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Workshop 57

# The Histone Code and Beyond

## New Approaches to Cancer Therapy

S. L. Berger
O. Nakanishi
B. Haendler
(Editors)

Springer

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# *Preface*

In recent years, it has been recognized that epigenetic marks play an essential role in the control of gene expression by modulating the access of regulatory factors to chromatin. Evidence is now accumulating that deregulation of these control mechanisms is linked to cancer initiation and progression.

In the chromatin, DNA is packaged around histone proteins and generally in the default state of repression. DNA regions that are to be transcribed are temporarily made accessible by decondensing the structure. For the uncoiling of chromatin, histones can be post-translationally modified at many positions, mainly located in their N-terminal tails. A range of histone modifications including phosphorylation, acetylation, methylation and ubiquitylation, all carried out by different families of specific enzymes, have been identified in recent years.

Another epigenetic event is DNA methylation, which often takes place on cytosine residues in CpG islands found in gene promoter regions. Aberration in DNA methylation is now recognized as a fundamental aspect of human cancer, each tumor type having its own DNA methylation pattern.

Besides histones, post-translational modifications affect and regulate a variety of additional proteins with important functions in signal transduction and gene transcription. Ubiquitylation, sumoylation and neddylation represent three related processes that may dramatically affect the function of targeted proteins, for instance by regulating their stability, subcellular localization or interactions with other proteins.

All these exciting developments in the area of chromatin research prompted us to organize a meeting to discuss the most recent breakthroughs made in this fast-moving field, with a focus on aspects relevant to cancer research. The following topics were selected for the workshop: chromatin dynamics, histone and DNA methylation, histone code and covalent modifications of nonhistone proteins.

We were pleased to be able to bring together internationally highly recognized experts who study this field using a variety of biochemical, genetic and structural approaches. We are grateful to all of them for their willingness to participate and for their excellent contributions.

We hope that the proceedings of this workshop will promote a better understanding of the mechanisms responsible for epigenetic control of gene expression and help to identify novel targets for tumor treatment in the near future.

*Shelley L. Berger*
*Osamu Nakanishi*
*Bernard Haendler*

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# 1 The Role of Histone Modifications in Epigenetic Transitions During Normal and Perturbed Development

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**Abstract.** Epigenetic mechanisms control eukaryotic development beyond DNA-stored information. DNA methylation, histone modifications and variants, nucleosome remodeling and noncoding RNAs all contribute to the dynamic make-up of chromatin under distinct developmental options. In particular, the great diversity of covalent histone tail modifications has been proposed to be ideally suited for imparting epigenetic information. While most of the histone tail modifications represent transient marks at transcriptionally permissive chromatin, some modifications appear more robust at silent chromatin regions, where they index repressive epigenetic states with functions also outside transcriptional

regulation. Under-representation of repressive histone marks could be indicative of epigenetic plasticity in stem, young and tumor cells, while committed and senescent (old) cells often display increased levels of these more stable modifications. Here, we discuss profiles of normal and aberrant histone lysine methylation patterns, as they occur during the transition of an embryonic to a differentiated cell or in controlled self-renewal vs pro-neoplastic or metastatic conditions. Elucidating these histone modification patterns promises to have important implications for novel advances in stem cell research, nuclear reprogramming and cancer, and may offer novel targets for the combat of tumor cells, potentially leading to new diagnostic and therapeutic avenues in human biology and disease.

## 1.1 The Distinction Between Genome and Epigenomes

The DNA double helix is the prime macromolecule to store and propagate genetic information. The key genetic principle is mutation of one or many of the four nucleotides, resulting in a change in the DNA sequence that can ultimately define a new species barrier. The genome of the unicellular eukaryote *Saccharomyces cerevisiae* consists of around 6,000 genes (Goffeau et al. 1996), a number sufficient for controlling basic cellular processes, such as metabolism, cell division, and DNA damage repair. Mammals have five times as many genes (Lander et al. 2001; Waterston et al. 2002), and most of these additional genes can be associated with specialized functions to determine cell type identities and lineage commitment. This results in the phenotypical discrimination of more than 200 cell types. How can these distinct cell types be defined and stably propagated from a common genome?

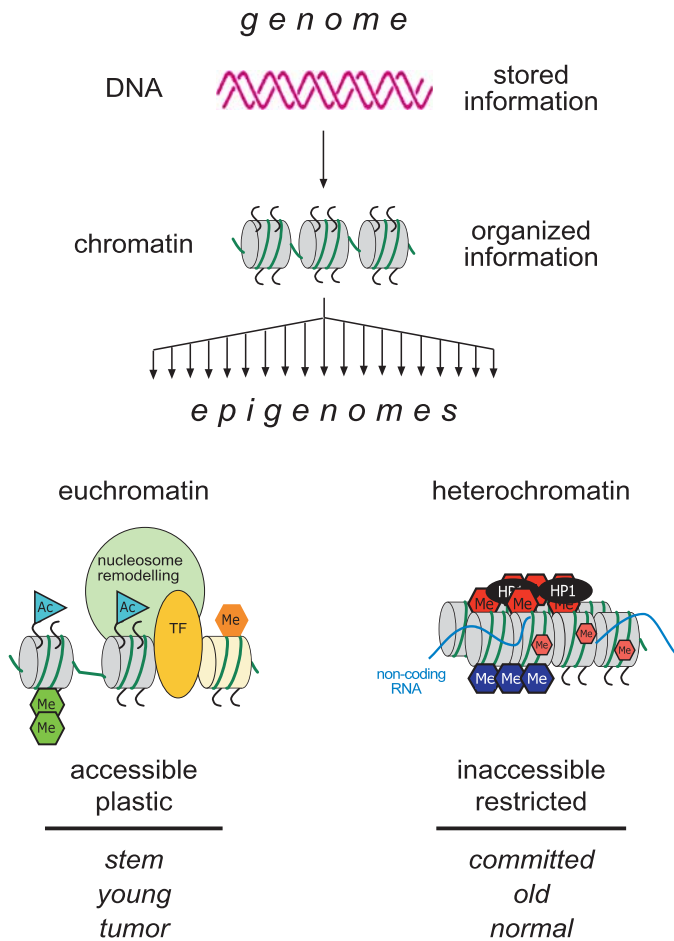
In eukaryotes, the DNA molecule is not naked, but is organized in chromatin – the dynamic template of the genetic information. The basic repeating unit of chromatin is the nucleosome, consisting of 147 bp of DNA wrapped around an octamer of the core histones H2A, H2B, H3, and H4 (Luger et al. 1997). One of the key epigenetic principles for altering chromatin states are covalent modifications, both of the DNA (methylation) and of the flexible histone N-termini (histone “tails”). An epigenome can thus be defined by the sum of biochemical modifications of the chromatin template. Epigenomes will greatly differ between distinct cell types and among resting vs proliferative cells, and it has

been suggested that histone modifications could reflect a histone code that may index diverse developmental or proliferative options (Strahl and Allis 2000; Turner 2000; Jenuwein and Allis 2001). Currently, more than 30 amino acid positions have been described in the N-termini of the four core histones to be subject to distinct post-translational modifications. Although this large number may be counter-intuitive for a histone code and rather reflect biochemical affinities for chromatin-associated factors (Schreiber and Bernstein 2002), several histone modifications, and in particular distinct combinations of selective histone marks, appear to be of predictive value (see Sects. 1.2 and 1.3).

In addition to DNA methylation, histone modifications and variants (Sarma and Reinberg 2005), nucleosome remodeling factors (Narlikar et al. 2002) and noncoding RNAs (Lippman and Martienssen 2004) all cooperate to organize chromatin into accessible (euchromatic) or inaccessible (heterochromatic) subdomains. These epigenetic mechanisms considerably extend the information potential of the genetic code and are important for the differential usage of gene function during cell type specification. Thus, one genome can generate many epigenomes (Fig. 1), as the fertilized egg progresses through development and translates its information into a multitude of cell fates. As a general pattern, activating epigenetic mechanisms appear to prevail in stem, young, and tumor cells, since these cells would allow access to the nearly full gene comple-

**Fig. 1.** Genome vs epigenomes. The figure illustrates the distinction between genome (DNA sequence) and epigenome (the collective modification pattern of chromatin). Diverse mechanisms including DNA methylation, histone modifications and variants, chromatin-remodeling activities, and noncoding RNAs contribute to the generation of distinct epigenomes, as the fertilized egg progresses during development. The various epigenetic modifications are indicated as histone acetylation (*blue flag*), histone lysine methylation (*green hexagons* for active and *red* or *blue hexagons* for repressive marks), histone arginine methylation (*orange hexagon*) DNA methylation (*small brown hexagons*), histone variants (*yellow nucleosome*) and remodeling complexes (e.g., ISWI). Euchromatin allows transcription factors (TF) to access the underlying DNA sequence, while repressive modifications that may be induced by small noncoding RNAs could lead to the further compaction of heterochromatic domains

ment. A transcriptionally permissive state would therefore be indicative of cellular plasticity and pluripotency. By contrast, repressive mechanisms accumulate in committed and old cells and stabilize developmental options by restricting expression of lineage-inappropriate genes. Both activating and repressive epigenetic mechanisms are important for transcriptional control, but also play major roles in overall chromatin



organization to partition eukaryotic chromosomes into centromeric and telomeric domains, to index recombination sites and to respond to DNA damage repair. The implications of epigenetic mechanisms for human biology and disease, including genome stability, cancer, stem cells, and aging are far-reaching.

## 1.2 Activating Histone Modifications

Histone modifications include acetylation, phosphorylation, methylation (of arginines and lysines), ubiquitination, sumoylation and ADP-ribosylation (van Holde 1988). These modifications are predominantly found in the flexible N-termini of the small (102–135 amino acids) histone proteins. One nucleosome contains two copies of the four core histones, and approximately 30 amino acids (the histone tails) of each histone molecule protrude from one nucleosome. If only these amino acids are considered, this would result in the potential of $2 \times 4 \times 30$ positions conferring epigenetic information per nucleosome. That each amino acid is important is suggested by the extremely high evolutionary conservation of the histone sequences. However, histone modifications do not appear to occur in isolation, but rather in a combinatorial manner as proposed for modification cassettes (Fischle et al. 2003) and trans-histone pathways (Briggs et al. 2002). Intriguingly, almost all of the known histone modifications can either have an activating or a repressive function, dependent on which amino acid position(s) in the histone N-termini are modified (see Sect. 1.3). Both synergistic and antagonistic pathways have been described (Berger 2002) that can progressively induce combinations of active marks while simultaneously counteracting repressive modifications. It is, however, not known how many distinct combinations for modifications of the various N-terminal histone positions indeed exist in any given nucleosome, although some of the major sites can be modified at 60%–80% in bulk histone preparations (Peters et al. 2003; Rice et al. 2003). In addition to the N-termini, modifications in the globular histone fold domains have recently been shown to also impact chromatin structure and assembly, thereby influencing gene expression and DNA damage repair (van Leeuwen et al. 2002; Ng et al. 2002; Freitas et al. 2003; Xu et al. 2005, Ye et al. 2005).

Activating histone tail modifications have been shown to comprise general H3 and H4 acetylation, H3S10 phosphorylation, arginine methylation at H3R17 and lysine methylation at H3K4 and H3K36 positions (Lachner et al. 2003), although there are many others. Histone acetyltransferases (HATs), kinases, protein arginine methyltransferases (PRMTs), and histone methyltransferases (HMTases) are the enzymes that confer these modifications (Fig. 2). The various activating histone modifications are induced by these enzymatic activities, which can either be associated in multi-protein complexes or are recruited to the chro-

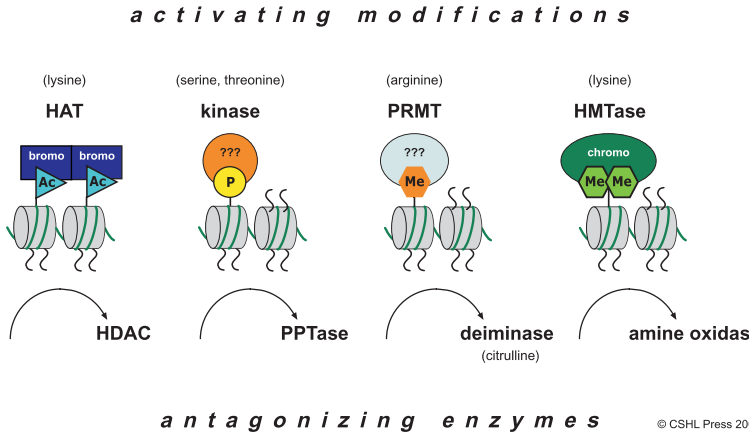



**Fig. 2.** Activating histone modifications and associated enzymes. Activating histone modifications comprise lysine acetylation (*blue flag*), serine and threonine phosphorylation (*yellow circle*), arginine methylation (*orange hexagon*), and lysine methylation at positions H3K4, H3K36, and H3K79 (*green hexagons*). Lysine acetylation is recognized by bromo-domain factors, while lysine methylation can recruit chromo-domain proteins. Specific binders for histone phosphorylation and arginine methylation are currently not known. Activating modifications are induced by histone acetyltransferases (*HATs*), kinases, protein arginine methyltransferases (*PRMTs*), and histone lysine methyltransferases (*HMTases*). Most of these marks are transient and can be actively removed by histone deacetylases (*HDACs*), protein phosphatases (*PPTases*), deiminases and amine oxidases

matin template in a sequential order by transcription factors, nucleosome remodelers or the RNA-PolII machinery (Sarma and Reinberg 2005). Histone modifications can result in *cis*-effects, which alter electrostatic interactions of the DNA polymer with nucleosomes. For example, both acetylation and phosphorylation will reduce the net positive charge of the basic histone molecules, thereby increasing overall accessibility of nucleosomal DNA. Alternatively, histone modifications can generate affinities for chromatin-associated factors, which then mediate transitions of the chromatin template by *trans*-mechanisms. This has been shown for histone acetylation in providing a recruiting signal for positively acting bromo domain factors (Dhalluin et al. 1999; Jacobson et al. 2000) and for histone lysine methylation, which represents binding sites for chromo domain (Daniel et al. 2005) or WDR5 proteins (Wysocka et al. 2005). Currently, no protein modules that selectively recognize histone arginine methylation or H3S10 phosphorylation are known.

Most activating modifications are transient and can be actively removed, for example by histone deacetylases (HDACs), protein phosphatases (PPTases) and peptidyl arginine deiminases (PADs). PADs convert unmodified or monomethylated arginine to citrulline, thereby inducing an alteration in amino acid composition (Wang et al. 2004; Cuthbert et al. 2004). The function and stability of citrulline in histones or nucleosomes is currently not clear. In contrast to arginine methylation, lysine methylation is considered a chemically very stable modification, as the amino-methyl bond cannot be cleaved directly. However, recently a lysine specific "demethylase" (LSD1) was described as an amine oxidase that is able to remove H3K4 methylation (Shi et al. 2004). The enzyme acts by oxidative destabilization of the amino-methyl bond, resulting in unmodified lysine and formaldehyde. LSD1 was shown to be selective for the activating H3K4 methylation mark and can only destabilize mono- and di-, but not trimethylation. This demethylase is part of a large repressive protein complex that also contains HDACs and other enzymes (Shi et al. 2004). Similar to the collaboration of activating enzymes (e.g., HATs and PRMTs) in inducing combinations of positive histone modifications, there is synergy between antagonizing enzymes (e.g., HDACs and PADs) in removing or counteracting these activating marks.

## 1.3 Repressive Histone Modifications

In addition to DNA methylation (Jaenisch and Bird 2003), many of the known histone modifications can also exert a repressive function on the chromatin template. In particular, histone lysine methylation has emerged as a central epigenetic modification that can index silenced chromatin regions. There are five well-characterized and prominently methylated lysine positions in the histone H3 and H4 N-termini, although there are additional methylation sites also in the globular domains (van Leeuwen et al. 2002; Ng et al. 2002) and in the linker histone H1 (Kuzmichev et al. 2004). Whereas H3K4 and H3K36 methylation are activating marks (see Sect. 1.2), histone H3 lysine 9 (H3K9), lysine 27 (H3K27) and histone H4 lysine 20 (H4K20) methylation represent OFF marks that are mainly involved in the organization of repressive chromatin structures (Peters et al. 2003; Schotta et al. 2004). All of the five major histone lysine methylation sites can exist in three distinct states: mono-, di- and trimethylation (Fig. 3).

The existence of three lysine methylation states provides an additional layer of regulatory control, since mono-, di- and trimethylation may serve diverse biological functions. For example, there are more than 50 SET-domain HMTases in mammals (Schneider et al. 2002; Schotta et al. 2004), which differ in their potential to induce mono- vs di- vs trimethylation. Dependent on a distributive or processive activity (Zhang et al. 2003), some of these enzymes can only establish monomethylation (e.g., PR-SET7) (Nishioka et al. 2002; Xiao et al. 2005), while others convert an unmodified substrate to several methylation states (e.g., EZH2) (Kuzmichev et al. 2004) or are specialized to induce the fully methylated end state of trimethylation (e.g., Suv39h) (Peters et al. 2003). Methylation at distinct histone lysine positions generates selective binding sites for chromatin-associated factors containing a chromo domain, as was first demonstrated for HP1 to interact with H3K9me3 and for Polycomb to display affinity toward H3K27me3 (Daniel et al. 2005; Ringrose and Paro 2004).

Recently, the chromo-domain superfamily of “royal proteins” (Maurer-Stroh et al. 2003) has been shown to comprise additional methyllysine binders, such as tudor domain (Sanders et al. 2004) or malignant brain tumor (MBT) (W. Fischle and C.D. Allis, personal communica-