Cell. Mol. Life Sci. 66 (2009) 1419 – 1433
1420-682X/09/081419-15
DOI 10.1007/s00018-008-8605-1

Cellular and Molecular Life Sciences

Review

Histone methylation and ubiquitination with their cross-talk and roles in gene expression and stability

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Received 24 September 2008; received after revision 21 November 2008; accepted 28 November 2008
Online First 30 December 2008

Abstract: Methylation of lysine residues of histones is associated with functionally distinct regions of chromatin, and, therefore, is an important epigenetic mark. Over the past few years, several enzymes that catalyze this covalent modification on different lysine residues of histones have been discovered. Intriguingly, histone lysine methylation has also been shown to be cross-regulated by histone ubiquitination or the enzymes that catalyze this modification. These covalent modifications and their cross-talks play important roles in regulation of gene expression, heterochromatin formation, genome stability, and cancer. Thus, there has been a very rapid progress within past several years towards elucidating the molecular basis of histone lysine methylation and ubiquitination, and their aberrations in human diseases. Here, we discuss these covalent modifications with their cross-regulation and roles in controlling gene expression and stability.

Keywords. Cross-talk, histone, lysine, methylation, ubiquitination, gene expression, DNA repair.

Introduction

In eukaryotes, DNA is packaged into chromatin which is an array of nucleosomes. Each nucleosome consists of a histone octamer that is wrapped by 146 base pairs of DNA [1, 2]. Two molecules of each histone, H2A, H2B, H3 and H4 form a histone octamer [2]. Nucleosomal arrays fold into a 30 nm solenoid fiber with the help of linker histone H1 [3, 4], and then these fibers fold further to form a higher-order chromatin structure. The DNA transacting processes such as DNA replication, transcription, repair and recombination occur on the chromatinized DNA template, and thus, chromatin undergoes structural reorganization as well as dynamic changes during these important cellular events [5 – 10]. Conversely, folding and unfolding of higher-order chromatin structure also influence genome functions.

Nucleosomal core histones are evolutionarily conserved. These core histones have flexible amino-terminal tails that protrude outward from the nucleosome and globular carboxy-terminal domains which make up the nucleosome scaffold. Histones function as acceptors for a variety of post-translational modifications such as acetylation, phosphorylation, ubiquitination, methylation, sumoylation, and ADP ribosylation [8, 10 – 17]. Most modifications were originally observed on the amino-terminal tails of histones, with the exception of ubiquitination which occurs on the carboxy-terminal tails of histones H2A and H2B [14, 18]. However, several novel post-translational modifications have also been identified recently in the core region of histones H3 [15, 16, 19, 20]. Histone

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modifications modulate DNA–histone interactions within and between nucleosomes, thus altering higher-order chromatin structure. Therefore, combinations of post-translational modifications on single histone, nucleosome or nucleosomal domain establish specific local or global patterns of so-called "histone codes" for distinct downstream functions. However, these histone codes can be further altered by multiple extracellular and intracellular stimuli. Thus, chromatin serves as a signaling platform, and hence, functions as a genomic integrator of various signaling events or pathways [21–24].

Covalent modifications of histones are strongly correlated with gene expression [9, 15, 16]. For example, phosphorylation of serine on histone H3 and acetylation of lysines on histones H3 and H4 track with gene activation. Similarly, methylation of lysines on histones H3 and H4 either activates or represses gene transcription depending upon the position of the modified residues [15, 16]. Many of these covalent modifications are '*cis*-linked' sequential events on the same histone tail [15, 16, 25], thus generating different histone codes. However, a large number of recent studies have also demonstrated '*trans*-tail' regulation of histone H3 lysines 4 and 79 methylation by histone H2B ubiquitination [25–28]. Such trans-tail cross-talk is especially interesting since the tails of histones H2B and H3 are quite far from one another in the crystal structure [2]. Furthermore, histone H3 lysine 4/79 methylation and histone H2B ubiquitination, and their regulatory cross-talks play important roles in gene regulation and carcinogenesis [15, 18, 25, 29]. Thus, the cross-talk between histone H2B ubiquitination and histone H3 lysine 4/79 methylation with their functions in regulation of gene expression and genome stability is the focus of this review article. The enzymes that catalyze these modifications are also discussed here.

Histone H3 lysines 4 and 79 methylation/demethylation

Lysine methylation occurs in mono-, di-, or trimethylated forms. In higher eukaryotes, lysines 4, 9, 27, 36 and 79 of histone H3 and lysine 20 of histone H4 are methylated [15, 16]. Methylation of lysines 9 and 27 of histone H3 and lysine 20 of histone H4 are not observed in *Saccharomyces cerevisiae*. Distinct methylated-lysine residues of histones represent either a specific activated or repressed state [14, 15, 30–32]. Further, several studies have implicated histone lysine methylation in regulation of DNA repair and genome integrity. These distinct functions of methylated-lysines are mediated by differential "read-out" of the effector proteins [14, 15, 30–32]. We discuss below histone H3 lysines 4 and 79 methylation with their regulatory enzymes.

Histone H3 lysine 4 methylase

Methylation of lysine 4 on histone H3 is carried out by histone methylase that contains a 140 amino acid-long catalytic domain known as SET [Su(var)3–9, Enhancer-of-zeste, Trithorax]. The enzymatic activity of the SET domain-containing protein depends on its ability to assemble into a multiprotein complex. The first histone H3 lysine 4 methylase complex, COMPASS (Complex Proteins Associated with Set1) was identified in *Saccharomyces cerevisiae*. COMPASS consists of Set1 and seven other proteins (Cps60, Cps50, Cps40, Cps35, Cps30, Cps25, and Cps15) [33], and is essential for mono-, di-, and trimethylation of lysine 4 on histone H3 [14, 33–38]. However, COMPASS exists in various forms that differ in subunit compositions, and are associated with different methylase (mono, di and trimethylation) activities. For example, purified COMPASS lacking Cps60 can mono- and dimethylate, but is not capable of trimethylating lysine 4 of histone H3 [14, 32, 37]. The Cps25 component of COMPASS is essential for di- and trimethylation of lysine 4 on histone H3 [14, 32, 37]. Cps30 drastically affects COMPASS-mediated mono-, di, and trimethylation of histone H3 lysine 4 [14, 32, 37]. Following the discovery of COMPASS, the Set1's human homologues such as MLL1–4 (Mixed-lineage leukemia 1–4) and hSet1A and B (Table 1) were found in COMPASS-like complexes that are capable of methylating lysine 4 of histone H3 [14, 39, 40]. Further, other methylases such as SET7/9 and SMYD3 have been implicated to methylate lysine 4 of histone H3 in human (Table 1) [41, 42]. Similarly, several histone H3 lysine 4 methylases have been identified in mouse [32, 41, 42]. Although there are several histone H3 lysine 4 methylases in mammals, their functions are not redundant. For example, the deletion of either MLL1 or MLL2 causes embryonic lethality [43, 44]. So, why do mammals need different methylases for histone H3 lysine 4 methylation? Several recent studies have shown that some of the histone H3 lysine 4-specific methylases in mammals are only proficient in catalyzing either mono- or mono- and dimethylation, whereas other methylases are capable of catalyzing up to trimethylation [41, 42, 45, 46]. Such distinct functions of different histone H3 lysine 4 methylases are due to the presence of specific residues in the catalytic domain. Thus, these results provide some clues as to why mammals need multiple histone H3 lysine 4-specific methylases. However, further investigations will shed light on as to why

multiple methylases are essential for methylation of lysine 4 on histone H3 in mammals.

Table 1. The enzymes involved in the regulation of histone H3 lysine 4/79 methylation and histone H2B ubiquitination in budding yeast (*S. cerevisiae*) and human [15, 29, 32, 41, 42, 47, 48, 149, 150, 152–154, 161, 162, 179].

Modification site	Enzyme	Budding yeast	Human
H3-K4	Methylase	Set1 ($K4me_{1/2/3}$)	MLL1 ($K4me_{1/2}$) MLL2($K4me_{1/2/3}$) MLL3($K4me_{1/2/3}$) MLL4($K4me_{1/2/3}$) Set1A($K4me_{1/2/3}$) Set1B($K4me_{1/2/3}$) SMYD3($K4me_{2/3}$) SET7/9 ($K4me_{1/2}$)
	Demethylase	Jhd2 ($K4me_3$)	LSD1 ($K4me_{2/1}$) SMCX ($K4me_{3/2}$) SMCY ($K4me_{3/2}$) RBP2 ($K4me_{3/2}$) PLU-1 ($K4me_{3/2}$) JHDM1B ($K4me_3$)
H3-K79	Methylase	Dot1	DOT1L
	Demethylase	?	?
H2B-K120 (h) H2B-K123 (y)	E2 conjugase	Rad6	HR6A, H6RB
	E3 ligase	Bre1	RNF20, RNF40
	Ub-protease	Ubp8 Ubp10	USP22 ?

The methylated-lysine 4 substrates for demethylases are mentioned in the parenthesis. The methylated forms of lysine 4 on histone H3 generated by methylases are mentioned in the parenthesis. Abbreviations: K, lysine; Ub, ubiquitin; me_1, monomethyl; me_2, dimethyl; me_3, trimethyl; $me_{1/2}$, both mono- and dimethyl; $me_{1/2/3}$, mono-, di- and trimethyl; h, human; and y, budding yeast.

Histone H3 lysine 79 methylase

Unlike methylation of lysine 4, lysine 79 methylation on histone H3 is catalyzed by a non-Set protein, namely, Dot1 (Disruptor of telomeric silencing) [47, 48]. Dot1 has been originally identified via its role in genome silencing. It is highly conserved from yeast to human (Table 1). Dot1p methylates only nucleosomal substrates, but not free histone H3. Dot1 has a catalytic domain with a unique organization that resembles arginine methyltransferase. The location of lysine 79 in the globular domain of histone H3 may be accounted for the structural distinction of Dot1 from other lysine-specific methylases [48, 49].

Histone H3 lysine 4/79 demethylase

Histone H3 lysine 4 methylation is dynamic in nature, whereas methylated-lysine 79 on histone H3 is not actively reversed [15, 31, 50–54]. Lysine 4 methylation on histone H3 is reverted by lysine demethylase, LSD1, a FAD (flavin adenine dinucleotide)-dependent amine oxidase which produces an unmethylated-lysine 4 (Table 1) [31, 52, 54–67]. LSD1 is conserved from yeast (*Schizosaccharomyces pombe*, but not *Saccharomyces cerevisiae*) to human [31, 67, 68], and can specifically demethylate monomethyl as well as dimethyl lysine 4 of histone H3 (Table 1). LSD1 associates with several transcriptional repressor complexes [31, 64, 69, 70], and thus, has been shown to impair transcription of target genes [31, 63, 70–73]. For example, LSD1 interacts with the Co-REST complex to repress transcription of neuronal genes via histone H3 lysine 4 demethylation. Intriguingly, LSD1 also associates with transcriptional activation complex that contains histone H3 lysine 4-specific methylase, MLL1 [31, 74]. The presence of both methylase and demethylase within the same complex indicates the requirement of a very fine balance between methylated- as well as unmethylated-lysine 4 on histone H3 for transcriptional regulation of target genes.

Although LSD1 demethylates mono- and dimethylated-lysine 4 on histone H3, it is unable to remove methyl group from trimethylated-lysine 4 [31]. Demethylation of trimethylated-lysine 4 on histone H3 is carried out by a different class of histone demethylases that contain the JmjC (Jumonji-C) domain (Table 1) [31, 42]. In mammals, there are four members of JARID (Jumonji, AT rich interactive domain) family with a JmjC domain (e.g., JARID1A or RBP2, JARID1B or PLU-1, JARID1C or SMCX, and JARID1D or SMCY). These proteins are required for demethylation of trimethylated-lysine 4 on histone H3 in mammals [31]. "Lid" is the only known homologue of the JARID family in *Drosophila* [31, 66, 75]. Similarly, JMJ2 and YJR119C (or Jhd2) are the JARID homologues in fission and budding yeasts, respectively, for demethylation of trimethylated-lysine 4 on histone H3 [31, 53. 67, 76, 77].

Histone H3 lysine 4/79 methylation in transcriptional regulation, DNA repair and cancer

Both lysines 4 and 79 methylation on histone H3 play crucial roles in regulation of gene activation and silencing, and DNA repair as discussed below.

Histone H3 lysine 4 methylation

Methylation of lysine 4 on histone H3 is correlated with cellular functions and diseased states [15, 48, 52, 67, 76, 78–84]. Histone H3 lysine 4 methylation has been implicated in maintaining silencing at telomere, mating loci, and rDNA arrays. In contrast to its role in silencing, it is strongly associated with certain transcriptionally active regions of *Schizosaccharomyces pombe* and mammalian genomes [32, 85–89]. Trimethylation of lysine 4 on histone H3 occurs concomitantly with active transcription. Similarly, dimethyl

lysine 4 on histone H3 is predominantly found on active gene loci. However, dimethyl lysine 4 is also present on poised and inactive genes [90, 91]. Interestingly, several recent studies have demonstrated that lysine 4 methylation on histone H3 does not directly affect transcription, indicating the requirement for downstream effectors [30, 32, 41, 92–96]. Consistent with these studies, several transcription factors have been shown to interact with tri- and/or dimethyl lysine 4 on histone H3 (Table 2). For example, CHD1 (a factor that remodels nucleosomes in an ATP-hydrolysis dependent manner), bromodomain PHD (plant homeodomain) transcription factor BPTF (a component of NURF (nucleosome remodeling factor), which mobilizes nucleosomes in an ATP-dependent fashion) and TAF3 (a component of the general transcription factor IID that is involved in gene activation) interact with tri- and/or dimethylated-lysine 4 of histone H3 (Table 2). However, a demethylase, JMJD2A (JHDM3A), which is involved in gene silencing as well as activation in a context-dependent manner, has also been found to interact with trimethyl lysine 4 on histone H3. Similarly, ING2 (inhibitor of growth 2), that is associated with histone deacetylase involved in gene silencing, has been shown to interact with trimethyl lysine 4 on histone H3. Collectively, these results demonstrate that the "readers" of di- and trimethyl lysine 4 fall into two categories: one that non-covalently modulates chromatin structure while the other, that covalently modifies chromatin structure. It may be possible that the two different readers function in a cascade on di- and trimethyl lysine 4 within a nucleosome. However, it is not known whether all nucleosomes within the transcriptionally active gene loci recruit both categories of readers or effectors. Furthermore, what provides the selectivity for recognition of methylated-lysine 4 on histone H3 by these effectors remains to be elucidated.

As mentioned above, methylated-lysine 4 on histone H3 interacts with CHD1. This interaction has recently been shown to be required for recruitment of the components of the spliceosome [97], thus implicating histone H3 lysine 4 methylation in regulation of pre-mRNA splicing (Table 2). In fact, Sims et al. [97] have demonstrated that the depletion of CHD1 from nuclear extracts significantly impairs splicing efficiency *in vitro*. Further, RNAi-based knockdown of CHD1 dramatically reduces pre-mRNA splicing on active genes *in vivo* [97]. In addition, significant loss of histone H3 lysine 4 methylation using RNAi-based Ash2 knockdown leads to the defects in pre-mRNA splicing, resulting in a reduced association of the U2 snRNP components with chromatin at active genes. These results indicate that histone H3 lysine 4 methylation facilitates pre-mRNA splicing through recruitment of CHD1 and spliceosome components to the actively transcribing genes.

In addition to its role in transcription and splicing, histone H3 lysine 4 methylation participates in DNA repair through recruitment of ING1 protein that is a member of a family of tumor suppressors (Table 2) [41, 98–106]. ING1 has been implicated in DNA repair, cellular senescence, apoptosis, and oncogenesis [98–106]. The expression of ING1 is significantly reduced in cancer cells [98, 105, 107]. Further, mutations in ING1 with altered expression levels are found in multiple human cancers. Both DNA repair and apoptotic functions of ING1 depends on the interaction of its C-terminal PHD finger with trimethylated-lysine 4 on histone H3. Further, the ING1 proteins that bear mutations on several amino acids (e.g., N216S, V218L, and G221V) are unable to interact with methylated-lysine 4 on histone H3, and hence, do not stimulate DNA repair and cell death [105, 106, 108–111]. Notably, these mutant versions of the ING1 protein are found in human cancer cells. Thus, histone H3 lysine 4 methylation plays a crucial role in recruitment of ING1 for protecting cells against genotoxic stress.

As discussed above, lysine 4 methylation on histone H3 is an important regulator of transcription, splicing and DNA repair (Table 2). Thus, an altered activity of histone H3 lysine 4-specific methylase or demethylase can cause cell proliferation, abnormal growth and cancer (Table 3). For example, the translocation of histone H3 lysine 4-specific methylase, MLL1 (myeloid/lymphoid or mixed lineage leukemia-1), impairs expression of homeotic (*Hox*) genes. Such impaired *Hox* expression leads to the progression of leukemia [14, 15, 46, 112]. Approximately 80 % of infant acute leukemia and therapy-related leukemia are caused by chromosomal rearrangement involving MLL1 [14, 46, 113]. Similarly, MLL3 has also been found to be translocated into chromosome 7q36 in leukemia and developmental defects [114, 115]. Another histone H3 lysine 4-specific methylase, SMYD3, is over-expressed in the majority of colorectal and hepatocellular carcinomas, and enhances cell proliferation [116]. Furthermore, downregulation of a histone H3 lysine 4-specific SET7/9 methylase in monocytes attenuates expression of the key NF-kB (nuclear factor kappa-B)-dependent genes induced by inflammatory stimuli [117], thus revealing a new biological role of SET7/9 or histone H3 lysine 4 methylation in monocytes in response to inflammation. Therefore, overexpression, downregulation, translocation or rearrangement of genes expressing methylases for histone H3 lysine 4 methylation lead to aberrant gene expression, hence causing cellular transforma-

Table 2. The proteins that interact with methylated-lysine 4/79 on histone H3 or ubiquitinated-H2B, and their functions in gene expression and stability [14, 15, 18, 19, 29, 30, 32, 41, 42, 96, 104, 111, 118–122, 126–128, 161, 162, 164, 170, 176, 178–181].

Modifications	Interacting proteins	Functions
H3-K4 methylation	BPTF (me_3)	Chromatin remodeling
	CHD1 ($me_{2/3}$)	Chromatin remodeling, and splicing
	JMJD2A (me_3)	Demethylation, and gene activation and silencing
	ING2 (me_3)	Gene silencing
	ING1 (me_3)	DNA repair
	TAF3 (me_3)	Gene activation
H3-K79 methylation	h53BP1/yRad9	Checkpoint activation and DNA repair
	Inhibit Sir binding	Regulate telomeric silencing and transcriptional activation, and demarcation of euchromatin
H2B-ubiquitination	hWdr82/yCps35 (?)	Transcriptional stimulation
	FACT (?)	Transcriptional elongation, and nucleosome reassembly
	19S proteasome (?)	H3 K4/79 methylation
	Inhibit Sir binding	Regulate telomeric silencing

The question marks in the parenthesis represent the possibility. The methylated forms of lysine 4 on histone H3 that interact with other proteins are mentioned in the parenthesis. Abbreviations: K, lysine; me_3, trimethyl; $me_{2/3}$, di- and trimethyl; h, human; and y, budding yeast.

Table 3. The connections of the enzymes associated with histone H3 lysine 4/79 methylation and histone H2B ubiquitination to diseases [14, 15, 31, 46, 52, 54, 67, 78–84, 112–117, 125, 161, 162, 182, 183].

Enzymes	Diseases
LSD1	Overexpressed in prostate cancer.
	High levels correlated with cancer and tumor relapse during therapy.
PLU-1	Overexpressed in breast, prostate and testis cancers.
SMCX	Multiple missense mutations in X-linked mental retardation.
	Regulates dendrite growth.
RBP2	Downregulated in sporadic human melanoma skin cancer.
MLL1	Translocated in leukemia.
	Partial duplication in acute myoblastic leukemia and in gastric cancer.
	Deletions in exon 8 in acute lymphoblastic leukemia.
MLL2	Amplified in solid tumor cell lines.
MLL3	Chromosomal aberrations in hematological neoplasia, holoprosensephaly, and leukemia.
MLL4	Integration of hepatitis B virus DNA into MLL4 gene in human hepatocellular carcinoma.
SMYD3	Overexpressed in colorectal, liver, breast and cervical cancers.
SET7/9	Defective inflammatory response in the absence of SET7/9.
DOT1L	DOT1L fuses with MLL in leukemia.
	Growth and developmental defect in the absence of DOT1L.
	Defect in double-stranded DNA break repair in the absence of DOT1L.
HR6A	Developmental defect in the absence of HR6A.
HR6B	Damaged synaptonemal complex structure and increased crossing-over frequency during the male meiotic prophase in the absence of HR6B.
RNF20	Mutated in colorectal cancer.
USP22	Cancer stem cell marker.

tion and various types of cancers (Table 3). Like methylases, histone H3 lysine 4-specific demethylases have also been shown to be misregulated in various cancers and human diseases (Table 3). Thus, histone H3 lysine 4-specific demethylases and methylases are potential therapeutic targets for treatment of cancer.

Histone H3 lysine 79 methylation

Methylation of lysine 79 occurs within the globular domain of histone H3, that is conserved in eukaryotic organisms. Several studies [14, 15, 19, 118, 119] have demonstrated that lysine 79 methylation of histone H3 regulates association of the Sir (silent information regulator) proteins with telomeres to keep heterochromatin genes silent (Table 2). The elimination of lysine 79 methylation on histone H3 abolishes the telomere position effect by spreading Sir proteins from a silenced region to a non-silenced region [15, 19, 118–120].

In addition to its role in silencing, histone H3 lysine 79 methylation plays an important role in DNA repair through the recruitment of checkpoint protein, 53BP1 (Rad9 in *Saccharomyces cerevisiae*) (Table 2) [121–123]. The histone H3 lysine 79 methylation level is not altered in response to DNA double-stranded break. However, DNA double-stranded break rapidly alters the higher-order chromatin structure. Such structural alteration exposes methylated-lysine 79 on histone H3 for interaction with 53BP1 [121–123]. The Tudor domain of 53BP1 binds to methylated-lysine 79 on histone H3, and, hence, regulates DNA repair [124]. Thus, histone H3 lysine 79 methylation plays a pivotal

role to activate checkpoint pathway in response to DNA damage.

Several studies have also implicated histone H3 lysine 79 methylation or methylase in leukemogenesis (Table 3). For example, the MLL-AF10 fusion protein is involved in acute myeloid leukemia. Histone H3 lysine 79 methylase, hDOT1L, interacts with the MLL-AF10 fusion protein, and results in leukemic transformation [125]. Further, hDOT1L has also been shown to cause leukemia through its interaction with MLL in a methylase activity-dependent manner [125]. In fact, upregulation of a number of leukaemia-associated genes (e.g., *Hoxa9*) with hypermethylation of lysine 79 on histone H3 was observed in MLL-hDOT1L and MLL-AF10 transformed cells. Furthermore, several recent studies have demonstrated the role of histone H3 lysine 79 methylation in stimulation of transcription in mammals (Table 2) [126–128]. Together, these studies have thus implicated the crucial role of histone H3 lysine 79 methylation or methylase in transcriptional regulation, DNA repair, and carcinogenesis.

Lysine 4/79 methylation of histone H3 variants

All nucleosomal core histones, with the exception of H4, have variant counterparts which often differ only by a few amino acids [129, 130]. These variants can be tissue-specific, replication-dependent or replication and cell cycle phase-independent, and are incorporated into DNA by different chromatin assembly pathways. It was initially thought that histone variants function in a redundant manner with the parental histones. However, several recent studies have revealed that histone variants have specific features that distinguish them from parental histones, and have profound impact on cellular functions [129, 130].

Among these, histone H3 variants are most studied. The number of histone H3 variants differs from species to species [129, 130]. For example, mammals have four histone H3 variants, namely H3.1, H3.2, H3.3, and centromeric protein A (CENP-A). In addition, another histone H3 variant, namely H3t, is found in the testis. On the other hand, H3.3 and Cse4 (cetromere-specific) are the two histone H3 variants in *S. cerevisiae*. The histone H3.1, H3.2, H3.3 and H3t variants show remarkable similarity in their amino acid sequences. In human, H3.1 and H3.2 are 99% identical. H3.3 shows 96% identity with H3.1. Similarly, H3t differs from H3.1 by just four amino acids. However, CENP-A and H3.1 share only 46% identity. Similar to histone H3, its variants are post-translationally modified [129, 130]. However, covalently modified histone H3 variants have differential effects on transcription. Histone H3.3 variant is enriched in methylation of lysine 4/79 and acetylation of lysines 9, 18 and 23, and these modifications are generally associated with transcriptionally active states. Histone H3.2 variant is covalently modified by lysine 27 methylation which is associated with transcriptional repression. Histone H3.1 carries covalent modification marks involved in gene activation as well as repression. Thus, these histone H3 variants carry distinct marks including lysine 4/79 methylation for differential regulation of gene expression, even though their amino acid sequences are quite identical. However, the molecular basis for such differential covalent modifications of histone H3 variants remains to be elucidated.

Histone H2B ubiquitination/deubiquitination

Ubiquitin (76 amino acids) is attached to cellular proteins through formation of an isopeptide bond between the C-terminus of ubiquitin and a lysine side chain of the target protein by sequential actions of E1 activating, E2 conjugating, and E3 ligase enzymes [131–133]. This ubiquitin molecule is further conjugated to other ubiquitin molecules through its lysine residues to generate structurally distinct polyubiquitin chains [131–133]. The ubiquitin molecule has seven lysine residues (lysines 6, 11 27, 29, 33, 48, and 63), and thus, seven isopeptide linkages can be formed with these residues. The nature of the ubiquitin linkage determines the specific fate of a protein. The 26S proteasome complex degrades the proteins marked by a chain of more than four ubiquitin molecules. Lysine 48-linked polyubiquitin chain constitutes the "kiss of death" for a protein by the 26S proteasome complex [133–135]. Although most of the proteins are targeted for proteasomal degradation by the lysine 48-linked polyubiquitin chain, it has been recently demonstrated that polyubiquitin chain linked through lysine 63 may also serve as a signal for the proteasome-dependent degradation of some proteins [136]. The existence of forked-ubiquitin chains containing all possible isopeptide linkages has also been demonstrated [136]. These chains are resistant to proteolytic degradation by the proteasome, and play important roles in the signaling and other regulatory pathways [136–140].

Ubiquitination occurs on histones H1, H2A, H2B and H3 [14, 18, 29]. This modification on different histones plays distinct roles in regulation of chromatin structures, and hence gene expression and genome stability [15, 16, 29]. Histone H2B ubiquitination is observed throughout eukaryotic organisms from budding yeast to human. In budding yeast, lysine 123 on histone H2B is ubiquitinated, while ubiquitination occurs at lysines 120, 119, 143 on histone H2B in human, *Schizosac-*

charomyces pombe and *Arabidopsis*, respectively [18, 29, 141–144]. Only single ubiquitin is conjugated to histone H2B. Thus, ubiquitinated-histone H2B is resistant to proteasomal degradation, and plays an important role in maintaining chromatin structure and signaling gene regulation. Furthermore, monoubiquitination of histone H2B is reversible. The ubiquitin moiety from ubiquitinated-H2B is removed by ubiquitin specific-proteases. While previous studies have demonstrated monoubiquitination of histone H2B, a very recent study from the laboratory of William Tansey has implicated polyubiquitination of lysine 123 on histone H2B in *Saccharomyces cerevisiae* [145]. Such polyubiquitination is not associated with the destruction of histone H2B by the 26S proteasome [145]. Further, this study demonstrates the presence of polyubiquitination on other lysine residues of histone H2B in *Saccharomyces cerevisiae* [145]. Intriguingly, these ubiquitination events are under the influence of different ubiquitin-specific proteases, and, therefore, seem to have distinct biological functions [145]. Thus, it appears that some of the biological functions of histone H2B are exerted by ubiquitin chains, rather than a single ubiquitin moiety.

Histone H2B ubiquitination occurs via the sequential catalytic actions of E1-activating, E2-conjugating and E3-ligase enzymes. The E1 activating enzyme activates ubiquitin in an ATP-dependent manner. The activated-ubiquitin is then conjugated to a cysteine residue of E2-conjugating enzyme via a thioester bond. Finally, ubiquitin moiety is transferred to a lysine reside within the substrate protein by E3-ligase enzyme. Generally, E3-ligase enzymes belong to the family of either HECT (Homologous to E6AP C-Terminus) or RING (Really Interesting New Gene) proteins. The HECT E3-ligase enzyme bears a conserved HECT domain with an essential cystein that forms a thiol ester with ubiquitin, and then ubiquitin is transferred to the lysine residue of substrate [131, 146]. In contrast, RING E3-ligase enzyme functions as a scaffold to recruit ubiquitin-charged E2 conjugating enzyme and substrate for stimulation of direct attack of ubiquitin from ubiquitinated-E2 to the lysine residue of substrate [131, 146]. Thus, the E2 and E3 enzymes play crucial roles in selection of the substrate proteins. Therefore, an E1-activating enzyme is involved for ubiquitination of histones and all other target proteins, while different E2-conjugating and E3-ligase enzymes are required for ubiquitination of different proteins [131, 146]. However, the E3-ligase enzymes are more specific to the substrate proteins [131, 146].

Rad6 was first identified in *Saccharomyces cerevisiae* as an E2 ubiquitin-conjugase for histone H2B monoubiquitination at lysine 123 (Table 1) [141]. Very recently, Geng and Tansey [145] have demonstrated that Rad6 is also involved in the process of lysine 123 polyubiquitination on histone H2B in *Saccharomyces cerevisiae*. Rad6 functions in conjunction with RING finger E3 ligase, Bre1, to ubiquitinate histone H2B (Table 1). These two enzymes are evolutionary conserved from yeast to human (Table 1). In *S. pombe*, Rhp6 and Brl1 are the homologues of Rad6 and Bre1, respectively, and are required for histone H2B ubiquitination [144]. HR6A and HR6B are the mammalian homologues of yeast Rad6 [147, 148]. Two putative Bre1 homologues, namely RNF20 and RNF40, have been identified in humans [149, 150]. However, only RNF20 affects histone H2B ubiquitination in human cells, even though RNF20 has been shown to form complex with RNF40 *in vivo* [149, 150].

The ubiquitin moiety from ubiquitinated-histone H2B is removed by the action of deubiquitinating enzymes. These enzymes consist of ubiquitin C-terminal hydrolases and ubiquitin-specific processing proteases (UBP). The sixteen UBPs with molecular weight ranging from 50 to 250 kDa have been found in budding yeast. The variable N-terminal extensions in these UBPs are believed to contribute to the substrate specificity [18, 151]. In budding yeast, the ubiquitin proteases, namely Ubp8 and Ubp10, remove ubiquitin moiety from ubiquitinated-histone H2B (Table 1). Ubp8 is a component of coactivator SAGA (Spt-Ada-Gcn5-acetyltransferase), and its function is dependent on the global structural integrity of SAGA [152–154]. Further, two relatively new components of SAGA, namely Sgf11 and Sus1 that do not maintain SAGA integrity, are essential for deubiquitinase activity of Ubp8 [152–160]. Since Ubp8 functions in a SAGA-dependent manner, its role in removing ubiquitin from ubiquitinated-histone H2B is thus restricted to euchromatin [152–154]. Ubp8 is conserved from yeast to human (Table 1). "Nonstop" and USP22 are the orthologs of yeast Ubp8 in *Drosophila* and human, respectively [160–163]. Like in yeast, these orthologs are the integral components of *Drosophila* SAGA and human TFTC/STAGA [160–163]. Further, the orthologs of yeast Sgf11 and Sus1 are present in *Drosophila* and human, and are essential for deubiquitination of ubiquitinated-histone H2B [159–163]. Like Ubp8, Ubp10 deubiquitinates ubiquitinated-histone H2B [164, 165]. However, the function of Ubp10 is independent of SAGA, but depends on the Sir silencing complex [142, 164]. Ubp10 interacts with the silencing protein Sir4 [142, 164, 166]. Such interaction allows Ubp10 to enrich at silenced loci for removal of ubiquitin from ubiquitinated-histone H2B [142]. Thus, Ubp10 plays an important role for histone H2B deubiquitination at telomere to maintain

heterochromatic structure (and hence silencing) [142]. The orthologs of yeast Ubp10 in higher eukaryotes are not yet clearly known. Recently, Weake et al. [63] have suggested CG15817 as a *Drosophila* orthologue of yeast Ubp10. However, further characterization of CG15817 is necessary to define its role in regulation of deubiquitination of ubiquitinated-histone H2B in *Drosophila.*

Histone H2B ubiquitination in transcriptional regulation

Histone H2B ubiquitination plays an important role in regulation of gene expression (Table 2) [14, 18, 29, 142]. The coactivator SAGA-associated Ubp8 regulates the levels of ubiquitinated-histone H2B at the active genes [152–154]. Such regulation of histone H2B ubiquitination has been shown to control transcriptional activation at *GAL1*, *SUG2* and *PHO84* in *Saccharomyces cerevisiae* [8, 152–154]. Further, histone H2B ubiquitination has been implicated in stimulation of transcriptional elongation in both yeast and human [144, 167–170]. Such stimulation is mediated via the facilitated deposition of histones at the active coding sequence by ubiquitinated-histone H2B [170]. Like in yeast, ubiquitinated-histone H2B has also been found to be enriched around transcriptionally active sequences in human, bovine thymus, chicken erythrocytes, and *Tetrahymena* macronuclei [18]. Thus, the stimulatory role of histone H2B ubiquitination in transcription seems to be conserved from yeast to human. Such transcriptional stimulation by histone H2B ubiquitination plays crucial roles in development, cell cycle progression, and other important cellular processes [18, 29]. For example, histone H2B ubiquitination has been implicated to regulate the development of neuronal connectivity in the visual system through transcriptional modulation of genes involved in axon targeting in *Drosophila* [163]. The human homologue (USP22) of yeast Ubp8 regulates transcription of specific genes for appropriate cell cycle progression [161, 162]. Moreover, dynamic nature of histone H2B ubiquitination modulates the transcriptional activities of target genes. For example, the transcriptional activity of tumor suppressor p53 is accompanied by fast change in the level of histone H2B ubiquitination at its target gene, *p21* [29]. Similarly, transcription in yeast is also regulated by the dynamic nature of histone H2B ubiquitination at SAGA-regulated gene [152]. Thus, the enzymes that regulate histone H2B ubiquitination are involved in the etiology of cancers and other human diseases (Table 3).

Although there is a positive correlation between transcription and histone H2B ubiquitination, H2B associated with several active genes (e.g., immunoglobulin κ chain) have also been found to be non-ubiquitinated [18]. Furthermore, in contrast to stimulation, histone H2B ubiquitination has been intriguingly implicated to silence certain euchromatic gene (e.g., *ARG1*) in yeast [156]. Similarly, ubiquitinated-H2B histones are also found in the transcriptionally inactive compartments, such as *Tetrahymena* micronuclei or the sex body of mouse spermatids [18]. Thus, histone H2B ubiquitination plays a differential role in regulation of transcription of euchromatic genes, depending on gene location. In addition to its role in controlling euchromatic gene expression, histone H2B ubiquitination plays a crucial role in regulating telomeric silencing (Table 2) [18, 29, 164]. As mentioned above, the ubiquitin protease, Ubp10, targets ubiquitinated-histone H2B for deubiquitination. Such deubiquitination helps to recruit Sir2 (histone deacetylase) at telomere for silencing [164]. Thus, Ubp10 serves to maintain low level of ubiquitinated-histone H2B at telomere for gene silencing [164].

A spermatid-specific histone H2B (ssH2B) variant with additional 12 amino acids at the carboxy terminus as compared to somatic H2B has been identified in mouse round spermatids [171]. Similarly, H2B histone variant has been found to be exclusively expressed in human testis, and is present in sperm [172]. This histone H2B variant has the conserved lysine 120 for ubiquitination. However, its ubiquitination status and function in spermatogenesis remain largely unknown.

Cross-talk between histone H2B ubiquitination and histone H3 lysine 4/79 methylation

A large number of studies [14, 25–29, 152, 173] have implicated the trans-tail regulation of histone H3 lysine 4 methylation by histone H2B ubiquitination. These studies have demonstrated that mutation of the E2 conjugating enzyme, Rad6, completely abolishes the methylase activity of COMPASS for histone H3 lysine 4 di- and trimethylation. However, monomethylation activity of COMPASS is not dependent on histone H2B ubiquitination [14, 28, 174]. Like COMPASS, Dot1 which is essential for histone H3 lysine 79 methylation is also regulated by Rad6 activity or histone H2B ubiquitination [118, 175]. Therefore, the deletion of *RAD6* abolishes Dot1-mediated methylation of lysine 79 on histone H3. Thus, the works from several laboratories have demonstrated the existence of cross-talks between histone H2B ubiquitination and histone H3 lysine 4/79 methylation. Such a regulatory network between histone ubiquitination

and methylation has been shown to be controlled by the proteasomal ATPases, namely Rpt6 and Rpt4. For example, mutations in the *RPT6* and *RPT4* genes disrupt methylation of lysines 4 and 79 on histone H3, but not histone H2B ubiquitination [176]. Consistently, the proteasome components are recruited to the active gene via histone H2B ubiquitination, and possibly reconfigure chromatin for access of Set1 and Dot1 methylases during transcription [176]. However, Shahbazian et al. [28] have demonstrated that lysine 123 ubiquitination of histone H2B does not regulate recruitment of these methylases, but controls their processivities. Therefore, the molecular mechanisms for cross-talks between histone H2B ubiquitination and histone H3 lysine 4/79 methylation are not clearly known.

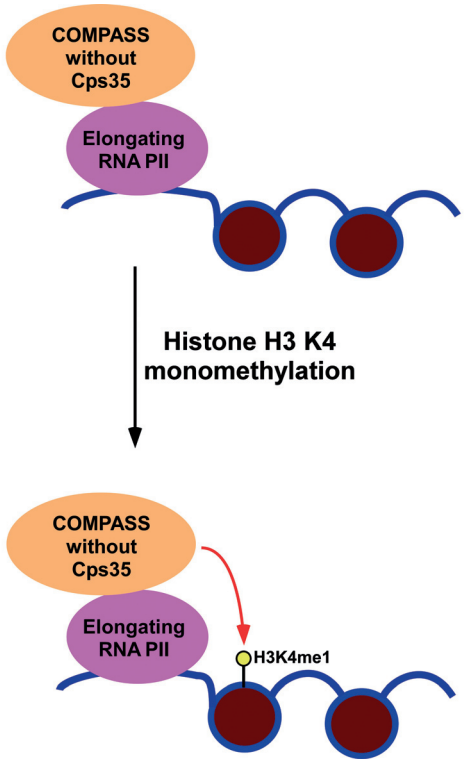

Figure 1. Schematic diagram showing monomethylation of lysine 4 on histone H3 in the absence of histone H2B ubiquitination (or Rad6/Bre1) in *S. cerevisiae*. COMPASS that lacks Cps35 is recruited to the active gene in an elongating RNA polymerase II-dependent manner in the absence of histone H2B ubiquitination [177]. COMPASS without Cps35, thus recruited, monomethylates lysine 4 on histone H3 [177]. Abbreviations: K4, lysine 4; me1, monomethyl; and RNA PII, RNA polymerase II.

We have recently shown that histone H2B ubiquitination plays a crucial role to regulate the subunit composition and methylase activity of COMPASS in *Saccharomyces cerevisiae* [177]. COMPASS that lacks its WD-repeat containing subunit Cps35 is recruited to the active gene in an elongating RNA polymerase II-dependent manner (Fig. 1). This COMPASS has histone H3 lysine 4 mono-, but not di- and tri-, methylase activity (Fig. 1). However, COMPASS containing Cps35 has mono-, di- and trimethylase activities (Fig. 2). We show that Cps35 interacts with active chromatin in a histone H2B ubiquitination-dependent manner, but independently of Set1 [177]. Thus, the fully functional form of COMPASS is assembled on the active gene by histone H2B ubiquitination and elongating RNA polymerase II, resulting in mono-, di- and trimethylation of lysine 4 on histone H3 (Fig. 2). Importantly, Cps35 also interacts with histone H3 lysine 79-specific methylase, Dot1. Further, histone H3 lysine 79 methylation is significantly reduced in the absence of Cps35. Thus, histone H2B ubiquitination-dependent recruitment of Cps35 is essential for lysine 79 methylation on histone H3. Therefore, Cps35 plays an important role for cross-regulation of histone H3 lysines 4 and 79 methylation by histone H2B ubiquitination. However, it is still not known whether Cps35 directly interacts with ubiquitinated-histone H2B. Furthermore, whether cross-talk between histone H2B ubiquitination and histone H3 lysine 4/79 methylation is mediated by Cps35 in mammals remained unknown until recently. However, a very recent study of Wu et al. [178] has nicely demonstrated that the human homologue of yeast Cps35 is essential to translate the signal from histone H2B ubiquitination to histone H3 lysine 4 methylation. Thus, the cross-talk mechanism between histone H2B ubiquitination and histone H3 lysine 4/79 methylation appears to be conserved from yeast to human.

Concluding remarks

The rapid progress within last few years towards understanding the molecular basis of histone H3 lysine 4/79 methylation and histone H2B ubiquitination with their cross-talk has been instrumental to our knowledge of epigenetics in regulation of gene expression, heterochromatin formation, and DNA repair. Although much has been learned, there are still several unanswered questions regarding how histone H3 lysine 4/79 methylation and histone H2B ubiquitination events translate into tightly controlled gene expression, heterochromatin formation, and DNA repair. For example, why do several methylases methylate the same lysine 4 residue on histone H3? How histone H2B ubiquitination and histone H3 lysine 4/79 methylation signal a transcriptional response in living cells is not clearly understood. How does Cps35 interact with ubiquitinated-histone H2B?

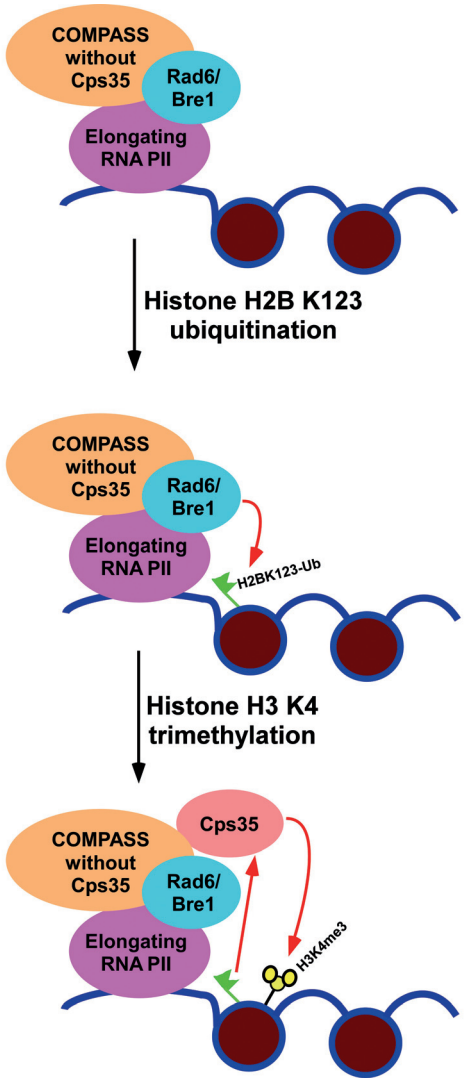

Figure 2. Schematic diagram showing trimethylation of lysine 4 on histone H3 in the presence of histone H2B ubiquitination (or Rad6/ Bre1) in *S. cerevisiae.* COMPASS lacking Cps35 is recruited to active gene in an elongating RNA polymerase II-dependent manner [177]. Rad6/Bre1 ubiquitinates histone H2B at lysine 123. Ubiquitinated-histone H2B recruits Cps35 independently of Set1 [177]. Cps35, thus recruited, associates with the rest of COMPASS, and provides mono-, di- and trimethylase activities to COMPASS, hence leading to trimethylation of lysine 4 on histone H3 [177]. Cps35 also interacts with Dot1 for methylation of lysine 79 on histone H3 (not shown in the diagram for clarity) [177]. Similar mechanism of cross-talk between histone H2B ubiquitination and histone H3 lysine 4 methylation has been recently demonstrated in human [178]. The tri-, but not mono-, methylated-lysine 4 of histone H3 interacts with several proteins involved in various cellular functions such as gene activation and silencing, pre-mRNA splicing, chromatin remodeling, and DNA repair (Table 2). Abbreviations: K4, lysine 4; K123, lysine 123; ub, ubiquitin; me3, trimethyl; and RNA PII, RNA polymerase II.

Understanding the mechanisms governing these basic epigenetic phenomena will surely represent a very attractive target for drug discovery to prevent the onset of cancers and other human diseases, since a large number of studies have shown histone H3 lysines 4 and 79 methylation and histone H2B ubiquitination as important players in controlling gene expression, genome stability, cell proliferation and cancer.

Acknowledgements. We apologize to the authors whose work could not be cited owing to space limitations. The work in Bhaumik's laboratory was supported by a National Scientist Development Grant (0635008N) from American Heart Association, a Research Scholar Grant (06–52) from American Cancer Society, a Mallinckrodt Foundation award, and several internal grants from Southern Illinois University. A.S. is supported by a predoctoral fellowship (0710187Z) from American Heart Association.

1 Kornberg, R. D. (1977) Structure of chromatin. Annu. Rev. Biochem. 46, 931–954.
2 Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F. and Richmond, T. J. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature 389, 251–260.
3 Kornberg, R. D., and Lorch, Y. (1999) Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. Cell 98, 285–294.
4 Woodcock, C. L., Skoultchi, A. I., and Y. Fan. (2006) Role of linker histone in chromatin structure and function: H1 stoichiometry and nucleosome repeat length. Chromosome Res. 14, 17–25.
5 Berger, S. L. (2002) Histone modifications in transcriptional regulation.Curr. Opin. Genet. Dev. 12, 142–148.
6 Bryk, M., Briggs, S. D., Strahl, B. D., Curcio, M. J., Allis, C. D., and Winston, F. (2002) Evidence that Set1, a factor required for methylation of histone H3, regulates rDNA silencing in S. cerevisiae by a Sir2-independent mechanism, Curr. Biol. 12, 165–170.
7 Fischle, W., Wang, Y., and Allis, C. D. (2003) Histone and chromatin cross-talk. Curr. Opin. Cell Biol. 15, 172–183.
8 Li, B., Carey, M., and Workman, J. L. (2007) The role of chromatin during transcription. Cell 128, 707–719.
9 Razin, S.V., Iarovaia, O.V., Sjakste, N., Sjakste, T., Bagdoniene, L., Rynditch, A.V., Eivazova, E. R., Lipinski, M., and Vassetzky, Y. S. (2007) Chromatin domains and regulation of transcription. J. Mol. Biol. 369, 597–607.
10 Groth, A., Rocha, W., Verreault, A., and Almouzni, G. (2007) Chromatin challenges during DNA replication and repair. Cell 128, 721–733.
11 Rice, J. C., Briggs, S. D., Ueberheide, B., Barber, C. M., Shabanowitz, J., Hunt, D. F., Shinkai, Y., and Allis, C. D. (2003) Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. Mol. Cell 12, 1591–1598.
12 Ehrenhofer-Murray, A. E. (2004) Chromatin dynamics at DNA replication, transcription and repair. Eur. J. Biochem. 271, 2335–2349.
13 Cosgrove, M. S., and Wolberger, C. (2005) How does the histone code work? Biochem. Cell Biol. 83, 468–476.
14 Shilatifard, A. (2006) Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. Annu. Rev. Biochem. 75, 243–269.
15 Bhaumik, S. R., Smith, E., and Shilatifard, A. (2007) Covalent modifications of histones during development and disease pathogenesis. Nat. Struct. Mol. Biol. 14, 1008–1016.
16 Kouzarides, T. (2007) Chromatin modifications and their function. Cell 128, 693–705.
17 Kusch, T., and Workman, J. L. (2007) Histone variants and complexes involved in their exchange. Subcell. Biochem. 41, 91–109.
18 Zhang, Y. (2003) Transcriptional regulation by histone ubiquitination and deubiquitination Genes Dev. 17, 2733–2740.

19 Ng, H. H., Feng, Q., Wang, H., Erdjument-Bromage, H., Tempst, P., Zhang, Y., and Struhl, K. (2002) Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. Genes Dev. 16, 1518–1527.
20 Schneider, J., and Shilatifard, A. (2006) Histone demethylation by hydroxylation: chemistry in action. ACS Chem. Biol. 1, 75–81.
21 Strahl, B. D., and Allis, C. D. (2000) The language of covalent histone modifications. Nature 403, 41–45.
22 Ruthenburg, A. J., Li, H., Patel, D. J., and Allis, C. D. (2007) Multivalent engagement of chromatin modifications by linked binding modules. Nat. Rev. Mol. Cell Biol. 12, 983–994.
23 Turner, B. M.(2000) Histone acetylation and an epigenetic code. Bioessays. 22, 836–45.
24 Turner, B. M.(2007) Defining an epigenetic code. Nat. Cell Biol. 1, 2–6.
25 Latham, J. A., and Dent, S. Y. (2007) Cross-regulation of histone modifications. Nat. Struct. Mol. Biol.14, 1017–1024.
26 Sun, Z. W., and Allis. C. D. (2002) Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. Nature 418, 104–108.
27 Dover, J., Schneider, J., Tawiah-Boateng, M. A., Wood, A., Dean, K., Johnston, M., and Shilatifard, A. (2002) Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by Rad6. J. Biol. Chem. 277, 28368–28371.
28 Shahbazian, M. D., Zhang, K., and Grunstein, M. (2005) Histone H2B ubiquitylation controls processive methylation but not monomethylation by Dot1 and Set1. Mol. Cell 19, 271–277.
29 Weake, V. M., and Workman, J. L. (2008) Histone ubiquitination: triggering gene activity. Mol. Cell 29, 653–663.
30 Sims III, R. J., and Reinberg, D. (2006) Histone H3 Lys 4 methylation: caught in a bind? Genes Dev. 20, 2779–2786.
31 Klose, R. J., and Zhang, Y. (2007) Regulation of histone methylation by demethylimination and demethylation. Nat. Rev. Mol. Cell Biol. 8, 307–318.
32 Shilatifard, A. (2008) Molecular implementation and physiological roles for histone H3 lysine 4 (H3K4) methylation. Curr. Opin. Cell Biol. 3, 341–348.
33 Miller, T., Krogan, N. J., Dover, J., Erdjument-Bromage, H., Tempst, P., Johnston, M., Greenblatt, J. F., and Shilatifard, A. (2001) COMPASS: a complex of proteins associated with a trithorax-related SET domain protein, Proc. Natl. Acad. Sci. USA 98, 12902–12907.
34 Roguev, A., Schaft, D., Shevchenko, A., Pijnappel, W. W., Wilm, M., Aasland, R., and Stewart, A. F. (2001) The *Saccharomyces cerevisiae* Set1 complex includes an Ash2 homologue and methylates histone 3 lysine 4. EMBO J 20, 7137–7148.
35 Nagy, P. L., Griesenbeck, J., Kornberg, R. D., and Cleary, M. L. (2002) A trithorax-group complex purified from *Saccharomyces cerevisiae* is required for methylation of histone H3. Proc. Natl. Acad. Sci. USA 99, 90–94.
36 Krogan, N. J., Dover, J., Khorrami, S., Greenblatt, J. F., Schneider, J., Johnston, M., and Shilatifard, A.(2002) COMPASS, a histone H3 (Lysine 4) methyltransferase required for telomeric silencing of gene expression. J. Biol. Chem. 277, 10753–10755.
37 Schneider, J., Wood, A., Lee, J. S., Schuster, R., Dueker, J., Maguire, C., Swanson, S. K., Florens, L., Washburn, M. P. and Shilatifard, A. (2005) Molecular regulation of histone H3 trimethylation by COMPASS and the regulation of gene expression. Mol. Cell 19, 849–856.
38 Wood, A., Shukla, A., Schneider, J., Lee, J. S., Stanton, J. D., Dzuiba, T., Swanson, S. K., Florens, L., Washburn, M. P., and Wyrick, J., Bhaumik, S. R., and Shilatifard, A. (2007) Ctk complex-mediated regulation of histone methylation by COMPASS. Mol. Cell. Biol. 27, 709–720.
39 Hughes, C. M., Rozenblatt-Rosen, O., Milne, T. A., Copeland, T. D., Levine, S. S., Lee, J. C., Hayes, D. N., Shanmugam, K. S., Bhattacharjee, A., and Biondi, C.A., Kay, G. F., Hayward, N. K., Hess, J. L., and Meyerson, M. (2004) Menin associates with a trithorax family histone methyltransferase complex and with the hoxc8 locus. Mol. Cell 13, 587–597.
40 Tenney, K., and Shilatifard, A. (2005) A COMPASS in the voyage of defining the role of trithorax/MLL-containing complexes: linking leukemogensis to covalent modifications of chromatin. J. Cell. Biochem. 95, 429–436.
41 Ruthenburg, A. J., Allis, C. D., and Wysocka, J. (2007) Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. Mol. Cell 25, 15–30.
42 Shi, Y., and Whetstine, J. R. (2007) Dynamic regulation of histone lysine methylation by demethylases. Mol. Cell 25, 1–14.
43 Yu, B. D., Hess, J. L., Horning, S. E., Brown, G. A., and Korsmeyer, S. J. (1995) Altered Hox expression and segmental identity in Mll-mutant mice. Nature 378, 505–508.
44 Lubitz, S., Glaser, S., Schaft, J., Stewart, A. F., and Anastassiadis, K. (2007) Increased apoptosis and skewed differentiation in mouse embryonic stem cells lacking the histone methyltransferase Mll2. Mol. Biol. Cell 18, 2356–2366.
45 Dreijerink, K. M., Mulder, K. W., Winkler, G. S., Höppener, J. W., Lips, C. J., and Timmers, H. T. (2006) Menin links estrogen receptor activation to histone H3K4 trimethylation. Cancer Res. 66, 4929–4935.
46 Krivtsov, A. V., and Armstrong, S. A. (2007) MLL translocations, histone modifications and leukemia stem-cell development. Nat. Rev. Cancer 7, 823–833.
47 Feng, Q., Wang, H., and Ng, H. H., Erdjument-Bromage, H., Tempst, P., Struhl, K., and Zhang, Y. (2002) Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. Curr. Biol. 12 , 1052–1058.
48 Santos-Rosa, H., and Caldas, C. (2005) Chromatin modifier enzymes, the histone code and cancer. Eur. J. Cancer 41, 2381–2402.
49 Min, J., Feng, Q., and Li, Z., Zhang Y, and Xu, R. M. (2003) Structure of the catalytic domain of human DOT1L, a non-SET domain nucleosomal histone methyltransferase. Cell 112, 711–723.
50 Bannister, A. J., Schneider, R., and Kouzarides, T. (2002) Histone methylation: dynamic or static? Cell 109, 801–806.
51 Bannister, A. J., and Kouzarides, T. (2005) Reversing histone methylation. Nature 436, 1103–1106.
52 Benevolenskaya, E. V. (2007) Histone H3K4 demethylases are essential in development and differentiation. Biochem. Cell Biol. 85, 435–443.
53 Agger, K., Christensen, J., Cloos, P. A., and Helin, K. (2008) The emerging functions of histone demethylases. Curr. Opin. Genet. Dev. 18, 159–168.
54 Lan, F., Nottke, A.C., and Shi, Y. (2008) Mechanisms involved in the regulation of histone lysine demethylases. Curr. Opin. Cell Biol. 20, 316–325.
55 Kim, S., Benoiton, L., and Paik, W. K. (1964) ε-Alkyllysinase. Purification and properties of the enzyme. J. Biol. Chem. 239, 3790–3796.
56 Paik, W. K., and Kim, S. (1973) Enzymatic demethylation of calf thymus histones. Biochem. Biophys. Res. Commun. 51, 781–788.
57 Paik, W. K., and Kim, S. (1974) ε-alkyllysinase. New assay method, purification, and biological significance. Arch. Biochem. Biophys. 165, 369–378.
58 Tong, J. K., Hassig, C. A., Schnitzler, G. R., Kingston, R. E., and Schreiber, S. L. (1998) Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex. Nature 395, 917–921.
59 Humphrey, G. W., Wang, Y., Russanova, V. R., Hirai, T., Qin, J., Nakatani, Y., and Howard, B. H. (2001) Stable histone deacetylase complexes distinguished by the presence of SANT domain proteins CoREST/kiaa0071 and Mta-L1. J. Biol. Chem. 276, 6817–6824.
60 You, A., Tong, J. K., Grozinger, C. M., and Schreiber, S. L. (2001) CoREST is an integral component of the CoRESThu-

man histone deacetylase complex. Proc. Natl Acad. Sci. USA 98, 1454–1458.
61 Hakimi, M. A., Dong, Y., Lane, W. S., Speicher, D. W., and Shiekhattar, R. (2003) A candidate X-linked mental retardation gene is a component of a new family of histone deacetylase-containing complexes. J. Biol. Chem. 278, 7234–7239.
62 Shi, Y., Sawada, J., Sui, G., Affar el B., Whetstine, J. R, Lan, F., Ogawa, H., Luke, M. P., Nakatani, Y., and Shi, Y. (2003) Coordinated histone modifications mediated by a CtBP co-repressor complex. Nature 422, 735–738.
63 Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J. R., Cole, P. A., Casero, R. A., and Shi, Y. (2004) Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell 119, 941–953
64 Lee, M. G., Wynder, C., Cooch, N., and Shiekhattar, R. (2005) An essential role for CoREST in nucleosomal histone 3 lysine 4 demethylation. Nature 437, 432–435.
65 Lee, M. G., Wynder, C., Bochar, D. A., Hakimi, M. A., Cooch, N., and Shiekhattar, R. (2006) Functional interplay between histone demethylase and deacetylase enzymes. Mol. Cell Biol. 26, 6395–6402.
66 Lee, N., Zhang, J., Klose, R. J., Erdjument-Bromage, H., Tempst, P., Jones, R. S., and Zhang, Y. (2007) The trithorax group protein Lid is a trimethyl-H3K4 demethylase. Nature Struct. Mol. Biol. 14, 341–343.
67 Shi, Y. (2007) Histone lysine demethylases: emerging roles in development, physiology and disease. Nat. Rev. Genet. 8, 829–833.
68 Culhane, J. C., and Cole, P. A. (2007) LSD1 and the chemistry of histone demethylation. Curr. Opin. Chem. Biol. 11, 561–568.
69 Kim, B. J., Kang, K. M., Jung, S. Y., Choi, H. K., Seo, J. H., Chae, J. H., Cho, E. J., Youn, H. D., Qin, J., and Kim, S. T. (2008) Esco2 is a novel corepressor that associates with various chromatin modifying enzymes. Biochem. Biophys. Res. Commun. 372, 298–304.
70 Yokoyama, A., Takezawa, S., Schüle, R., Kitagawa, H., and Kato, S. (2008) Transrepressive function of TLX requires the histone demethylase LSD1. Mol. Cell. Biol. 28, 3995–4003.
71 Ballas, N., Battaglioli, E., Atouf, F., Andres, M. E., Chenoweth, J., Anderson, M. E., Burger, C., Moniwa, M., Davie, J. R., Bowers, W. J., Federoff, H. J., Rose, D. W., Rosenfeld, M. G., Brehm, P., and Mandel, G. (2001) Regulation of neuronal traits by a novel transcriptional complex. Neuron 31, 353–365.
72 Zhu, Q., Liu, C., Ge, Z., Fang, X., Zhang, X., Stråat, K., Björkholm, M., and Xu, D. (2008) Lysine-specific demethylase 1 (LSD1) is required for the transcriptional repression of the telomerase reverse transcriptase (hTERT) gene. PLoS ONE 3, e1446.
73 Tsai, W. W., Nguyen, T. T., Shi, Y., and Barton, M. C. (2008) p53-targeted LSD1 functions in repression of chromatin structure and transcription in vivo. Mol. Cell. Biol. 28, 5139–5146.
74 Wang, J., Scully, K., Zhu, X., Cai, L., Zhang, J., Prefontaine, G. G., Krones, A., Ohgi, K. A., Zhu, P., Garcia-Bassets, I., Liu, F., Taylor, H., Lozach, J., Jayes, F. L., Korach, K. S., Glass, C. K., Fu, X. D., and Rosenfeld, M. G. (2007) Opposing LSD1 complexes function in developmental gene activation and repression programmes. Nature 446, 882–887.
75 Gildea, J. J., Lopez, R., and Shearn, A. (2000) A screen for new trithorax group genes identified little imaginal discs, the Drosophila melanogaster homologue of human retinoblastoma binding protein 2. Genetics 156, 645–663.
76 Secombe, J., and Eisenman, R. N. (2007) The function and regulation of the JARID1 family of histone H3 lysine 4 demethylases: the Myc connection. Cell Cycle 6, 1324–1328.
77 Liang, G., Klose, R. J., Gardener, K. E., and Zhang, Y. (2007) Yeast Jhd2 is an H3-K4 tri-methyl demethylase. Nat. Struct. Mol. Biol. 14, 243–245.
78 Sawan, C., Vaissière, T., Murr, R., and Herceg, Z. (2008) Epigenetic drivers and genetic passengers on the road to cancer. Mutat. Res. 642, 1–13.
79 Cortez, C. C., and Jones, P. A. (2008) Chromatin, cancer and drug therapies. Mutat. Res. 647: 44–51.
80 Saigo, K., Yoshida, K., Ikeda, R., Sakamoto, Y., Murakami, Y., Urashima, T., Asano, T., Kenmochi, T., and Inoue, I. (2008) Integration of hepatitis B virus DNA into the myeloid/lymphoid or mixed-lineage leukemia (MLL4) gene and rearrangements of MLL4 in human hepatocellular carcinoma. Hum. Mutat. 29, 703–8
81 Hamamoto, R., Silva, F. P., Tsuge, M., Nishidate, T., Katagiri, T., Nakamura, Y., and Furukawa, Y. (2006) Enhanced SMYD3 expression is essential for the growth of breast cancer cells. Cancer Sci. 97, 113–8.
82 Silva, F. P., Hamamoto, R., Kunizaki, M., Tsuge, M., Nakamura, Y., and Furukawa, Y. (2008) Enhanced methyltransferase activity of SMYD3 by the cleavage of its N-terminal region in human cancer cells. Oncogene 27, 2686–2692.
83 Schneider, R., Bannister, A. J., and Kouzarides, T. (2002) Unsafe SETs: histone lysine methyltransferases and cancer. Trends Biochem. Sci. 27, 396–402.
84 Xiang, Y., Zhu, Z., Han, G., Ye, X., Xu, B., Peng, Z., Ma, Y., Yu, Y., Lin, H., Chen, A. P., and Chen, C. D. (2007) JARID1B is a histone H3 lysine 4 demethylase up-regulated in prostate cancer. Proc. Natl. Acad. Sci. USA. 104, 19226–19231.
85 Litt, M. D., Simpson, M., Gaszner, M., Allis, C. D., and Felsenfeld, G. (2001) Correlation between histone lysine methylation and developmental changes at the chicken beta-globin locus. Science 293, 2453–2455.
86 Boggs, B. A., Cheung, P., Heard, E., Spector, D. L., Chinault, A. C., and Allis, C. D. (2002) Differentially methylated forms of histone H3 show unique association patterns with inactive human X chromosomes. Nat. Genet. 30, 73–76.
87 Noma, K. I., and Grewel, S. I. (2002) Histone H3 lysine 4 methylation is mediated by Set1 and promotes maintenance of active chromatin states in fission yeast. Proc. Natl. Acad. Sci. USA 99, 16438–16445.
88 Bernstein, B. E., Kamal, M., Lindblad-Toh, K., Bekiranov, S., Bailey, D. K., Huebert, D. J., McMahon, S., Karlsson, E. K., Kulbokas III, E. J. 3rd, and Gingeras, T. R., Schreiber, S. L., and Lander, E. S. (2005) Genomic maps and comparative analysis of histone modifications in human and mouse, Cell 120, 169–181.
89 Bernstein, B. E., Mikkelsen, T. S., Xie, X., Kamal, M., Huebert, D. J., Cuff, J., Fry, B., Meissner, A., Wernig, M., and Plath, K., Jaenisch, R., Wagschal, A., Feil, R., Schreiber, S. L., and Lander, E. S. (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125, 315–326.
90 Khalil, A. M., Boyar, F. Z., and Driscoll, D.J. (2004) Dynamic histone modifications mark sex chromosome inactivation and reactivation during mammalian spermatogenesis. Proc. Natl. Acad. Sci. USA. 101, 16583–16587.
91 Khalil, A. M., and Driscoll, D.J. (2006) Histone H3 lysine 4 dimethylation is enriched on the inactive sex chromosomes in male meiosis but absent on the inactive X in female somatic cells. Cytogenet. Genome Res. 112, 11–5.
92 Sims III, R. J., Chen, C. F., Santos-Rosa, H., Kouzarides, T., Patel, S. S., and Reinberg, D. (2005) Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. J. Biol. Chem 280, 41789–41792.
93 Pray-Grant, M. G., Daniel, J. A., Schieltz, D., Yates III, J. R., and P. A. Grant. (2005) Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent Acetylation. Nature 433, 434–438.
94 Shi, X., Hong, T., Walter, K. L., Ewalt, M., Michishita, E., Hung, T., Carney, D., Pena, P., Lan, F., and Kaadige, M. R. (2006) ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. Nature 442, 96–99.

95 Wysocka, J., Swigut, T., Xiao, H., Milne, T. A., Kwon, S. Y., Landry, J., Kauer, M., Tackett, A. J., Chait, B. T., Badenhorst, P., Chait, B. T., Badenhorst, P., Wu, C., and Allis, C. D. (2006) A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodeling. Nature 442, 86–90.

96 Vermeulen, M., Mulder, K. W., Denissov, S., Pijnappel, W. W., van Schaik, F. M., Varier, R. A., Baltissen, M. P., Stunnenberg, H. G., Mann, M., and Timmers, H. T. (2007) Selective anchoring of TFIID to nucleosomes by trimethylation of histone H3 lysine 4. Cell 131, 58–69.

97 Sims III R. J., Millhouse, S., Chen, C. F., Lewis, B. A., Erdjument-Bromage, H,. Tempst, P., Manley, J. L., and Reinberg, D. (2007) Recognition of trimethylated histone H3 lysine 4 facilitates the recruitment of transcription post-initiation factors and pre-mRNA splicing. Mol. Cell 28, 665–676.

98 Garkavtsev, I., Kazarov, A., Gudkov, A., and Riabowol, K. (1996) Suppression of the novel growth inhibitor p33ING1 promotes neoplastic transformation, Nat. Genet. 14, 415–420.

99 Garkavtsev, I., Grigorian, I. A., Ossovskaya, V. S., Chernov, M. V., Chumakov, P. M., and Gudkov, A. V. (1998) The candidate tumour suppressor p33ING1 cooperates with p53 in cell growth control. Nature 391, 295–298.

100 Garkavtsev, I., and Riabowol, K. (1997) Extension of the replicative life span of human diploid fibroblasts by inhibition of the p33ING1 candidate tumor suppressor. Mol. Cell. Biol. 17, 2014–2019.

101 Helbing, C. C., Veillette, C., Riabowol, K., Johnston, R. N., and Garkavtsev, I. (1997) A novel candidate tumor suppressor, ING1, is involved in the regulation of apoptosis. Cancer Res. 57, 1255–1258.

102 Kuzmichev, A., Zhang, Y., Erdjument-Bromage, H., Tempst, P., and Reinberg, D. (2002) Role of the Sin3-histone deacetylase complex in growth regulation by the candidate tumor suppressor p33(ING1). Mol. Cell. Biol. 22, 835–848.

103 Cheung Jr., K. J., Mitchell, D., Lin, P., and Li, G. (2001) The tumor suppressor candidate p33(ING1) mediates repair of UV-damaged DNA. Cancer Res. 61, 4974–4977.

104 Peña, P. V., Hom, R. A., Hung, T., Lin, H., Kuo, A. J., Wong, R. P., Subach, O. M., Champagne, K. S., Zhao, R., Verkhusha, V. V., Li, G., Gozani, O., and Kutateladze, T. G. (2008) Histone H3K4me3 binding is required for the DNA repair and apoptotic activities of ING1 tumor suppressor. J. Mol. Biol. 380, 303–312.

105 Campos, E. I., Chin, M. Y., Kuo, W. H., and Li, G. (2004) Biological functions of the ING family tumor suppressors. Cell. Mol. Life Sci. 61, 2597–2613.

106 Gong, W., Suzuki, K., Russell, M., and Riabowol, K. (2005) Function of the ING family of PHD proteins in cancer. Int. J. Biochem. Cell Biol. 37, 1054–1065.

107 Toyama, T., Iwase, H., Watson, P., Muzik, H., Saettler, E., and Magliocco, A., DiFrancesco, L., Forsyth, P., Garkavtsev, I., Kobayashi, S., and Riabowol, K. (1999) Suppression of ING1 expression in sporadic breast cancer, Oncogene 18, 5187–5193.

108 Campos, E. I., Martinka, M., Mitchell, D. L., Dai, D. L., and Li, G. (2004) Mutations of the ING1 tumor suppressor gene detected in human melanoma abrogate nucleotide excision repair. Int. J. Oncol. 25, 73–80.

109 Gunduz, M., Ouchida, M., Fukushima, K., Hanafusa, H., Etani, T., and Nishioka, S., Nishizaki, K., Shimizu, K. (2000) Genomic structure of the human ING1 gene and tumor-specific mutations detected in head and neck squamous cell carcinomas. Cancer Res. 60, 3143–3146.

110 Chen, L., Matsubara, N., Yoshino, T., Nagasaka, T., Hoshizima, N., and Shirakawa, Y., Naomoto, Y., Isozaki, H., Riabowol, K., and Tanaka, N. (2001) Genetic alterations of candidate tumor suppressor ING1 in human esophageal squamous cell cancer. Cancer Res. 61, 4345–4349.

111 Soliman, M. A., and Riabowol, K. (2007) After a decade of study-ING, a PHD for a versatile family of proteins. Trends Biochem. Sci. 32, 509–519.

112 Argiropoulos, B., and Humphries, R. K. (2007) Hox genes in hematopoiesis and leukemogenesis. Oncogene 26, 6766–6776.

113 Ayton, P. M., and Cleary, M. L. (2001) Molecular mechanisms of leukaemogenesis mediated by MLL fusion proteins. Oncogene 20, 5695–5707.

114 Tan, Y. C., and Chow, V. T. (2001) Novel human HALR (MLL3) gene encodes a protein homologous to ALR and to ALL-1 involved in leukemia, and maps to chromosome 7q36 associated with leukemia and developmental defects. Cancer Detect. Prev. 25, 454–469.

115 Ruault, M., Brun, M. E., Ventura, M., Roizès, G., and De Sario, A. (2002) MLL3, a new human member of the TRX/MLL gene family, maps to 7q36, a chromosome region frequently deleted in myeloid leukemia. Gene 284, 73–81

116 Hamamoto, R., Furukawa, Y., and Morita, M., Iimura, Y., Silva, F. P., Li, M., Yagyu, R., and Nakamura, Y. (2004) SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cell. Nat. Cell Biol. 6, 731–740.

117 Li, Y., Reddy, M. A., Miao, F., Shanmugam, N., Yee, J. K., Hawkins, D., Ren, B., and Natarajan R. (2008) Role of the histone H3 lysine 4 methyltransferase, SET7/9, in the regulation of NF-kappa B dependent inflammatory genes: Relevance to diabetes and inflammation. J. Biol. Chem. 283, 26771–26781.

118 van Leeuwen, F., Gafken, P. R., and Gottschling, D. E. (2002) Dot1p modulates silencing in yeast by methylation of the nucleosome core. Cell 109, 745–756.

119 Altaf, M., Utley, R.T., Lacoste, N., Tan, S., Briggs, S. D., and Côté, J. (2007) Interplay of chromatin modifiers on a short basic patch of histone H4 tail defines the boundary of telomeric heterochromatin. Mol. Cell 28, 1002–1014.

120 Fingerman, I. M., Li, H. C., and Briggs, S. D. (2007) A charge-based interaction between histone H4 and Dot1 is required for H3K79 methylation and telomere silencing: identification of a new trans-histone pathway. Genes Dev. 21, 2018–2029.

121 Huyen, Y., Zgheib, O., Ditullio, R. A. Jr, Gorgoulis, V. G., Zacharatos, P., Petty, T. J., Sheston, E. A., Mellert, H. S., Stavridi, E. S., and Halazonetis, T. D. (2004) Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. Nature 432, 406–11.

122 Wysocki, R., Javaheri, A., Allard, S., Sha, F., Côté, J., and Kron, S. J. (2005) Role of Dot1-dependent histone H3 methylation in G1 and S phase DNA damage checkpoint functions of Rad9. Mol. Cell. Biol. 19, 8430–43.

123 Grenon, M., Costelloe, T., Jimeno, S., O'Shaughnessy, A., Fitzgerald, J., Zgheib, O., Degerth, L., and Lowndes, N. F. (2007) Docking onto chromatin via the *Saccharomyces cerevisiae* Rad9 Tudor domain. Yeast 24, 105–119.

124 Karagiannis, T. C., and El-Osta, A. (2007) Chromatin modifications and DNA double-strand breaks: the current state of play. Leukemia 21, 195–200.

125 Okada, Y., Feng, Q., Lin, Y., Jiang, Q., Li, Y., Coffield, V.M., Su, L., Xu, G., and Zhang, Y. (2005) hDOT1L links histone methylation to leukemogenesis. Cell 121, 167–178.

126 Bitoun, E., Oliver, P. L., and Davies, K. E. (2007) The mixed-lineage leukemia fusion partner AF4 stimulates RNA polymerase II transcriptional elongation and mediates coordinated chromatin remodeling. Hum Mol Genet. 16, 92–106.

127 Sawado, T., Halow, J., Im, H., Ragoczy, T., Bresnick, E. H., Bender, M. A., and Groudine, M. (2008) H3 K79 dimethylation marks developmental activation of the beta-globin gene but is reduced upon LCR-mediated high-level transcription. Blood 112, 406–414.

128 Steger, D. J., Lefterova, M. I., Ying, L., Stonestrom, A. J., Schupp, M., Zhuo, D., Vakoc, A. L., Kim, J. E., Chen, J., Lazar, M. A., Blobel, G. A., and Vakoc, C. R.(2008) DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously

coupled with gene transcription in mammalian cells. Mol. Cell. Biol. 8, 2825–2839.
129 Hake, S. B., and Allis, C. D. (2006) Histone H3 variants and their potential role in indexing mammalian genomes: the "H3 barcode hypothesis". Proc. Natl. Acad. Sci. USA. 103, 6428–6435.
130 Loyola, A., and Almouzni, G. (2007) Marking histone H3 variants: how, when and why? Trends Biochem. Sci. 32, 425–33.
131 Pickart, C. M., and Eddins, M. J. (2004) Ubiquitin: structures, function, mechanisms. Biochem. Biophys. Acta 1695, 55–72.
132 Pickart, C. M., and Fushman, D. (2004) Polyubiquitin chains: polymeric protein signals. Curr. Opin. Chem. Biol 8, 610–616.
133 Wang, M., Cheng, D., Peng, J., and Pickart, C. M. (2006) Molecular determinants of polyubiquitin linkage selection by an HECT ubiquitin ligase. EMBO J. 25, 1710–9.
134 Chau, V., Tobias, J. W., Bachmair, A., Marriott, D., Ecker, D. J., Gonda, D. K., and Varshavsky, A. (1989) A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. Science 243, 1576–1583.
135 Finley, D., Sadis, S., Monia, B. P., Boucher, P., Ecker, D. J., Crooke, S. T., and Chau, V. (1994) Inhibition of proteolysis and cell cycle progression in a multiubiquitination-deficient yeast mutant. Mol. Cell. Biol. 14, 5501–5509.
136 Kim, H. T., Kim, K. P., Lledias, F., Kisselev, A. F., Scaglione, K. M., Skowyra, D., Gygi, S. P., and Goldberg, A. L. (2007) Certain pairs of ubiquitin-conjugating enzymes (E2 s) and ubiquitin-protein ligases (E3 s) synthesize nondegradable forked ubiquitin chains containing all possible isopeptide linkages. J. Biol. Chem. 282, 17375–17386.
137 Spence, J., Sadis, S., Haas, A. L., and Finley, D. (1995) A ubiquitin mutant with specific defects in DNA repair and multiubiquitination. Mol. Cell. Biol. 15, 1265–1273
138 Deng, L., Wang, C., Spencer, E., Yang, L., Braun, A., You, J., Slaughter, C., Pickart, C., and Chen, Z. J. (2000) Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. Cell 103, 351–361.
139 Hicke, L., and Dunn, R. (2003) Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. Annu. Rev. Cell Dev. Biol. 19, 141–172.
140 Pickart, C. M., and Cohen, R. E. (2004) Proteasomes and their kin: proteases in the machine age. Nat. Rev. Mol. Cell. Biol. 5, 177–187.
141 Robzyk, K., Recht, J., and Osley, M.A. (2000) Rad6-dependent ubiquitination of histone H2B in yeast. Science 287, 501–504.
142 Osley, M.A. (2006) Regulation of histone H2A and H2B ubiquitylation. Brief. Funct. Genomic. Proteomic. 5, 179–189.
143 Sridhar, V. V., Kapoor, A., Zhang, K., Zhu, J., Zhou, T., Hasegawa, P. M., Bressan, R. A., and Zhu, J. K. (2007) Control of DNA methylation and heterochromatic silencing by histone H2B deubiquitination. Nature 447, 735–738.
144 Tanny, J. C., Erdjument-Bromage, H., Tempst, P., and Allis, C. D. (2007) Ubiquitylation of histone H2B controls RNA polymerase II transcription elongation independently of histone H3 methylation. Genes Dev. 21, 835–847.
145 Geng, F., and Tansey, W. P. (2008) Polyubiquitylation of Histone H2B. Mol. Biol. Cell 9, 3616–3624.
146 Pickart, C. M. (2001) Mechanisms underlying ubiquitination. Annu. Rev. Biochem.70, 503–533.
147 Koken, M. H., Reynolds, P., Jaspers-Dekker, I., Prakash, L., Prakash, S., Bootsma, D., and Hoeijmakers, J. H. (1991) Structural and functional conservation of two human homologs of the yeast DNA repair gene RAD6. Proc. Natl. Acad. Sci. USA 88, 8865–8869.
148 Roest, H. P., van Klaveren, J., de Wit, J., van Gurp, C. G., Koken, M. H., Vermey, M., van Roijen, J. H., Hoogerbrugge, J. W., Vreeburg, J. T., and Baarends, W. M., Bootsma, D., Grootegoed, J. A., Hoeijmakers, J. H. (1996) Inactivation of the HR6B ubiquitin-conjugating DNA repair enzyme in mice causes male sterility associated with chromatin modification. Cell 86, 799–810.
149 Zhu, B., Zheng, Y., Pham, A. D., Mandal, S. S., Erdjument-Bromage, H., Tempst, P., and Reinberg, D. (2005) Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation. Mol. Cell 20, 601–611.
150 Kim, J., Hake, S. B., and Roeder, R. G. (2005) The human homolog of yeast BRE1 functions as a transcriptional coactivator through direct activator interactions. Mol. Cell 20, 759–770.
151 Hurley, J. H., Lee, S., and Prag, G. (2006) Ubiquitin-binding domains. Biochem. J. 399, 361–372.
152 Henry, K.W., Wyce, A., Lo, W. S., Duggan, L. J., Emre, N. C., Kao, C. F., Pillus, L., Shilatifard, A., Osley, M.A., and Berger, S.L. (2003) Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. Genes Dev. 17, 2648–2663.
153 Daniel, J. A., Torok, M. S., Sun, Z. W., Schieltz, D., Allis, C. D., Yates, J. R., 3rd, and Grant, P. A. (2004) Deubiquitination of histone H2B by a yeast acetyltransferase complex regulates transcription. J. Biol. Chem. 279, 1867–1871.
154 Shukla, A., Stanojevic, N., Duan, Z., Sen, P., and Bhaumik, S. R. (2006) Ubp8p, a histone deubiquitinase whose association with SAGA is mediated by Sgf11p, differentially regulates lysine 4 methylation of histone H3 *in vivo*. Mol. Cell. Biol. 26, 3339–3352.
155 Powell, D. W., Weaver, C. M., Jennings, J. L., McAfee, K. J., He, Y., Weil, P. A., and Link, A. J. (2004) Cluster analysis of mass spectrometry data reveals a novel component of SAGA. Mol. Cell. Biol. 24, 7249–7259.
156 Lee, K. K., Florens, L., Swanson, S. K., Washburn, M. P., and Workman, J. L. (2005) The deubiquitylation activity of Ubp8 is dependent upon Sgf11 and its association with the SAGA complex. Mol. Cell. Biol. 25, 1173–1182.
157 Ingvarsdottir, K., Krogan, N. J., Emre, N. C., Wyce, A., Thompson, N. J., Emili, A., Hughes, T. R., Greenblatt, J. F., and Berger, S. L. (2005) H2B ubiquitin protease Ubp8 and Sgf11 constitute a discrete functional module within the *Saccharomyces cerevisiae* SAGA complex. Mol. Cell. Biol. 25, 1162–1172.
158 Kohler, A., Pascual-Garcia, P., Llopis, A., Zapater, M., Posas, F., Hurt, E., and Rodriguez-Navarro, S. (2006) The mRNA export factor Sus1 is involved in Spt/ Ada/Gcn5 acetyltransferase-mediated H2B deubiquitinylation through its interaction with Ubp8 and Sgf11. Mol. Biol. Cell 17, 4228–4236.
159 Kurshakova, M. M., Krasnov, A. N., Kopytova, D. V., Shidlovskii, Y. V., Nikolenko, J. V., Nabirochkina, E. N., Spehner, D., Schultz, P., Tora, L., and Georgieva, S. G. (2007) SAGA and a novel Drosophila export complex anchor efficient transcription and mRNA export to NPC. EMBO J. 26, 4956–4965.
160 Zhao, Y., Lang, G., Ito, S., Bonnet, J., Metzger, E., Sawatsubashi, S., Suzuki, E., Le Guezennec, X., Stunnenberg, H. G., and Krasnov, A., Georgieva, S. G., Schüle, R., Takeyama, K., Kato, S., Tora, L., and Devys, D. (2008) A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. Mol. Cell 29, 92–101.
161 Zhang, X. Y., Varthi, M., Sykes, S. M., Phillips, C., Warzecha, C., Zhu, W., Wyce, A., Thorne, A. W., Berger, S. L., and McMahon, S. B. (2008) The putative cancer stem cell marker USP22 Is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression. Mol. Cell 29, 102–111.
162 Zhang, X. Y., Pfeiffer, H. K., Thorne, A. W., and McMahon, S. B. (2008) USP22, an hSAGA subunit and potential cancer stem cell marker, reverses the polycomb-catalyzed ubiquitylation of histone H2A. Cell Cycle 7, 1522–1524.
163 Weake, V. M., Lee, K. K., Guelman, S., Lin, C. H., Seidel, C., Abmayr, S. M., and Workman, J. L. (2008) SAGA-mediated H2B deubiquitination controls the development of neuronal

connectivity in the Drosophila visual system. EMBO J. 27, 394–405.
164 Emre, N. C., Ingvarsdottir, K., Wyce, A., Wood, A., Krogan, N. J., Henry, K. W., Li, K., Marmorstein, R., Greenblatt, J. F., Shilatifard, A., and Berger, S. L. (2005) Maintenance of low histone ubiquitylation by Ubp10 correlates with telomere-proximal Sir2 association and gene silencing. Mol. Cell 17, 585–594.
165 Gardner, R. G., Nelson, Z. W., and Gottschling, D. E. (2005) Ubp10/Dot4p regulates the persistence of ubiquitinated histone H2B: distinct roles in telomeric silencing and general chromatin. Mol. Cell. Biol. 25, 6123–6139.
166 Gasser, S. M., and Cockell, M. M. (2001) The molecular biology of the SIR proteins. Gene 279, 1–16.
167 Xiao, T., Kao, C. F., Krogan, N. J., Sun, Z. W., Greenblatt, J. F., Osley, M. A., and Strahl, B. D. (2005) Histone H2B ubiquitylation is associated with elongating RNA polymerase II. Mol. Cell. Biol. 25, 637–651.
168 Pavri, R., Zhu, B., Li, G., Trojer, P., Mandal, S., Shilatifard, A., and Reinberg, D. (2006) Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. Cell 125, 703–717.
169 Shukla, A., and Bhaumik, S. R. (2007) H2B-K123 ubiquitination stimulates RNAPII elongation independent of H3-K4 methylation. Biochem. Biophys. Res. Commun. 359, 214–220.
170 Fleming, A. B., Kao, C. F., Hillyer, C., Pikaart, M., and Osley, M. A. (2008) H2B ubiquitylation plays a role in nucleosome dynamics during transcription elongation. Mol. Cell 31, 57–66.
171 Emmanual, U., Zhang, Y., Kangasniemi, M., Saperstein, W., Moss, S. B., and Meistrich, M. L. (1995) Stage-Specific Distribution of the Spermatid-Specific Histone 2B in the Rat Testis'. Biol. Reprod. 53, 820–826.
172 Li, A., Maffey, A. H., Abbott, W. D., Conde e Silva, N., Prunell, A., Siino, J., Churikov, D., Zalensky, A. O., and Ausió, J. (2005) Characterization of nucleosomes consisting of the human testis/sperm-specific histone H2B variant (hTSH2B). Biochemistry 44, 2529–2535.
173 Shukla, A., Stanojevic, N., Duan, Z., Shadle, T., and Bhaumik, S. R. (2006) Functional analysis of H2B-Lys-123 ubiquitination in regulation of H3-Lys-4 methylation and recruitment of RNA polymerase II at the coding sequences of several active genes in vivo. J. Biol. Chem. 281, 19045–19054.
174 Dehé, P. M., Pamblanco, M., Luciano, P., Lebrun, R., Moinier, D., Sendra, R., Verreault, A., Tordera, V., and Géli, V. (2005) Histone H3 lysine 4 mono-methylation does not require ubiquitination of histone H2B. J. Mol. Biol. 353, 477–484.
175 Ng, H. H., Xu, R. M., Zhang, Y., and Struhl, K. (2002b) Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine. J. Biol. Chem. 277, 34655–34657.
176 Ezhkova, E., and Tansey, W. P. (2004) Proteasomal ATPases link ubiquitylation of histone H2B to methylation of histone H3. Mol. Cell 13, 435–442.
177 Lee, J. S., Shukla, A., Schneider, J., Swanson, S. K., Washburn, M. P., Florens, L., Bhaumik, S. R., and Shilatifard A. (2007) Histone Crosstalk between H2B Monoubiquitination and H3 Methylation Mediated by COMPASS. Cell 131, 1084–1096.
178 Wu, M., Wang, P. F., Lee, J. S., Martin-Brown, S., Florens, L., Washburn, M., and Shilatifard, A. (2008) Molecular regulation of H3K4 trimethylation by Wdr82, a component of the human Set1/COMPASS. Mol. Cell. Biol. 28, 7337–7344.
179 Dillon, S. C., Zhang, X., Trievel, R. C., and Cheng, X. (2005) The SET-domain protein superfamily: protein lysine methyltransferases. Genome Biol. 6, 227.
180 Lachner, M., O'Sullivan, R. J., and Jenuwein, T. (2003) An epigenetics road map for histone lysine methylation. J. Cell Sci. 116, 2117–2124.
181 Shi, X., Kachirskaia, I., Walter, K. L., Kuo, J. H., Lake, A., Davrazou , F., Chan, S. M., Martin, D. G., Fingerman, I. M., and Briggs, S. D., Howe, L., Utz, P. J., Kutateladze, T. G., Lugovskoy, A. A., Bedford, M. T., Gozani, O. (2007) Proteome-wide analysis in *Saccharomyces cerevisiae* identifies several PHD fingers as novel direct and selective binding modules of histone H3 methylated at either lysine 4 or lysine 36. J. Biol. Chem 282, 2450–2455.
182 Barber, T. D., McManus, K., Yuen, K. W., Reis, M., Parmigiani, G., Shen, D., Barrett, I., Nouhi, Y., Spencer, F., Markowitz, S., Velculescu, V. E., Kinzler, K. W., Vogelstein, B., Lengauer, C., and Hieter, P. (2008) Chromatid cohesion defects may underlie chromosome instability in human colorectal cancers. Proc. Natl. Acad. Sci. USA 105, 3443–3448.
183 Lydall, D., and Whitehall, S. (2005) Chromatin and the DNA damage response. DNA Repair (Amst). 4, 1195–1207.