from the internal 5′ UTR. Although the mechanisms of transcriptional control are not clear, the YY1 binding site is important for L1 transcription (Becker et al. 1993; Athanikar et al. 2004), and runt-domain transcription factors 1 and 2 (RUNX1 and RUNX2) increase L1 transcription and retrotransposition frequency in cell culture assays (Yang et al. 2003). In studies to characterize L1Md-A5, we characterized two ARE/EpRE (anti-oxidant/electrophile response elements)-like sequences within the mouse L1 promoter that are activated by oxidative stress under homologous (Lu and Ramos 2003) and heterologous conditions (Teneng et al. 2007). Proteins shown to be present in the macromolecular complex assembled on ARE/EpREs include Nrf-2, junD, Hsp90, and phiAP3 (Holderman et al. 2002; Miller and Ramos 2005). In addition, the aryl hydrocarbon receptor (AHR) and CCAAT/enhancer-binding protein (C/EBP) were

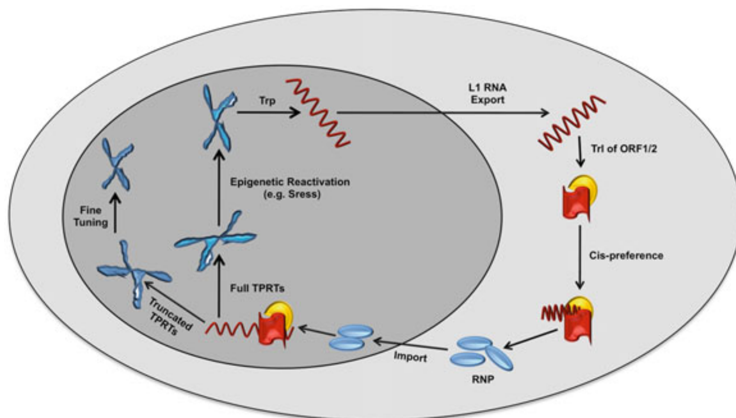

Fig. 6.3 Life cycle of L1 retrotransposon. L1 in the chromosome (white band) is transcribed, and the resulting RNA exported to the cytoplasm. Following protein synthesis, the proteins (ORF1 and -2 protein) preferentially bind to the parent RNA to form a ribonucleoprotein complex (*RNP*). Upon activation, the RNP gets imported into the nucleus where the associated RNA is reverse transcribed at the target site via target-primed reverse transcription (*TPRT*). If this new insertion is full length, it retains the potential to serve as a start site for subsequent generations of retrotransposition events. If it is truncated, it can either negatively or positively fine-tune the genome transcriptome

identified in the stress-activated multimeric complex (Chen and Ramos 2000; Kerzee and Ramos 2000). The similarities in response between mouse and human cell lines indicate that key elements of the L1 response are conserved across evolutionary lines. However, by virtue of differences in their genetic and epigenetic programs, and structural differences between the mouse and human L1 5′ UTR regions, the cellular response to stress is regulated in a context-specific manner.

After transcription, the L1 RNA transcript is exported to the cytoplasm where translation occurs and the RNA forms a ribonucleoprotein complex with its cognate ORF1 and ORF2 proteins (Feng et al. 1996). L1 reinsertion involves target-primed reverse transcription in which ORF2 nicks DNA at the target site, and uses the 3′ -OH to prime reverse transcription of L1 RNA (Morrish et al. 2002). Due to the non-processive nature of ORF2, and the presence of premature cryptic polyadenylation sites on the L1 RNA, reverse transcriptase often fails to reach the 5 prime (5′) end during first-strand synthesis resulting in 5′-truncated L1s (Han and Boeke 2004; Goodier et al. 2001). These complex relationships are depicted in Fig. 6.3.

6.4 Benzo(a)pyrene (BaP), the Aryl Hydrocarbon Receptor (AHR), and LINE-1

BaP is a ubiquitously distributed polycyclic aromatic hydrocarbon (PAH) and environmental pollutant generated by the incomplete combustion of fossil fuels, cigarettes, and cooking oil (Davis 1968; Lee et al. 1972; Kazerouni et al. 2001).

A potent carcinogen found in several animal species, BaP is metabolized by cytochrome P450 enzymes to mutagenic derivatives that form DNA adducts and cause oxidative stress (Miller et al. 2000; Johnson et al. 2003). BaP metabolic intermediates bind covalently to DNA, induce redox stress, inhibit DNA repair, and alter networks of gene expression in mammalian cells (Lu et al. 2000; Stribinskis and Ramos 2006; Ramos et al. 2007; Ramos and Nanez 2009). Humans are exposed daily to BaP in the air, upon consumption of contaminated foods and directly or indirectly from smoking. The average daily intake of BaP in the US population has been estimated at 2.2 μg/day (Hattemer-Frey and Travis 1991). BaP and related hydrocarbons bind to the aryl hydrocarbon receptor (AHR) to activate transcription of genes involved in development, homeostasis, and cellular metabolism (Miller and Ramos 2001; Falahatpisheh and Ramos 2003).

The AHR is a basic helix-loop-helix (bHLH) and PAS homology domain transcription factor involved in developmental control and cellular homeostasis (Vogel et al. 2004). AHR binds with high affinity to PAHs, most notably 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and regulates expression of several genes including those encoding for phases 1 and 2 drug-metabolizing enzymes (Xu et al. 2005). The relative expression of AHR is cell context specific and regulated by ligand binding to the PAS domain (Brauze et al. 2006) and redox stress (Teneng et al. 2007; Ramos and Nanez 2009). Most of the effects of exposure to PAHs are mediated by the AHR (Machala et al. 2001; Falahatpisheh and Ramos 2003; Ramos et al. 2006; Pollenz and Buggy 2006; Ramos 2006).

In the absence of ligand, AHR exists in a cytosolic complex with two 90 kDa heat shock protein (HSP90) (Ramadoss and Perdew 2005; Beischlag et al. 2008). This association is required for AHR to assume a conformation conducive to ligand binding. When bound to a ligand, AHR dissociates from this heteromeric complex, translocates into the nucleus where it dimerizes with the AHR nuclear translocator (ARNT) (Whitelaw et al. 1993; Rowlands and Gustafsson 1997). The ligand-bound AHR/ARNT complex acts as a transcription factor and binds to specific xenobiotic response elements (TNGCGTG) located within the promoters of several genes (Reisz-Porszasz et al. 1994; Fukunaga et al. 1995). Xenobiotic metabolizing enzyme products are highly carcinogenic and can cause DNA damage and oxidative stress (Miller et al. 2000; Johnson et al. 2003).

After ligand-dependent gene activation and nuclear export, the AHR is targeted for degradation via the 26S proteasome pathway (Pollenz 2002; Pollenz and Buggy 2006). Blocking AHR degradation in cell culture increases both the magnitude and duration of gene regulation by the AHR (Pollenz 2002). Ligand-dependent degradation of the receptor is important for signaling and has been conserved from fish to humans (Song and Pollenz 2002; Pollenz and Buggy 2006). Interestingly, it has been reported that AHR acts as a ligand-dependent E3 ubiquitin ligase in estrogen receptor α (ERα) and androgen receptor (AR) signaling (Ohtake et al. 2009). Other proteins targeted by the AHR for degradation remain to be identified, with emerging evidence from this laboratory implicating DNA methyltransferases (DNMTs) as putative targets for AHR regulation (Teneng et al. 2011).

AHR-null mice die prematurely, while AHR-deficient transgenic animals have kidney and reproductive system defects and are protected against BaP/PAH-induced skin tumors (Shimizu et al. 2000; Falahatpisheh and Ramos 2003). Transgenic mice expressing constitutively active AHR have higher incidence of glandular stomach tumors (Andersson et al. 2002) and exhibit increased susceptibility to carcinogens (Moennikes et al. 2004). Previous studies showed that BaP reactivates L1 transcription and retrotransposition rates in mammalian cells via mechanisms involving AHR (Lu and Ramos 2003; Stribinskis and Ramos 2006).

Significant species-specific differences have been noted in the reactivation of L1 by DNA damage. Induction of endogenous L1 by UV irradiation is often limited to transformed cells, as documented for human embryonic carcinoma NTera2D1 cells (Deragon et al. 1990), human keratinocyte HaCaT cells (Banerjee et al. 2005), human cervical cancer HeLa cells (Teneng et al. 2007), and L1 of *Rattus norvegicus* (L1Rn) chloroleukemia cells (Servomaa and Rytomaa 1990). In contrast, human mammary epithelial (HMEC) cells, murine vascular smooth muscle cells, or embryonic kidney cells are refractory to L1 induction by UV irradiation (Teneng et al. 2007). UV irradiation is associated with DNA single- and double-strand breaks and double-helix distortions (Squires et al. 2004) and, therefore, may facilitate L1 propagation by providing pre-nicked sites for reverse transcription and reinsertion. This interpretation is consistent with the dependence of propagation on endonuclease activity at initiation and insertion sites (Feng et al. 1996). Farkash et al. (2006) have shown that L1 retrotransposition by gamma irradiation causes genomic instability by induction of phosphorylated H2AX foci. Cells respond to DNA damage by triggering DNA repair mechanisms involving recruitment and phosphorylation of H2AX and ATM to lesion sites (Suzuki et al. 2006). These proteins play important roles in DNA damage sensing and repair response (Skalka and Katz 2005). Differences in H2AX foci and sensitivity to irradiation between different cells and species may in fact be accounted for by differences in cellular ATM levels (Kato et al. 2006). ATM has been implicated in L1 retrotransposition and gamma irradiation-induced H2AX foci in HeLa cells (Gasior et al. 2006). Additional work is needed to elucidate the role of DNA repair mechanisms in control of L1.

In studies of L1, a focus on the AHR is warranted based on its role as a sensitive molecular sensor of environmental stress in response to PAHs (Pollenz 2002; Song and Pollenz 2002). In previous studies to compare the ability of different AHR ligands to activate L1, we found that BaP was the only stressor that uniformly activates L1 irrespective of cell type. TCDD or indole-3-pyruvate, non-genotoxic AHR ligands did not induce L1 across cell lines, suggesting that contextual specificity and DNA damage are critical regulators of L1 reactivation (Teneng et al. 2007). Using the competitive AHR inhibitor α-naphthoflavone, AHR-null cells, or siRNA targeting the AHR, we showed that activation of L1 by BaP requires the AHR. Interestingly, induction of L1 by UV could be inhibited by pretreatment with NAC, linking L1 activation to redox-dependent mechanisms (Lu and Ramos 2003). On the basis of these and other findings, we concluded that reactivation of L1 in mammalian cells involves AHR and redox-dependent pathways.

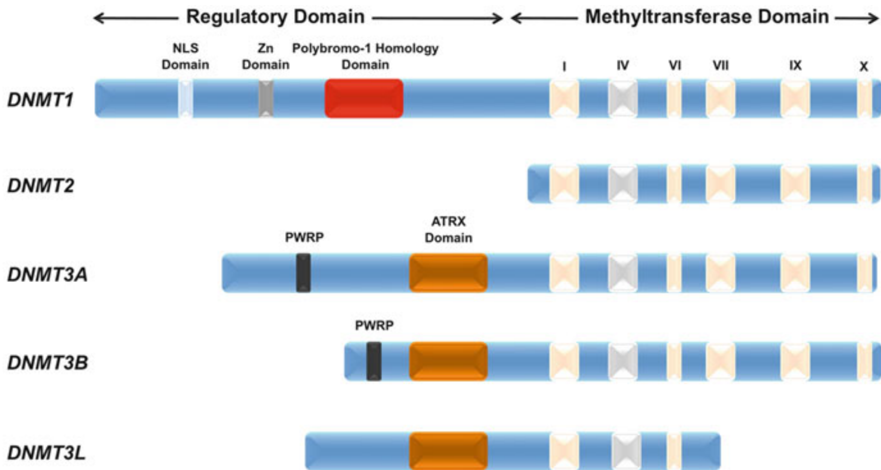

Fig. 6.4 Classification of mammalian DNMTs. DNMT1 is a maintenance methyltransferase, while DNMT 3A and 3B are "de novo" methyltransferases. Other less characterized transferases include DNMT2 and DNMT3L. The functional classification of the DNMTs is correlated to the structural organization of the DNMTs

6.5 Epigenetic Regulation of LINE-1

Epigenetics is the transmission of heritable states of gene expression through cell division in the absence of changes in DNA sequence. To date, multiple mechanisms including DNA methylation (Esteller 2000; Reik et al. 2001), histone acetylation (Roth et al. 2001), histone methylation (Ben-Porath and Cedar 2001), histone citrullination (Mastronardi et al. 2006; Thompson and Fast 2006), histone phosphorylation (Kim et al. 2008), and ADP ribosylation (Realini and Althaus 1992) have been recognized as critical regulators of epigenetic inheritance. Although cross talk among these mechanism has been described (Ben-Porath and Cedar 2001), relatively little is understood about the complexity of these interactions.

By far the best characterized epigenetic modification is the methylation of DNA at 5′ cytosines by DNMTs. Three major DNMTs are known to catalyze this reaction, namely, DNMT1, DNMT3A, and DNMT3B (Fig. 6.4) (Siedlecki and Zielenkiewicz 2006; Cheng and Blumenthal 2008). DNMT1 is a maintenance methyltransferase that ensures that patterns of DNA methylation are transferred from parent to daughter cells during mitotic cell division. DNMT3A and 3B are referred to as "de novo" methyltransferases and, as such, help to establish new patterns of DNA methylation in response to appropriate signals. DNMT2 and DNMT3L are two additional DNA methyltransferases without methyltransferase enzymatic activity towards DNA as the primary substrate. DNMT2 has little DNA methylation activity (Okano et al. 1998) and functions to methylate tRNA outside the nucleus (Goll et al. 2006), while DNMT3L is a homolog of DNMT3 that lacks the catalytic domain common to other members and functions to stimulate the

enzymatic activities of the other DNMTs (Chen et al. 2005a; Bourc'his et al. 2001). Importantly, loss of DNMT3L leads to loss of de novo methylation of both LTR and non-LTR retrotransposons (LTR region of intracisternal A-particle (IAP) and 5′ UTR region of L1MdA, respectively) in mouse testis which is followed by retrotransposon reactivation and formation of abnormal meiotic structures (Bourc'his and Bestor 2004). Factors that affect DNA methylation status include the activity of DNMT itself (Majumder et al. 2006), the pattern of histone-tail modifications (Ben-Porath and Cedar 2001), diet, and environmental insults (Friso and Choi 2002; Maier et al. 2002; Wichmann et al. 2003; Zhou et al. 2008). The latter may be mediated by loss or excess of DNA methyltransferase activity (Rountree et al. 2001; Damiani et al. 2008; Leng et al. 2008).

Studies by Feinberg and others established a link between cancer and DNA hypomethylation (Feinberg and Vogelstein 1983), and this area of investigation has guided much of the tumor biology research carried out during the past 28 years. The impact of DNA hypomethylation includes aberrant gene expression (Clark and Melki 2002), loss of imprinting (Rugg-Gunn et al. 2005; Chen et al. 2006; Byun et al. 2007; Linhart et al. 2007), microsatellite instability (Tanaka et al. 2006), chromosomal instability (Ehrlich 2002), and reactivation of retrotransposons (Bourc'his and Bestor 2004; Roman-Gomez et al. 2005). The finding that hypermethylation at promoter sites in tumor suppressor genes is prevalent in several cancer-associated genes has also received increasing attention (Kleymenova et al. 1998; Mirmohammadsadegh et al. 2006) due to their relevance in the diagnosis and prognosis of clinical outcomes in disease as well as for the treatment of human malignancies through the so-called epigenetic drug therapy (Claus and Lubbert 2003; Lubbert 2000). Thus, neoplasia is now recognized as a pathological process characterized by global variations in DNA methylation that correlate with altered patterns of gene expression and acquisition of tumorigenic and metastatic phenotypes (Baylin et al. 2001).

As noted earlier, the covalent methylation and acetylation of histone tails have been identified as critical mechanisms of epigenetic control. This is because most eukaryotic DNA is extensively folded and compacted with histone and non-histone proteins into a heteromeric polymer called chromatin, and this process is regulated by histone modifications (Fig. 6.5). The basic unit of chromatin is called the nucleosome which is made of highly basic histone (H) proteins, with each nucleosome containing two copies each of H2A, H2B, H3, and H4. Covalent modifications of histones have distinct roles in gene expression, and hence, a "histone code" has been proposed (Guil and Esteller 2009). Of note are the modifications that involve different degrees of methylation or acetylation on histone H3 lysine residues (H3K) and histone H2B acetylation (H2BK). They involve H3K20Me3, K3K4Me, H3K9Me, H3K27Me, H3K79Me, H3K79Me2, H3K79Me3, H2BK5Me, and H3K14Ac (Barski et al. 2007; Jones et al. 2008; Lindroth et al. 2008; Lu et al. 2008; Riclet et al. 2009). H3K4 trimethylation (H3K4Me3) and H3K9 acetylation (H3K9Ac) are both characteristic of transcriptionally active chromatin (Nightingale et al. 2007; Ruthenburg et al. 2007), while H3K9 trimethylation (H3K9Me3) and H3K27 trimethylation (H3K27Me3) are

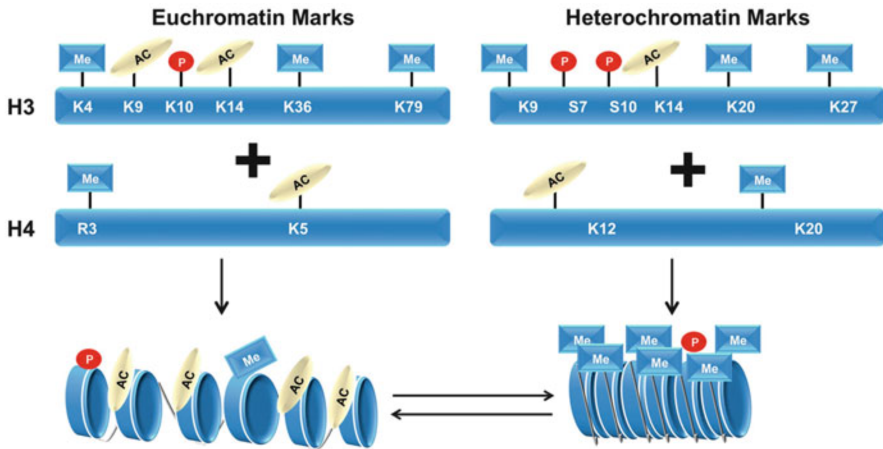

Fig. 6.5 Examples of histone modifications and typical consequences in mammalian cells. Histones H3 and H4 elicit important modifications which affect chromatin structure and gene expression including methylation (*Me*), acetylation (*Ac*), and phosphorylation (*P*). Combinatorial effects of different modifications could have different outcomes from cell to cell, hence a "histone code." Depicted in Fig. 6.5 is cross talk between epigenetic marks on H3 and H4. Cross talk between H3K4me, H3K36Me and H3K79Me, H3K9Ac, H3K14Ac, H3K14P, and H4R3Me and H4K5Ac leads to the formation of open chromatin. On the other hand, H3K9Me, H3K20Me, H3K27Me, H3K14Ac, H3S5P, H3S10P and H4K12Ac, and H4K20Me marks lead to the formation of compact chromatin

mainly found in regions of transcriptionally silent chromatin (Pan et al. 2007; Kondo et al. 2008).

The cross talk between DNA methylation and chromatin modifications is evidenced by the finding that methylation of cytosine, if transferred along the gene axis, stimulates recruitment of methyl-binding proteins (MBDs), which in turn recruit other proteins responsible for chromatin remodeling. MBDs represent a family of five well-characterized proteins (MeCP2, MBD1, MBD2, MBD3, and MBD4) whose primary function is the recruitment of histone deacetylases (HDACs) and histone methyltransferases (HMTs), leading to transcriptional inactivation (Ben-Porath and Cedar 2001). HMTs methylate histones, a mark that is recognized and bound by chromatin silencers such as heterochromatin protein-1 (HP1).

During embryonic development, the genome undergoes reprogramming events that involve global cytosine demethylation, later followed by a specific pattern of DNA methylation established de novo after implantation (Santos et al. 2002). The maintenance of DNA methylation in somatic cells is critical as aberrant changes in methylation status have been linked to tumorigenesis and genomic instability (Roman-Gomez et al. 2005). A significant part of this methylation is targeted to repetitive sequences. Given that L1 is a genetic element that can move throughout the genome, mammalian cells have evolved sophisticated silencing systems to regulate L1 retroelement expression. These silencing systems include the following:

(1) DNA hypermethylation of the L1 promoter to maintain chromatin in a compacted state (Woodcock et al. 1997), (2) bidirectional processing of small L1 transcripts to siRNAs that suppress L1 (Yang and Kazazian 2006), (3) transcriptional elongation deficiencies caused by premature polyadenylation sites (Perepelitsa-Belancio and Deininger 2003; Han and Boeke 2004), (4) truncation of 5′-UTR sequences that inhibit reverse transcription during retrotransposition (Myers et al. 2002), and (5) recruitment of repressor proteins that interact with the L1 promoter or internal ribosome entry sites (Li et al. 2006; Montoya-Durango et al. 2009; Muotri et al. 2010).

To date, DNA methylation is the best characterized mechanism for silencing of retroelements in mammalian cells (Hata and Sakaki 1997; Steinhoff and Schulz 2003; Harony et al. 2006). L1 promoter hypomethylation has been described in several cancers, including testicular tumors (Bratthauer and Fanning 1992), urothelial bladder carcinoma (Florl et al. 1999), prostate carcinoma (Santourlidis et al. 1999), hepatocellular carcinoma (Lin et al. 2001), chronic lymphocytic leukemia (Dante et al. 1992), and chronic myeloid leukemia (Roman-Gomez et al. 2005). Previous studies in our laboratory have shown that chemical stressors such as BaP, dioxin, ultraviolet exposure, and gamma irradiation reactivate L1 transcription (Lu and Ramos 1998; Lu et al. 2000; Lu and Ramos 2003; Farkash et al. 2006; Stribinskis and Ramos 2006; Teneng et al. 2007), and this response is partly mediated via epigenetic mechanisms. The modulation of L1 by environmental stressors is biologically relevant since both BaP (Stribinskis and Ramos 2006) and gamma irradiation (Farkash et al. 2006) increase retrotransposition rates in mammalian cells.

Of note, within the realm of L1 epigenetics is the involvement of E2F/RB family complexes in the recruitment of transcriptional regulatory complexes to the L1 promoter (Montoya-Durango et al. 2009). The RB family of tumor suppressors (p107, pRB, and p130) plays a critical role in cellular senescence by forming transcriptional inhibitory complexes with E2F transcription factors that repress S-phase gene expression (Narita et al. 2003; Sebastian et al. 2005). The characterization of RB as a tumor suppressor was achieved through genetic studies of children affected by retinoblastoma (for review, see DeCaprio (2009)). pRb mutations lead to development of different tumors including osteosarcoma (Goodrich and Lee 1993), soft-tissue sarcomas (Moll et al. 2001), and DNA virus-induced cancers. Rb is a paramount regulator of cell cycle progression, and its mutation is required for cellular transformation (Wikenheiser-Brokamp 2006; Macpherson 2008). We have shown that removal of Rb protein family members is associated with both increased acetylation of nucleosomal histones at the L1 retroelement promoter and decreased histone epigenetic silencing marks, which together lead to endogenous L1 reactivation (Montoya-Durango et al. 2009). Using a tamoxifen-inducible system to remove E2F proteins from E2F targets on DNA, we have shown that E2F regulation of L1 is exerted primarily through epigenetic silencing rather than transcriptional activation, suggesting that E2F/Rb complexes have adapted to evolutionarily silence L1 sequences along with other repetitive elements. Thus, another

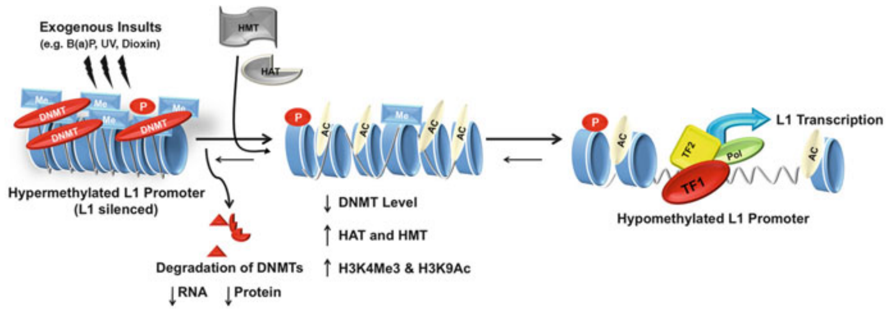

Fig. 6.6 Proposed model for epigenetic cross talk in the regulation of L1. Early events in L1 activation involve chromatin modifications with enrichment for H3K4Me3 and H3K9Ac which are hallmarks of active gene expression. During this phase we observe reduced DNMT RNA and protein levels probably due to degradation. As cells divide with reduced levels of DNA methyltransferase, L1 promoter becomes hypomethylated. Transcription factors then bind to the hypomethylated promoter, leading to transcriptional activation of L1 followed by retrotransposition. The plus sign depicts cross talk between the epigenetic marks on the histones

mechanism for Rb-induced tumorigenesis might involve L1 epigenetic reactivation and concomitant increased genomic instability (Montoya-Durango et al. 2009).

The human L1 promoter contains 34 CpGs spread over a 371bp CpG island within the promoter region (Fig. 6.6). As noted, methylated cytosines on CpG repeats are bound by methyl CpG-binding domain proteins (MBDs) to suppress gene expression by recruiting corepressor complexes (Brown et al. 2008). As such, these proteins act as scaffolds for recruitment of other chromatin-modifying proteins that promote further epigenetic silencing of target genomic regions (Hargreaves and Crabtree 2011) and serve as methylation-specific binding elements that promote epigenetic silencing of methylated DNA. Interestingly, we have shown that MBD2 is recruited to the human L1 promoter in human cervical cancer cells, and this recruitment is not significantly altered by short-term BaP treatment (Teneng et al. 2011). This is in agreement with our observation that CpG sites within the L1 promoter are subject to changes in methylation only following prolonged exposures to carcinogen treatment (Teneng et al. 2011). Our findings are consistent with previous work showing that BaP inhibits assembly of the DNA methylation machinery (Weisenberger and Romano 1999; Zhang et al. 2005) and deregulates DNA methylation in breast cancer cells (Sadikovic and Rodenhiser 2006). The modulation of L1 retrotransposon by BaP involves modulation of DNMT1 and DNMT3A protein levels via a proteasome-dependent pathway that may involve AHR (Teneng et al. 2011).

Of interest is the finding that BaP induces hypomethylation of cytosines across several transcriptionally active binding sites and has no effect on sites where putative transcription factor binding sites are absent. Further evidence has shown that genetic disruption of DNMT1 results in hypomethylation of several CpGs and increased steady-state levels of L1 transcripts. One issue that has not been addressed is whether the E3 ligase activity of AHR is required for DNMT1

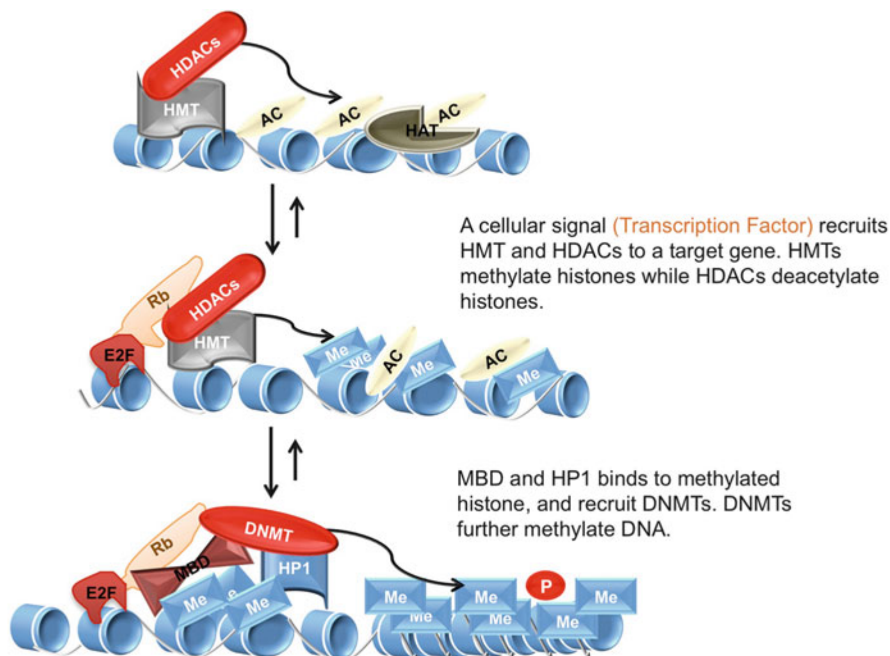

Fig. 6.7 Suggested mechanism for induction of DNA methylation by chromatin signals. Cellular signals cause recruitment of histone deacetylases (*HDAC*) and histone methylases (*HMT*). These modify histones in the vicinity, causing them to be recognized by factors including HP1 that in turn recruits DNMTs to methylate DNA. MBDs bind to methylated DNA and recruit HDAC and HMTs to modify surrounding histones, thus restarting the cycle

degradation (Ohtake et al. 2009). Given that there exists considerable cross talk between DNA methylation and chromatin covalent modifications on histones (Ben-Porath and Cedar 2001; Vaissiere et al. 2008), we have also analyzed chromatin isolated from HeLa cells and demonstrated that BaP enriched the L1 promoter for H3K4Me3 and H3K9Ac, hallmarks of transcriptionally active chromatin (Robertson et al. 2008). Time-course analyses indicated that while histone modifications were early events (12 h), DNA demethylation occurred late (after 96 h). These kinetic profiles indicate that reactivation likely involves early chromatin remodeling followed by decrease DNA methylation. A schematic representation of these relationships is depicted in Fig. 6.7. Thus, L1 elements, once thought to be silenced primarily by DNA methylation, are subject to complex regulatory mechanisms involving histone acetylation and methylation, and transcription factor recruitment for silencing and/or activation. This novel mechanism for retrotransposon activation by environmental stress and the role of changes in nucleosomal histone codes, DNMT availability, and CpG hypomethylation need to be further explored.

6.6 LINE-1, Differentiation, and Transformation

The most straightforward detrimental effect of L1 on the genome is believed to be insertion into functional sequences like exons, promoters, and enhancers, leading to severe compromise of gene function (Feng et al. 1996; Cost et al. 2002; Szak et al. 2002). Furthermore, L1 insertion into introns of genes can cause exon skipping or introduce alternate splicing sites (Narita et al. 1993; Mulhardt et al. 1994; Takahara et al. 1996). Recently, it was demonstrated that L1 sequences within transcriptional units affect both the structure of the target DNA and the amount of mRNA transcribed (Han and Boeke 2005). Of interest is the realization that over 75 % of human genes contain at least one L1 insertion, most of which are part of introns or 5′ and 3′ untranslated regions (Han et al. 2004).

In efforts to evaluate the impact of L1 ORF2 sequences within a transcription unit, L1 sequences have been fused to a LacZ construct downstream of a green fluorescent protein (GFP) reading frame to show that the presence of L1 ORF2 sequence reduced steady-state protein levels and led to a 70-fold decrease in RNA levels relative to LacZ upstream (Han et al. 2004). After introduction of frameshift mutations, this reduction was not affected, meaning that the RNA deficit results from the presence of ORF2 nucleotide sequence, and not the protein itself (Han et al. 2004; Belancio et al. 2006). Additionally, highly expressed genes contain small amounts of L1 sequences, while poorly expressed genes contained large amounts of L1 sequence (Belancio et al. 2006). The underrepresentation of L1 sequences in highly expressed genes suggests that L1 might “fine-tune” the genome, a hypothesis that we are pursuing in the laboratory at this time. Interestingly, L1 “carries” chunks of flanking DNA on retrotransposition (Cordaux and Batzer 2009) and, hence, is believed to provide promoter sequences to normally silenced genes, or repressor binding sites to new targets, which would in turn affect genes proximal to insertion sites. We have proposed that L1 is a center for formation of heterochromatin in regions outside of pericentromeric regions, hence making these elements an evolutionarily conserved mechanism for global regulation of chromatin status during development and disease.

Changes in L1 activity may be causative in some form or merely the result of generalized dysregulation of genetic programming. In keeping with this concepts, retroelements are transcriptionally repressed in terminally differentiated cells and being highly active in embryonic (undifferentiated) and transformed (dedifferentiated) cells (Poznanski and Calarco 1991; Packer et al. 1993). Additionally, RT activity is high in embryonic and transformed cells and low or undetectable in terminally differentiated cells (Deragon et al. 1990; Medstrand and Blomberg 1993). This inverse correlation between L1 expression and differentiation phenotype indicates that expression of retroelements may influence differentiation programming.

Using computational biology methodology, we identified genes predictive of L1 expression that may help to define L1 regulatory dynamic networks (Ramos et al. 2007). Genes within the network included sphingosine phosphate lyase 1 (SGPL1),

syndecan binding protein (SynBP), and microsomal glutathione S-transferase 1 (Mgst 1). Interestingly, members of the AHR superfamily were identified as a critical node, and siRNA targeting AHR established the biological connectivity of several genes within the network. Evidence for an *AHR-L1* axis includes the inhibition of BaP inducibility by α-NP, the refractoriness of AHR-null mouse vascular smooth muscle cells to BaP challenge, the inhibition of both endogenous L1 and *L1Md-A5* promoter activity by genetic knockdown of AHR in HeLa cells, and the restoration of promoter responsiveness following ectopic re-expression of the receptor.

L1 expression causes genomic instability, a hallmark of neoplasia (Gilbert et al. 2004). As noted earlier, increased L1 has been reported in urothelial bladder carcinoma, testicular tumors, hepatocellular carcinoma, chronic lymphocytic leukemia, prostate carcinoma, and chronic myeloid leukemia (Bratthauer and Fanning 1992; Dante et al. 1992; Florl et al. 1999; Santourlidis et al. 1999; Lin et al. 2001; Roman-Gomez et al. 2005). Full-length L1 mRNAs have been identified in human teratocarcinoma cells, but not in differentiated cell line counterparts (Skowronski and Singer 1985).

Of interest, within the context of L1 carcinogenesis is the reverse transcriptase (RT) encoded by L1 ORF2. Expression of RT coding genes is active in early embryos, germ cells, and tumor tissues, all characterized by high proliferative activity (Pittoggi et al. 2003). Generally, terminally differentiated cells do not express RT (Spadafora 2004), with inhibition of L1-encoded RT causing reduced tumor sizes in nude mice (Landriscina et al. 2005; Oricchio et al. 2007). Further, siRNA or pharmacological inhibition targeting RT reduces proliferation rates and reprograms gene expression in human tumorigenic cell lines (Mangiacasale et al. 2003; Oricchio et al. 2007). Together, these findings suggest that expression of L1 may inhibit or reverse differentiation programming in mammalian cells. In keeping with this hypothesis, we recently showed that kidney embryonic cells undergo L1 reactivation followed by changes in genetic programming of differentiation networks, suggesting that L1 is a master regulator of nephrogenesis by regulation of WT1 and AHR signaling networks (Ramos et al. 2011). Interestingly, L1 re-expression induced changes of genetic networks of differentiation that resemble the gene expression patterns induced by PAH treatment, suggesting that (i) L1 and PAHs target similar sets of genes and/or signaling pathways and (ii) L1-induced genetic network disruption mimics the effects of environmental carcinogens (Ramos et al. 2011). It is possible that ectopic expression of $L1^{RP}$ in mK4 cells causes reintegration of L1 proximal to differentiation marker genes which in turn silences the target gene by methylation. Although it is unknown how or why L1 would target these promoters, L1 retrotransposon insertions are "target-primed" events mostly targeting intron and noncoding genomic locations.

It has been reported that the human telomerase reverse transcriptase (TERT) activity is silent in terminally differentiated cells but reactivated in the majority of cancer cells (Liu et al. 2004). In fact, 90 % of tumors and cancers display high telomerase activity and readily detectable TERT expression (Meyerson 2000; Granger et al. 2002). Retrotransposons and viruses share the requirement of RT

for propagation. Interestingly, it has been reported that measles virus selectively induces apoptosis in terminally differentiated thymus epithelial cells while allowing nonterminally differentiated cells a survival advantage (Valentin et al. 1999). The mechanism by which selection for nonterminally differentiated cells is achieved remains unsolved, but it is possible that similar mechanisms are involved in the L1 RT model.

Interestingly, the effects of $L1^{RP}$ expression on renal cell differentiation did not require functional RT, leading to the conclusion that modulation of cellular phenotypes by L1 involves retrotransposition-dependent, as well as retrotransposition-independent, events. The ability of L1 to influence differentiation markers via retrotransposition-independent mechanisms suggests that the presence of L1 transcripts in cells may in of itself be sufficient to mediate effects on cellular phenotype. Detailed understanding of how L1-encoded proteins drive the loss of differentiation could identify L1-encoded proteins as therapeutic targets for inducing differentiation control. Lastly, it is important to acknowledge that L1 may influence cellular differentiation at the noncoding level, with the presence of fragments of L1 promoter sequences within intergenic regions triggering DNA methylation that spreads to neighboring genes.

6.7 Personalized Medicine

Personalized medicine is an emerging field that utilizes information about a person's genetic makeup to prevent, diagnose, and treat disease. Its application is touted by some as a means to establish earlier intervention and improve medical outcomes and more efficient drug development. As L1s continue to be active within the human genome, as evidenced by novel L1 insertion polymorphisms in human populations (Wang et al. 2006; Rouchka et al. 2010), mobile elements provide a likely mechanism of structural variation through de novo insertions and post-insertional rearrangements. Comparative analyses of the human and chimpanzee genomes indicate that recombination-associated genomic deletions represent a significant source of structural variation occurring since divergence of these two lineages around 6 million years ago (Han et al. 2008; Sen et al. 2006). L1 has produced approximately 8,000 human processed pseudogenes and one million human Alu, which are primate specific and created after the divergence between rodents and primates approximately 75 million years ago (Zhang et al. 2003). Although most new L1 insertions are found within introns and intergenic regions (Szak et al. 2002), some of these elements are located within genes. The activation and reintegration of retrotransposons into the genome has been linked to several diseases in human and rodents.

Greater than 50 retrotransposon or retrotransposon-mediated insertions have been linked to human disease (for review, see Belancio et al. 2008; Chen et al. 2005b). Many of the active L1 retrotransposons are polymorphic with respect to insertion presence or absence and have been used to study human evolution

(Sheen et al. 2000; Myers et al. 2002). Individual variation in retrotransposition capability may be an important contributor to human genetic diversity through processes such as recombination, insertional mutagenesis, gene conversion, and sequence transduction (Hedges and Deininger 2007; Seleme et al. 2006).

Variations in genotype and environment constitute the basis for the phenotypic differences that define individuals. In some instances, genotypic variation is directly responsible for disease phenotypes, while in others, variants coupled with environmental insult produce a disease phenotype. Research in the area of "personalized medicine" has been focused on understanding an individual's disease phenotype in terms of their genetic and epigenetic profile. Specifically, how these profiles relate to the etiology of their specific diseases and how this knowledge may be used to guide treatment are regarded as paramount.

This chapter, along with other contributions in the primary literature, has demonstrated that L1 insertions can have a profound impact on primary genetic structure and that coupled with epigenetic status, can dramatically affect the transcription of gene loci within or adjacent to sites of L1 insertion. The insertion of LINE-1s within the intronic regions of specific gene loci can result in functionally altered splice isoforms (Han et al. 2004; Han and Boeke 2005) for the corresponding transcript. Similarly, L1 insertions upstream of a gene, especially within the promoter region of the gene, may result, depending upon the epigenetic status of the L1, in either increased or decreased expression of that gene relative to normal levels (Han and Boeke 2005; Montoya-Durango et al. 2009). Also, antisense insertion of full-length L1 within a promoter relative to the orientation of that locus could drive ectopic expression of that locus off of the antisense promoter within the L1 element (Speek 2001).

It has been estimated that a new L1 insertion within the human genome occurs at between 1/95 and 1/270 live births (Ewing and Kazazian 2010). This activity has resulted in considerable variation between individuals and occurs on average, at 287 sites within the genome. Such heterogeneity, coupled with the impact of L1 on gene function and regulation, makes L1 an obvious and essential target of study for personalized medicine. Technological advances, most notably next-generation sequencing, have made it possible to assess an individual's L1 insertion profile. Within this context, work has been done on two fronts. First, the development of techniques that selectively amplify and sequence genomic regions that contain L1 elements (Beck et al. 2010; Ewing and Kazazian 2010, 2011; Huang et al. 2010) and, second, the characterization of L1 insertion profiles from whole-genome sequencing data (Ewing and Kazazian 2010, 2011; Rouchka et al. 2010). The latter approach is the one likely to be most viable on a long-term basis given that whole-genome sequencing will almost certainly become a clinical tool, and an individual's genome will likely be analyzed for any number of genetic variants, L1 included. Initial analysis of the newly released data from the 1,000 Genomes Project identified 22 previously unreported L1 insertion within the sequence data reported for a mother/father/daughter trio, suggesting that polymorphic expression of L1 in human populations may account for individual differences in disease susceptibility (Rouchka et al. 2010).

Therapeutic manipulation of L1 may prove particularly valuable, as successfully shown in the medical management of the AIDS epidemic using nucleoside reverse transcriptase inhibitors (nRTIs) and non-nucleoside reverse transcriptase inhibitors (nnRTIs). The human immunodeficiency virus (HIV), like L1, encodes reverse transcriptase. Nevirapine, an nnRTI, has been shown to inhibit embryonic endogenous reverse transcriptase in a murine model (Pittoggi et al. 2003), while other reverse transcriptase inhibitors have been shown to induce cell death and senescence in prostatic (Chen et al. 2005b), cervical (Bourc'his et al. 2001), and renal (Okano et al. 1998) tumor cell lines in vitro. Nude mice inoculated with melanoma, prostate, small cell, and colon carcinoma cells all exhibit tumor growth arrest or significant arrest (Moran et al. 1996). The antitumor effects of nRTIs may extend beyond just L1 inhibition as one group theorized that AZT's (an nRTI) ability to radiosensitize human malignant glioma cells was attributable to its effect on telomerase activity, another enzyme with reverse transcriptase activity.

Epigenomic therapy has a great deal of potential for a new wave of cancer treatments. Of particular interest is the emergence of histone deacetylase inhibitors (HDACi) and histone acetylase inhibitors (HATi) as drugs to regulate tumor biology. HDACi are being used for the treatment of leukemias caused by aberrant expression of chimeric proteins that repress differentiation genes. For instance, in acute promyelocytic leukemia (APL), a fusion between retinoic acid receptor alpha (RAR) and promyelocytic leukemia (PML) or promyelocytic leukemia zinc finger protein (PLZF) produces a chimeric form of RAR that abnormally recruits HDACs and represses transcription of myeloid lineage genes. As such, combined treatment with retinoic acid (RA) and HDAC inhibitors (HDACi) can lead to clinical remission. Likewise, the formation of a chimeric protein from AML and eight-twenty-one (AML-ETO) has been associated with recruitment of the N-CoR-mSin3-HDAC corepressor complex (Amann et al. 2001) and disruption of hematopoietic differentiation programs. Of relevance within the context of L1 biology is that overexpression of AML-ETO markedly decreases L1 transcriptional activity and retrotransposition in human cancer cell lines (Yang et al. 2003). Since gene expression requires changes in chromatin structure, namely, histone acetylation, and L1 is extensively regulated at the epigenetic level, treatment with HDACi may lead to global L1 reactivation and reopening of long stretches of chromatin that alter genes involved in regulation of differentiation. In principle, HATi should inhibit the uncontrolled reactivation of L1. The utility of these agents in the treatment of cancer has been examined in studies testing the effects of curcumin, a naturally occurring HATi, in the prevention and treatment of colorectal, prostate, kidney, lung, ovarian, breast, cervical, and liver cancers (Balasubramanyam et al. 2004). A major challenge that remains for many of these agents is their low permeability to the intracellular compartment. Likewise, isothiazolone has been shown to inhibit the enzymatic activity of both pCAF and p300 and to reduce cell proliferation of human ovarian and colon cancer cell lines (Stimson et al. 2005).

Continued elucidation of both upstream controls and downstream consequences of L1 and further research into the effects of reverse transcriptase inhibitors, HDACi, and HATi should soon open the door to clinical trials exploring the utility

of epigenetic therapies in disorders mediated by L1. These efforts in turn should promote awareness and interest among researchers, clinicians, and patients.

Clearly, the increase in L1 expression seen in diseased and undifferentiated cells represents a logical target for exploration both diagnostically and therapeutically. Precedence exists for measurement of embryonic markers in the serum, most notably in alpha-fetoprotein for hepatocellular carcinoma and yolk sac tumors as well as beta-HCG for some testicular carcinomas. Both these proteins are expressed in early development stages and are subsequently switched off in differentiated cell lines only to be reexpressed in the event of neoplasia. LINE-1's control over the expression of many elements of the genome may, in theory, suggest that increased retrotransposition may precede elevations in the aforementioned established biomarkers. The clear implication here is that L1 may hold potential as a biomarker whose elevation predates current markers, thus allowing for earlier diagnosis, treatment, and the potential for better outcomes. The contributions of personalized medicine may extend beyond individualized care, to improve population-wide disease outcomes by improving diagnostic prevention strategies.

6.8 Closing Remarks

One could conceive that retroelements are critically involved in a "survival-for-the-fittest" battle within the mammalian genome. Under healthy conditions, the mammalian genome has the upper hand due to silencing of L1 expression in terminally differentiated, somatic cells. Conversely, under stressful conditions the uncontrolled reactivation of L1 elements can wreak havoc in the genome. Continued progress in understanding L1 biology will allow for better understanding of L1-mediated effects on gene expression, gene disruption, genome evolution, differentiation, and disease.

References

Adhikary G, Crish JF, Bone F, Gopalakrishnan R, Lass J, Eckert RL (2005) An involucrin promoter AP1 transcription factor binding site is required for expression of involucrin in the corneal epithelium in vivo. Invest Ophthalmol Vis Sci 46:1219–1227

Amann JM, Nip J, Strom DK, Lutterbach B, Harada H, Lenny N, Downing JR, Meyers S, Hiebert SW (2001) ETO, a target of t(8;21) in acute leukemia, makes distinct contacts with multiple histone deacetylases and binds mSin3A through its oligomerization domain. Mol Cell Biol 21:6470–6483

Andersson P, McGuire J, Rubio C, Gradin K, Whitelaw ML, Pettersson S, Hanberg A, Poellinger L (2002) A constitutively active dioxin/aryl hydrocarbon receptor induces stomach tumors. Proc Natl Acad Sci U S A 99:9990–9995

Athanikar JN, Badge RM, Moran JV (2004) A YY1-binding site is required for accurate human LINE-1 transcription initiation. Nucleic Acids Res 32:3846–3855

Babushok DV, Kazazian HH Jr (2007) Progress in understanding the biology of the human mutagen LINE-1. Hum Mutat 28:527–539

Balasubramanyam K, Altaf M, Varier RA, Swaminathan V, Ravindran A, Sadhale PP, Kundu TK (2004) Polyisoprenylated benzophenone, garcinol, a natural histone acetyltransferase inhibitor, represses chromatin transcription and alters global gene expression. J Biol Chem 279:33716–33726

Banerjee G, Gupta N, Tiwari J, Raman G (2005) Ultraviolet-induced transformation of keratinocytes: possible involvement of long interspersed element-1 reverse transcriptase. Photodermatol Photoimmunol Photomed 21:32–39

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K (2007) High-resolution profiling of histone methylations in the human genome. Cell 129:823–837

Baylin SB, Esteller M, Rountree MR, Bachman KE, Schuebel K, Herman JG (2001) Aberrant patterns of DNA methylation, chromatin formation and gene expression in cancer. Hum Mol Genet 10:687–692

Beck CR, Collier P, Macfarlane C, Malig M, Kidd JM, Eichler EE, Badge RM, Moran JV (2010) LINE-1 retrotransposition activity in human genomes. Cell 141:1159–1170

Becker KG, Swergold GD, Ozato K, Thayer RE (1993) Binding of the ubiquitous nuclear transcription factor YY1 to a cis regulatory sequence in the human LINE-1 transposable element. Hum Mol Genet 2:1697–1702

Beischlag TV, Luis Morales J, Hollingshead BD, Perdew GH (2008) The aryl hydrocarbon receptor complex and the control of gene expression. Crit Rev Eukaryot Gene Expr 18:207–250

Belancio VP, Hedges DJ, Deininger P (2006) LINE-1 RNA splicing and influences on mammalian gene expression. Nucleic Acids Res 34:1512–1521

Belancio VP, Hedges DJ, Deininger P (2008) Mammalian non-LTR retrotransposons: for better or worse, in sickness and in health. Genome Res 18:343–358

Ben-Porath I, Cedar H (2001) Epigenetic crosstalk. Mol Cell 8:933–935

Bourc'his D, Bestor TH (2004) Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. Nature 431:96–99

Bourc'his D, Xu GL, Lin CS, Bollman B, Bestor TH (2001) Dnmt3L and the establishment of maternal genomic imprints. Science 294:2536–2539

Bratthauer GL, Fanning TG (1992) Active LINE-1 retrotransposons in human testicular cancer. Oncogene 7:507–510

Brauze D, Widerak M, Cwykiel J, Szyfter K, Baer-Dubowska W (2006) The effect of aryl hydrocarbon receptor ligands on the expression of AhR, AhRR, ARNT, Hif1alpha, CYP1A1 and NQO1 genes in rat liver. Toxicol Lett 167:212–220

Brouha B, Schustak J, Badge RM, Lutz-Prigge S, Farley AH, Moran JV, Kazazian HH Jr (2003) Hot L1s account for the bulk of retrotransposition in the human population. Proc Natl Acad Sci U S A 100:5280–5285

Brown SE, Suderman MJ, Hallett M, Szyf M (2008) DNA demethylation induced by the methyl-CpG-binding domain protein MBD3. Gene 420:99–106

Byun HM, Wong HL, Birnstein EA, Wolff EM, Liang G, Yang AS (2007) Examination of IGF2 and H19 loss of imprinting in bladder cancer. Cancer Res 67:10753–10758

Casavant NC, Hardies SC, Funk FD, Comer MB, Edgell MH, Hutchison CA 3rd (1988) Extensive movement of LINES ONE sequences in beta-globin loci of Mus caroli and Mus domesticus. Mol Cell Biol 8:4669–4674

Chen YH, Ramos KS (2000) A CCAAT/enhancer-binding protein site within antioxidant/electrophile response element along with CREB-binding protein participate in the negative regulation of rat GST-Ya gene in vascular smooth muscle cells. J Biol Chem 275:27366–27376

Chen ZX, Mann JR, Hsieh CL, Riggs AD, Chedin F (2005a) Physical and functional interactions between the human DNMT3L protein and members of the de novo methyltransferase family. J Cell Biochem 95:902–917

Chen JM, Stenson PD, Cooper DN, Ferec C (2005b) A systematic analysis of LINE-1 endonuclease-dependent retrotranspositional events causing human genetic disease. Hum Genet 117:411–427

Chen HL, Li T, Qiu XW, Wu J, Ling JQ, Sun ZH, Wang W, Chen W, Hou A, Vu TH, Hoffman AR, Hu JF (2006) Correction of aberrant imprinting of IGF2 in human tumors by nuclear transfer-induced epigenetic reprogramming. EMBO J 25:5329–5338

Cheng X, Blumenthal RM (2008) Mammalian DNA methyltransferases: a structural perspective. Structure 16:341–350

Clark SJ, Melki J (2002) DNA methylation and gene silencing in cancer: which is the guilty party? Oncogene 21:5380–5387

Claus R, Lubbert M (2003) Epigenetic targets in hematopoietic malignancies. Oncogene 22:6489–6496

Cordaux R, Batzer MA (2009) The impact of retrotransposons on human genome evolution. Nat Rev Genet 10:691–703

Cost GJ, Feng Q, Jacquier A, Boeke JD (2002) Human L1 element target-primed reverse transcription in vitro. EMBO J 21:5899–5910

Damiani LA, Yingling CM, Leng S, Romo PE, Nakamura J, Belinsky SA (2008) Carcinogen-induced gene promoter hypermethylation is mediated by DNMT1 and causal for transformation of immortalized bronchial epithelial cells. Cancer Res 68:9005–9014

Dante R, Dante-Paire J, Rigal D, Roizes G (1992) Methylation patterns of long interspersed repeated DNA and alphoid repetitive DNA from human cell lines and tumors. Anticancer Res 12:559–563

Davis HJ (1968) Gas chromatographic determination of benzo(a)pyrene in cigarette smoke. Anal Chem 40:1583–1585

DeCaprio JA (2009) How the Rb tumor suppressor structure and function was revealed by the study of Adenovirus and SV40. Virology 384:274–284

Deininger PL, Batzer MA (2002) Mammalian retroelements. Genome Res 12:1455–1465

Deragon JM, Sinnett D, Labuda D (1990) Reverse transcriptase activity from human embryonal carcinoma cells NTera2D1. EMBO J 9:3363–3368

Dombroski BA, Mathias SL, Nanthakumar E, Scott AF, Kazazian HH Jr (1991) Isolation of an active human transposable element. Science 254:1805–1808

Ehrlich M (2002) DNA methylation in cancer: too much, but also too little. Oncogene 21:5400–5413

Esteller M (2000) Epigenetic lesions causing genetic lesions in human cancer: promoter hypermethylation of DNA repair genes. Eur J Cancer 36:2294–2300

Ewing AD, Kazazian HH Jr (2010) High-throughput sequencing reveals extensive variation in human-specific L1 content in individual human genomes. Genome Res 20:1262–1270

Ewing AD, Kazazian HH Jr (2011) Whole-genome resequencing allows detection of many rare LINE-1 insertion alleles in humans. Genome Res 21:985–990

Fabbro C, Braghetta P, Girotto D, Piccolo S, Volpin D, Bressan GM (1999) Cell type-specific transcription of the alpha1(VI) collagen gene. Role of the AP1 binding site and of the core promoter. J Biol Chem 274:1759–1766

Falahatpisheh MH, Ramos KS (2003) Ligand-activated Ahr signaling leads to disruption of nephrogenesis and altered Wilms' tumor suppressor mRNA splicing. Oncogene 22:2160–2171

Fanning TG (1983) Size and structure of the highly repetitive BAM HI element in mice. Nucleic Acids Res 11:5073–5091

Farkash EA, Kao GD, Horman SR, Prak ET (2006) Gamma radiation increases endonuclease-dependent L1 retrotransposition in a cultured cell assay. Nucleic Acids Res 34:1196–1204

Farrar GJ, Kenna PF, Humphries P (2002) On the genetics of retinitis pigmentosa and on mutation-independent approaches to therapeutic intervention. EMBO J 21:857–864

Fawcett TW, Martindale JL, Guyton KZ, Hai T, Holbrook NJ (1999) Complexes containing activating transcription factor (ATF)/cAMP-responsive-element-binding protein (CREB)

interact with the CCAAT/enhancer-binding protein (C/EBP)-ATF composite site to regulate Gadd153 expression during the stress response. Biochem J 339(Pt 1):135–141

Feinberg AP, Vogelstein B (1983) Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature 301:89–92

Feng Q, Moran JV, Kazazian HH Jr, Boeke JD (1996) Human L1 retrotransposon encodes a conserved endonuclease required for retrotransposition. Cell 87:905–916

Florl AR, Lower R, Schmitz-Drager BJ, Schulz WA (1999) DNA methylation and expression of LINE-1 and HERV-K provirus sequences in urothelial and renal cell carcinomas. Br J Cancer 80:1312–1321

Friso S, Choi SW (2002) Gene-nutrient interactions and DNA methylation. J Nutr 132:2382S–2387S

Fukunaga BN, Probst MR, Reisz-Porszasz S, Hankinson O (1995) Identification of functional domains of the aryl hydrocarbon receptor. J Biol Chem 270:29270–29278

Furano AV, Robb SM, Robb FT (1988) The structure of the regulatory region of the rat L1 (L1Rn, long interspersed repeated) DNA family of transposable elements. Nucleic Acids Res 16:9215–9231

Gasior SL, Wakeman TP, Xu B, Deininger PL (2006) The human LINE-1 retrotransposon creates DNA double-strand breaks. J Mol Biol 357:1383–1393

Gilbert N, Doucet AJ, Bucheton A (2004) Genomic instability associated with human LINE-1 retrotransposition. J Soc Biol 198:419–424

Goll MG, Kirpekar F, Maggert KA, Yoder JA, Hsieh CL, Zhang X, Golic KG, Jacobsen SE, Bestor TH (2006) Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. Science 311:395–398

Goodier JL, Ostertag EM, Du K, Kazazian HH Jr (2001) A novel active L1 retrotransposon subfamily in the mouse. Genome Res 11:1677–11685

Goodrich DW, Lee WH (1993) Molecular characterization of the retinoblastoma susceptibility gene. Biochim Biophys Acta 1155:43–61

Granger MP, Wright WE, Shay JW (2002) Telomerase in cancer and aging. Crit Rev Oncol Hematol 41:29–40

Guil S, Esteller M (2009) DNA methylomes, histone codes and miRNAs: tying it all together. Int J Biochem Cell Biol 41:87–95

Han JS, Boeke JD (2004) A highly active synthetic mammalian retrotransposon. Nature 429:314–318

Han JS, Boeke JD (2005) LINE-1 retrotransposons: modulators of quantity and quality of mammalian gene expression? Bioessays 27:775–784

Han JS, Szak ST, Boeke JD (2004) Transcriptional disruption by the L1 retrotransposon and implications for mammalian transcriptomes. Nature 429:268–274

Han K, Lee J, Meyer TJ, Remedios P, Goodwin L, Batzer MA (2008) L1 recombination-associated deletions generate human genomic variation. Proc Natl Acad Sci U S A 105:19366–19371

Hann SR, Dixit M, Sears RC, Sealy L (1994) The alternatively initiated c-Myc proteins differentially regulate transcription through a noncanonical DNA-binding site. Genes Dev 8:2441–2452

Hargreaves DC, Crabtree GR (2011) ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. Cell Res 21:396–420

Harony H, Bernes S, Siman-Tov R, Ankri S (2006) DNA methylation and targeting of LINE retrotransposons in Entamoeba histolytica and Entamoeba invadens. Mol Biochem Parasitol 147:55–63

Hata K, Sakaki Y (1997) Identification of critical CpG sites for repression of L1 transcription by DNA methylation. Gene 189:227–234

Hattemer-Frey HA, Travis CC (1991) Benzo-a-pyrene: environmental partitioning and human exposure. Toxicol Ind Health 7:141–157

Hedges DJ, Deininger PL (2007) Inviting instability: transposable elements, double-strand breaks, and the maintenance of genome integrity. Mutat Res 616:46–59

Holderman MT, Miller KP, Dangott LJ, Ramos KS (2002) Identification of albumin precursor protein, Phi AP3, and alpha-smooth muscle actin as novel components of redox sensing machinery in vascular smooth muscle cells. Mol Pharmacol 61:1174–1183

Huang CR, Schneider AM, Lu Y, Niranjan T, Shen P, Robinson MA, Steranka JP, Valle D, Civin CI, Wang T, Wheelan SJ, Ji H, Boeke JD, Burns KH (2010) Mobile interspersed repeats are major structural variants in the human genome. Cell 141:1171–1182

Johnson CD, Balagurunathan Y, Lu KP, Tadesse M, Falahatpisheh MH, Carroll RJ, Dougherty ER, Afshari CA, Ramos KS (2003) Genomic profiles and predictive biological networks in oxidant-induced atherogenesis. Physiol Genomics 13:263–275

Jones B, Su H, Bhat A, Lei H, Bajko J, Hevi S, Baltus GA, Kadam S, Zhai H, Valdez R, Gonzalo S, Zhang Y, Li E, Chen T (2008) The histone H3K79 methyltransferase Dot1L is essential for mammalian development and heterochromatin structure. PLoS Genet 4:e1000190

Kato TA, Nagasawa H, Weil MM, Genik PC, Little JB, Bedford JS (2006) gamma-H2AX foci after low-dose-rate irradiation reveal ATM haploinsufficiency in mice. Radiat Res 166:47–54

Kazazian HH Jr, Moran JV (1998) The impact of L1 retrotransposons on the human genome. Nat Genet 19:19–24

Kazazian HH Jr, Wong C, Youssoufian H, Scott AF, Phillips DG, Antonarakis SE (1988) Haemophilia A resulting from de novo insertion of L1 sequences represents a novel mechanism for mutation in man. Nature 332:164–166

Kazerouni N, Sinha R, Hsu CH, Greenberg A, Rothman N (2001) Analysis of 200 food items for benzo[a]pyrene and estimation of its intake in an epidemiologic study. Food Chem Toxicol 39:423–436

Kedar PS, Widen SG, Englander EW, Fornace AJ Jr, Wilson SH (1991) The ATF/CREB transcription factor-binding site in the polymerase beta promoter mediates the positive effect of N-methyl-N′-nitro-N-nitrosoguanidine on transcription. Proc Natl Acad Sci U S A 88:3729–3733

Kerzee JK, Ramos KS (2000) Activation of c-Ha-ras by benzo(a)pyrene in vascular smooth muscle cells involves redox stress and aryl hydrocarbon receptor. Mol Pharmacol 58:152–158

Kim HG, Lee KW, Cho YY, Kang NJ, Oh SM, Bode AM, Dong Z (2008) Mitogen- and stress-activated kinase 1-mediated histone H3 phosphorylation is crucial for cell transformation. Cancer Res 68:2538–2547

Kimberland ML, Divoky V, Prchal J, Schwahn U, Berger W, Kazazian HH Jr (1999) Full-length human L1 insertions retain the capacity for high frequency retrotransposition in cultured cells. Hum Mol Genet 8:1557–1560

Kleymenova EV, Yuan X, LaBate ME, Walker CL (1998) Identification of a tumor-specific methylation site in the Wilms tumor suppressor gene. Oncogene 16:713–720

Kondo Y, Shen L, Cheng AS, Ahmed S, Boumber Y, Charo C, Yamochi T, Urano T, Furukawa K, Kwabi-Addo B, Gold DL, Sekido Y, Huang TH, Issa JP (2008) Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. Nat Genet 40:741–750

Kumar A, Bennetzen JL (1999) Plant retrotransposons. Annu Rev Genet 33:479–532

Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T,

Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Raymond C, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blöcker H, Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kasprzyk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ (2001) Initial sequencing and analysis of the human genome. Nature 409:860–921

Landriscina M, Fabiano A, Altamura S, Bagala C, Piscazzi A, Cassano A, Spadafora C, Giorgino F, Barone C, Cignarelli M (2005) Reverse transcriptase inhibitors down-regulate cell proliferation in vitro and in vivo and restore thyrotropin signaling and iodine uptake in human thyroid anaplastic carcinoma. J Clin Endocrinol Metab 90:5663–5671

Lee RF, Sauerheber R, Benson AA (1972) Petroleum hydrocarbons: uptake and discharge by the marine mussel Mytilus edulis. Science 177:344–346

Lee J, Cordaux R, Han K, Wang J, Hedges DJ, Liang P, Batzer MA (2007) Different evolutionary fates of recently integrated human and chimpanzee LINE-1 retrotransposons. Gene 390:18–27

Leng S, Stidley CA, Bernauer AM, Picchi MA, Sheng X, Frasco MA, Van Den Berg D, Gilliland FD, Crowell RE, Belinsky SA (2008) Haplotypes of DNMT1 and DNMT3B are associated with mutagen sensitivity induced by benzo[a]pyrene diol epoxide among smokers. Carcinogenesis 29:1380–1385

Li W, Zhang P, Fellers JP, Friebe B, Gill BS (2004) Sequence composition, organization, and evolution of the core Triticeae genome. Plant J 40:500–511

Li PW, Li J, Timmerman SL, Krushel LA, Martin SL (2006) The dicistronic RNA from the mouse LINE-1 retrotransposon contains an internal ribosome entry site upstream of each ORF: implications for retrotransposition. Nucleic Acids Res 34:853–864

Lin CH, Hsieh SY, Sheen IS, Lee WC, Chen TC, Shyu WC, Liaw YF (2001) Genome-wide hypomethylation in hepatocellular carcinogenesis. Cancer Res 61:4238–4243

Lindroth AM, Park YJ, McLean CM, Dokshin GA, Persson JM, Herman H, Pasini D, Miro X, Donohoe ME, Lee JT, Helin K, Soloway PD (2008) Antagonism between DNA and H3K27 methylation at the imprinted Rasgrf1 locus. PLoS Genet 4:e1000145

Linhart HG, Lin H, Yamada Y, Moran E, Steine EJ, Gokhale S, Lo G, Cantu E, Ehrich M, He T, Meissner A, Jaenisch R (2007) Dnmt3b promotes tumorigenesis in vivo by gene-specific de novo methylation and transcriptional silencing. Genes Dev 21:3110–3122

Liu L, Saldanha SN, Pate MS, Andrews LG, Tollefsbol TO (2004) Epigenetic regulation of human telomerase reverse transcriptase promoter activity during cellular differentiation. Genes Chromosomes Cancer 41:26–37

Lovsin N, Gubensek F, Kordi D (2001) Evolutionary dynamics in a novel L2 clade of non-LTR retrotransposons in Deuterostomia. Mol Biol Evol 18:2213–2224

Lu KP, Ramos KS (1998) Identification of genes differentially expressed in vascular smooth muscle cells following benzo[a]pyrene challenge: implications for chemical atherogenesis. Biochem Biophys Res Commun 253:828–833

Lu KP, Ramos KS (2003) Redox regulation of a novel L1Md-A2 retrotransposon in vascular smooth muscle cells. J Biol Chem 278:28201–28209

Lu KP, Hallberg LM, Tomlinson J, Ramos KS (2000) Benzo(a)pyrene activates L1Md retrotransposon and inhibits DNA repair in vascular smooth muscle cells. Mutat Res 454:35–44

Lu X, Simon MD, Chodaparambil JV, Hansen JC, Shokat KM, Luger K (2008) The effect of H3K79 dimethylation and H4K20 trimethylation on nucleosome and chromatin structure. Nat Struct Mol Biol 15:1122–1124

Lubbert M (2000) DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action. Curr Top Microbiol Immunol 249:135–164

Machala M, Vondracek J, Blaha L, Ciganek M, Neca JV (2001) Aryl hydrocarbon receptor-mediated activity of mutagenic polycyclic aromatic hydrocarbons determined using in vitro reporter gene assay. Mutat Res 497:49–62

Macpherson D (2008) Insights from mouse models into human retinoblastoma. Cell Div 3:9

Maier A, Schumann BL, Chang X, Talaska G, Puga A (2002) Arsenic co-exposure potentiates benzo[a]pyrene genotoxicity. Mutat Res 517:101–111

Majumder S, Ghoshal K, Datta J, Smith DS, Bai S, Jacob ST (2006) Role of DNA methyltransferases in regulation of human ribosomal RNA gene transcription. J Biol Chem 281:22062–22072

Mangiacasale R, Pittoggi C, Sciamanna I, Careddu A, Mattei E, Lorenzini R, Travaglini L, Landriscina M, Barone C, Nervi C, Lavia P, Spadafora C (2003) Exposure of normal and transformed cells to nevirapine, a reverse transcriptase inhibitor, reduces cell growth and promotes differentiation. Oncogene 22:2750–2761

Mastronardi FG, Wood DD, Mei J, Raijmakers R, Tseveleki V, Dosch HM, Probert L, Casaccia-Bonnefil P, Moscarello MA (2006) Increased citrullination of histone H3 in multiple sclerosis brain and animal models of demyelination: a role for tumor necrosis factor-induced peptidylarginine deiminase 4 translocation. J Neurosci 26:11387–11396

McClintock B (1950) The origin and behavior of mutable loci in maize. Proc Natl Acad Sci U S A 36:344–355

Medstrand P, Blomberg J (1993) Characterization of novel reverse transcriptase encoding human endogenous retroviral sequences similar to type A and type B retroviruses: differential transcription in normal human tissues. J Virol 67:6778–6787

Meyerson M (2000) Role of telomerase in normal and cancer cells. J Clin Oncol 18:2626–2634

Miller KP, Ramos KS (2001) Impact of cellular metabolism on the biological effects of benzo[a] pyrene and related hydrocarbons. Drug Metab Rev 33:1–35

Miller KP, Ramos KS (2005) DNA sequence determinants of nuclear protein binding to the c-Ha-ras antioxidant/electrophile response element in vascular smooth muscle cells: identification of Nrf2 and heat shock protein 90 beta as heterocomplex components. Cell Stress Chaperones 10:114–125

Miller KP, Chen YH, Hastings VL, Bral CM, Ramos KS (2000) Profiles of antioxidant/electrophile response element (ARE/EpRE) nuclear protein binding and c-Ha-ras transactivation in vascular smooth muscle cells treated with oxidative metabolites of benzo[a]pyrene. Biochem Pharmacol 60:1285–1296

Mills RE, Bennett EA, Iskow RC, Luttig CT, Tsui C, Pittard WS, Devine SE (2006) Recently mobilized transposons in the human and chimpanzee genomes. Am J Hum Genet 78:671–679

Minakami R, Kurose K, Etoh K, Furuhata Y, Hattori M, Sakaki Y (1992) Identification of an internal cis-element essential for the human L1 transcription and a nuclear factor(s) binding to the element. Nucleic Acids Res 20:3139–3145

Mirmohammadsadegh A, Marini A, Nambiar S, Hassan M, Tannapfel A, Ruzicka T, Hengge UR (2006) Epigenetic silencing of the PTEN gene in melanoma. Cancer Res 66:6546–6552
Moennikes O, Loeppen S, Buchmann A, Andersson P, Ittrich C, Poellinger L, Schwarz M (2004) A constitutively active dioxin/aryl hydrocarbon receptor promotes hepatocarcinogenesis in mice. Cancer Res 64:4707–4710
Moll AC, Imhof SM, Schouten-Van Meeteren AY, Kuik DJ, Hofman P, Boers M (2001) Second primary tumors in hereditary retinoblastoma: a register-based study, 1945–1997: is there an age effect on radiation-related risk? Ophthalmology 108:1109–1114
Montoya-Durango DE, Liu Y, Teneng I, Kalbfleisch T, Lacy ME, Steffen MC, Ramos KS (2009) Epigenetic control of mammalian LINE-1 retrotransposon by retinoblastoma proteins. Mutat Res 665:20–28
Moran JV, Holmes SE, Naas TP, DeBerardinis RJ, Boeke JD, Kazazian HH Jr (1996) High frequency retrotransposition in cultured mammalian cells. Cell 87:917–927
Morrish TA, Gilbert N, Myers JS, Vincent BJ, Stamato TD, Taccioli GE, Batzer MA, Moran JV (2002) DNA repair mediated by endonuclease-independent LINE-1 retrotransposition. Nat Genet 31:159–165
Mulhardt C, Fischer M, Gass P, Simon-Chazottes D, Guenet JL, Kuhse J, Betz H, Becker CM (1994) The spastic mouse: aberrant splicing of glycine receptor beta subunit mRNA caused by intronic insertion of L1 element. Neuron 13:1003–1015
Muotri AR, Marchetto MC, Coufal NG, Oefner R, Yeo G, Nakashima K, Gage FH (2010) L1 retrotransposition in neurons is modulated by MeCP2. Nature 468:443–446
Musova Z, Hedvicakova P, Mohrmann M, Tesarova M, Krepelova A, Zeman J, Sedlacek Z (2006) A novel insertion of a rearranged L1 element in exon 44 of the dystrophin gene: further evidence for possible bias in retroposon integration. Biochem Biophys Res Commun 347:145–149
Myers JS, Vincent BJ, Udall H, Watkins WS, Morrish TA, Kilroy GE, Swergold GD, Henke J, Henke L, Moran JV, Jorde LB, Batzer MA (2002) A comprehensive analysis of recently integrated human Ta L1 elements. Am J Hum Genet 71:312–326
Narita N, Nishio H, Kitoh Y, Ishikawa Y, Minami R, Nakamura H, Matsuo M (1993) Insertion of a 5′ truncated L1 element into the 3′ end of exon 44 of the dystrophin gene resulted in skipping of the exon during splicing in a case of Duchenne muscular dystrophy. J Clin Invest 91:1862–1867
Narita M, Nunez S, Heard E, Lin AW, Hearn SA, Spector DL, Hannon GJ, Lowe SW (2003) Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 113:703–716
Nightingale KP, Gendreizig S, White DA, Bradbury C, Hollfelder F, Turner BM (2007) Cross-talk between histone modifications in response to histone deacetylase inhibitors: MLL4 links histone H3 acetylation and histone H3K4 methylation. J Biol Chem 282:4408–4416
Ohshima K, Hattori M, Yada T, Gojobori T, Sakaki Y, Okada N (2003) Whole-genome screening indicates a possible burst of formation of processed pseudogenes and Alu repeats by particular L1 subfamilies in ancestral primates. Genome Biol 4:R74
Ohtake F, Fujii-Kuriyama Y, Kato S (2009) AhR acts as an E3 ubiquitin ligase to modulate steroid receptor functions. Biochem Pharmacol 77:474–484
Okano M, Xie S, Li E (1998) Dnmt2 is not required for de novo and maintenance methylation of viral DNA in embryonic stem cells. Nucleic Acids Res 26:2536–2540
Oricchio E, Sciamanna I, Beraldi R, Tolstonog GV, Schumann GG, Spadafora C (2007) Distinct roles for LINE-1 and HERV-K retroelements in cell proliferation, differentiation and tumor progression. Oncogene 26:4226–4233
Ostertag EM, Kazazian HH Jr (2001) Twin priming: a proposed mechanism for the creation of inversions in L1 retrotransposition. Genome Res 11:2059–2065
Packer AI, Manova K, Bachvarova RF (1993) A discrete LINE-1 transcript in mouse blastocysts. Dev Biol 157:281–283

Pan G, Tian S, Nie J, Yang C, Ruotti V, Wei H, Jonsdottir GA, Stewart R, Thomson JA (2007) Whole-genome analysis of histone H3 lysine 4 and lysine 27 methylation in human embryonic stem cells. Cell Stem Cell 1:299–312

Perepelitsa-Belancio V, Deininger P (2003) RNA truncation by premature polyadenylation attenuates human mobile element activity. Nat Genet 35:363–366

Pittoggi C, Sciamanna I, Mattei E, Beraldi R, Lobascio AM, Mai A, Quaglia MG, Lorenzini R, Spadafora C (2003) Role of endogenous reverse transcriptase in murine early embryo development. Mol Reprod Dev 66:225–236

Pollenz RS (2002) The mechanism of AH receptor protein down-regulation (degradation) and its impact on AH receptor-mediated gene regulation. Chem Biol Interact 141:41–61

Pollenz RS, Buggy C (2006) Ligand-dependent and -independent degradation of the human aryl hydrocarbon receptor (hAHR) in cell culture models. Chem Biol Interact 164:49–59

Poznanski AA, Calarco PG (1991) The expression of intracisternal A particle genes in the preimplantation mouse embryo. Dev Biol 143:271–281

Proffitt J, Crabtree G, Grove M, Daubersies P, Bailleul B, Wright E, Plumb M (1995) An ATF/CREB-binding site is essential for cell-specific and inducible transcription of the murine MIP-1 beta cytokine gene. Gene 152:173–179

Ramadoss P, Perdew GH (2005) The transactivation domain of the Ah receptor is a key determinant of cellular localization and ligand-independent nucleocytoplasmic shuttling properties. Biochemistry 44:11148–11159

Ramos KS, Falahatpisheh HM, Nanez A, He Q (2006) Modulation of biological regulatory networks during nephrogenesis. Drug Metab Rev 8(4):677–683

Ramos KS (2006) Transcriptional profiling and functional genomics reveal a role for AHR transcription factor in nephrogenesis. Ann N Y Acad Sci 1076:728–735

Ramos KS, Nanez A (2009) Genetic regulatory networks of nephrogenesis: deregulation of WT1 splicing by benzo(a)pyrene. Birth Defects Res C Embryo Today 87:192–197

Ramos KS, He Q, Kalbfleisch T, Montoya-Durango DE, Teneng I, Stribinskis V, Brun M (2007) Computational and biological inference of gene regulatory networks of the LINE-1 retrotransposon. Genomics 90:176–185

Ramos KS, Montoya-Durango DE, Teneng I, Nanez A, Stribinskis V (2011) Epigenetic control of embryonic renal cell differentiation by L1 retrotransposon. Birth Defects Res A Clin Mol Teratol 91:693–702

Realini CA, Althaus FR (1992) Histone shuttling by poly(ADP-ribosylation). J Biol Chem 267:18858–18865

Reik W, Dean W, Walter J (2001) Epigenetic reprogramming in mammalian development. Science 293:1089–1093

Reisz-Porszasz S, Probst MR, Fukunaga BN, Hankinson O (1994) Identification of functional domains of the aryl hydrocarbon receptor nuclear translocator protein (ARNT). Mol Cell Biol 14:6075–6086

Reznikoff WS (2003) Tn5 as a model for understanding DNA transposition. Mol Microbiol 47:1199–1206

Riclet R, Chendeb M, Vonesch JL, Koczan D, Thiesen HJ, Losson R, Cammas F (2009) Disruption of the interaction between transcriptional intermediary factor 1{beta} and heterochromatin protein 1 leads to a switch from DNA hyper- to hypomethylation and H3K9 to H3K27 trimethylation on the MEST promoter correlating with gene reactivation. Mol Biol Cell 20:296–305

Robertson AG, Bilenky M, Tam A, Zhao Y, Zeng T, Thiessen N, Cezard T, Fejes AP, Wederell ED, Cullum R, Euskirchen G, Krzywinski M, Birol I, Snyder M, Hoodless PA, Hirst M, Marra MA, Jones SJ (2008) Genome-wide relationship between histone H3 lysine 4 mono- and tri-methylation and transcription factor binding. Genome Res 18:1906–1917

Roepman R, van Duijnhoven G, Rosenberg T, Pinckers AJ, Bleeker-Wagemakers LM, Bergen AA, Post J, Beck A, Reinhardt R, Ropers HH, Cremers FP, Berger W (1996) Positional cloning

of the gene for X-linked retinitis pigmentosa 3: homology with the guanine-nucleotide-exchange factor RCC1. Hum Mol Genet 5:1035–1041

Roman-Gomez J, Jimenez-Velasco A, Agirre X, Cervantes F, Sanchez J, Garate L, Barrios M, Castillejo JA, Navarro G, Colomer D, Prosper F, Heiniger A, Torres A (2005) Promoter hypomethylation of the LINE-1 retrotransposable elements activates sense/antisense transcription and marks the progression of chronic myeloid leukemia. Oncogene 24:7213–7223

Roth SY, Denu JM, Allis CD (2001) Histone acetyltransferases. Annu Rev Biochem 70:81–120

Rouchka E, Montoya-Durango DE, Stribinskis V, Ramos K, Kalbfleisch T (2010) Assessment of genetic variation for the LINE-1 retrotransposon from next generation sequence data. BMC Bioinformatics 11(Suppl 9):S12

Rountree MR, Bachman KE, Herman JG, Baylin SB (2001) DNA methylation, chromatin inheritance, and cancer. Oncogene 20:3156–3165

Rowlands JC, Gustafsson JA (1997) Aryl hydrocarbon receptor-mediated signal transduction. Crit Rev Toxicol 27:109–134

Rugg-Gunn PJ, Ferguson-Smith AC, Pedersen RA (2005) Epigenetic status of human embryonic stem cells. Nat Genet 37:585–587

Ruthenburg AJ, Allis CD, Wysocka J (2007) Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. Mol Cell 25:15–30

Sadikovic B, Rodenhiser DI (2006) Benzopyrene exposure disrupts DNA methylation and growth dynamics in breast cancer cells. Toxicol Appl Pharmacol 216:458–468

Santos F, Hendrich B, Reik W, Dean W (2002) Dynamic reprogramming of DNA methylation in the early mouse embryo. Dev Biol 241:172–182

Santourlidis S, Florl A, Ackermann R, Wirtz HC, Schulz WA (1999) High frequency of alterations in DNA methylation in adenocarcinoma of the prostate. Prostate 39:166–174

Sassaman DM, Dombroski BA, Moran JV, Kimberland ML, Naas TP, DeBerardinis RJ, Gabriel A, Swergold GD, Kazazian HH Jr (1997) Many human L1 elements are capable of retrotransposition. Nat Genet 16:37–43

Schwahn U, Lenzner S, Dong J, Feil S, Hinzmann B, van Duijnhoven G, Kirschner R, Hemberger M, Bergen AA, Rosenberg T, Pinckers AJ, Fundele R, Rosenthal A, Cremers FP, Ropers HH, Berger W (1998) Positional cloning of the gene for X-linked retinitis pigmentosa 2. Nat Genet 19:327–332

Sebastian T, Malik R, Thomas S, Sage J, Johnson PF (2005) C/EBPbeta cooperates with RB:E2F to implement Ras(V12)-induced cellular senescence. EMBO J 24:3301–3312

Seleme MC, Vetter MR, Cordaux R, Bastone L, Batzer MA, Kazazian HH Jr (2006) Extensive individual variation in L1 retrotransposition capability contributes to human genetic diversity. Proc Natl Acad Sci U S A 103:6611–6616

Sen SK, Han K, Wang J, Lee J, Wang H, Callinan PA, Dyer M, Cordaux R, Liang P, Batzer MA (2006) Human genomic deletions mediated by recombination between Alu elements. Am J Hum Genet 79:41–53

Servomaa K, Rytomaa T (1990) UV light and ionizing radiations cause programmed death of rat chloroleukaemia cells by inducing retropositions of a mobile DNA element (L1Rn). Int J Radiat Biol 57:331–343

Sheen FM, Sherry ST, Risch GM, Robichaux M, Nasidze I, Stoneking M, Batzer MA, Swergold GD (2000) Reading between the LINEs: human genomic variation induced by LINE-1 retrotransposition. Genome Res 10:1496–1508

Shimizu Y, Nakatsuru Y, Ichinose M, Takahashi Y, Kume H, Mimura J, Fujii-Kuriyama Y, Ishikawa T (2000) Benzo[a]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. Proc Natl Acad Sci U S A 97:779–782

Siedlecki P, Zielenkiewicz P (2006) Mammalian DNA methyltransferases. Acta Biochim Pol 53:245–256

Skalka AM, Katz RA (2005) Retroviral DNA integration and the DNA damage response. Cell Death Differ 12(Suppl 1):971–978

Skowronski J, Singer MF (1985) Expression of a cytoplasmic LINE-1 transcript is regulated in a human teratocarcinoma cell line. Proc Natl Acad Sci U S A 82:6050–6054

Smit AF, Toth G, Riggs AD, Jurka J (1995) Ancestral, mammalian-wide subfamilies of LINE-1 repetitive sequences. J Mol Biol 246:401–417

Smith GP (1976) Evolution of repeated DNA sequences by unequal crossover. Science 191:528–535

Song Z, Pollenz RS (2002) Ligand-dependent and independent modulation of aryl hydrocarbon receptor localization, degradation, and gene regulation. Mol Pharmacol 62:806–816

Spadafora C (2004) Endogenous reverse transcriptase: a mediator of cell proliferation and differentiation. Cytogenet Genome Res 105:346–350

Speek M (2001) Antisense promoter of human L1 retrotransposon drives transcription of adjacent cellular genes. Mol Cell Biol 21:1973–1985

Squires S, Coates JA, Goldberg M, Toji LH, Jackson SP, Clarke DJ, Johnson RT (2004) p53 prevents the accumulation of double-strand DNA breaks at stalled-replication forks induced by UV in human cells. Cell Cycle 3:1543–1557

Steinhoff C, Schulz WA (2003) Transcriptional regulation of the human LINE-1 retrotransposon L1.2B. Mol Genet Genomics 270:94–402

Stimson L, Rowlands MG, Newbatt YM, Smith NF, Raynaud FI, Rogers P, Bavetsias V, Gorsuch S, Jarman M, Bannister A, Kouzarides T, McDonald E, Workman P, Aherne GW (2005) Isothiazolones as inhibitors of PCAF and p300 histone acetyltransferase activity. Mol Cancer Ther 4:1521–1532

Stribinskis V, Ramos KS (2006) Activation of human long interspersed nuclear element 1 retrotransposition by benzo(a)pyrene, an ubiquitous environmental carcinogen. Cancer Res 66:2616–2620

Suzuki K, Okada H, Yamauchi M, Oka Y, Kodama S, Watanabe M (2006) Qualitative and quantitative analysis of phosphorylated ATM foci induced by low-dose ionizing radiation. Radiat Res 165:499–504

Szak ST, Pickeral OK, Makalowski W, Boguski MS, Landsman D, Boeke JD (2002) Molecular archeology of L1 insertions in the human genome. Genome Biol 3:research0052

Takahara T, Ohsumi T, Kuromitsu J, Shibata K, Sasaki N, Okazaki Y, Shibata H, Sato S, Yoshiki A, Kusakabe M, Muramatsu M, Ueki M, Okuda K, Hayashizaki Y (1996) Dysfunction of the Orleans reeler gene arising from exon skipping due to transposition of a full-length copy of an active L1 sequence into the skipped exon. Hum Mol Genet 5:989–993

Tanaka H, Deng G, Matsuzaki K, Kakar S, Kim GE, Miura S, Sleisenger MH, Kim YS (2006) BRAF mutation, CpG island methylator phenotype and microsatellite instability occur more frequently and concordantly in mucinous than non-mucinous colorectal cancer. Int J Cancer 118:2765–2771

Teneng I, Stribinskis V, Ramos KS (2007) Context-specific regulation of LINE-1. Genes Cells 12:1101–1110

Teneng I, Montoya-Durango DE, Quertermous JL, Lacy ME, Ramos KS (2011) Reactivation of L1 retrotransposon by benzo(a)pyrene involves complex genetic and epigenetic regulation. Epigenetics 6:355–367

Thompson PR, Fast W (2006) Histone citrullination by protein arginine deiminase: is arginine methylation a green light or a roadblock? ACS Chem Biol 1:433–441

Vaissiere T, Sawan C, Herceg Z (2008) Epigenetic interplay between histone modifications and DNA methylation in gene silencing. Mutat Res 659:40–48

Valentin H, Azocar O, Horvat B, Williems R, Garrone R, Evlashev A, Toribio ML, Rabourdin-Combe C (1999) Measles virus infection induces terminal differentiation of human thymic epithelial cells. J Virol 73:2212–2221

Vogel CF, Sciullo E, Matsumura F (2004) Activation of inflammatory mediators and potential role of ah-receptor ligands in foam cell formation. Cardiovasc Toxicol 4:363–373

Wang J, Song L, Grover D, Azrak S, Batzer MA, Liang P (2006) dbRIP: a highly integrated database of retrotransposon insertion polymorphisms in humans. Hum Mutat 27:323–329

Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, Agarwala R, Ainscough R, Alexandersson M, An P, Antonarakis SE, Attwood J, Baertsch R, Bailey J, Barlow K, Beck S, Berry E, Birren B, Bloom T, Bork P, Botcherby M, Bray N, Brent MR, Brown DG, Brown SD, Bult C, Burton J, Butler J, Campbell RD, Carninci P, Cawley S, Chiaromonte F, Chinwalla AT, Church DM, Clamp M, Clee C, Collins FS, Cook LL, Copley RR, Coulson A, Couronne O, Cuff J, Curwen V, Cutts T, Daly M, David R, Davies J, Delehaunty KD, Deri J, Dermitzakis ET, Dewey C, Dickens NJ, Diekhans M, Dodge S, Dubchak I, Dunn DM, Eddy SR, Elnitski L, Emes RD, Eswara P, Eyras E, Felsenfeld A, Fewell GA, Flicek P, Foley K, Frankel WN, Fulton LA, Fulton RS, Furey TS, Gage D, Gibbs RA, Glusman G, Gnerre S, Goldman N, Goodstadt L, Grafham D, Graves TA, Green ED, Gregory S, Guigó R, Guyer M, Hardison RC, Haussler D, Hayashizaki Y, Hillier LW, Hinrichs A, Hlavina W, Holzer T, Hsu F, Hua A, Hubbard T, Hunt A, Jackson I, Jaffe DB, Johnson LS, Jones M, Jones TA, Joy A, Kamal M, Karlsson EK, Karolchik D, Kasprzyk A, Kawai J, Keibler E, Kells C, Kent WJ, Kirby A, Kolbe DL, Korf I, Kucherlapati RS, Kulbokas EJ, Kulp D, Landers T, Leger JP, Leonard S, Letunic I, Levine R, Li J, Li M, Lloyd C, Lucas S, Ma B, Maglott DR, Mardis ER, Matthews L, Mauceli E, Mayer JH, McCarthy M, McCombie WR, McLaren S, McLay K, McPherson JD, Meldrim J, Meredith B, Mesirov JP, Miller W, Miner TL, Mongin E, Montgomery KT, Morgan M, Mott R, Mullikin JC, Muzny DM, Nash WE, Nelson JO, Nhan MN, Nicol R, Ning Z, Nusbaum C, O'Connor MJ, Okazaki Y, Oliver K, Overton-Larty E, Pachter L, Parra G, Pepin KH, Peterson J, Pevzner P, Plumb R, Pohl CS, Poliakov A, Ponce TC, Ponting CP, Potter S, Quail M, Reymond A, Roe BA, Roskin KM, Rubin EM, Rust AG, Santos R, Sapojnikov V, Schultz B, Schultz J, Schwartz MS, Schwartz S, Scott C, Seaman S, Searle S, Sharpe T, Sheridan A, Shownkeen R, Sims S, Singer JB, Slater G, Smit A, Smith DR, Spencer B, Stabenau A, Stange-Thomann N, Sugnet C, Suyama M, Tesler G, Thompson J, Torrents D, Trevaskis E, Tromp J, Ucla C, Ureta-Vidal A, Vinson JP, Von Niederhausern AC, Wade CM, Wall M, Weber RJ, Weiss RB, Wendl MC, West AP, Wetterstrand K, Wheeler R, Whelan S, Wierzbowski J, Willey D, Williams S, Wilson RK, Winter E, Worley KC, Wyman D, Yang S, Yang SP, Zdobnov EM, Zody MC, Lander ES (2002) Initial sequencing and comparative analysis of the mouse genome. Nature 420:520–562

Weisenberger DJ, Romano LJ (1999) Cytosine methylation in a CpG sequence leads to enhanced reactivity with Benzo[a]pyrene diol epoxide that correlates with a conformational change. J Biol Chem 274:23948–23955

Whitelaw M, Pongratz I, Wilhelmsson A, Gustafsson JA, Poellinger L (1993) Ligand-dependent recruitment of the Arnt coregulator determines DNA recognition by the dioxin receptor. Mol Cell Biol 13:2504–2514

Wichmann AE, Thomson NM, Peterson LA, Wattenberg EV (2003) Genotoxic methylating agents modulate extracellular signal regulated kinase activity through MEK-dependent, glutathione-, and DNA methylation-independent mechanisms in lung epithelial cells. Chem Res Toxicol 16:87–94

Wikenheiser-Brokamp KA (2006) Retinoblastoma family proteins: insights gained through genetic manipulation of mice. Cell Mol Life Sci 63:767–780

Woodcock DM, Lawler CB, Linsenmeyer ME, Doherty JP, Warren WD (1997) Asymmetric methylation in the hypermethylated CpG promoter region of the human L1 retrotransposon. J Biol Chem 272:7810–7816

Xu C, Li CY, Kong AN (2005) Induction of phase I, II and III drug metabolism/transport by xenobiotics. Arch Pharm Res 28:249–268

Yang N, Kazazian HH Jr (2006) L1 retrotransposition is suppressed by endogenously encoded small interfering RNAs in human cultured cells. Nat Struct Mol Biol 13:763–771

Yang N, Zhang L, Zhang Y, Kazazian HH Jr (2003) An important role for RUNX3 in human L1 transcription and retrotransposition. Nucleic Acids Res 31:4929–4940

Yang N, Zhang L, Kazazian HH Jr (2005) L1 retrotransposon-mediated stable gene silencing. Nucleic Acids Res 33:e57

Zhang Z, Harrison PM, Liu Y, Gerstein M (2003) Millions of years of evolution preserved: a comprehensive catalog of the processed pseudogenes in the human genome. Genome Res 13:2541–2558

Zhang N, Lin C, Huang X, Kolbanovskiy A, Hingerty BE, Amin S, Broyde S, Geacintov NE, Patel DJ (2005) Methylation of cytosine at C5 in a CpG sequence context causes a conformational switch of a benzo[a]pyrene diol epoxide-N2-guanine adduct in DNA from a minor groove alignment to intercalation with base displacement. J Mol Biol 346:951–965

Zhou X, Sun H, Ellen TP, Chen H, Costa M (2008) Arsenite alters global histone H3 methylation. Carcinogenesis 29:1831–1836

Part III
Epigenetics, Gene Regulation, and Stem Cells

Chapter 7
Environmental Impact on Epigenetic Histone Language

John M. Denu

Abstract The epigenome of a cell or organism consists of chemical instructions acting with and upon genetic information to dictate gene expression. Such information exists in the form of noncoding RNAs, 5′ modifications at the DNA base cytosine, and combinatorial posttranslational modifications (PTMs) on histones. In this chapter, we explore the idea of a histone language written within the combinatorial complexity of PTMs. Examples of cross talk between DNA methylation and histone PTMs are presented. Environmental factors such as drugs and diet have profound impact on epigenetic information. Enzyme-catalyzed chromatin modification requires co-substrates that are key intermediates from major metabolic pathways. Links between metabolism and epigenetic mechanisms are discussed. Lastly, we discuss targeting the epigenome as a new paradigm for drug development.

Keywords Histone code • Histone language • Metabolism • Posttranslational modification • Methyltransferase • Acetyltransferase • Deacetylase • Drugs • Epigenetic • Epigenome

Abbreviations

AceCS	Acetyl-CoA synthetase
ACL	ATP citrate lyase
ATRX	Alpha-thalassemia X-linked mental retardation

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BET	Bromodomain and extra-terminal
BHC80	BRAF-histone deacetylase complex 80
ChIP	Chromatin immunoprecipitation
DNMT	DNA methyltransferase
HAT/KAT	Histone/lysine acetyltransferase
HDAC	Histone deacetylase
HP1	Heterochromatin protein 1
ING2	Inhibitor of growth 2
LSD1	Lysine-specific demethylase 1
NTDs	Neural tube closure defects
NUP98	Nucleoporin 98
PHD	Plant homeodomain
PRC	Polycomb repressive complex
PTM	Posttranslational modification
RAG2	Recombination activating gene 2
SAHA	Suberoylanilide hydroxamic acid
SAM	*S*-Adenosyl-L-methionine
SILAC	Stable isotope labeling with amino acids in cell culture
TAF3	TBP-associated factor 3
THF	Tetrahydrofolate
TSA	Trichostatin A
UHRF1	Ubiquitin-like containing PHD and RING finger domains 1

7.1 Introduction

Epigenetics is a rapidly growing field of study that refers to chemical instructions acting with and upon genetic information to dictate gene expression. The field of epigenetics is expanding at an exponential rate, owing in large part to the completion of the human genome-sequencing project (Lander et al. 2001; Venter et al. 2001). After this monumental task was accomplished, researchers soon realized that the strict DNA sequence was of limited use to explain the diversity of species, programs of development, cellular identity, and control of gene expression patterns. Epigenetic research aims to uncover how differences in cellular or organismal phenotypes are manifested through mechanisms other than differences in DNA sequences (Bernstein et al. 2007). Although the full extent and complexity of this epigenetic information remains to be discovered, it is generally agreed that this information exists in the form of noncoding RNAs, 5′ modifications at the DNA base cytosine, and a vast array of posttranslational modifications (PTMs) on

histones (Bonasio et al. 2010). In eukaryotic organisms, histones function as DNA spools, permitting the amazing compaction of DNA.

The definition of epigenetics has evolved since its original use by Waddington to refer aspects of differentiation and canalization of cells and tissues in a developing organism. Waddington refined his definition of epigenetics as "… the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being" (Waddington 1968). In 1990, Holliday wrote "Epigenetics can be defined as the study of the mechanisms of temporal and spatial control of gene activity during development of complex organisms. Mechanisms of epigenetic control must include the inheritance of particular spectrum of gene activities in each specialized cell. In addition to the classical DNA code, it is necessary to envisage the superimposition of an additional layer of information which comprises part of the hereditary material, and in many cases this is very stable. The term epigenetic inheritance has been introduced to describe this situation" (Holliday 1990).

A number of recent publications have attempted to define a more contemporary meaning (Berger et al. 2009; Bonasio et al. 2010; Riddihough and Zahn 2010). An encompassing definition comes from Jablonka and Lamb: "Epigenetics: The study, in both prokaryotes and eukaryotes, of the stochastic and inducible developmental processes that lead to long-term, persistent changes in gene activities and organismal states. Epigenetic inheritance is a component of epigenetics. It occurs when phenotypic variations (e.g., in gene expression or in the architecture of cellular complexes) that do not stem from variations in DNA base sequence are transmitted to subsequent generations of cells or organisms" (Jablonka and Lamb 2010). No doubt there has been considerable print devoted to defining the term "epigenetics," but as the field continues to grow and mature, narrow usage of the term will inevitably decline. To a great extent, epigenetics is "mechanistic biology." Therefore, it is most instructive to define epigenetics by simply describing the specific process under study.

In this chapter, we will focus on how histone modifications are dynamically controlled to elicit changes in gene expression. The discussions will explore histone PTMs as a coded language, the intimate connection between histone PTMs and DNA methylation, the influence of diet/metabolism on histone PTMs, and the therapeutic potential of targeting epigenetic mechanisms as drug development platforms.

7.2 Histone Modifications: A PTM Language

Epigenetic mechanisms involve chemical-based information that controls the expression of genes. In addition to 5′ modifications on the DNA base cytosine, histone proteins harbor an extensive set of dynamically controlled PTMs, which include acetylation, methylation, phosphorylation, and ubiquitylation (Kouzarides 2007) (Fig. 7.1). Chromatin consists of histone proteins (core histones H2A, H2B,

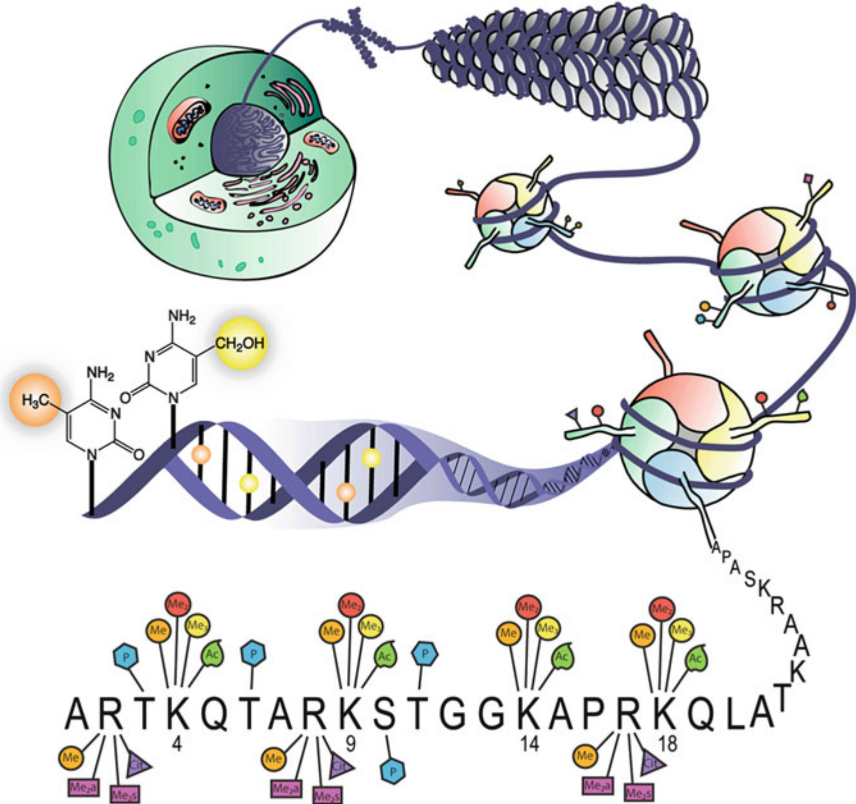

Fig. 7.1 Epigenetic information stored in DNA and histone modifications. DNA is wrapped around core histone proteins (H2A, H2B, H3, and H4), permitting compacting within the eukaryotic cell nucleus. The ability to access DNA for gene expression depends on the modification state of DNA and histones. The cytosine base of DNA can be modified by methylation and hydroxymethylation. Among the known posttranslational modifications, phosphorylation, methylation, and acetylation are shown on the first 18 amino acids of the H3 tail domain

H3, H4, and linker histones) that spool DNA and restrict the access of protein machines, which transcribe the DNA sequence into functional gene products (Luger 2003; Luger et al. 1997). The addition and removal of histone PTMs are catalyzed by enzymes such as histone/lysine acetyltransferases (HATs or KATs), histone deacetylases (HDACs), lysine/arginine methyltransferases and lysine demethylases, and protein kinases and phosphatases. The extensive modifications have suggested that histone PTMs may represent a code or a language that can be interpreted by the cell to elicit proper transcriptional responses (Jenuwein and Allis 2001; Strahl and Allis 2000; Turner 2000). The N-terminal tail domain of histone H3 is a prime example of the potential complexity of PTM combinations (Fig. 7.1). Seven of the first 10 amino acids of H3 can be modified by phosphorylation, acetylation, or methylation, yielding a possible 5,000 unique PTM states (Garske

et al. 2010). If one considers the remaining modifiable residues on the H3 tail domain, the number of permutations exceeds millions. Add to this number the occurrence of PTMs on other core histones H4, H2A, and H2B, as well as the existence of histone variants, and the number of possibilities becomes almost astronomical. Does nature functionally utilize this level of complexity? Is there built-in redundancy? These questions remain unanswered.

Over the last several years, a number of histone PTMs have been tied to specific molecular processes of gene expression or repression. The majority of these insights were distilled from genome-wide and locus-specific analyses employing ChIP (chromatin immunoprecipitation). Antibodies raised against a peptide that includes the modified form of the amino acid in question are used to enrich chromatin that harbors the specific PTM of interest. The associated DNA is determined by quantitative PCR, DNA arrays, or DNA sequencing. A number of interesting trends emerged from genome-wide studies (Barski et al. 2007; Wang et al. 2008). Acetylated lysine 9 and lysine 14 of H3 and acetylated lysine 8 and 16 on H4 are associated with “active” chromatin. Methylated lysine 4, 36, and 79 of H3 are associated with “active” chromatin. Lysine 9 and lysine 27 on histone H3 are associated with repressed genes. Lysine residues can be mono-, di-, or trimethylated, and the degree of methylation can signal different states. For instance, H3K4me1 marks enhancers of active genes, while H3K4me2/3 is associated with promoters and is important for transcriptional initiation. Although our understanding of the combinatorial complexity of the histone language is in its infancy, there are examples of connections between different PTMs (Table 7.1). This so-called cross talk has been noted between H2B ubiquitination at K120, which requires H3K79 methylation. Phosphorylation of H3T3 prevents the binding and recognition of H3K4me3 by chromatin-binding proteins. Similarly antagonism was observed with phosphorylation of serine 10 of histone H3 (H3S10ph), which is critical for the dissociation of HP1 (heterochromatin protein 1) from trimethylated lysine 9 of H3 (H3K9me3).

How do histone PTMs control chromatin function? The mapping of various histone PTMs within chromatin-enshrouded genes has provided important associations with gene expression, but the mechanisms by which PTMs control these functions are still poorly understood. Currently two general models can be proposed to help explain the functional outcomes of histone PTMs. The first is that histone PTMs directly affect the intrinsic dynamics of chromatin, allowing either less or greater access to the underlying DNA. Repressive chromatin (often referred to as heterochromatin) is characterized by chromatin structures that restrict DNA access, while open chromatin (euchromatin) is more permissive for gene expression. An example of this model comes from the acetylation of lysine 16 of histone H4 that completely abolishes the ability of the tail domains to mediate nucleosome-nucleosome interactions, which are required for chromatin condensation (Shogren-Knaak et al. 2006). Other support for altered intrinsic nucleosome dynamics comes from H3K56 acetylation, which is linked to replication-coupled nucleosome assembly, DNA damage repair, and stem cell regulatory genes. K56 sits at the end of helix 1 within the histone core domain and participates in electrostatic interactions with

Table 7.1 Abbreviated list of histone modifications, purported functional role(s), and their interconnection with other modifications

Histone modification	Functional role	Cross talk
Histone H3		
H3R2me2a	Transcriptional repression (Guccione et al. 2007)	Antagonizes H3K4 methylation (Guccione et al. 2007)
H3T3ph	Positioning of kinetochore during mitosis (Wang et al. 2010)	Antagonizes PHD finger binding of H3K4me3 (Garske et al. 2010)vp
H3K4me1	Marks enhancers of active genes (Heintzman et al. 2007)	
H3K4me2/3	Transcriptional activation (initiation) (Heintzman et al. 2007)	Antagonizes methylation of H3K9 (Binda et al. 2010)
H3K9me2/3	Transcriptional repression (Nakayama et al. 2001)	
H3K9ac	Transcriptional activation (Agalioti et al. 2002)	Promotes BRD4 bromodomain binding in concert with S10ph (Zippo et al. 2009)
H3S10ph	Chromosomal condensation and segregation during mitosis and meiosis (Wei et al. 1999); transcriptional activation (Ivaldi et al. 2007)	Antagonizes HP1 binding at H3K9me3 (Fischle et al. 2005). Promotes H3K14 and H4K16 acetylation (Zippo et al. 2009; Lo et al. 2000)
H3K14ac	Transcriptional activation (Agalioti et al. 2002; Kuo et al. 1996)	Required for 14-3-3 binding of H3S10ph (Walter et al. 2008)
H3K27me2/3	Transcriptional repression (Cao et al. 2002)	Forms bivalent domains with H3K4me3 (Bernstein et al. 2006)
H3K36me2	Transcriptional activation (elongation) (Kizer et al. 2005)	Coupled to deacetylation of transcribed genes (Keogh et al. 2005); antagonizes H3K27 methylation (Yuan et al. 2011)
H3K56ac	Replication-coupled nucleosome assembly (Han et al. 2007), DNA damage repair (Li et al. 2008), hESC core transcriptional network regulation (Xie et al. 2009)	
H3K79 me1/2/3	Transcriptional activation (elongation), where increasing methylation correlates to greater transcriptional activity (Steger et al. 2008), DNA damage (Huyen et al. 2004)	
Histone H4		
H4K5ac	DNA damage repair (Ikura et al. 2000)	
H4K8ac	Transcriptional activation (Agalioti et al. 2002)	
H4K12ac	Telomere plasticity (Zhou et al. 2011)	
H4K16ac	Transcriptional activation (Kapoor-Vazirani et al. 2011), chromatin structure (Shogren-Knaak et al. 2006), dosage compensation (Lavender et al. 1994)	

(continued)

Table 7.1 (continued)

Histone modification	Functional role	Cross talk
H4K20me3	Transcriptional repression (Congdon et al. 2010), genome stability (Tardat et al. 2007)	Antagonizes deposition of H4K16 acetylation during transcription3 (Kapoor-Vazirani et al. 2011)
H2A		
H2AK5ac	Stimulates H2A to H2A.Z exchange (Altaf et al. 2010)	
H2B		
H2BK120 ubiquitination	Transcriptional activation (elongation) (Kim et al. 2009)	Required for methylation of H3K79 (Ng et al. 2002); stimulates H3K4 methylation (Kim et al. 2009)

nucleosomal DNA. A biophysical investigation of nucleosomes containing H3K56 acetylation reported that acetylation increased DNA "breathing" near the H3K56 contact area (Neumann et al. 2009). An alternative conclusion was reached when Watanabe et al. characterized nucleosomes and nucleosomal arrays containing the H3K56Q substitution (Watanabe et al. 2010). Watanabe et al. observed no significant structural effects on nucleosomes but did report defects in interactions between nucleosome arrays.

A second model to explain the functional role of histone PTMs implicates "reader" proteins. Here, the model proposes that histone PTMs, in their proper context and with combinatorial complexity, direct the binding and action of enzyme/protein complexes that subsequently alter chromatin structure. There is now considerable evidence for the existence of a diverse set of "reader" proteins that recognize and bind to specific PTMs in a contextual manner (Garske et al. 2010; Taverna et al. 2007). These specialized histone-binding modules include bromodomains, chromodomains, and PHD (plant homeodomain) fingers, which recognize histones in a modification-dependent manner. There have been a number of excellent reviews on this topic, and the reader is directed to these papers for a detailed discussion (Lee et al. 2010; Oliver and Denu 2011; Taverna et al. 2007; Winter and Fischle 2010). Briefly, a few examples will be given. Chromatin-modifying enzymes are usually found in large multi-subunit complexes, and many of the individual proteins contain multi-domains. These modular domains, which are distinct for the catalytic domains, mediate additional interactions with chromatin, largely in a PTM-specific manner. One of the most prevalent modules are the PHD fingers, which are zinc-fingerlike domains containing ~60 amino acids and a Cys4-His-Cys3 motif that binds two zinc atoms (Musselman and Kutateladze 2009). There are ~100 PHD fingers predicted in the human genome and ~20 have been characterized with respect to their preferred binding specificity (Chi et al. 2010; Musselman and Kutateladze 2009). The tail domain of H3 is a preferred interaction site among analyzed PHD fingers. Generally, the characterized PHD

fingers read the methylation state of H3K4, with some preferring the unmodified state, while others prefer the hypermethylated form. In support for critical roles in transcriptional regulation, mutants in the PHD domain cause loss of function in a number of genes associated with cancer, immunodeficiency syndromes, and neurological disorders (Baker et al. 2008). A W453R mutation of the recombination activating gene 2 (RAG2)-PHD finger (found in patients with Omenn syndrome) impairs recognition of trimethylated H3K4 and proper antigen-receptor gene assembly during V(D)J recombination (Matthews et al. 2007). The TBP-associated factor 3 (TAF3), a component of RNA polymerase II (Vermeulen et al. 2007), and inhibitor of growth 2 (ING2) (Shi et al. 2006) contain PHD fingers that recognize the hypermethylated state of H3K4. In contrast, the PHD finger 1 of the autoimmune regulator binds unmethylated H3K4 and might function in targeting weakly expressed genes (Org et al. 2008). Interestingly, binding of the BRAF-histone deacetylase complex 80 (BHC80)-PHD finger to unmethylated H3K4 results in lysine-specific demethylase 1 (LSD1)-mediated gene repression (Lan et al. 2007). In a genetically well-defined acute myeloid leukemia, the PHD finger from the JARID1A lysine demethylase is fused to nucleoporin 98 (NUP98). This chromosomal translocation creates a NUP98-JARID1A fusion that inappropriately binds to genes harboring H3K4me3, preventing their demethylation and impairing proper differentiation of hematopoietic and progenitor cells (Wang et al. 2009a).

Several members of the jumonji family of lysine demethylases (namely, PHF8 and KIAA1718) contain an N-terminal PHD finger domain that directs substrate selection on the H3 tail domain (Horton et al. 2010). Both PHD fingers display strong preference for H3K4me3; however, PHF8 demethylates H3K9me2 and KIAA1718 demethylates H3K27me2. The distinct specificity stems from the structural differences with the linker region between the catalytic jumonji domain and the PHD finger. Engagement of the PHD finger at H3K4me3 places the catalytic domain of PHF8 at H3K9me2, whereas the engagement of the PHD finger from KIAA1718 places the catalytic domain at H3K27me2.

Chromodomains (chromatin organization modifiers) are another large group of chromatin-binding modules and are members of the so-called "Royal" family which include double chromodomain, chromo barrel, tudor, double/tandem tudor, and MBT (Taverna et al. 2007). The chromodomain was originally described as a sequence motif common to Drosophila proteins, polycomb (Pc), and heterochromatin protein 1 (HP1). It is now established that HP1 and Pc recognize the repressive H3 marks H3K9me3 and H3K27me3, respectively. HP1 mediates heterochromatin formation through the specific binding at H3K9me3. The ability to self-associate and bridge unique internucleosome contacts is important to induce repressive chromatin (Canzio et al. 2011). Methylation at H3K27 has been implicated in X-chromosome inactivation, stem cell pluripotency, and germ line development (Casanova et al. 2011). The polycomb repressive complex (PRC2) methylates H3K27 and, in cooperation with PRC1, binds to H3K27me3, inducing a heterochromatin-like state in repressed loci.

Bromodomains represent a chromatin-binding module that preferentially binds acetylated lysine residues within certain histone sequences. Accordingly,

bromodomains are generally associated with active chromatin. The human genome encodes 42 bromodomain-containing proteins (Sanchez and Zhou 2009). A number of HATs, ATP-remodeling complexes, and the transcriptional protein TAF1 (transcription initiation factor TFIID subunit 1) contain bromodomains. Generally, bromodomain-containing proteins are proposed to recruit transcriptional co-activators, ATP-remodeling complexes, and transcription initiation factors to activated genes (Taverna et al. 2007).

7.3 Cross Talk Between DNA Methylation and Histone PTMs

There is substantial evidence that certain DNA methylation patterns can be stably inherited through the germ line (transgenerational inheritance) (Lange and Schneider 2010; Skinner et al. 2010). Although the direct evidence for transgenerational inheritance of histone PTMs (marks) remains scarce, there is strong evidence for mitotic inheritance in which specific histone marks are maintained during replication of somatic cells (Probst et al. 2009; Xu and Zhu 2010). What is undeniable is the profound interconnectedness of DNA methylation, specific histone PTMs, and histone-modifying enzymes. Trimethylation of H3K4 inversely correlates with the occurrence of methyl-CpG. Functional studies of two H3K4 demethylases, LSD1 and LSD2, revealed an essential role in global DNA methylation and establishment of maternal DNA genomic imprints (Ciccone et al. 2009; Wang et al. 2009b). In plants, the histone H2A variant H2A.Z and DNA methylation do not coexist (Zilberman et al. 2008). In mammals, H2A.Z plays an essential role during development, but the mechanisms and the role of DNA methylases are unknown. In ES cells deficient in two H3K9 methyltransferases (Suv39h1 and Suv39h2), disruption of methylation in mouse major satellite tandem repeat DNA sequences was noted, suggesting a link between H3K9me and DNA methylation (Lehnertz et al. 2003). Alterations in methylation status of several imprinted genes were observed in mouse embryos deficient in EED (embryonic ectoderm development), a member of the PRC2 family that mediates H3K27methylation (Mager et al. 2003).

Recent studies have begun to provide a molecular understanding of the links between methyl DNA and histone PTM. Using nucleosomes methylated on DNA and/or on histone H3, Bartke et al. performed a detailed SILAC-based proteome analysis to interrogate the cross talk between DNA methylation and methylation at H3K4, H3K9, and H3K27 (Bartke et al. 2010). Most significantly, the quantitative proteomic analysis revealed that the binding of several chromatin complexes to nucleosomes are affected by the methylation status of both DNA and H3, while the binding of some complexes are affected by only one type of modification. Thus, chromatin-binding complexes can read the modification state of DNA and histones in the context of the nucleosome. The PRC2 complex was enriched on H3K27me3 nucleosomes, but the presence of methyl-CpG DNA antagonized binding. For UHRF1 (ubiquitin-like, containing PHD and RING finger domains 1), stronger

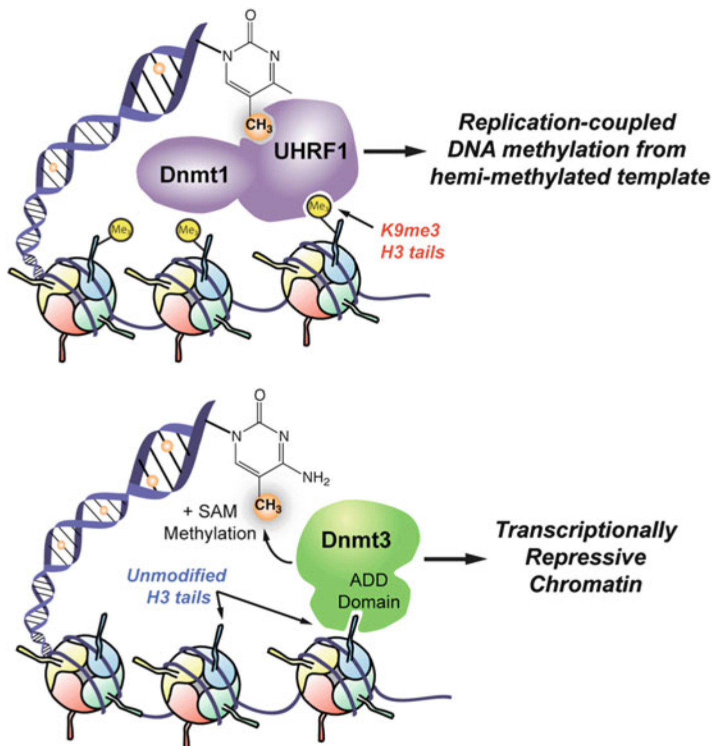

Fig. 7.2 Interconnections between DNA methylation and histone methylation. The *upper panel* shows the dual recognition of DNA methylation and histone methylation during replication-coupled DNA methylation from hemimethylated DNA. The *lower panel* shows histone PTM status directs DNA methylation to form repressive chromatin

cooperative binding was observed when H3K9me3 and methylated DNA were present in the nucleosome. Mice lacking the UHRF1 gene phenocopy those deficient in the DNA methyltransferase DNMT1 (Bostick et al. 2007; Sharif et al. 2007). UHRF1 is required for targeting DNMT1 to hemimethylated DNA and provides a mechanism for the accurate transmission of methylated DNA patterns during replication. At the molecular level, the tandem tudor domain of UHRF1 binds H3K9me3, while the SRA domain binds methylated CpG (Nady et al. 2011) (Fig. 7.2). The ability of UHRF1 complexes to recognized both H3K9me3 and methylated DNA might ensure faithful transmission of actively repressed genes during mitotic inheritance.

The DNMt3 family of DNA methyltransferases comprise DNMT3a and DNMT3b, as well as the DNMT3L (DNMT3-like), protein that lacks the catalytic domain and instead functions as an activator of DNMT3a and DNMT3b (Chedin 2011). These proteins contain a PHD-like ADD domain that specifically interacts with the unmodified state of H3K4, preferentially methylating linker DNA within nucleosomal substrates (Zhang et al. 2010) (Fig. 7.2). These mechanistic studies

corroborate genome-wide investigations that indicate DNA methylation inversely correlates with H3K4me3 (Meissner et al. 2008) as well as the observation that H3K4me demethylases (LSD1-2) are required for maintaining global DNA methylation patterns (Ciccone et al. 2009; Wang et al. 2009b). In the latter case, unmethylated H3K4 is critical for the ADD domain of DNMT3 proteins to access imprinted loci. Interestingly, the ATRX (alpha-thalassemia X-linked mental retardation) protein shares a similar ADD domain, in addition to its C-terminal ATPase/helicase domain. Nearly half of the mutations in the ATRX gene occur with the ADD domain, leading to mental retardation, facial dysmorphia, urogenital abnormalities, and alpha-thalassemia (Gibbons et al. 2008). Together, these observations suggest that ATRX function relies on the ability to recognize the PTM state of the H3 tail. Recently, Dhayalan et al. demonstrated that the ADD domain of ATRX displays preferential binding to H3 tail peptides that lack methylation on H3K4 but contain methylation of H3K9 (Dhayalan et al. 2011). The combination of H3K4/H3K9me3 modifications is characteristic of heterochromatin. Known disease-causing mutations in the ADD domain were shown to disrupt interactions with H3 (Dhayalan et al. 2011).

7.4 Chromatin Modification Requires Key Metabolites

The enzymes responsible for modifying histones and chromatin rely on co-substrates and coenzymes that are derived from central metabolism (Denu 2003; Smith and Denu 2009) (Table 7.2). Histone acetyltransferases use acetyl-CoA as the acyl donor, lysine and arginine methyltransferases use SAM (*S*-adenosyl-L-methionine) as the methyl donor, protein kinases transfer the terminal phosphate of ATP, and non-covalent nucleosome remodeling enzymes employ the energy from ATP hydrolysis to alter nucleosome structure or position. Enzymes that remove PTMs are also dependent on central metabolites (Table 7.2). For example, sirtuin deacetylases are completely dependent on NAD^+. Lysine demethylases LSD1/KDM1 use molecular oxygen and oxidized FAD to perform removal of lysine methyl groups, producing formaldehyde and H_2O_2. The lysine demethylase reaction catalyzed by the JHDM enzymes requires alpha-ketoglutarate and produces succinate and formaldehyde. Although there have been few studies that link histone PTMs with the levels of relevant metabolites, this will be an essential future area of investigation if we are to understand the full extent of these epigenetic modifications and how they are influence by diet, lifestyle, and environmental exposures. A few examples underscore the potentially profound impact on epigenetic mechanisms.

Table 7.2 Reactants and products of chromatin- and histone-modifying enzymes

Modification	Chromatin modifier	Reactants	Products
Acetylation	Acetyltransferases		
	GNAT family	Lysine, acetyl-CoA	Acetyl-lysine, CoA-SH
	MYST family		
	p300/CBP family		
Deacetylation	Histone deacetylases		
	Class I, II, IV HDACs	Acetyl-lysine	Lysine, acetate
	Class III HDACs: sirtuins	Acetyl-lysine, NAD^+	Lysine, O-acetyl-ADP ribose, nicotinamide
Lysine methylation	Lysine methyltransferases		
	SET domain methyltransferase	Lysine, *S*-adenosyl-L-methionine (SAM)	mono/di/trimethylated lysine, *S*-adenosyl-L-homocysteine (SAH)
	Dot1/KMT4 methyltransferase		
Lysine	demethylation	Demethylases	
	LSD1/KDM1 family	Methyl-lysine, FAD	Lysine, FADH2, formaldehyde
	JHDM family	Methyl-lysine, 2-oxoglutarate	Lysine, succinate, formaldehyde
Arginine methylation	Arginine methyltransferases		
	PRMTs	Arginine, *S*-adenosyl-L-methionine (SAM)	Methyl-arginine, *S*-adenosyl-L-homocysteine (SAH)
Arginine deimination	Protein arginine deiminases		
	PADs	Arginine	Citrulline, ammonia
Phosphorylation	Kinases		
	Haspin, Mst1, MSK1, CKII	Serine/threonine/tyrosine, ATP	Phospho-serine/threonine/tyrosine, ADP
Ubiquitination			
	Bmi/ring1A	Lysine, ubiquitin	Mono-ubiquitylated lysine
	RNF20/RNF40		
ADP-ribosylation			
	ADP-ribosyltransferases	Glutamate/arginine, NAD^+	ADP-ribosyl-glutamine/arginine, nicotinamide
Chromatin remodeling			
	SWI/SNF	ATP	ADP, remodeled chromatin

7.5 Acetyl-CoA and Histone Acetyltransferases

Histone acetyltransferases use acetyl-CoA as the acetyl donor during the transfer reaction. As with any enzymatic reaction, the rate of product formation depends on the concentration (or availability) of substrates, in this case, acetyl-CoA and histones. Acetyl-CoA is an essential intermediate in many catabolic and anabolic pathways. The acetyl-CoA pool in mitochondria functions mainly in energy-generating pathways and is generated primarily from pyruvate dehydrogenases, which provides 2-carbon fuels for the TCA cycle. In mammalian cytoplasm and

nuclei, ACL (ATP citrate lyase) and AceCS1 (acetyl-CoA synthetase) catalyze distinct reactions that generate acetyl-CoA (Wellen et al. 2009). ACL converts ATP and citrate to acetyl-CoA and oxaloacetate. AceCS1 produces acetyl-CoA from ATP, CoA, and acetate. The production of acetyl-CoA in the cytoplasm is utilized for lipid biosynthesis, but, more recently reported, this pool of acetyl-CoA appears to supply nuclear HATs with substrates for histone acetylation. SiRNA knockdown of ACL or AceCS1 reduced the global levels of histone acetylation, and the effect was additive when both enzymes were reduced in cells grown in glucose-rich media. When cells were treated with high levels of acetate, AceCS1 was able to compensate for loss of ACL. These observations suggest that histone acetylation depends not only to the levels of AceCS1 and ACL but on the levels of citrate, acetate, ATP, and CoA, which are determined by the metabolic state of the cell.

7.6 Folate Metabolism and Histone Methylation

It is well established that methyl-group donor metabolism plays an essential role in DNA methylation (Dolinoy et al. 2007; Stover 2009). However, the biochemical understanding of these connections remains elusive, and the extent to which we understand methyl-group donor metabolism and its regulation of histone methylation is even less. Treatment with methyl donors (e.g., folate) has the capacity to modulate DNA methylation, correcting aberrant methylation patterns of imprinted genes (Dolinoy et al. 2007; Dolinoy and Jirtle 2008) and affecting DNA methylation-based mechanisms of central nervous system repair after injury (Iskandar et al. 2010). During early human embryonic development, neural tube closure defects (NTDs) are some of the most common human birth defects, and clinical trials indicate that 70 % of NTD might be preventable by maternal folate supplementation (Beaudin and Stover 2009). Given our recent understanding of the epigenetic information contained within DNA methylation, these birth defects might be the consequence of inappropriate gene expression during normal developmental programs. Folate is a B vitamin that exists in cells as a group of enzyme cofactors that mediate many different 1-carbons reactions (Fox and Stover 2008; Tibbetts and Appling 2010). Folate cofactors are required for de novo synthesis of purines and thymidylate as well as the remethylation of homocysteine to methionine. The essential amino acid methionine is adenosylated to *S*-adenosylmethionine (SAM), which serves as the methyl-group donor for histones and cytosine bases of DNA, among other important reactions.

The inability to remethylate homocysteine elevates plasma homocysteine, decreases SAM, and increases *S*-adenosylhomocysteine (SAH). This decrease in SAM/SAH ratio is thought to be an indicator of methylation capacity, leading to hypomethylation of DNA (Finkelstein 2000; Friso et al. 2002), and presumably histones as well. Because of the tight connections among folate and methyl-donor metabolic networks, it has been difficult to isolate the direct influence of individual impairments on this complex pathway. Adding to the difficulty of resolving

aberrant effects, several studies suggest that cellular levels of folate cofactors are limiting, because the concentration of folate-dependent enzymes/proteins is greater than the concentration of folate cofactors, and the nanomolar binding affinity would predict that the available folate cofactors are protein bound (Herbig et al. 2002). Thus, the general lack of free folate metabolites would suggest that there is a highly complex competition among diverse pathways, creating a metabolic network that is exquisitely sensitive to various folate deficiencies.

Surprisingly little is known concerning the connection between folate metabolism and the control of histone methylation. However, a few recent studies suggest a critical link. Piyathilake et al. report an association between folate fortification and changes in histone methylation in cervical tissues (Piyathilake et al. 2010). The levels of H3K9me were assessed from cervical specimens obtained before (1990–1992) and after the mandatory folic acid fortification in the USA (2000–2002). Higher H3K9me was associated with progression to higher-grade cervical intraepithelial neoplasia (CIN). Among CIN grade 3 samples, higher levels of H3K9me were observed from the post-fortification group compared to that of the pre-fortification. However, among women free of CIN, the post-fortification group displayed lower H3K9me levels compared to those from the pre-fortification. It would appear that the effect of folate fortification yielded different effects depending on the stage of carcinogenesis. The mechanism behind these observations is unclear, but Piyathilake et al. suggest that DNMT1 may be involved (Piyathilake et al. 2010). DNMT expression is higher in CIN 3 lesions and may contribute to enhanced H3K9 methylation. The DNMT inhibitor 5-Aza-dC has been shown to reduce the levels of H3K9me and stimulate the re-expression of tumor-suppressor genes (Kondo et al. 2003). DNMT was shown to associate with HP1 and the K9 methyltransferase SUV39H1 (Fuks et al. 2003), providing a possible connection between DNA methylation, H3K9me, and carcinogenesis.

Using a genetic mouse model for neural tube development, Ichi et al. propose that the ability of folate to rescue the neural tube defects of *splotch*$^{-/-}$ involves the upregulation of a H3K27 demethylase (KDM6B), which lowers H3K27me2 levels at the promoters of two essential genes for neural tube development Hes1 and Neurog2 (Ichi et al. 2010). Neural tube defects of *splotch*$^{-/-}$ embryos can be prevented with addition of either folate or thymidylate, suggesting that the defect involves the arm of the folate pathway leading to nucleotide biosynthesis. These observations implicate an important relationship between folate metabolism and a role for H3K27 demethylation during neural tube development.

It is interesting to note that histone demethylase reactions produce formaldehyde. Formaldehyde is known to condense with tetrahydrofolate (THF) to yield methylene-THF, a key intermediate in folate-mediated 1-carbon metabolism. This suggests a possibility mechanism for replenishing one-carbon folate cofactors in the nucleus. In support of this idea, Luka et al. recently demonstrated that lysine demethylase LSD1 is capable of binding to folate metabolites, with the highest affinity observed with the natural pentaglutamate form of THF (Luka et al. 2011). The authors point out that demethylation reactions performed by dimethylglycine dehydrogenase and sarcosine dehydrogenase involve the condensation of

formaldehyde with protein-bound THF (Luka et al. 2011). Additional studies will be needed to test whether LSD1 operates in a similar manner and more generally to explore whether other histone demethylase reactions are coupled to methylene-THF formation. This would be a highly appealing model that recycles 1-carbon metabolites and provides yet another intricate link between (de)methylation and folate metabolism.

7.7 HDAC Inhibition from Metabolism

Aberrant HDAC activity has been associated with cancer and a number of other human pathologies. There is now considerable evidence that inhibition of the zinc-dependent HDACs has tremendous therapeutic potential against cancer (Kim and Bae 2011; Ma et al. 2009), neurodegeneration (Li et al. 2011), inflammatory diseases (Glauben and Siegmund 2011), autoimmune disorders (Faraco et al. 2011), memory loss (Guan et al. 2009; Dash et al. 2009), and anxiety disorders (Kaplan and Moore 2011). With respect to cancer, the therapeutic benefits of HDAC inhibition are proposed to operate through the derepression of tumor suppression genes. HDACs generally function to mediate gene repression by deacetylation histones and inducing chromatin structures that are repressive for transcription. Inhibition of HDACs tips the balance towards histone acetylation and promotes transcriptionally permission chromatin. HDAC inhibitors can trigger growth arrest, differentiation, and apoptosis. A discussion of pharmacological strategies is provided in the following section, but here, the role of diet and metabolism as a means to affect HDAC activity will be presented. It is important to note that HDACs have a number of nonhistone substrates, which does complicate a clear interpretation of the phenotypes associated with cellular inhibition of HDACs. There are newly emerging reports that the human diet offers a number of potential HDAC inhibitors, either acting directly or indirectly through metabolic conversion. Examples of these compounds include short-chain fatty acids, seleno-alpha-keto acids, thiol-containing small molecules, mercapturic acid metabolites, indoles, and polyphenols. For a detailed review, see (Rajendran et al. 2011). Three decades ago, the short-chain fatty acid butyrate, which is produced in the gut via fermentation of dietary fiber, was shown to promote cell differentiation and increase histone acetylation (Leder and Leder 1975). Butyrate functions as a competitive inhibitor of HDACs with a K_i of 46 mM (Sekhavat et al. 2007). Diets high in fiber have been linked to increased plasma butyrate levels, leading the authors of the study to postulate that such short-chain fatty acids might afford protection against cardiovascular disease and type 2 diabetes (Nilsson et al. 2010). A structural analog of butyrate, keto-methylselenobutyrate, and methylseleno-pyruvate was identified as competitive HDAC inhibitors (Lee et al. 2009; Nian et al. 2009). These seleno-alpha-keto acids are generated from dietary organoselenium compounds and might function in vivo as natural HDAC inhibitors. These observations suggested that other naturally occurring alpha-keto acids

might act as HDAC inhibitors. Indeed, the glycolytic product pyruvate inhibited HDAC activity, while lactate did not (Thangaraju et al. 2009). Interestingly, sodium-coupled monocarboxylate transporters (SCMTs) exert a tumor-suppressor function by controlling the intracellular concentrations of pyruvate, butyrate, and propionate (Ganapathy et al. 2008). Silencing of SCMTs in cancer cells and limiting pyruvate to lactate conversion led to increased HDAC activity and reduced apoptosis. Ganapathy et al. hypothesized that cancer cells might actively silence SCMTs and promote the reactions of pyruvate to lactate, thereby bypassing inhibiting of HDAC and induction apoptosis (Ganapathy et al. 2008).

Cruciferous vegetables are a rich source of glucosinolates. Broccoli is an abundant source of glucoraphanin, which is the precursor of sulforaphane (SFN), a well-established anticancer agent (Higdon et al. 2007). Metabolites of SFN inhibit HDAC activity and derepress tumor-suppressor genes, leading to cell cycle arrest and apoptosis. The active SFN metabolites are generated through the mercapturic acid pathway, which generates SFN-glutathione, SFN-cysteine and SFN-N-acetylcysteine (reviewed in Rajendran et al. 2011). Inhibition is most potent with SFN-cysteine, followed by SFN-N-acetylcysteine and SFN-glutathione. Molecular modeling suggests that SFN-cysteine binds to the active site of HDACs in analogous fashion to that of SAHA (suberoylanilide hydroxamic acid) and TSA (trichostatin A). TSA is a natural product hydroxamic acid from *Streptomyces platensis* and is a potent HDAC inhibitor (Yoshida et al. 1990). SAHA (vorinostat) is an FDA-approved HDAC inhibitor used to treat cutaneous T cell lymphoma (Duvic and Vu 2007; Marks and Breslow 2007).

7.8 Targeting the Epigenome as a New Paradigm for Drug Development: Epi-drugs

Rapidly accelerating research in chromatin and epigenetics has provided new opportunities for the development of novel therapeutics that target epigenetic mechanisms. The tremendous potential in focusing on dysregulated epigenetic pathways as a drug development strategy is supported by current FDA-approved drugs, vorinostat (SAHA), azacitidine (5-azacytidine), and decitabine (2′-deoxy-5-azacytidine) (Marks and Xu 2009; Scott and Deeg 2010). As noted above, SAHA is an inhibitor of histone deacetylases (HDACs) for the treatment of cutaneous T cell lymphoma (Duvic and Vu 2007; Marks and Breslow 2007). Azacitidine and decitabine are DNA methyltransferase inhibitors used as chemotherapeutics towards myelodysplastic syndromes (Keating 2009; Scott and Deeg 2010). The mechanism of action for such drugs is believed to involve the transcriptional derepression of tumor-suppressor genes (Xu et al. 2007). SAHA effectively increases histone acetylation and azacitidine induces hypomethylation, both mechanisms promoting activation of critical gene required for cell cycle arrest and/or apoptosis. The success of such compounds provides a compelling argument

for a new paradigm in developing other "epi-drugs" that take advantage of the specificity of the histone language written within the epigenome. As discussed earlier, the combination of PTMs in the proper context provides a "histone code" or "histone language" that is read and interpreted by an array of protein "readers" (Gardner et al. 2011; Oliver and Denu 2011). Among the protein "readers" are the bromodomain family that function as acetyl-lysine recognition motifs (Mujtaba et al. 2007; Sanchez and Zhou 2009). The bromodomain and extra-terminal (BET) subfamily are represented by BRD2, BRD3, BRD4, and BRDT (Zeng and Zhou 2002). Two recent investigations reported the development of small-molecule compounds that specifically disrupt the interactions between these bromodomains and acetylated histone tails (Filippakopoulos et al. 2010; Nicodeme et al. 2010). These studies offer proof of concept that pharmacological disruption of histone readers might be another strategy for the development of "epi-drugs."

Filippakopoulos et al. reported a cell-permeable small molecule, (+)-JQ1, that binds a subset of human bromodomains (e.g., BRD4) with nanomolar affinity and competes with acetyl-lysine-modified histone peptides (Filippakopoulos et al. 2010). BRD4 is a component of a recurrent chromosomal translocation found in aggressive human squamous carcinoma (Engleson et al. 2006; French et al. 2001, 2003). The translocations create an oncogenic fusion protein involving the tandem N-terminal bromodomains of BRD4 and the nuclear protein in testis (NUT). This genetically defined fatal malignancy offered a unique opportunity to investigate the ability of (+)-JQ1 to block the action of this misregulated chromatin "reader." Using several patient-derived xenograft mouse models, (+)-JQ1 treatment showed marked tumor regression and prolonged survival after 18 days of therapy. In a separate study, Nicodeme et al. describe the identification of I-BET, an optimized derivative of a benzodiazepine compound (Nicodeme et al. 2010) that targets inflammatory gene expression by disrupting the interaction of acetylated histones with the BET family of chromatin readers. The compound bound BRD2, BRD3, and BRD4 with similar low nanomolar affinity. Using a mouse model, I-BET treatment provided protection against lipopolysaccharide-induced endotoxic shock and bacteria-induced sepsis (Nicodeme et al. 2010). Gene expression and epigenetic analyses revealed I-BET-sensitive gene repression was characterized by low basal levels of histone H3 and H4 acetylation, trimethylation of Lys4 on histone H3 (H3K4me3), RNA Pol II, and low CpG content. The identification of new small molecules, (+)-JQ1 and I-BET, is encouraging prospects for new therapeutic approaches that directly target "readers" of chromatin PTMs.

Given our current understanding of epigenetic mechanisms, there is abundant rationale for the development of new "epi-drugs" that block the action of other dysregulated histone "readers" and modifying enzymes, particularly in cancer. For example, if one assumes that re-expression of inappropriately silenced tumor-suppressor genes is a major goal in antitumor therapies, then "epi-drugs" should be designed to target the lysine methyltransferases of H3K9 and H3K27 or lysine demethylases that target H3K4me3. Moreover, disrupting the "readers" of H3K9me3 and H3K27me3 offers another viable strategy to derepress tumor-suppressor genes. In genetically defined cancers like acute myeloid leukemia caused by

the NUP98-JARID1A fusion (Wang et al. 2009a), small-molecule disruptors that specifically target the interaction between the PHD finger from NUP98-JARID1A and H3K4me3 might provide a novel therapeutic for affected patients.

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References

Agalioti T, Chen G, Thanos D (2002) Deciphering the transcriptional histone acetylation code for a human gene. Cell 111:381–392

Altaf M, Auger A, Monnet-Saksouk J, Brodeur J, Piquet S, Cramet M, Bouchard N, Lacoste N, Utley RT, Gaudreau L, Cote J (2010) NuA4-dependent acetylation of nucleosomal histones H4 and H2A directly stimulates incorporation of H2A.Z by the SWR1 complex. J Biol Chem 285:15966–15977

Baker LA, Allis CD, Wang GG (2008) PHD fingers in human diseases: disorders arising from misinterpreting epigenetic marks. Mutat Res 647:3–12

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K (2007) High-resolution profiling of histone methylations in the human genome. Cell 129:823–837

Bartke T, Vermeulen M, Xhemalce B, Robson SC, Mann M, Kouzarides T (2010) Nucleosome-interacting proteins regulated by DNA and histone methylation. Cell 143:470–484

Beaudin AE, Stover PJ (2009) Insights into metabolic mechanisms underlying folate-responsive neural tube defects: a minireview. Birth Defects Res A Clin Mol Teratol 85:274–284

Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A (2009) An operational definition of epigenetics. Genes Dev 23:781–783

Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125:315–326

Bernstein BE, Meissner A, Lander ES (2007) The mammalian epigenome. Cell 128:669–681

Binda O, LeRoy G, Bua DJ, Garcia BA, Gozani O, Richard S (2010) Trimethylation of histone H3 lysine 4 impairs methylation of histone H3 lysine 9: regulation of lysine methyltransferases by physical interaction with their substrates. Epigenetics 5:767–775

Bonasio R, Tu S, Reinberg D (2010) Molecular signals of epigenetic states. Science 330:612–616

Bostick M, Kim JK, Esteve PO, Clark A, Pradhan S, Jacobsen SE (2007) UHRF1 plays a role in maintaining DNA methylation in mammalian cells. Science 317:1760–1764

Canzio D, Chang EY, Shankar S, Kuchenbecker KM, Simon MD, Madhani HD, Narlikar GJ, Al-Sady B (2011) Chromodomain-mediated oligomerization of HP1 suggests a nucleosome-bridging mechanism for heterochromatin assembly. Mol Cell 41:67–81

Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y (2002) Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 298:1039–1043

Casanova M, Preissner T, Cerase A, Poot R, Yamada D, Li X, Appanah R, Bezstarosti K, Demmers J, Koseki H, Brockdorff N (2011) Polycomblike 2 facilitates the recruitment of PRC2 Polycomb group complexes to the inactive X chromosome and to target loci in embryonic stem cells. Dev Suppl 138:1471–1482

Chedin F (2011) The DNMT3 family of mammalian de novo DNA methyltransferases. Prog Mol Biol Transl Sci 101:255–285

Chi P, Allis CD, Wang GG (2010) Covalent histone modifications–miswritten, misinterpreted and mis-erased in human cancers. Nat Rev Cancer 10:457–469

Ciccone DN, Su H, Hevi S, Gay F, Lei H, Bajko J, Xu G, Li E, Chen T (2009) KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. Nature 461:415–418

Congdon LM, Houston SI, Veerappan CS, Spektor TM, Rice JC (2010) PR-Set7-mediated monomethylation of histone H4 lysine 20 at specific genomic regions induces transcriptional repression. J Cell Biochem 110:609–619

Dash PK, Orsi SA, Moore AN (2009) Histone deacetylase inhibition combined with behavioral therapy enhances learning and memory following traumatic brain injury. Neuroscience 163:1–8

Denu JM (2003) Linking chromatin function with metabolic networks: Sir2 family of NAD(+)-dependent deacetylases. Trends Biochem Sci 28:41–48

Dhayalan A, Tamas R, Bock I, Tattermusch A, Dimitrova E, Kudithipudi S, Ragozin S, Jeltsch A (2011) The ATRX-ADD domain binds to H3 tail peptides and reads the combined methylation state of K4 and K9. Hum Mol Genet 20:2195–2203

Dolinoy DC, Jirtle RL (2008) Environmental epigenomics in human health and disease. Environ Mol Mutagen 49:4–8

Dolinoy DC, Huang D, Jirtle RL (2007) Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci USA 104:13056–13061

Duvic M, Vu J (2007) Vorinostat: a new oral histone deacetylase inhibitor approved for cutaneous T-cell lymphoma. Expert Opin Investig Drugs 16:1111–1120

Engleson J, Soller M, Panagopoulos I, Dahlen A, Dictor M, Jerkeman M (2006) Midline carcinoma with t(15;19) and BRD4-NUT fusion oncogene in a 30-year-old female with response to docetaxel and radiotherapy. BMC Cancer 6:69

Faraco G, Cavone L, Chiarugi A (2011) The therapeutic potential of HDAC inhibitors in the treatment of multiple sclerosis. Mol Med 17:442–447

Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I, Philpott M, Munro S, McKeown MR, Wang Y, Christie AL, West N, Cameron MJ, Schwartz B, Heightman TD, La Thangue N, French CA, Wiest O, Kung AL, Knapp S, Bradner JE (2010) Selective inhibition of BET bromodomains. Nature 468:1067–1073

Finkelstein JD (2000) Homocysteine: a history in progress. Nutr Rev 58:193–204

Fischle W, Tseng BS, Dormann HL, Ueberheide BM, Garcia BA, Shabanowitz J, Hunt DF, Funabiki H, Allis CD (2005) Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. Nature 438:1116–1122

Fox JT, Stover PJ (2008) Folate-mediated one-carbon metabolism. Vitam Horm 79:1–44

French CA, Miyoshi I, Aster JC, Kubonishi I, Kroll TG, Dal Cin P, Vargas SO, Perez-Atayde AR, Fletcher JA (2001) BRD4 bromodomain gene rearrangement in aggressive carcinoma with translocation t(15;19). Am J Pathol 159:1987–1992

French CA, Miyoshi I, Kubonishi I, Grier HE, Perez-Atayde AR, Fletcher JA (2003) BRD4-NUT fusion oncogene: a novel mechanism in aggressive carcinoma. Cancer Res 63:304–307

Friso S, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, Olivieri O, Jacques PF, Rosenberg IH, Corrocher R, Selhub J (2002) A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci U S A 99:5606–5611

Fuks F, Hurd PJ, Deplus R, Kouzarides T (2003) The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. Nucleic Acids Res 31:2305–2312

Ganapathy V, Thangaraju M, Gopal E, Martin PM, Itagaki S, Miyauchi S, Prasad PD (2008) Sodium-coupled monocarboxylate transporters in normal tissues and in cancer. AAPS J 10:193–199

Gardner KE, Allis CD, Strahl BD (2011) Operating on chromatin, a colorful language where context matters. J Mol Biol 409:36–46

Garske AL, Oliver SS, Wagner EK, Musselman CA, LeRoy G, Garcia BA, Kutateladze TG, Denu JM (2010) Combinatorial profiling of chromatin binding modules reveals multisite discrimination. Nat Chem Biol 6:283–290

Gibbons RJ, Wada T, Fisher CA, Malik N, Mitson MJ, Steensma DP, Fryer A, Goudie DR, Krantz ID, Traeger-Synodinos J (2008) Mutations in the chromatin-associated protein ATRX. Hum Mutat 29:796–802

Glauben R, Siegmund B (2011) Inhibition of histone deacetylases in inflammatory bowel diseases. Mol Med 17:426–433

Guan JS, Haggarty SJ, Giacometti E, Dannenberg JH, Joseph N, Gao J, Nieland TJ, Zhou Y, Wang X, Mazitschek R, Bradner JE, DePinho RA, Jaenisch R, Tsai LH (2009) HDAC2 negatively regulates memory formation and synaptic plasticity. Nature 459:55–60

Guccione E, Bassi C, Casadio F, Martinato F, Cesaroni M, Schuchlautz H, Luscher B, Amati B (2007) Methylation of histone H3R2 by PRMT6 and H3K4 by an MLL complex are mutually exclusive. Nature 449:933–937

Han J, Zhou H, Li Z, Xu RM, Zhang Z (2007) Acetylation of lysine 56 of histone H3 catalyzed by RTT109 and regulated by ASF1 is required for replisome integrity. J Biol Chem 282:28587–28596

Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, Barrera LO, Van Calcar S, Qu C, Ching KA, Wang W, Weng Z, Green RD, Crawford GE, Ren B (2007) Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. Nat Genet 39:311–318

Herbig K, Chiang EP, Lee LR, Hills J, Shane B, Stover PJ (2002) Cytoplasmic serine hydroxymethyltransferase mediates competition between folate-dependent deoxyribonucleotide and *S*-adenosylmethionine biosyntheses. J Biol Chem 277:38381–38389

Higdon JV, Delage B, Williams DE, Dashwood RH (2007) Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. Pharmacol Res 55:224–236

Holliday R (1990) Mechanisms for the control of gene activity during development. Biol Rev Camb Philos Soc 65:431–471

Horton JR, Upadhyay AK, Qi HH, Zhang X, Shi Y, Cheng X (2010) Enzymatic and structural insights for substrate specificity of a family of jumonji histone lysine demethylases. Nat Struct Mol Biol 17:38–43

Huyen Y, Zgheib O, Ditullio RA Jr, Gorgoulis VG, Zacharatos P, Petty TJ, Sheston EA, Mellert HS, Stavridi ES, Halazonetis TD (2004) Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. Nature 432:406–411

Ichi S, Costa FF, Bischof JM, Nakazaki H, Shen YW, Boshnjaku V, Sharma S, Mania-Farnell B, McLone DG, Tomita T, Soares MB, Mayanil CS (2010) Folic acid remodels chromatin on Hes1 and Neurog2 promoters during caudal neural tube development. J Biol Chem 285:36922–36932

Ikura T, Ogryzko VV, Grigoriev M, Groisman R, Wang J, Horikoshi M, Scully R, Qin J, Nakatani Y (2000) Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis. Cell 102:463–473

Iskandar BJ, Rizk E, Meier B, Hariharan N, Bottiglieri T, Finnell RH, Jarrard DF, Banerjee RV, Skene JH, Nelson A, Patel N, Gherasim C, Simon K, Cook TD, Hogan KJ (2010) Folate regulation of axonal regeneration in the rodent central nervous system through DNA methylation. J Clin Invest 120:1603–1616

Ivaldi MS, Karam CS, Corces VG (2007) Phosphorylation of histone H3 at Ser10 facilitates RNA polymerase II release from promoter-proximal pausing in Drosophila. Genes Dev 21:2818–2831

Jablonka E, Lamb MJ (2010) Transgenerational epigenetic inheritance. MIT Press, Cambridge, MA

Jenuwein T, Allis CD (2001) Translating the histone code. Science 293:1074–1080

Kaplan GB, Moore KA (2011) The use of cognitive enhancers in animal models of fear extinction. Pharmacol Biochem Behav 99:217–228

Kapoor-Vazirani P, Kagey JD, Vertino PM (2011) SUV420H2-mediated H4K20 trimethylation enforces RNA polymerase II promoter-proximal pausing by blocking hMOF-dependent H4K16 acetylation. Mol Cell Biol 31:1594–1609

Keating GM (2009) Azacitidine: a review of its use in higher-risk myelodysplastic syndromes/ acute myeloid leukaemia. Drugs 69:2501–2518

Keogh MC, Kurdistani SK, Morris SA, Ahn SH, Podolny V, Collins SR, Schuldiner M, Chin K, Punna T, Thompson NJ, Boone C, Emili A, Weissman JS, Hughes TR, Strahl BD, Grunstein M, Greenblatt JF, Buratowski S, Krogan NJ (2005) Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. Cell 123:593–605

Kim HJ, Bae SC (2011) Histone deacetylase inhibitors: molecular mechanisms of action and clinical trials as anti-cancer drugs. Am J Transl Res 3:166–179

Kim J, Guermah M, McGinty RK, Lee JS, Tang Z, Milne TA, Shilatifard A, Muir TW, Roeder RG (2009) RAD6-mediated transcription-coupled H2B ubiquitylation directly stimulates H3K4 methylation in human cells. Cell 137:459–471

Kizer KO, Phatnani HP, Shibata Y, Hall H, Greenleaf AL, Strahl BD (2005) A novel domain in Set2 mediates RNA polymerase II interaction and couples histone H3 K36 methylation with transcript elongation. Mol Cell Biol 25:3305–3316

Kondo Y, Shen L, Issa JP (2003) Critical role of histone methylation in tumor suppressor gene silencing in colorectal cancer. Mol Cell Biol 23:206–215

Kouzarides T (2007) Chromatin modifications and their function. Cell 128:693–705

Kuo MH, Brownell JE, Sobel RE, Ranalli TA, Cook RG, Edmondson DG, Roth SY, Allis CD (1996) Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. Nature 383:269–272

Lan F, Collins RE, De Cegli R, Alpatov R, Horton JR, Shi X, Gozani O, Cheng X, Shi Y (2007) Recognition of unmethylated histone H3 lysine 4 links BHC80 to LSD1-mediated gene repression. Nature 448:718–722

Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Raymond C, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blöcker H, Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kaspryzk A, Kennedy S,

Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ (2001) Initial sequencing and analysis of the human genome. Nature 409:860–921

Lange UC, Schneider R (2010) What an epigenome remembers. Bioessays 32:659–668

Lavender JS, Birley AJ, Palmer MJ, Kuroda MI, Turner BM (1994) Histone H4 acetylated at lysine 16 and proteins of the Drosophila dosage compensation pathway co-localize on the male X chromosome through mitosis. Chromosome Res 2:398–404

Leder A, Leder P (1975) Butyric acid, a potent inducer of erythroid differentiation in cultured erythroleukemic cells. Cell 5:319–322

Lee JI, Nian H, Cooper AJ, Sinha R, Dai J, Bisson WH, Dashwood RH, Pinto JT (2009) Alpha-keto acid metabolites of naturally occurring organoselenium compounds as inhibitors of histone deacetylase in human prostate cancer cells. Cancer Prev Res (Phila) 2:683–693

Lee JS, Smith E, Shilatifard A (2010) The language of histone crosstalk. Cell 142:682–685

Lehnertz B, Ueda Y, Derijck AA, Braunschweig U, Perez-Burgos L, Kubicek S, Chen T, Li E, Jenuwein T, Peters AH (2003) Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. Curr Biol 13:1192–1200

Li Q, Zhou H, Wurtele H, Davies B, Horazdovsky B, Verreault A, Zhang Z (2008) Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. Cell 134:244–255

Li G, Jiang H, Chang M, Xie H, Hu L (2011) HDAC6 alpha-tubulin deacetylase: a potential therapeutic target in neurodegenerative diseases. J Neurol Sci 304:1–8

Lo WS, Trievel RC, Rojas JR, Duggan L, Hsu JY, Allis CD, Marmorstein R, Berger SL (2000) Phosphorylation of serine 10 in histone H3 is functionally linked in vitro and in vivo to Gcn5-mediated acetylation at lysine 14. Mol Cell 5:917–926

Luger K (2003) Structure and dynamic behavior of nucleosomes. Curr Opin Genet Dev 13:127–135

Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature 389:251–260

Luka Z, Moss FR, Loukachevitch LV, Bornhop DJ, Wagner C (2011) Histone demethylase LSD1 is a folate-binding protein. Biochemistry 50:4750–4756

Ma X, Ezzeldin HH, Diasio RB (2009) Histone deacetylase inhibitors: current status and overview of recent clinical trials. Drugs 69:1911–1934

Mager J, Montgomery ND, de Villena FP, Magnuson T (2003) Genome imprinting regulated by the mouse Polycomb group protein Eed. Nat Genet 33:502–507

Marks PA, Breslow R (2007) Dimethyl sulfoxide to vorinostat: development of this histone deacetylase inhibitor as an anticancer drug. Nat Biotechnol 25:84–90

Marks PA, Xu WS (2009) Histone deacetylase inhibitors: potential in cancer therapy. J Cell Biochem 107:600–608

Matthews AG, Kuo AJ, Ramon-Maiques S, Han S, Champagne KS, Ivanov D, Gallardo M, Carney D, Cheung P, Ciccone DN, Walter KL, Utz PJ, Shi Y, Kutateladze TG, Yang W, Gozani O, Oettinger MA (2007) RAG2 PHD finger couples histone H3 lysine 4 trimethylation with V(D)J recombination. Nature 450:1106–1110

Meissner A, Mikkelsen TS, Gu H, Wernig M, Hanna J, Sivachenko A, Zhang X, Bernstein BE, Nusbaum C, Jaffe DB, Gnirke A, Jaenisch R, Lander ES (2008) Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature 454:766–770

Mujtaba S, Zeng L, Zhou MM (2007) Structure and acetyl-lysine recognition of the bromodomain. Oncogene 26:5521–5527

Musselman CA, Kutateladze TG (2009) PHD fingers: epigenetic effectors and potential drug targets. Mol Interv 9:314–323

Nady N, Lemak A, Walker JR, Avvakumov GV, Kareta MS, Achour M, Xue S, Duan S, Allali-Hassani A, Zuo X, Wang YX, Bronner C, Chedin F, Arrowsmith CH, Dhe-Paganon S (2011) Recognition of multivalent histone states associated with heterochromatin by UHRF1. J Biol Chem 286:24300–24311

Nakayama J, Rice JC, Strahl BD, Allis CD, Grewal SI (2001) Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science 292:110–113

Neumann H, Hancock SM, Buning R, Routh A, Chapman L, Somers J, Owen-Hughes T, van Noort J, Rhodes D, Chin JW (2009) A method for genetically installing site-specific acetylation in recombinant histones defines the effects of H3 K56 acetylation. Mol Cell 36:153–163

Ng HH, Xu RM, Zhang Y, Struhl K (2002) Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79. J Biol Chem 277:34655–34657

Nian H, Bisson WH, Dashwood WM, Pinto JT, Dashwood RH (2009) Alpha-keto acid metabolites of organoselenium compounds inhibit histone deacetylase activity in human colon cancer cells. Carcinogenesis 30:1416–1423

Nicodeme E, Jeffrey KL, Schaefer U, Beinke S, Dewell S, Chung CW, Chandwani R, Marazzi I, Wilson P, Coste H, White J, Kirilovsky J, Rice CM, Lora JM, Prinjha RK, Lee K, Tarakhovsky A (2010) Suppression of inflammation by a synthetic histone mimic. Nature 468:1119–1123

Nilsson AC, Ostman EM, Knudsen KE, Holst JJ, Bjorck IM (2010) A cereal-based evening meal rich in indigestible carbohydrates increases plasma butyrate the next morning. J Nutr 140:1932–1936

Oliver SS, Denu JM (2011) Dynamic interplay between histone H3 modifications and protein interpreters: emerging evidence for a "histone language". Chembiochem 12:299–307

Org T, Chignola F, Hetenyi C, Gaetani M, Rebane A, Liiv I, Maran U, Mollica L, Bottomley MJ, Musco G, Peterson P (2008) The autoimmune regulator PHD finger binds to non-methylated histone H3K4 to activate gene expression. EMBO Rep 9:370–376

Piyathilake CJ, Macaluso M, Celedonio JE, Badiga S, Bell WC, Grizzle WE (2010) Mandatory fortification with folic acid in the United States appears to have adverse effects on histone methylation in women with pre-cancer but not in women free of pre-cancer. Int J Womens Health 1:131–137

Probst AV, Dunleavy E, Almouzni G (2009) Epigenetic inheritance during the cell cycle. Nat Rev Mol Cell Biol 10:192–206

Rajendran P, Williams DE, Ho E, Dashwood RH (2011) Metabolism as a key to histone deacetylase inhibition. Crit Rev Biochem Mol Biol 46:181–199

Riddihough G, Zahn LM (2010) Epigenetics. What is epigenetics? Introduction. Science 330:611

Sanchez R, Zhou MM (2009) The role of human bromodomains in chromatin biology and gene transcription. Curr Opin Drug Discov Devel 12:659–665

Scott BL, Deeg HJ (2010) Myelodysplastic syndromes. Annu Rev Med 61:345–358

Sekhavat A, Sun JM, Davie JR (2007) Competitive inhibition of histone deacetylase activity by trichostatin A and butyrate. Biochem Cell Biol 85:751–758

Sharif J, Muto M, Takebayashi S, Suetake I, Iwamatsu A, Endo TA, Shinga J, Mizutani-Koseki Y, Toyoda T, Okamura K, Tajima S, Mitsuya K, Okano M, Koseki H (2007) The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. Nature 450:908–912

Shi X, Hong T, Walter KL, Ewalt M, Michishita E, Hung T, Carney D, Pena P, Lan F, Kaadige MR, Lacoste N, Cayrou C, Davrazou F, Saha A, Cairns BR, Ayer DE, Kutateladze TG, Shi Y, Cote J, Chua KF, Gozani O (2006) ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. Nature 442:96–99

Shogren-Knaak M, Ishii H, Sun JM, Pazin MJ, Davie JR, Peterson CL (2006) Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science 311:844–847

Skinner MK, Manikkam M, Guerrero-Bosagna C (2010) Epigenetic transgenerational actions of environmental factors in disease etiology. Trends Endocrinol Metab 21:214–222

Smith BC, Denu JM (2009) Chemical mechanisms of histone lysine and arginine modifications. Biochim Biophys Acta 1789:45–57

Steger DJ, Lefterova MI, Ying L, Stonestrom AJ, Schupp M, Zhuo D, Vakoc AL, Kim JE, Chen J, Lazar MA, Blobel GA, Vakoc CR (2008) DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously coupled with gene transcription in mammalian cells. Mol Cell Biol 28:2825–2839

Stover PJ (2009) One-carbon metabolism-genome interactions in folate-associated pathologies. J Nutr 139:2402–2405

Strahl BD, Allis CD (2000) The language of covalent histone modifications. Nature 403:41–45

Tardat M, Murr R, Herceg Z, Sardet C, Julien E (2007) PR-Set7-dependent lysine methylation ensures genome replication and stability through S phase. J Cell Biol 179:1413–1426

Taverna SD, Li H, Ruthenburg AJ, Allis CD, Patel DJ (2007) How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. Nat Struct Mol Biol 14:1025–1040

Thangaraju M, Carswell KN, Prasad PD, Ganapathy V (2009) Colon cancer cells maintain low levels of pyruvate to avoid cell death caused by inhibition of HDAC1/HDAC3. Biochem J 417:379–389

Tibbetts AS, Appling DR (2010) Compartmentalization of Mammalian folate-mediated one-carbon metabolism. Annu Rev Nutr 30:57–81

Turner BM (2000) Histone acetylation and an epigenetic code. Bioessays 22:836–845

Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J, Wei M, Wides R, Xiao C, Yan C, Yao A, Ye J, Zhan M, Zhang W, Zhang H, Zhao Q, Zheng L, Zhong F, Zhong W, Zhu S, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T, Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferriera S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C, Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D, McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigó R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays A, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, Nodell M, Pan S, Peck J, Peterson M, Rowe W, Sanders R, Scott J, Simpson M, Smith T, Sprague A, Stockwell T, Turner R, Venter E, Wang M, Wen M, Wu D, Wu M, Xia A, Zandieh A, Zhu X (2001) The sequence of the human genome. Science 291:1304–1351

Vermeulen M, Mulder KW, Denissov S, Pijnappel WW, van Schaik FM, Varier RA, Baltissen MP, Stunnenberg HG, Mann M, Timmers HT (2007) Selective anchoring of TFIID to nucleosomes by trimethylation of histone h3 lysine 4. Cell 131:58–69

Waddington CH (1968) The basic ideas of biology. Edinburgh University Press, Edinburgh

Walter W, Clynes D, Tang Y, Marmorstein R, Mellor J, Berger SL (2008) 14-3-3 interaction with histone H3 involves a dual modification pattern of phosphoacetylation. Mol Cell Biol 28:2840–2849

Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, Cui K, Roh TY, Peng W, Zhang MQ, Zhao K (2008) Combinatorial patterns of histone acetylations and methylations in the human genome. Nat Genet 40:897–903

Wang GG, Song J, Wang Z, Dormann HL, Casadio F, Li H, Luo JL, Patel DJ, Allis CD (2009a) Haematopoietic malignancies caused by dysregulation of a chromatin-binding PHD finger. Nature 459:847–851

Wang J, Hevi S, Kurash JK, Lei H, Gay F, Bajko J, Su H, Sun W, Chang H, Xu G, Gaudet F, Li E, Chen T (2009b) The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. Nat Genet 41:125–129

Wang F, Dai J, Daum JR, Niedzialkowska E, Banerjee B, Stukenberg PT, Gorbsky GJ, Higgins JM (2010) Histone H3 Thr-3 phosphorylation by Haspin positions Aurora B at centromeres in mitosis. Science 330:231–235

Watanabe S, Resch M, Lilyestrom W, Clark N, Hansen JC, Peterson C, Luger K (2010) Structural characterization of H3K56Q nucleosomes and nucleosomal arrays. Biochim Biophys Acta 1799:480–486

Wei Y, Yu L, Bowen J, Gorovsky MA, Allis CD (1999) Phosphorylation of histone H3 is required for proper chromosome condensation and segregation. Cell 97:99–109

Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB (2009) ATP-citrate lyase links cellular metabolism to histone acetylation. Science 324:1076–1080

Winter S, Fischle W (2010) Epigenetic markers and their cross-talk. Essays Biochem 48:45–61

Xie W, Song C, Young NL, Sperling AS, Xu F, Sridharan R, Conway AE, Garcia BA, Plath K, Clark AT, Grunstein M (2009) Histone H3 lysine 56 acetylation is linked to the core transcriptional network in human embryonic stem cells. Mol Cell 33:417–427

Xu M, Zhu B (2010) Nucleosome assembly and epigenetic inheritance. Protein Cell 1:820–829

Xu WS, Parmigiani RB, Marks PA (2007) Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene 26:5541–5552

Yoshida M, Kijima M, Akita M, Beppu T (1990) Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. J Biol Chem 265:17174–17179

Yuan W, Xu M, Huang C, Liu N, Chen S, Zhu B (2011) H3K36 Methylation antagonizes PRC2-mediated H3K27 methylation. J Biol Chem 286:7983–7989

Zeng L, Zhou MM (2002) Bromodomain: an acetyl-lysine binding domain. FEBS Lett 513:124–128

Zhang Y, Jurkowska R, Soeroes S, Rajavelu A, Dhayalan A, Bock I, Rathert P, Brandt O, Reinhardt R, Fischle W, Jeltsch A (2010) Chromatin methylation activity of Dnmt3a and Dnmt3a/3L is guided by interaction of the ADD domain with the histone H3 tail. Nucleic Acids Res 38:4246–4253

Zhou BO, Wang SS, Zhang Y, Fu XH, Dang W, Lenzmeier BA, Zhou JQ (2011) Histone H4 lysine 12 acetylation regulates telomeric heterochromatin plasticity in Saccharomyces cerevisiae. PLoS Genet 7:e1001272

Zilberman D, Coleman-Derr D, Ballinger T, Henikoff S (2008) Histone H2A.Z and DNA methylation are mutually antagonistic chromatin marks. Nature 456:125–129

Zippo A, Serafini R, Rocchigiani M, Pennacchini S, Krepelova A, Oliviero S (2009) Histone crosstalk between H3S10ph and H4K16ac generates a histone code that mediates transcription elongation. Cell 138:1122–1136

Chapter 8
Chromatin Structure and Gene Expression: Function Follows Form

Aleksandra B. Adomas and Paul A. Wade

Abstract Chromatin by its nature presents a major obstacle to all processes occurring in the nucleus: transcription, DNA repair, and replication. At local level, intimate interactions of DNA with the core histones render it less accessible to proteins that read sequence. Higher levels of chromatin organization may act to form a barrier hindering access of large macromolecular complexes to specific sequences. In this way, the spatial organization of chromatin within the nucleus constitutes the most basic level at which gene expression is regulated. These levels of structure are impacted by multiple different systems. Histone modifications play important roles in regulating transcription by affecting local and long-range chromatin structure. Accessibility of DNA binding sites to transcription factors is modulated by ATP-dependent nucleosome remodeling complexes which can translocate nucleosomes over considerable distances. Here, we discuss how histone and DNA modifications affect chromatin conformation and ultimately gene expression, and we provide examples where alterations in chromatin structure impact phenotype in development, disease, and adaptation to the environment.

Keywords Chromatin structure • DNA loop • Environment • Gene expression • Histone modification • Nucleosome remodeling • Stem cell • Transcription regulation

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Abbreviations

Ac	Acetylation
ATP	Adenosine triphosphate
AVP	Arginine vasopressin
BPA	Bisphenol A
CpG	Dinucleotide 5′-cytosine phosphate guanine – 3′
CTCF	CCCTC-binding transcription factor
EDC	Endocrine disrupting compound
ESC	Embryonic stem cell
HAT	Histone acetyltransferase
HCP	CpG-rich promoter
HMGB	High-mobility-group box
LAD	Lamina associated domain
LCP	CpG-poor promoter
Me	Methylation

8.1 Introduction

Eukaryotic chromatin is a highly dynamic structure which compacts the genome within the cell in a series of organizational layers. The DNA is wrapped around a histone octamer, thus creating a fundamental unit of chromatin, a nucleosome. The DNA sequence can be modified by inclusion of a direct chemical modification, cytosine methylation. The nucleosomal histones H2A, H2B, H3, and H4 can be chemically modified (Fig. 8.1) and canonical histones can be exchanged with histone variants. A 10 nm chromatin fiber consists of arrays of regularly spaced histone–DNA complexes with their modifications, making up the primary structure of the chromatin (Fig. 8.2a). Three-dimensional models of chromatin in nuclei have emerged that propose additional sophisticated layers of genome regulation through higher-order organization and nuclear compartmentalization (Zhou et al. 2011). While it seems obvious that chromatin by its nature should present a major obstacle to all processes occurring in the nucleus, it is the physiologic substrate for enzymes dedicated to nuclear physiology. The resolution of this paradox requires intimate knowledge of the relationship of chromatin structure to DNA function at both the local and global levels.

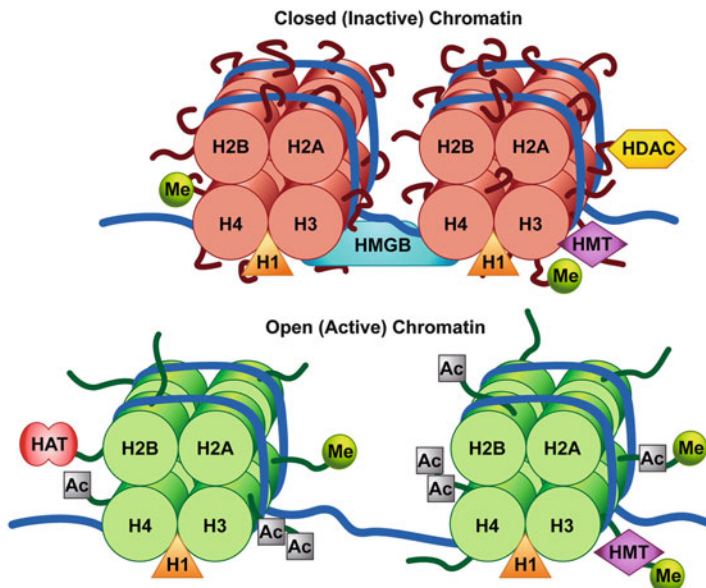

Fig. 8.1 Nucleosome structure. A nucleosome consists of a histone octamer (two copies of *H2A*, *H2B*, *H3*, and *H4*, each) wrapped by DNA. The linker histone H1 binds to DNA wrapped around the core, locking it in place and stabilizing higher-order chromatin structures. The nucleosomal histones can be chemically modified, with acetylation (*Ac*) and methylation (*Me*) being the most studied marks. Acetylation has the greatest potential to unfold chromatin since it neutralizes the basic charge of the lysine and decreases histone tail affinity for DNA. Open chromatin structure that is accessible to the transcription machinery is characterized by hyperacetylation. Histone acetyltransferases (*HATs*) use acetyl-CoA to transfer an acetyl group to the amino groups on the N-terminal tails of histones. The reaction is reversed by histone deacetylases (*HDACs*). Histone methyltransferases (*HMT*) are responsible for methylation. Nonhistone architectural proteins, like high-mobility group box (*HMGB*), play a crucial role in the maintenance of the chromatin structure

8.2 Higher-Order Chromatin Organization in the Nucleus

Several models have been proposed to describe the molecular details of the first layer of chromatin secondary structure, the 30 nm fiber, including the one-start solenoid, two-start helix zigzag, the crosslinker, and the supranucleosome models (Fussner et al. 2011). New experimental approaches, however, including chromatin conformation capture and cryoelectron microscopy, call into question the in situ evidence for the 30 nm chromatin fiber (Fussner et al. 2011; Tremethick 2007). While the relevance of the 30 nm fiber remains controversial, it is clear that higher-order organization of chromatin does occur in nuclei and that these organizational layers have important relationships to DNA function.

A large number of chromatin domains have been identified in the mammalian genome, showing that the eukaryotic genome is highly compartmentalized, with functionally distinct segments (Guelen et al. 2008; Handoko et al. 2011; Zhou et al. 2011). For example, a large fraction of the genome, known as LADs,

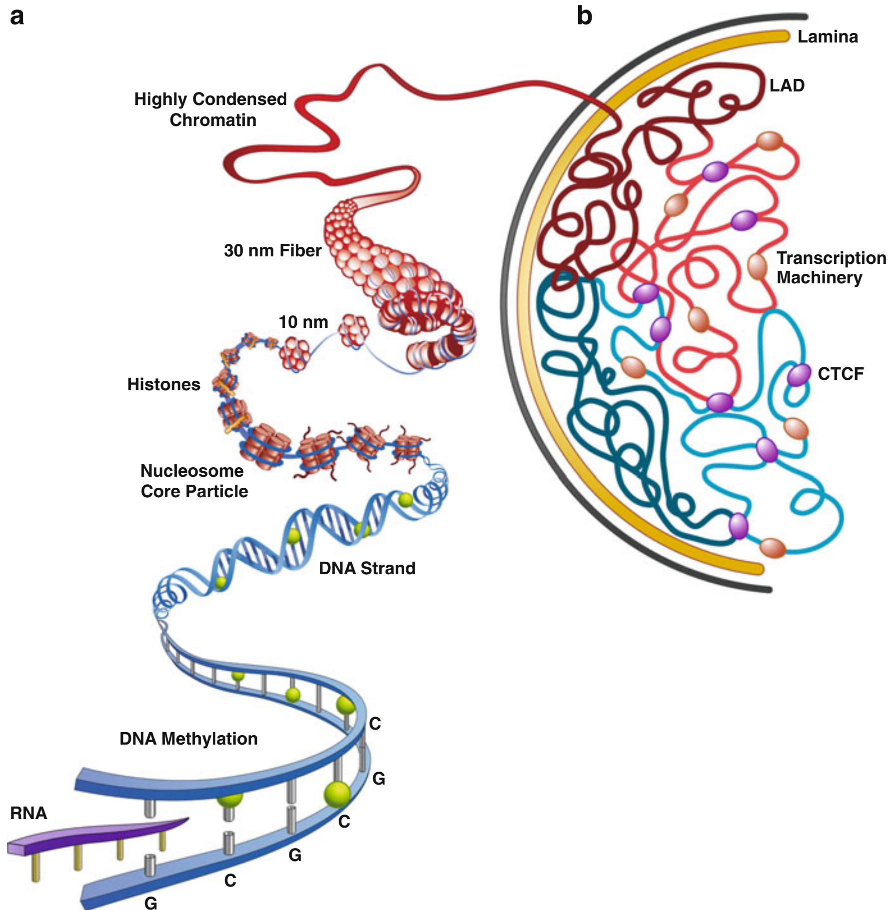

Fig. 8.2 Chromatin structure. (**a**) A 10 nm chromatin fiber consists of arrays of regularly spaced histone–DNA complexes with their modifications, making up the primary structure of the chromatin. Further compaction and association of nonhistone proteins lead to formation of transcriptionally inactive heterochromatin. (**b**) The eukaryotic genome is highly compartmentalized, with functionally distinct segments. A large fraction of the genome, known as LADs, for lamina-associated domains, is associated with the nuclear membrane and correlates with low gene-expression levels, indicating that LADs represent a repressive chromatin environment. The borders are demarcated by the insulator protein CTCF. Chromatin loops separate active (*light blue* or *red*) from repressive (*dark blue* or *red*) regions and LADs from euchromatin regions. Interactions occur within chromosomes, and relatively few occur between chromosomes (two chromosomes shown in two different colors)

for lamina-associated domains, is associated with the nuclear membrane and correlates with low gene-expression levels, indicating that LADs represent a repressive chromatin environment (Guelen et al. 2008). The borders are demarcated by the insulator protein CTCF by promoters that are oriented away from LADs or by CpG islands, suggesting possible mechanisms of LAD confinement (Fig. 8.2b).

CTCF, one of the most extensively studied insulator-binding proteins in vertebrates, is known to demarcate boundaries between accessible euchromatin and silent heterochromatin (Felsenfeld et al. 2004). It has also been proposed as one of the leading candidates to coordinate higher-order chromatin structures and to regulate gene expression at the global level. Handoko et al. (2011) identified over 1,800 chromatin loops in mouse embryonic stem cells (ESCs), with about 5/6 of them connecting loci located on the same chromosome and the rest occurring between chromosomes. Most importantly, the CTCF-mediated chromatin loops (Fig. 8.2b) comprised a diverse array of chromatin domains and included not only gene clusters that are coordinately transcribed in mouse ESCs but also gene clusters that are coordinately silenced. The transcription of genes in higher eukaryotes is frequently regulated through communication between cis-regulatory DNA elements such as promoters and enhancers, which can be located hundreds of kilobases away from each other along the linear chromatin fiber in vivo. Handoko et al. (2011) analyzed location of the key tissue-specific enhancer binding protein p300 which can direct active transcription across long distances from its corresponding promoters (Visel et al. 2009). They concluded that CTCF-associated DNA looping can facilitate communication between regulatory elements, such as enhancers and promoters, thus influencing transcription. These interaction events can reinforce the effect of distal regulatory elements specifically on their corresponding promoters through organizing proper chromatin configuration (Fig. 8.3) (Handoko et al. 2011). Additionally, the authors found chromatin loops that separate active from repressive regions and LADs from euchromatin. These findings illustrate a fundamental principle of structural conformation of chromatin in cells: many previously identified functional properties of chromatin domains are actually arranged in a looped configuration, with the CTCF protein as the “glue” connecting the ends of the domains (Espinoza and Ren 2011). Given that there are approximately 10–15,000 bona fide CTCF sites within a given cell (Espinoza and Ren 2011), it is currently unknown whether CTCF is unique in its ability to partition functional domains of chromatin or whether other proteins may have similar functions.

It has been suggested that the long-range interactions between active functional elements are sufficient to drive folding of local chromatin domains into compact globular states (Sanyal et al. 2011). Such structures have been observed along chromosomes as a result of frequent long-range interactions among active genes and nearby regulatory elements (Baù et al. 2011). Sanyal et al. (2011) speculate that this pattern could be cell-type specific and that increasingly longer range interactions could drive aggregation of groups of globules into larger domains. Taken together, these results suggest that complex genomes are subdivided into large, discrete domains that constitute fundamental units of chromosome organization within the nucleus. This concept supports the hypothesis that the spatial organization of chromosomes within the nucleus has an essential regulatory function.

Several models have been proposed by which distant genomic elements contact each other (Dekker 2008). Passive diffusion models are based on the assumption

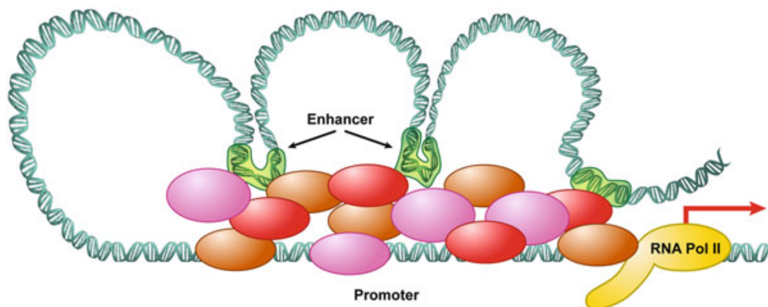

Fig. 8.3 Gene transcription in higher eukaryotes. The transcription of genes in higher eukaryotes is frequently regulated through communication between cis-regulatory DNA elements such as promoters and enhancers, which can be located hundreds of kilobases away from each other along the chromatin fiber in vivo. DNA looping can facilitate communication between regulatory elements and influence transcription. These interaction events can reinforce the effect of distal regulatory elements specifically on their corresponding promoters through organizing proper chromatin configuration

that the mobility of loci provides opportunities for random collisions that are then converted into productive interactions; whether they are productive is dependent on the affinity and specificity of bound protein complexes. On the active side, enhancers have been proposed to actively track along chromatin fibers, in actin-dependent fashion (Dundr et al. 2007).

8.3 Regulation of Chromatin Structure by Posttranslational Modification of Histones

Elucidating just how a nucleosomal array can be compacted into higher-order chromatin structures is central to understanding how the dynamics of chromatin structure functionally translates into the regulation of the genome. A wealth of literature has demonstrated that core histone modifications and histone variants play important roles in modulating higher-order chromatin structures and in regulating transcription by affecting the contact between different histones in adjacent nucleosomes or the interaction of histones with DNA (Li and Reinberg 2011). For the purposes of transcription, modifications can be divided into those that correlate with activation and those that correlate with repression. Acetylation, methylation, phosphorylation, and ubiquitination have been implicated in activation, whereas methylation, ubiquitination, sumoylation, deimination, and proline isomerization have been implicated in repression (Kouzarides 2007). However, any given modification might have the potential to activate or repress under different conditions. Of all the known modifications, acetylation has the most potential to unfold chromatin since it neutralizes the basic charge of the lysine and decreases histone tail affinity for DNA (Fig. 8.1) (Grunstein 1997). For example, Hansen and

colleagues reported that increasing acetylation of nucleosomal arrays inhibited Mg^{2+}-dependent higher-order folding while substantially enhancing transcription (Tse et al. 1998). More specifically, acetylation of histone H4 on a particular residue (lysine 16 (H4K16ac)) has a remarkably similar impact on salt-induced folding (Shogren-Knaak et al. 2006). In the same way, phosphorylation of linker histones H1 results in weakening their binding to the nucleosome (Hendzel et al. 2004). These and related experiments illustrate the concept that histone modification status is intimately linked to structural organization of the chromatin fiber and to function of the underlying DNA.

The binding of nonhistone architectural proteins with chromatin also plays a crucial role in chromatin structural dynamics. The high-mobility-group box (HMGB) family of proteins (Fig. 8.1) is a major class of nonhistone chromatin architectural proteins in eukaryotes involved in transcription regulation (Agresti and Bianchi 2003; Ueda and Yoshida 2010). Studies with the progesterone receptor showed that protein–protein interactions between HMGB proteins and transcription factors do not merely recruit the protein to the target DNA but also effectively induce conformational changes in the transcription factor that are required for its binding to DNA (Edwards et al. 2008). The ability of HMGB proteins to bend DNA may induce structural changes in local chromatin structure, providing binding sites for transcription factors. Finally, the competition between HMGB proteins and histone H1 for binding sites on chromatin (Catez et al. 2004) may be required for chromatin structural changes and the formation of stable transcription machinery on the promoter (Ueda and Yoshida 2010).

8.4 Chromatin Remodeling and Histone Chaperones

Nucleosomal DNA, despite being the physiologic substrate for eukaryotic RNA polymerases, is largely believed to be repressive for transcription (Bondarenko et al. 2006). The mechanisms that mobilize nucleosomes to permit unfettered access to proteins intimately involved in the initiation and progression of transcription must overcome a considerable energy barrier, as more than 120 direct atomic interactions are formed between histones and DNA within a single nucleosome (Luger 2003). Despite the considerable energetic cost, nucleosomes are known to translocate over considerable distances along DNA, modifying the accessibility of DNA binding sites, in a process catalyzed by ATP-dependent nucleosome remodeling complexes (Becker and Horz 2002). Nucleosome remodeling includes translational movements of the histone octamer and alterations in nucleosome composition, such as replacement of canonical histones with specialized variants. The molecular mechanisms that regulate the histone–DNA interactions that control nucleosome positioning and mobility are not completely understood, although the advent of high-throughput sequencing technologies has facilitated recent progress in this area (see below).

The role of chromatin remodeling in transcription has been recently studied in the transcription of ribosomal DNA (rDNA), which constitutes the major transcriptional activity in eukaryotic cells. The ATP-dependent B-WICH complex, consisting of a core of William syndrome transcription factor (WSTF), SNF2h, and nuclear myosin 1 (NM1), maintains an open chromatin structure over a 200 bp region at rRNA promoters (Vintermist et al. 2011). The remodeling is restricted to the region including the upstream control element (to which transcription factor upstream binding factor (UBF) associates), the core element (where pre-initiation complex assembles), and the transcription start site (TSS). The proposed mechanism of B-WICH action is to actively prevent compaction of chromatin and thereby to both permit and facilitate for recruitment of histone acetyltransferases (HATs), resulting in acetylated histone H3 (Vintermist et al. 2011).

The transcription of rDNA is also controlled by the B23/nucleophosmin histone chaperone association with chromatin. Histone chaperones are a class of chromatin binding proteins, which associate with histones upon their synthesis, escort them into the nucleus, and aid in their specific association with DNA (Avvakumov et al. 2011). Interestingly, the RNA binding activity of B23 is crucial for its association with rDNA chromatin and this activity is regulated during mitosis by cdc2-cyclin B-mediated phosphorylation (Hisaoka et al. 2010). Depletion of UBF decreases the chromatin binding affinity of B23, which in turn leads to an increase in histone density at the rDNA chromatin. These results suggest a plausible mechanism for inactivation of rRNA transcription during mitosis.

Another histone chaperone shown to bind to rDNA chromatin and stimulate rRNA transcription, FACT, is purported to reorganize nucleosomes through destabilization of dimer–tetramer contacts and possibly evicting one H2A–H2B dimer to allow passage of the transcribing RNA polymerase through a nucleosomal template (Birch et al. 2009; Reinberg and Sims 2006; Winkler and Luger 2011; Xin et al. 2009). Two major models exist in the literature for FACT-mediated nucleosome reorganization (Winkler and Luger 2011). The "dimer eviction model" proposes that the FACT complex utilizes its histone chaperone function to actively displace a single H2A–H2B dimer from a nucleosome to promote DNA accessibility (Reinberg and Sims 2006). The "global accessibility/non-eviction model" suggests that FACT-induced H2A–H2B dimer displacement is a nonessential by-product of FACT action and not essential for nucleosome reorganization (Xin et al. 2009). This model depicts FACT loosening internal contacts within the nucleosome and in effect changing its dynamic nature to allow sufficient access to the DNA template. Further experiments will be required to resolve these issues.

8.5 Nucleosome Position

Recent evidence indicates that nucleosomes are strategically positioned throughout the genome by nucleosome-positioning elements encoded within the DNA sequence (Ioshikhes et al. 2006; Kaplan et al. 2009; Segal et al. 2006). In an attempt

to estimate the importance of DNA sequence in the nucleosome positioning, Kaplan and colleagues (2009) compared the in vivo yeast nucleosome organization with an in vitro analysis of purified chicken erythrocyte histone octamers assembly on purified yeast genomic DNA. The in vitro and in vivo maps were notably similar and showed stereotypic nucleosome depletion at translation end sites, indicating that this depletion is largely encoded by nucleosome sequence preferences. However, the level of depletion around transcription start sites in vitro was smaller than in vivo, indicating that transcription factors, chromatin remodelers, the transcription initiation machinery, and other mechanisms also contribute (Kaplan et al. 2009). The nucleosome-positioning sequences favor the sharp bending required for the wrapping of DNA around histones (Widom 2001). Indeed, it has been shown in *Saccharomyces cerevisiae* that these DNA sequence elements are present in the promoters of some genes that are maintained in a transcriptionally poised state by the positioning of nucleosomes over TATA-box elements (Choi and Kim 2009; Ioshikhes et al. 2006; Tirosh and Barkai 2008). TATA-containing genes tend to be repressed by nucleosomes and tend to lack activating histone modifications (Choi and Kim 2008). However, they are associated with increased expression variability and divergence (Landry et al. 2007). The capacity of genes to alter their expression levels, i.e., transcriptional plasticity, is essential for cellular adaptation to changes in the environment and has been correlated with promoter nucleosome occupancy (Tirosh and Barkai 2008). Promoters were found to be enriched with two patterns of nucleosome occupancy, corresponding to low or high plasticity. In particular, high-plasticity genes tend to have high nucleosome occupancy directly upstream of their TSS and dynamic nucleosome positioning with high turnover rate. Variable expression is likely to occur during removal of the nucleosome from the promoter for gene activation (Choi and Kim 2009). The high-plasticity genes also differ in other regulatory properties, including the frequency and distribution of transcription factor binding sites, the presence of histone variant H2A.Z, and their sensitivity to chromatin regulation. Notably, these features appear to be conserved from yeast to humans (Tirosh and Barkai 2008).

8.6 ES Cells and Differentiation

Plasticity understood as an ability of cells to change their behavior in response to internal or external environmental cues is most pronounced in stem cells, whose developmental potential is infinite. The recent focus on genome-wide distribution of histone marks and DNA methylation and their correlation with transcriptional activity in embryonic stem cells, lineage-committed ESCs, and adult cells has provided a better understanding of plasticity in stem cells and differentiated tissues (Barrero et al. 2010; Ernst et al. 2011; Fouse et al. 2008; Hawkins et al. 2010; Meissner et al. 2008; Mikkelsen et al. 2007). During differentiation, stem cells lose their potential and refine their identity into specialized tissue-specific cell type. The pluripotent ESCs feature hyperactive transcriptional activity and express high

levels of chromatin-remodeling factors involved in maintaining an open chromatin structure. The permissive chromatin state characterized by the presence of large H3K27me3 domains and smaller regions of H3K4m3e is progressively and selectively closed during differentiation (Barrero et al. 2010). H3K4me3 is catalyzed by trithorax-group (trxG) proteins and associated with activation, while H3K27me3 is catalyzed by polycomb-group (PcG) proteins and associated with silencing. The presence of these two antagonistic marks defines bivalent genes and has been proposed to play a role in silencing differentiation genes in ESCs while keeping them poised for activation upon initiation of specific developmental pathways (Bernstein et al. 2006; Mikkelsen et al. 2007). The poised nature of these domains is further reinforced by the absence of DNA methylation, despite the presence of numerous CpG islands (Fouse et al. 2008; Meissner et al. 2008). DNA methylation is one of the most prevalent mechanisms employed in the genome to maintain inactive regions in a repressed state (especially when present in high density at CpG islands) (Suzuki and Bird 2008). Bivalent genes often encode master transcriptional regulators that are expressed very early during development, usually in a cell-type-specific manner, and control transcriptional programs leading to differentiation. Ultimately, the function of bivalent domains could be to maintain important regulatory sequences accessible and responsive at very early stages of differentiation (Barrero et al. 2010).

The analysis of genome-wide distribution of histone and DNA modifications has distinguished two major types of mammalian promoters (Barrero et al. 2010). While CpG-rich promoters (HCP) are associated with both ubiquitously expressed housekeeping genes and genes with more complex expression patterns, particularly those expressed during embryonic development, CpG-poor promoters (LCP) are generally associated with highly tissue-specific genes. In ES cells, HCP genes are usually devoid of DNA methylation and include self-renewal and housekeeping genes that are usually enriched with H3K4me3, as well as developmental genes containing bivalent marks. In turn, LCP genes are not expressed, show DNA methylation, and mostly do not carry H3K4 or H3K27 methylation (Meissner et al. 2008; Mikkelsen et al. 2007). These genes are likely to be targets of early master transcription regulators and are induced late during differentiation in a tissue-specific manner (Barrero et al. 2010).

Focusing on differentiated human cell types and nine chromatin marks, Ernst et al. (2011) characterized transcription regulatory elements, their cell-type specificities, and functional interactions. The overall patterns of variability indicate that although enhancers and promoters vary drastically in activity level across cell types, they tend to preserve their chromatin identity as regions of regulatory potential. Promoters and enhancers differ in their overall specificities, with the majority of promoters showing activity in multiple cell types, and enhancers being significantly more cell-type specific, with most elements active in a single cell type. Genes belonging to diverse functional classes appear to be controlled by different transcriptional systems. Developmental genes seem to be strongly regulated by both enhancers and promoters, showing the highest number of proximal enhancers and diverse promoter states, including poised RNA polymerase and polycomb-repressed

genes. Tissue-specific genes (e.g., immune genes and steroid metabolism genes) seem to be more dependent on enhancer regulation, showing multiple tissue-specific enhancers but less diverse promoter states. Lastly, housekeeping genes are primarily promoter regulated, with few enhancers in their vicinities.

8.7 Disease States

Interestingly, single-nucleotide polymorphisms (SNPs) associated with disease are frequently positioned within enhancer elements specifically active in relevant cell types and, in some cases, affect a binding motif for a predicted transcription factor (Ernst et al. 2011). Epigenetic defects have been linked with many diseases and developmental disorders. For example, as a consequence of mutations in the methyl CpG-binding protein 2 (MeCP2) gene in Rett syndrome, DNA methylation proceeds normally, but epigenetic silencing is impaired due to failure to recognize this mark (Bienvenu and Chelly 2006). Strikingly, prenatal and early infant development is normal, and an onset of neurodevelopmental symptoms does not occur until later in childhood. The delay in disease onset is likely associated with the developmental pathways of neurons, as MeCP2 has the inherent capacity to fold chromatin into higher-order structures (Georgel et al. 2003) and likely acts in the same capacity as histone H1 in adult neurons (Skene et al. 2010).

A strong argument can be made for disruptions in chromatin modifications affecting cancer development (Feinberg 2007; Jones and Baylin 2002). DNA hypomethylation at a global level was documented approximately 30 years ago (Feinberg and Vogelstein 1983). This loss of covalent DNA modification can have broad impact on genome function, leading to activation of many growth-promoting genes, and has been described in many cancers. In addition, cancer cells frequently display aberrant DNA methylation at tumor suppressors (Dammann et al. 2000). A number of protein-coding genes are overexpressed in ovarian cancer because of loss of DNA methylation, including maspin, claudin-3, and claudin-4 (Barton et al. 2008). Also, mutations in *NSD1*, an H3K36/H4K20 methyltransferase, lead to Sotos syndrome, leukemia, and Wilms tumor (Feinberg 2007).

Diabetes is a chronic metabolic disease associated with both genetic and environmental factors. In addition to histone lysine acetylation, active epigenetic changes, such as histone methyltransferase SET7/9 recruitment and H3K4me marks, appear to be characteristic of the insulin gene promoter activation only in cells associated with insulin production. Prior exposure to hyperglycemia can lead to epigenetic changes in target cells and altered chromatin structure resulting in persisting patterns of gene expression associated with the pathology of diabetic micro- and macrovascular complications (Villeneuve et al. 2011).

8.8 The Environment

The epigenome is an important target of the environment signals. Gene–environment interactions are thought to be mediated by epigenetic modifications, and epigenetic changes often arise in response to changes in the environment (Jaenisch and Bird 2003). Toxins such as heavy metals disrupt DNA methylation and chromatin structure (Sutherland and Costa 2003). Biochemical and genome-wide evidence implies that chronic cocaine administration causes chromatin structure in the brain to be in a generally more permissive state (LaPlant and Nestler 2011). Following a single cocaine injection, total levels of acetylated histone H4 (acH4), phosphoacetylated histone H3, but not acH3 alone, are increased within 30 min (Kumar et al. 2005). Accordingly, most global gene-expression changes observed are consistent with a state of increased gene activation in key brain reward regions. Blocking cocaine's ability to create more open chromatin structure has been suggested as a potential therapeutic treatment of addiction and remains an attractive hypothesis that needs to be tested (LaPlant and Nestler 2011).

There is now increasing evidence that epigenome is modified by endocrine-disrupting compounds (EDCs), such as bisphenol A (BPA). The observed adverse effects of exposure of rodents to BPA include altered development of male and female reproductive tracts, altered development and organization of mammary gland, increased prostate gland volume, disruption of sexual differentiation in the brain, accelerated growth and puberty, higher incidence of breast and prostate cancer, increased body weight, altered reproductive function and sexual behavior, and immune disregulation (reviewed in Kundakovic and Champagne 2011). Dolinoy et al. (2007) used the Agouti, viable, yellow mouse model to show that maternal exposure to BPA causes hypomethylation of several loci and that maternal dietary supplementation with methyl donors negates the effect of BPA. Significant demethylation of specific CpG sites was also associated with inappropriate expression of several genes, including developmental homeobox *Hox10* (Bromer et al. 2010).

Other environmental influences, including stress, have been proposed to lead to epigenetic changes with impact on health. Periodic infant–mother separation during early postnatal life in mice alters expression of arginine vasopressin (AVP) that is associated with sustained DNA hypomethylation (Murgatroyd et al. 2009). Notably this early life stress-induced phenotype lasts for at least 1 year following the initial experience. The research following up the Dutch famine in 1945 showed that adverse environmental conditions can affect mammalian development not only when occurring prenatally, or neonatally, but during periconception as well. Methylation of seven loci implicated in growth, metabolic, and cardiovascular diseases was affected in adults conceived during the famine. Moreover, changes in DNA methylation might be sex and timing specific which matches the difference in obesity, adult schizophrenia, and neural defects at birth observed in men and women exposed to famine during gestation (Heijmans et al. 2009; Tobi et al. 2009).

This and many other lines of evidence, including epidemiological data, indicate that early life events play a powerful role in susceptibility to chronic diseases later on in life (Barker 2007; Gluckman et al. 2008; Walker 2011). Chromatin modifications are propagated from one generation of cells to the next, and normal patterns of inheritance can be modified by environmental signals. It has been demonstrated that histone modifications are direct targets for developmental reprogramming by environmental estrogens that promotes tumors in adulthood. Activation of estrogen-receptor signaling in the developing reproductive tract leads to phosphorylation of the histone lysine methyltransferase EZH2. Phosphorylation inactivates EZH2 (responsible for deposition of repressive H3K27me3 mark) and represses gene expression (Bredfeldt et al. 2010). As a result of this developmental reprogramming, many estrogen-responsive genes become hypersensitive to estrogen in adulthood, exhibiting elevated expression throughout the estrus cycle and resulting in a hyper-estrogenized phenotype in the adult uterus that promotes development of hormone-dependent tumors (Walker 2011).

Thus, environmental cues can activate cell signaling leading to modification of chromatin-remodeling programs and, in turn, to changes in histone and DNA modifications, chromatin conformation, and changes in gene expression. It is important to stress that the presence of a particular histone modification at a given regulatory element may impact local gene expression. Affecting a histone-modifying enzyme activity, genetically or pharmacologically, has global effects and some of the observed changes in gene expression could be indirect.

8.9 Concluding Remarks

The genome is an intrinsically dynamic, highly complicated assembly, regulated by architectural proteins actively remodeling chromatin or modifying it chemically. The principal function of many modifications is not only to directly alter structure but also to embed information within chromatin recognizable by protein complexes whose function is to disassemble, reassemble, and remodel nucleosome structures. Chromatin is the substrate of eukaryotic nuclear enzymes that accomplish the tasks associated with nuclear physiology. That the building blocks used to compact the genome are also utilized to regulate its function presents an intellectual challenge to biologists. Deciphering the structures and chemistry of chromatin and how these are impacted by environment and disease hold promise for understanding the fundamental principles of gene regulation.

References

Agresti A, Bianchi ME (2003) HMGB proteins and gene expression. Curr Opin Genet Dev 13:170–178

Avvakumov N, Nourani A, Cote J (2011) Histone chaperones: modulators of chromatin marks. Mol Cell 41:502–514

Barker DJP (2007) The origins of the developmental origins theory. J Intern Med 261:412–417

Barrero MJ, Boue S, Izpisua Belmonte JC (2010) Epigenetic mechanisms that regulate cell identity. Cell Stem Cell 7:565–570

Barton CA, Hacker NF, Clark SJ, O'Brien PM (2008) DNA methylation changes in ovarian cancer: implications for early diagnosis, prognosis and treatment. Gynecol Oncol 109:129–139

Baù D, Sanyal A, Lajoie BR, Capriotti E, Byron M, Lawrence JB, Dekker J, Marti-Renom MA (2011) The three-dimensional folding of the alpha-globin gene domain reveals formation of chromatin globules. Nat Struct Mol Biol 18:107–114

Becker PB, Horz W (2002) ATP-dependent nucleosomere modeling. Annu Rev Biochem 71:247–273

Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125:315–326

Bienvenu T, Chelly J (2006) Molecular genetics of Rett syndrome: when DNA methylation goes unrecognized. Nat Rev Genet 7:415–426

Birch JL, Tan BC, Panov KI, Panova TB, Andersen JS, Owen-Hughes TA, Russell J, Lee SC, Zomerdijk JC (2009) FACT facilitates chromatin transcription by RNA polymerases I and III. EMBO J 28:854–865

Bondarenko VA, Steele LM, Ujvári A, Gaykalova DA, Kulaeva OI, Polikanov YS, Luse DS, Studitsky VM (2006) Nucleosomes can form a polar barrier to transcript elongation by RNA polymerase II. Mol Cell 24:469–479

Bredfeldt TG, Greathouse KL, Safe SH, Hung MC, Bedford MT, Walker CL (2010) Xenoestrogen-induced regulation of EZH2 and histone methylation via estrogen receptor signaling to PI3K/AKT. Mol Endocrinol 24:993–1006

Bromer JG, Zhou Y, Taylor MB, Doherty L, Taylor HS (2010) Bisphenol-A exposure in utero leads to epigenetic alterations in the developmental programming of uterine estrogen response. FASEB J 24:2273–2280

Catez F, Yang H, Tracey KJ, Reeves R, Misteli T, Bustin M (2004) Network of dynamic interactions between histone H1 and high-mobility-group proteins in chromatin. Mol Cell Biol 24:4321–4328

Choi JK, Kim YJ (2008) Epigenetic regulation and the variability of gene expression. Nat Genet 40:141–147

Choi JK, Kim YJ (2009) Intrinsic variability of gene expression encoded in nucleosome positioning sequences. Nat Genet 41:498–503

Dammann R, Li C, Yoon JH, Chin PL, Bates S, Pfeifer GP (2000) Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. Nat Genet 25:315–319

Dekker J (2008) Gene regulation in the third dimension. Science 319:1793–1794

Dolinoy DC, Huang D, Jirtle RL (2007) Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci U S A 104:13056–13061

Dundr M, Ospina JK, Sung MH, John S, Upender M, Ried T, Hager GL, Matera AG (2007) Actin-dependent intranuclear repositioning of an active gene locus in vivo. J Cell Biol 179:1095–1103

Ernst J, Kheradpour P, Mikkelsen TS, Shoresh N, Ward LD, Epstein CB, Zhang X, Wang L, Issner R, Coyne M, Ku M, Durham T, Kellis M, Bernstein BE (2011) Mapping and analysis of chromatin state dynamics in nine human cell types. Nature 473:43–49

Espinoza CA, Ren B (2011) Mapping higher order structure of chromatin domains. Nat Genet 43:615–616

Feinberg AP (2007) Phenotypic plasticity and the epigenetics of human disease. Nature 447:433–440

Feinberg AP, Vogelstein B (1983) Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature 301:89–92

Felsenfeld G, Burgess-Beusse B, Farrell C, Gaszner M, Ghirlando R, Huang S, Jin C, Litt M, Magdinier F, Mutskov V, Nakatani Y, Tagami H, West A, Yusufzai T (2004) Chromatin boundaries and chromatin domains. Cold Spring Harb Symp Quant Biol 69:245–250

Fouse SD, Shen Y, Pellegrini M, Cole S, Meissner A, Van Neste L, Jaenisch R, Fan G (2008) Promoter CpG methylation contributes to ES cell gene regulation in parallel with Oct4/Nanog, PcG complex, and histone H3K4/K27 trimethylation. Cell Stem Cell 2:160–169

Fussner E, Ching RW, Bazett-Jones DP (2011) Living without 30 nm chromatin fibers. Trends Biochem Sci 36:1–6

Georgel PT, Horowitz-Scherer RA, Adkins N, Woodcock CL, Wade PA, Hansen JC (2003) Chromatin compaction by human MeCP2 – assembly of novel secondary chromatin structures in the absence of DNA methylation. J Biol Chem 278:32181–32188

Gluckman PD, Hanson MA, Cooper C, Thornburg KL (2008) Effect of in utero and early-life conditions on adult health and disease. N Engl J Med 359:61–73

Grunstein M (1997) Histone acetylation in chromatin structure and transcription. Nature 389:349–352

Guelen L, Pagie L, Brasset E, Meuleman W, Faza MB, Talhout W, Eussen BH, de Klein A, Wessels L, de Laat W, van Steensel B (2008) Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature 453:948–951

Handoko L, Xu H, Li G, Ngan CY, Chew E, Schnapp M, Lee CW, Ye C, Ping JL, Mulawadi F, Wong E, Sheng J, Zhang Y, Poh T, Chan CS, Kunarso G, Shahab A, Bourque G, Cacheux-Rataboul V, Sung WK, Ruan Y, Wei CL (2011) CTCF-mediated functional chromatin interactome in pluripotent cells. Nat Genet 43:630–638

Hawkins RD, Hon GC, Lee LK, Ngo Q, Lister R, Pelizzola M, Edsall LE, Kuan S, Luu Y, Klugman S, Antosiewicz-Bourget J, Ye Z, Espinoza C, Agarwahl S, Shen L, Ruotti V, Wang W, Stewart R, Thomson JA, Ecker JR, Ren B (2010) Distinct epigenomic landscapes of pluripotent and lineage-committed human cells. Cell Stem Cell 6:479–491

Heijmans BT, Tobi EW, Lumey LH, Slagboom PE (2009) The epigenome archive of the prenatal environment. Epigenetics 4:526–531

Hendzel MJ, Lever MA, Crawford E, Th'ng JP (2004) The C-terminal domain is the primary determinant of histone H1 binding to chromatin in vivo. J Biol Chem 279:20028–20034

Hisaoka M, Ueshima S, Murano K, Nagata K, Okuwaki M (2010) Regulation of nucleolar chromatin by B23/nucleophosmin jointly depends upon its RNA binding activity and transcription factor UBF. Mol Cell Biol 30:4952–4964

Ioshikhes IP, Albert I, Zanton SJ, Pugh BF (2006) Nucleosome positions predicted through comparative genomics. Nat Genet 38:1210–1215

Jaenisch R, Bird A (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 33:245–254

Jones PA, Baylin SB (2002) The fundamental role of epigenetic events in cancer. Nat Rev Genet 3:415–428

Kaplan N, Moore IK, Fondufe-Mittendorf Y, Gossett AJ, Tillo D, Field Y, LeProust EM, Hughes TR, Lieb JD, Widom J, Segal E (2009) The DNA-encoded nucleosome organization of a eukaryotic genome. Nature 458:362–366

Kouzarides T (2007) Chromatin modifications and their function. Cell 128:693–705

Kumar A, Choi KH, Renthal W, Tsankova NM, Theobald DE, Truong HT, Russo SJ, Laplant Q, Sasaki TS, Whistler KN, Neve RL, Self DW, Nestler EJ (2005) Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. Neuron 48:303–314

Kundakovic M, Champagne FA (2011) Epigenetic perspective on the developmental effects of bisphenol A. Brain Behav Immun 25:1084–1093

Landry CR, Lemos B, Rifkin SA, Dickinson WJ, Hartl DL (2007) Genetic properties influencing the evolvability of gene expression. Science 317:118–121

LaPlant Q, Nestler EJ (2011) CRACKing the histone code: cocaine's effects on chromatin structure and function. Horm Behav 59:321–330
Li G, Reinberg D (2011) Chromatin higher-order structures and gene regulation. Curr Opin Genet Dev 21:175–186
Luger K (2003) Structure and dynamic behavior of nucleosomes. Curr Opin Genet Dev 13:127–135
Meissner A, Mikkelsen TS, Gu H, Wernig M, Hanna J, Sivachenko A, Zhang X, Bernstein BE, Nusbaum C, Jaffe DB, Gnirke A, Jaenisch R, Lander ES (2008) Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature 454:766–770
Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim TK, Koche RP, Lee W, Mendenhall E, O'Donovan A, Presser A, Russ C, Xie X, Meissner A, Wernig M, Jaenisch R, Nusbaum C, Lander ES, Bernstein BE (2007) Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature 448:553–560
Murgatroyd C, Patchev AV, Wu Y, Micale V, Bockmühl Y, Fischer D, Holsboer F, Wotjak CT, Almeida OF, Spengler D (2009) Dynamic DNA methylation programs persistent adverse effects of early-life stress. Nat Neurosci 12:1559–1566
Reinberg D, Sims RJ III (2006) de FACTo nucleosome dynamics. J Biol Chem 281:23297–23301
Sanyal A, Baù D, Martí-Renom MA, Dekker J (2011) Chromatin globules: a common motif of higher order chromosome structure? Curr Opin Cell Biol 23:325–331
Segal E, Fondufe-Mittendorf Y, Chen L et al (2006) A genomic code for nucleosome positioning. Nature 442:772–778
Shogren-Knaak M, Ishii H, Sun JM, Pazin MJ, Davie JR, Peterson CL (2006) Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science 311:844–847
Skene PJ, Illingworth RS, Webb S, Kerr AR, James KD, Turner DJ, Andrews R, Bird AP (2010) Neuronal MeCP2 is expressed at near histone-octamer levels and globally alters the chromatin state. Mol Cell 37:457–468
Sutherland JE, Costa M (2003) Epigenetics and the environment. Ann N Y Acad Sci 983: 151–160.
Suzuki MM, Bird A (2008) DNA methylation landscapes: provocative insights from epigenomics. Nat Rev Genet 9:465–476
Tirosh I, Barkai N (2008) Two strategies for gene regulation by promoter nucleosomes. Genome Res 18:1084–1091
Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD, Slagboom PE, Heijmans BT (2009) DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. Hum Mol Genet 18:4046–4053
Tremethick DJ (2007) Higher-order structures of chromatin: the elusive 30 nm fiber. Cell 128:651–654
Tse C, Sera T, Wolffe AP, Hansen JC (1998) Disruption of higher-order folding by core histone acetylation dramatically enhances transcription of nucleosomal arrays by RNA polymerase III. Mol Cell Biol 18:4629–4638
Ueda T, Yoshida M (2010) HMGB proteins and transcriptional regulation. Biochim Biophys Acta-Gene Regul Mech 1799:114–118
Villeneuve LM, Reddy MA, Natarajan R (2011) Epigenetics: deciphering its role in diabetes and its chronic complications. Clin Exp Pharmacol Physiol 38:401–409
Vintermist A, Böhm S, Sadeghifar F, Louvet E, Mansén A, Percipalle P, Ostlund Farrants AK (2011) The chromatin remodelling complex B-WICH changes the chromatin structure and recruits histone acetyl-transferases to active rRNA genes. PLoS One 6:e19184
Visel A, Blow MJ, Li Z, Zhang T, Akiyama JA, Holt A, Plajzer-Frick I, Shoukry M, Wright C, Chen F, Afzal V, Ren B, Rubin EM, Pennacchio LA (2009) ChIP-seq accurately predicts tissue-specific activity of enhancers. Nature 457:854–858
Walker CL (2011) Epigenomic reprogramming of the developing reproductive tract and disease susceptibility in adulthood. Birth Defects Res A Clin Mol Teratol 91:666–671

Widom J (2001) Role of DNA sequence in nucleosome stability and dynamics. Q Rev Biophys 34:269–324

Winkler DD, Luger K (2011) The histone chaperone FACT: structural insights and mechanisms for nucleosome reorganization. J Biol Chem 286:18369–18374

Xin H, Takahata S, Blanksma M, McCullough L, Stillman DJ, Formosa T (2009) yFACT induces global accessibility of nucleosomal DNA without H2A-H2B displacement. Mol Cell 35:365–376

Zhou VW, Goren A, Bernstein BE (2011) Charting histone modifications and the functional organization of mammalian genomes. Nat Rev Genet 12:7–18

Chapter 9
Epigenetics of Pluripotency

R. David Hawkins and Bing Ren

Abstract Every cell in a multicellular organism carries the same set of genetic material, but their function and differentiation potential differ greatly. Covalent modifications to DNA or the histone proteins at specific genomic regions are important for cell type-specific gene expression programs. The genomic landscape of such covalent modifications is commonly referred to as the epigenome. How is the epigenome of each cell established and maintained? In recent years, scientists have tried to answer this fundamental question by using embryonic stem cells as a unique in vitro model of cellular differentiation. This chapter reviews recent studies that demonstrate a critical role for epigenetic processes in establishing and safeguarding the pluripotency of embryonic stem cells.

Keywords Transcription factors • Cis-regulatory elements • Promoters • Enhancers • Repressive sequences • Chromatin modifications • DNA methylation • Histone methylation • Histone acetylation • Embryonic stem cells • Pluripotency • H3K4me3 • H3K4me1 • H3K9me3 • H3K27me3

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Abbreviations

BAF	Brg1-associated factor
BS	Bisulfite sequencing
ChIP	Chromatin immunoprecipitation
ChIP-chip	Chromatin immunoprecipitation followed by microarray analysis
ChIP-seq	Chromatin immunoprecipitation followed by sequencing
ES	Embryonic stem cells
ICM	Inner cell mass
LIF	Leukemia inhibitory factor
lincRNAs	Long intergenic noncoding RNAs
MLL	Mixed-lineage leukemia
NFR	Nucleosome free region
RNAi	RNA interference
RRBS	Reduced representation bisulfite sequencing

9.1 Introduction

The life cycle of all multicellular organisms begins with a single cell, which gives rise to a diverse set of cell types during development. Each cell type carries out specialized functions that contribute to the survival and propagation of the individual species. Execution of developmental programs in different species, while varying greatly in form and duration, all depend on the same kind of genetic material, DNA, which is passed from one generation to the next. For decades it has been known that identical genetic material exists in the nuclei of different cell types in individual organisms. This observation has raised a fundamental question in biology: how does the same DNA sequence direct the genesis of different cell types?

Developmental biologists have known for some time that some cells possess the ability to differentiate into multiple cell types under proper conditions. Early zygotic cells and primordial germ cells, for example, have the potential to produce all (or nearly all) cell types in an organism. This property is referred to as pluripotency. Hematopoietic stem cells and neural stem cells, for example, can give rise to a limited number of cell types, a property referred to as multipotency. On the other hand, most somatic cells, such as neurons and epithelial cells, are in a terminally differentiated state and cannot give rise to further cell types.

During normal development, pluripotent cells exist only transiently and therefore are, very difficult to study. The discovery of mouse embryonic stem (ES) cells

30 years ago made it possible to study the molecular mechanisms of pluripotency (Evans and Kaufman 1981; Martin 1981). Subsequently, successful isolation of the human ES cells further permitted investigation of this problem in human cells (Thomson et al. 1998; Reubinoff et al. 2000). Isolated from the inner cell mass (ICM) of the blastocyst, ES cells can propagate indefinitely in vitro without senescence and can give rise to nearly all cell types in the body if reintroduced back into the embryos. Further, the possibility to genetically engineer ES cells in vitro has enabled the creation of hundreds of mouse models of human diseases, making ES cells one of the most powerful genetic tools for biologists. As an experimental system, ES cells have also provided a valuable means for study of mechanisms of pluripotency and cell fate determination. Indeed, the last decade has witnessed tremendous progress in our understanding of the molecular mechanisms that regulate pluripotency and cellular differentiation potential. It is now increasingly clear that the establishment and maintenance of cellular identity requires the interplay between both the epigenetic processes and the network of transcription factors (Meissner 2010; Young 2011).

9.2 Transcriptional Networks Regulate Pluripotency of ES Cells

In multicellular organisms, each cell is identified by a unique set of cellular functions that are stably maintained during the life cycle. What controls cellular identity and how is it maintained? At the molecular level, cellular identity is dictated by the collection of RNA transcripts. The levels of RNA transcripts, in turn, are primarily determined by the transcriptional process, which is regulated by the concerted action of a large number of DNA-binding proteins, that is, transcription factors, and specific DNA sequences known as *cis*-regulatory elements. Transcription factors bind to *cis*-elements and act to promote or inhibit transcription of nearby genes along the genome. How do transcription factors control the pluripotency of ES cells?

Scientists have now uncovered a number of transcription factors that are essential for either establishment or maintenance of pluripotency. Among these regulators, three are considered to be central (Heng and Ng 2010; Young 2011). First, Oct4 (also known as POU5f1, Oct3) was identified over 20 years ago (Scholer et al. 1990). Oct4 belongs to a family of POU domain containing transcription factors and recognizes an octamer motif ATGC(A/T)AAT. Oct4 is expressed at high levels in the ICM and in ES cells and is downregulated upon ES cell differentiation. Genetic ablation of the Oct4 gene results in embryos consisting of trophoblasts but completely devoid of embryonic structures, supporting its key function in establishing or maintaining pluripotency (Nichols et al. 1998). Second, Sox2, an HMG-box family transcription factor, complexes with Oct4 and binds to

enhancers of a number of stem cell genes, including the growth factor Fgf4, and activates their expression (Botquin et al. 1998; Chen et al. 2008) (Boyer et al. 2005). The third key transcription factor found to be critical for pluripotency is Nanog (Chambers et al. 2003; Mitsui et al. 2003). First identified in a screen for LIF-independent transcriptional regulator of pluripotency, Nanog is a homeodomain family transcription factor expressed specifically in the ICM and ES cells. Upon deletion of the Nanog gene, ES cells undergo differentiation towards parietal endoderm-like cells.

According to a well-accepted model, Oct4, Sox2, and Nanog maintain pluripotency of ES cells by regulating each other's transcription and transcription of additional regulatory proteins (Young 2011). Such a network is often referred to as a feed-forward circuit, which has the unique properties of producing two bistable states. In the presence of appropriate levels of all three proteins, the network is able to maintain the stable expression of each gene. On the other hand, if any of these proteins is absent or inappropriately expressed, ES cells would then exit from this pluripotency state and take on another stable state where all three proteins are repressed. Besides regulating themselves, Oct4, Sox2, and Nanog also control the expression of a large number of other pluripotency genes to further stabilize the pluripotent state. Additionally, Oct4, Nanog, and Sox2 also bind to promoters of a number of developmental regulators and inhibit their expression. Thus, in the absence of any of these proteins, the differentiation pathways would be turned on, leading to cellular differentiation.

While it is clear that the transcriptional regulatory network plays a critical role in establishing and maintaining pluripotency of the embryonic stem cells, it alone is insufficient to maintain pluripotency of ES cells (Meissner 2010). As discussed below, there is now clear evidence that epigenetic processes also play an essential role.

9.3 Epigenomes of ES Cells Differ from Those of Lineage-Committed Cell Types

It has been known for over half a century that covalent modifications to histone proteins and DNA exist in most eukaryotes, but the relevance of these epigenetic marks to gene regulation and animal development has become apparent only recently. This is due in part to the development of high-throughput technologies for mapping chromatin modification or DNA methylation genome wide. With the use of a chromatin immunoprecipitation assay followed by microarray (ChIP-chip) or high-throughput sequencing (ChIP-seq), investigators can accurately determine localization of specifically modified nucleosomes in the mammalian genome and, through integrative analysis, can associate function of particular histone

modifications to activation or repression of gene expression (Hawkins et al. 2010b). Similarly, via treatment of genomic DNA with sodium bisulfite followed by high-throughput DNA sequencing either for the whole genome (known as MethylC-seq or BS-seq) or selectively captured loci (reduced representation bisulfite sequencing or RRBS), one may interrogate the methylation status for each cytosine comprehensively (Lister and Ecker 2009). Application of these techniques in recent years has led to an explosion in our knowledge of distinct epigenomic landscapes in various mammalian cell types and a better understanding of the critical role that epigenetics may play in establishment and maintenance of cellular identity.

Human CD4+ T cells were among the first mammalian cell types whose chromatin states have been comprehensively characterized (Barski et al. 2007; Wang et al. 2008). Genomic profiles for 39 different histone modifications for this cell type have provided a plethora of information on their biological function and potential link to gene regulation. Maps of chromatin states in mouse ES cells and neural progenitor cells, also among the very first epigenomic maps to be generated, have further highlighted dynamics of chromatin modifications during lineage specification (Mikkelsen et al. 2007). Human ES cells and a primary lung fibroblast cell line (IMR90) were the first mammalian cells for which DNA methylation has been determined at base resolution (Lister et al. 2009). To date, epigenomes of numerous mammalian cell types (>100), including ES cells, have been examined (Hawkins et al. 2010a; Zhou et al. 2010). Integrative analysis of the epigenomic landscapes in these cells, with the help of newly developed pattern recognition tools such as ChromaSig and ChromHMM reveals characteristic chromatin modification patterns at distinct classes of transcriptional regulatory sequences in the genome (Hon et al. 2008; Ernst and Kellis 2010). Additionally, comparative analysis of pluripotent and lineage-committed cells has further underscored the role of epigenetic marks in cellular differentiation and cell fate specification (Hawkins et al. 2010a).

Cis-regulatory elements in the mammalian genome can be classified as promoters, enhancers, silencing/repressive elements, as well as the boundary elements (Heintzman and Ren 2009). These sequences function to regulate transcription of target genes by recruitment of transcription factors, which then interact with the general transcription machinery and nucleosome remodeling complexes to affect various steps of the transcription process. As summarized on the next page, it is now well recognized that activities of most of these elements are accompanied by characteristic chromatin modifications (Hon et al. 2009) (Fig. 9.1).

9.3.1 Promoters

Transcription of a gene begins at the promoter, where the RNA polymerase complex and associated general transcription factors assemble into a pre-initiation complex before initiating the synthesis of RNA. An active promoter is typically characterized by a ~300-bp-long sequence free of nucleosomes (nucleosome free

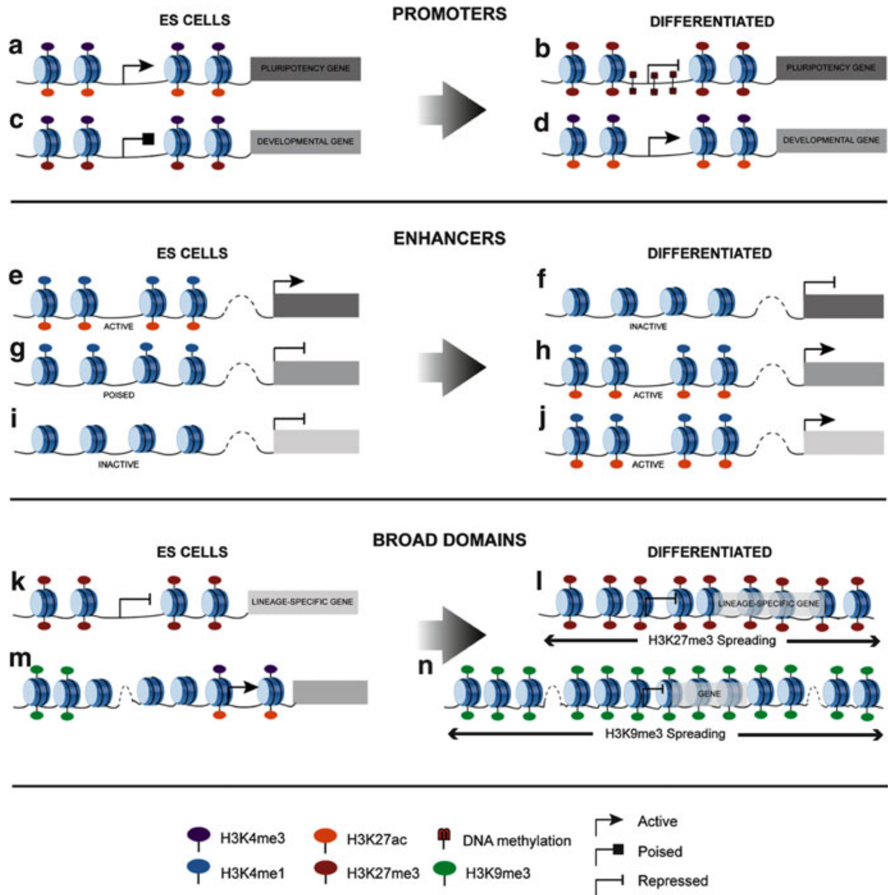

Fig. 9.1 Epigenetic states at regulatory elements. (**a**–**d**) Promoters have distinct modifications in ES and differentiated cells. (**a**–**b**) Pluripotency genes have an active promoter structure indicated by H3K4me3 and H3K27ac in ES cells. These promoters become epigenetically repressed following differentiation. (**c**–**d**) In ES cells developmental regulators have a poised chromatin structure at promoters, as indicated by co-localization of the "active" H3K4me3 and "repressive" H3K27me3 modifications, which often resolve to an active state following differentiation to the appropriate lineage. (**e**–**j**) Enhancers also have a unique chromatin structure. (**e**–**f**) Active ES cell enhancers are marked by H3K4me1 and accompanied by H3K27ac. Following differentiation, enhancers lose modifications, as inactive enhancers do not appear to be marked or exhibit DNA accessibility. (**g**–**h**) ES cells contain poised enhancers, which are solely marked by H3K4me1, and become activated (acquisition of acetylation) with differentiation. (**i**–**j**) Enhancers for lineage-specific gene expression are unmarked prior to differentiation. (**k**–**n**) Differentiated cells exhibit broad domains of repressive histone modifications. (**k**–**l**) H3K27me3 is found primarily in promoter regions. However, in differentiated cells large domains of tens of kilobases are formed. (**m**–**n**) H3K9me3 has a limited, peak-like pattern throughout ES cells. In differentiated cells it forms large repressive domains, over 1 Mb at times. The limited repressive structure of ES cells may provide a more "plastic" epigenome

region or NFR) (Cairns 2009). The NFR at a promoter is immediately flanked by histones that carry a number of modifications. The most prominent, promoter-associated histone modification is trimethylation of lysine 4 on histone H3 (denoted as H3K4me3). This mark is found not only at all active promoters but also at many inactive but poised promoters (Guenther et al. 2007). In fact, nearly 80 % of the known promoters are associated with this mark in ES cells, and promoter occupancy by H3K4me3 is generally invariant between cell types (Heintzman et al. 2009). However, during differentiation, this mark is erased at the promoter of many pluripotency genes (Hawkins et al. 2011). As discussed later in this chapter, maintenance of H3K4me3 is critical for sustained expression of the pluripotency genes and the self-renewal of ES cells. Besides H3K4me3, other histone modification marks are also present at promoters, including mono-ubiquitylation of lysine 120 on histone H2B (H2BK120ub) and acetylation of many lysine residues on the four core histones. Unlike H3K4me3, however, presence of lysine acetylation is strongly correlated with promoter activities as measured by RNA transcript levels of genes (Barski et al. 2007). At inactive promoters, methylation of several different lysine residues is often found, including H3K9me3, H3K27me3, or H4K20me3 (Barski et al. 2007). Interestingly, the promoters of many genes encoding key developmental regulators are frequently associated with a bivalent state of chromatin modification in the ES cells—they are occupied by both H3K4me3 and H3K27me3 (Bernstein et al. 2006). It has been proposed that this unique chromatin state allows development regulators to be silenced in ES cells but poised for activation during differentiation. Indeed, comparison between ES cells and lineage-committed cells shows that a large fraction of bivalent promoters change from a bivalent state in the ES cells to a monovalent state (either H3K4me3 alone or H3K27me3 alone), reflecting the fully activated or repressed state of the genes (Mikkelsen et al. 2007; Hawkins et al. 2010a).

In addition to characteristic chromatin modifications, promoters also display dynamic DNA methylation during differentiation (Lister et al. 2009). Most mammalian promoters are located in stretch of CG-rich sequences known as CpG islands (Deaton and Bird 2011). Methylation status of these promoters is inversely related to the promoter activities—active promoters are typically hypomethylated, while silenced promoters exhibit hypermethylation. During ES cell differentiation, the promoters of many pluripotent genes, including Oct4, become hypermethylated and silenced. It has been proposed that DNA methylation is critical for maintenance of the silenced state of these genes in lineage-committed cells (Hattori et al. 2004).

9.3.2 Enhancers

Transcriptional activities of most genes are subject to regulation in a cell type-specific or development stage-dependent manner, and this is driven in large part by long-range acting elements such as enhancers. Integrative analysis of chromatin modifications in several human cell lines showed that enhancers are typically

associated with the chromatin mark H3K4me1 (Heintzman et al. 2007). Presence of this mark is well correlated with transcriptional activation of nearby genes (Heintzman et al. 2009). Additionally, active enhancers also share a number of histone marks with promoters such as H3K4me2 and H3K27ac. Recently, it has been recognized that H3K4me1 marks not only active enhancers but also poised ones (Creyghton et al. 2010; Hawkins et al. 2011; Rada-Iglesias et al. 2011). Comparative analysis of chromatin states at enhancers indicates that these elements are associated with dynamic and cell type-specific chromatin modifications (Heintzman et al. 2009; Hawkins et al. 2011) (Koch et al. 2007; Ernst et al. 2011), consistent with their role in driving cell type-specific gene expression.

9.3.3 Repressive/Silencing Regions

In most mammalian cell types, especially terminally differentiated cells such as fibroblasts or T cells, a large fraction of the genome exists in contiguous blocks of silenced chromatin state characterized by the presence H3K27me3 (Hawkins et al. 2010a). Genes located within these regions are not transcribed, or transcribed at very low levels. This silenced state appears to be very stable and can be maintained through cell divisions as a form of cellular memory. Consistent with a potential role in maintaining the identity of each cell type, the landscape of these silencing domains varies among different cell types. Most interestingly, H3K27me3 covers only a small fraction of the ES cell genome (up to 8 %) as compared to nearly 30 % in differentiated cell types such as fibroblasts. This observation suggests that the ability of ES cells to respond to stimuli and differentiate into the three germ layers may partly be explained by a less repressive chromatin state (Meshorer and Misteli 2006).

9.4 Epigenetic Factors Involved in Pluripotency

The distinct epigenome of ES cells as compared to lineage-committed cell types strongly suggests that epigenetic factors play a crucial role in establishment or maintenance of pluripotency. Indeed, a number of genes coding for histone modification enzymes and chromatin regulators are known to be essential for ES cell maintenance or self-renewal (Niwa 2007). Additional epigenetic regulators are also necessary for ES cells to differentiate properly despite not being required for viability. It is now clear that epigenetic processes maintain ES cell pluripotency in at least three different ways: (1) sustaining active transcription of key genes involved in pluripotency via the chromatin modification H3K4me3 at promoters, (2) stably silencing lineage-specific transcriptional regulators through the formation of repressive chromatin domains featuring H3K27me3, and (3) ensuring the repression

of pluripotency genes upon differentiation through de novo DNA methylation at the promoters.

9.4.1 Sustained Expression of Pluripotency Genes Requires the H3K4me3 Chromatin State at the Promoter

As previously mentioned, the chromatin modification H3K4me3 marks active and bivalent states at gene promoters. Recent experiments have demonstrated that the H3K4me3 chromatin state at promoters of many pluripotency genes is necessary for the maintenance of pluripotency in ES cells. A comprehensive RNAi screening identified Ctr9 and Rtf1, two components of the Paf1C complex, as essential for self-renewal and pluripotency of the mouse ES cells (Ding et al. 2009). Initially identified as an RNA polymerase II interacting protein complex in yeast, Paf1C is necessary for recruitment of histone H3K4 methyltransferase activities and establishment of H3K4me3 at the promoter. Mammalian Paf1C complex contains six subunits including Paf1, Rtf1, Ctr9, Leo, Cdc73, and Ski8. Knockdown of any/all components of the Paf1C complex led to the ES cells exiting pluripotency, as demonstrated by loss of expression of the pluripotency genes such as Oct4 and increased expression of genes marking a differentiated state. Paf1C interacts with BRE1, a E3 ubiquitin ligase, which mono-ubiquitinylates H2BK120 at the promoter. Ubiquitinylation of H2BK120, in turn, is required for recruitment of the Setd1 histone methyltransferase complex, which establishes H3K4me3. In mouse ES cells depleted of Paf1C components, H3K4me3 is reduced at pluripotency genes along with decreased transcription. Furthermore, knockdown of the Setd1 protein as well as Cxxc1, a component of the Setd1 complex, enhances the phenotype of Ctr9 or Rtf1 knockdown in ES cells (Fig. 9.2). Therefore, establishment or maintenance of H3K4me3 at promoters is critical for transcription of pluripotency genes and ES cell self-renewal.

9.4.2 Suppression of Lineage-Specific Transcription Regulators Through the Formation of a Stable, Repressive Chromatin State

Comparative analysis of epigenomes between the ES cells and differentiated cells types has revealed dynamic chromatin modifications at many lineage-specific transcriptional regulators. In ES cells promoters of these genes are kept in a stable, repressive state featuring either H3K9me3 or H3K27me3 or in a bivalent state featuring both H3K4me3 and H3K27me3. Upon differentiation, a fraction of these promoters lose the repressive chromatin mark, allowing the genes to be activated in specific lineages. So it has been hypothesized that maintenance of the chromatin

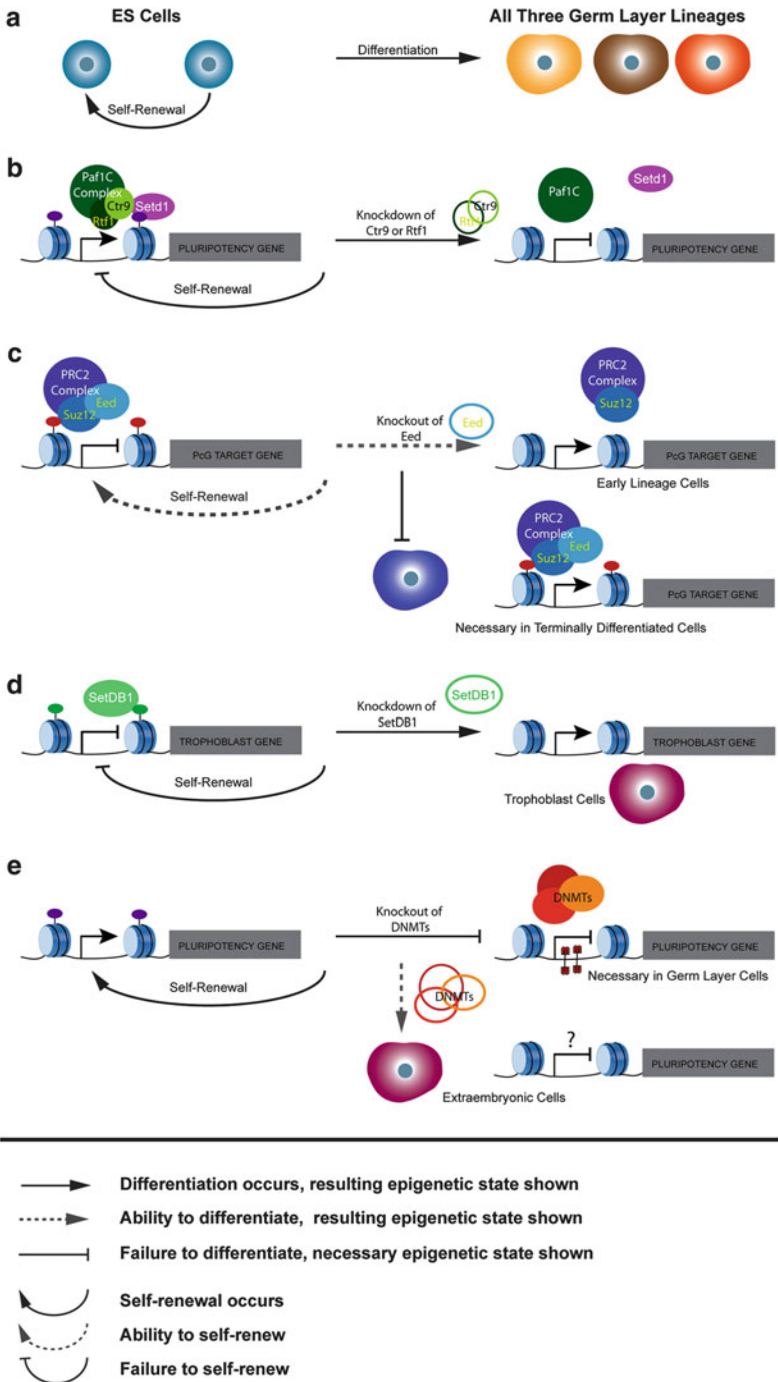

Fig. 9.2 Role of epigenetic modifiers in pluripotency. (**a**) Pluripotency of ES cells is largely defined by two key properties: self-renewal and differentiation to lineage-committed cells. (**b**) Failure to maintain H3K4me3 at pluripotency gene promoters through the loss of Paf1C complex

mark H3K9me3 or H3K27me3 at these promoters is critical for ES cell pluripotency.

To test whether H3K27me3 plays a role in maintenance of ES cell pluripotency, Boyer et al. studied localization of the PRC2 complex, which is responsible for trimethylation of H3K27, in the ES cell genome (Boyer et al. 2006; Lee et al. 2006). PRC2 consists of Ezh2, Suz12, and Eed. All three components were found to co-localize at the promoters of 500 genes enriched for transcription factors involved in lineage specification and differentiation. In wild-type ES cells, PRC2 target genes are associated with the H3K27me3 mark at the promoter and are transcriptionally repressed. By contrast, in ES cells lacking the Eed gene, close to 90 % of the PRC2 target genes become derepressed. This is accompanied by a higher propensity for the mutant ES cells to differentiate (Boyer et al. 2006). Interestingly, while ES cells lacking the Eed gene can differentiate to all three germ layers in vivo, later differentiation processes are impaired, which implies that PRC2 is necessary for multipotency (Chamberlain et al. 2008). Surprisingly, depletion of the other components of PRC2 leads to a different phenotype. For example, the Suz12 knockout ES cells can proliferate, but cannot differentiate to neurons (Pasini et al. 2007). Taken together, these studies indicate that the PRC2 complex and H3K27me3 chromatin modifications are necessary for maintaining the repressed transcription state of differentiation regulator genes.

H3K9me3 appears to be also involved in the silencing of a different group of genes. Two independent groups determined that the H3K9 methyltransferase SetDB1/Eset plays an important role in maintaining pluripotency in mouse ES cells. They illustrated through RNAi that SetDB1 was necessary for a stable stem cell state (Bilodeau et al. 2009; Yuan et al. 2009). One of the key lineages this modification restricts is that of the trophoblast. Eset appears to be targeted to these genes by interacting with Oct4 (Yuan et al. 2009), which explains earlier transcriptional network results showing that repressed gene promoters are also bound by Oct4 (Boyer et al. 2005). A later study using an ES cells conditionally null for SetDB1 confirmed that H3K9me3 is important for repressing certain lineage-specific master regulators (Lohmann et al. 2010).

Fig. 9.2 (continued) stability leads to increased differentiation and failure to self-renew. (**c**) Knockout ES cell lines for PRC2 subunits, such as Eed, can self-renew but have a propensity to differentiate to early lineages. They fail to form more committed cell types because PRC2 repression is necessary to silence lineage-specific genes of an opposing lineage. (**d**) SetDB1 is responsible for H3K9me3 in ES cells. Knockdown results in differentiation to trophoblasts since SetDB1 targets these gene promoters. (**e**) Knockout ES cell lines for DNMT1, DNMT3a, and DNMT3b and the triple knockout all self-renew but fail to differentiate properly. This is because epigenetic silencing of pluripotency genes by DNA methyltransferases is essential for differentiation. These cells for extra embryonic cells and pluripotency genes are thought to be silenced. Legend: Epigenetic modifications are as in Fig. 9.1. *PcG* Polycomb group

9.4.3 Lock-in the Exit from Pluripotency Through De Novo DNA Methylation of Pluripotency Genes

For ES cells to exit the pluripotency state and differentiate into specific cell lineages, Oct4, Nanog, and other transcription factors of the core pluripotency network must be stably silenced while lineage-specific genes are activated. Silencing of some of these genes requires DNA methylation and methylation of H3K9 (Cedar and Bergman 2009). DNA methylation is catalyzed by three DNA methyltransferases—DNMT1, DNMT3a, and DNMT3b (Li 2002). DNMT1 is primarily involved in maintenance of DNA methylation during DNA replication, while DNMT3a and DNMT3b are responsible for de novo DNA methylation. ES cells deleted for all three genes are viable, but do not differentiate properly, suggesting that DNA methylation is essential for ES cells to exit the pluripotency state and differentiate (Sakaue et al. 2010). The exact role of DNA methylation in the differentiation of ES cells is still under investigation.

9.4.4 Propagation of the Epigenetic Memory

During cellular division epigenetic modifications must be transmitted to the daughter cell in order to maintain stable gene expression patterns. It is not fully understood how histone modification patterns, especially H3K9me3 and H3K27me3, are maintained through mitosis. However, recent work studying the propagation H3K27me3 may provide insight for a general mechanism of epigenetic inheritance. Two groups found that components of PRC2 remain bound to regions it marks with H3K27me3. In doing so, the complex is able to modify new nucleosomes added to the daughter strand during replication (Hansen and Helin 2009; Margueron et al. 2009). These events are likely occurring during S phase and possibly at the replication fork (Hansen and Helin 2009). It remains to be determined how other chromatin modifications are propagated, but these studies provide an interesting mechanism to be tested.

In the case of DNA methylation, propagation is thought to be more straightforward. The maintenance methyltransferase DNMT1 makes use of CpG symmetry to modify the daughter strand. Still, this mechanism fails to explain how the high degree of non-CG methylation found in human ES cells is maintained (Lister et al. 2009). New insights into the mechanism of DNA methylation maintenance may help shed light on this problem. It is now clear that DNMT1 requires the action of the de novo DNMTs, 3A and 3B, in mouse ES cells and other cell types (Rhee et al. 2002; Lehnertz et al. 2003). Moreover, recent work took advantage of the fact that DNMT3A/B forms a stable complex with nucleosomes containing methylated DNA to demonstrate a homeostatic mode of inheritance, whereby the amount of de novo methyltransferase in the cell is regulated by the amount of methylated cytosine (Sharma et al. 2011). This could provide a means of accurately preserving non-CG methylation.

9.5 Perspectives

It is now clear that ES cells have unique epigenomes with patterns of histone modifications and general open chromatin structure contributing to their pluripotency. Even ubiquitous chromatin remodeling complexes, such as BAF, have been shown to have ES cell-specific components (for review, see Lessard and Crabtree (2010)). However, it remains unresolved how these modifications form the ES cell state and how they are repositioned during differentiation. For active and poised promoters, H3K4me3 is made by histone methyltransferases such as Setd1 or MLLs that are recruited by the transcriptional machinery. But how are these promoters targeted? For other modifications such as H3K4me1, which marks active and poised enhancers, and H3K27me3, which marks repressed and poised/bivalent promoters, more insight is needed. For H3K27me3, the answer may lie with noncoding RNAs (ncRNAs). Long intergenic ncRNAs, or lincRNAs, are important for pluripotency and differentiation, with knockdown of many lincRNAs resulting in an exit from the pluripotent state (Guttman et al. 2011). LincRNAs can associate with PRC2 (O'Geen et al. 2007; Khalil et al. 2009; Tsai et al. 2010), but may act as a scaffolding structure instead of a targeting mechanism. However, a study using RIP-seq, a method of identifying RNAs associated the proteins using next-generation sequencing, showed that several types of ncRNAs associate with the PRC2 subunit Ezh2 in mouse ES cells (Zhao et al. 2010). The idea of various RNAs targeting these complexes to the genome may make sense in light of no common sequence motif for H3K27me3-marked promoters and given that H3K27me3 can spread to form large domains beyond the promoter region.

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References

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K (2007) High-resolution profiling of histone methylations in the human genome. Cell 129:823–837

Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125:315–326

Bilodeau S, Kagey MH, Frampton GM, Rahl PB, Young RA (2009) SetDB1 contributes to repression of genes encoding developmental regulators and maintenance of ES cell state. Genes Dev 23:2484–2489

Botquin V, Hess H, Fuhrmann G, Anastassiadis C, Gross MK, Vriend G, Scholer HR (1998) New POU dimer configuration mediates antagonistic control of an osteopontin preimplantation enhancer by Oct-4 and Sox-2. Genes Dev 12:2073–2090

Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, Guenther MG, Kumar RM, Murray HL, Jenner RG, Gifford DK, Melton DA, Jaenisch R, Young RA (2005) Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 122:947–956

Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK, Bell GW, Otte AP, Vidal M, Gifford DK, Young RA, Jaenisch R (2006) Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature 441:349–353

Cairns BR (2009) The logic of chromatin architecture and remodelling at promoters. Nature 461 (7261):193–198

Cedar H, Bergman Y (2009) Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet 10:295–304

Chamberlain SJ, Yee D, Magnuson T (2008) Polycomb repressive complex 2 is dispensable for maintenance of embryonic stem cell pluripotency. Stem Cells 26:1496–1505

Chambers I, Colby D, Robertson M, Nichols J, Lee S, Tweedie S, Smith A (2003) Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. Cell 113:643–655

Chen X, Xu H, Yuan P, Fang F, Huss M, Vega VB, Wong E, Orlov YL, Zhang W, Jiang J, Loh YH, Yeo HC, Yeo ZX, Narang V, Govindarajan KR, Leong B, Shahab A, Ruan Y, Bourque G, Sung WK, Clarke ND, Wei CL, Ng HH (2008) Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell 133:1106–1117

Creyghton MP, Cheng AW, Welstead GG, Kooistra T, Carey BW, Steine EJ, Hanna J, Lodato MA, Frampton GM, Sharp PA, Boyer LA, Young RA, Jaenisch R (2010) Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc Natl Acad Sci U S A 107:21931–21936

Deaton AM, Bird A (2011) CpG islands and the regulation of transcription. Genes Dev 25:1010–1022

Ding L, Paszkowski-Rogacz M, Nitzsche A, Slabicki MM, Heninger A-K, de Vries I, Kittler R, Junqueira M, Shevchenko A, Schulz H, Hubner N, Doss MX, Sachinidis A, Hescheler J, Iacone R, Anastassiadis K, Stewart AF, Pisabarro MT, Caldarelli A, Poser I, Theis M, Buchholz F (2009) A genome-scale RNAi screen for Oct4 modulators defines a role of the Paf1 complex for embryonic stem cell identity. Cell Stem Cell 4:403–415

Ernst J, Kellis M (2010) Discovery and characterization of chromatin states for systematic annotation of the human genome. Nat Biotechnol 28:817–825

Ernst J, Kheradpour P, Mikkelsen TS, Shoresh N, Ward LD, Epstein CB, Zhang X, Wang L, Issner R, Coyne M, Ku M, Durham T, Kellis M, Bernstein BE (2011) Mapping and analysis of chromatin state dynamics in nine human cell types. Nature 473:43–49

Evans MJ, Kaufman MH (1981) Establishment in culture of pluripotential cells from mouse embryos. Nature 292:154–156

Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA (2007) A chromatin landmark and transcription initiation at most promoters in human cells. Cell 130:77–88

Guttman M, Donaghey J, Carey BW, Garber M, Grenier JK, Munson G, Young G, Lucas AB, Ach R, Bruhn L, Yang X, Amit I, Meissner A, Regev A, Rinn JL, Root DE, Lander ES (2011) lincRNAs act in the circuitry controlling pluripotency and differentiation. Nature 477:295–300

Hansen KH, Helin K (2009) Epigenetic inheritance through self-recruitment of the polycomb repressive complex 2. Epigenetics 4:133–138

Hattori N, Nishino K, Ko Y-G, Hattori N, Ohgane J, Tanaka S, Shiota K (2004) Epigenetic control of mouse Oct-4 gene expression in embryonic stem cells and trophoblast stem cells. J Biol Chem 279:17063–17069

Hawkins RD, Hon GC, Lee LK, Ngo Q, Lister R, Pelizzola M, Edsall LE, Kuan S, Luu Y, Klugman S, Antosiewicz-Bourget J, Ye Z, Espinoza C, Agarwahl S, Shen L, Ruotti V, Wang W, Stewart R, Thomson JA, Ecker JR, Ren B (2010a) Distinct epigenomic landscapes of pluripotent and lineage-committed human cells. Cell Stem Cell 6(5):479–491

Hawkins RD, Hon GC, Ren B (2010b) Next-generation genomics: an integrative approach. Nat Rev Genet 11:476–486
Hawkins RD, Hon GC, Yang C, Antosiewicz-Bourget JE, Lee LK, Ngo QM, Klugman S, Ching KA, Edsall LE, Ye Z, Kuan S, Yu P, Liu H, Zhang X, Green RD, Lobanenkov VV, Stewart R, Thomson JA, Ren B (2011) Dynamic chromatin states in human ES cells reveal potential regulatory sequences and genes involved in pluripotency. Cell Res 21:1393–1409
Heintzman ND, Ren B (2009) Finding distal regulatory elements in the human genome. Curr Opin Genet Dev 19:541–549
Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, Barrera LO, Van Calcar S, Qu C, Ching KA, Wang W, Weng Z, Green RD, Crawford GE, Ren B (2007) Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. Nat Genet 39:311–318
Heintzman ND, Hon GC, Hawkins RD, Kheradpour P, Stark A, Harp LF, Ye Z, Lee LK, Stuart RK, Ching CW, Ching KA, Antosiewicz-Bourget JE, Liu H, Zhang X, Green RD, Lobanenkov VV, Stewart R, Thomson JA, Crawford GE, Kellis M, Ren B (2009) Histone modifications at human enhancers reflect global cell-type-specific gene expression. Nature 459:108–112
Heng JC, Ng HH (2010) Transcriptional regulation in embryonic stem cells. Adv Exp Med Biol 695:76–91
Hon G, Ren B, Wang W (2008) ChromaSig: a probabilistic approach to finding common chromatin signatures in the human genome. PLoS Comput Biol 4:e1000201
Hon GC, Hawkins RD, Ren B (2009) Predictive chromatin signatures in the mammalian genome. Hum Mol Genet 18(R2):R195–R201
Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, Thomas K, Presser A, Bernstein BE, van Oudenaarden A, Regev A, Lander ES, Rinn JL (2009) Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci U S A 106:11667–11672
Koch CM, Andrews RM, Flicek P, Dillon SC, Karaoz U, Clelland GK, Wilcox S, Beare DM, Fowler JC, Couttet P, James KD, Lefebvre GC, Bruce AW, Dovey OM, Ellis PD, Dhami P, Langford CF, Weng Z, Birney E, Carter NP, Vetrie D, Dunham I (2007) The landscape of histone modifications across 1 % of the human genome in five human cell lines. Genome Res 17:691–707
Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, Chevalier B, Johnstone SE, Cole MF, K-i I, Koseki H, Fuchikami T, Abe K, Murray HL, Zucker JP, Yuan B, Bell GW, Herbolsheimer E, Hannett NM, Sun K, Odom DT, Otte AP, Volkert TL, Bartel DP, Melton DA, Gifford DK, Jaenisch R, Young RA (2006) Control of developmental regulators by Polycomb in human embryonic stem cells. Cell 125:301–313
Lehnertz B, Ueda Y, Derijck AA, Braunschweig U, Perez-Burgos L, Kubicek S, Chen T, Li E, Jenuwein T, Peters AH (2003) Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. Curr Biol 13:1192–1200
Lessard JA, Crabtree GR (2010) Chromatin regulatory mechanisms in pluripotency. Annu Rev Cell Dev Biol 26:503–532
Li E (2002) Chromatin modification and epigenetic reprogramming in mammalian development. Nat Rev Genet 3:662–673
Lister R, Ecker JR (2009) Finding the fifth base: genome-wide sequencing of cytosine methylation. Genome Res 19:959–966
Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, Nery JR, Lee L, Ye Z, Ngo QM, Edsall L, Antosiewicz-Bourget J, Stewart R, Ruotti V, Millar AH, Thomson JA, Ren B, Ecker JR (2009) Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 462:315–322
Lohmann F, Loureiro J, Su H, Fang Q, Lei H, Lewis T, Yang Y, Labow M, Li E, Chen T, Kadam S (2010) KMT1E mediated H3K9 methylation is required for the maintenance of embryonic stem cells by repressing trophectoderm differentiation. Stem Cells 28:201–212

Margueron R, Justin N, Ohno K, Sharpe ML, Son J, Drury WJ III, Voigt P, Martin SR, Taylor WR, De Marco V, Pirrotta V, Reinberg D, Gamblin SJ (2009) Role of the polycomb protein EED in the propagation of repressive histone marks. Nature 461:762–767

Martin GR (1981) Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci U S A 78:7634–7638

Meissner A (2010) Epigenetic modifications in pluripotent and differentiated cells. Nat Biotechnol 28:1079–1088

Meshorer E, Misteli T (2006) Chromatin in pluripotent embryonic stem cells and differentiation. Nat Rev Mol Cell Biol 7:540–546

Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim TK, Koche RP, Lee W, Mendenhall E, O'Donovan A, Presser A, Russ C, Xie X, Meissner A, Wernig M, Jaenisch R, Nusbaum C, Lander ES, Bernstein BE (2007) Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature 448:553–560

Mitsui K, Tokuzawa Y, Itoh H, Segawa K, Murakami M, Takahashi K, Maruyama M, Maeda M, Yamanaka S (2003) The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell 113:631–642

Nichols J, Zevnik B, Anastassiadis K, Niwa H, Klewe-Nebenius D, Chambers I, Scholer H, Smith A (1998) Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell 95:379–391

Niwa H (2007) How is pluripotency determined and maintained? Development 134:635–646

O'Geen H, Squazzo SL, Iyengar S, Blahnik K, Rinn JL, Chang HY, Green R, Farnham PJ (2007) Genome-wide analysis of KAP1 binding suggests autoregulation of KRAB-ZNFs. PLoS Genet 3:e89

Pasini D, Bracken AP, Hansen JB, Capillo M, Helin K (2007) The polycomb group protein Suz12 is required for embryonic stem cell differentiation. Mol Cell Biol 27:3769–3779

Rada-Iglesias A, Bajpai R, Swigut T, Brugmann SA, Flynn RA, Wysocka J (2011) A unique chromatin signature uncovers early developmental enhancers in humans. Nature 470:279–283

Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A (2000) Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. Nat Biotechnol 18:399–404

Rhee I, Bachman KE, Park BH, Jair K-W, Yen R-WC, Schuebel KE, Cui H, Feinberg AP, Lengauer C, Kinzler KW, Baylin SB, Vogelstein B (2002) DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. Nature 416:552–556

Sakaue M, Ohta H, Kumaki Y, Oda M, Sakaide Y, Matsuoka C, Yamagiwa A, Niwa H, Wakayama T, Okano M (2010) DNA methylation is dispensable for the growth and survival of the extraembryonic lineages. Curr Biol 20:1452–1457

Scholer HR, Ruppert S, Suzuki N, Chowdhury K, Gruss P (1990) New type of POU domain in germ line-specific protein Oct-4. Nature 344:435–439

Sharma S, De Carvalho DD, Jeong S, Jones PA, Liang G (2011) Nucleosomes containing methylated DNA stabilize DNA methyltransferases 3A/3B and ensure faithful epigenetic inheritance. PLoS Genet 7:e1001286

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science 282:1145–1147

Tsai M-C, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E, Chang HY (2010) Long noncoding RNA as modular scaffold of histone modification complexes. Science 329:689–693

Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, Cui K, Roh T-Y, Peng W, Zhang MQ, Zhao K (2008) Combinatorial patterns of histone acetylations and methylations in the human genome. Nat Genet 40:897–903

Young RA (2011) Control of the embryonic stem cell state. Cell 144(6):940–954

Yuan P, Han J, Guo G, Orlov YL, Huss M, Loh Y-H, Yaw L-P, Robson P, Lim B, Ng H-H (2009) Eset partners with Oct4 to restrict extraembryonic trophoblast lineage potential in embryonic stem cells. Genes Dev 23:2507–2520

Zhao J, Ohsumi TK, Kung JT, Ogawa Y, Grau DJ, Sarma K, Song JJ, Kingston RE, Borowsky M, Lee JT (2010) Genome-wide identification of Polycomb-associated RNAs by RIP-seq. Mol Cell 40:939–953

Zhou VW, Goren A, Bernstein BE (2010) Charting histone modifications and the functional organization of mammalian genomes. Nat Rev Genet 12:7–18

Part IV
Epigenetic Transgenerational Inheritance

Chapter 10
Transgenerational Epigenetic Inheritance in Drosophila

Luan Wang, Xiangyi Lu, and Douglas M. Ruden

Abstract Transgenerational epigenetic inheritance involves the inheritance of a phenotype across at least one generation that does not involve any changes in the DNA sequence. The primary mark of transgenerational epigenetic inheritance is thought to be DNA methylation, such as in imprinting in mammals and in the inheritance of coat color in agouti viable yellow (A^{vy}) mice. However, while most studies of *Drosophila melanogaster* indicate that there is no DNA cytosine methylation, nevertheless several systems of transgenerational epigenetic inheritance have been demonstrated in this organism. In this chapter, we review several Drosophila transgenerational epigenetic systems, including a system that we developed in our laboratory that involves the transgenerational epigenetic inheritance of an ectopic large bristle outgrowth (ELBO) in the eyes of *D. melanogaster* that can be passed from generation to generation for hundreds of generations. Understanding transgenerational epigenetic inheritance mechanisms in Drosophila can have a profound impact in understanding similar processes in humans in which environmental exposures can affect the health of future generations.

Keywords DNA looping • DNA methylation • *Drosophila melanogaster* • Epigenetics • Histone modifications • Transgenerational inheritance

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Abbreviations

A^{vy}	Agouti viable yellow
DCC	Dosage compensation complex
DMR	Differentially methylated region
dsRNA	Double-stranded RNA
ELBO	Ectopic large bristle outgrowth
E(var)	Enhancer of variegation
E(z)	Enhancer of zeste
FISH	Fluorescent in situ hybridization
H3K9	Histone 3 lysine 9
H3K27	Histone 3 lysine 27
H4K20	Histone 4 lysine 20
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
HMT	Histone methyltransferases
HP1	Heterochromatin protein 1
Hsp90	Heat shock protein 90
IGF2	Insulin growth factor 2
IAP	Intracisternal A particle
JAK	Janus kinase
KrIf-1	Kruppel Irregular-facets 1
LacZ	Beta galactosidase
miRNA	microRNA
nm	Nanometer
PcG	Polycomb group
PEV	Position-effect variegation
PGC	Primordial germ cells
PRE	Polycomb response elements
PSTVd	Potato spindle tuber viroid
PTGS	Posttranscriptional gene silencing
rasiRNA	Repeat-associated small interfering RNA
RdDM	RNA-directed DNA methylation
RISC	RNA induced silencing complex
RNAi	RNA interference
siRNA	Small interfering RNA
Su(var)	Suppressor of variegation
TGS	Transcriptional gene silencing
TRE	Trithorax response elements
TrxG	Trithorax group
Ubx	Ultrabithorax

Wg	Wingless
Xic	X inactivation center
Xist	X inactive-specific transcript

10.1 Introduction

The field of epigenetics is the study of heritable changes of gene expression and phenotypes that involve no DNA sequence alteration. Epigenetics plays essential roles in many biology activities, especially in cellular differentiation and embryogenesis, during which the totipotent stem cells differentiate into various pluripotent cell lines. The term epigenetic reprogramming is defined as mitotic or meiotic alterations that cause relatively permanent changes in phenotypes without DNA sequence modifications (Reik and Walter 2001). The role of epigenetics during development was famously raised by Waddington in 1942 when he tried to explain how one genome could create many different tissue types (Waddington 1942).

The initiation of epigenetic modifications starts at early development stages. Currently, there are two key periods of genome-wide reprogramming that have been identified. The earlier reprogramming stage is during gametogenesis, and the latter occurs during early embryogenesis (Morgan et al. 2005). Epigenetic reprogramming can also occur in other critical occasions such as dedifferentiation during cancer development or the transfer of somatic cell nuclei when cloning mammals (Rideout et al. 2001; Santos and Dean 2004), such as occurred famously with Dolly the sheep (Ashworth et al. 1998; Bulfield et al. 1998; Shiels et al. 1999; Wilmut 2003, 2005; Wilmut et al. 2009). The epigenetic marks are relatively stable and can be transmitted across mitosis, but are mostly erased in primordial germ cells (PGC) and in the zygote to ensure totipotency. The epigenetic modifications are considered vital for the development and determination of multicellular organisms and then cleared until the next generation. If an epigenetic mark is not cleared, then this is referred to as "transgenerational epigenetic inheritance."

Reports of "heritable germline epimutations" of several tumor suppressor genes in humans have suggested that transgenerational epigenetic inheritance occurs in higher organisms. A related phenomenon, failure of imprinting, in which DNA methylated marks at imprinted loci are lost, has also been associated with the likelihood that cancer will develop (Falls et al. 1999; Walter and Paulsen 2003). The germline inheritance of an epimutation was also documented in many human diseases such as Prader-Willi and Angelman syndromes and hereditary nonpolyposis colorectal cancer (Buiting et al. 2003; Chan et al. 2006). However, since the precise mechanisms of epigenetic regulation and the erasure processes are still obscure, the mechanism of transgenerational epigenetic inheritance in these systems is not understood.

Some of the most important questions in epigenetics refer to the inheritance of epigenetic patterns across generations: What are the epigenetic marks? How are

they maintained through meiosis? Are epigenetic marks such as DNA methylation, histone methylation, histone acetylation, and DNA looping involved in transgenerational epigenetic inheritance? Studies of transgenerational epigenetic inheritance in model organisms have begun to address these questions.

One of the most thoroughly studied examples of transgenerational epigenetic inheritance is the inheritance of the coat color in the agouti viable yellow (A^{vy}) strain of mice (Duhl et al. 1994). An intracisternal A particle (IAP) retrotransposon is located upstream of the *agouti* gene. The methylation status of the IAP alters the phenotypes of the genetically identical individuals. The unmethylated IAP maintains the activation of agouti gene and displays a yellow coat for the progeny, while the methylated IAP silenced the agouti gene and shows a brown "pseudo-agouti" coat color (Duhl et al. 1994). Further research found that the stochastic methylation of the IAP results in a wide range of different coat colors (Blewitt et al. 2006).

The environment, such as diet, can affect transgenerational epigenetic inheritance. The supplementation of methyl donors in the diet of the pregnant mother can change the coat color in the progeny to brown, evidently by IAP hyper-methylation (Wolff et al. 1998). It also has been reported that environmental stress can alter the epigenetic pattern of progeny after their birth. Weaver and colleague observed that the maternal behaviors such as licking and grooming and arched-back nursing in rats changed the DNA methylation pattern of a glucocorticoid receptor promoter in the hippocampus (Weaver et al. 2004). This methylation pattern can be reversed by cross-fostering (Weaver et al. 2004). Until recently, researchers have proposed that a certain number of epigenetic marks remain "uncleared" (i.e., not erased) in yeast, plants, Drosophila, and mice.

Recent research reveals that transgenerational epigenetic inheritance has been associated with a few families of human tumor suppressor genes such as MLH1 (Hitchins et al. 2005, 2007; Suter et al. 2004, 2007) and MSH2 (Chan et al. 2006), which correlates to increased risk of colorectal cancer. Those studies have focused on DNA methylation, such as 5-methylcytosine (5meC) at CpG dinucleotide sequences. But it is now supported by the research conducted on *Schizosaccharomyces pombe* (Dalgaard and Klar 2001; Grewal et al. 1998; Thon et al. 2005; Yamada-Inagawa et al. 2007) and *D. melanogaster* (Goll and Bestor 2005; Goll et al. 2006; Schaefer and Lyko 2010; Zemach et al. 2010), which shows little or no DNA methylation but still shows transgenerational transfer of nongenetic information via the gametes.

The existence of 5meC in the DNA of Drosophila remains debatable because it only contains an aspartic acid tRNA methyltransferase MT2, which is the only cytosine methyl transferase homolog, but without DNA methyltransferase activity (Goll et al. 2006). However, several reports have shown that low amounts of 5meC exist during the early embryo stages (Koryakov et al. 2011; Krauss and Reuter 2011; Rudolph et al. 2007; Schotta et al. 2002). However, since histone modifications are required for the initiation and maintenance of imprints in mammals, it is likely that DNA methylation, if it is present at all, is downstream

of Polycomb group (PcG) and Trithorax group (TrxG) complexes that modify histones (see later sections).

Several theories are proposed to explain the transgenerational epigenetic inheritance and related processes such as imprinting. It is suggested that histone modification appears to be a more ancient but less stable mark for imprinting compared to DNA methylation (Suter et al. 2004; Dalgaard et al. 2001).

10.2 Role of Chromatin Marks in Transgenerational Epigenetic Inheritance

Chromatin consists of histones and other proteins to form the chromatin fiber which wraps around the DNA for packaging and various functions. There are four types of histones, H2A, H2B, H3, and H4. Two copies of each histone wraps around ~146 base pairs of DNA to form a nucleosome. The nucleosomes are connected through 20–60 base pairs linker DNA and linker histone, H1, to form 10 nm "beads-on-a-string" fibers. Then the 10 nm fibers are wrapped into 30 nm diameter helical filaments.

The chromatin structure functions not only in the organizing of DNA but also in regulating gene transcription through epigenetic modifications. It is well established that the tails of several histones can have various posttranslational modifications, such as lysine acetylation, lysine and arginine methylation, phosphorylation, and ubiquitination. The regulatory effects of these modifications are carried out by a group of site-specific enzymes. For example, the mono-, di-, and trimethylation are executed by the histone methyltransferases (HMTs). Another important modification, acetylation, is expressed under the effect of two opposing enzyme groups: histone acetyltransferases (HATs) and histone deacetylases (HDACs). The euchromatin is often associated with lysine residue hyperacetylation, while the heterochromatin features deacetylated core histones, repressive marks, and formation of repressive effector complexes such as heterochromatin protein 1(HP1).

One of the important features of heterochromatin is that it can spread along the chromosomes and negatively regulate the expression of the neighboring genes through position-effect variegation (PEV), and this feature could be stably inherited during both mitosis and meiosis (Schotta et al. 2003a). Heterochromatin is rich in special regions of the genome such as centromeric, pericentromeric, and telomeric regions. It is assumed that the heterochromatin could segregate those special subnuclear regions to maintain repressive functions. In general, chromatin is classified into two categories: euchromatin and heterochromatin. Euchromatin is a loosely packed, gene-rich form of chromatin which is more accessible for transcription, whereas the heterochromatin is more condensed, gene-poor form of chromatin with less transcription activity.

10.3 Role of Small RNAs in Transgenerational Epigenetic Inheritance

It is thought that the noncoding RNAs play a vital role in transgenerational epigenetic inheritance, as well as in chromosomal dynamics. The small interfering RNAs (siRNAs) regulate DNA and histone methylation processes and are thus involved in the initiation and the maintenance of gene silencing. The RNA interference (RNAi) process is not only involved in eliminating or blocking the target mRNA in posttranscriptional gene silencing (PTGS) but also in the transcriptional gene silencing (TGS). Transcriptional gene silencing is the result of histone modifications, creating an environment of heterochromatin around a gene that makes it inaccessible to transcriptional machinery (RNA polymerase, transcription factors, etc.).

The term "small RNA" includes small interfering RNAs (siRNAs), repeat-associated small interfering RNAs (rasiRNAs), and microRNAs (miRNAs), which share similar proteins in the pathways. Among those proteins, some members of the Argonaute protein family are the core players in the effector complexes in the pathways (Shi et al. 2004). One example of epigenetic regulation by small RNAs is RNA-directed DNA methylation (RdDM) observed in recombinant viroid-infected plants (Bernstein and Allis 2005). It is first reported that the potato spindle tuber viroid (PSTVd)-transfected tobacco plants results in extensively sequence-specific de novo methylation of the transgene DNA (Wassenegger et al. 1994).

Jones and colleagues found that double-stranded RNA (dsRNA) produced by the infected RNA virus can cause methylation and transcriptional gene silencing (TGS), which is inherited independent of a RNA trigger. The initiation of the RdDM is Met1 DNA methyltransferase expression independent, but the maintenance and transgenerational inheritance is Met1 dependent when the RNA trigger is absent (Wassenegger et al. 1994). One theory is proposed that the DNA methylation is triggered by special DNA structure to initiate the methylation, and this is followed by TGS (Muskens et al. 2000). But recent evidence suggests the TGS could be triggered by RNA through RNA–DNA interaction. Similarly, noncoding RNA was found helping the establishment of heterochromatin in fission yeast through histone methylation and DNA methylation (Hall et al. 2002).

Noncoding RNAs are also used in dosage compensation. The organization of a noncoding RNA at a specific DNA locus is demonstrated by the roX RNAs in the dosage compensation complex (DCC) in Drosophila. The DCC complex consists of five core proteins, MSL1, MSL2, MSL3, MLE, and MOF, and two noncoding RNAs, roX1 and roX2. It is thought that the roX RNA can help the DCC complex target the X chromosomes (Gu et al. 1998). Research shows that the removal of both roX1 and roX2 results in the mislocalization of the DCC complex and the loss of acetylation of histone 4 at lysine 16 (H4K16Ac) by the MOF histone acetyltransferase on the X chromosome, but the details of the mechanism are still unclear (Meller and Rattner 2002).

It is interesting that several members of the DCC complex are RNA-interacting proteins. The maleless (MLE) protein contains motifs similar to RNA-dependent ATPases family and is associated with the male X chromosome (Richter et al. 1996). The association of the histone acetyltransferase MOF and the X chromosome is also dependent on the noncoding roX RNA, as well as the MSL-3 proteins (Akhtar et al. 2000). Considering the importance of noncoding RNAs in the normal function of DCC complex, it is necessary to consider the role of noncoding RNA in other types of epigenetic inheritance.

In mammals, one of the X chromosomes in females needs to be inactivated by a random mechanism (Heard 2004). The X chromosome inactivation starts at a single site called *X inactivation center* (*Xic*), and *Xist* (X inactive-specific transcript) gene inside *Xic* produces a 17 kb noncoding RNA that coats the X chromosome and promotes inactivation (Avner and Heard 2001). The *Xist* RNA is essential for the initiation of X chromosome activation, and its absence will cause embryonic lethality in embryonic stem (ES) cells (Penny et al. 1996). The inactivation process driven by *Xist* RNA includes hypoacetylation of histones H3 and H4 and methylation of H3K9, H3K27, and H4K20, which in turn promotes the alteration of chromatin structures (Heard 2004). One of the enzymes involved in this process is the E(z) histone methylation transferase, a member of the Polycomb group (PcG) that is required to maintain the repression status of certain genes (Silva et al. 2003).

Transgenerational epigenetic inheritance is also present in *Drosophila* Y chromosome imprinting, described by Maggert and colleagues (2002). They found that reporter genes within 23 independent P-elements insertions on the heterochromatic Y chromosome of *Drosophila* showed differential expression levels according to the parental sources of chromosome, while the autosomal heterochromatin insertion showed similar expression levels. The Y chromosome from *Drosophila* males suppresses (PEV), in which the insertion or translocation of a gene near heterochromatin causes variegated expression (Wakimoto 1998). For example, the In(1) wm4h rearrangement, which was isolated by Muller in 1930, has eyes with a strong white-mottled phenotype (Muller 1930). This rearrangement, which juxtaposes the white locus to centric X heterochromatin, has frequently been used for isolating PEV-modifying mutations, such as Su(var) (suppressor of variegation) and E(var) (enhancer of variegation) mutations (Moore et al. 1983; Reuter and Wolff 1981).

As with the PcG and TrxG proteins, many of the Su(var) and E(var) proteins are involved in posttranslational modifications of the histones. For example, Su(var)3-9 is a histone 3 lysine 9 (H3K9) methyl transferase, and the H3K9me3 epigenetic mark is associated with regions of condensed chromatin that do not allow transcription of most genes (Schotta et al. 2003b). It is worth noticing that *mod(mdg4),* also called *E(var)3-3,* affects the Y chromosome imprinting for several generations. The mutation of *mod* reduced the effect of Y chromosome on suppressing variegation by imprinting the Y chromosome. Dorn and colleagues have shown that the Y chromosome from *mod*(*mdg4*) males does not suppress variegation even in male offspring that do not inherit the *mod*(*mdg4*) mutation. This "paternal effect"

phenotype is stable and lasts for at least 11 generations through the male germline, which is as long as the experiment was carried out.

Hannon's laboratory discovered that the Piwi proteins, which are in the Argonaute family of slicer proteins, and piRNAs, which are small RNAs similar to microRNAs, may be the carrier of epigenetic information (Brennecke et al. 2008). The Piwi proteins are associated with *Drosophila* hybrid dysgenesis, in which the phenotypes of different intercrosses are dependent on the parental phenotypes. The Piwi proteins will be discussed in more details later in terms of how they are involved in transgenerational epigenetic inheritance in Drosophila.

10.4 Polycomb Group (PcG) and Trithorax Group (TrxG) Complexes in Drosophila

The *Polycomb* and *Trithorax* group proteins are essential to maintain the expression patterns of critical developmental genes during cellular development (Kennison 1995). The *Polycomb* group proteins maintain the repressed transcriptional states of developmental important genes while their antagonists, the *Trithorax* group proteins, exert their effect by keeping active transcriptional states of target genes. The first *Polycomb* (*Pc*) mutation was identified over 60 years ago. Ed Lewis proposed that the Pc proteins repress the genes in the Bithorax gene complex (Lewis 1978). Currently, there are 18 Polycomb group (PcG) protein members identified.

The Polycomb proteins are conservative during evolution from Drosophila to humans. The precise mechanisms of controlling the repression states of target genes are still unclear, but it is suggested that the PcG proteins may reduce the transcriptional activity by establishing the repressive chromatin marks on the histones like histone 3 lysine 27 tri-methylation (H3K27me3) (Schotta et al. 2003a; Shi et al. 2004). On the other hand, the TrxG proteins could introduce the active domain marks on histones like histone 3 lysine 4 tri-methylation (H3K4me3) for prolonged active transcription states of target genes (Klymenko and Muller 2004; Poux et al. 2002).

Both the PcG and TrxG complexes associate with Polycomb response elements/Trithorax response elements (PREs/TREs) which are several hundred base pairs long and have multiple transcription factor binding sites (Muller and Kassis 2006). These sites are scattered throughout the genome at precise locations in Drosophila, but distinct locations of PREs/TREs in mammalian cells have not yet been identified (Muller and Kassis 2006). Recently, long noncoding RNAs (lncRNAs) have been shown to form complexes with Polycomb proteins and have been proposed to direct these proteins to PREs (Aguilo et al. 2011; Kogo et al. 2011).

10.5 Drosophila Transgenerational Epigenetic Inheritance System 1: PcG and TrxG

PcG and TrxG complexes are apparently involved in transgenerational epigenetic inheritance in Drosophila. In 1998, Cavalli and Paro demonstrated that the TrxG complex on a transgene carrying a PRE/TRE sequence is maintained in both the soma and the germline (Cavalli and Paro 1998, 1999). By employing an artificial P-element transgene reporter system which a PRE/TRE preceding GAL4 binding sites regulates the expression level of *LacZ*, they showed that overexpression of a strong transcriptional activator GAL4 in the embryo overcame the repressive PcG state of the PRE/TRE and switch to the active TrxG state (Cavalli and Paro 1998, 1999).

Cavalli and Paro's system is an excellent system to begin to understand the mechanism of transgenerational epigenetic inheritance in Drosophila. In addition to the GAL4/LacZ reporter gene mentioned above, there is also a mini-w+ (mini-white) gene which is also regulated by PRE/TRE (presumably via spreading of inactive chromatin over the entire region), and its expression level can be visualized by measuring the redness of the eyes. They found that the active expression status of both LacZ and mini-w+ is maintained throughout the larval mitotic division and a certain percentage of mothers can transmit this status to their progenies, while the hsp70-GAL4 gene is absent (Cavalli and Paro 1998, 1999).

It is likely that GAL4-mediated transcriptional activation of the reporter genes requires the participation of Rvb1p/Rvb2p chromatin remodeling proteins in order to remove the PcG complex because they can provide the energy needed for chromatin remodeling process (Heard 2004; Penny et al. 1996). Recent studies also show that Rvb1p/Rvb2p proteins are required for epigenetic regulation of nonpermissive chromatin near the telomeres in yeast (Silva et al. 2003). The evidence from Cavalli and Paro supported transgenerational epigenetic inheritance because the active/TrxG state is inherited in more than one generation and no major DNA sequence changes are introduced to affect the PRE/TRE switch (Cavalli and Paro 1998, 1999). They also showed that the presence of GAL4 will remove the PcG group proteins from the PRE/TRE sites in the artificial construct. They also showed that a mutation in the TrxG gene Trithorax diminishes the transgenerational epigenetic inheritance, thereby validating the cross-inhibitory interactions of the TrxG and PcG groups (Cavalli and Paro 1998, 1999).

In 2003, the Bantignies lab discovered the Polycomb-dependent chromosome interactions between a PRE in the transgene and a PRE in the endogenous Ubx gene can be stably meiotically inherited (Bantignies et al. 2003). By three-dimensional fluorescent in situ hybridization (FISH), they showed that the mutation of the Ubx PRE could abolish the "three-dimensional interactions" between the PREs and cause stable epigenetic activation of the transgene for several generations. This phenotype could not be rescued by restoring the Ubx PRE in the F2 generation by backcrossing the PRE-mutated flies to wild-type flies (Bantignies et al. 2003). It is intriguing that elevated temperature restores repression of the transgene (Bantignies

et al. 2003), which suggests a role for Hsp90 in switching from and active/TrxG state to a repressive/PcG state (see below).

10.6 Transgenerational Epigenetic Inheritance System 2: Gametic Epigenetic Inheritance of Tumors

Xing and colleagues recently worked on suppressors and enhancers of $Hop^{Tum\text{-}1}$, a dominant Janus kinase (JAK), which causes a hematopoietic phenotype in adult flies by a transgenerational epigenetic inheritance system similar to that reported by Cavalli and Paro (1998, 1999; Shi et al. 2006; Xing et al. 2007). The global signaling of JAK was observed counteracting the formation of heterochromatin (Shi et al. 2006). Several enhancers of $Hop^{\text{Tum-1}}$ were identified with parental inheritance, including a loss-of-function allele of the Zn-finger transcription factor Krüppel, Kr^1 (Xing et al. 2007). They found that $Hop^{\text{Tum-1}}$/+ females mated to Kr^1/+ males produced F1 progeny with significantly enhanced size and number of hematopoietic tumors, regardless of whether or not they inherited the Kr^1 mutation (Xing et al. 2007).

The inheritance of Kr^1-like phenotype in Kr + progeny was attributed to the DNA methylation at Kr target sites (Xing et al. 2007). They found increased DNA methylation in an ftz promoter region which is regulated by Kr and conclude that the aberrant ftz transcription and promoter methylation are both transgenerationally inheritable. They came to the conclusion that the role of $Hop^{\text{Tum-1}}$ is the over-activation of JAK that disrupts epigenetic reprogramming and allows inheritance of methylation Kr target sequences that influence tumorigenesis in future generation (Xing et al. 2007).

However, as mentioned earlier, most laboratories do not see any DNA methylation in Drosophila (Goll and Bestor 2005; Goll et al. 2006). Nevertheless, even if DNA methylation occurs in Drosophila, the DNA methylation would likely be downstream of PcG function because PcG proteins are more likely to establish and maintain DNA methylation marks. We think that it is more likely that Rvb1p/Rvb2p or SMYD3/Trithorax inactivation by $Hop^{\text{Tum-1}}$ (which might reduce Hsp90 levels, see below) mediates the switch from active to inactive chromatin at the Kr PRE/TRE.

The findings from Xing and colleagues suggest that there would be increased susceptibility to cancers in the offspring of cancer patients, regardless of whether they inherited any of their parent's tumor susceptibility genes. Such a non-Mendelian inheritance system in epidemiological studies would generally be passed off as an "environmental" condition. To test this hypothesis, Feinberg and colleagues are developing a more rigorous statistical system to account for transgenerational epigenetic inheritance in cancer etiology (Feinberg 2007; Feinberg et al. 2006).

10.7 Transgenerational Epigenetic Inheritance System 3: Multigenerational Epigenetic Inheritance of Ectopic Large Bristle Outgrowths (ELBOs) in Drosophila

The transgenerational epigenetic inheritance system that we developed in our laboratory used another allele of Krüppel, KrIf-1 (Sollars et al. 2003). KrIf-1 is a spontaneous allele that was generated by the insertion of a repetitive sequence into the Kr promoter that causes ectopic overexpression of Kr mRNA and Kr protein in the eye imaginal disc during larval development (Carrera et al. 1998; Preiss et al. 1985; Ruden et al. 2005). We identified modifiers of KrIf-1 "small eye" phenotype with unusual properties (Ruden et al. 2005). We found that the maternal reduction of either Hsp90 (Hsp83 in Drosophila) or any member of TrxG genes will cause dramatic ectopic large bristle outgrowths (ELBOs) that resembled proximal appendages protruding from the ventral regions of one or both eyes.

Similar to the results observed by Li and colleagues with Kr^1 in their transgenerational epigenetic inheritance system, the maternal loss of Hsp83 is required to cause the ELBO phenotype. Interestingly, in both systems, the presence of the Hsp83 mutation is not required to maintain the phenotype in the affected progeny. It is interesting that the ELBO phenotype can also be induced by feeding geldanamycin to an isogenic strain of parental Drosophila with the KrIf-1 mutation. Since the geldanamycin is a specific and potent Hsp90 inhibitor, it indicates that loss of Hsp90 activity and not something else in the genetic background has established a postulated KrIf-1 metastable epiallele (Ruden et al. 2005).

Gangaraju and colleagues used the ELBO transgenerational epigenetic system to show that Piwi also suppresses epigenetic variation in a process that requires Hsp90 (Gangaraju et al. 2011), and they propose that the combined genetic and epigenetic increase in diversity in times of stress can dramatically increase phenotypic variation that can be selected. Gangaraju and colleagues show that combined maternal and zygotic loss of Piwi causes the ELBO phenotype in the F1 offspring and that Hsp90 activates Piwi by binding to it and allowing it to be phosphorylated at unknown sites by unidentified kinases (Gangaraju et al. 2011). They also show that flies with restored Piwi function that are selected for eight generations can be either epigenetically or genetically "canalized" (buffered) for the ELBO phenotype (Gangaraju et al. 2011), similar to what we showed in our original paper (Sollars et al. 2003). Furthermore, they show, as we did (Sollars et al. 2003), that Wg (Wingless/Wnt), is a downstream target of Kr that is apparently ectopically expressed in the wing imaginal discs (we later determined that Wg is not actually expressed in the wing imaginal discs, but rather in the hemocytes which are on the surface of the discs and are likely involved in tissue reengineering to generate the ELBOs (Ruden et al. 2003)).

Piwi prevents transposon mobilization in the male germline by "slicing" (chopping up) transposon RNAs (Brennecke et al. 2007; Zambon et al. 2006). Specchia and colleagues showed that Hsp90 and Piwi prevent phenotypic variation by suppressing transposon mobilization in the male germline (Specchia et al. 2010).

In this manner, Specchia and colleagues (2010) propose that Hsp90 is an "adaptively inducible canalizer" that can increase genetic variation in times of stress, and this newly induced genetic variation can be genetically assimilated over many generations to increase the fitness of the organism. In other words, they propose that Piwi is normally preventing transposon mobilization, but stress inactivates Piwi and the new transposon insertions and genomic rearrangements creates new genetic variability that can be selected from the random chaos. This is not a new idea either – Barbara McClintock proposed in her Nobel Prize seminar in 1983 that transposon mobilization in times of stress can be a last-ditch effort for an organism to reorganize its genome to save a sinking ship (McClintock 1984).

What is unique to the work of Gangaraju and colleagues (2011), other than the exciting finding that Piwi is involved in this transgenerational epigenetic process, is that Kr mRNA expression itself increases in the heads of the flies with the ELBOs. Since KrIf-1 is a spontaneous mutation that is presumably caused by a transposon insertion, an attractive model is that active Piwi-RISC (RNA induced silencing complexes) silences the KrIf-1 allele in a similar manner as it silences transposons (Fig. 10.1). Piwi-RISC is thought to silence transposons in two sequential steps: first by slicing the transposon transcripts, and then by interacting with heterochromatin protein 1 (HP1), and directly repressing transcription of the transposons (Fig. 10.1a). If KrIf-1 is caused by a transposon insertion, then recruitment of Piwi-RISC and HP1 to the KrIf-1 promoter can repress expression of Kr in the eye imaginal discs and prevent ELBO formation (Fig. 10.1a). If Hsp90 is inactivated, either by the specific inhibitor geldanamycin or by mutating Hsp83, the Drosophila gene that encodes Hsp90, then the transposons are expressed and mobilized and KrIf-1 is expressed and ectopic Kr protein in the eyes causes the ELBO phenotype (Fig. 10.1b).

While this is an attractive hypothesis to explain how inactive Piwi might cause transposon mobilization, we believe that a more complex mechanism must be responsible to explain the transgenerational epigenetic phenomena. We found, for instance, that Kr is expressed in the eye imaginal disc indistinguishably in the KrIf-1/+ flies regardless of whether the ELBOs were present or not. Indeed, since KrIf-1/KrIf-1 flies survive and have almost no eye tissues, increasing the expression of Kr mRNA, as shown by Gangaraju and colleagues should only cause the eyes to be smaller and not induce ELBOs (Gangaraju et al. 2010).

We propose instead that ELBOs are induced by a more complex mechanism. For example, Piwi might be involved in DNA looping or some other topological conformation such as localization of Polycomb response elements (PREs) or insulators to "Polycomb bodies" which are distinct nuclear loci where Polycomb complexes aggregate (Saurin et al. 1998). These complexes, we propose, are what are selected for and stabilized in the transgenerational selection experiments. In our original paper, for instance, we found that the strongest inducer of the ELBO phenotype was maternal deficiency of the TrxG gene verthandi (vtd), which was recently found to encode the Rad21 subunit of Drosophila cohesin (Hallson et al. 2008). This supports our hypothesis because Rad21-cohesin was found to associate with the CTCF sites at the IGF2-H19 DMR (differentially methylated region) and

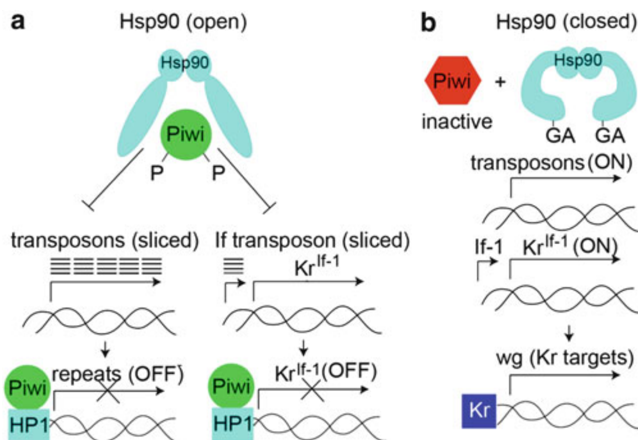

Fig. 10.1 Model for Piwi repression of Kruppel expression in the female germline. (**a**) Hsp90 binds to Piwi and this leads to its activation by phosphorylation. *Left*, Active Piwi slices transposon RNA into small fragments and the Piwi-RNA forms a complex to repress transcription of the transposons. *Right*, The KrIf-1 locus has a repeat sequence in the promoter that gets sliced by active Piwi. Repression of the repeat at this locus leads to repression of Kruppel (*Kr*) expression in the female germline. (**b**) In the absence of Hsp90, Piwi is not phosphorylated and is not active. Kruppel expression is ectopically present in many tissues, such as the eye imaginal discs, where it leads to the formation of ectopic large bristle outgrowths. However, we do note that this model explains some of the genetic data (Fig. 10.2)

siRNA knockdown of Rad21-cohesin causes both disruption of large DNA loops and loss of imprinting at this locus (Stedman et al. 2008). Also, Piwi has been shown to be required for long-range interactions at Drosophila PREs which control long-term expression or repression of the Hox genes by the TrxG and PcG genes, respectively (Grimaud et al. 2006). Based on these results, we propose an alternative model that Piwi, possibly in a complex with vtd, is forming a loop or some other structure among various Kr target genes, such as Wg or the Kr locus itself, and this looping somehow prevents Kr from activating expression of the target genes (Fig. 10.2a). When Piwi is inactive, the loop is broken and Kr can now activate its target genes and induce ELBOs (Fig. 10.2b). This mechanism can be tested, for instance, by mutating the various domains of Piwi, such as the slicing catalytic function, which should not affect chromatin looping, or the HP1-interaction site, which might.

In summary, the results of Gangaraju and colleagues (2011) provide evidence for a more general model of Piwi functioning as an “adaptively inducible canalizer” to both suppress transposon-mediated mutagenesis and stabilize metastable epialleles. In other words, the environment is feeding back onto the genome more than what was ever thought to occur in the past.

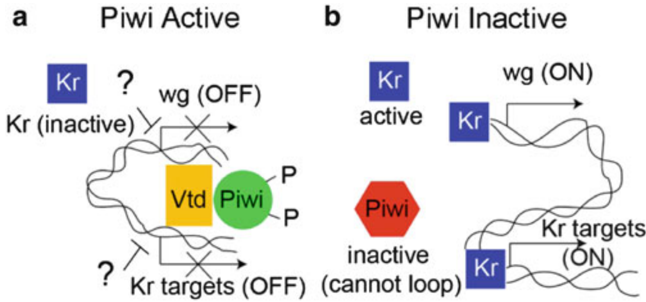

Fig. 10.2 Model for Piwi action involving long DNA loops. (**a**) Active Piwi forms a complex with proteins involved in DNA loop formation, such as the verthandi (*vtd*) protein which encodes the Rad21 subunit of cohesin. The looping complex prevents the Kruppel protein (*Kr*) from activating its target genes, such as the Wingless (Wg) gene. According to this model, the loops are inhibitory of Kruppel action and occur in tissues where Kruppel is not required for development, such as in the eye imaginal discs. The loops prevent Kruppel from activating its downstream genes. (**b**) When Piwi is inactive, the DNA loops do not occur. This allows Kruppel protein to activate its target genes, such as Wingless. Activated targets in the eye imaginal discs cause ectopic large bristle outgrowths (*ELBOs*)

10.8 Conclusions and Future Directions

We have described three transgenerational epigenetic inheritance systems in Drosophila, all of which likely function in the absence of DNA methylation. Common features, we believe, involve small RNAs, Polycomb and Trithorax complexes, and DNA looping. Further development of third-generation DNA-sequencing technologies that can map histone patterns and DNA loops at single cell resolution in the germline is needed to fully understand transgenerational epigenetic inheritance in Drosophila. The answers that are eventually obtained in model organisms such as what we describe in this chapter will help address the role of the environment in shaping evolution in humans.

An analogous system in humans would be a multigenerational increase in the severity of cancer in a manner that is independent on the accumulation of tumor-causing genes. While speculative, such an accumulative transgenerational epigenetic inheritance system in humans might explain the generational increase in environmentally sensitive diseases, such as diabetes, cardiovascular disease, autism, and cancer. Genetics alone cannot explain the dramatic increases in some of these diseases during that past few decades, but an epigenetics approach might help us finally reach a better understanding of the processes involved.

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References

Aguilo F, Zhou MM, Walsh MJ (2011) Long noncoding RNA, polycomb, and the ghosts haunting INK4b-ARF-INK4a expression. Cancer Res 71:5365–5369

Akhtar A, Zink D, Becker PB (2000) Chromodomains are protein-RNA interaction modules. Nature 407:405–409

Ashworth D, Bishop M, Campbell K, Colman A, Kind A, Schnieke A, Blott S, Griffin H, Haley C, McWhir J, Wilmut I (1998) DNA microsatellite analysis of Dolly. Nature 394:329

Avner P, Heard E (2001) X-chromosome inactivation: counting, choice and initiation. Nat Rev Genet 2:59–67

Bantignies F, Grimaud C, Lavrov S, Gabut M, Cavalli G (2003) Inheritance of polycomb-dependent chromosomal interactions in Drosophila. Genes Dev 17:2406–2420

Bernstein E, Allis CD (2005) RNA meets chromatin. Genes Dev 19:1635–1655

Blewitt ME, Vickaryous NK, Paldi A, Koseki H, Whitelaw E (2006) Dynamic reprogramming of DNA methylation at an epigenetically sensitive allele in mice. PLoS Genet 2:e49

Brennecke J, Aravin AA, Stark A, Dus M, Kellis M, Sachidanandam R, Hannon GJ (2007) Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila. Cell 128:1089–1103

Brennecke J, Malone CD, Aravin AA, Sachidanandam R, Stark A, Hannon GJ (2008) An epigenetic role for maternally inherited piRNAs in transposon silencing. Science 322:1387–1392

Buiting K, Gross S, Lich C, Gillessen-Kaesbach G, el-Maarri O, Horsthemke B (2003) Epimutations in Prader-Willi and Angelman syndromes: a molecular study of 136 patients with an imprinting defect. Am J Hum Genet 72:571–577

Bulfield G, Campbell K, James R, Wilmut I (1998) Voices from Roslin: the creators of Dolly discuss science, ethics, and social responsibility. interview by Arlene Judith Klotzko. Camb Q Healthc Ethics 7:121–140

Carrera P, Abrell S, Kerber B, Walldorf U, Preiss A, Hoch M, Jackle H (1998) A modifier screen in the eye reveals control genes for Kruppel activity in the Drosophila embryo. Proc Natl Acad Sci U S A 95:10779–10784

Cavalli G, Paro R (1998) The Drosophila Fab-7 chromosomal element conveys epigenetic inheritance during mitosis and meiosis. Cell 93:505–518

Cavalli G, Paro R (1999) Epigenetic inheritance of active chromatin after removal of the main transactivator. Science 286:955–958

Chan TL, Yuen ST, Kong CK, Chan YW, Chan AS, Ng WF, Tsui WY, Lo MW, Tam WY, Li VS, Leung SY (2006) Heritable germline epimutation of MSH2 in a family with hereditary nonpolyposis colorectal cancer. Nat Genet 38:1178–1183

Dalgaard JZ, Klar AJ (2001) Does *S. pombe* exploit the intrinsic asymmetry of DNA synthesis to imprint daughter cells for mating-type switching? Trends Genet 17:153–157

Duhl DM, Vrieling H, Miller KA, Wolff GL, Barsh GS (1994) Neomorphic agouti mutations in obese yellow mice. Nat Genet 8:59–65

Falls JG, Pulford DJ, Wylie AA, Jirtle RL (1999) Genomic imprinting: implications for human disease. Am J Pathol 154:635–647

Feinberg AP (2007) An epigenetic approach to cancer etiology. Cancer J 13:70–74

Feinberg AP, Ohlsson R, Henikoff S (2006) The epigenetic progenitor origin of human cancer. Nat Rev Genet 7:21–33

Gangaraju VK, Yin H, Weiner MM, Wang J, Huang XA, Lin H (2011) Drosophila Piwi functions in Hsp90-mediated suppression of phenotypic variation. Nat Genet 43:153–158

Goll MG, Bestor TH (2005) Eukaryotic cytosine methyltransferases. Annu Rev Biochem 74:481–514

Goll MG, Kirpekar F, Maggert KA, Yoder JA, Hsieh CL, Zhang X, Golic KG, Jacobsen SE, Bestor TH (2006) Methylation of tRNA Asp by the DNA methyltransferase homolog Dnmt2. Science 311:395–398

Grewal SI, Bonaduce MJ, Klar AJ (1998) Histone deacetylase homologs regulate epigenetic inheritance of transcriptional silencing and chromosome segregation in fission yeast. Genetics 150:563–576

Grimaud C, Bantignies F, Pal-Bhadra M, Ghana P, Bhadra U, Cavalli G (2006) RNAi components are required for nuclear clustering of Polycomb group response elements. Cell 124:957–971

Gu W, Szauter P, Lucchesi JC (1998) Targeting of MOF, a putative histone acetyl transferase, to the X chromosome of *Drosophila melanogaster*. Dev Genet 22:56–64

Hall IM, Shankaranarayana GD, Noma K-i, Ayoub N, Cohen A, Grewal SIS (2002) Establishment and maintenance of a heterochromatin domain. Science 297:2232–2237

Hallson G, Syrzycka M, Beck SA, Kennison JA, Dorsett D, Page SL, Hunter SM, Keall R, Warren WD, Brock HW, Sinclair DA, Honda BM (2008) The Drosophila cohesin subunit Rad21 is a trithorax group (trxG) protein. Proc Natl Acad Sci U S A 105:12405–12410

Heard E (2004) Recent advances in X-chromosome inactivation. Curr Opin Cell Biol 16:247–255

Hitchins M, Williams R, Cheong K, Halani N, Lin VA, Packham D, Ku S, Buckle A, Hawkins N, Burn J, Gallinger S, Goldblatt J, Kirk J, Tomlinson I, Scott R, Spigelman A, Suter C, Martin D, Suthers G, Ward R (2005) MLH1 germline epimutations as a factor in hereditary nonpolyposis colorectal cancer. Gastroenterology 129:1392–1399

Hitchins MP, Wong JJ, Suthers G, Suter CM, Martin DI, Hawkins NJ, Ward RL (2007) Inheritance of a cancer-associated MLH1 germ-line epimutation. N Engl J Med 356:697–705

Kennison JA (1995) The Polycomb and trithorax group proteins of Drosophila: trans-regulators of homeotic gene function. Annu Rev Genet 29:289–303

Klymenko T, Muller J (2004) The histone methyltransferases Trithorax and Ash1 prevent transcriptional silencing by Polycomb group proteins. EMBO Rep 5:373–377

Kogo R, Shimamura T, Mimori K, Kawahara K, Imoto S, Sudo T, Tanaka F, Shibata K, Suzuki A, Komune S, Miyano S, Mori M (2011) Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. Cancer Res 71:6320–6326

Koryakov DE, Walther M, Ebert A, Lein S, Zhimulev IF, Reuter G (2011) The SUUR protein is involved in binding of SU(VAR)3-9 and methylation of H3K9 and H3K27 in chromosomes of *Drosophila melanogaster*. Chromosome Res 19:235–249

Krauss V, Reuter G (2011) DNA methylation in Drosophila – a critical evaluation. Prog Mol Biol Transl Sci 101:177–191

Lewis EB (1978) A gene complex controlling segmentation in Drosophila. Nature 276:565–570

Maggert KA, Golic KG (2002) The Y chromosome of *Drosophila melanogaster* exhibits chromosome-wide imprinting. Genetics 162:1245–1258

McClintock B (1984) The significance of responses of the genome to challenge. Science 226:792–801

Meller VH, Rattner BP (2002) The roX genes encode redundant male-specific lethal transcripts required for targeting of the MSL complex. EMBO J 21:1084–1091

Moore GD, Sinclair DA, Grigliatti TA (1983) Histone gene multiplicity and position effect variegation in *Drosophila melanogaster*. Genetics 105:327–344

Morgan HD, Santos F, Green K, Dean W, Reik W (2005) Epigenetic reprogramming in mammals. Hum Mol Genet 14:R47–R58

Muller HJ (1930) Types of visible variations induced by X-rays in Drosophila. J Genet 22:299–334

Muller J, Kassis JA (2006) Polycomb response elements and targeting of polycomb group proteins in Drosophila. Curr Opin Genet Dev 16:476–484

Muskens MW, Vissers AP, Mol JN, Kooter JM (2000) Role of inverted DNA repeats in transcriptional and post-transcriptional gene silencing. Plant Mol Biol 43:243–260

Penny GD, Kay GF, Sheardown SA, Rastan S, Brockdorff N (1996) Requirement for Xist in X chromosome inactivation. Nature 379:131–137

Poux S, Horard B, Sigrist CJ, Pirrotta V (2002) The Drosophila trithorax protein is a coactivator required to prevent re-establishment of polycomb silencing. Development 129:2483–2493

Preiss A, Rosenberg UB, Kienlin A, Seifert E, Jackle H (1985) Molecular genetics of Kruppel, a gene required for segmentation of the Drosophila embryo. Nature 313:27–32
Reik W, Walter J (2001) Genomic imprinting: parental influence on the genome. Nat Rev Genet 2:21–32
Reuter G, Wolff I (1981) Isolation of dominant suppressor mutations for position-effect variegation in *Drosophila melanogaster*. Mol Gen Genet 182:516–519
Richter L, Bone JR, Kuroda MI (1996) RNA-dependent association of the Drosophila maleless protein with the male X chromosome. Genes Cells 1:325–336
Rideout WM III, Eggan K, Jaenisch R (2001) Nuclear cloning and epigenetic reprogramming of the genome. Science 293:1093–1098
Ruden DM, Garfinkel MD, Sollars VE, Lu X (2003) Waddington's widget: Hsp90 and the inheritance of acquired characters. Semin Cell Dev Biol 14:301–310
Ruden DM, Xiao L, Garfinkel MD, Lu X (2005) Hsp90 and environmental impacts on epigenetic states: a model for the trans-generational effects of diethylstibesterol on uterine development and cancer. Hum Mol Genet 14 Spec No 1:R149–R155
Rudolph T, Yonezawa M, Lein S, Heidrich K, Kubicek S, Schäfer C, Phalke S, Walther M, Schmidt A, Jenuwein T, Reuter G (2007) Heterochromatin formation in Drosophila is initiated through active removal of H3K4 methylation by the LSD1 homolog SU(VAR)3-3. Mol Cell 26:103–115
Santos F, Dean W (2004) Epigenetic reprogramming during early development in mammals. Reproduction 127:643–651
Saurin AJ, Shiels C, Williamson J, Satijn DP, Otte AP, Sheer D, Freemont PS (1998) The human polycomb group complex associates with pericentromeric heterochromatin to form a novel nuclear domain. J Cell Biol 142:887–898
Schaefer M, Lyko F (2010) Lack of evidence for DNA methylation of Invader4 retroelements in Drosophila and implications for Dnmt2-mediated epigenetic regulation. Nat Genet 42:920–921
Schotta G, Ebert A, Krauss V, Fischer A, Hoffmann J, Rea S, Jenuwein T, Dorn R, Reuter G (2002) Central role of Drosophila SU(VAR)3-9 in histone H3-K9 methylation and heterochromatic gene silencing. EMBO J 21:1121–1131
Schotta G, Ebert A, Dorn R, Reuter G (2003a) Position-effect variegation and the genetic dissection of chromatin regulation in Drosophila. Semin Cell Dev Biol 14:67–75
Schotta G, Ebert A, Reuter G (2003b) SU(VAR)3-9 is a conserved key function in heterochromatic gene silencing. Genetica 117:149–158
Shi H, Djikeng A, Tschudi C, Ullu E (2004) Argonaute protein in the early divergent eukaryote *Trypanosoma brucei*: control of small interfering RNA accumulation and retroposon transcript abundance. Mol Cell Biol 24:420–427
Shi S, Calhoun HC, Xia F, Li J, Le L, Li WX (2006) JAK signaling globally counteracts heterochromatic gene silencing. Nat Genet 38:1071–1076
Shiels PG, Kind AJ, Campbell KH, Wilmut I, Waddington D, Colman A, Schnieke AE (1999) Analysis of telomere length in Dolly, a sheep derived by nuclear transfer. Cloning 1:119–125
Silva J, Mak W, Zvetkova I, Appanah R, Nesterova TB, Webster Z, Peters AH, Jenuwein T, Otte AP, Brockdorff N (2003) Establishment of histone h3 methylation on the inactive X chromosome requires transient recruitment of Eed-Enx1 polycomb group complexes. Dev Cell 4:481–495
Sollars V, Lu X, Xiao L, Wang X, Garfinkel MD, Ruden DM (2003) Evidence for an epigenetic mechanism by which Hsp90 acts as a capacitor for morphological evolution. Nat Genet 33:70–74
Specchia V, Piacentini L, Tritto P, Fanti L, D'Alessandro R, Palumbo G, Pimpinelli S, Bozzetti MP (2010) Hsp90 prevents phenotypic variation by suppressing the mutagenic activity of transposons. Nature 463:662–665
Stedman W, Kang H, Lin S, Kissil JL, Bartolomei MS, Lieberman PM (2008) Cohesins localize with CTCF at the KSHV latency control region and at cellular c-myc and H19/Igf2 insulators. EMBO J 27:654–666

Suter CM, Martin DI, Ward RL (2004) Germline epimutation of MLH1 in individuals with multiple cancers. Nat Genet 36:497–501

Suter CM, Martin DI, Ward RL (2007) Addendum: germline epimutation of MLH1 in individuals with multiple cancers. Nat Genet 39:1414

Thon G, Hansen KR, Altes SP, Sidhu D, Singh G, Verhein-Hansen J, Bonaduce MJ, Klar AJ (2005) The Clr7 and Clr8 directionality factors and the Pcu4 cullin mediate heterochromatin formation in the fission yeast *Schizosaccharomyces pombe*. Genetics 171:1583–1595

Waddington CH (1942) Canalization of development and the inheritance of acquired characters. Nature 150:563–565

Wakimoto BT (1998) Beyond the nucleosome: epigenetic aspects of position-effect variegation in Drosophila. Cell 93:321–324

Walter J, Paulsen M (2003) Imprinting and disease. Semin Cell Dev Biol 14:101–110

Wassenegger M, Heimes S, Riedel L, Sänger HL (1994) RNA-directed de novo methylation of genomic sequences in plants. Cell 76:567–576

Weaver ICG, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ (2004) Epigenetic programming by maternal behavior. Nat Neurosci 7:847–854

Wilmut I (2003) Dolly-her life and legacy. Cloning Stem Cells 5:99–100

Wilmut I (2005) The search for cells that heal the creator of Dolly the cloned sheep asks that society look past the controversies to the ultimate payoff. Sci Am 293:A35

Wilmut I, Sullivan G, Taylor J (2009) A decade of progress since the birth of Dolly. Reprod Fertil Dev 21:95–100

Wolff GL, Kodell RL, Moore SR, Cooney CA (1998) Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. FASEB J 12:949–957

Xing Y, Shi S, Le L, Lee CA, Silver-Morse L, Li WX (2007) Evidence for transgenerational transmission of epigenetic tumor susceptibility in Drosophila. PLoS Genet 3:1598–1606

Yamada-Inagawa T, Klar AJ, Dalgaard JZ (2007) *Schizosaccharomyces pombe* switches mating type by the synthesis-dependent strand-annealing mechanism. Genetics 177:255–265

Zambon RA, Vakharia VN, Wu LP (2006) RNAi is an antiviral immune response against a dsRNA virus in *Drosophila melanogaster*. Cell Microbiol 8:880–889

Zemach A, McDaniel IE, Silva P, Zilberman D (2010) Genome-wide evolutionary analysis of eukaryotic DNA methylation. Science 328:916–919

Chapter 11
Environmental Epigenetics and Epigenetic Transgenerational Inheritance

Michael K. Skinner

Abstract The developing role of environmental epigenetics in biology and medicine is discussed in relationship to its impact on transgenerational inheritance. The ability of environmental factors, such as nutrition and toxicants, to promote transgenerational adult-onset disease through alterations in the germline epigenome is reviewed. Observations suggest epigenetic transgenerational inheritance has a significant impact on evolutionary biology, disease etiology, and toxicology.

Keywords Epigenetics • Developmental origin of disease • Environment • Germline • Inheritance • Toxicants • Transgenerational

Abbreviations

BPA Bisphenol A

11.1 Introduction

Although the influence of environmental factors on biology has been appreciated and investigated for hundreds of years, the basic molecular mechanisms by which the environment regulates biology have for the most part been lacking.

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The majority of environmental factors from nutrition, temperature, and toxicants cannot alter DNA sequence. Nevertheless, they are known to have long-lasting effects on phenotype and disease for most organisms. Early observations by Lamarck and others suggested phenotypic changes resulted from environmental exposures (Jirtle and Skinner 2007). Potential mechanisms for these interactions were initially proposed in the 1940s by Conrad Waddington, the person who started the field of epigenetics (Waddington 1940, 1956). The specific molecular mechanisms involved, however, were not elucidated until more recently (Chen and Riggs 2005; Holliday and Pugh 1975).

Epigenetics is defined as molecular factors and processes around DNA that regulate genome activity independent of DNA sequence and that are mitotically stable (Skinner et al. 2010). These factors include DNA methylation, histone modifications, chromatin structure, and some noncoding RNAs (Jirtle and Skinner 2007; Skinner et al. 2010). Epigenetics is a critical element in the regulation of genome activity, and it enables the environment to interact with genetic factors in the regulation of biology. Thus, in contrast to genetic factors and classic Mendelian genetics that are generally resistant to environmental influences, epigenetic factors provide an efficient means by which the environment can influence genomic activity and have long-lasting effects on physiology, phenotype, and biology (Skinner 2011).

Epidemiology studies for decades have suggested significant environmental impacts on biology that could not be explained by genetics alone. Examples are as follows:

1. Regional differences in specific disease types and frequencies worldwide. If individuals are moved early in life to a different region, they will often acquire the disease types and frequencies associated with that region.
2. The majority of diseases have a rather small component that can be attributed to genetic abnormalities or mutations. This suggests a potential alternate mechanism for disease etiology other than genetics.
3. Nearly all diseases have shown a dramatic increase in frequency over the past several decades; this observation cannot be explained by genetic mechanisms.
4. Hundreds of environmental compounds or toxicants are associated with the onset of disease, but many do not have the ability to alter DNA sequence or promote mutations.
5. With regard to evolutionary biology, the same organism does not evolve at the same rate in different parts of the world, indicating environmental impacts on the process.

These are just a few of the biological observations that cannot be easily explained through classical genetic mechanisms. Epigenetics provides a molecular mechanism that can help explain all these phenomena, providing a general mechanism of how the environment can influence biology. This scientific field of research is called environmental epigenetics (Jirtle and Skinner 2007).

The majority of environmental exposures will act on somatic cells to influence biology. This includes nutrition, temperature, stress, and toxicological agents.

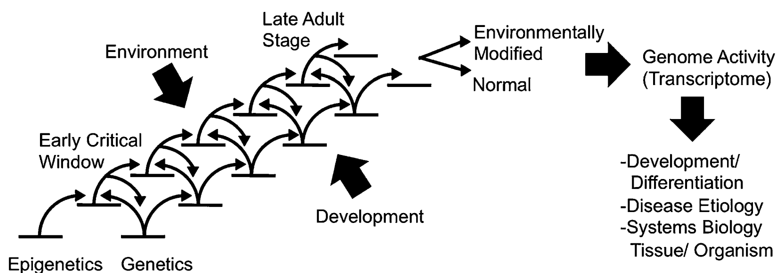

Fig. 11.1 Epigenetic and genetic cascade of events in development (Skinner 2011)

At critical windows of exposure for a given somatic cell type or tissue, these environmental exposures can shift the normal differentiation through modifications of the epigenome. The altered epigenome then interacts with genome and results in an abnormal state of cellular or tissue differentiation (Fig. 11.1). The primary reason this phenomena occurs is because of the mitotic stability of epigenetic modifications and the presence of a critical window of susceptibility during early development. Thus, the ability to replicate the cellular epigenome during mitosis results in cells that are influenced by environmental exposures early in development to form an altered epigenome that persists throughout the life of the individual.

This provides the mechanism for the early life basis of adult-onset disease and phenotype. Environmental exposures throughout life affect various somatic cell types at their specific critical windows of development to promote a later-life disease or phenotypes associated with that somatic cell type. For example, a fetal exposure to abnormal nutrition promotes metabolic disease later in life due to the critical developmental windows of the associated organs. In contrast, a pubertal exposure affects developing organs such as the mammary gland and prostate gland since their somatic cells are developing during puberty. After the individual has become an adult and the developmental process is complete, the individual develops a resistance to environmental exposures and shifts in phenotype. Thus, environmental exposures of somatic cells during development will provide the majority of altered phenotypes and disease states that are observed in humans. These somatic cell effects generally are restricted to isolated cell types and will not be able to be transmitted to the next generation (Skinner 2010; Skinner et al. 2010).

However, it is possible to also transmit environmental exposure information to the next generation through epigenetic modifications in the germlines (i.e., sperm and egg). An environmental exposure that affects the germline has a relatively narrow critical window of development, while the germ cell programs its epigenome and development. If the germline epigenome is modified, impacts on the generation derived from that germline can occur; however, this is still not considered transgenerational since the germline was directly exposed. Nevertheless, if the epigenetic modification becomes permanently programmed, a potential transgenerational phenotype can be inherited. Thus, the majority of environmental exposures affect somatic cells that cannot produce transgenerational effects.

Direct germline exposures can influence the individual developed from that germ cell, but the effects primarily end with that individual. In rare instances, environmental exposures that permanently modify the germline epigenome and promote a transgenerational phenotype can profoundly affect the incidence of human diseases.

11.2 Epigenetic Transgenerational Inheritance

The transmission and inheritance of epigenetic information transgenerationally can have significant impacts on biology. The definition of epigenetic transgenerational inheritance is as follows: *Epigenetic transgenerational inheritance involves the germline transmission of epigenetic information between generations in the absence of direct environmental exposures*. An environmental exposure of the germline that produces a phenotype in the next generation that germline develops is not a transgenerational phenomenon (Fig. 11.2).

The concept and clarifications of an environmental exposure involving a multigenerational exposure or a transgenerational phenomenon have been previously described (Skinner 2008, 2010). Exposure of a gestating female (F0 generation) also exposes the fetus (F1 generation) and the germline that will generate the F2 generation (Fig. 11.2). Therefore, exposure of a gestating female involves a multiple-generation exposure influencing the F0, F1, and F2 generations. The F3 generation is the first generation that is not directly exposed and is considered to potentially result from epigenetic transgenerational inheritance. The exposure of an adult male involves the exposure of the adult individual (F0 generation) and the germline that will generate the F1 generation (Fig. 11.2). Therefore, it is not until the F2 generation that epigenetic transgenerational inheritance can be considered. Since direct environmental exposure simply involves classic pharmacological or toxicological effects of the exposure, only a phenotype that appears in an unexposed generation is considered to potentially result from epigenetic transgenerational inheritance.

The epigenetic transgenerational inheritance requires the involvement of the germline. The critical windows of environmental exposure for the germline primarily involve the development of primordial germ cells and cell fate determination in the sperm or egg during embryonic gonadal sex determination. When the primordial germ cells migrate down the genital ridge during embryonic development in mammals, DNA demethylation of the primordial germ cell genome occurs such that upon colonization of the gonad prior to gonadal sex determination, the primordial germ cell is in a nearly unmethylated, pluripotent state. At the onset of gonadal sex determination, the primordial germ cell initiates DNA remethylation to develop a male- or female-specific DNA methylation pattern and commitment to a sperm or egg cell lineage (Fig. 11.3) (Allegrucci et al. 2005; Durcova-Hills et al. 2006). Due to this DNA demethylation and remethylation prior to and during gonadal sex determination, the germline is sensitive to disruption in epigenetic programming from environmental agents. This results in epigenetic alterations having the

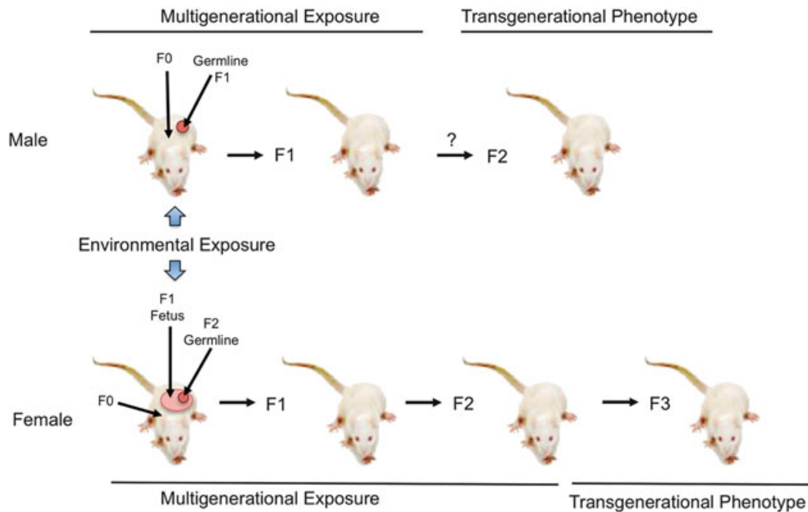

Fig. 11.2 Transgenerational epigenetic inheritance in males and females in response to environmental agents. In a gestating mother, there is multiple-generation exposure of the F0 female, the F1 embryo, and the F2 generation germline to environmental factors. The transgenerational transmission of disease phenotypes through the male germline (*labeled red*) is indicated. Both male and female offspring develop disease, but the transgenerational phenotype is transmitted only paternally after exposure to vinclozolin (Skinner 2010)

capacity to become permanently programmed in an abnormal manner in the germ cells (Anway et al. 2005; Guerrero-Bosagna et al. 2010). These imprinted-like sites act like imprinted genes that have a parent-of-origin allele-specific DNA methylation program that is transgenerationally inherited (Guerrero-Bosagna et al. 2010).

The other developmental stage where the germ cell epigenome undergoes demethylation is after fertilization. The early developing embryo then initiates remethylation around the blastula stage of development prior to implantation. Genomically imprinted genes escape this demethylation while the rest of the genome, for the most part, is demethylated and reset to erase the prior generation's effects on the germ cell epigenome (Fig. 11.3) (Morgan et al. 2005). Therefore, the programming of the germline epigenome during gonadal sex determination is the most critical window of exposure to permanently alter the epigenome for transmission transgenerationally (Jirtle and Skinner 2007; Morgan et al. 2005; Skinner et al. 2010). Although epigenetic alterations during gametogenesis in the adult gonads are possible, these changes do not appear to become permanently programmed. They can promote multigenerational effects in the next generation the exposed germline generates, but these epigenetic sites seem to be corrected at subsequent

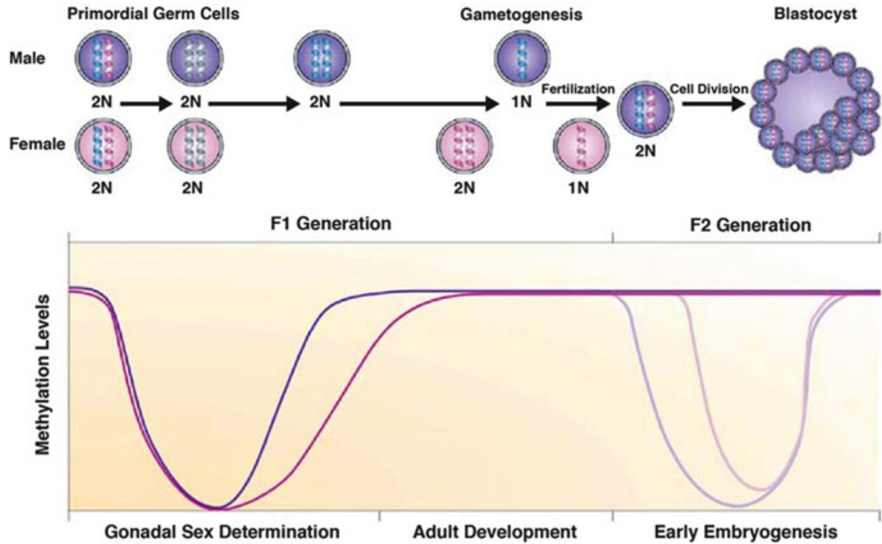

Fig. 11.3 Alterations in methylation status during development. During embryonic development and gonadal sex determination, primordial germ cells undergo genome-wide demethylation, which erases previous parental-specific methylation marks that regulate imprinted gene expression. In the male germline (*colored purple*), paternal methylation marks in imprinted genes are laid down in developing gonocytes that will develop into spermatogonia. The female germline (*colored pink*) establishes maternal methylation marks in imprinted genes at a later stage of development. After fertilization, the paternal genome is actively demethylated (indicated by the *lighter purple line* in the graph), whereas the maternal genome undergoes passive demethylation (indicated by the *lighter pink line* in the graph). Genome-wide remethylation occurs on both parental genomes before implantation; however, imprinted genes maintain their methylation marks throughout this reprogramming. This allows for the inheritance of parental-specific monoallelic expression in somatic tissues throughout adulthood (Jirtle and Skinner 2007)

generations (Skinner 2010). More extensive research in this area is required to determine if unique epigenetic modifications allow for the formation of direct or indirect transgenerational phenotypes.

The environmental exposures that alter the epigenome of the germline to promote epigenetic transgenerational inheritance do not follow classic genetics or Mendelian processes. Therefore, epigenetic inheritance provides an alternate mechanism of heritability not previously appreciated. An important aspect of this process is that it is responsive to environmental factors and exposures. Therefore, epigenetic transgenerational inheritance provides a mechanism by which the environment can alter biology and fills a void in classic genetics. Non-Mendelian and familial inheritance of different disease states and phenotypes can now include epigenetic modifications as potential mechanisms for transgenerational inheritance.

11.3 Environmental Induction of Epigenetic Transgenerational Inheritance

One of the initial demonstrations of environmental toxicants producing epigenetic transgenerational inheritance involved the actions of the endocrine disruptors, vinclozolin and methoxychlor (Anway et al. 2005). Vinclozolin is one of the most commonly used fungicides in agriculture and is an antiandrogenic endocrine disruptor (Wong et al. 1995). Methoxychlor is a commonly used pesticide and has a combination of estrogenic, antiestrogenic, and antiandrogenic endocrine disruptor activities (Tiemann 2008). These compounds promote transgenerational adult-onset disease and spermatogenic defects in the F1 to F4 generations (Anway et al. 2005). Vinclozolin also promotes a series of adult-onset diseases, including infertility, prostate disease, kidney disease, and mammary gland tumors in aging rats in the F1 to F3 generations (Anway et al. 2006; Nilsson et al. 2008). Recently, the transgenerational epigenetic modifications in the sperm of the male F3 generation animals were mapped (Guerrero-Bosagna et al. 2010). Significant effects on the DNA methylation of different promoters were identified genome-wide. The actions of these epigenetic transgenerational effects are mediated through the male germline (Anway et al. 2005) and affect the transcriptomes of all developing tissues examined (Anway et al. 2008). In addition to an epigenetic transgenerational effect on the differential DNA methylation marks in sperm (Guerrero-Bosagna et al. 2010), all tissues derived from that sperm have transgenerational effects on tissue-specific transcriptomes.

Thus, endocrine disruptor-induced epigenetic transgenerational inheritance involves actions that alter gonadal sex determination programming by modifying DNA methylation in the developing male germline which permanently programs the sperm so that the altered epigenome can be transmitted to subsequent generations. This ultimately leads to all tissues that developed from the male germline to develop altered transcriptomes and increased susceptibility to develop adult-onset disease (Fig. 11.4) (Skinner et al. 2010). Therefore, environmental exposures can promote transgenerational inheritance of adult-onset disease or phenotypes through this elucidated epigenetic mechanism.

A number of other studies from different laboratories have now also identified environmentally induced epigenetic transgenerational inheritance of adult-onset disease phenotypes (Table 11.1) (Skinner et al. 2010). These studies involve the actions of environmental endocrine disruptors and toxicants, such as bisphenol A (BPA) to promote testis abnormalities (F3 generation) (Salian et al. 2009), dioxin to promote uterus abnormalities (F3 generation) (Bruner-Tran and Osteen 2011), and vinclozolin to promote imprinted gene DNA methylation abnormalities (F3 generation) (Guerrero-Bosagna et al. 2010; Stouder and Paoloni-Giacobino 2010). Likewise, pharmaceutical agents, such as thyroxine and morphine, can promote behavioral abnormalities in the F1 to F3 generations (Vyssotski 2011). In addition to environmental toxicant exposures, abnormal nutrition also promotes epigenetic transgenerational inheritance of disease states (Bertram et al. 2008; Kaati et al. 2007;

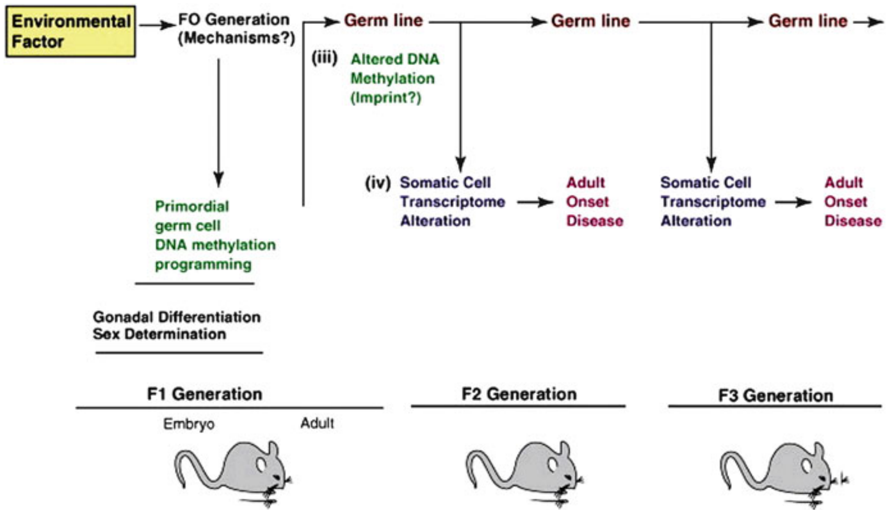

Fig. 11.4 Role of the germline in epigenetic transgenerational inheritance. An environmental factor acts on the F0 generation gestating female to influence the developing F1 generation fetus and alter gonadal development to reprogram the primordial germ cell DNA methylation. This altered DNA methylation in the germline becomes permanently programmed similar to an imprinted-like gene and is transferred through the germline to subsequent generations. The embryo generated from this germline starts with an altered epigenome that causes developing somatic cells and tissues to have an altered transcriptome. This altered somatic cell transcriptome can then promote adult-onset disease associated with the transgenerational phenotype (Skinner et al. 2010)

Table 11.1 Epigenetic transgenerational inheritance

Environmental epigenetic transgenerational inheritance	Reference
Vinclozolin induced epigenetic transgenerational adult-onset disease in rats (F1–F4)	Anway et al. (2005)
BPA-induced transgenerational testicular abnormality (F1–F3)	Salian et al. (2009)
Dioxin induced transgenerational uterine abnormality (F1–F4)	Bruner-Tran and Osteen (2011)
Morphine induced	Vyssotski (2011)
Stress induced behavior alterations (F0–F2)	Matthews and Phillips (2010, 2011)
Nutrition induced transgenerational obesity in mice (F1–F3)	Waterland et al. (2008), Bertram et al. (2008), Pentinat et al. (2010), Kaati et al. (2007)
Transgenerational response in longevity to nutrition (F0–F2)	Waterland et al. (2008), Bertram et al. (2008), Pentinat et al. (2010), Kaati et al. (2007)
Tumor susceptibility in Drosophila (F1–F3)	Xing et al. (2007)
Stem cell culture-induced adult-onset disease (F0–F4)	Lee et al. (2009)

Pentinat et al. 2010; Waterland et al. 2008). This includes caloric restriction promoting metabolic disease phenotypes (F3 generation) (Waterland et al. 2008) and high-fat diets promoting adult-onset metabolic disease and obesity (Pentinat et al. 2010). Furthermore, factors such as stress (Matthews and Phillips 2010, 2012), temperature (Xing et al. 2007), and cell culture (Lee et al. 2009) influence transgenerational phenotypes (Table 11.1). A number of endocrine (Walker and Gore 2011) and genetic (Nadeau 2009) influences also effect epigenetic transgenerational inheritance. All these environmental exposures used the critical window of germline programming during gonadal sex determination. Moreover, several epidemiology studies provide evidence for epigenetic inheritance of disease phenotypes in humans (Pembrey et al. 2006). It is anticipated that any environmental exposure that significantly alters normal fetal gonadal development will promote epigenetic transgenerational inheritance. The biological impacts of this phenomenon are significant and are a previously unappreciated regulatory factor in biology.

11.4 Conclusions and Biological Impacts

The ability of environmental exposures to influence generational effects significantly alters our understanding of the basic regulation of biology. Consequently, a number of different areas now need to be considered with regard to how the environment can have long-lasting effects, not only on the individual exposed but on subsequent progeny for generations to come. Environmental epigenetics provides a molecular mechanism by which environmental factors promote immediate and long-term effects on the individual exposed (Skinner et al. 2010). If the normal germline program is permanently altered, epigenetic transgenerational inheritance of disease or phenotypes can also be promoted. Due to the continued effect on subsequent programming and generations, several basic biological phenomena need to be reevaluated.

Basic developmental biology processes are currently thought to utilize primarily classic genetic mechanisms. Environmental epigenetics at critical windows of development can promote a different path for a developmental system and provide a mechanism of plasticity by which the environment can directly impact development (Fig. 11.1). This could help explain biological variation in the phenotypes of specific organisms and even tissues within an organism. Since an organism does not develop in a vacuum, but instead needs to respond to its environment, environmental epigenetics and transgenerational inheritance of epigenetic changes have a profound impact on developmental biology.

A large number of environmental compounds are known to be associated with the onset of disease, but how these compounds cause disease is often unknown. Molecular toxicology has been focused on the initial signal transduction processes without consideration of later-life effects. Environmental epigenetics enables molecular toxicologists to explain how the initial signaling events can promote both short- and long-term effects on disease susceptibility without causing DNA

mutations. Again, if the germline is modified, epigenetic transgenerational inheritance of these disease states and phenotypes will also need to be considered. The degree by which toxicological effects are transgenerational needs to be established within the population. Clearly, the impacts not only on the individual exposed but on subsequent generations raise the potential biohazards of environmental toxicants.

The current paradigm for the causal factor in disease etiology involves genetic mutations and chromosomal abnormalities. Nevertheless, only a small percentage of nearly all disease states are associated with known genetic mutations. Epigenetics provides a molecular mechanism that is anticipated to have a significant role in disease etiology and can respond to environmental factors to directly influence the onset of disease. As discussed, the prenatal and early postnatal exposures are likely more critical in disease etiology than the adult exposures that are more resistant to epigenetic change due to the mature differentiated state of the cells. The mitotic stability of the early life epigenetic alterations allows these transient environmental exposures to influence adult-onset disease. Epigenetic phenomena also can occur at high frequency and are reproducible compared to the extremely low frequency and nonreproducible nature of genetic mutations. Environmental epigenetics will likely play a critical role in disease etiology and cooperate with genetic processes and susceptibilities to influence disease.

The ability to permanently alter the epigenome of the germline to promote transgenerational disease or phenotypes also has significance with regard to evolutionary biology. Environmental epigenetics can play a significant role in the induction of phenotypic variation that facilitates adaptation events and natural selection. If these epigenetic alterations are transgenerational and the phenotypes appear in subsequent generations, the natural selection process is also facilitated and the inheritance of such phenotypes explained. Epigenetic transgenerational inheritance of environmentally induced phenotypic variation can explain rapid evolutionary processes and how the environment can influence evolution. This provides a solution for the time period issue that is problematic when only random genetic mutations are considered as the primary evolutionary events. Therefore, environmental epigenetics and epigenetic transgenerational inheritance are anticipated to impact most areas of biology and medicine. This should not be seen as a challenge to classic genetics and genomics, but instead seen as a complementary molecular mechanism to regulate genomic activity and provide a mechanism by which environmental factors can influence biology and disease formation.

References

Allegrucci C, Thurston A, Lucas E, Young L (2005) Epigenetics and the germline. Reproduction 129:137–149

Anway MD, Cupp AS, Uzumcu M, Skinner MK (2005) Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308:1466–1469

Anway MD, Leathers C, Skinner MK (2006) Endocrine disruptor vinclozolin induced epigenetic transgenerational adult-onset disease. Endocrinology 147:5515–5523

Anway MD, Rekow SS, Skinner MK (2008) Transgenerational epigenetic programming of the embryonic testis transcriptome. Genomics 91:30–40

Bertram C, Khan O, Ohri S, Phillips DI, Matthews SG, Hanson MA (2008) Transgenerational effects of prenatal nutrient restriction on cardiovascular and hypothalamic-pituitary-adrenal function. J Physiol 586:2217–2229

Bruner-Tran KL, Osteen KG (2011) Developmental exposure to TCDD reduces fertility and negatively affects pregnancy outcomes across multiple generations. Reprod Toxicol 31:344–350

Chen ZX, Riggs AD (2005) Maintenance and regulation of DNA methylation patterns in mammals. Biochem Cell Biol 83:438–448

Durcova-Hills G, Hajkova P, Sullivan S, Barton S, Surani MA, McLaren A (2006) Influence of sex chromosome constitution on the genomic imprinting of germ cells. Proc Natl Acad Sci U S A 103:11184–11188

Guerrero-Bosagna C, Settles M, Lucker BJ, Skinner MK (2010) Epigenetic transgenerational actions of vinclozolin on promoter regions of the sperm epigenome. PLoS One 5:e13100

Holliday R, Pugh JE (1975) DNA modification mechanisms and gene activity during development. Science 187:226–232

Jirtle RL, Skinner MK (2007) Environmental epigenomics and disease susceptibility. Nat Rev Genet 8:253–262

Kaati G, Bygren LO, Pembrey M, Sjostrom M (2007) Transgenerational response to nutrition, early life circumstances and longevity. Eur J Hum Genet 15:784–790

Lee J, Kanatsu-Shinohara M, Ogonuki N, Miki H, Inoue K, Morimoto T, Morimoto H, Ogura A, Shinohara T (2009) Heritable imprinting defect caused by epigenetic abnormalities in mouse spermatogonial stem cells. Biol Reprod 80:518–527

Matthews SG, Phillips DI (2010) Minireview: transgenerational inheritance of the stress response: a new frontier in stress research. Endocrinology 151:7–13

Matthews SG, Phillips DI (2012) Transgenerational inheritance of stress pathology. Exp Neurol 233(1):95–101

Morgan HD, Santos F, Green K, Dean W, Reik W (2005) Epigenetic reprogramming in mammals. Hum Mol Genet 14(1):R47–R58

Nadeau JH (2009) Transgenerational genetic effects on phenotypic variation and disease risk. Hum Mol Genet 18:R202–R210

Nilsson EE, Anway MD, Stanfield J, Skinner MK (2008) Transgenerational epigenetic effects of the endocrine disruptor vinclozolin on pregnancies and female adult onset disease. Reproduction 135:713–721

Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjostrom M, Golding J (2006) Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet 14:159–166

Pentinat T, Ramon-Krauel M, Cebria J, Diaz R, Jimenez-Chillaron JC (2010) Transgenerational inheritance of glucose intolerance in a mouse model of neonatal overnutrition. Endocrinology 151:5617–5623

Salian S, Doshi T, Vanage G (2009) Impairment in protein expression profile of testicular steroid receptor coregulators in male rat offspring perinatally exposed to Bisphenol A. Life Sci 85:11–18

Skinner MK (2008) What is an epigenetic transgenerational phenotype? F3 or F2. Reprod Toxicol 25:2–6

Skinner MK (2010) Metabolic disorders: fathers' nutritional legacy. Nature 467:922–923

Skinner MK (2011) Role of epigenetics in developmental biology and transgenerational inheritance. Birth Defects Res C Embryo Today 93:51–55

Skinner MK, Manikkam M, Guerrero-Bosagna C (2010) Epigenetic transgenerational actions of environmental factors in disease etiology. Trends Endocrinol Metab 21:214–222

Stouder C, Paoloni-Giacobino A (2010) Transgenerational effects of the endocrine disruptor vinclozolin on the methylation pattern of imprinted genes in the mouse sperm. Reproduction 139:373–379

Tiemann U (2008) In vivo and in vitro effects of the organochlorine pesticides DDT, TCPM, methoxychlor, and lindane on the female reproductive tract of mammals: a review. Reprod Toxicol 25:316–326

Vyssotski D (2011) Transgenerational epigenetic compensation. Evolocus 1:1–6

Waddington CH (1940) Organisers and genes. Cambridge University Press, Cambridge

Waddington CH (1956) Principles of embryology. George Allen & Unwin Ltd, London

Walker DM, Gore AC (2011) Transgenerational neuroendocrine disruption of reproduction. Nat Rev Endocrinol 7:197–207

Waterland RA, Travisano M, Tahiliani KG, Rached MT, Mirza S (2008) Methyl donor supplementation prevents transgenerational amplification of obesity. Int J Obes 32:1373–1379

Wong C, Kelce WR, Sar M, Wilson EM (1995) Androgen receptor antagonist versus agonist activities of the fungicide vinclozolin relative to hydroxyflutamide. J Biol Chem 270:19998–20003

Xing Y, Shi S, Le L, Lee CA, Silver-Morse L, Li WX (2007) Evidence for transgenerational transmission of epigenetic tumor susceptibility in Drosophila. PLoS Genet 3:1598–1606

Chapter 12
The Nature of Human Transgenerational Responses

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Abstract We review in this chapter what is known about transgenerational responses (TGRs) in humans, that is, a defined exposure in one generation producing a measurable outcome in the next, *unexposed* generation(s). The nature of the transgenerational signalling in humans is unknown, but epigenetic inheritance is one candidate. Human studies have focused on transmission down the male line because the Russian doll nature of the female line introduces many confounding influences that are difficult to take into account statistically. We summarise the key findings from TGR studies in three populations: ancestral food supply on grandchild's mortality rate in Överkalix in Northern Sweden, early paternal smoking on offspring body mass index in the Avon Longitudinal Study of Parents and Children (ALSPAC) in the UK, and paternal betel quid chewing on offspring metabolic syndrome risk in Taiwan. Exposure-sensitive periods in childhood and sex-specific transmissions are features that support biological rather than cultural transmission. Drawing on the results of animal experiments, we consider some

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possible mechanisms, including the ‘Y dipstick’ hypothesis, by which ancestral experience might impact on the health of future generations, and how these ideas can inform the design of future studies in humans.

Keywords Betel quid • Food supply • Human • Smoking • Transgenerational response (TGR) • Y chromosome

Abbreviations

ALSPAC	Avon Longitudinal Study of Parents and Children
BMI	Body mass index
TGR	Transgenerational response

12.1 Introduction

This chapter will briefly review what is known about transgenerational responses (TGRs) in humans. Drawing on the results of animal experiments, we consider some possible mechanisms by which ancestral experience might impact on the health of future generations, and how these ideas can inform the design of future studies in humans.

We use the term TGR to refer to any situation where a defined exposure in one generation produces a measurable outcome in the next, *unexposed* generation(s). We are not concerned here with the outcome, if any, in the exposed individual (including those exposed in utero through the mother). We use the word transgenerational ‘response’ rather than ‘effect’ because the human observations to date suggest that an existing mechanism is being induced.

Understanding the determinants of human health and disease depends on understanding the potential contributions to interindividual variation in development, physiology, and behaviour, particularly in response to the environment. To date studies of common complex disorders have tended to focus on genetic variation and the period from conception onwards. Past parental and ancestral experience is usually regarded as contributing only indirectly (i.e. culturally) to the prevailing social and physical environment of the family. Clearly, cultural transmission through parental nurturing behaviour and social patterning of all kinds also plays a part in human health.

Biological inheritance from one generation to the next is usually regarded as the result of transmission of genes and other DNA variation from both parents, plus ‘maternal effects’ carried either within the egg cytoplasm such as in mitochondria and RNA molecules or through the transplacental passage of toxins, nutrients, and

metabolic signals. There is growing evidence, however, both in animals (Carone et al. 2010; Franklin et al. 2010; Ng et al. 2010; Anderson et al. 2006; Drake and Walker 2004; Boucher et al. 1994) and humans (Bygren et al. 2001; Kaati et al. 2002; Pembrey et al. 2006; Bygren et al. 2006; Chen et al. 2006; Kaati et al. 2007) that sperm carry information about the ancestral environment that can influence the development and health of the next generation(s), presumably through enduring alterations in gene expression. Our use of the term TGR implies nothing about the mediating mechanisms or the nature of the transgenerational signal. The latter is unknown, but epigenetic inheritance is one candidate.

What follows concentrates on transmission down the male line because the Russian doll nature of the female line introduces many confounding influences that are difficult to take into account statistically. This makes interpretation of results, both negative and positive, hazardous. It should be noted that exposure of a woman carrying a 25-week-old female fetus exposes not only that daughter but also the fetal ovaries containing the recombining genomes destined for potential grandchildren.

Our focus on male line transmission does not mean that we dismiss the idea of TGR inheritance down the female line, only that it is more difficult to study. Indeed, our own results include evidence of the transmission of some kind of response by the paternal grandmother. In many ways, the female line already represents a system for the transgenerational flow of information about the past environment. Kuzawa (2005) suggests that the flow of nutrients reaching the fetus provides an integrated signal of nutrition as experienced by recent matrilineal ancestors, which effectively limits the responsiveness to short-term ecologic fluctuations during any given pregnancy. He calls this capacity intergenerational ‘phenotypic inertia’, suggesting that it allows the fetus to read the signal of longer-term ecologic trends.

12.2 Human Studies of Transgenerational Responses

There are human epidemiological male line TGR data from three populations: Överkalix in Northern Sweden, the Avon Longitudinal Study of Parents and Children (ALSPAC) in the UK, and betel quid chewers in Taiwan. In addition, there are some human studies that include exposure in the peri-conceptional period (Helgason and Jonasson 1981; Heijmans et al. 2008; Tobi et al. 2009), but they are not discussed further in this chapter.

12.2.1 Taiwan

An observed TGR to betel nut (*Areca catechu*) in CD1 mice indicates that paternal exposure transmitted an increased risk of hyperglycaemia and obesity to non-betel-fed first-generation offspring, especially male offspring (Boucher et al. 1994).

This led to an investigation within the Keelung Community-based Integrated Screening Program in Taiwan where Boucher and colleagues studied TGR with paternal betel quid (*paan*) chewing. They found a similar association to that found in mice, with a dose-dependent association of paternal betel quid use with early metabolic syndrome in the adult offspring who had never chewed betel quid themselves (Chen et al. 2006).

12.2.2 Sweden

The Överkalix studies are based on samples of individuals born in the town in specified years. Their longevity and other health outcomes were linked to detailed historical records of harvests and food supply experienced by their ancestors. Bygren's group initially looked for associations between the food supply in the childhood of the parents and grandparents and longevity of the study individuals born in 1905 (Bygren et al. 2001). They showed that the paternal grandfathers' food supply during mid-childhood (i.e. 'slow growth' period during the few years leading up to the prepubertal growth spurt) was associated with longevity in their grandchildren. Subsequent analyses, using three independent cohorts (Kaati et al. 2002), showed that the paternal grandfather's plentiful food supply in mid-childhood was associated with a fourfold increased chance of diabetes on the grandchild's death certificate (see also Bygren et al. 2006). This study also found that transgenerational responses to the parent's environment were similar to those in the grandparent's environment when the proband's (i.e. grandchildren's) own social and cultural circumstances were taken into account (Kaati et al. 2007).

12.2.3 United Kingdom

Marcus Pembrey in collaboration with Bygren's team in Sweden and with Jean Golding devised a study using data from ALSPAC to look for a TGR induced by the onset of smoking in the father's childhood, that is, starting with the exposed ancestor. The outcomes examined in the offspring were those found to be relevant in the Swedish study and included birth characteristics and body mass index (BMI) at ages 7 and 9 years. The ALSPAC data showed that the earlier the father started smoking, the shorter the gestational duration and greater the BMI at 9 years in sons but not daughters. The greatest association was found for sons of men who had started smoking before the age of 11.

12.2.4 Further Swedish Analyses

The ALSPAC results led to sex-specific analysis of the Överkalix data with dramatic results. It became apparent that the mortality rate of the men born in the target years was linked to just their paternal *grandfather's* food supply in mid-childhood, whereas the mortality rate of the women studied was associated solely with the paternal *grandmother's* food supply (Pembrey et al. 2006). This association was shown in 2 of 3 independent cohorts. The exposure-sensitive periods were both in paternal grandparents' mid-childhood and also in the fetal/infant period for the paternal grandmothers, but there was no triggering of TGR during adolescence in either sex (Fig. 12.1). The TGRs even persisted when the grandchild's early life circumstances were taken into account (Kaati et al. 2007). With this latter adjustment, a father-to-son effect was also revealed, with a good food supply in the fathers' mid-childhood, leading to increased mortality rate in his male offspring.

12.2.5 Subsequent Information

The accumulation of evidence for human TGR with sex-specific transmission patterns from the ALSPAC-Överkalix collaboration have been summarised recently (Pembrey 2010). Further ALSPAC analyses show that paternal *onset* of smoking before age 11 continues to be associated with greater BMI in sons (but not daughters to the same extent) up to the age of 15, with the most striking findings at age 13 (unpublished results).

12.2.6 Key Features of the Human TGR Data to Date

There are several features of these human TGR data that are worth noting (Fig. 12.1):

- There are exposure-sensitive periods in childhood in terms of inducing a TGR in the next generation(s). Both the Överkalix and ALSPAC data show that exposure of boys in mid-childhood before puberty, but not during puberty itself, is associated with a TGR. There are currently no published data on the age of onset of paternal betel quid chewing in the Taiwan study. The three generations of data from Överkalix show two exposure-sensitive periods in the paternal grandmother, the fetal/infant period, and mid-childhood.
- These paternal transmissions show sex-specific effects, but the outcomes are not sex limited, with granddaughters and grandsons affected depending on the ancestral exposure.

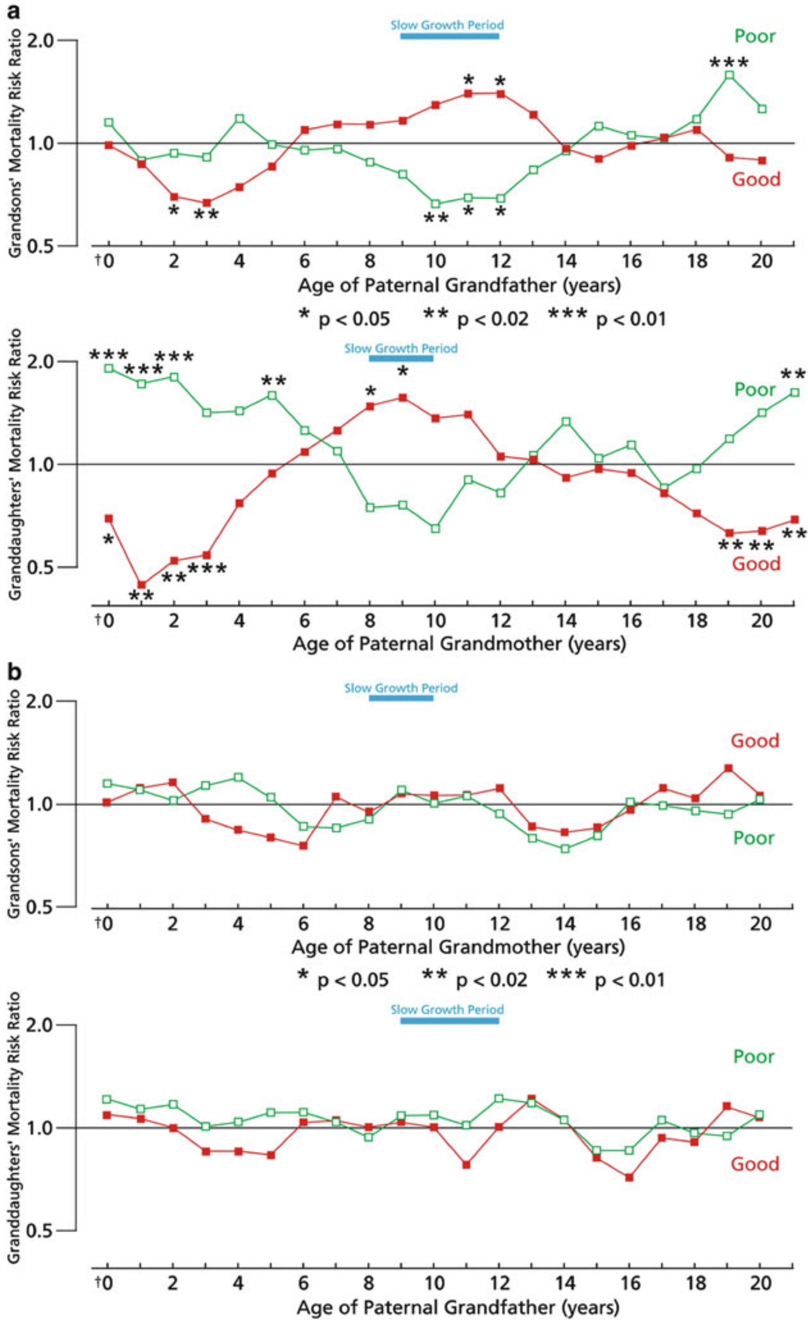

Fig. 12.1 Överkalix data on the effect of paternal grandparental food supply at different times in their early life on the mortality rate of their grandchildren. On the y-axis is a measure of mortality rate in the grandsons and granddaughters. The age at which the paternal grandparent was exposed

- Although the paternal grandparent-to-grandchild effects in the Överkalix data are the most striking, father-to-son effects are also observed in the Överkalix studies in line with the ALSPAC and Taiwan data.
- Figure 12.1 also illustrates a dose-dependent association. The mortality risk ratio of the grandchild (y-axis) portrayed in red (solid squares) is for good ancestral food supply compared to moderate ancestral food supply. The mortality risk ratio portrayed in green (open squares) is for poor ancestral food supply compared to moderate ancestral food supply. A dose-dependent association is revealed by the red and green lines going in opposite directions. This helps to define the exposure-sensitive periods in ancestral childhood. There was also a dose-dependent association between paternal betel chewing and offspring risk of metabolic syndrome (Chen et al. 2006).
- Taken together, the evidence points to sperm carrying information about paternal or ancestral exposure rather than the transgenerational outcomes being just the consequence of social patterning and cultural transmission. The latter possibility would not easily explain the dose dependency and why the timing of exposure in childhood would be so critical for triggering a TGR and its direction. It also does not explain the Överkalix results where the same set of fathers transmit a TGR to just children of one sex depending on ancestral exposure.
- While paternal grandmothers do show an exposure-sensitive period in mid-childhood like paternal grandfathers, the most striking TGR comes from exposure in the fetal/infant period. Since TGRs represent information capture and transmission by the germ line, this process would not be expected to have the same timing in males and females. Gametogenesis is much earlier in the female, as is the most exposure-sensitive period.
- The patterns in Fig. 12.1, including the reversal in the direction of the TGR depending on the exposure period of the paternal grandmother, suggest that the transgenerational effects observed are a feature of a pre-evolved response mechanism. If TGRs have indeed evolved, at least in part, as a means of transgenerational adaptation to the prevailing physical and social environment, one would expect TGRs to be triggered before puberty, so that all future offspring benefit.

Fig. 12.1 (continued) to a good (*solid red squares*) or a poor food supply (*open green squares*) is given along the x-axis. The grandparent's food supply is associated with a shorter life for the grandchild when the risk ratio is significantly *above* 1 (Fig. 12.2a). In contrast, the grandparent's food supply is associated with a longer lifespan in the grandchild when the risk ratio is *below* 1 (Fig. 12.2a). There is no significant effect of grandparent's food supply on the grandchild's longevity when the risk ratio is 1 (Fig. 12.2b) (Figure reproduced with permission (Pembrey et al. 2006)) ($^{*}p$ <0.05; $^{**}p$ <0.02; $^{***}p$ <0.01)

12.3 Possible TGR Mechanisms

There has been interest in the impact of experience before breeding on subsequent generations for centuries, although in the second half of the twentieth century, anything that hinted at Lamarckism was treated with great suspicion. In fact, many mammalian experiments documented transgenerational effects (reviewed by Campbell and Perkins 1988), but there were no plausible biological mechanisms until the discovery of genomic imprinting in the 1980s. This normal phenomenon of parent-of-origin-dependent differential gene expression establishes the principle of an epigenetic mark (DNA methylation) placed in one generation influencing gene expression in the next. This led to speculation that environmentally induced epigenetic changes at imprinted genes might constitute a form of transgenerational adaptation (Pembrey 1996). The idea of a 'feedforward loop' provided a theoretical background to the transgenerational studies of ancestral food supply by Bygren's group already done in Sweden (Bygren et al. 2001).

12.3.1 Sex-Specific Transmissions: Is the Y Chromosome Involved?

The Swedish study provides evidence of TGRs through the male line, such that fathers are able to transmit a signal from their own father to just their sons and/or a signal from their own mother to just their daughters. This segregation suggests a chromosome-associated signal rather than transmission of free-lying elements in sperm, such as microRNAs (Rassoulzadegan et al. 2006; Anderson and Kedersha 2009), although both might be involved. The most parsimonious explanation is that the human TGRs described to date are transmitted across the generations by the Y chromosome and possibly the X (Fig. 12.2). DNA sequences within the non-recombining part of the Y chromosome (perhaps encoding microRNAs) might accumulate epigenetic marks in response to early experiences which persist more easily through meiosis than elsewhere in the genome because of the lack of chromosome pairing and recombination. It is also possible that this part of the Y chromosome could tolerate the evolution of a section of 'responsive DNA' that could change *reversibly* in response to toxic exposures, in effect a genotoxic sensor. Whatever the mechanism, we refer to this preferential transmission of the memory of early experience to the next generation by the Y chromosome as the 'Y dipstick' hypothesis.

Mammalian experiments on TGRs have reported numerous sex-specific effects after paternal exposure before breeding. This can lead to affected offspring of both sexes (Carone et al. 2010; Boucher et al. 1994), solely or mainly in males (Anderson et al. 2006; Drake and Walker 2004; Anway et al. 2005), and solely or mainly in females (Ng et al. 2010). Multiple-generation TGRs through the male line

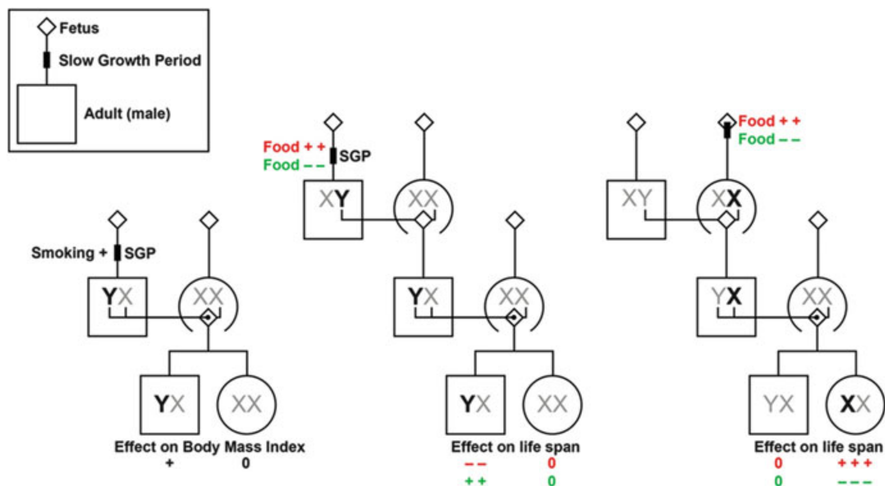

Fig. 12.2 Representative developmental family pedigrees of the life course from fetus to adulthood. They illustrate the relationship between paternal or grandparental exposure and the outcomes in children or grandchildren. The sex chromosomes are included to show the correspondence between the transmission of the TGRs and the Y chromosome (*bold*) or the X chromosome (*bold*). In the latter case, there is the potential for the grandmother's fetal/infant food supply to indirectly influence her son's X chromosome up until his birth by modifying metabolism and transplacental effects

include examples of transmission by intermediate males who are not themselves overtly affected by the outcome (Franklin et al. 2010).

12.3.2 What 'State' Is Transmitted by the Sperm?

The triggers for the above experimental mammalian TGRs include low-protein diet, chronic high-fat diet, preconceptional fasting, preconceptional betel nut chewing, fetal exposure to the endocrine disrupter, vinclozolin, fetal exposure to the glucocorticoid, dexamethasone, and early trauma caused by chronic and unpredictable maternal separation postnatally. How can such different exposures induce a TGR? What is the common pathway?

In the past, paternal effects were thought to be through DNA mutations, and indeed some are. Extensive studies of low-dose radiation (and some mutagens) in mice and rats show that TGRs induced in males result in widespread genomic instability in all descendants of both sexes, even in chromosomes that were never irradiated (Barber et al. 2002; de Boer et al. 2010). This indicates the existence of a radiation-sensing mechanism that induces a cellular response. It is this response mode that is initially transmitted via sperm. It is thought that the downstream genomic instability in offspring of males exposed before breeding contributes to

their increased sensitivity to mutagens and tumour risk (de Boer et al. 2010; Lord et al. 1998). Exposures such as tobacco smoke and betel nut (or even possibly increased toxin intake at times of starvation or plenty) raise the question of direct toxic effects on the germ line, leading to extreme sperm selection, for example. Even in this scenario, however, the surviving sperm are likely to carry protective genomic adjustments, epigenetic or otherwise, implicating transmission of the 'response mode' rather than direct damage. Further support for this notion comes from so-called transgenerational genetic effects [in distinction to 'transgenerational environmental effects'] (Nelson and Nadeau 2010).

In specifically engineered congenic (cross-bred) strains of mice, the event inducing a TGR is a DNA mutation (probably of a particular sort), yet comparable phenotypic changes in subsequent generations can occur *without* transmission of the mutation. For example, C57BL/6J mice, which are normally obesity-susceptible with high food intake, were created to carry a mutation (Obrq2aA/J allele) that made them obesity resistant. Interestingly, breeding such a male led to offspring that did *not* inherit the mutation but still became resistant to obesity with high food intake for at least two generations; only male descendants were studied (Yazbek et al. 2010). Using a similar experimental approach (with controlled physical and social environment where appropriate), congenic male mice were created with different Y chromosomes (Nelson et al. 2010). Daughters of the males with a substituted Y chromosome and daughters of host strain males are genetically identical. The only difference is in the genetic constitution of their father's Y chromosome, which they do not inherit. Remarkably, across a broad panel of biochemical, physiological, and behavioural traits, frequent and large phenotypic differences occurred between these two classes of females, including mean platelet volume, triglycerides, bone mineral density, and startle reflex (Nelson et al. 2010). Thus, there may be mechanisms where it would not be necessary to invoke the X chromosome in the transmission of the TGR via father to granddaughter in the Överkalix study.

As we have noted above, another trigger in experimental TGR can be traumatic experiences in early life. Franklin et al. (2010) investigated the transgenerational effect of stress in mice induced by chronic and unpredictable maternal separation postnatally. As expected, when the offspring were adults, those exposed to separation were at increased risk of depressive-like behaviours, and their behavioural response to adverse environments was altered. Importantly, most of the behavioural alterations were expressed by the next generation in the offspring of males subjected to maternal separation, despite the fact that these males were reared normally. Again, the enduring response to stress is likely to be the trigger for the TGRs, possibility through glucocorticoid hormone mediators (Harris and Seckl 2010). Furthermore, chronic stress leads to DNA damage (Hara et al. 2011). Thus, it is plausible that all the various exposures mentioned above might lead to genotoxic stress, and it is the response to this that is transmitted and induces the TGR.

12.3.3 Outcomes in the Offspring

There are currently considerable restrictions in the range of offspring outcomes studied for any given experimental exposure in the published mammalian literature. Exceptions are descendant's adult pathology after vinclozolin exposure during rat development (Anway et al. 2006) and the phenotypic screen in the Y substitution congenic mouse experiment mentioned above (Nelson et al. 2010) that found both metabolic and behavioural differences. Usually, if the research group's interest is in diet, then a particular diet is chosen as the exposure, and growth and metabolism tend to be the offspring outcomes studied. If the interest is in behavioural response to social stress, then offspring outcomes relate to behavioural states.

Restriction to specific candidate associations already established in single-generation studies is understandable given our rudimentary knowledge of TGRs and the paradigm shift it represents. Nevertheless, there is no reason to assume there are only specific associations between a particular exposure and an outcome in offspring. It is just as plausible that one of several adverse exposures might induce a broad generic outcome in the next generation(s). Certainly, in single-generation fetal 'programming' studies, fetal adversity is associated with a wide range of physical and psychological disorders in adulthood (Harris and Seckl 2010).

Considering the three human TGR studies, there was a deliberate alignment of offspring outcomes studied in ALSPAC with those observed in the initial Överkalix studies. Inclusion of diabetic deaths in the 2002 Överkalix study (Kaati et al. 2002) may have been influenced by the knowledge of early mammalian TGR experiments. This includes Boucher's betel nut study in mice (Boucher et al. 1994), a forerunner of the Taiwan study on early metabolic syndrome (Chen et al. 2006). That said, there is some coherence between the TGR outcomes of the three studies: adult cardiovascular and diabetic risks and increased body mass index in children 9 to 15 years of age. All are features of the metabolic syndrome, which some regard as a maladaptation to a modern world (Wilkin and Voss 2004). With this perspective, the question becomes: what is the evolved adaptive system that can impact so strongly on health and development in many present day human societies? It is perhaps worth bearing this question in mind in planning future human studies of TGR.

12.3.4 Some Models of Possible Transgenerational Signals

Figure 12.3 provides three possible models for human TGRs. The first portrays the inheritance of acquired epigenetic characteristics, where some DNA methylation marks occur in somatic cells and in the germ line in response to an environmental exposure. All or some of these are transmitted intact by sperm to the next generation, thereby modifying gene expression in the offspring. The second model portrays the idea of one of several adverse exposures inducing epigenetic or DNA

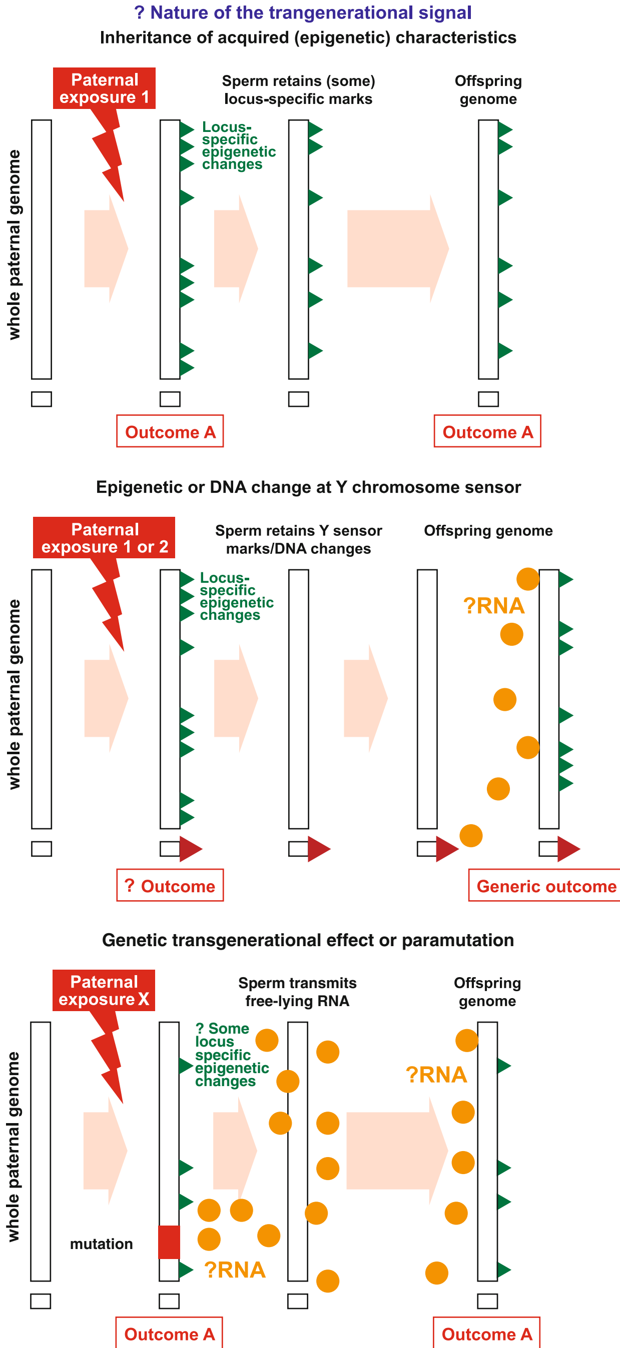

Fig. 12.3 Three possible models of TGRs. A schematic representation from *left to right* of the paternal genome; the paternal genome after an exposure showing acquired epigenetic marks (*green triangles*); the sperm genome; and the son's genome with epigenetic marks. The Y chromosome is represented as a box separate from the rest of the genome. The *red triangle* represents an acquired change at the proposed Y chromosome genotoxic sensor (see text)

changes in a genotoxic sensor on the Y chromosome, in addition to genome-wide DNA methylation changes in somatic cells. But in this scenario, these soma-wide DNA methylation marks are erased from the germ line even though some may be reset in the next generation. This resembles what has been observed with epimutations of *MLH1* in some cancer-affected families with Lynch syndrome (Hitchins et al. 2007, 2011). Only the epigenetic or DNA changes at the Y chromosome genotoxic sensor are transmitted on sperm, but this is a sufficient cue to induce a generic genome-wide epigenetic change in the offspring, possibly through microRNAs. The final model represents what seems to underlie the 'transgenerational genetic effects' revealed in the congenic mice experiments described above (Nelson and Nadeau 2010). There may well be other systems with various combinations likely. The intention is to provide a framework for the next stage of human TGR studies, including DNA methylation studies of blood, other tissues, and sperm.

While not ruling out gametic inheritance of specific acquired epigenetic characteristics, we favour a model that channels the response to a range of adverse exposures through a common transgenerational signal and generic response in the offspring. Thus, our simplest working hypothesis is that a range of adverse exposures, including 'uncertainty stress', lead to genotoxic stress which can induce a cellular/genomic response in the germ line (including sperm selection), particularly at critical times during development. Transmission of this genomic response to the next generation unleashes a generic, default 'survival mode' pattern of gene expression during development of the offspring. And it is against this setting that other determinants of health operate for better or worse. The sex-specific nature of the transmissions suggests that a major transgenerational signal is carried on the Y chromosome, possibly at a genotoxic sensor.

We think the time has come to carry out human studies that assess a range of early life exposures in terms of inducing TGRs and include a range of outcomes in the next generation(s), so as to define the elements of a generic outcome if indeed one exists. This broad approach increases the risk of false-positive associations; however, there are features of human male germ-line TGRs that can be exploited to reduce this risk. These include sex-specific transmissions, exposure during the sensitive mid-childhood period, and, for paternal grandparents, most notably the grandmother, the reversal in the direction of the association between exposure during their fetal/infant life and exposure in mid-childhood. An important challenge in the future will be to determine if matrilineal exposures can modify the male germ-line TGRs we have discussed.

References

Anderson P, Kedersha N (2009) RNA granules: post-transcriptional and epigenetic modulators of gene expression. Nat Rev Mol Cell Biol 10:430–436

Anderson LM, Riffle L, Wilson R, Travlos GS, Lubomirski MS, Alvord WG (2006) Preconceptional fasting of fathers alters serum glucose in offspring of mice. Nutrition 22:327–331

Anway MD, Cupp AS, Uzumcu M, Skinner MK (2005) Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308:1466–1469

Anway MD, Leathers C, Skinner MK (2006) Endocrine disruptor vinclozolin induced epigenetic transgenerational adult-onset disease. Endocrinology 147:5515–5523

Barber R, Plumb MA, Boulton E, Roux I, Dubrova Y (2002) Elevated mutation rates in the germ line of first- and second-generation offspring of irradiated male mice. Proc Natl Acad Sci U S A 99:6877–6882

Boucher BJ, Ewen SW, Stowers JM (1994) Betel nut (Areca catechu) consumption and the induction of glucose intolerance in adult CD1 mice and in their F1 and F2 offspring. Diabetologia 37:49–55

Bygren LO, Kaati G, Edvinsson S (2001) Longevity determined by ancestors' over nutrition during their slow growth period. Acta Biotheor 49:53–59

Bygren LO, Kaati G, Edvinsson S, Pembrey ME (2006) Reply to Senn. Eur J Hum Genet 14:1149–1150

Campbell JH, Perkins P (1988) Transgenerational effects of drug and hormonal treatments in mammals: a review of observations and ideas. Prog Brain Res 73:535–553

Carone BR, Fauquier L, Habib N, Shea JM, Hart CE, Li R, Bock C, Li C, Gu H, Zamore PD, Meissner A, Weng Z, Hofmann HA, Friedman N, Rando OJ (2010) Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. Cell 143:1084–1096

Chen TH, Chiu YH, Boucher BJ (2006) Transgenerational effects of betel-quid chewing on the development of the metabolic syndrome in the Keelung Community-based Integrated Screening Program. Am J Clin Nutr 83:688–692

de Boer P, Ramos L, de Vries M, Gochhait S (2010) Memoirs of an insult: sperm as a possible source of transgenerational epimutations and genetic instability. Mol Hum Reprod 16:48–56

Drake AJ, Walker BR (2004) The intergenerational effects of fetal programming: non-genomic mechanisms for the inheritance of low birth weight and cardiovascular risk. J Endocrinol 180:1–16

Franklin TB, Russig H, Weiss IC, Gräff J, Linder N, Michalon A, Vizi S, Mansuy IM (2010) Epigenetic transmission of the impact of early stress across generations. Biol Psychiatry 68:408–415

Hara MR, Kovacs JJ, Whalen EJ, Rajagopal S, Strachan RT, Grant W, Towers AJ, Williams B, Lam CM, Xiao K, Shenoy SK, Gregory SG, Ahn S, Duckett DR, Lefkowitz RJ (2011) A stress response pathway regulates DNA damage through beta-2 adrenoreceptors and beta-arrestin-1. Nature 477:349–353

Harris A, Seckl J (2010) Glucocorticoids prenatal stress and programming of disease. Horm Behav. doi:10.1016/j.yhbeh.2010.06.007

Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE, Lumey L (2008) Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A 105:17046–17049

Helgason T, Jonasson MR (1981) Evidence for a food additive as a cause of ketosis-prone diabetes. Lancet 2:716–720

Hitchins MP, Wong JJ, Suthers G, Suter CM, Martin DI, Hawkins NJ, Ward RL (2007) Inheritance of a cancer-associated MLH1 germ-line epimutation. N Engl J Med 356:697–705

Hitchins MP, Rapkins RW, Kwok CT, Srivastava S, Wong JJ, Khachigian LM, Polly P, Goldblatt J, Ward RL (2011) Dominantly inherited constitutional epigenetic silencing of MLH1 in a cancer-affected family is linked to a single nucleotide variant within the 5'UTR. Cancer Cell 20:200–213

Kaati G, Bygren LO, Edvinsson S (2002) Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. Eur J Hum Genet 10:682–688

Kaati G, Bygren LO, Pembrey M, Sjostrom M (2007) Transgenerational response to nutrition, early life circumstances and longevity. Eur J Hum Genet 15:784–790

Kuzawa CW (2005) Fetal origins of developmental plasticity: are fetal cues reliable predictors of future nutritional environments? Am J Hum Biol 17:5–21

Lord B, Woolford L, Wang L, McDonald D, Lorimore S, Stones V, Wright E, Scott D (1998) Induction of lympho-haemopoietic malignancy: impact of preconception paternal irradiation. Int J Radiat Biol 74:721–728

Nelson VR, Nadeau JH (2010) Transgenerational genetic effects. Epigenomics 2:797–806

Nelson V, Spiezio S, Nadeau J (2010) Transgenerational genetic effects of the paternal Y chromosome on daughter's phenotypes. Epigenomics 2:513–521

Ng SF, Lin RC, Laybutt DR, Barres R, Owens JA, Morris MJ (2010) Chronic high-fat diet in fathers programs beta-cell dysfunction in female rat offspring. Nature 467:963–966

Pembrey ME (1996) Imprinting and transgenerational modulation of gene expression; human growth as a model. Acta Genet Med Gemellol 45:111–125

Pembrey ME (2010) Male-line transgenerational responses in humans. Hum Fertil 13:268–271

Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjostrom M, Golding J (2006) Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet 14:159–166

Rassoulzadegan M, Grandjean V, Gounon P, Vincent S, Gillot I, Cuzin F (2006) RNA-mediated non-mendelian inheritance of an epigenetic change in the mouse. Nature 441:469–474

Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD, Slagboom PE, Heijmans BT (2009) DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. Hum Mol Genet 18:4046–4053

Wilkin TJ, Voss LD (2004) Metabolic syndrome: maladaptation to a modern world. J R Soc Med 97:511–520

Yazbek SN, Spiezio SH, Nadeau JH, Buchner DA (2010) Ancestral paternal genotype controls body weight and food intake for multiple generations. Hum Mol Genet 19:4134–4144

Biography

Randy L. Jirtle, Ph.D., directed the epigenetics and imprinting laboratory at Duke University, Durham, NC, until 2012. He is currently a Visiting Professor at McArdle Laboratory for Cancer Research at the University of Wisconsin-Madison, Madison, WI, where he received his B.S. in nuclear engineering in 1970 and Ph.D. in radiation biology in 1976. Jirtle's research interests include epigenetics, genomic imprinting, and the fetal origins of disease susceptibility, and he has published more than 190 journal articles. He was a featured scientist on the *NOVA* television program on epigenetics entitled *Ghost in Your Genes*. He was invited to speak on epigenetics in 2004 and 2011 at Nobel symposia in Stockholm, Sweden. In 2006, he received the Distinguished Achievement Award from the College of Engineering, University of Wisconsin-Madison. Jirtle was nominated for *Time Magazine's* "Person of the Year" in 2007. He was the inaugural recipient of the Epigenetic Medicine Award in 2008 and received the STARS Lecture Award in Nutrition and Cancer from the National Cancer Institute in 2009. In 2011, Jirtle organized the Keystone meeting *Environmental Epigenomics and Disease Susceptibility*. For more information about Professor Jirtle, visit his website (http://www.geneimprint.com).

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Glossary

2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD) A highly toxic polychlorinated dibenzo-p-dioxin compound that is classified as a human carcinogen. It was also a contaminant in Agent Orange, a herbicide used in the Vietnam War.

5-Aza-2-deoxy-cytidine (Decitabine) A cytosine in which the 5 carbon of the cytosine ring has been replaced with nitrogen. Decitabine is exclusively incorporated into DNA, inhibiting mammalian DNA methyltransferases.

5-Azacytidine (AZA) A cytidine RNA analog in which the 5 carbon of the cytosine ring has been replaced with nitrogen. 5-Azacytidine can be incorporated into RNA, and after metabolic activation also into DNA, where it functions as an inhibitor of mammalian DNA methyltransferases.

Acetylation (ac) The enzymatic introduction of an acetyl group to an organic compound, for instance histones.

Acquired immune deficiency syndrome (AIDS) A disease of the human immune system caused by the human immunodeficiency virus (HIV). Presently, there is no cure or vaccine for AIDS; however, antiretroviral treatment can slow the course of the disease, and can lead to a near-normal life expectancy.

Acute myeloid leukemia (AML) A cancer of the myeloid line of white blood cells whose rapid growth interferes with the production of normal blood cells in the bone marrow. AML is the most common acute leukemia affecting adults, and its incidence increases with age.

Acute promyelocytic leukemia (APL) A subtype of AML, a cancer of the blood and bone marrow. Since there is an abnormal accumulation of immature granulocytes called promyelocytes in APL, it is also known as acute pro-granulocytic leukemia. APL is responsive to all trans retinoic acid therapy.

Adrenocorticotropic hormone (ACTH) A polypeptide tropic hormone secreted by the anterior pituitary gland in response to biological stress.

Agouti gene The murine *Agouti* gene controls fur color through the deposition of yellow pigment in developing hairs. Several variants of this gene exist, and in the Agouti viable yellow (A^{vy}) mouse strain, *Agouti* expression can be heritably modified by epigenetic modifications.

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Alleles Different variants or copies of a gene. For most genes on the chromosomes, there are two copies: one copy inherited from the mother and the other from the father. The DNA sequence of each of these copies may be different because of genetic polymorphisms.

Alpha-thalassemia/mental retardation syndrome X-linked (ATRX) A protein that belongs to the switch/sucrose nonfermentable (SWI/SNF) family of chromatin remodeling molecules that facilitates gene expression by allowing transcription factors to gain access to their targets in chromatin. Mutations in *ATRX* alter DNA methylation, and are associated with an X-linked mental retardation syndrome that is often accompanied by ATRX syndrome.

Alzheimer's Disease (AD) AD is the most common form of dementia. There is presently no cure for this disease, and it worsens as it progresses, eventually leading to death. AD is diagnosed most commonly in people who are over 65.

Androgenote Embryos that develop from two paternal haploid nuclei. These embryos do not develop to term.

Angelman syndrome (AS) A rare pediatric disease caused by chromosomal aberrations or epigenetic inactivation of genes on maternal chromosome 15.

Antioxidant (AO) An antioxidant inhibits the oxidation of other molecules. Oxidation reactions produce free radicals, which can damage DNA and lead to cellular death. Antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E inhibit these oxidation reactions.

Assisted reproduction technologies (ART) The combination of approaches that are being applied in the fertility clinic, including *in vitro* fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI).

Ataxia telangiectasia mutated (ATM) A serine/threonine protein kinase that is recruited and activated by DNA double-strand breaks. It phosphorylates several key proteins, thereby activating DNA damage checkpoint delay. This results in cell cycle arrest, and subsequent DNA repair or apoptosis.

Autism A neuropsychiatric disorder characterized by impaired social interaction and communication, and by restricted and repetitive behavior.

Autism spectrum disorder (ASD) A range of conditions classified as pervasive developmental disorders that include autism, Asperger syndrome, pervasive developmental disorder not otherwise specified (PDD-NOS), childhood disintegrative disorder, and Rett syndrome. These disorders are typically characterized by social deficits, communication difficulties, stereotyped or repetitive behaviors and interests, and in some cases, cognitive delays.

Avon Longitudinal Study of Parents and Children (ALSPAC) A cohort study of children born in the former county of Avon, England during 1991 and 1992; the initial recruits were 14,000 pregnant women. This study population is used by researchers in health, education, and other social science disciplines. The study is hosted at the University of Bristol, and was initially led by Jean Golding.

Axin fused ($Axin^{Fu}$) mouse $Axin^{Fu}$ mice have an IAP element inserted into intron 6 of the *Axin* gene, resulting in a number of alternative transcripts. Complete demethylation of the IAP site results in the formation of severely kinked tails,

whereas, the mice have straight tails when the IAP site is fully methylated; partial methylation of this IAP site causes intermediate tail kinking. Supplementation of the mother's diet with methyl donors during pregnancy reduces the incidence of kinked tails in the offspring by increasing DNA methylation at this locus.

Azidothymidine (AZT) A nucleoside analog that functions as a reverse-transcriptase inhibitor. It is used as an antiretroviral drug for the treatment of patients with HIV/AIDS.

Basic helix loop helix (bHLH) The basic helix loop helix motif is characterized by two α-helices connected by a loop. bHLH proteins normally bind to a consensus sequence called an E-box. The canonical E-box, CACGTG, is palindromic; however some bHLH transcription factors bind to related non-palindromic sequences that are similar to the E-box.

Beckwith-Wiedemann syndrome (BWS) An overgrowth disorder usually present at birth that is characterized by an increased risk of childhood cancer and congenital features, such as macroglossia, macrosomia, midline abdominal wall defects, ear creases/ear pits, and neonatal hypoglycemia. More than five distinct errors involving 11p15 have been identified in different BWS patients. Imprinted genes involved in the etiology of this syndrome are *IGF2, CDKN1C, H19*, and *KCNQ1OT1*.

Benzo(a)pyrene (BaP) A polycyclic aromatic hydrocarbon found in coal tar that is classified as a human carcinogen. It was found to be responsible for scrotal cancers in chimney sweeps in the eighteenth century, and skin cancers among the fuel industry workers in the nineteenth century.

Bipolar disorder (BPD) A psychiatric disease defined by the presence of one or more episodes of abnormally elevated energy levels, cognition, and mood with or without one or more depressive episodes.

Bisphenol A (BPA) An organic compound manufactured to make polycarbonate polymers and epoxy resins used in the production of plastics. BPA exhibits hormone-like properties, and studies indicate it increases the incidence of cancer and reproductive problems. Concerns have been raised about its use in consumer products and food containers. Thus, the European Union, Canada, and the United States have banned BPA use in baby bottles.

Bisulfite sequencing (BS) A procedure in which sodium bisulfite is used to deaminate cytosine to uracil in genomic DNA. Conditions are chosen so that 5-methylcytosine is not changed. PCR amplification and subsequent DNA sequencing then reveals the exact position of cytosines that are methylated in genomic DNA.

Bivalent chromatin A chromatin region that is modified by a combination of histone modifications such that it represses gene transcription, but at the same time retains the potential of acquiring gene expression.

Body mass index (BMI) A measure of human body shape defined as the body mass divided by the square of the height (i.e. kg/m^2). The BMI for a healthy weight individual ranges from 18.5 to 25.

Borderline Personality Disorder (BPD) A personality disorder characterized by unusual variability and depth of moods, which may also affect cognition and interpersonal relationships.

Brain-derived neurotrophic factor (BDNF) A protein that acts on neurons of the central and peripheral nervous system, supporting their survival and encouraging the growth and differentiation of new neurons and synapses.

Bromo domain Protein motif found in a variety of nuclear proteins, including transcription factors and HATs involved in transcriptional activation. Bromo domains bind to histone tails carrying acetylated lysine residues.

cAMP response element binding protein (CREB) A transcriptional activator for many immediate early genes.

Cell fate The programmed path of cell differentiation. Although all cells have the same DNA, their cell fate can be different. Some cells develop into the brain, whereas others are the precursors of blood. Cell fate is determined in part by the organization of chromatin – DNA and the histone proteins – in the nucleus.

Cellular memory (epigenetic) Specific active and repressive organizations of chromatin can be maintained from one cell to its daughter cells; this is called epigenetic inheritance. It ensures that specific states of gene expression are inherited over many cell generations.

Cerebellum Region of the brain that plays a role in motor control, as well as language, attention, and some elements of emotion.

Cerebral cortex A sheet of neural tissue that is 2–4 mm thick, and comprised of up to six layers. It covers the cerebrum and cerebellum, and is divided into left and right hemispheres. The cerebral cortex plays a critical role in memory, attention, perceptual awareness, thought, language, and consciousness. It has a grey color in preserved brains, and is also referred to as grey matter.

ChIP-chip After chromatin immunoprecipitation, DNA is purified from the immunoprecipitated chromatin fraction and hybridized on arrays of short DNA fragments representing specific regions of the genome.

ChIP-seq Sequencing of the totality of DNA fragments obtained by ChIP using next-generation sequencing to quantify patterns of enrichment across the genome.

Chromatid In each somatic cell generation, the genomic DNA is replicated in order to make two copies of each individual chromosome. During the M phase of the cell cycle, these copies – called chromatids – are microscopically visible and next to each other before they get distributed to the daughter cells.

Chromatin The nucleo-protein-complex constituting the chromosomes in eukaryotic cells. Structural organization of chromatin is complex and involves different levels of compaction. The lowest level of compaction is represented by an extended array of nucleosomes.

Chromatin conformation capture assay (Hi-C) A high-throughput next generation sequencing technique used to analyze the organization of chromosomes and their interactions. This technique is useful for better understanding gene regulation, nuclear partitioning, and chromatin dynamics.

Chromatin immunoprecipitation (ChIP) This is a method for examining protein–DNA interactions occurring in the cell. DNA-binding proteins are cross-linked to the DNA and enriched using antibodies with specific affinity to particular proteins (e.g. histones) or covalent modifications on proteins. After ChIP, the genomic DNA is purified from the chromatin fragments brought down by the antiserum and analyzed by qPCR, microarray (ChIP-chip), or next-generation sequencing (ChIP-seq).

Chromatin remodeling Locally, the organization and compaction of chromatin can be altered by different enzymatic machineries. This is called chromatin remodeling. Several chromatin remodeling proteins move nucleosomes along the DNA and require ATP for their action.

Chromo domain (chromatin organization modifier domain) A protein–protein interaction motif first identified in *Drosophila melanogaster* HP1 and polycomb group proteins. It is also found in other nuclear proteins involved in transcriptional silencing and heterochromatin formation. Chromo domains consist of approximately 50 amino acids that bind to histone tails methylated at certain lysine residues.

Chromosomal domain It is often observed in higher eukaryotes that chromatin is organized (e.g. by histone methylation) the same way across hundreds to thousands of kilobases of DNA. These "chromosomal domains" can comprise multiple genes that are similarly expressed. Some chromosomal domains are controlled by genomic imprinting.

Chromosomal instability (CIN) An increased rate of chromosome mis-segregation in mitosis that results in a failure to maintain the correct chromosomal complement, thereby causing an increased risk of developing cancer.

Colorectal cancer (CRC) A cancer from uncontrolled cell growth in the colon or rectum. Symptoms of colorectal cancer typically include rectal bleeding and anemia. Screening by colonoscopy is effective at decreasing the chance of dying from colorectal cancer.

Conditioned place preference (CPP) assay This assay is used to evaluate preferences for environmental stimuli that have been associated with a positive or negative reward.

Copy number variation (CNV) Alterations in the DNA of a genome that results in a cell having an increased or decreased number of copies of one or more sections of the DNA. These variations range from kilobases to megabases in size.

CpG dinucleotide A cytosine followed by a guanine in the sequence of bases of the DNA. Cytosine methylation in mammals occurs primarily at CpG dinucleotide positions.

CpG island (CGI) A small stretch of DNA, several hundred bases up to several kilobases in size, that is particularly rich in CpG dinucleotides, and is also relatively enriched in cytosines and guanines. Most CpG islands comprise promoter sequences that drive the expression of genes.

CpG island methylator phenotype (CIMP) Cancers can be classified according to the degree of methylation in their genome. Those with high degrees of methylation are referred to as having a CpG island methylator phenotype, and are characterized by epigenetic instability.

CREB-binding protein (CBP) A protein involved in transcriptional regulation that is often associated with histone acetyltransferases such as p300.

Cytomegalovirus (CMV) A member of the viral genus known as *Herpesviridae*. Herpesviruses can remain latent within the body over long periods. Although they can be found throughout the body, CMV infections are frequently associated with the salivary glands in humans.

Cytosine methylation DNA methylation in mammals occurs at cytosines that are part of CpG dinucleotides. As a consequence of the palindromic nature of the CpG sequence, methylation is symmetrical, and affects both strands of DNA at a methylated target site. When present at promoters, it is usually associated with transcriptional repression.

Deacetylation The removal of acetyl groups from proteins. Deacetylation of histones is often associated with gene repression, and is mediated by histone deacetylases (HDACs).

de novo DNA methylation The addition of methyl groups to a stretch of DNA that is not yet methylated.

Deoxyribonucleic acid (DNA) A molecule encoding the genetic instructions used in the development and function of all known living organisms and many viruses.

Developmental origins of health and disease (DOHaD) A theory that postulates that environmental exposure during early developmental interacts with genotypic variation to change the ability of the organism to cope with its environment in later life, thereby altering the incidence of chronic diseases and neurological disorders in adulthood.

Diabetes Control and Complications Trial (DCCT) A major clinical study conducted from 1983 to 1993. It showed that keeping blood glucose levels as close to normal as possible slows the onset and progression of the eye, kidney, and nerve damage caused by diabetes.

Diethylstilbestrol (DES) A synthetic non-steroidal estrogen first synthesized in 1938; it is also classified as an endocrine disruptor. DES was given to pregnant women between 1940 and 1970 in the mistaken belief it would reduce the risk of pregnancy complications and losses. DES was subsequently shown to cause rare vaginal tumors in the daughters, and developmental malformations in both the daughters and sons who were exposed in utero. Studies are now being conducted to determine if an increased incidence of developmental abnormalities are also present in the grandchildren of the women who were given DES during pregnancy.

Differentially methylated region (DMR) A segment of DNA generally rich in cytosine and guanine nucleotides, with the cytosine nucleotides methylated on only one parental allele. DNA methylation of these regulatory elements is parent-of-origin dependent when they regulate the mono-allelic expression of imprinted genes.

Disomy The occurrence in the cell of two copies of a chromosome, or part of a chromosome, that are identical and of the same parental origin (i.e. uniparental disomy).

Dizygotic twins Fraternal or dizygotic twins develop from two separate eggs that are fertilized by two separate sperm.

DNA demethylation Removal of methyl groups from the DNA. This can occur actively by an enzymatically mediated process, or passively when methylation is not maintained after DNA replication.

DNA methylation A biochemical modification of DNA resulting from the addition of a methyl group to either adenine or cytosine bases. Methylation in mammals is essentially confined to cytosines that are in CpG dinucleotides. Methyl groups can be removed from DNA by DNA demethylation.

DNA methyltransferase The enzyme that adds new (de novo) methylation to the DNA, or maintains existing patterns of DNA methylation.

Dopamine A catecholamine neurotransmitter that has an important role in cognitive function, voluntary movement, reward, motivation, and prolactin production.

Dosage compensation In mammals, the X-chromosome is normally present in two copies in females and only one copy in males. Dosage compensation, by random inactivation of one of the X-chromosomes in females, ensures that in spite of this copy number difference X-linked genes are expressed at the same level in both sexes.

Double strand break (DSB) A break in double-stranded DNA in which both strands are cleaved can result in mutagenic events or cell death if left unrepaired or repaired inappropriately.

Double stranded RNA (dsRNA) RNA with two complementary strands; it is similar to the DNA found in all cells. dsRNA forms the genetic material of double-stranded RNA viruses.

Down syndrome A chromosomal condition caused by the presence of all or part of a third copy of chromosome 21. This syndrome is named after John Langdon Down, the British physician who described it in 1866. Down syndrome is the most common chromosome abnormality in humans. It is typically associated with a delay in cognitive ability and physical growth, and a particular set of facial characteristics.

Dutch famine (Hunger winter) A famine that took place in the German-occupied part of the Netherlands during the winter of 1944–1945 near the end of World War II. A German blockade cut off food and fuel shipments from farm areas to punish the reluctance of the Dutch to aid the Nazi war effort. About 22,000 people died because of the famine. Subsequently, it was determined that the children of pregnant women exposed to the famine were more susceptible to cardiovascular disease, diabetes, obesity, micro-albuminuria, and schizophrenia.

Eight-twenty-one (ETO) This gene derives its name from its association with many cases of acute myelogenous leukemia (AML) in which a reciprocal translocation, t(8;21), brings together a large portion of the *ETO* gene from chromosome eight and part of the *AML1* gene from chromosome 21.

Embryo (EMB) A multicellular diploid eukaryote in its earliest stage of development. In humans, it is called an embryo until about 8 weeks after fertilization, and then it is called a fetus.

Embryonic stem (ES) cells Cultured cells obtained from the inner cell mass of the blastocyst. These cells are totipotent, and can be differentiated into all of the different somatic cell lineages.

Endocrine disruptor A chemical compound that has an antagonistic effect on the action of a hormone to which it is structurally similar. Some pesticides act as endocrine disruptors, and in animal studies are found to have adverse effects on development by altering DNA methylation at specific loci. A well-characterized endocrine disruptor is bisphenol A (BPA), a chemical used for the productions of certain plastics.

Enhancer A small, specialized sequence of DNA which, when recognized by specific regulatory proteins, can enhance the activity of the promoter of a gene(s) located in close proximity.

Enhancer RNA (eRNA) Enhancer regions can produce their own RNA or eRNA that can intensify the ability of cells to produce specific protein coding transcripts.

Epialleles Copies of a DNA sequence or a gene that differ in their epigenetic or expression states without the occurrence of a genetic mutation.

Epigenesis The development of an organism from fertilization through a sequence of steps leading to a gradual increase in complexity through differentiation of cells and formation of organs.

Epigenetic code Patterns of DNA methylation and histone modifications can modify the way genes on the chromosomes are expressed. This led to the idea that combinations of epigenetic modifications constitute a code on top of the genetic code that modulates gene expression, and can be recognized by specific non-histone proteins.

Epigenetic inheritance The somatic inheritance, or inheritance through the germ line, of epigenetic information (i.e. changes that affect gene function without the occurrence of an alteration in the DNA sequence).

Epigenetic marks Regional modifications of DNA and chromatin proteins. This includes DNA methylation and histone methylation that can be maintained from one cell generation to the next, and may affect the way genes are expressed.

Epigenetic reprogramming The resetting of epigenetic marks on the genome so that they become like those of another cell type or of another developmental stage. Epigenetic reprogramming occurs in primordial germ cells brought back to a 'ground state'. Epigenetic reprogramming and dedifferentiation also occur after somatic cell nuclear transfer.

Epigenetics The study of heritable changes in gene function that arise without an apparent change in the genomic DNA sequence. Epigenetic mechanisms are involved in the formation and maintenance of cell lineages during development and in X-inactivation and genomic imprinting; they are frequently perturbed in diseases.

Epigenome The epigenome is the overall epigenetic state of a particular cell. In the developing embryo, each cell type has a different epigenome. Epigenome maps represent the presence of DNA methylation, histone modification, and other chromatin modifications along the chromosomes.

Epigenome-wide association studies (EWAS) The principle of epigenome-wide association studies involves scanning cases and controls to identify epigenetic variations associated with a specific trait or disease.

Epigenotype The totality of epigenetic marks that are found along the DNA sequence of the genome in a particular cell lineage or at a particular developmental stage.

Epimutation A change in the normal epigenetic marking of a gene or regulatory DNA sequence (e.g. DNA methylation) that affects gene expression.

Escape of X-inactivation Regions and genes on the X-chromosomes that are not affected by the dosage compensation/X-inactivation mechanism, and remain active on both X-chromosomes in females.

Euchromatin A type of chromatin that appears lightly stained when observed through the microscope at interphase. Euchromatic chromosomal domains are loosely compacted and relatively rich in genes. The opposite type of chromatin organization is heterochromatin.

Fluorescent in situ hybridization (FISH) A cytogenetic technique that uses fluorescent probes to detect and localize the presence or absence of specific DNA sequences on chromosomes.

Folate A methyl donor obtained primarily from the diet that influences nucleotide synthesis and methylation reactions, including DNA methylation.

Fragile X syndrome A genetic syndrome that is the most common single-gene cause of autism and inherited mental retardation among boys. It is associated with the expansion of the CGG trinucleotide repeat affecting the *Fragile X mental retardation 1 (FMR1)* gene on the X chromosome.

γ-Aminobutyric acid (GABA) The chief inhibitory neurotransmitter in the mammalian central nervous system. It helps control neuronal excitability throughout the nervous system, and is also involved in the regulation of muscle tone.

Genome The entirety of an organism's hereditary information that is encoded either in DNA or in RNA for many types of viruses. The genome includes both the genes and the non-coding sequences of the DNA.

Genome-wide association study (GWAS) An examination of all or most of the genes in groups of individuals different for a specific trait or disease in order to identify DNA sequence-based factors that contribute to the origin of such phenotypes.

Genomic imprinting An epigenetic phenomenon that affects a small subset of genes in the genome of Therian mammals, and results in mono-allelic gene expression in a parent-of-origin dependent manner.

Glucocorticoid receptor (GR) A receptor encoded by *NR3C1* that glucocorticoids (e.g. cortisol) bind to it. The GR regulates genes that modulate development, metabolism, immune functions, and stress response.

Glucocorticoids Steroid hormones that bind to the glucocorticoid receptor (GR), and affect development, immunological functions, metabolic processes, and stress response.

Green fluorescent protein (GFP) A protein composed of 238 amino acids, and first isolated from the jellyfish, *Aequorea victoria*. It exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range. GFP is frequently used as a reporter of gene expression.

Gynogenote Embryos that develop from two maternal haploid nuclei; these embryos do not develop to term.

Heterochromatin A type of chromatin that appears dark when observed through the microscope at interphase. Heterochromatic chromosomal domains, found in all cell types, are highly compacted, rich in repeat sequences, and show little or no gene expression. Extended regions of heterochromatin are found close to centromeres and at telomeres.

Hippocampus A region of the brain belonging to the limbic system that plays a role in long-term memory and spatial navigation.

Histone acetylation Posttranslational modification of the ε-amino group of lysine residues in histones that is catalyzed by a family of enzymes called histone acetyltransferases (HATs). Acetylation contributes to the formation of decondensed, transcriptionally permissive chromatin structures, and facilitates interaction with proteins containing bromo domains.

Histone acetyltransferase (HAT) An enzyme that acetylates specific lysine amino acids on histone proteins.

Histone code A theory that specific combinations of histone modifications are recognized by non-histone proteins through specific protein domains, such as bromo and chromo domains, thereby bring about a specific chromatin configuration and expression state (see epigenetic code).

Histone deacetylase (HDAC) An enzyme that removes acetyl groups from histone proteins. This increases the positive charge of histones, and enhances their attraction to the negatively charged phosphate groups in DNA, resulting in chromatin condensation.

Histone deacetylase inhibitor (HDACi) A class of compounds that interferes with the function of histone deacetylases. These compounds are used in psychiatry and neurology as mood stabilizers and anti-epileptics. They are also being investigated as possible treatments for cancer and inflammatory disease.

Histone demethylase (HDM) Proteins catalyzing the active enzymatic removal of methyl groups from either lysine or arginine residues of histones. Prominent examples are LSD1 and Jumonji proteins.

Histone methylation Posttranslational methylation of amino acid residues in histones catalyzed by histone methyltransferases (HMTs). Histone methylation is found at arginine as mono- or dimethylation and lysine as mono-, di-, or trimethylation. Different types of methylation can be found in either open transcriptionally active or closed transcriptionally silent chromatin. Methylated lysine residues are recognized by proteins containing chromo domains.

Histone methyltransferase (HMT) Enzymes catalyzing the transfer of methyl groups from S-adenosyl-methionine (SAM) to lysine or arginine residues in histones.

Histone variants Canonical histones with distinct amino acid changes accumulating at specific chromatin regions associated with the activating or silencing of transcription.

Human chorionic gonadotropin (HCG) A hormone produced during pregnancy that is made by the developing placenta after conception, and later by the placental component syncytiotrophoblast. Measurement of HCG in the blood or urine can be used to test for pregnancy.

Human mammary epithelial cell (HMEC) Mammary epithelial cells line the ducts and lobes of the breast, and they produce milk. Breast cancer most often originates in these epithelial cells.

Hypothalamic-pituitary-adrenal (HPA) axis A complex set of direct influences and feedback interactions between the hypothalamus, the pituitary gland, and the adrenal glands. Interactions between the organs in the HPA axis control the reactions to stress, and regulate body processes, including digestion, the immune system, mood and emotions, sexuality, and energy usage.

Human immunodeficiency virus (HIV) A lentivirus that causes acquired immunodeficiency syndrome (AIDS), an infectious disease in which progressive failure of the human immune system leads to life-threatening opportunistic infections and/or cancer.

Hypothalamus A portion of the brain that links the nervous system to the endocrine system via the pituitary gland and controls body temperature, hunger, thirst, sleep, and circadian cycles.

Immunodeficiency, centromeric region instability, facial anomalies syndrome (ICF) A rare autosomal recessive disease characterized by immunodeficiency and characteristic rearrangements in the vicinity of the centromeres of chromosomes 1 and 16 and sometimes 9. Symptoms of this syndrome include mild facial dysmorphism, growth retardation, failure to thrive, and psychomotor retardation. ICF always involves limited hypomethylation of DNA, and often arises from mutations in one of the DNA methyltransferase genes.

Impaired glucose tolerance (IGT) A pre-diabetic state of hyperglycemia that is associated with insulin resistance and increased risk of cardiovascular disease.

Imprinted brain theory A theory that proposes that autism spectrum disorder (ASD) represents a paternal bias in the expression of imprinted genes, whereas psychotic spectrum disorder (PSD) results from an imbalance in favor of maternal and/or X-chromosome gene expression.

Imprinted genes Genes that show a parent-of-origin specific gene expression pattern controlled by epigenetic marks that originate from the germ line.

Imprinting See genomic imprinting

Imprinting control region (ICR) Region of the DNA that shows germ-line derived, parent-of-origin dependent epigenetic marks that control the parental-specific allelic expression of neighboring imprinted genes.

Imprintome The complete repertoire of differentially methylated imprint regulatory elements in the genome defines the imprintome. Because imprinting is a direct consequence of epigenetic regulation, the imprintome is a subset of the epigenome rather than the genome or transcriptome.

Induced pluripotent stem cells (iPS) Cells derived from differentiated somatic cells by *in vitro* reprogramming. Reprogramming is triggered by the activation of pluripotency factor genes and cultivation in ES cell medium. iPS cells are capable of generating all cell types of an embryo.

Inner cell mass (ICM) In early embryogenesis, the inner cell mass of cells will eventually give rise to the fetus. This structure forms before implantation into the endometrium of the uterus. The ICM lies within the blastocyst cavity, and is entirely surrounded by a single layer of cells called the trophoblast.

Intracisternal A particle (IAP) A family of retrovirus-like elements that encode for virus-like particles found regularly in early rodent embryos. They are also transcribed in a wide variety of neoplasms because of DNA hypomethylation.

Intra-uterine environment The collective conditions affecting a fetus in the uterus.

Intra-uterine growth restriction (IUGR) IUGR refers to poor growth of a baby during pregnancy often resulting from poor maternal nutrition or lack of adequate oxygen supply to the fetus.

In vitro fertilization (IVF) Fertilization of a surgically retrieved oocyte in the laboratory, followed by a short period of *in vitro* cultivation before the embryo is transferred back into the uterus to allow development to term.

Ionizing radiation (IR) Particulate (e.g. electrons and alpha particles) or electromagnetic radiation with enough energy to remove tightly bound electrons from the orbit of an atom, thereby causing the atom to become ionized.

Kinship theory of imprinting An evolutionary theory that attempts to explain the origin and evolution of imprinted genes.

Lamarck Jean-Baptiste Pierre Antoine de Monet, Chevalier de Lamarck (1744–1829) was a French naturalist known best for his theory of inheritance of acquired characteristics or Lamarckism.

Large intervening non-coding RNA (lincRNA) A molecule of RNA 200 to many thousands of nucleotides in length that is transcribed by non-protein coding areas of DNA. These ribonucleotides may play a role in a variety of biological processes, such as cancer formation.

Long interspersed elements (LINE) Highly repeated sequences, 6,000–8,000 base pairs in length, that contain RNA polymerase II promoters. They also have an open reading frame that is related to the reverse transcriptase of retroviruses, but they do not contain LTRs (long terminal repeats). Copies of the LINE1 family form about 15 % of the human genome. LINE elements are usually transcriptionally silent and marked by DNA methylation.

Long non-coding RNA (lncRNA) Non-protein coding transcripts longer than 200 nucleotides. This limit distinguishes long ncRNAs from microRNAs (miRNAs), short interfering RNAs (siRNAs), Piwi-interacting RNAs (piRNAs), and small nucleolar RNAs (snoRNAs).

Long terminal repeat (LTR) Sequences of DNA that repeat hundreds or thousands of times. They are found in retroviral DNA and in retrotransposons that flank functional genes. They are used by viruses to insert their genetic sequences into the host genome.

Major depressive disorder (MDD) A mental disorder characterized by episodes of all-encompassing low mood accompanied by low self-esteem and loss of interest or pleasure in normally enjoyable activities.

Maternal effects Long-term effects on the development of the embryo triggered by factors in the cytoplasm of the oocyte.

Medial prefrontal cortex (mPFC) A part of the prefrontal cortex in the mammalian brain that is implicated in decision making and the processing of risk and fear.

Medial pre-optic area (MPOA) A region in the forebrain rostral to the hypothalamus that is involved in sexual and parenting behaviors.

Messenger RNA (mRNA) A large family of RNA molecules that convey genetic information from DNA to the ribosome, where they specify the amino acid sequence of the protein products of gene expression.

Metatherians Pouched mammals found mainly in Australia and the Americas, such as the opossum.

Methyl-CpG-binding protein 2 (MeCP2) A protein that is essential for the normal function of nerve cells; mutations in this gene cause Rett syndrome.

Methylated DNA immunoprecipitation-microarray (MeDIP-chip) A genome-wide, high-resolution approach to detect DNA methylation in the whole genome or CpG islands. The method utilizes anti-methylcytosine antibody to immunoprecipitate DNA that contains highly methylated CpG sites. The enriched methylated DNA is then interrogated using DNA microarrays.

Methylated DNA immunoprecipitation-sequencing (MeDIP-seq) A genome-wide, high-resolution approach to detect DNA methylation in the whole genome or CpG islands. The method utilizes anti-methylcytosine antibody to immunoprecipitate DNA that contains highly methylated CpG sites. The enriched methylated DNA is then interrogated using massive parallel sequencing techniques.

Methyl-CpG binding domain (MBD) Protein domain in methyl-CpG-binding proteins (MBPs) responsible for recognizing and binding to methylated cytosine residues in DNA. Proteins containing MBDs form a specific family of proteins with various molecular functions.

Methyl-CpG-binding proteins (MBPs) Proteins containing domains (such as MBD) that bind to 5-methyl-cytosine in the context of CpG dinucleotides. MBPs mostly act as mediators for molecular functions such as transcriptional control or DNA repair.

Methyl-DNA binding domain capture-sequencing (MethylCap-seq) A recently developed technique for the genome-wide profiling of DNA methylation. This technique consists of capturing the methylated DNA fragments by their methyl-CpG binding domains (MBDs), and the subsequent deep sequencing of eluted DNA.

Methyl tetrahydrofolate reductase (MTHFR) A key enzyme in the folate S-adenosylmethionine (SAM) pathway.

micro RNA (miRNA) A small non-coding RNA molecule about 22 nucleotides in length found in plants and animals. It functions in transcriptional and post-transcriptional regulation of gene expression.

Microsatellite instability (MSI) A condition manifested by damaged DNA due to defects in DNA repair. Sections of DNA called microsatellites, which consist of a sequence of repeating units of 1–6 base pairs in length, become unstable and can shorten or lengthen during cell division.

Mitogen-activated protein kinase (MAPK) A protein in a cellular signaling pathway that transduces signals from the cell surface to the nucleus, and modifies gene expression by affecting the activities of transcription factors.

Mixed-lineage leukemia (MLL) A type of childhood leukemia in which a piece of chromosome 11 is translocated to another chromosome. Children with this type of leukemia have a particularly poor prognosis. The name comes from the gene expression profiles in this disease being differ than those seen in ALL and AML.

Monozygotic twins Two individuals developing from one zygote that split and formed two embryos, also known as identical twins.

Neural tube closure defect (NTD) One of the most common birth defects, occurring in approximately one in 1,000 live births in the United States. A NTD is an opening in the spinal cord or brain that occurs very early in human development. During gastrulation, specialized cells on the dorsal side of the fetus begin to fuse and form the neural tube. When the neural tube does not close completely, a NTD develops.

Neuronal plasticity The ability of the brain to change as a result of one's experience.

Newborn epigenetics study (NEST) A study initiated at Duke University by Cathrine Hoyo and Susan Murphy in 2004 to prospectively test the influence of in utero environmental exposures on the epigenetic profiles in newborns.

Next-generation sequencing (NGS) A technology similar to capillary electrophoresis-based Sanger sequencing where the bases of a small fragment of DNA are sequentially identified from signals emitted as each fragment is resynthesized from a DNA template strand. NGS extends this process across millions of reactions in a massively parallel fashion, rather than being limited to a single or a few DNA fragments.

N-methyl-D-aspartate (NMDA) receptor An ionotropic glutamate receptor that stimulates intracellular signaling cascades that affect gene transcription, synaptic plasticity, and learning and memory.

Noncoding RNA (ncRNA) RNA transcripts that do not encode for a protein. ncRNA generation frequently involves RNA processing.

Non-Mendelian inheritance The inheritance of traits that do not follow Mendelian rules, and cannot be explained by simple genetic models.

Nucleolus Specific compartments within the nucleus formed by rDNA repeat domains. Nucleoli are marked by specific heterochromatic structures and active gene expression.

Nucleosome Fundamental organizational unit of chromatin consisting of 147 base pairs of DNA wound around a histone octamer.

Nucleosome Free Region (NFR) Regions in the DNA with an increased accessibility to micrococcal nuclease digestion. Thus, NFR refers to a deficiency in experimentally determined nucleosomes, but it does not imply a complete lack of histones. NFRs at the 5′ and 3′ ends of genes are sites of transcription initiation for mRNA and noncoding RNA.

Nucleus accumbens (Nac) A collection of neurons that forms the main part of the ventral striatum. It is thought to play an important role in reward, pleasure, laughter, addiction, aggression, fear, and the placebo effect.

Open reading frame (ORF) An open reading frame is a portion of a DNA molecule that, when translated into amino acids, contains no stop codons.

Paraventricular nucleus (PVN) A neuronal nucleus in the hypothalamus containing neurons that are activated by stressful or physiological changes.

Parthenogenetic (PG) A form of asexual reproduction in which growth and development of embryos occur without fertilization.

Persistent organic pollutants (POPs) Organic compounds that are resistant to environmental degradation through chemical, biological, and photolytic processes.

Phenylketonuria (PKU) An autosomal recessive metabolic genetic disorder characterized by a mutation in the gene for the hepatic enzyme phenylalanine hydroxylase (PAH), rendering it nonfunctional. Untreated PKU can lead to mental retardation, seizures, and other serious medical problems. The primary treatment for PKU is a strict phenylalanine-restricted diet supplemented by a medical formula containing amino acids and other nutrients.

Pituitary An endocrine gland protruding from the hypothalamus that secretes hormones involved in the homeostasis of an organism.

piwi RNA (piRNA) The largest class of small non-coding RNA molecules expressed in animal cells. They form RNA-protein complexes through interactions with piwi proteins. These piRNA complexes are linked to both epigenetic and post-transcriptional gene silencing of retrotransposons and other genetic elements in germ cells.

Plant homeodomain (PHD) The PHD finger is a Cys_4-His-Cys_3 zinc-finger-like motif found in nuclear proteins thought to be involved in epigenetics and chromatin-mediated transcriptional regulation.

Polycomb group proteins A family of proteins initially discovered in fruit flies that can remodel chromatin such that epigenetic silencing of genes takes place. Polycomb-group proteins are well known for silencing *Hox* genes through modulation of chromatin structure during embryonic development.

Polycomb response elements (PREs) *cis*-regulatory DNA elements that recruit both the Polycomb group (PcG) and Trithorax group (TrxG) proteins that are required for gene silencing and activation, respectively.

Polycyclic aromatic hydrocarbon (PAH) Potent atmospheric pollutants that consist of fused aromatic rings. PAHs occur in oil, coal, and tar deposits, and are produced as byproducts of fuel burning. PAHs are also found in meat cooked at high temperatures such as in grilling or barbecuing, and in smoked fish. As a pollutant, they are of concern because some compounds have been identified as carcinogenic, mutagenic, and teratogenic.

Position effect variegation (PEV) Cell/tissue specific variability of gene expression controlled by the temporal inheritance of certain epigenetic states. PEV is a consequence of variable formation of heterochromatin across the respective gene. A classical example of PEV is found in certain mutations leading to variegated eye pigmentation in fruit flies.

Positron emission tomography (PET) A nuclear medical imaging technique that produces a three-dimensional image of functional processes in the body. The system detects pairs of gamma rays emitted by a positron-emitting radionuclide that is introduced into the body on a biologically active molecule. Three-dimensional images of the radionuclide concentration within the body are then constructed by computer analysis.

Post-translational modification (PTM) Proteins are created by ribosomes translating mRNA into polypeptide chains that then undergo post-translational modifications such as folding and cutting before becoming mature proteins.

Post-traumatic stress disorder (PTSD) A severe anxiety disorder that can develop after exposure to any event that results in psychological trauma.

Potato spindle tuber viroid (PSTVd) A small, circular RNA molecule that contains a pospiviroid RY motif stem loop within the viroidal RNA. All potatoes and tomatoes are susceptible to PSTVd, and there is no form of natural resistance.

Prader-Willi syndrome (PWS) A rare pediatric developmental disorder caused by chromosomal aberrations or epigenetic misregulation of imprinted genes on paternal chromosome 15. Characteristics of PWS are low muscle tone, short stature, incomplete sexual development, cognitive disabilities, problem behaviors, and a chronic feeling of hunger that can lead to excessive eating and life-threatening obesity.

Predictive adaptive response (PAR) A form of developmental plasticity that evolved as adaptive responses to environmental cues acting early in the life cycle, but where the advantage of the induced phenotype is primarily manifested in a later phase of the life cycle.

Prefrontal cortex (PFC) The anterior part of the frontal lobes of the brain, lying in front of the motor and premotor areas. This brain region is implicated in planning complex cognitive behavior, personality expression, decision making, and moderating social behavior. The basic activity of this brain region is considered to be orchestration of thoughts and actions in accordance with internal goals.

Primordial germ cell (PGC) Diploid germ cell precursors that exist briefly in the developing embryo before differentiating to become germ cells.

Prolactin (PRL) Prolactin is a single-chain protein hormone closely related to growth hormone. It is secreted from the anterior pituitary, but it is also synthesized and secreted by a broad range of cells in the body, most prominently various immune cells, the brain, and the decidua of the pregnant uterus. The mammary gland is a major target organ for prolactin where it stimulates mammary gland development and milk production.

Promyelocytic leukemia (PML) A subtype of acute myelogenous leukemia (AML). It is a cancer of the blood and bone marrow with an abnormal accumulation of immature granulocytes called promyelocytes. The disease is characterized by a chromosomal translocation involving the *retinoic acid receptor alpha (RARA)* gene, and is unique from other forms of AML in its responsiveness to all trans retinoic acid therapy.

Protamines Small, arginine-rich proteins that replace histones late in the haploid phase of spermatogenesis during spermiogenesis. They are thought to be essential for sperm head condensation and DNA stabilization. Protamines are removed from paternal chromosomes in the mammalian zygote after fertilization.

Prototherians Egg-laying mammals (i.e. platypus and echidna) are the most ancestral mammals; they are only found in Australia, Tasmania, and New Guinea.

Psychosis An abnormal condition of the mind, described as involving a loss of contact with reality.

Psychotic spectrum disorder (PSD) A group of psychiatric diagnoses that share several clinical features, typically involving reality distortion.

Quantitative real time polymerase chain reaction (qPCR) A laboratory technique based on PCR that is used to amplify and simultaneously quantify a targeted DNA molecule.

Reelin A protein that regulates neuronal migration in the developing brain, and is also involved in important neuronal cell functions in the adult brain like synaptic plasticity, dendrite development, and adult neurogenesis.

Reduced Representation Bisulfite Sequencing (RRBS) A technique that couples bisulfite conversion and next generation sequencing. It is an innovative method that enriches genomic regions with a high density of potential methylation sites, and allows for the determination of DNA methylation at a single-nucleotide resolution.

Regions of altered methylation (RAMs) Persistent RAMs seen in precancerous tissues are thought to play a critical role in the genesis of cancer.

Retinitis pigmentosa (RP2) An inherited, degenerative eye disease that causes severe vision impairment and often blindness.

Retinoblastoma (RB) A rapidly growing cancer that develops in the retina of the eye. There are two forms of this disease, a heritable form and a non-heritable form. The heritable form involves mutations in *RB1*, an imprinted tumor suppressor gene on chromosome 13 that is expressed preferentially from the maternal allele.

Rett syndrome (RTT) A neurodevelopmental disorder of the grey matter of the brain that almost exclusively affects females. Rett syndrome is caused by mutations in *MECP2* located on the X chromosome, and can arise both sporadically or from germline mutations.

Reverse transcriptase (RT) An enzyme used to generate complementary DNA (cDNA) from an RNA template, a process termed reverse transcription. RT is needed for the replication of retroviruses, and RT inhibitors are widely used as antiretroviral drugs. Reverse transcriptase was discovered independently by Howard Temin at the University of Wisconsin–Madison and David Baltimore at MIT; a discovery for which they shared the 1975 Nobel Prize in Physiology or Medicine.

Ribonucleic acid (RNA) A ubiquitous family of large biological molecules that perform multiple vital roles in the coding, decoding, regulation, and expression of genes. RNA is assembled as a chain of nucleotides, but it is usually single-stranded.

RNA-directed DNA methylation (RdDM) An epigenetic process first elucidated in plants whereby small double-stranded RNA (dsRNA) is processed to guide methylation to complementary DNA loci.

RNA-induced silencing complex (RISC) A multiprotein complex that incorporates one strand of a small interfering RNA (siRNA) or microRNA (miRNA). RISC uses the siRNA or miRNA as a template for recognizing complementary mRNA, which is then cleaved by activating RNase. This process is important in both gene regulation and the defense against viral infections.

RNA interference (RNAi) Posttranscriptional regulatory effects on mRNAs (i.e. control of translation or stability) triggered by processed dsRNA and ssRNA. Effects are propagated by enzymatic complexes such as RISC containing the small RNAs bound by Argonaute proteins.

Rubinstein-Taybi syndrome (RTS) A disorder caused by mutations in the *CREBBP* that is characterized by short stature, moderate to severe learning difficulties, distinctive facial features, and broad thumbs and first toes.

S-Adenosyl methionine (SAM) A cofactor for all DNA methyltransferases (DNMTs) and histone methyltransferases (HMTs), providing the methyl group added to either cytosines (DNA) or histones (arginine or lysine).

S-Adenosylhomocysteine (SAH) Hydrolyzed product formed after the methylation reaction catalyzed by DNA and histone methyltransferases using SAM as a methyl group donor. SAH is a competitive inhibitor of SAM for most methyltransferases.

Schizophrenia A mental disorder characterized by disintegration of thought processes and of emotional responsiveness, involving hallucinations, paranoia, delusions, and disorganized speech and thinking.

Serotonin A neurotransmitter produced in the brain that regulates mood, appetite, sleep, and impulse control. It is also known to influence the functioning of the cardiovascular, renal, immune, and gastrointestinal systems.

SET domain A domain found in virtually all lysine-specific histone methyltransferases (HMTs). A protein–protein interaction domain required for HMT activity and modulation of chromatin structure that is frequently associated with cysteine-rich Pre-SET and Post-SET domains.

Sex-determining region Y (SRY) A sex-determining gene on the Y chromosome in Therian mammals.

Short interspersed nuclear element (SINE) Non-long terminal repeat retrotransposons are highly abundant and heterogeneous; their length is about 300 base pairs. The most abundant SINEs in humans are in the Alu family.

Silver-Russell syndrome (SRS) A growth disorder that is one of 200 types of dwarfism. Evidence indicates that one cause results from hypomethylation of the imprinting control region which controls the monoallelic expression of *H19* and *IGF2*. Like other imprinting disorders, the incidence of Silver–Russell syndrome may be increased with the use of assisted reproductive technologies such as IVF.

Single nucleotide polymorphism (SNP) A DNA sequence variation occurring when a single nucleotide in the genome differs between members of a biological species or paired chromosomes.

Small interfering RNAs (siRNAs) RNAs that range in the size between 21 and 24 nucleotides, and are derived from double-stranded long RNAs cleaved by Dicer. siRNAs are incorporated into the RISC complex to be targeted to complementary RNAs to promote cleavage of these mRNAs.

snoRNAs Small nucleolar RNAs involved in processing of small RNAs such as ribosomal RNAs.

Social environment The people and institutions with whom people interact.

Spermatogonia Immature diploid sperm cells that develop into mature spermatozoa or sperm. Major epigenetic changes occur in spermatogonia cells.

Stable isotope labeling with amino acids in cell culture (SILAC) A popular non-radioactive isotopic labeling technique for quantitative proteomics. It is based on mass spectrometry for detecting differences in protein abundance among samples.

Stem cell Non committed cell that has the capacity to self-renew. Stem cells also have the capacity to differentiate into specialized cells.

Suberoylanilide hydroxamic acid (SAHA) An inhibitor of certain histone deacetylases, leading to enhanced levels of histone acetylation.

Sumoylation Addition of a small ubiquitin-like modifier or SUMO group to histone residues that is associated with transcriptional modification.

Supra-optic nucleus (SON) A nucleus of magnocellular neurosecretory cells in the hypothalamus of the mammalian brain; the cells produce antidiuretic hormone.

Tempero-parietal junction (TPJ) An area of the brain where the temporal and parietal lobes meet at the posterior end of the Sylvian fissure. This area is known to play a crucial role in self-other distinction processes and theory of mind. Damage to this area of the brain is implicated in producing out-of-body experiences.

Tetrahydrofolate (THF) A co-enzyme in many reactions, especially in the metabolism of amino acids and nucleic acids. It is produced from dihydrofolic acid by dihydrofolate reductase. It acts as the donor of a group with one carbon atom. A shortage of THF can cause megaloblastic anemia.

Therians A subclass of mammals that give birth to live young without using a shelled egg, consisting of the eutherians (true placental mammals) and the metatherians (marsupials). The only omitted extant mammalian group is the egg-laying prototherians (monotremes).

Totipotency Capacity of stem cells to produce all cell types required to form a mammalian embryo, i.e., embryonic and extra embryonic cells. Totipotent cells are formed during the first cleavages of the embryo.

Tourette syndrome An inherited neuropsychiatric disorder with onset in childhood that is characterized by physical and vocal tics. The exact cause of Tourette's is unknown, but both genetic and environmental factors are involved.

Transgenerational response (TGR) The transmittance of information from one generation to the next that affects the traits of offspring without altering the primary structure of DNA.

Transcriptional gene silencing (TGS) The stable repression of transcription that mainly affects transposons, chromosomal repeats, and transgenic inserts; however, it can also involve protein encoding genes. It results from epigenetic modifications of DNA and histones that create an environment of heterochromatin around a gene, making it inaccessible to transcriptional machinery.

Transcriptome The set of all RNA molecules, including mRNA, rRNA, tRNA, and other non-coding RNA produced in a cell.

Trichostatin A (TSA) An inhibitor of certain types of histone deacetylases.

Trithorax group proteins (TRX) Proteins containing a trithorax-like bromo domain: They are usually involved in recognizing histone modifications marking transcriptionally active regions and contributing to the maintenance of activity.

Trithorax response elements (TRE) Chromosomal regions, a few hundred base pairs long, that maintain the active or silent transcriptional state of their associated genes after the initial determining activators and repressors have disappeared.

Trophoblasts (TB) Cells forming the outer layer of a blastocyst that provide nutrients to the embryo; they develop into the placenta.

Tuberous sclerosis A rare multisystem genetic disease that causes non-malignant tumors in the brain and in other organs such as the kidneys, heart, eyes, lungs, and skin.

Turner syndrome A disorder affecting women that is caused by a chromosomal abnormality in which all or part of one of the X-chromosomes is absent.

Type 2 diabetes mellitus (T2DM) A metabolic disorder that is characterized by high blood glucose coupled with insulin resistance and relative insulin deficiency. The development of type 2 diabetes is caused by a combination of lifestyle and genetic factors.

Ultrabithorax (Ubx) A member of the homeobox gene family. In fruit flies, it is expressed in the third thoracic and first abdominal segments where it represses wing formation.

Untranslated region (UTR) The sections on each side of a coding sequence on a strand of mRNA. It is called the 5′ UTR if it is the leader sequence and the 3′ UTR if it is trailer sequence.

Vascular smooth muscle cell (VSMC) The stromal cells of the vascular wall are involved in the physiological functions, and the pathological changes taking place in the vascular wall. VSMCs of resistance vessels participate in the regulation of blood pressure and also in hypertension.

Ventral tegmental area (VTA) A group of neurons located close to the midline on the floor of the midbrain or mesencephalon. The VTA is the origin of the dopaminergic cell bodies of the mesocorticolimbic dopamine system. It is important in cognition, motivation, drug addiction, intense emotions relating to love, and several psychiatric disorders.

Waddington Conrad Hal Waddington (1905–1975) was a developmental biologist, paleontologist, geneticist, embryologist and philosopher who coined the word epigenetics. He used the term epigenetic landscape as a metaphor for how gene regulation modulates development.

Williams syndrome A rare neurodevelopmental disorder caused by a deletion of about 26 genes from the long arm of chromosome 7. It is characterized by a distinctive facial appearance, along with a low nasal bridge, an unusually cheerful demeanor and ease with strangers, developmental delay coupled with strong language skills, and cardiovascular problems, such as supravalvular aortic stenosis and transient hypercalcemia.

Wilms' tumor (WT) A cancer of the kidney that typically occurs in children. Loss of imprinting (LOI) and overexpression of *IGF2* is the most common epigenetic alteration in Wilms' tumor.

X-chromosome inactivation Epigenetically controlled form of dosage compensation in female mammals resulting in transcriptional silencing of genes on the surplus X-chromosome. X-chromosome inactivation is triggered by the noncoding RNA *Xist*, and it is manifested by various epigenetic modifications, including histone methylation, histone deacetylation, and DNA methylation.

X-inactivation center (XIC) Region at which the XIST-mediated inactivation starts. Allelic differences in the XIC may lead to skewed X-chromosome inactivation.

X-inactive specific transcript (XIST) The mammalian XIST gene encodes for a nonprotein encoding RNA that coats the inactive X-chromosome.

X trisomy A form of chromosomal variation characterized by the presence of an extra X chromosome in each cell of a female. There is usually no distinguishable difference between women with triple X and the rest of the female population.

Yolk sac (YS) A membranous sac attached to the embryo, providing early nourishment in the form of yolk in bony fishes, sharks, reptiles, birds, and primitive mammals. It functions as the developmental circulatory system of the human embryo before internal circulation begins.

Zinc finger (ZNF) A small protein structural motif that is formed by the coordination of one or more zinc ions in order to stabilize the fold. The vast majority of zinc finger proteins function as interaction modules that bind DNA, RNA, proteins, or other small molecules.

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