Epigenetic Modifications Regulate Gene Expression

Introduction

Epigenetics is defined as heritable changes in gene activity and expression that occur without alteration in DNA sequence [1, 2]. It is known these non-genetic alternations are tightly regulated by two major epigenetic modifications: chemical modifications to the cytosine residues of DNA (DNA methylation) and histone proteins associated with DNA (histone modifications) [1, 3]. Functionally, the patterns of epigenetic modifications can serve as epigenetic markers to represent gene activity and expression as well as chromatin state [3-6].

Epigenetic modifications are crucial for packaging and interpreting the genome under the influence of physiological factors [4-8]. Epigenetics is one of the fastest-growing areas of science and has now become a central issue in biological studies of development and disease [3, 7-11]. In recent years, there have been rapid advancees in the understanding of epigenetic mechanisms [12-14], which include histone modifications [4-6, 15], DNA methylation [10, 11, 16, 18], small and non-coding RNAs [20, 56], and chromatin architecture [13, 15, 21]. These mechanisms, in addition to other transcriptional regulationary events [15, 17], ultimately regulate gene activity and expression during development and differentiation, or in response to environmental stimuli [16, 17].

Epigenetic research can help explain how cells carrying identical DNA differentiate into different cell types, and how they maintain differentiated cellular states [14, 17]. Epigenetics is thus considered a bridge between genotype and phenotype [1-3, 7, 9].

While epigenetics refers to the changes of single genes or sets of genes, the term epigenome reflects the overall epigenetic state of a cell, and refers to global analyses of epigenetic markers across the entire genome [3, 14]. It is therefore critically important to map the epigenetic modification patterns or profile the epigenome in a given cell, which then can be used as epigenetic biomarkers for clinical prediction, diagnosis, and therapeutic development [8, 11, 18, 45]. International human epigenome projects are currently working to catalog all the epigenetic markers in all major tissues across the entire genome. The resulting reference maps will usher in epigenetics as an exciting new era of medical science [14]. As an example of the research community's commitment to classifying epigenetic markers, the National Institutes of Health (NIH) has recently launched a $190 million research effort to learn more about epigenetics.

This mini-review focuses on DNA methylation and the predominant histone modifications, with emphasis on their dynamic interactions within the chromatin environment to form the complex epigenetic mechanisms that orchestrate the regulation of genes at the molecular level in mammalian cells.

Chromatin Structure

Genomic DNA in eukaryotic cells is packaged with special proteins termed histones to form protein/DNA complexes called chromatin. The basic unit of chromatin is the nucleosome, which is composed of ~ 146 base pairs (bp) of DNA wrapped around an octamer of the four core histones (H2A, H2B, H3, and H4). The core histones are tightly packed in globular regions, with amino-terminal tails that extend from the globular region, making them accessible to histone modifying enzymes (Figure 1) [22]. Another protein, termed linker histone H1, interacts with DNA links between nucleosomes. It functions in the compaction of chromatin into higher-order structures that comprise chromosomes. This organization of chromatin allows DNA to be tightly packaged, accurately replicated, and sorted into daughter cells during cellular division (Figure 2) [22-24].

In a non-dividing cell, chromatin can be divided into two functional states: euchromatin or heterochromatin, which are transcriptionally active or inactive states of chromatin, or areas of the chromosomes, respectively [4, 6, 13]. Euchromatin is the region where DNA is accessible, representing an open conformation due to the relaxed state of nucleosome arrangement. The genomic regions of euchromatin are more flexible, and contain genes in active and inactive transcriptional states [4, 13, 25]. Conversely, heterochromatin are areas where DNA is packaged into highly condensed forms that are inaccessible to transcription factors or chromatin-associated proteins [4, 26, 28]. The genomic regions within heterochromatin primarily consist of repetitive sequences and the repressed genes associated with morphogenesis or differentiation (imprinting or X chromosome inactivation) [9, 10]. Heterochromatin is also a prevalent chromatin state of DNA sequences that have critical functions in controlling chromosomal stability and the prevention of mutations and translocations [26, 27, 28].

The functions of chromatin currently extend beyond simply packaging DNA and regulating genetic information. Instead, the dynamic states of chromatin structures tightly govern activation and function of the genome to further influence cellular behavior [4, 6, 15]. Results from the ENCyclopedia of DNA Elements (ENCODE) pilot project provided insight into the shape of chromatin architecture, in which the genome is organized into "open" or "closed" chromatin territories representing higher-order functional domains with distinctive distribution patterns of the epigenetic markers (Figure 2, 3, Table 1) [13, 14, 25]. The dynamic

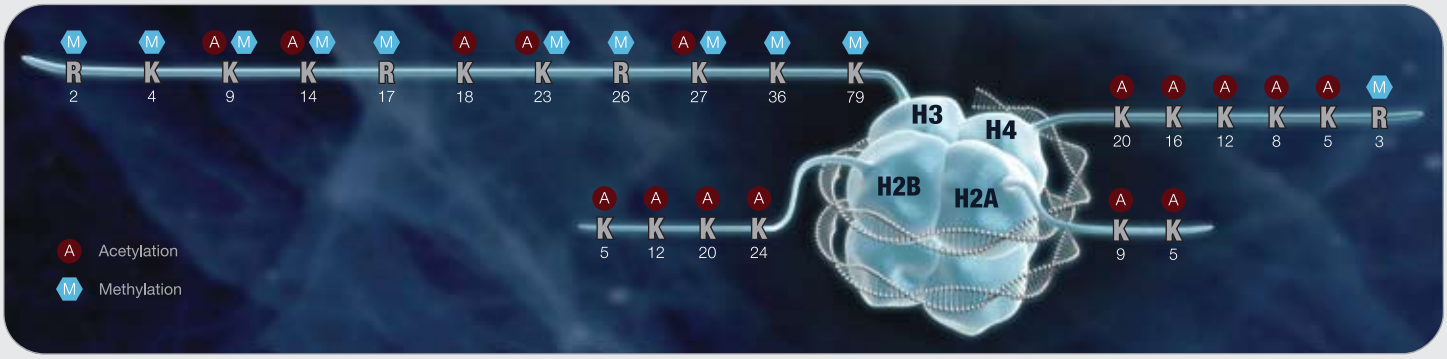

Figure 1: Nucleosome - The Fundamental Packing Unit of Chromatin Representing Primary Structure That Determines DNA Accessibility. A single nucleosome is composed of ~146 basepairs (bp) of double-stranded helical DNA wrapped around a core of histone proteins (an octomer), from which histone tails protrude, and are subjected to different post-translational modifications (PTM) at different residues: acelylation of histone tails [A] including histone H3 at lysine 9, 14, 18, or 23 (H3K9Ac, K14, K18, or K23), histone H4 at lysine 9, 14,18, or 23 (H3K9Ac, K14, K18, or K23), and methylation of histone tails [M] occurring on lysine residue 4, 9, 27, 36, or 79 of H3 (H3K4me, H3K9me, H3K27me, H3K36me, or H3K79me, respectively), and on lysine residue 20 of H4 (H4K20me).

composition of chromatin during different stages of the cell cycle, or from one cell type to another, is regulated through multiple epigenetic mechanisms. How higher-order structures of chromatin are formed, regulated, and their affects on genomic activity and function continues to remain elusive [1, 21, 24].

DNA Methylation

DNA methylation, the first recognized and most well-characterized epigenetic modification, is linked to transcriptional silencing, and is important for gene regulation, development, and tumorigenesis [10, 11, 16]. In mammalian cells, DNA methylation occurs at the 5' position of the cytosine ring within CpG dinucleotides via addition of a methyl group to create a 5-methylcytosine (m^5C). The modification at m^5C is catalyzed by DNA methyltransferases (DNMTs), including DNMT1, DNMT3a, and DNMT3b [29, 30]. DNMT3a and DNMT3b are *de novo* methyltransferases, preferentially targeting unmethylated CpGs to initiate methylation. The process of *de novo* methylation can occur in early embryonic stem cells or cancer cells [31]. During DNA replication, DNMT1 acts as a maintenance methyltransferase predominantly recognizing and methylating hemimethylated CpGs, thereby copying DNA methylation patterns from parental to daughter strands [24, 32]. DNMTs can function co-operatively to methlylate DNA [29]. Moreover, it has been observed that knockout DNMTs in mouse models are embryonically lethal, indicting the importance of DNA methylation for embryonic development of mammalian cells [31, 32].

As DNA methylation is predominantly found in CpG sites of the mammalian genome, these sites tend to cluster in regions of large repetitive sequences such as centromeric repeats or at the 5' ends of many genes, called CpG Islands (CpGIs) [3,33]. In humans, 50-70% of all CpGs are methylated, primarily in heterochromatic regions. In contrast, euchromatic CpGIs remain locally unmethylated, with the exception of genes involved in imprinting, X chromosome inactivation, and tissue-specific differentiation [10, 11, 16, 18]. Distinctive distribution patterns of CpG methylation are believed critical for the control of gene silencing and chromosomal stability [27, 28]. For example, hypermethylation in repetitive sequences combined with histone modifications can result in the condensation of chromatin DNA into inaccessible heterochromatin states (Figure 2). Conversely, the CpGIs in promoter-associated regions of DNA are frequently unmethylated, remaining accessible to transcription factors and chromatin-associated proteins for the expression of most housekeeping genes and many regulated genes [2, 10, 16, 18, 34, 53, 55].

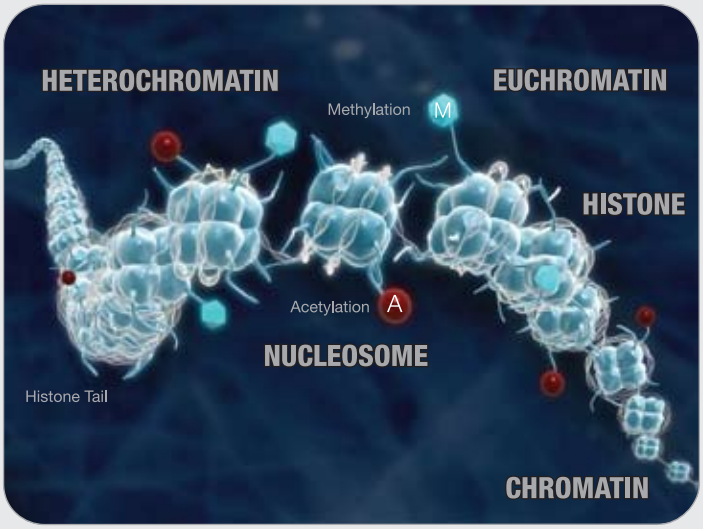

Figure 2: The Epigenetic Control of Chromatin Organization. DNA associates with histone proteins packaged into chromatin to form high-order structures that comprise chromosomes. This composition of chromatin is crucial for regulating the accessibility and function of the genome, and is controlled by epigenetic modifications, such as DNA methylation and histone modifications.

Aberrant patterns of DNA methylation influence many aspects of disease processes [8, 10, 18], especially in many human tumors [11, 16, 55]. Cancers have the unique property in which global hypomethylation alters chromatin architecture, leading to inappropriate activation of oncogenes and transposable elements, whereas local hypermethylation of CpGIs at the promoter region of tumor suppressor genes (TSGs) prevents activation of these genes. This epigenetic alteration can serve as an additional oncogenic mechanism, contributing to tumorgenesis. In fact, CpGI hypermethylation at TSG promoter regions has been recognized as a hallmark of many types of cancer cells. This finding has greatly advanced the field of epigenetics [10, 18, 19]. Today, many genome-wide approaches for identifying candidate TSGs have been developed to detect changes in DNA methylation, existing genome-wide or within localized regions, that may prove useful as markers for risk assessment, early prognosis, and treatment of disease [14, 18, 19, 34]. In an article on page 6 of this publication, we describe a new technique to study DNA methylation.

Histone Modification

The amino terminals of the core histone are subjected to several types of multivalent modifications, including acetylation, methylation, phosphorylation, ubiquitination, sumoylation, etc. [6, 21]. Histone modifications, recognized as a post-translational modification (PTM), are critical for regulating chromatin structure and function, which can in turn affect many DNA-related processes, such as transcription [13, 25], recombination [50], DNA repair and replication [13, 24], and chromosomal organization [4, 21-23].

Acetylation and methylation of lysines at histone tails are the two most common PTMs with distinct distributions along both euchromatin and heterochromatin (Figure 1, 2) [4, 13, 14]. In contrast to DNA methylation, which is relatively stable, histone modifications are more dynamic and difficult to analyze [12-14]. The Chromatin Immunoprecipitation (ChIP) Assay is a popular technique used to characterize the distribution of histone modifications and analyze the occupancy of transcription factors at specific loci or throughout the genome of a live cell [3, 12-14]. In an article on page 10 of this publication, we describe a newly developed ChIP system for the study of histone modifications and their role in regulating gene expression.

Acetylation of Histones H3 and H4 (H3ac, H4ac)

The acetylation of histones was the first epigenetic modification connected with biological activity [10, 21]. The lysine residues at the N-terminal of histone tails are subjected to either acetylation by histone acetyltransferase enzymes (HATs), or deacetylation by histone deacetylases (HDACs) [4, 21]. These lysine residues have a positive charge that can bind tightly to the negatively charged DNA to condense nucleosomes and form a closed chromatin structure, which is inaccessible to transcription factors. Acetylation removes positive charges and reduces the affinity between histones and DNA, thereby opening the condensed chromatin structure to allow transcriptional machines easier access to promoter regions. Histone hyperacetylation is thus considered a more reliable epigenetic regulator of transcriptional activation, through either direct disruption of the physical structure of chromatin or recruitment of other chromatin associated factors (Figure 2) [4, 10, 21].

Methylation of Histones H3 and H4 (H3me, H4me)

In contrast to the well-studied hyperacetylation of histones that is positively correlated with actively transcribed genes [41], histone methylation is associated with either transcriptional activation, inactivation, or silent genomic regions [4, 13]. Histone methylation occurs on different lysine residues, with the potential addition of one, two, or three methyl groups. The effect of histone methylation on gene function and chromatin state is dependent not only on the specific lysine residue modified, but also on its degree of methylation. Histone methylation has been characterized as *the* distinguishing pattern associated with different effects on gene activity [5, 6, 51, 52]. Generally, in activated gene regions, there is an

enrichment of histone methylation at H3K4me, H3K36me, or H3K79me [25, 40, 47, 49, 50, 52]. Therefore, these epigenetic markers are associated with transcriptional activation. In contrast, the enrichment of histone methylation at H3K9me, H3K20me, or H4K27me is implicated in gene inactivation or silencing [6, 14, 21]. However, an exception to such patterns has been observed in genes that regulate development of stem cells, in which H3K4me3 and H3K27me3 co-exist as "bivalent domains", keeping these key genes in poised states for later activation [34-35, 37, 53, 54].

The complexity inherent in histone methylation is further demonstrated by the degree of methylation at identical lysine residues associated with opposite effects on gene activity. It is well-documented that H3K9me3, H3K27me3, and K4K20me3 are associated with transcriptional repression or heterochromatin formation [14, 21, 35-36, 46]. Recent studies revealed that mono-methylation at these residues (H3K9me1, H3K27me1, & K4K20me1) was distributed mostly in euchromatin regions and linked with gene activation [36, 46].

Interpretation of Epigenetic Modifications

The hope that completion of the Human Genome Project did reveal some secrets of the genome, but it did not fundamentally alter our perspective on how the expression of genes are regulated as much as initially hoped [3, 13]. Gene regulation is under the control of multiple influences, ranging from those passed down from each generation, to those responsive to environmental stimuli, and has been recognized as important for genomic function [1, 7, 8]. To understand the biological significance of epigenetic markers, it is necessary to identify the distribution of DNA methylation and histone modifications, where they occur (globally or regionally) among different tissue or cell types, and when they occur (normal development or disease processes).

The basic blueprint of epigenetic modification distributions among the genome and across a variety of mammalian cells indicates that genomic features underlie epigenetics. The recently completed global genome-analyses by the ENCODE Project [13, 25] and other studies [38-41] have characterized histone modifications in more detail. For example, H3K4me3, H3K4me2, and H3ac are heavily enriched around the transcriptional start sites (TSSs) of genes, with slightly lower enrichment of H3K4me1 and H4ac. H3K4me3, H3K4me2, and H3ac enrichment at TSSs are positively related to the extent of gene activity. For expressed genes, the enrichment of H3K4me3 and H3ac occurs just downstream from the TSS, with lower levels of enrichment of H3K4me2, H3K4me1, and H4ac occuring farther downstream. For genes that are not expressed or are expressed at low levels, H3K4me3 and H3ac enrichment is weaker, but still centered on the TSSs, while H4ac enrichment is more broadly distributed [25].

In addition to obtaining similar distribution patterns for H3K4me1, 2, and 3 by utilizing ChIP-Seq technology, Barski et al [36] further identified some unexpected distributions of histone modifications: H3K4me1, 2, 3, and H3K36me3 correlated with gene activation (high level of enrichment around the TSSs); H3K9me1, H3K20me1, and H3K27me1 correlated with gene expression due to elevated levels of these markers localized downstream of TSSs and throughout the entire transcribable region. In contrast, high levels of H3K27me2, 3, H3K79me3, and modest levels of H3K9me2 and 3 were linked to gene repression or silencing. Relative enrichment of H3K9me2 and 3 indicates heterochromatin formation, and H4K20me3 is associated with repressive chromatin. Similiar results were obtained from other human and mouse cell lines [38-40]. Studies of the relationships between epigenetic modifications and transcriptional activation are expected to employ these genetic tools to identify additional functional genomic elements that impact gene expression [13, 14].

Recent studies have provided insights into epigenetic regulation of development. For instance, two typical epigenetic markers indicative of gene activity, H3K4me3 and H3K27me3, are found to co-exist on key developmental genes expressed at low levels in mouse embryonic stem (ES) cells [37] and on some development-associated genes in human induced pluripotent ES cells [42]. The bivalent cluster of modifications could be thought of as a pair of "Yin-Yang" markers regulating the differentiation of ES cells by maintaining a "poised" low level of expression in the ES cells [17]. This finding has important implications for the preservation of pluripotency in ES cells. It may be possible to manipulate differentiation of ES cells by selective regulation of modification pathways. In our upcoming issue of Pathways™, we will feature exciting new developments in the stem cell field.

In global mapping analysis of histone modifications in human T cells, Zhao and colleagues [38, 41] reported that H3K9/14ac and H3K4me3, associated with constitutively expressed or rapidly inducible genes, are required for T cell development and function. Conversely, H3K27me3 is associated with permanently silenced genes that are involved in the development of other cell types. Importantly, they found that both H3K4me3 and H3K27me3 localized at many gene promoter regions in differentiated T cells, suggesting counteracting effects on gene regulation at the bivalent domains. However, genes within bivalent domains were expressed at much lower levels than other genes in the genome [38], thereby confirming the conclusion derived from ES cells [28, 37, 42].

The complexity of epigenetic regulation was underscored *in vivo* by Fraga et al. [43], who first defined that loss of H4K16ac and H4K20me3 is a common hallmark of human cancer cells associated with DNA hypermethylation at repetitive sequences. Kondo, Shen, and colleagues [44], studying the relationship between DNA methylation and histone modification in prostate cancers, found that most of the genes enriched with H3K27me3 had no detectable DNA methylation, and most genes with hypermethylation had no enrichment of H3K27me3. This finding indicates that the role of H3K27me3 on silencing TSGs in cancer cells is tissue- and cell-specific, and is potentially independent of DNA methylation at the promoter region. The H3K27me3-mediated gene silencing in cancer cells might be a promising therapeutic target. These examples highlight the reciprocal role for epigenetic modifications to create vast regulatory epigenetic codes that serve as "readouts" for information encoded in the genome, and to further characterize unique features across the genome [4, 5, 13]. The dynamic status of epigenetic modifications can regulate the epigenetic state of chromatin, favoring an "euchromatic" (on) or "heterochromatic" (off) state [5, 14, 21].

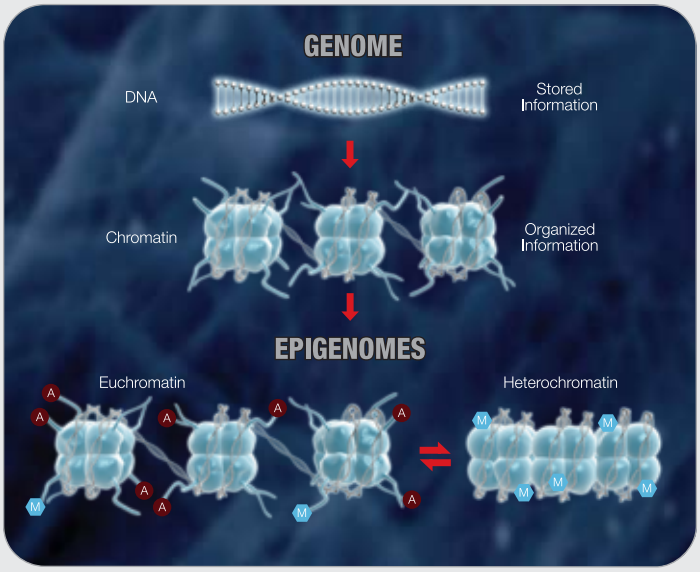

Figure 3: Multiple Levels of Organization Control Access to the Genetic Information Contained Within the DNA, Including Heritable Epigenetic Modifications that Regulate Gene Expression.

Table 1: Epigenetic Modification Signatures Distinguish Chromatic Status Into Two States - Active Euchromatin or Repressed Heterochromatin.

		EUCHROMATIN	HETEROCHROMATIN
CHROMATIN FEATURES	Structure	Less Condensed, Open, Accessible	Condensed, Closed Inaccessible
	DNA Sequence	Gene Rich	Repetitve Elements
	Activity	Expressed, Active	Repressed, Silent
EPIGENETIC MARKERS	DNA Methylation	Hypomethylation	Hypermethylation
	Histone Acetylation	Hyperacetylation of Histone H3, H4	Hypoacetylation of Histone H3, H4
	Histone Methylation	H3K4me2, H3K4me3 H3K9me1	H3K27me2, H3K27me3 H3K9me2, H3K9me3

This balancing effect, in which the dynamic status of DNA methylation and histone modifications are interacting, is illustrated in Figure 3 (Table 1) [34, 44, 53, 55]. Among the modifications with gene silencing effects, H3K9me3 is also critical for heterochromatin formation. It recruits repressors and related co-factors, including HDAC and heterochromatin protein-1 (HP-1), resulting in further deacetylation and DNA methylation, ultimately leading to DNA condensation into heterochromatin [4, 10,]. It is still not completely understood how such different epigenetic states are generated and maintained.

Concluding Remarks

There are several points that characterize epigenetic modifications which should be considered collectively for understanding their biological implications. Although epigenetic modifications are heritable in somatic cells, they are reversible and may serve as, or are linked to, potential targets for drug treatment [8, 26]. This last feature has significant implications for the prevention, diagnosis, and treatment of human diseases, and the effects of aging [18, 45]. In contrast to discrete genetic mutations, epigenetic modifications are progressive processes that can be quantitatively analyzed [34, 36, 37]. The degree of epigenetic modifications, as measured by intensity or distribution, may ultimately influence their roles on gene regulation [13, 25]. Therefore, obtaining quantitative information about epigenetic modifications is important for the implications highlighted above.

Epigenetic modifications, resulting from a dynamic process controlled by multiple mechanisms responsive to environmental stimuli can remain stable and heritable [7-11, 34]. However, the complexity of the interplay among these markers has to be clarified under varying conditions. The distributions of epigenetic modifications are not only associated with specific tissue/cell types, but they are also linked to the sites in which they are localized [13, 25]. For example, DNA hypomethylation exists throughout the genome while localized hypermethylation has been observed on TSGs in cancer cells [10]. In addition, the patterns of H3K4me3 and H3K9me3 define distinctive chromatin domains [37]. This position effect of epigenetic markers regulates gene expression under specific chromatin microenvironments.

Epigenetics refers to the "orchestration" of genomic regulation by dynamic and coordinated modifications. This orchestrated series of events involves unraveling of specific regions of DNA, performing functions (such as transcription), and then restoring DNA back to its original state. Combining epigenetic markers with known regulatory functions to define reference epigenomes was recommended by the Alliance for the Human Epigenome and Disease (AHEAD) project [14]. The major goal of the AHEAD project is to map the epigenetic markers in defined sets of epigenomes so that comparison of disease states to the epigenomic patterns present in normal tissues and differentiated cells may provide insights into factors responsible for regulating disease and aging. Significant progress has been made towards identifying the global distribution of epigenetic modifications. The challenge that remains is to develop the innovative tools or platforms to decode the human epigenome effectively [3, 14, 18, 19].

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