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Gene-Environment Interactions and Epigenetic Basis of Human Diseases

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Abstract

Most human diseases are related in some way to the loss or gain in gene functions. Regulation of gene expression is a complex process. In addition to genetic mechanisms, epigenetic causes are gaining new perspectives in human diseases related to gene deregulation. Most eukaryotic genes are packed into chromatin structures, which lead to high condensations of the genes that require dynamic chromatin remodeling processes to facilitate their transcription. DNA methylation and histone modifications represent two of the major chromatin remodeling processes. They also serve to integrate environmental signals for the cells to modulate the functional output of their genome. Complex human diseases such as cancer and type 2 diabetes are believed to have a strong environmental component in addition to genetic causes. Aberrancies in chromatin remodeling are associated with both genetically and environmentally-related diseases. We will focus on recent findings of the epigenetic basis of human metabolic disorders to facilitate further exploration of epigenetic mechanisms and better understandings of the molecular cues underlying such complex diseases.

Introduction

The human genome encodes approximately 30,000 genes. It is estimated that over 8,000 human diseases are caused by defects in single genes (Kaplan, 2002). These unifactorial or monogenic diseases, such as cystic fibrosis and hemophilia, are individually rare and affect approximately one percent of the human population. In contrast, the causes of major common diseases, such as cancer and diabetes, are much more complex. They often involve both susceptibility genes and their interactions with the environment. Gene-environment interactions are thought to be mediated by epigenetic modifications of the genome, and epigenetic changes of the genome often arise in response to changes in the environment (Jaenisch and Bird, 2003). Unlike genetic changes, epigenetic changes are more dynamic and are often reversible, depending on the existence or removal of the inducing factors. Gene-environment interactions can alter gene activities and lead to cascades of cellular events to facilitate the adaptation of an individual cell to its environment. Among various cellular events, gene transcription regulation is a central mechanism, which controls the RNA and subsequent protein productions that are essential in cell functions. The interactions between genes and intracellular protein factors can be viewed as an endogenous gene-environment interaction (Figure 1). Furthermore, external environmental cues can inflict additional impact on this endogenous interaction by modulating the availability or conformation of a specific protein factor. Such effects are subsequently manifested by the onsets of developmental abnormalities and chronic diseases at the organismal level (Figure 1).

In the past two decades, epigenetic control of the genome function has drawn widespread interest with a focus on gene regulation. Epigenetic inheritance describes the heritable changes in gene expression from a cell or multicellular organism to its descendants without that information being encoded in the DNA sequence (Waterland and Jirtle, 2003). During the differentiation of embryonic stem (ES) cells into various tissue stem cells such as neural stem (NS) cells, epigenetic processes are involved to selectively activate a subset of tissue-specific gene expression. Although the DNA content between ES and NS cells is identical, neuronal cells usually maintain the expression of neural-specific genes throughout development. The transmission of such tissue-specific gene expression pattern is also referred to as cellular memory (Cavalli and Paro, 1999), which allows cells of different phenotype but identical genotype to transmit a specific phenotype to their progeny cells even when the phenotype-inducing stimulus is absent. At the organismal level, epigenetic inheritance has been reported recently in different species including plants, yeast, *Drosophila*, and mammals (Chong and Whitelaw, 2004). This particular form of epigenetic inheritance also raises the possibility of non-genetic inheritance of human diseases from the parents to their offspring, which will be discussed in detail below.

Epigenetic mechanisms in development and diseases

The term epigenetics was first used to describe gene-environment interactions that lead to manifestations of various phenotypes during development. Epigenetic mechanisms are often involved to switch on or off the genes that produce permanent changes associated with the differentiation of diverse cell types. The importance of epigenetics was not recognized until recent two decades following the observation that abnormal DNA methylation events are associated with cancer (Feinberg and Vogelstein, 1983). For a long time, DNA methylation was believed to be the only epigenetic mechanism (Holliday and Pugh, 1975; Riggs, 1975). Subsequently, chromatin remodeling was identified as another major epigenetic mechanism via posttranslational modifications of histone proteins (Hebbes et al., 1988; Landsberger and Wolffe, 1997). Initial studies of chromatin remodeling were focused on histone acetylation, a reversible biochemical process that confers either open or condensed chromatin conformations to alter gene expressions (Brehm et al., 1998; Magnaghi-Jaulin et al., 1998). Recent advances in the field of epigenetics have also uncovered other histone-based epigenetic codes such

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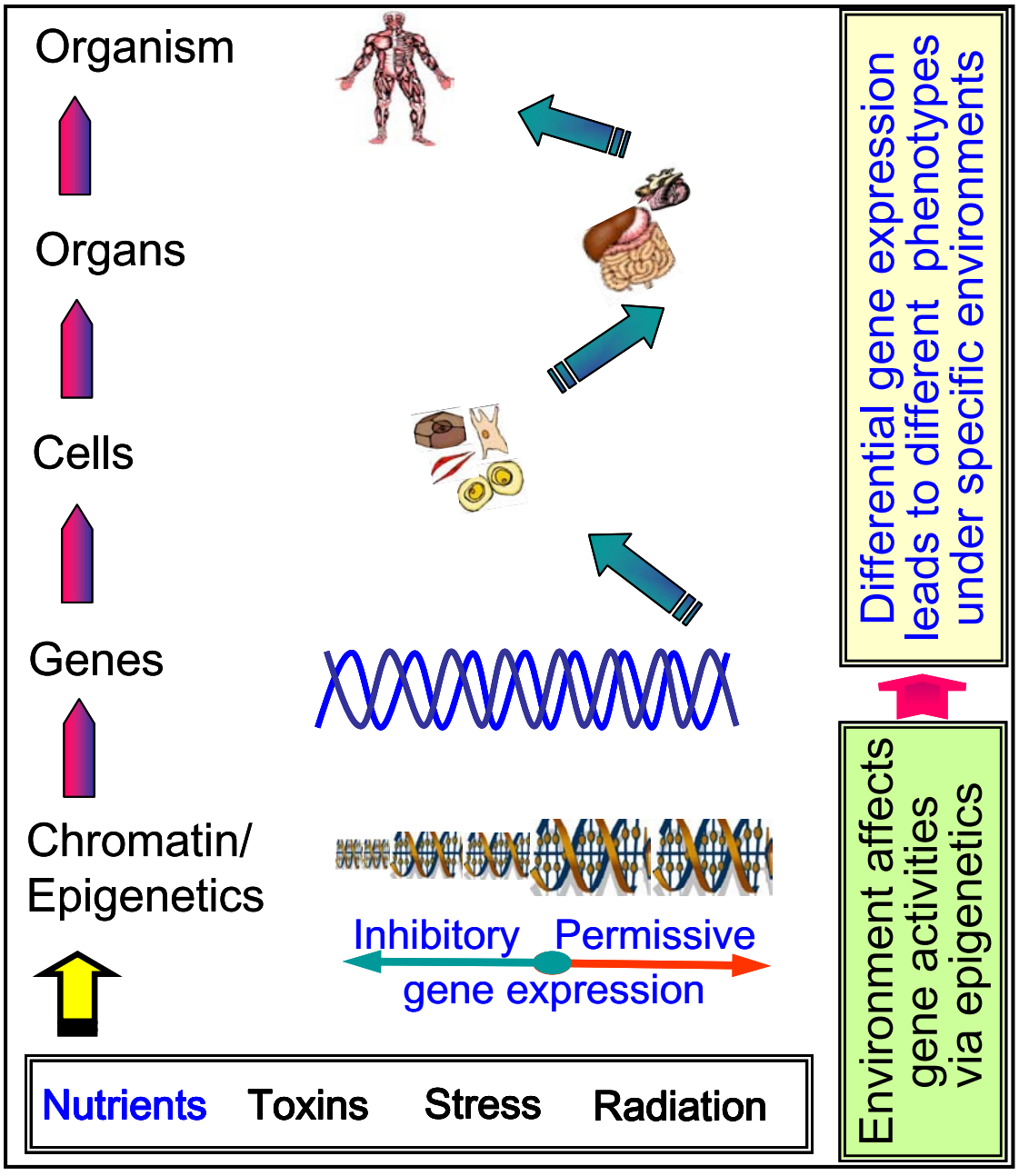

Figure 1. A hierarchical view of gene-environment interactions during development. As depicted, environmental effects are integrated by epigenetic process including chromatin remodeling to either allow or inhibit gene expressions at the molecular level. Such effects will be manifested at the organismal level via ultimate functional output of the genome.

as methylation, sumoylation, ubiquitination, ADP-ribosylation, and biotinylation (Hassan and Zempleni, 2006; Nicholson et al., 2004; Peterson and Laniel, 2004) (Table 1). Although DNA methylation modification of the genome occurs primarily on cytosines located in CpG dinucleotides, post-translational modifications of histone proteins are much more complex and affect multiple residues (Arg, Lys, Pro, Ser) at over 30 sites within the N-terminal tails of histones (Iizuka and Smith, 2003; Jenuwein and Allis, 2001) (Figure 2). Histone methylation alone can appear in the form of mono-, di-, and tri-methylation (Cheung and Lau, 2005). Even more complex, these different forms of methylation can occur on different amino acid residues that are located at different positions (e.g., H3 Lys 4, 9, 27, 36, 79; H3 Arg 2, 17, 16) (Nicholson et al., 2004). Multiple studies have shown that DNA methylation and histone deacetylation can regulate gene expression synergistically through protein mediators such as the methyl-CpG binding protein MeCP2 (Jones et al., 1998; Nan et al., 1997). Furthermore, epigenetic gene regulation through RNA-associated pathways have been discovered (Fire et al., 1998; Matzke et al., 2001; Matzke and Birchler, 2005). Altogether, these changes are now collectively referred to as epigenetic mechanisms.

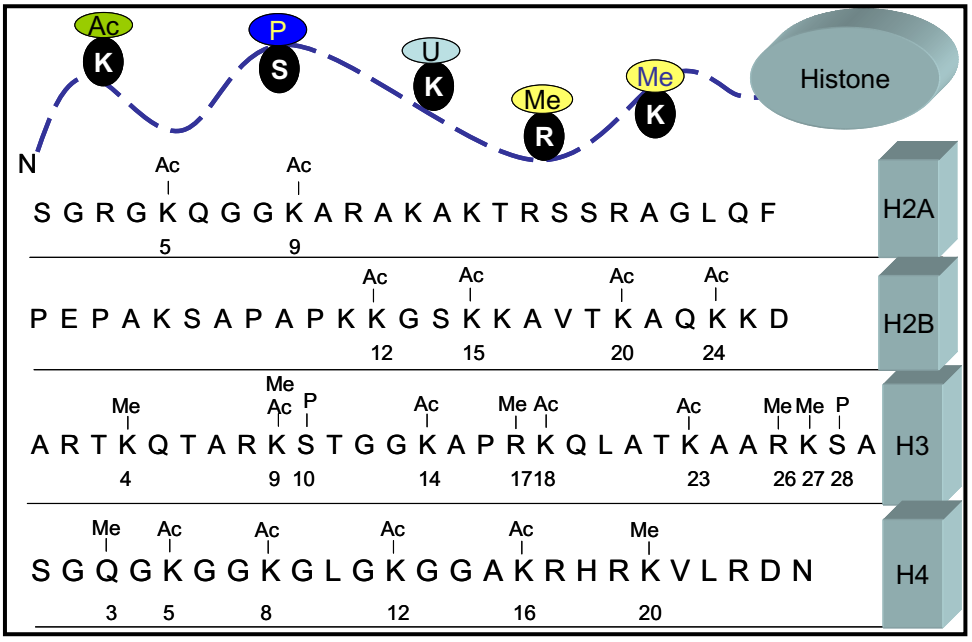

Figure 2. Target sites of the core histone protein tails that are subject to different posttranslational modifications. Ac: acetylation; Me: methylation; P: Phosphorylation; U: ubiquitination; K: lysine; S: serine; R: Arginine. A complete and frequently updated map of histone modifications is available from http://www.histone.com/modification_map.htm.

Histone posttranslational modifications

The tight interactions between DNA and histone proteins lead to a high level of structure condensation of genes into chromosomes, which impede gene transcription by default (Han and Grunstein, 1988; Kornberg, 1974; Lorch et al., 1987). The core histones, H2A, H2B, H3, and H4, together with 147 base pairs of genomic DNA wrapped around them compose the nucleosomes, the basic units of chromatin. In eukaryotic cells, gene activation is closely associated with covalent modifications of histone N-terminal tails, which are often different between active and silenced chromatins. Acetylation is the most extensively studied modification that can effectively influence gene expression. Acetylation of lysine 14 (K14) or 9 (K9) in histone H3 by histone acetyltransferase enzymes (HATs) is generally associated with active gene transcription (Nicholson et al., 2004). Furthermore, other modifications such as methylation of histone H3 at the arginine 17 position (H3meR17), or phosphoacetylation of H3 at the serine 10/lysine 14 position (H3pS10-acK14), also confer an open chromatin conformation that facilitates transcription (Clayton and Mahadevan, 2003; Valls et al., 2005). The functional relationship between different histone modifications and their effects on gene expression is summarized in Table 1.

Table 1. A summary of the functional relationship between chromatin modification and gene expression*

Chromatin molecules	Modifications	Reversibility	Effect on gene expression
DNA	Methylation	Yes	Repression
Histones	Acetylation	Yes	Activation
	Methylation	Yes	Diverse
	Sumoylation	Yes	Repression
	Phosphorylation	Yes	Activation+
	Ubiquitination	Yes	Activation+
	ADP ribosylation	Yes	Repression
	Biotinylation	Yes	Repression

*An inverse correlation between DNA methylation and gene expression, and positive correlation between histone acetylation and gene expression are well established with only a few exceptions. Other modes of histone modifications can either repress or activate gene expression depending on the modified residue and the status of each modification. Methylation of H3-K4 and H3-K36 are generally associated with transcription activation, whereas H3-K9 methylation is associated with transcription repression. The functional consequences for modifications of other residues are not conserved across species (Ebert et al., 2006). Such differences may be related to different mechanisms of recognition and interpretation of histone marks in different species. Other modes of histone modifications have been less well studied, and there also exist opposing results among different studies regarding their effect on gene expression (indicated by "+") (Garcia-Salcedo et al., 2003; Hassan and Zempleni, 2006; Peterson and Laniel, 2004; Zhang, 2003).

Gene activation by histone acetylation is thought to be biophysical in nature. The lysine has a positive charge at its end and can bind tightly to the negatively charged DNA to form a closed chromatin structure to impede the access of transcription factors. Acetylation of lysine residues removes their positive charge and attenuates the charge interaction between histone tails and DNA. This view is initially supported by chromatin immunoprecipitation studies showing that acetylated histones were preferentially associated with actively transcribed beta–globin gene sequences but not with repressed ovalbumin sequences (Hebbes et al., 1988). Furthermore, acetylated chromatin loci are easily accessed and digested by DNA nuclease, whereas the chromatin structure of silenced genes is resistant to DNase digestion (Hebbes et al., 1994; Howe et al., 1998). However, it has been

argued that acetylation of a small number of residues in histone tails are not sufficient to diminish their ionic interactions with DNA (Peterson and Laniel, 2004). An alternative mechanism proposes that various histone modifications act as docking modules to recruit other chromatin modifying enzymes and basal transcription machinery (Santos-Rosa and Caldas, 2005; Zhang, 2006). For example, a protein segment known as bromodomain can specifically bind to acetylated lysines. The bromodomain is often found in enzymes that help activate transcription including SWI/SNF, an ATP-dependent chromatin remodeling complex (Hassan et al., 2001). Lysine acetylation can recruit the SWI/SNF complex to facilitate transcription activation (Santos-Rosa and Caldas, 2005). Histone H3-K9 methylation is associated with transcriptionally silent chromatin (Mathieu et al., 2005). Proteins with a chromodomain, such as HP1, can specifically bind to methylated-lysine. HP1 is a transcription silencing protein that interacts with histone deacetylase (HDAC) (Nielsen et al., 1999; Zhang et al., 2002). Its binding to methylated H3-K9 results in histone deacetylation that eventually leads to gene silencing (Bannister et al., 2001; Zhang et al., 2002). On the other hand, H3-K4 methylation is recognized by the chromodomain protein CHD1, which can further recruit HATs to activate target gene transcription (Sims et al., 2005). Several recent studies have simultaneously reported that trimethylated H3-K4 serves as the recognition site for the plant homeodomain-containing transcription factors such as the ING (inhibitor of growth) tumor suppressor protein and the BPTF (bromodomain and PHD domain transcription factor) (Li et al., 2006; Pena et al., 2006; Shi et al., 2006). These findings have greatly added to our understandings of how different histone modifications modulate gene transcription.

DNA methylation

DNA methylation is a fundamental DNA modification that not only modulates gene expression, but also is important for regulating chromosomal stability (Holliday, 2006; Robertson, 2005). DNA methylation is catalyzed by a family of DNA methyltransferases (DNMTs), and is generally associated with gene silencing. The cytosines in CpG dinucleotides appear to be the favorite substrate for DNMTs. Although a major portion of the genome is unmethylated, CpG islands associated with gene promoters are subject to dynamic methylation modifications during development (Reik et al., 2001). During DNA replication, the pattern of DNA methylation is copied to the daughter strand by the maintenance DNA methyltransferase DNMT1. There are other DNMTs that can create new DNA methylation patterns in the genome without preexisting methylation information (Lei et al., 1996). These *de novo* DNMT activities are indicated to play important roles in early development by generating tissue-specific methylation patterns during organogenesis (Okano et al., 1999). Methylation modification of mammalian genome undergoes dramatic changes during early development, which is linked to the rapid differentiation and formation of various tissues and organs (Reik et al., 2001). As differentiation approaches completion, tissues-specific methylation patterns will be established and maintained during later development. Aging in mammals is associated with alterations in the amount and patterns of DNA methylation in somatic cells (Liu et al., 2003). Total genomic deoxymethylcytosine (dMC) generally decrease during aging in various organisms (Mays-Hoopes, 1989). Given that most somatic cells have the potential for a finite number of cell divisions, the loss of overall dMC content in the genome has been proposed to function as a cellular counting mechanism to trigger senescence (Neumeister et al., 2002).

Transcriptional silencing by DNA methylation involves a variety of regulatory proteins including DNMTs, methyl-binding domain proteins (MBDs), histone deacetylases (HDACs) and other chromatin remodeling factors. Accumulating evidence points to the fact that DNA methylation and histone modifications are interrelated in gene regulation in many scenarios (Berger, 2007; Geiman and Robertson, 2002). DNMT1, for example, is shown to associate with HDAC2 and DNMT-associated protein 1 to form a silencing complex, which is recruited to replication foci through interactions with proliferating cell nuclear antigen (Chuang et al., 1997; Rountree et al., 2000). Interestingly, HDAC2 is only associated with DNMT1 during late S phase of the cell cycle, during which period the bulk of transcriptionally inactive heterochromatin is replicated (Rountree et al., 2000). It is possible that HDAC2 is the major player involved in keeping the heterochromatin hypoacetylated. In addition, direct binding of DNMT3a and DNMT1 to HDAC1 was observed (Fuks et al., 2000; Fuks et al., 2001), and histone methylation is also coupled with methylated human Alu elements (Kondo and Issa, 2003). Although many studies have suggested that DNA methylation may act as a primary player in silencing target genes preceding other epigenetic pathways (Hashimshony et al., 2003; Padjen et al., 2005; Schubeler et al., 2000), there are also scenarios where DNA methylation seems to act as a secondary event to stabilize gene silencing status initiated by existing chromatin modifications (Bachman et al., 2003; Mutskov and Felsenfeld, 2004; Tamaru and Selker, 2001). Studies on the kinetics of silencing of transgenes show that loss of histone acetylation and H3-K4 methylation is the first step leading to reversible transcriptional repression, which is followed by H3-K9 methylation and promoter DNA methylation to stabilize the silenced chromatin state (Mutskov and Felsenfeld, 2004). Similarly, it has been shown in *Neurospora crassa* that trimethylated H3-K9 marks chromatin regions for subsequent DNA methylation (Tamaru and Selker, 2001). These scenarios together indicate a strong cooperation between DNA methylation and histone modifications in regulating gene activities during development. In many cases, disruption of either of those two processes may lead to aberrant gene expression seen in complex human diseases (Rodenhiser and Mann, 2006).

Epigenetic developmental phenomena

As aforementioned, epigenetic mechanisms participate in mammalian development by facilitating the formation of multiple cell types from a single fertilized egg cell. The DNA content in the fertilized egg contains the core developmental information. As development proceeds, the information is manifested step by step through selective gene expression that is regulated by various factors including epigenetic mechanisms. Classical studies of gene regulation focus on identifying regulatory DNA elements and their interacting transcription factors. Several biological phenomena manifested during mammalian development appear to contradict with the classical Mendelian genetic rules and thus were quite confusing before the knowledge of epigenetics. Examples of such epigenetic developmental phenomena include genomic imprinting, X-inactivation and metastable

Table 2. A representative list of human diseases associated with epigenetic anomalies.

Disorder & estimated incidence (per $1X10^5$)	Candidate genes	Epigenetic anomalies
Angelman's syndrome (6)	Deregulation of the imprinted UBE3A locus on 15q11–13	Disruption of the parental DNA methylation markers
ATR-X syndrome (130)	Loss of ATRX function	Hypomethylation of certain repeat and satellite sequences
Beckwith-Wiedeman syndrome (10)	Disruption of the imprinted IGF2/CDKN1C loci on 11p15.5	Loss of genomic imprinting
Coffin-Lowry syndrome (2)	Mutation in RSK genes	Disrupted chromatin remodeling via activation of CBP
Fragile X syndrome (50)	Loss of FMR1/FMR2 function	Promoter methylation due to an expansion of the CGG repeat
ICF syndrome (0.5)	DNMT3b mutation	Centromeric DNA hypomethylation
Klinefelter syndrome (100)	Extra X chromosome in males	Abnormal X-inactivation/imprinting
Prader-Willi syndrome (15)	Disruption of the imprinted SNRF/SNRPN locus on 15q11–13	Disruption of genomic imprinting
Rett syndrome (8)	MeCP2 mutation	Deregulated promoter activities
Rubinstein-Taybi syndrome (1)	Mutations in the gene encoding CREB -binding protein	Reduced histone H3 acetylation
Williams syndrome (10)	Loss of WSTF function	Condensed chromatin structures

epialleles, which will be discussed below. Epigenetic studies in the past two decades have greatly enhanced our understanding of the complex mechanisms underling gene transcription regulation, and have shed insights into the molecular mechanisms underlying such developmental paradoxes that otherwise cannot be addressed solely by genetic studies.

Genomic imprinting is a well-characterized developmental phenomenon that describes a unique form of gene regulation that leads to only one parental allele being expressed depending on its parental origin (Delaval and Feil, 2004; Surani, 1991). The imprinting phenomenon was first observed in early nuclear transplantation studies, where diploid androgenotes derived from two male pronuclei or diploid gynogenotes derived from two female pronuclei failed to develop properly (McGrath and Solter, 1984; Surani et al., 1984). Subsequently, uniparental disomies, in which a single chromosome inherited solely through the maternal or the paternal germline, have been studied in mice to identify regions of the genome that carry imprinted genes (Cattanach, 1986). Insulin-like growth factor 2 (*Igf2*) and its receptor *Igf2r* are two of the first reported genes subject to imprinting regulation (Barlow et al., 1991; DeChiara et al., 1991). Currently there are more than 80 genes known to be imprinted in mice and humans, with about one third of those being imprinted in both species (Morison et al., 2005). 600 genes are predicted to be imprinted in the mouse genome (Luedi et al., 2005). Among the identified imprinted genes, a major common feature is that they are associated with at least one regulatory DNA element, often referred to as the imprinting control region (ICR), that is essential in regulating the parental origin-specific expression status via interaction with specific transcription factors (Kim et al., 2007; Yang et al., 2003). Furthermore, these ICRs are often differentially modified epigenetically. Differential DNA methylation of the parental ICRs is one of the most common features associated with imprinted genes (Kim et al., 2003; Liang et al., 2000; Mancini-DiNardo et al., 2003). Such epigenetic imprints undergo erasure and reestablishment during germline development (Kerjean et al., 2000; Reik et al., 2001). Since imprinted genes are functionally haploid, a single mutation or deletion of the functionally active allele would predispose affected individuals to developmental abnormalities. Typical disorders associated with imprinted genes include Prader-Willi and Angelman syndromes, Beckwith-Wiedemann syndrome and multiple forms of neoplasia (Weksberg et al., 2003; Zeschnigk et al., 1997) (Table 2). Conversely, aberrant co-expression of both parental alleles, a condition defined as loss of imprinting, has been linked with tumorigenesis (Cui et al., 2003; Jelinic and Shaw, 2007).

X inactivation is a mechanism by which mammals adjust the genetic imbalance that arises from the different numbers of gene-rich X-chromosomes between XX females and XY males. The dosage difference of X-linked genes between the two sexes is functionally equalized by silencing one of the two X chromosomes in females. Mammalian X inactivation appears in two forms: imprinted and random X inactivation. Imprinted X activation occurs in early mammals such as marsupials (Graves, 1996). This form of dosage compensation is achieved by inactivating the paternally inherited X chromosome. Imprinted X inactivation also occurs in the extra-embryonic tissues of placental mammals, whereas the cells forming the embryo undergo random X inactivation through which either the paternal or the maternal X chromosome is inactivated (Huynh and Lee, 2001). To achieve random X-inactivation, each cell ensures that only one X chromosome remains active and that the other X chromosome is inactivated in a random manner. The differential treatment of two X chromosomes in the same female nucleus results in an active X (Xa) and an inactive X (Xi). X-inactivation is a typical developmental phenomenon involving RNA-mediated epigenetic silencing. The underlying mechanism is linked with a noncoding RNA called Xist, which is unique to placental mammals (Duret et al., 2006). The *Xist* RNA, which is expressed exclusively from and coats the Xi, can spread over and encompass the Xi. *Tsix*, an antisense

gene of *Xist*, also participates in maintaining inactivation. *Tsix* overlaps with the *Xist* gene and is transcribed in the antisense orientation (Boumil et al., 2006; Lee et al., 1999). On Xi, Xist RNA coats the chromosome to repress *Tsix* expression; on Xa, Tsix RNA blocks Xist transcription and the *Xist*-mediated X-inactivation is therefore inhibited. Multiple studies have established that differential DNA methylation and histone modification states exist between Xi and Xa (Goto et al., 2002; Hansen, 2003; Heard et al., 2001; O'Neill et al., 1999). Xi is hypermethylated in CpG islands and in gene promoter regions, and displays histone H4 hypoacetylation (Allaman-Pillet et al., 1998; Jeppesen and Turner, 1993; Keohane et al., 1998). However, Xist is hypermethylated on Xa and unmethylated on Xi (Allaman-Pillet et al., 1998), which allows its exclusive expression from the Xi. Tsix is also regulated by differential methylation of its enhancers between the Xi and Xa in mice (Boumil et al., 2006). Taken together, differential chromatin modifications and RNA-mediated gene silencing appear to constitute the core molecular basis for mammalian X-inactivation.

Metastable epiallele (ME) is another example of epigenetic developmental phenomenon. It refers to the fact that some mammalian alleles display an unusual characteristic of variable expressivity in the absence of genetic heterogeneity (Rakyan et al., 2002). Two extensively studied examples of ME are the mouse *agouti viable yellow* (A^{vy}) and *axin fused* ($Axin^{Fu}$) alleles (Rakyan et al., 2002). ME formation is often associated with the insertions of retroelements such as the intracisternal A particle (IAP) retrotransposons (Dolinoy et al., 2006; Vasicek et al., 1997). The A^{vy} allele is formed by an IAP insertion into the mouse *agouti* gene transcription start site (Duhl et al., 1994), and the $Axin^{Fu}$ allele is formed after an IAP insertion into intron 6 of the mouse *Axin* gene (Vasicek et al., 1997). The long terminal repeats (LTRs) of IAPs function as the promoter to initiate its transcription following its insertion into the host sequences. It is noteworthy, however, that no single-copy endogenous gene in mammals has been shown to behave in the same way as the IAP insertion alleles. Because IAP transcription is tightly controlled by its LTR methylation status (Mietz and Kuff, 1990; Walsh et al., 1998), expression of the resulting ME alleles also becomes sensitive to DNA methylation. DNA methylation in the A^{vy} IAP correlates inversely with ectopic *Agouti* expression. Since DNA methylation states are easily perturbed by environmental factors such as nutrients, phenotypic mosaicism will arise from methylation differences among individuals (variable expressivity) due to different environment exposure. A^{vy} allele in mice has become a useful experimental marker for studying epigenetic inheritance (Waterland and Jirtle, 2003; Wolff et al., 1998). Since the degree of methylation varies among individual isogenic A^{vy}/a mice, there is a wide variation in coat color ranging from yellow phenotype with maximum ectopic agouti overexpression (unmethylated), to an array of varied agouti/yellow phenotypes due to partial agouti overexpression (partial methylation), and to a pseudoagouti phenotype with minimal agouti expression (methylated) (Morgan et al., 1999). Yellow (A^{vy}/a) mice are often larger, obese, hyperinsulinemic, and more susceptible to tumorigenesis as compared with their non-yellow siblings. Pseudoagouti A^{vy}/a mice are lean, healthy, and long lived as compared with their yellow siblings. A^{vy} expression and subsequent fur color in the offspring have been shown to be modulated by maternal diet (Wolff et al., 1998), providing an mechanistic insight into how nutrient supplies during pregnancy affect the health and longevity of the offspring. Using this unique ME experimental system, a recent study demonstrated that such inheritable maternal effects are mediated by epigenetic changes in the germline induced by nutritional interference, and has led to an intriguing research area known as transgenerational epigenetic inheritance (Cooney, 2006; Cropley et al., 2006).

Transgenerational epigenetic inheritance

Transgenerational epigenetic inheritance refers to the biological process that an epigenetic state established in the parent either stochastically or in response to the environment can be inherited by the offspring (Chong and Whitelaw, 2004). It was first described in plants (Brink et al., 1968), and was later observed in yeast, *Drosophila*, and mouse both at transgenes and endogenous alleles (Cavalli and Paro, 1999; Grewal and Klar, 1996; Rakyan et al., 2002), although it is not yet clear whether it occurs in humans (Rakyan et al., 2002). Epigenetic inheritance is thought to result from the incomplete clearing of epigenetic marks during either primordial germ cell development or early embryonic development (Chong and Whitelaw, 2004). Many nutrients, especially those involved in biological methylation pathways, have been shown to influence DNA methylation stability either locally or genome-wide (Liu et al., 2003). To elucidate whether nutritional changes in grandparents may affect offspring development via altered DNA methylation, Cropley and colleagues took advantage of the A^{vy} ME mouse model by crossing the a/a female mice with the A^{vy}/a males (P1). During mid-gestation between E8.5 and E15.5, they supplemented the pregnant P1 females with methyl donors such as betaine, folic acid and methionine, all of which are known to enhance genomic methylation. The resulting F1 pseudoagouti females were mated to a/a males and fed with normal diet without further supplementation of methyl donors. It turned out that the ratio of agouti fur color was significantly higher in F2 offspring from supplemented grandmothers than those from grandmothers fed with control diet (Cropley et al., 2006). This increased agouti ratio is likely due to the inheritance of methylated A^{vy} alleles from the grandmother supplemented with methyl donor. The degree of change in offspring fur color is similar between the F1 and F2 generations, indicating that methylation changes at the A^{vy} locus is stably maintained throughout germline development in both F1 and F2 generations (Cropley et al., 2006). This study demonstrated for the first time that specific nutrients can induce epigenetic change at a specific gene locus and confirmed that epigenetic mechanism is a major player in transgenerational inheritance of early environmental effects.

Evidence supporting epigenetic inheritance also comes from studies of the biological effects of endocrine disruptors, which are environmental toxins that interfere with the endocrine system and disrupt the physiological function of hormones by acting as estrogens or antiestrogens or antiandrogens. Examples of environmental endocrine disruptors include pesticides (such as methoxychlor), fungicides (vinclozolin), insecticides (trichlorfon), and various xenoestrogens (Anway and Skinner, 2006). A recent study suggests that environmental endocrine disruptors are detrimental to reproduction, and may promote abnormalities such as a decrease in sperm count, and an increase in testicular cancer (Anway et al., 2005). Transient exposure of a gestating female rat during the period of gonadal sex

determination to vinclozolin (an antiandrogenic compound) or methoxychlor (an estrogenic compound) induced an adult phenotype in the F1 generation of decreased spermatogenic capacity (cell number and viability) and increased the incidence of male infertility (Anway et al., 2005). These effects were transferred through the male germ line to nearly all males of subsequent generations from F1 to F4. Interestingly, the effects on reproduction appear to correlate with altered DNA methylation patterns in the germ line, as comparative analysis of DNA methylation revealed that vinclozolin exposure altered methylation patterns in the testis (Anway et al., 2005). Although it is not determined which genes are responsible for the resulting phenotypes, a subset of imprinted genes are proposed to be the candidate carriers of such transgenerational effects because germline reprogramming of their methylation markers are more prone to environmental changes (Reik et al., 2001). In addition to methyl donors and endocrine disruptors, glucose has also been implicated to trigger transgenerational inheritance phenotypes (Aerts and Van Assche, 2006; Gauguier et al., 1990). Altogether, these observations highlight the biological importance of epigenetic actions inflicted by environmental agents, and provide a mechanistic view on how epigenome-environment interactions can lead to developmental abnormalities independent of genetic changes. The ability of an environmental factor to reprogram the germ line and to promote a transgenerational disease state has significant implications for disease etiology. These recent progresses lay important foundations for future efforts to elucidate the etiology of complex human diseases. Apparently, identifying specific candidate genes involved in these processes will be a major focus for future studies.

Epigenetic mechanisms in metabolic disorders

Metabolic syndrome (MetS) is a combination of medical disorders that increase the risk for common chronic diseases such as obesity, cardiovascular disease, and diabetes. It is also known as syndrome X, insulin resistance syndrome, obesity syndrome, or Reaven's syndrome. While the MetS term is getting widely accepted in the research literature, there is still an ongoing debate over its clinical utility and whether MetS qualifies to stand alone as a disease condition for clinical diagnoses (Kahn et al., 2005). It is estimated that over 20% of adults in the US have MetS and the incidence is rising at an elevated rate (Zarich, 2006). The recent explosion of MetS poses a severe health threat to the general population (Grundy, 2004). The fetal basis of adult disease (FeBAD) theory proposes that metabolic disorders have a developmental origin and are related to early nutrition during gestation and lactation (Hales and Barker, 2001). This theory has gained extensive support from recent epidemiological studies showing that fetal undernutrition, lower birth weights, and obesity in humans are associated with an increased risk of diabetes, congestive heart failure, and stroke (Lawlor et al., 2005). Traditional genetic tools are sufficient for uncovering the underlying causes for monogenic diseases; however, the genetic basis underlying the pathogenesis of complex MetS diseases largely remains unknown so far. Major metabolic diseases such as type 2 diabetes (T2D) and obesity are often polygenic and multifactorial in nature. Development of T2D is a multistep process with strong genetic and environmental influences (Smith and Ravussin, 2005; Speakman, 2004). T2D is associated with older age and obesity. Although genetic components underlying obesity development are supported by overwhelming evidence (Rankinen et al., 2006), non-genetic mechanisms are gaining new perspectives (Koza et al., 2006; Speakman, 2004), including a growing interest in epigenetic mechanisms such as DNA methylation and genomic imprinting (Gallou-Kabani and Junien, 2005; Smith et al., 2006). Despite some sporadic studies exploring the epigenetic control of several individual genes related to diabetes and obesity, more systematic efforts are needed for this emerging area of research that holds the promise for unraveling the complex molecular events leading to human MetS (Gallou-Kabani and Junien, 2005).

Many imprinted genes are implicated to be involved in regulating growth and metabolism (Charalambous et al., 2003; Smith et al., 2006; Varrault et al., 2006). Interestingly, disrupted expressions of imprinted genes are found in developmental syndromes displaying features typical of diabetes and obesity, two of the hallmarks of MetS (Delrue and Michaud, 2004). For example, Beckwith–Wiedemann syndrome **(**BWS**)** is a developmental disorder that is caused by a variety of genetic or epigenetic alterations within two imprinted domains (Maher and Reik, 2000; Sparago et al., 2007). BWS patients display fetal and postnatal overgrowth, which is associated with defective expression of imprinted genes such as *IGF2*, *H19* and *CDNK1C* (Smith et al., 2006). Imprinted genes control the supply of nutrients in the placenta and regulate tissue growth in the fetus (Constancia et al., 2004). Imprinted genes also play a key role in postnatal development. The imprinted GNAS domain encodes multiple gene products including the G-protein □ (G_s□) subunit, Nesp55 (maternally expressed) and the neuroendocrine-specific G_s□, isoform XL□s (paternally expressed). Maternal or paternal transmission of the mouse Gnas knockout produces opposite effects on energy metabolism: loss of the paternal Gnas function causes a decrease in adiposity, hypermetabolic function, and hypoglycemia, whereas loss of the maternal Gnas function leads to greater adiposity (Plagge et al., 2004). The preadipocyte factor-1 (Pref-1) is a paternally expressed gene encoding a transmembrane protein. Disruption of Pref-1 in mice results in reduced body weight at birth (Moon, 2002). Paradoxically, transgene-based Pref-1 overexpression also causes lower birth weight and has been suggested to function as a paternal inhibitor of adipogenesis (Lee et al., 2003). Other imprinted genes that are involved in regulating growth and metabolism include *Peg1*, *Peg3*, *Plag4* and *Hyma1* (Curley et al., 2004). In a high fat-induced obesity mouse model, Peg1 expression showed a 23-fold increase in obese mice as compared to normal controls (Koza et al., 2006). Interestingly, both Peg1 and Peg3 are strongly expressed in the hypothalamus and septum (Constancia et al., 2004; Keverne, 2001). Given the key role of hypothalamic neurons in regulating energy homeostasis (hunger/satiety and food intake), these genes are believed to regulate growth and metabolism through neural programming. Furthermore, Peg1 and Peg3 also appear to regulate adipose tissue growth. Oligonucleotide microarray analyses reveal that Peg1 and Peg3 are upregulated in the adipose tissues from diet-induced obesity mice (Moraes et al., 2003). Consistent with these findings, overexpression of Peg1 can increase the size of adipocytes (Takahashi et al., 2005). Because the expression of imprinted genes is strictly controlled by epigenetic mechanisms, these studies will lead to further characterizations of the underlying epigenetic changes in

energy metabolism as related to the etiology of MetS.

Adipogenesis refers to the formation of adipocytes from preadipocytes (Rosen and Spiegelman, 2000). It is one of the most intensely studied models of cellular differentiation due to the availability of in vitro models that faithfully recapitulate most of the critical aspects of adipocyte formation in vivo. More recently, studies of adipogenesis have proceeded with the hope that manipulation of this process in humans may facilitate the battle against obesity and diabetes. Several studies have investigated epigenetic regulation of key adipogenic gene activities during *in vitro* adipogenesis, including the adipose most abundant transcript 1 (apM1), the glucose transporter Glut4, and glycerol phosphate dehydrogenase Gpd1 (Musri et al., 2006). H3 hyperacetylation and H3-K4 trimethylation, for example, are positively correlated with apM1 transcription during early adipogenesis; whereas treatment with methylthioadenosine (an inhibitor of H3-K4 methylation) decreases apM1 expression as well as adipogenesis (Musri et al., 2006). The obese gene leptin is highly expressed in mature adipocytes but not in preadipocytes (MacDougald et al., 1995), which is consistent with the observation that its promoter is highly methylated in human preadipocytes and becomes demethylated in adipocytes (Melzner et al., 2002). Promoter demethylation and consequent expression of the *leptin* and *glut4* genes are also observed during mouse preadipocyte differentiation (Yokomori et al., 1999; Yokomori et al., 2002). The secreted frizzled related protein (SFRP) 5 is an inhibitor of Wnt signaling in adipogenesis. In a high-fat induced obese mouse model, SFRP5 expression was upregulated significantly to promote adipogenesis, and its upregulation was implicated to be due to epigenetic modifications (Koza et al., 2006). These findings together argue for an epigenetic regulation of adipogenesis and obesity development. Dietary components such as folic acid and choline can interact with biological DNA methylation process (Ghoshal et al., 2006; McCabe and Caudill, 2005; Niculescu et al., 2006). Nutrition imbalance can thus lead to DNA methylation aberrancies that affect the activities of key adipogenic genes, which facilitates the development of obesity and other MetS.

Conclusions

Epigenetic modifications are key regulators of developmental processes including differentiation, growth, and aging. Variations in epigenetic modifications can contribute to genetic diversity, whereas abnormal epigenetic changes often lead to developmental abnormalities and diseases. Advances in understanding epigenetic mechanisms have shed important insights into the complex mechanisms underlying tumorigenesis and also have opened up new therapeutic options and targets for treating cancer patients. Although the role of epigenetic alterations in cancer is widely-acknowledged, the relevance of epigenetics to common metabolic diseases remains less conspicuous to date. Epigenetic studies of obesity, T2D and other related metabolic disorders are still in its early stage. We have summarized some converging data that highlight the significance of epigenetic mechanisms in modulating gene activities in response to environmental influences during development. Due to the polygenic and multifactorial nature of most complex human diseases, complementing classical genetic research with emerging epigenetic tools will be the ultimate method of choice to explore the molecular causes underling the development of complex human diseases. Understanding how small molecules (nutrients and chemicals) interact with the epigenome will allow us to design a new generation of epigenetic drugs for curing complex diseases such as cancer and MetS.

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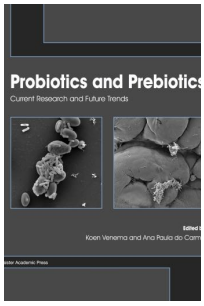

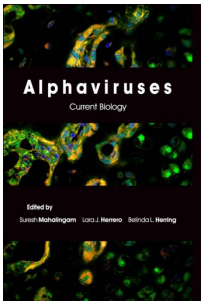

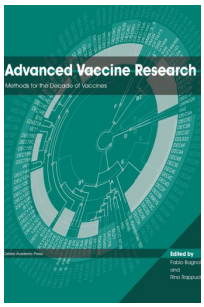

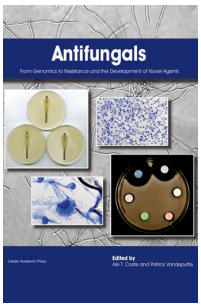

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