

Histone Modifiers in Cancer: Friends or Foes?

Idan Cohen¹, Elżbieta Poręba², Kinga Kamieniarz¹, and Robert Schneider¹

Abstract

Covalent modifications of histones can regulate all DNA-dependent processes. In the last few years, it has become more and more evident that histone modifications are key players in the regulation of chromatin states and dynamics as well as in gene expression. Therefore, histone modifications and the enzymatic machineries that set them are crucial regulators that can control cellular proliferation, differentiation, plasticity, and malignancy processes. This review discusses the biology and biochemistry of covalent histone posttranslational modifications (PTMs) and evaluates the dual role of their modifiers in cancer: as oncogenes that can initiate and amplify tumorigenesis or as tumor suppressors.

Keywords: histone modifications, methylation, acetylation, phosphorylation, chromatin, cancer

Introduction

Covalent posttranslational modifications of proteins are a major mechanism by which protein function is regulated.^{1–4} These modifications can, for example, facilitate protein interactions or determine protein stability and thereby regulate specific cellular responses. They can be highly dynamic or have a function in epigenetic memory as stable marks. These properties enable precise regulation of key processes by these modifications such as cell cycle, apoptosis, signal transduction, and chromatin structure.

Currently, the best-characterized substrates for multisite protein modifications are the histone proteins. Although histones were originally considered simply as a static scaffold for DNA packaging, it is now becoming evident that they are dynamic proteins, which undergo multiple types of posttranslational modifications (PTMs) in order to play a fundamental role in regulating chromatin function.⁵ Nucleosomes are the basic units that compose the chromatin fiber. Each of these primary building units consists of 4 core histones (H3, H4, H2A, and H2B), which form a histone octamer, assembled from 2 heterodimers of the core histones H3 and H4 together with 2 heterodimers of H2A and H2B, around

which 146 base pairs of DNA are wrapped.⁶ The unstructured N- and C-terminals of the histone tails extend from the DNA gyres and are heavily posttranslationally modified with several different modifications such as acetylation, methylation, phosphorylation, ubiquitylation, sumoylation, ADP ribosylation, deimination, biotinylation, butyrylation, N-formylation, and proline isomerization (Fig. 1A).⁴ These covalent modifications are added or removed by enzymes often termed as “writers” and “erasers.” By establishing different combinations of histone PTMs, they can form the “histone code.”^{1,3} In addition, recent reports also show a substantial impact of PTMs occurring on residues within the histone-structured globular domain.^{7,8} While histone tail modifications serve mainly as “signaling platforms” and determine the interactions of histones with other proteins (Fig. 1B), modifications in the globular domain may directly affect the DNA-binding surface of the nucleosomes by altering the physical interaction between the histone body and DNA.⁹

In recent years, chromatin research focused mainly on the core histones, whereas the linker histone H1, which binds to the nucleosome at the entry/exit point of DNA, has been largely overlooked. Similar to the core histones, H1

histones are targets of multiple posttranslational modifications, including phosphorylation, acetylation, methylation, ubiquitination, and ADP ribosylation. Analysis with modern mass spectrometry has revealed a large number of modified residues, both in the tails and the globular domain of H1.^{10,11} H1 modifications are likely to be involved in the regulation of the multiple linker histone functions¹²; therefore, their characterization is necessary in order to better understand higher-order chromatin structure and dynamics.

The potential complexity of histone PTMs in regulating chromatin-dependent processes is reflected by more than 10 different types of modifications (listed above) at nearly 80 different sites that were reported on histone tails and the core domains (including the linker histone H1).⁴ The number of histone PTMs is likely to rise in the following

¹Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

²Department of Molecular Virology, Institute of Experimental Biology, Adam Mickiewicz University, Poznan, Poland

Corresponding Author:

Robert Schneider, Max Planck Institute of Immunobiology and Epigenetics, Stuebeweg 51, 79108 Freiburg, Germany
Email: schneider@immunbio.mpg.de

years because a huge effort is being made in searching for novel PTM sites, with significant improvement of several detection and genome-wide mapping techniques.¹³

Because PTMs occur on distinct amino acid residues on specific histones and within chromatin at certain genomic regions, this allows a vast range of flexibility in regulating chromatin dynamics and signaling transmission. By employing different combinatorial patterns of modifications, it is possible to regulate two opposite processes like transcriptional activation and repression using the same amino acid sequence within a histone. For example, acetylation of key lysine residues of histone H3 and H4 by histone acetyltransferases (HATs) is commonly associated with transcriptional activation, whereas low levels of acetylation are found at transcriptionally inactive chromatin (Fig. 1A).⁴ Another level of complexity and regulation can be found in the cross-talk between histone marks, where modifications regulate one another.^{2,14} A specific modification can directly influence the occurrence of another PTM either in cis, within the same histone, or in trans, between 2 adjacent histone molecules or even across different nucleosomes.¹⁵ This additional regulatory layer adds a greater combinatorial intricacy and larger diversity to the histone language.

Additionally, posttranslationally modified chromatin can also serve as a selective binding platform for regulatory proteins, which can then translate this signal into changes in chromatin structure or dynamics. Evolutionarily conserved specialized proteins, termed “histone readers,” possess the ability to specifically bind certain histone modifications and mediate the initiation of a defined nuclear process such as transcription, DNA repair, or replication (Fig. 1B).¹⁶ For example, it is now well established that trimethylated H3K9 (H3K9me3) can recruit and be bound by a protein known as heterochromatin protein 1 (HP1) via its evolutionarily conserved chromodomain.^{17,18} This interaction with H3K9me3 was shown to occur in “repressed”

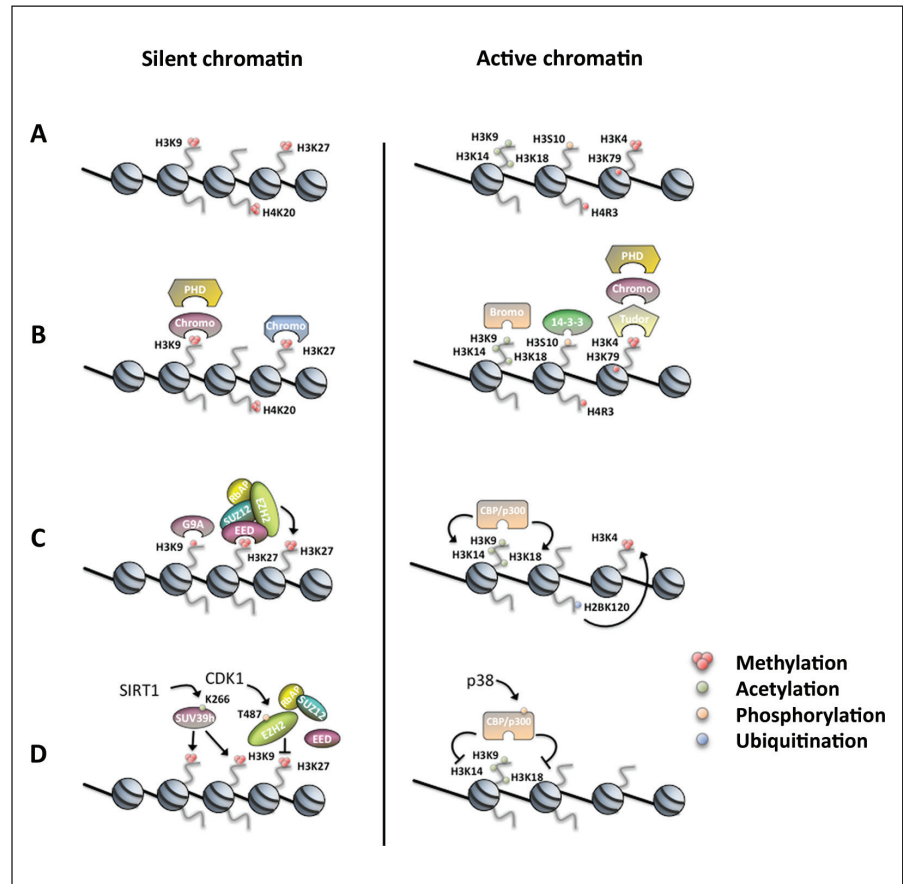


Figure 1. Histone PTMs regulating chromatin-dependent processes. **(A)** Specific histone PTMs can be found in either active (right panel) or silent (left panel) chromatin compartments. **(B)** These modifications can directly alter nucleosome/DNA interactions and/or mediate the binding of particular histone readers. **(C)** Modifications can regulate each other and block or promote the binding of histone modifiers. For example, the binding of the PRC2 component EED to H3K27me3 stimulates the enzymatic activity of EZH2 to place more H3K27me3 on neighboring H3 tails.^{20,21} In addition, also the HMT G9a is capable of binding its own substrate H3K9me1, showing both the ability of reading and writing the same epigenetic mark within the same polypeptide²⁶⁹ (left panel). Similarly, the p300 Bromo domain enables the setting of multiple acetyl marks within the H3 tail by binding to preacetylated sites, or the H2BK120ub mark is required for the propagation of H3K4me3 (right panel). **(D)** Histone modifiers can be also modified by PTMs, which regulate their activity. For example, HDAC SIRT1 stimulates the enzymatic activity of SUV39h by deacetylation of SUV39h K266 (left panel)¹³⁰; phosphorylation of EZH2 T487 by CDK1 interrupts PRC2 component assembly, thereby inhibiting EZH2 methyltransferase activity,²⁴³ and p300 C-terminal phosphorylation by p38 regulates its stability, which directly affects the histone acetylation state.^{22,23} For details, see text.

chromatin (i.e., heterochromatin) and leads to formation of compacted chromatin that can physically inhibit the access of the transcriptional machinery. In addition to H3K9me3, K26-methylated H1 can also be bound by HP1.¹⁹ Interestingly, histone-modifying complexes that set or remove these marks were recently shown to possess specific “reader” capabilities. For example, binding of trimethylated H3K27 (H3K27me3) by the

Polycomb repressive complex 2 (PRC2) stimulates the activity of the enzymatic PRC2 subunit enhancer of zeste homolog 2 (EZH2), which in turn places more H3K27me3 marks on the neighboring nucleosomes.^{20,21} These findings show that patterns of histone modifications can be “read and written” by the same interacting factor(s) or complexes (Fig. 1C).

Recently, several reports demonstrated regulation of the “writers” and

“readers” themselves by PTMs, which adds an additional layer of regulation. For example, p300 that acts as a crucial element in the eukaryotic gene regulation network was shown to be regulated by phosphorylation, which greatly reduces its HAT activity.^{22,23} The mitogen-activated protein kinase p38 associates with p300 and is implicated in the phosphorylation-mediated degradation of p300 stability and function (Fig. 1D).²⁴ Also, EZH2 is now known to be phosphorylated by cyclin-dependent kinase 1 (CDK1) in a cell cycle-dependent manner (Fig. 1D).^{25,26} These sequential PTMs were shown to affect either EZH2 enzymatic activity or its recruitment to target genes by increasing the binding to non-coding RNA (also see below).

While it is still under discussion whether histone modifications form a true “code,” it has become clear that they control (almost) all chromatin-mediated processes such as gene expression, DNA repair, chromosome condensation, development, and differentiation as well as disease processes. Numerous reports show a tight link between age-related diseases, birth defects, and several types of cancer with disruption of certain histone PTMs or patterns of PTMs.²⁷⁻³⁰ Therefore, we believe that one of the major goals in postgenomic biology is to unravel the molecular basis and the physiological role of covalent protein modifications. Identifying novel histone modifications, the modifying enzymes, and the downstream effector proteins that can bind to these marks and translate them into specific biological outcomes sets a fascinating challenge. This has the potential to identify novel disease pathways and thereby new therapeutic targets as well as diagnostic markers.

In this review, we discuss the different types of covalent histone modifications (in particular, acetylation, phosphorylation, and lysine methylation) and the corresponding modifiers with a specific focus on their role in cancer. In addition to the modifications of histones, we also describe modifications of the modifiers that regulate their

activity, adding another level of complexity to the language of PTMs.

Histone Acetylation and Acetyltransferases

Lysine acetylation is a reaction in which an acetyl group from the acetyl-coenzyme A (acetylCoA) cofactor is transferred to the ϵ -amino nitrogen of lysine residues. This reaction is catalyzed by histone acetyltransferases (HATs), whereas the reverse reaction is performed by histone deacetylases (HDACs).

Already in the early 1960s, it has been observed that histones can be covalently modified by acetyl groups,^{31,32} and very soon, this modification has been linked to transcriptional activation.³³ However, the first HAT catalytic proteins were isolated and cloned only in the 1990s.³⁴⁻³⁶ The revolutionary finding that a transcriptional coactivator protein, Gcn5, is a nuclear HAT³⁵ led to a surge in HAT-related research and the identification of numerous enzymes and their substrates. HATs can be grouped on the basis of their catalytic domains into 3 families: Gcn5 N-acetyltransferases or GNATs (including Gcn5, PCAF, Hat1, and others); MYST HATs (named for the founding members of this family: MOZ/Morf, Ybf2, Sas2, and Tip60); and an “orphan class” of HAT enzymes lacking a true consensus HAT domain, featuring, among others, p300/CBP and Taf1.³⁷ Representative members of the families, with important physiological roles as well as putative links to carcinogenesis, are subject to a more detailed discussion below.

In general, opposite to HATs, HDACs negatively regulate gene expression. Four HDAC classes have been identified: class I HDACs (HDAC1-3 and 8) are related to yeast Rpd3, class II HDACs (HDAC4-7, 9, and 10) are related to yeast Hda1p, and class IV HDACs contain one unrelated member (HDAC11). The enzymes in those classes are zinc dependent. Class III HDACs (sirtuins) are NAD-dependent enzymes related to yeast Sir2.³⁸ Sirtuins and their

involvement in cancer are the subject of another review in this issue (see Bosch-Presegué & Vaquero).

A common feature of HATs and HDACs is that they operate within multi-protein complexes and often the same enzyme resides within more than one complex. The complex in which a given HAT or HDAC is incorporated determines in many cases its substrate specificity and/or its interaction with other protein factors—and as such has a profound effect on its function.^{37,39} In general, HATs, and to an even greater extent, HDACs, are promiscuous enzymes when compared to the highly specific histone methyltransferases (HMTs).

HATs and HDACs were initially found to target histones as substrates but subsequently were also shown to target many nonhistone proteins, a fact that eventually led to a change in their nomenclature and renaming as lysine acetyltransferases and lysine deacetylases (the new nomenclature is not commonly employed in the field yet; therefore, in this review, we use the traditional nomenclature).⁴⁰

Apart from the core histones, linker histone H1 can also be acetylated. The first demonstration of H1 lysine acetylation *in vivo* came for H1.4K26.⁴¹ In this study, it has been shown that SirT1, a HDAC involved in establishing repressive chromatin, interacts with H1.4 and is able to deacetylate H1.4K26 *in vitro*. However, it is still unknown which enzyme acetylates H1.4K26 and what is the biological function of this mark. A recent mass spectrometry study revealed that there are many acetylation sites on H1,¹¹ awaiting functional studies.

There is now a wealth of evidence pointing to a role of core histone acetylation in transcriptional activation, and two molecular mechanisms have been suggested. On the one hand, acetylation partially neutralizes the positive charge of histones, weakening their interaction with the nucleosomal DNA, thus probably “opening” chromatin structure and facilitating the access of transcription factors to their recognition elements.⁴²

On the other hand, acetylated lysines can create a specific signal for regulatory factors or chromatin-remodeling complexes and contribute to their targeting to a specific region. Consistent with this hypothesis, it has been found that bromodomains act as acetyl-lysine recognition modules, directing enzymes that contain them to particular chromosomal sites.^{43,44} Besides transcription, new roles for histone acetylation have been uncovered and include nucleosome assembly, chromatin folding, heterochromatic silencing,³⁹ DNA damage repair, and replication.^{37,45,46}

We now move from general functions of histone acetylation to a discussion of representative HAT enzymes, together with their substrates, and highlight their potential role in oncogenesis.

Gcn5 and the SAGA Complex

The *Tetrahymena thermophila* protein p55 was the first identified nuclear, transcription-related HAT enzyme and shown to be the homolog of yeast Gcn5 (general control nonderepressible 5).³⁵ An intriguing observation at that time was the inability of recombinant Gcn5 to acetylate nucleosomal histones, while it exhibited strong HAT activity on recombinant histones. It has been subsequently shown that native Gcn5 exists in high molecular weight complexes, whose subunits enable Gcn5 to acetylate nucleosomes.⁴⁷ The more notable complex is SAGA (named for Spt-Ada-Gcn5 acetyltransferase), a 2-MDa multiprotein, chromatin-modifying molecular machine, highly conserved between yeast and humans. Human SAGA was also named in the literature as TFTC (TATA-binding protein-free TAFII-containing complex).⁴⁸⁻⁵⁰ A second distinct Gcn5-containing complex in metazoans is ATAC (Ada2a containing).⁵¹

While most metazoan genomes code for one Gcn5-type factor, vertebrates have a second gene encoding pCAF (p300/CBP-associated factor), which has 73% identity to Gcn5.⁵⁰ Gcn5 is an essential protein both in *Drosophila*⁵² and mice⁵³; moreover, it is able to compensate for pCAF loss in mammals:

pCAF^{-/-} mice have elevated Gcn5 levels, allowing them to develop normally.⁵³

The substrate specificity of Gcn5 *in vitro* depends on the complex it is in and, to a lesser extent, on the organism from which it is purified. As part of the SAGA complex, Gcn5 acetylates preferentially 4 lysine residues in the N-terminal tail of histone H3: H3K9, K14, K18, and K23.⁵⁰ *In vivo*, *Drosophila* Gcn5 was found to be involved in acetylation of larval polytene chromosomes at H3K9 and H3K14, and surprisingly also H4K5 and H4K12, which was not predicted from biochemical studies.^{52,54} In chicken cells, Gcn5 loss leads to decreased acetylation levels of H3K9 and H2BK16.⁵⁵ Disruption of Gcn5 HAT activity in mice leads to decreased acetylation levels of H3K9/K18 and H4K12, but H3K14 remains unchanged.⁵⁶ In human cells, Gcn5 is involved in the acetylation of H3K9 and K14, both in the ATAC and SAGA complexes.⁵⁷ In addition to being a “writer” of the histone code, SAGA also contains multiple domains capable of recognizing acetylated and methylated histone PTMs so it can “read” the combinatorial histone code.^{48,58}

Apart from the well-established functions of Gcn5 and the SAGA complex in core histone acetylation and transcriptional activation, new roles are emerging for this complex. For example, while SAGA is mostly known for activating highly regulated genes that respond to environmental stresses^{59,60} and is required for transactivation of nuclear receptors,^{61,62} it can also play an inhibitory role in the transcription of some inducible genes, such as HIS3 and ARG1.^{63,64} In particular, while Gcn5 is required for normal HIS3 transcriptional start site selection and transcriptional activation, another SAGA subunit, Spt8, inhibits TBP binding to the HIS3 promoter.⁶³ Furthermore, SAGA is now emerging as a regulator of a whole set of mRNA-related processes, including transcription elongation, mRNA splicing, and export.⁶⁵⁻⁶⁷ Finally, Gcn5 and SAGA appear to be important for

the maintenance of genome integrity through promoting replication-coupled nucleosome assembly, assisting in DNA repair especially after UV damage, as well as participating in telomere maintenance.⁶⁸⁻⁷⁰

Taking into account the SAGA complex’s functional importance, it is not surprising that mutations or altered expression of SAGA subunits correlate with neurological disease and aggressive cancers in humans.^{49,71} SAGA functions as a cofactor for several proto-oncoproteins.⁵⁰ In particular, the subunits TRRAP and Gcn5 regulate the oncogenic activity of the oncoprotein c-Myc and tumor suppressor p53,⁷² and another SAGA subunit, SPT7, is implicated in the transcription of several Myc-dependent genes.⁷³ BRCA1 is a tumor suppressor protein with E3 ubiquitin ligase activity and is involved among others in DNA repair and cell cycle progression. Mutations in the C-terminal transactivation domain of BRCA1, which predispose women to early onset of breast cancer, result in a loss of interaction between BRCA1 and TRRAP, which in turn prevents Gcn5/TRRAP from coactivating BRCA1. In fact, it seems that the HAT activity of Gcn5 is necessary for BRCA1-mediated gene regulation and DNA repair.⁷⁴ Further on, the deubiquitinase subunit of SAGA, USP22, has been grouped by transcriptional profiling into an 11-gene signature of poor prognosis in multiple types of cancer.^{75,76} USP22, together with Gcn5, protects telomeres from DNA damage through the stabilization of the TRF1 component of shelterin, which is dependent on USP22-mediated deubiquitination of TRF1.⁷⁷

The Histone Acetyltransferase MOF

MOF (males absent on the first) is a member of the MYST family of HATs and has very particular characteristics. The *Drosophila* MOF protein is particularly well studied as a key component of the dosage compensation complex, which is required to upregulate X-linked gene expression in male flies having one X chromosome, in contrast to

females that have two.⁷⁸ While mammals use a different mechanism for dosage compensation (one of the two X chromosomes gets inactivated in females), the MOF enzyme is conserved between humans and flies, together with the multiprotein complexes in which it resides.⁷⁹⁻⁸¹

An additional striking feature of MOF is its high acetyltransferase specificity for lysine 16 on histone H4 (H4K16), a unique and unusual characteristic for a HAT enzyme.^{78,82} Moreover, MOF appears to be the main acetyltransferase for H4K16, as MOF knockout mice embryos exhibit a loss of acetylation specifically at this residue.⁸³ *Nota bene*, those MOF-null embryos die at implantation *in vivo*; hence, MOF is essential for mammalian development. The acetylation of H4K16 has profound effects on chromatin structure: it inhibits *in vitro* the formation of compact 30-nm-like fibers and impedes the ability of chromatin to form cross-fiber interactions.⁸⁴ Interestingly, acetylation of only 30% of H4K16 disrupts nucleosomal array folding to the same extent as the complete removal of all N-terminal histone tails and inhibits chromatin compaction to a greater extent than deletion of the H4 N-terminal tail altogether.⁸⁵ By now, no other single histone acetylation or methylation has been found to affect so profoundly the chromatin structure as H4K16ac.

MOF is involved in multiple cellular processes. Most notably, H4K16 acetylation mediated by MOF leads to increased transcription both *in vitro* and *in vivo*.^{81,82} However, apart from transcription, MOF participates in DNA damage response and, together with another MYST family HAT, Tip60, acetylates p53 on K120 to influence the cell's decision to undergo apoptosis versus cell cycle arrest.⁸⁶⁻⁸⁹ One could hypothesize that deregulation of this type of processes might influence cancer progression. Concomitantly with this, the loss of acetylation at H4K16 (as well as H4K20 methylation) has been found to be a common hallmark of human cancer.⁹⁰ This reduction occurs at

hypomethylated DNA repetitive regions, which have been previously linked to chromosomal instabilities.^{91,92} It is currently not clear whether and how MOF is deregulated in those cancers; mutations in MOF, downregulation of its enzymatic activity, or its mistargeting could all account for H4K16ac loss. For comprehensive reviews on MOF and other MYST family members' involvement in cancer, see Avvakumov and Côté⁹³ and Rea *et al.*⁹⁴

The Histone Acetyltransferases p300 and CBP

p300 and CBP were originally identified as binding partners of the adenovirus early region 1A (E1A) protein^{95,96} and the cyclic AMP response element (CRE)-binding protein,⁹⁷ respectively. They were then shown to function as transcriptional adaptors, and in 1996, 2 independent groups have demonstrated that p300 and CBP possess HAT activity.^{98,99}

p300 and CBP have a multidomain structure and contain, in addition to the enzymatic HAT domain, several protein interaction domains.¹⁰⁰ p300 and CBP are the most promiscuous HAT enzymes; they are capable of acetylating all the 4 core histones on multiple residues¹⁰¹ as well as at least 70 other proteins, including p53, Rb, E2F, and myb.¹⁰² Most of p300 and CBP target sites on histones can be also acetylated by other enzymes, and a recent study has identified H3K18ac and H3K27ac as the only histone acetylation sites significantly reduced upon CBP and p300 depletion in mouse embryonic fibroblasts.¹⁰³ p300 and CBP are highly homologous, in particular their HAT domains, which share >90% sequence identity, and are often referred to as p300/CBP, indicating a presumable functional redundancy. However, *in vivo* studies have revealed that CBP and p300 have both shared as well as unique properties. For example, while both CBP and p300 knockout mice die before birth, displaying an open neural tube defect,^{104,105} only p300^{-/-} (and not CBP^{-/-}) embryos exhibit

defective development of the heart. In contrast, CBP^{+/-} mice display growth retardation and craniofacial abnormalities as well as an increased incidence of hematological malignancies, which are not shared by their counterpart p300^{+/-} mice.^{104,105} Taken together, this suggests that CBP and p300 are not redundant and may have some unique functions.¹⁰⁶

p300 and CBP function primarily as transcriptional cofactors for a number of nuclear proteins. They are able to interact with the basal transcription factors TATA-binding protein (TBP) and TFIIB,^{107,108} and can form a complex with RNA polymerase II.^{109,110} They are also cofactors for oncoproteins (e.g., fos, jun, and myb), transforming viral proteins (e.g., E1A) and tumor suppressor proteins (e.g., p53, E2F, Rb, or BRCA1).¹¹¹ Likewise, p300 and CBP aid transcription in 2 ways: firstly, they act as a bridge, linking DNA-binding transcription factors to the basal transcriptional machinery, and secondly, by acetylating histones, they create a permissive chromatin environment for gene expression.

Apart from transcription, p300 and CBP are also implicated in other processes. They interact with (and often acetylate) multiple proteins involved in DNA replication and repair, such as proliferating cell nuclear antigen (PCNA), DNA polymerase β , or thymine DNA glycosylase.^{45,112,113} CBP and p300 have been also implicated in the regulation of the progression of the cell cycle via interactions with cyclin E and cyclin-dependent kinase 2¹¹⁴⁻¹¹⁶ as well as in nuclear import by acetylation of importin- α .¹¹⁷

Concomitant to their important physiological functions, p300 and CBP misregulation is strongly linked to cancer. p300 and CBP seem to have a dual role in oncogenesis; they can be either friends or foes. On the one hand, genetic studies show that they can act as tumor suppressors, as CBP- and p300-null chimeric mice develop hematological malignancies.¹¹⁸ In fact, mutations of both HATs have been reported in human tumors; in particular, loss-of-function

point mutations have been detected in colorectal, gastric, breast, ovarian, lung, and pancreatic carcinomas.¹¹⁹⁻¹²¹ On the other hand, aberrant activation or localization of those HATs can be oncogenic, and chromosomal translocations resulting in fusion proteins, including MLL-CBP, MLL-p300, MOZ-CBP, and MOZ-p300, have been identified in acute myeloid leukemia (AML), myeloid/lymphoid or mixed lineage leukemia (MLL).¹¹¹ The oncogenic potential of MLL-CBP has been confirmed in murine models too. A tumor suppressor function for p300 and CBP is also contradicted by a number of cell culture studies, as depletion or reduction of those enzymes leads to proliferation defects and/or activation of senescence checkpoints.¹¹¹

Nonhistone Targets of Acetyltransferase Activities

Even though core histones are the best-characterized acetylation substrates, they are not the only ones. For example, high mobility group (HMG) chromatin-associated proteins have been shown to be acetylated already in the 1970s¹²²; however, the *in vivo* importance of these acetylation events still remains to be studied. On the other hand, posttranslational modification with acetyl groups has been shown to be important for the regulation of numerous transcriptional activators including the tumor suppressor p53,¹²³ proto-oncogene c-Myb,¹²⁴ tissue-specific activators such as GATA-1 or MyoD,¹²⁵ the cell cycle-related factor E2F,¹²⁶ or Tat, a transcriptional activator encoded by HIV-1.¹²⁷ Interestingly, many chromatin-modifying enzymes seem to be regulated via acetylation as well: not only are HATs and HDACs acetylated themselves,^{128,129} but also, the activity of the HMT SUV39H1 is regulated by acetylation in its catalytic SET domain¹³⁰ (see below). Protein acetylation takes place also outside the nucleus, in the cytoplasm and mitochondria, and it has been found to be a major mechanism by which key proteins are regulated in physiological

processes such as cell migration, metabolism, and aging but also in pathological processes such as cancer and neurodegenerative disorders.¹³¹ It is currently not known whether the cytoplasmic substrates of HAT/HDAC proteins are also involved in oncogenesis. A recent mass spectrometry screen identified 3,600 lysine acetylation sites on 1,750 proteins,¹³² supporting the notion that acetylation is a widespread mechanism of regulating protein functions.¹³³ This also means that inhibiting HDACs does not only alter core histones' acetylation but also acetylation of many other factors, which should be taken into consideration. Therefore, further studies are needed to uncover the truly important mediators of HDAC's therapeutic effects.

Histone Phosphorylation

Histone phosphorylation is a dynamic modification in which a phosphate group is added to a specific amino acid's residue. It was first identified in the late 1960s,^{134,135} and the first histone kinase was discovered in 1968.¹³⁶ For a long time, serine and threonine were the only residues identified to be phosphorylated in histones, but recent data also uncovered histone tyrosine phosphorylation (Fig. 2).¹³⁷⁻¹⁴¹ Histones H1, H2A, H2B, H3, and H4 are all phosphorylated at multiple sites,¹⁴²⁻¹⁴⁷ but the most studied are so far the phosphorylations of H3. Histone phosphorylation reaction is catalyzed by several distinct kinases that are mostly specific for individual histone residues, although one histone residue can be phosphorylated by multiple kinases.¹⁴⁸

The precise mechanistic role of histone phosphorylation in the regulation of chromatin structure is not yet fully elucidated. There are several potential mechanisms how histone phosphorylation can affect chromatin-related processes. For example, it has been shown that histone phosphorylation can directly influence chromatin compaction *in vivo*.¹⁴⁹ Studies in *Tetrahymena* demonstrated that formation of a "negative patch,"

resulting from phosphorylation of many residues in linker histone H1, influences its binding to DNA and leads to an alteration in chromatin structure that facilitates transcription in this region.¹⁴⁹ Moreover, histone phosphorylation can also affect the binding of nonhistone proteins, excluding them from chromatin. The best-characterized example of this mechanism is the dissociation of HP1 protein from chromatin subsequent to mitosis-specific H3S10 phosphorylation (H3S10p).¹⁵⁰ In addition to this, phosphorylated histone residues can also be recognized by adapter proteins that serve as docking molecules for other nonhistone proteins.

Histone phosphorylation has been linked to many different cellular processes, for example, transcriptional activation, mitosis, meiosis, DNA damage repair, and apoptosis (Fig. 2). Therefore, a single phosphorylated residue within a histone can be linked to different chromatin states. For example, on one hand, H3S10p is associated with transcriptional activation of induced immediate-early genes,¹⁵¹ but on the other hand, it is also globally present at the highly condensed chromatin during mitosis.¹⁵²

Histone Phosphorylation in Transcription Regulation

A number of studies demonstrate an essential role of histone H3S10 phosphorylation in gene activation. H3S10 is phosphorylated by various kinases. Phosphorylation of H3S10 by mitogen- and stress-activated protein kinases 1 and 2 (MSK1 and MSK2) as well as RSK2 kinase has been shown to play a role in the activation of mitogen-stimulated immediate-early response genes, such as c-fos and c-jun.^{146,151,153-155} H3S10 phosphorylation mediated by I κ B kinase- α (IKK- α) occurs at NF- κ B-responsive promoters in response to inflammatory cytokines.¹⁵⁶ Furthermore, Pim1 kinase catalyzes H3S10 phosphorylation at the E-boxes in Myc-target genes, contributing to their transcriptional activation after stimulation with growth factor.¹⁵⁷ It is noteworthy

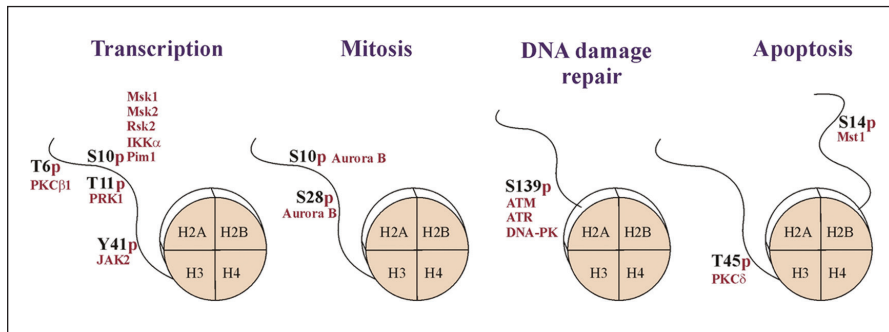


Figure 2. Histone phosphorylation is implicated in multiple cellular processes. Phosphorylation of the histone H3, H2AX, and H2B is involved in the regulation of transcription, mitotic chromatin condensation, and DNA damage response and apoptosis, respectively. Histone octamers and the modified tails are schematically shown. The enzymes responsible for these modifications (red) and the sites of phosphorylation are indicated. For details, see text.

that PIM1 kinase was demonstrated to cooperate with Myc in cell transformation and tumor growth.¹⁵⁷

Additionally, an important role in transcriptional regulation can be found in a cross-talk between H3S10p and other histone modifications. For example, H3S10 phosphorylation can stimulate acetylation of histone H3K14,^{158,159} inhibit acetylation of histone H3K9,¹⁶⁰ and affect methylation of histone H3K9,¹⁶¹ thus supporting an active transcriptional state. Gene activation by cooperative H3S10p and H3K14 acetylation has been broadly reported.^{158,162} In line with this simultaneous phosphorylation and acetylation, H3S10 and K14 can recruit 14-3-3 proteins concomitant with dissociation of heterochromatin proteins HP1 from adjacent H3K9me2.¹⁶³

Displacement of the HP1 α protein from chromatin occurs not only due to H3S10p but also in response to H3Y41 phosphorylation.¹⁴¹ This histone residue has been shown to be phosphorylated in hematopoietic cells¹⁴¹ and is catalyzed by Jak2 kinase (Janus kinase 2), an enzyme that plays an important role in hematopoiesis and hematological malignancies.^{141,164} H3Y41 phosphorylation and displacement of HP1 α lead to activation of oncogenes.¹⁴¹ The genome-wide mapping of H3Y41-phosphorylated chromatin identified >2,000 potential JAK2-target genes, including genes encoding JAK2 itself, c-Myc, and the histone demethylase JMJD2C.¹⁶⁵

Another remarkable example of interdependence between histone H3 methylation and phosphorylation, which occurs between 2 nonadjacent sites within H3, comes from recent studies showing androgen receptor (AR)-dependent transcriptional regulation by protein kinase C-related kinase 1 (PRK1) and protein kinase C β I (PKC β I).^{166,167} Phosphorylation of H3T11 by the PRK1, in an androgen receptor-dependent manner, activates transcription by enhancing demethylation of H3K9 by the demethylases LSD1 (demethylating H3K9me1/me2) and JMJD2C (demethylating H3K9me2/me3).¹⁶⁶ PRK1 is crucial to androgen receptor function because PRK1 knockdown inhibits the androgen receptor-dependent transcription. It is noteworthy that high PRK1 and H3T11p levels correlate with high Gleason scores of prostate carcinomas, suggesting a role for PRK1 in tumorigenesis.

The PKC β I-mediated phosphorylation of histone H3T6 plays also an important role in AR-dependent gene activation. In the presence of ligand, association of ligand-activated AR with PRK1 results in activation of PKC β I kinase that phosphorylates H3T6. Phosphorylation of H3T6 prevents the removal of H3K4 methyl marks by LSD1 (specific for H3K4me1/me2) and JARID1B (specific for H3K4me2/me3), which contributes to transcriptional activation.¹⁶⁷ Similar to H3T11p, the level of phosphorylated H3T6 also positively

correlates with high Gleason scores of prostate carcinomas.¹⁶⁷ Moreover, knockdown of PKC β I can inhibit tumor cell proliferation *in vitro* and cancer progression of tumor xenografts *in vivo*, emphasizing the important role of PKC β I in the control of prostate tumor cell growth.¹⁶⁷

Histone Phosphorylation and Mitosis

Apart from transcriptional regulation, histone phosphorylation also plays essential roles during mitosis. Several histone sites have been detected to be phosphorylated by mitotic kinases and to be involved in chromatin condensation as well as in controlling chromosome localization of the proteins that function in chromosome segregation during cell division. A hallmark of mitosis is the global phosphorylation of H3S10 that begins during prophase, reaches maximum levels in metaphase, and is followed by a decrease during cell cycle progression to telophase. Reversible phosphorylation of H1 histones is also linked to mitosis. Phosphorylation of H1 is strongly cell cycle regulated, reaching maximum during late G2 and mitosis, and abruptly decreases in telophase. It occurs predominantly in the C-terminal tail on (S/T) PXX consensus motifs recognized by cyclin-dependent kinases, CDKs.¹⁶⁸⁻¹⁷¹ Phosphorylation of both H1 and H3S10 specific for mitosis has been proposed to be required for proper condensation of mitotic chromatin.^{152,172} Mitotic H3S10 phosphorylation is mainly catalyzed by Aurora B. Apart from histones, Aurora B also phosphorylates a number of mitotic proteins. It is a catalytic component of the chromosomal passenger complex that regulates the spindle checkpoint and controls correct microtubule attachments to kinetochores.¹⁷³⁻¹⁷⁵ Although Aurora B has been found to be overexpressed in certain tumor types, its role in tumorigenesis is currently not clear. It is still not known whether the increased Aurora B expression in cancer cells reflects hyperproliferation or carcinogenesis.¹⁷⁶ Additionally, the evidence of genetic changes in *AURKB* locus in

cancers is minimal.¹⁷⁷ On the other hand, increased Aurora B levels in several cancers including advanced colorectal cancer, glioblastoma, and ovarian and hepatocellular cancers have been associated with poor prognosis.¹⁷⁸⁻¹⁸⁰ Furthermore, exogenous overexpression of Aurora B in Chinese hamster embryo cells, concomitant with increased mitosis-specific H3S10 phosphorylation, can enhance chromosome instability and tumor invasiveness.¹⁷⁸

Histone Phosphorylation in DNA Repair

Histone PTMs, such as phosphorylation, ubiquitylation, and acetylation, play an essential role in DNA damage response pathways and participate in recruitment of downstream DNA damage response and repair proteins, as well as in the amplification of DNA damage signals. The histone H2A variant, H2AX, is a key component of DNA damage response.^{181,182} H2AX accounts for 2% to 25% of the total histone H2A pool in mammalian cells.¹⁸³ In response to DNA damage, H2AX is rapidly phosphorylated (i.e., within minutes) at S139, and this modification is spread over megabases from the break site.¹⁸⁴ Depending on the way double-strand breaks (DSBs) are induced, this reaction is mediated by several enzymes belonging to a family of phosphatidylinositol-3 kinase-like kinases (PIKK). Phosphorylation of H2AXS139 is catalyzed by ATM and DNA PK kinases in response to ionizing irradiation (IR)-induced breaks, and ATR governs H2AXS139 phosphorylation after DNA replication stress.^{185,186} The S139-phosphorylated H2AX, named γ H2AX, plays an important role during the early stage of DNA damage response. It is necessary for the amplification of DNA damage signal and subsequent recruitment of a number of proteins involved in DNA damage response at the site of DSB. γ H2AX is directly bound by MDC1 (mediator of DNA checkpoint 1), which serves as a platform for recruiting other DNA damage response proteins, including 53BP1

(p53-binding protein 1), BRCA1, NBS1 (Nijmegen breakage syndrome), and microcephalin.¹⁸⁷⁻¹⁹² Subsequently, DNA damage response proteins transduce signals to Chk1 (checkpoint kinase 1) and Chk2 (checkpoint kinase 2) effector kinases that regulate many downstream targets, leading to the activation of DNA repair genes and a cell cycle delay.¹⁹³

Recent studies have shown that H2AX can also be phosphorylated at Y142.^{137,138} A novel kinase, WSTF (William syndrome transcription factor), is catalyzing this modification. H2AXY142 is constitutively phosphorylated at physiological conditions and is dephosphorylated by EYA (eyes absent homolog) phosphatases following the DNA damage.^{137,138} Dephosphorylation of H2AXY142p has been suggested to function as a switch mechanism determining cell fate after DNA damage, which enables the cell to choose between a DNA repair and an apoptotic response.¹³⁸ The proposed model is that H2AXY142 dephosphorylation, which occurs along with H2AXS139 phosphorylation (in response to ionizing irradiation), stimulates recruitment of proteins involved in DNA repair, such as MDC1, Mre11, and Rad50.¹³⁸ Conversely, when both H2AXS139p and H2AXY142p marks persist, apoptosis-inducing protein kinase JNK1 (Jun N-terminal kinase) is preferentially recruited and can stimulate an apoptotic response.¹³⁸ However, there are still unanswered questions concerning this hypothesis. For instance, whereas phosphorylation of S139 occurs very rapidly following DSB and reaches its maximum within minutes, the kinetics of H2AXY142p dephosphorylation are much slower and occur over several hours.¹⁹⁴ Further studies are needed to fully elucidate the role of Y142 phosphorylation and to completely understand the regulatory mechanisms to determine cell fate after DNA damage.

Considering the important role of H2AX phosphorylation in DNA damage response, it is not surprising that its deregulation is implicated in disease. Deficiency of both H2AX and DNA damage response proteins predisposes

to cancer. The human H2AX gene (*H2AFX*) is located on chromosome 11q23.2-23.3, a region that is frequently lost in human cancers.^{195,196} It has been demonstrated that cells deficient for H2AX are sensitive to ionizing radiation and exhibit genomic instability and enhanced susceptibility to cancer.^{195,196} Furthermore, defects in kinases responsible for H2AX phosphorylation have been implicated in certain cancers.¹⁹⁷

Histone Phosphorylation in Apoptosis

Besides H2AXY142 phosphorylation, which has been suggested to function as a switch for apoptosis, the phosphorylation of histone H2B at S14 is also implicated in apoptosis. This modification is mediated by caspase-cleaved MST1 (mammalian STE20-like kinase 1) kinase.¹⁹⁸ During apoptosis, upon caspase-mediated cleavage, MST1 becomes cleaved, and its activated form translocates to the nucleus.^{199,200} The MST1-mediated H2BS14 phosphorylation (H2BS14p) correlates with chromatin condensation and occurs immediately prior to DNA laddering. Therefore, H2BS14p might be involved in the regulation of apoptotic chromatin condensation. Its mechanistic role is still unknown, but the authors propose H2BS14p to function through regulation of higher-order chromatin structure.¹⁹⁸ In support of this, phosphorylation of H2BS14 inhibits nuclear transport by regulating the regulator of chromosome condensation 1 (RCC1). Likewise, apoptosis-related phosphorylation has also been detected to occur on histone H3T45, catalyzed by protein kinase C δ (PKC δ).²⁰¹ The functional role of H3T45 phosphorylation has not yet been elucidated; however, it has been suggested that it might induce structural changes within the nucleosome, thus enabling DNA fragmentation.

Histone Methylation

Methylation of histone tails occurs mainly at lysines (K) and arginines (R). In this review, we focus mainly on lysine methylation; for a recent review on

arginine methylation, see Di Lorenzo and Bedford.²⁰² Although, in the early days, histone methylation was considered to be a stable and irreversible epigenetic mark, the discovery of the lysine histone demethylases (HDMs) flipped over this idea and showed that it can be dynamic.²⁰³ This finding also provided the first indication of how methylation may regulate transcription and enable gene maintenance in an on or off state.

Methylation as a histone PTM has a number of fundamental differences compared to the above-described acetylation and phosphorylation. Lysines can be monomethylated, dimethylated, or trimethylated, whereas arginines can be monomethylated or dimethylated. For arginine methylation, symmetric or asymmetric dimethylation must be distinguished because the precise methyl states are relevant for functional outcome. In contrast to acetylation, addition of a methyl group on a side chain does not alter its charge, and therefore, it is unlikely that methylation would directly modulate nucleosomal interactions required for chromatin folding. Another distinct characteristic of histone methylation is that it has been implicated in both transcriptional activation and repression. For example, trimethylation of H4K20 (H4K20me₃), H3K9, and H3K27 was shown to be involved in transcriptional silencing, whereas trimethylation at H3K4 (H3K4me₃) and H3K36 act as active marks.¹⁵ Arginine methylation, catalyzed by a family of protein arginine methyltransferases (PRMTs), can act as a transcriptional activator or repressor.²⁰⁴ Methylation of lysines has been described on all 4 core histones but also on linker histone H1. H1.4K26 was the first methylation site studied in H1.²¹⁹ Recently, H1.2K187 has been identified as a novel G9a target on H1,²⁰⁵ and multiple other methylation sites have been mapped on the linker histone, awaiting functional characterization.¹¹

It is now well known that lysine methylation can provide a binding surface that coordinates the recruitment of histone “readers.” Hence, multiple protein domains

binding specifically to methyl lysine had been identified: PHD fingers (for binding to H3K4me₃ as in ING2 and the BPTF PHD finger of NURF),^{206,207} Tudor domains (for binding to H3K4me₃ and H4K20me₃ as in JMJD2A demethylase),²⁰⁸ chromodomains (for binding to H3K9me_{2/3} as in HP1),²⁰⁹ and WD-40 domains (for binding to H3K27me₃ as in EED).^{210,211} Proteins containing these domains can be found in many chromatin-remodeling and chromatin-modifying complexes as well as transcriptional activators/repressors. These specific “code-reading” abilities can help to either maintain or also rapidly rearrange local genomic regions in an active or inactive chromatin state. Lysine methylation may also regulate the depletion of specific methyl-sensitive binders that interact with unmethylated histone tails²¹² and by that may also prevent the propagation of adjacent modification on a following residue.

Taken together, because methylation has so many faces and is considered to be an essential step involved in many cell fate determinations such as developmental and differentiation processes,²¹³⁻²¹⁵ pluripotency,²¹⁶ and maintaining genome integrity,²¹⁷ it is no surprise that it is highly associated with cancer and other diseases (approximately 50% of the HMTs encoded by the human genome are now linked to diseases and in particular tumorigenesis). In the following part, we mainly focus on HMTs that have clear links to tumorigenesis and discuss the possible mechanisms and pathways by which histone methylation may contribute to cancer formation.

The Histone Methyltransferase EZH2, a Key Component of the Polycomb System

Enhancer of zeste homolog 2 (EZH2) is one of the best-studied HMT enzymes involved in oncogenesis. It is an evolutionarily conserved transcriptional repressor that specifically dimethylates and trimethylates H3K27 residues.²¹⁸ In addition, along with another HMT G9a, it has also methyltransferase activity towards the linker histone H1.4K26.^{219,220}

EZH2 belongs to the Polycomb repressive complex 2 (PRC2), which is 1 of the 2 classes of Polycomb group proteins (PcG), originally discovered in fruit flies and involved in the silencing of the HOX gene cluster.²¹⁸ Although EZH2 is the active enzyme within PRC2, it requires the other members of the complex, suppressor of zeste 12 (SUZ12) and embryonic ectoderm development (EED), for its enzymatic activity.²¹⁴ Through targeted trimethylation of H3K27 at regulatory regions in the genome (and potentially other targets), Polycomb group proteins affect a wide range of cellular processes that includes apoptosis, proliferation, differentiation, and cell cycle regulation.^{221,222} In mammals, Polycomb group gene expression is important in many aspects of development. It is required for early mouse development^{214,223} as well as for maintaining ES cell pluripotency and plasticity during embryonic development.^{224,225} Moreover, null mutations for the PRC2 genes SUZ12 and EZH2 lead to embryonic lethality in mice.^{214,223} Currently, there are numerous lines of evidence suggesting that Polycomb group proteins are dysregulated in cancer and may promote cancer progression.²²² Indeed, EZH2 is found to be overexpressed in many types of aggressive cancers such as the breast, lung, liver, nasopharyngeal, colon, and prostate as well as in many other types of carcinomas.²²⁶⁻²²⁹ Moreover, EZH2 can transform the growth of a normal prostate epithelial cell line both *in vitro* and *in vivo*.²³⁰ These findings show that EZH2 can potentially act as an oncogene rather than simply be a marker for hyperproliferation. Furthermore, RNAi-targeting EZH2 inhibits tumor growth and liver metastasis of pancreatic cancer, blocks prostate cancer metastasis, significantly decreases breast xenograft growth in addition to improved survival in breast cancer models, and reduces proliferation of human papillomavirus-positive tumor cells.²³¹⁻²³⁴

Despite the fact that the mechanism of EZH2 in oncogenesis is not yet clear,

it is believed that H3K27me3 methylation of promoters of tumor suppressor genes may be involved in their anomalous silencing. Indeed, it has been shown that EZH2 represses the expression of several tumor suppressor genes, including p16 INK4a,²²⁸ E-cadherin,²³⁵ DAB2IP,²³² RUNX3,²³⁶ BRCA1,²³¹ and the adrenergic receptor β 2, which is expressed at low levels in metastatic prostate cancer and tumors that exhibit poor prognosis.²³⁷ Additionally, oncoproteins can activate EZH2 to induce epigenetic silencing.²³⁸ In contrast to EZH2 activation, several inactivating mutations of EZH2 have been recently discovered in myeloid malignancies, suggesting that EZH2 can also act as a tumor suppressor.²³⁹ Likewise, recurrent EZH2 mutations were described in lymphomas of germinal center origin. These mutations replace a single tyrosine in the catalytic SET domain of the EZH2 protein (Y641) and reduce its enzymatic activity.²⁴⁰ Together, these findings show that although inhibition of EZH2 was proposed as a therapeutic strategy, inactivating EZH2 might promote other malignancies. Despite what we described above, it is still unclear whether EZH2 overexpression is a cause or a consequence of tumorigenesis. Dysregulation of EZH2 in cancer may well occur as a result of a secondary effect due to the high proliferative nature of solid tumor and metastatic cells, for instance, through the regulation of microRNAs (miR), which are known to regulate multiple target genes. Indeed, miR-101 microRNA was found to target EZH2 but also to regulate cell proliferation, invasion, and tumor growth. Interestingly, a loss of this miRNA leads to overexpression of EZH2 in several cancers.²⁴¹ In addition, miR-26a, which is downregulated in breast cancer specimens and cell lines, was also found to target EZH2. Upon miR-26a downregulation, EZH2 becomes significantly upregulated in breast cancer.²⁴²

In line with the concept of histone modifiers being themselves regulated by PTMs, CDK1/2-dependent phosphorylation at multiple residues (T345, T350,

and T487) can regulate EZH2 activity and levels.^{25,26} While phosphorylation at T345 increases the binding of EZH2 to the HOTAIR noncoding RNA that may facilitate its recruitment to target genes, the phosphorylation at T487 disrupts EZH2 binding to other PRC2 components SUZ12 and EED and thereby inhibits its methyltransferase activity, resulting in inhibition of cancer cell invasiveness.^{25,243} Moreover, phosphorylation of EZH2 at T350 is important for its recruitment and maintenance of H3K27me3 levels at EZH2 target loci. Therefore, blocking T350 phosphorylation not only diminishes the global effect of EZH2 on gene silencing but also mitigates EZH2-mediated cell proliferation and migration.²⁴⁴

Altogether, this clearly links EZH2 with oncogenesis and demonstrates that accurate and precise regulation of H3K27 methylation is needed in order to prevent cancer development and progression.

Histone H3K9 Methyltransferases

In concordance with other methyl-lysine marks, H3K9 monomethylation, dimethylation, or trimethylation can induce distinct chromatin states. H3K9me3 was found to correlate with transcriptional silent chromatin and to act as a specific binding platform for the heterochromatin protein 1 (HP1).¹⁷ The SUV39H1 and SUV39H2 enzymes specifically trimethylate H3K9 and use monomethylated H3K9 as a preferred substrate. By doing so, they play a critical role in the establishment of constitutive heterochromatin especially at pericentric heterochromatin.²⁴⁵ The combined disruption of both *Suv39h* genes severely impairs mice viability and induces chromosomal instabilities (primarily by impairing chromosome segregation) together with an increased risk of developing B cell lymphomas.²⁴⁶ It seems that H3K9 methylation protects cells from genome instability, which is a critical pathway in cancer progression, and its absence can result in tumorigenesis. Additionally, SUV39H1 is also involved in the repressive functions of the

retinoblastoma (Rb) tumor suppressor protein, which targets the enzyme to promoters of cell cycle control genes.²⁴⁷ Moreover, the HDAC SirT1 interacts with the N-terminal domain of SUV39H1 and deacetylates SUV39H1 on K266 to stimulate its methyltransferase activity. The binding of SirT1 alone can also activate SUV39H1 through additional mechanisms independent of its deacetylase activity. Therefore, the SUV39H1-SirT1 complex may regulate transcriptional silencing through the HDAC activity of SirT1 and the enhanced methylase activity of SUV39H1.¹³⁰ Interestingly, recent studies show that DBC1 (deleted in breast cancer 1) not only binds to and inhibits SirT1 but also binds to the catalytic domain of SUV39H1 and causes its complete inactivation.²⁴⁸

The HMT G9a (and the HMT Glp1, an interaction partner of G9a) is classified as a H3K9 methyltransferase. Indeed, H3K9 methylation levels decrease in G9a-deficient embryos, which displayed severe growth retardation and early lethality.²⁴⁹ However, recently, it had been shown that G9a could also contribute to H3K27 (H3K27me1 and H3K27me2) methylation *in vivo*.²⁵⁰ G9a was found to promote lung cancer invasion and metastasis by silencing the cell adhesion molecule Ep-CAM.²⁵¹ Besides this, G9a was upregulated in aggressive lung cancer cells, and its elevated expression correlated with poor prognosis. Additionally, RNAi-mediated knockdown of G9a in highly invasive lung cancer cells inhibited cell migration and invasion *in vitro* and metastasis *in vivo*.²⁵¹

Histone H3K4 Methyltransferases

A well-studied example of a H3K4-specific HMT involved in human cancer is the mixed lineage leukemia (MLL) protein. MLL is a mammalian trithorax group (Trx-G) protein that regulates Hox gene expression and counteracts PcG function. Rearrangements of the MLL gene, which is located at chromosome 11q23, are associated with aggressive acute leukemias in both children and

adults and include MLL fusion genes, partial tandem duplications, or amplification of MLL.²⁵² Whereas MLL-deficient mice are embryonic lethal and have defects in the hematopoietic system accompanied by Hox gene dysregulation,^{253,254} mice carrying MLL fusions and amplifications often upregulate Hox gene expression. They develop acute myeloid leukemia that apparently results in a block of hematopoietic differentiation. Therefore, MLL fusion proteins are thought to contribute to tumorigenesis by a gain-of-function mechanism rather than a loss-of-function mechanism.²⁵⁵ MLL tumorigenic activity is not necessarily related to its HMT activity because the loss of the SET domain in MLL1 N-terminal fusions upregulates the expression of several target genes, which are essential for the transforming activity of the MLL fusion proteins.²⁵⁶

The SET and MYND domain-containing protein 3 (SMYD3) specifically methylates H3K4 and plays an important role in transcriptional regulation as a member of the RNA polymerase complex.²⁵⁷ Through the interaction with the RNA helicase HELZ, SMYD3 can form a complex with RNA polymerase II and activates a set of genes that includes oncogenes, homeobox genes, and genes associated with cell cycle regulation. Concordantly, it can be commonly found upregulated in colorectal and hepatocellular carcinomas.²⁵⁷ Overexpression of SMYD3 enhances cell growth, whereas siRNA in cancer cells results in growth suppression.²⁵⁷ A parallel study from the same group shows that SMYD3 expression is elevated in a great majority of breast cancer tissues and its increased levels are essential for proliferation of the breast cancer cells.²⁵⁸ Additionally, SMYD3 can promote breast carcinogenesis by directly regulating the expression of the proto-oncogene WNT10B. In another study, it was found that human cancer cells express both the full-length and a cleaved (apparently proteolytic cleavage of the full-length protein) SMYD3 protein, resulting in a form of

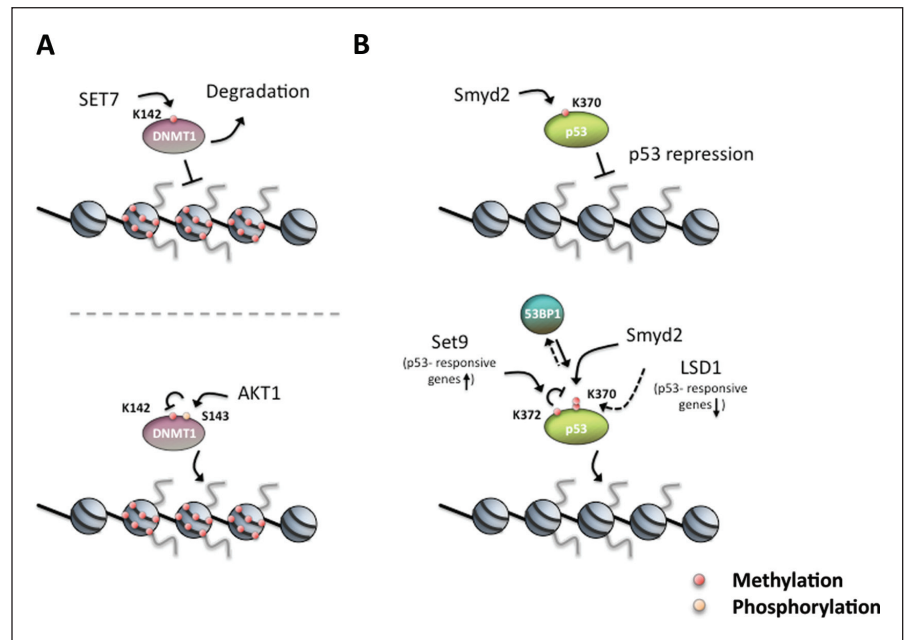


Figure 3. Nonhistone proteins are dynamically regulated by PTMs to control their activity. **(A)** During mitosis, DNMT1 stability is regulated by HMT SET7, which monomethylates DNMT1 K142 and promotes its degradation (upper panel). In interphase, DNMT1 S143 is phosphorylated by AKT1 kinase, which also prevents monomethylation of K142 (lower panel).^{260,261} **(B)** The tumor suppressor p53 is subject to both activating and repressing methylation. Smyd2 monomethylates p53 K370me1, which represses its activity (upper panel),²⁶² whereas the HMT Set9 (solid line) and the demethylase LSD1 (dashed line) regulate the methylation states of p53 K372me1 and K370me2, respectively. p53 K370me2 was shown to promote the association of the coactivator 53BP1.²⁶³ Thus, by removing the methylation, LSD1 represses p53 function through the inhibition of the interaction with 53BP1. Additionally, Set9 monomethylates p53 K372me1 that results in nuclear localization and increased stability and at the same time prevents the methylation by Smyd2 at K370 (e.g., K370me1). Note that p53 is also regulated by acetylation, not shown for simplicity.

SMYD3 protein that lacks 34 amino acids in the N-terminal region and has a higher HMTase activity compared to the full-length protein.²⁵⁹ This indicates that the N-terminal region of SMYD3 plays an important role in the regulation of its enzymatic activity and may serve as an attractive target to abolish SMYD3 methyltransferase activity.

Modifying the Modifiers by Methylation

Apart from histones, chromatin-modifying enzymes can also be methylated by HMTs and their activity modulated by this methylation. A recent example for this is the regulation of DNA methyltransferase-1 (DNMT1) stability through SET7-mediated lysine methylation (Fig. 3A). The HMT SET7 (a H3K4-

specific HMT) directly interacts with DNMT1 and specifically monomethylates DNMT1 at K142. This methylation peaks during the S and G2 phases of the cell cycle and promotes DNMT1 degradation.²⁶⁰ Moreover, as part of a complex cross-talk as shown for histones, the interplay between monomethylation of DNMT1 K142 by SET7 and phosphorylation of DNMT1 S143 by AKT1 kinase can regulate DNMT1 stability by a methylation/phosphorylation switch.²⁶¹

Similar to histones, p53 is subject to both activating and repressing lysine methylation (Fig. 3B). For instance, SMYD2 monomethylates p53 K370 (K370me1), which represses its activity.²⁶² On the other hand, p53 K370 dimethylation (K370me2) was shown to promote the association of the coactivator

p53-binding protein 1 (53BP1) through the tandem Tudor domains in 53BP1. Thus, by removing this methylation, the lysine-specific demethylase LSD1 represses p53 function through the inhibition of interaction of p53 and 53BP1.²⁶³ Likewise, the Set domain-containing protein Set9 monomethylates p53 at K372 (K372me1), resulting in the nuclear localization and increased stability of p53.²⁶⁴ In addition, Set9-mediated methylation of K372 was shown to inhibit Smyd2-mediated methylation of K370. All together, this demonstrates that p53 is dynamically regulated by lysine methylation and demethylation and that the methylation status may produce different regulatory outputs. The example of p53 shows that a similar regulatory complexity can exist for nonhistone proteins as for histones.^{265,266}

Concluding Remarks and Perspective

Although in the last decades the chromatin field provided a wealth of knowledge about the biology and function of histone modification together with its regulating mechanisms, the exact impact and influence of certain specific modifications on the tumorigenic process are still largely unclear. Several lines of evidence suggest that a dysregulation of the epigenetic machinery contributes to human cancers through disturbing the “histone code” and/or its readout, but it remains to be determined whether the dysregulation of either the epigenetic players or disruption of specific marks is the initiator or just the outcome effect of oncogenesis.

Histone modifications and their modifiers hold great promise as therapeutic targets because, in contrast to genetic mutations, PTMs are dynamic and potentially reversible. On top of that, many histone modifications are set by site-specific modifiers at a precise and targeted position, thereby most suitable for inhibition. Indeed, although we do not yet fully understand the underlying mechanism, HDAC inhibitors are proven

to be highly effective anticancer drugs that inhibit tumor growth, reactivate tumor suppressor genes, and lead to genomic instability by a variety of mechanisms. In line with histone acetylation as a potential therapy target, a recent article showed that inactivating mutations of acetyltransferase genes act as a major pathogenetic mechanism shared by common forms of B cell non-Hodgkin lymphoma, suggesting that reduction in HAT dosage is important for lymphomagenesis.²⁶⁷ Interestingly, this work also demonstrates specific defects in acetylation-mediated inactivation of the BCL6 oncoprotein and activation of the p53 tumor suppressor proteins.²⁶⁷ In addition to HDAC inhibitors, methyltransferase inhibitors have now been broadly used for cancer, inflammatory, and autoimmune diseases.²⁶⁸

We are currently only beginning to understand the diverse implications of histone PTMs and the corresponding modifying enzymes for biology and about disease. We do not know enough how PTMs integrate in normal biological processes and networks, for example cell proliferation, differentiation, or development, and therefore, we do not have enough insights into their deregulation in diseases such as cancer. The significance of studying posttranslational modifications extends beyond the field of chromatin research because changes in the modification patterns are likely to affect many aspects of protein functions. Therefore, apart from their role in chromatin function, it is important to see histone modifications as a model system to understand how covalent modifications regulate protein functions.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This work was supported by the Max Planck Society; the Deutsche Forschungsgemeinschaft [grant number SFB 746]; and a European Research Council starting grant. The Polish State Committee funds E.P. for Scientific Research [grant number NN303813140]. I.C. is a Humboldt fellow.

References

- Jenuwein T, Allis CD. Translating the histone code. *Science*. 2001;293:1074-80.
- Latham JA, Dent SY. Cross-regulation of histone modifications. *Nat Struct Mol Biol*. 2007;14:1017-24.
- Strahl BD, Allis CD. The language of covalent histone modifications. *Nature*. 2000;403:41-5.
- Kouzarides T. Chromatin modifications and their function. *Cell*. 2007;128:693-705.
- Zhou VW, Goren A, Bernstein BE. Charting histone modifications and the functional organization of mammalian genomes. *Nat Rev Genet*. 2011;12:7-18.
- Chodaparambil JV, Edayathumangalam RS, Bao Y, Park YJ, Luger K. Nucleosome structure and function. *Ernst Schering Res Found Workshop*. 2006;(57):29-46.
- Tropberger P, Schneider R. Going global: novel histone modifications in the globular domain of H3. *Epigenetics*. 2010;5:112-7.
- Cosgrove MS, Boeke JD, Wolberger C. Regulated nucleosome mobility and the histone code. *Nat Struct Mol Biol*. 2004;11:1037-43.
- Daujat S, Weiss T, Mohn F, *et al*. H3K64 trimethylation marks heterochromatin and is dynamically remodeled during developmental reprogramming. *Nat Struct Mol Biol*. 2009;16:777-81.
- Deterding LJ, Bunker MK, Banks GC, Tomer KB, Archer TK. Global changes in and characterization of specific sites of phosphorylation in mouse and human histone H1 isoforms upon CDK inhibitor treatment using mass spectrometry. *J Proteome Res*. 2008;7:2368-79.
- Wisniewski JR, Zougman A, Krüger S, Mann M. Mass spectrometric mapping of linker histone H1 variants reveals multiple, acetylations methylations, and phosphorylation as well as differences between cell culture and tissue. *Mol Cell Proteomics*. 2007;6:72-87.
- Izzo A, Kamiñiarz K, Schneider R. The histone H1 family: specific members, specific functions? *Biol Chem*. 2008;389:333-43.
- Garcia BA. Mass spectrometric analysis of histone variants and post-translational modifications. *Front Biosci (Schol Ed)*. 2009;1:142-53.
- Lee JS, Smith E, Shilatifard A. The language of histone crosstalk. *Cell*. 2010;142:682-5.
- Izzo A, Schneider R. Chatting histone modifications in mammals. *Brief Funct Genomics*. 2010;9:429-43.
- Taverna SD, Li H, Ruthenburg AJ, Allis CD, Patel DJ. How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. *Nat Struct Mol Biol*. 2007;14:1025-40.
- Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature*. 2001;410:116-20.
- Bannister AJ, Zegerman P, Partridge JF, *et al*. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature*. 2001;410:120-4.
- Daujat S, Zeissler U, Waldmann T, Happel N, Schneider R. HP1 binds specifically to

- Lys26-methylated histone H1.4, whereas simultaneous Ser27 phosphorylation blocks HP1 binding. *J Biol Chem.* 2005;280:38090-5.
20. Margueron R, Justin N, Ohno K, *et al.* Role of the polycomb protein EED in the propagation of repressive histone marks. *Nature.* 2009;461:762-7.
 21. Bonasio R, Tu S, Reinberg D. Molecular signals of epigenetic states. *Science (New York, NY).* 2010;330:612-6.
 22. Yuan LW, Gambee JE. Phosphorylation of p300 at serine 89 by protein kinase C. *J Biol Chem.* 2000;275:40946-51.
 23. Schwartz C, Beck K, Mink S, *et al.* Recruitment of p300 by C/EBP β triggers phosphorylation of p300 and modulates coactivator activity. *EMBO J.* 2003;22:882-92.
 24. Poizat C, Puri PL, Bai Y, Kedes L. Phosphorylation-dependent degradation of p300 by doxorubicin-activated p38 mitogen-activated protein kinase in cardiac cells. *Mol Cell Biol.* 2005;25:2673-87.
 25. Kaneko S, Li G, Son J, *et al.* Phosphorylation of the PRC2 component Ezh2 is cell cycle-regulated and up-regulates its binding to ncRNA. *Genes Dev.* 2010;24:2615-20.
 26. Zeng X, Chen S, Huang H. Phosphorylation of EZH2 by CDK1 and CDK2: a possible regulatory mechanism of transmission of the H3K27me3 epigenetic mark through cell divisions. *Cell Cycle.* 2011;10:579-83.
 27. Chi P, Allis CD, Wang GG. Covalent histone modifications: miswritten, misinterpreted and mis-erased in human cancers. *Nat Rev Cancer.* 2010;10:457-69.
 28. Nimura K, Ura K, Kaneda Y. Histone methyltransferases: regulation of transcription and contribution to human disease. *J Mol Med.* 2010;88:1213-20.
 29. Pedersen MT, Helin K. Histone demethylases in development and disease. *Trends Cell Biol.* 2010;20:662-71.
 30. Varier RA, Timmers HT. Histone lysine methylation and demethylation pathways in cancer. *Biochim Biophys Acta.* 2011;1815:75-89.
 31. Phillips DM. Proceedings of the Biochemical Society. *Biochem J.* 1961;80:40P.
 32. Phillips DM. The presence of acetyl groups in histones. *Biochem J.* 1963;87:258-63.
 33. Allfrey VG, Faulkner R, Mirsky AE. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc Natl Acad Sci U S A.* 1964;51:786-94.
 34. Brownell JE, Allis CD. An activity gel assay detects a single, catalytically active histone acetyltransferase subunit in Tetrahymena macronuclei. *Proc Natl Acad Sci U S A.* 1995;92:6364-8.
 35. Brownell JE, Zhou J, Ranalli T, *et al.* Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell.* 1996;84:843-51.
 36. Kleff S, Andrulis ED, Anderson CW, Sternglanz R. Identification of a gene encoding a yeast histone H4 acetyltransferase. *J Biol Chem.* 1995;270:24674-7.
 37. Lee KK, Workman JL. Histone acetyltransferase complexes: one size doesn't fit all. *Nat Rev Mol Cell Biol.* 2007;8:284-95.
 38. Hildmann C, Riestler D, Schwienhorst A. Histone deacetylases: an important class of cellular regulators with a variety of functions. *Appl Microbiol Biotechnol.* 2007;75:487-97.
 39. Shahbazian MD, Grunstein M. Functions of site-specific histone acetylation and deacetylation. *Ann Rev Biochem.* 2007;76:75-100.
 40. Allis CD, Berger SL, Cote J, *et al.* New nomenclature for chromatin-modifying enzymes. *Cell.* 2007;131:633-6.
 41. Vaquero A, Scher M, Lee D, *et al.* Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin. *Mol Cell.* 2004;16:93-105.
 42. Grunstein M. Histone acetylation in chromatin structure and transcription. *Nature.* 1997;389:349-52.
 43. Dhalluin C, Carlson JE, Zeng L, *et al.* Structure and ligand of a histone acetyltransferase bromodomain. *Nature.* 1999;399:491-6.
 44. Mujtaba S, Zeng L, Zhou MM. Structure and acetyl-lysine recognition of the bromodomain. *Oncogene.* 2007;26:5521-7.
 45. Hasan S, El-Andaloussi N, Hardeland U, *et al.* Acetylation regulates the DNA end-trimming activity of DNA polymerase beta. *Mol Cell.* 2002;10:1213-22.
 46. Kurdistani SK, Grunstein M. Histone acetylation and deacetylation in yeast. *Nat Rev Mol Cell Biol.* 2003;4:276-84.
 47. Grant PA, Duggan L, Côté J, *et al.* Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev.* 1997;11:1640-50.
 48. Baker SP, Grant PA. The SAGA continues: expanding the cellular role of a transcriptional coactivator complex. *Oncogene.* 2007;26:5329-40.
 49. Koutelou E, Hirsch CL, Dent SYR. Multiple faces of the SAGA complex. *Curr Opin Cell Biol.* 2010;22:374-82.
 50. Nagy Z, Tora L. Distinct GCN5/PCAF-containing complexes function as co-activators and are involved in transcription factor and global histone acetylation. *Oncogene.* 2007;26:5341-57.
 51. Guelman S, Suganuma T, Florens L, *et al.* Host cell factor and an uncharacterized SANT domain protein are stable components of ATAC, a novel dAda2A/dGcn5-containing histone acetyltransferase complex in *Drosophila*. *Mol Cell Biol.* 2006;26:871-82.
 52. Carré C, Szymczak D, Pidoux J, Antoniewski C. The histone H3 acetylase dGcn5 is a key player in *Drosophila melanogaster* metamorphosis. *Mol Cell Biol.* 2005;25:8228-38.
 53. Xu W, Edmondson DG, Evrard YA, *et al.* Loss of Gcn512 leads to increased apoptosis and mesodermal defects during mouse development. *Nat Genet.* 2000;26:229-32.
 54. Ciurciu A, Komonyi O, Pankotai T, Boros IM. The *Drosophila* histone acetyltransferase Gcn5 and transcriptional adaptor Ada2a are involved in nucleosomal histone H4 acetylation. *Mol Cell Biol.* 2006;26:9413-23.
 55. Kikuchi H, Takami Y, Nakayama T. GCN5: a supervisor in all-inclusive control of vertebrate cell cycle progression through transcription regulation of various cell cycle-related genes. *Gene.* 2005;347:83-97.
 56. Bu P, Evrard YA, Lozano G, Dent SYR. Loss of Gcn5 acetyltransferase activity leads to neural tube closure defects and exencephaly in mouse embryos. *Mol Cell Biol.* 2007;27:3405-16.
 57. Nagy Z, Riss A, Fujiiyama S, *et al.* The metazoan ATAC and SAGA coactivator HAT complexes regulate different sets of inducible target genes. *Cell Mol Life Sci.* 2010;67:611-28.
 58. Li S, Shogren-Knaak MA. The Gcn5 bromodomain of the SAGA complex facilitates cooperative and cross-tail acetylation of nucleosomes. *J Biol Chem.* 2009;284:9411-7.
 59. Huisinga KL, Pugh BF. A genome-wide house-keeping role for TFIID and a highly regulated stress-related role for SAGA in *Saccharomyces cerevisiae*. *Mol Cell.* 2004;13:573-85.
 60. Nagy Z, Riss A, Romier C, *et al.* The human SPT20-containing SAGA complex plays a direct role in the regulation of endoplasmic reticulum stress-induced genes. *Mol Cell Biol.* 2009;29:1649-60.
 61. Yanagisawa J, Kitagawa H, Yanagida M, *et al.* Nuclear receptor function requires a TFTC-type histone acetyl transferase complex. *Mol Cell.* 2002;9:553-62.
 62. Zhao Y, Lang G, Ito S, *et al.* A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. *Mol Cell.* 2008;29:92-101.
 63. Belotserkovskaya R, Sterner DE, Deng M, *et al.* Inhibition of TATA-binding protein function by SAGA subunits Spt3 and Spt8 at Gcn4-activated promoters. *Mol Cell Biol.* 2000;20:634-47.
 64. Ricci AR, Genereaux J, Brandl CJ. Components of the SAGA histone acetyltransferase complex are required for repressed transcription of ARG1 in rich medium. *Mol Cell Biol.* 2002;22:4033-42.
 65. Ginsburg DS, Govind CK, Hinnebusch AG. NuA4 lysine acetyltransferase Esa1 is targeted to coding regions and stimulates transcription elongation with Gcn5. *Mol Cell Biol.* 2009;29:6473-87.
 66. Gunderson FQ, Johnson TL. Acetylation by the transcriptional coactivator gcn5 plays a novel role in co-transcriptional spliceosome assembly. *PLoS Genet.* 2009;5:e1000682.
 67. Rodríguez-Navarro S. Insights into SAGA function during gene expression. *EMBO Rep.* 2009;10:843-50.
 68. Atanassov BS, Evrard YA, Multani AS, *et al.* Gcn5 and SAGA regulate shelterin protein turnover and telomere maintenance. *Mol Cell.* 2009;35:352-64.
 69. Burgess RJ, Zhang Z. Roles for Gcn5 in promoting nucleosome assembly and maintaining genome integrity. *Cell Cycle (Georgetown, Tex).* 2010;9:2979-85.
 70. Burgess RJ, Zhou H, Han J, Zhang Z. A role for Gcn5 in replication-coupled nucleosome assembly. *Mol Cell.* 2010;37:469-80.
 71. Helmlinger D, Hardy S, Abou-Sleymane G, *et al.* Glutamine-expanded ataxin-7 alters TFTC/STAGA recruitment and chromatin structure leading to photoreceptor dysfunction. *PLoS Biol.* 2006;4:e67.
 72. McMahon SB, Wood MA, Cole MD. The essential cofactor TRRAP recruits the histone acetyltransferase hGCN5 to c-Myc. *Mol Cell Biol.* 2000;20:556-62.

73. Liu X, Vorontchikhina M, Wang Y-L, Faiola F, Martinez E. STAGA recruits mediator to the MYC oncoprotein to stimulate transcription and cell proliferation. *Mol Cell Biol.* 2008;28:108-21.
74. Oishi H, Kitagawa H, Wada O, *et al.* An hGCN5/TRRAP histone acetyltransferase complex co-activates BRCA1 transactivation function through histone modification. *J Biol Chem.* 2006;281:20-6.
75. Glinsky GV, Berezovska O, Glinskii AB. Microarray analysis identifies a death-from-cancer signature predicting therapy failure in patients with multiple types of cancer. *J Clin Invest.* 2005;115:1503-21.
76. Zhang X-Y, Varthi M, Sykes SM, *et al.* 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.* 2008;29:102-11.
77. Atanassov BS, Evrard YA, Multani AS, *et al.* Gcn5 and SAGA regulate shelterin protein turnover and telomere maintenance. *Mol Cell.* 2009;35:352-64.
78. Hilfiker A, Hilfiker-Kleiner D, Pannuti A, Lucchesi JC. *mof*, a putative acetyl transferase gene related to the Tip60 and MOZ human genes and to the SAS genes of yeast, is required for dosage compensation in *Drosophila*. *EMBO J.* 1997;16:2054-60.
79. Dou Y, Milne TA, Tackett AJ, *et al.* Physical association and coordinate function of the H3 K4 methyltransferase MLL1 and the H4 K16 acetyltransferase MOF. *Cell.* 2005;121:873-85.
80. Mendjan S, Taipale M, Kind J, *et al.* Nuclear pore components are involved in the transcriptional regulation of dosage compensation in *Drosophila*. *Mol Cell.* 2006;21:811-23.
81. Smith ER, Pannuti A, Gu W, *et al.* The *drosophila* MSL complex acetylates histone H4 at lysine 16, a chromatin modification linked to dosage compensation. *Mol Cell Biol.* 2000;20:312-8.
82. Akhtar A, Becker PB. Activation of transcription through histone H4 acetylation by MOF, an acetyltransferase essential for dosage compensation in *Drosophila*. *Mol Cell.* 2000;5:367-75.
83. Thomas T, Dixon MP, Kueh AJ, Voss AK. *Mof* (MYST1 or KAT8) is essential for progression of embryonic development past the blastocyst stage and required for normal chromatin architecture. *Mol Cell Biol.* 2008;28:5093-105.
84. Shogren-Knaak M, Ishii H, Sun JM, *et al.* Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science.* 2006;311:844-7.
85. Robinson PJJ, An W, Routh A, *et al.* 30 nm chromatin fibre decompaction requires both H4-K16 acetylation and linker histone eviction. *J Mol Biol.* 2008;381:816-25.
86. Gupta A, Sharma GG, Young CSH, *et al.* Involvement of human MOF in ATM function. *Mol Cell Biol.* 2005;25:5292-305.
87. Sykes SM, Mellert HS, Holbert MA, *et al.* Acetylation of the p53 DNA-binding domain regulates apoptosis induction. *Mol Cell.* 2006;24:841-51.
88. Tamburini BA, Tyler JK. Localized histone acetylation and deacetylation triggered by the homologous recombination pathway of double-strand DNA repair. *Mol Cell Biol.* 2005;25:4903-13.
89. Tang Y, Luo J, Zhang W, Gu W. Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. *Mol Cell.* 2006;24:827-39.
90. Fraga MF, Ballestar E, Villar-Garea A, *et al.* Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nat Genet.* 2005;37:391-400.
91. Eden A, Gaudet F, Waghmare A, Jaenisch R. Chromosomal instability and tumors promoted by DNA hypomethylation. *Science (New York, NY).* 2003;300:455.
92. Ehrlich M. DNA methylation in cancer: too much, but also too little. *Oncogene.* 2002;21:5400-13.
93. Avvakumov N, Côté J. The MYST family of histone acetyltransferases and their intimate links to cancer. *Oncogene.* 2007;26:5395-407.
94. Rea S, Xouri G, Akhtar A. Males absent on the first (MOF): from flies to humans. *Oncogene.* 2007;26:5385-94.
95. Eckner R, Ewen ME, Newsome D, *et al.* Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. *Genes Dev.* 1994;8:869-84.
96. Yee SP, Branton PE. Detection of cellular proteins associated with human adenovirus type 5 early region 1A polypeptides. *Virology.* 1985;147:142-53.
97. Chrivia JC, Kwok RP, Lamb N, *et al.* Phosphorylated CREB binds specifically to the nuclear protein CBP. *Nature.* 1993;365:855-9.
98. Bannister AJ, Kouzarides T. The CBP co-activator is a histone acetyltransferase. *Nature.* 1996;384:641-3.
99. Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y. The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell.* 1996;87:953-9.
100. Wang L, Tang Y, Cole PA, Marmorstein R. Structure and chemistry of the p300/CBP and Rtt109 histone acetyltransferases: implications for histone acetyltransferase evolution and function. *Curr Opin Struct Biol.* 2008;18:741-7.
101. Schiltz RL, Mizzen CA, Vassilev A, *et al.* Overlapping but distinct patterns of histone acetylation by the human coactivators p300 and PCAF within nucleosomal substrates. *J Biol Chem.* 1999;274:1189-92.
102. Wang H, Walsh STR, Parthun MR. Expanded binding specificity of the human histone chaperone NASP. *Nucleic Acids Res.* 2008;36:5763-72.
103. Jin Q, Yu L-R, Wang L, *et al.* Distinct roles of GCN5/PCAF-mediated H3K9ac and CBP/p300-mediated H3K18/27ac in nuclear receptor transactivation. *EMBO J.* 2011;30:249-62.
104. Kung AL, Rebel VI, Bronson RT, *et al.* Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP. *Genes Dev.* 2000;14:272-7.
105. Yao TP, Oh SP, Fuchs M, *et al.* Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. *Cell.* 1998;93:361-72.
106. Kalkhoven E. CBP and p300: HATs for different occasions. *Biochem Pharmacol.* 2004;68:1145-55.
107. Kwok RP, Lundblad JR, Chrivia JC, *et al.* Nuclear protein CBP is a coactivator for the transcription factor CREB. *Nature.* 1994;370:223-6.
108. Yuan W, Condorelli G, Caruso M, Felsani A, Giordano A. Human p300 protein is a coactivator for the transcription factor MyoD. *J Biol Chem.* 1996;271:9009-13.
109. Nakajima T, Uchida C, Anderson SF, Parvin JD, Montminy M. Analysis of a cAMP-responsive activator reveals a two-component mechanism for transcriptional induction via signal-dependent factors. *Genes Dev.* 1997;11:738-47.
110. Neish AS, Anderson SF, Schlegel BP, Wei W, Parvin JD. Factors associated with the mammalian RNA polymerase II holoenzyme. *Nucleic Acids Res.* 1998;26:847-53.
111. Iyer NG, Ozdag H, Caldas C. p300/CBP and cancer. *Oncogene.* 2004;23:4225-31.
112. Hasan S, Hassa PO, Imhof R, Hottiger MO. Transcription coactivator p300 binds PCNA and may have a role in DNA repair synthesis. *Nature.* 2001;410:387-91.
113. Tini M, Benecke A, Um S-J, *et al.* Association of CBP/p300 acetylase and thymine DNA glycosylase links DNA repair and transcription. *Mol Cell.* 2002;9:265-77.
114. Ait-Si-Ali S, Poleskaya A, Filleur S, *et al.* CBP/p300 histone acetyl-transferase activity is important for the G1/S transition. *Oncogene.* 2000;19:2430-7.
115. Ait-Si-Ali S, Ramirez S, Barre FX, *et al.* Histone acetyltransferase activity of CBP is controlled by cycle-dependent kinases and oncoprotein E1A. *Nature.* 1998;396:184-6.
116. Perkins ND, Felzien LK, Betts JC, *et al.* Regulation of NF-kappaB by cyclin-dependent kinases associated with the p300 coactivator. *Science (New York, NY).* 1997;275:523-7.
117. Bannister AJ, Miska EA, Görlich D, Kouzarides T. Acetylation of importin-alpha nuclear import factors by CBP/p300. *Curr Biol.* 2000;10:467-70.
118. Rebel VI, Kung AL, Tanner EA, *et al.* Distinct roles for CREB-binding protein and p300 in hematopoietic stem cell self-renewal. *Proc Natl Acad Sci U S A.* 2002;99:14789-94.
119. Muraoka M, Konishi M, Kikuchi-Yanoshita R, *et al.* p300 gene alterations in colorectal and gastric carcinomas. *Oncogene.* 1996;12:1565-9.
120. Kishimoto M, Kohno T, Okudela K, *et al.* Mutations and deletions of the CBP gene in human lung cancer. *Clin Cancer Res.* 2005;11:512-9.
121. Bryan EJ, Jokubaitis VJ, Chamberlain NL, *et al.* Mutation analysis of EP300 in colon, breast and ovarian carcinomas. *Int J Cancer.* 2002;102:137-41.
122. Sterner R, Vidali G, Heinrichson RL, Allfrey VG. Postsynthetic modification of high mobility group proteins: evidence that high mobility group proteins are acetylated. *J Biol Chem.* 1978;253:7601-4.
123. Gu W, Roeder RG. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell.* 1997;90:595-606.
124. Tomita A, Towatari M, Tsuzuki S, *et al.* c-Myb acetylation at the carboxyl-terminal conserved domain by transcriptional co-activator p300. *Oncogene.* 2000;19:444-51.

125. Boyes J, Byfield P, Nakatani Y, Ogryzko V. Regulation of activity of the transcription factor GATA-1 by acetylation. *Nature*. 1998;396:594-8.
126. Martínez-Balbás MA, Bauer UM, Nielsen SJ, Brehm A, Kouzarides T. Regulation of E2F1 activity by acetylation. *EMBO J*. 2000;19:662-71.
127. Kiernan RE, Vanhulle C, Schiltz L, *et al.* HIV-1 tat transcriptional activity is regulated by acetylation. *EMBO J*. 1999;18:6106-18.
128. Brandl A, Heinzl T, Krämer OH. Histone deacetylases: salesmen and customers in the post-translational modification market. *Biol Cell*. 2009;101:193-205.
129. Sterner DE, Berger SL. Acetylation of histones and transcription-related factors. *Microbiol Mol Biol Rev*. 2000;64:435-59.
130. Vaquero A, Scher M, Erdjument-Bromage H, *et al.* SIRT1 regulates the histone methyltransferase SUV39H1 during heterochromatin formation. *Nature*. 2007;450:440-4.
131. Close P, Creppe C, Gillard M, *et al.* The emerging role of lysine acetylation of non-nuclear proteins. *Cell Mol Life Sci*. 2010;67:1255-64.
132. Choudhary C, Kumar C, Gnäd F, *et al.* Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* (New York, NY). 2009;325:834-40.
133. Kouzarides T. Acetylation: a regulatory modification to rival phosphorylation? *EMBO J*. 2000;19:1176-9.
134. Gutierrez RM, Hnilica LS. Tissue specificity of histone phosphorylation. *Science*. 1967;157:1324-5.
135. Kleinsmith LJ, Allfrey VG, Mirsky AE. Phosphoprotein metabolism in isolated lymphocyte nuclei. *Proc Natl Acad Sci U S A*. 1966;55:1182-9.
136. Langan TA. Histone phosphorylation: stimulation by adenosine 3',5'-monophosphate. *Science*. 1968;162:579-80.
137. Xiao A, Li H, Shechter D, *et al.* WSTF regulates the H2A.X DNA damage response via a novel tyrosine kinase activity. *Nature*. 2009;457:57-62.
138. Cook PJ, Ju BG, Telese F, *et al.* Tyrosine dephosphorylation of H2AX modulates apoptosis and survival decisions. *Nature*. 2009;458:591-6.
139. Krishnan N, Jeong DG, Jung SK, *et al.* Dephosphorylation of the C-terminal tyrosyl residue of the DNA damage-related histone H2A.X is mediated by the protein phosphatase eyes absent. *J Biol Chem*. 2009;284:16066-70.
140. Singh RK, Kabbaj MH, Paik J, Gunjan A. Histone levels are regulated by phosphorylation and ubiquitylation-dependent proteolysis. *Nat Cell Biol*. 2009;11:925-33.
141. Dawson MA, Bannister AJ, Gottgens B, *et al.* JAK2 phosphorylates histone H3Y41 and excludes HP1 α from chromatin. *Nature*. 2009;461:819-22.
142. Baker SP, Phillips J, Anderson S, *et al.* Histone H3 Thr 45 phosphorylation is a replication-associated post-translational modification in *S. cerevisiae*. *Nat Cell Biol*. 2010;12:294-8.
143. Ahn SH, Henderson KA, Keeney S, Allis CD. H2B (Ser10) phosphorylation is induced during apoptosis and meiosis in *S. cerevisiae*. *Cell Cycle*. 2005;4:780-3.
144. Foster ER, Downs JA. Histone H2A phosphorylation in DNA double-strand break repair. *FEBS J*. 2005;272:3231-40.
145. Krishnamoorthy T, Chen X, Govin J, *et al.* Phosphorylation of histone H4 Ser1 regulates sporulation in yeast and is conserved in fly and mouse spermatogenesis. *Genes Dev*. 2006;20:2580-92.
146. Nowak SJ, Corces VG. Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. *Trends Genet*. 2004;20:214-20.
147. Thiriet C, Hayes JJ. Linker histone phosphorylation regulates global timing of replication origin firing. *J Biol Chem*. 2009;284:2823-9.
148. Oki M, Aihara H, Ito T. Role of histone phosphorylation in chromatin dynamics and its implications in diseases. *Subcell Biochem*. 2007;41:319-36.
149. Dou Y, Gorovsky MA. Regulation of transcription by H1 phosphorylation in *Tetrahymena* is position independent and requires clustered sites. *Proc Natl Acad Sci U S A*. 2002;99:6142-6.
150. Fischle W, Tseng BS, Dormann HL, *et al.* Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. *Nature*. 2005;438:1116-22.
151. Mahadevan LC, Willis AC, Barratt MJ. Rapid histone H3 phosphorylation in response to growth factors, phorbol esters, okadaic acid, and protein synthesis inhibitors. *Cell*. 1991;65:775-83.
152. Hendzel MJ, Wei Y, Mancini MA, *et al.* Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation. *Chromosoma*. 1997;106:348-60.
153. Thomson S, Mahadevan LC, Clayton AL. MAP kinase-mediated signalling to nucleosomes and immediate-early gene induction. *Semin Cell Dev Biol*. 1999;10:205-14.
154. Sassone-Corsi P, Mizzen CA, Cheung P, *et al.* Requirement of Rsk-2 for epidermal growth factor-activated phosphorylation of histone H3. *Science*. 1999;285:886-91.
155. Soloaga A, Thomson S, Wiggin GR, *et al.* MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14. *EMBO J*. 2003;22:2788-97.
156. Anest V, Hanson JL, Cogswell PC, *et al.* A nucleosomal function for IkappaB kinase-alpha in NF-kappaB-dependent gene expression. *Nature*. 2003;423:659-63.
157. Zippo A, De Robertis A, Serafini R, Oliviero S. PIM1-dependent phosphorylation of histone H3 at serine 10 is required for MYC-dependent transcriptional activation and oncogenic transformation. *Nat Cell Biol*. 2007;9:932-44.
158. Cheung P, Tanner KG, Cheung WL, *et al.* Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. *Mol Cell*. 2000;5:905-15.
159. Lo WS, Trievel RC, Rojas JR, *et al.* Phosphorylation of serine 10 in histone H3 is functionally linked in vitro and in vivo to Gcn5-mediated acetylation at lysine 14. *Mol Cell*. 2000;5:917-26.
160. Edmondson DG, Davie JK, Zhou J, *et al.* Site-specific loss of acetylation upon phosphorylation of histone H3. *J Biol Chem*. 2002;277:29496-502.
161. Rea S, Eisenhaber F, O'Carroll D, *et al.* Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature*. 2000;406:593-9.
162. Clayton AL, Rose S, Barratt MJ, Mahadevan LC. Phosphoacetylation of histone H3 on c-fos- and c-jun-associated nucleosomes upon gene activation. *EMBO J*. 2000;19:3714-26.
163. Winter S, Simboeck E, Fischle W, *et al.* 14-3-3 proteins recognize a histone code at histone H3 and are required for transcriptional activation. *EMBO J*. 2008;27:88-99.
164. Tefferi A, Lasho TL, Gilliland G. JAK2 mutations in myeloproliferative disorders. *N Engl J Med*. 2005;353:1416-7.
165. Rui L, Emre NC, Kruhlak MJ, *et al.* Cooperative epigenetic modulation by cancer amplicon genes. *Cancer Cell*. 2010;18:590-605.
166. Metzger E, Yin N, Wissmann M, *et al.* Phosphorylation of histone H3 at threonine 11 establishes a novel chromatin mark for transcriptional regulation. *Nat Cell Biol*. 2008;10:53-60.
167. Metzger E, Imhof A, Patel D, *et al.* Phosphorylation of histone H3T6 by PKC β (I) controls demethylation at histone H3K4. *Nature*. 2010;464:792-6.
168. Bradbury EM, Cary PD, Crane-Robinson C, Rattle HW. Conformations and interactions of histones and their role in chromosome structure. *Ann N Y Acad Sci*. 1973;222:266-89.
169. Bradbury EM, Inglis RJ, Matthews HR. Control of cell division by very lysine rich histone (F1) phosphorylation. *Nature*. 1974;247:257-61.
170. Langan TA, Gautier J, Lohka M, *et al.* Mammalian growth-associated H1 histone kinase: a homolog of cdc2+/CDC28 protein kinases controlling mitotic entry in yeast and frog cells. *Mol Cell Biol*. 1989;9:3860-8.
171. Boggs BA, Allis CD, Chinault AC. Immunofluorescent studies of human chromosomes with antibodies against phosphorylated H1 histone. *Chromosoma*. 2000;108:485-90.
172. Roth SY, Allis CD. Chromatin condensation: does histone H1 dephosphorylation play a role? *Trends Biochem Sci*. 1992;17:93-8.
173. Lens SM, Medema RH. The survivin/Aurora B complex: its role in coordinating tension and attachment. *Cell Cycle*. 2003;2:507-10.
174. Wilkinson RW, Odedra R, Heaton SP, *et al.* AZD1152, a selective inhibitor of Aurora B kinase, inhibits human tumor xenograft growth by inducing apoptosis. *Clin Cancer Res*. 2007;13:3682-8.
175. Hsu JY, Sun ZW, Li X, *et al.* Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. *Cell*. 2000;102:279-91.
176. Katayama H, Brinkley WR, Sen S. The Aurora kinases: role in cell transformation and tumorigenesis. *Cancer Metastasis Rev*. 2003;22:451-64.
177. Tchatchou S, Wirttenberger M, Hemminki K, *et al.* Aurora kinases A and B and familial breast cancer risk. *Cancer Lett*. 2007;247:266-72.
178. Ota T, Suto S, Katayama H, *et al.* Increased mitotic phosphorylation of histone H3 attributable to AIM-1/Aurora-B overexpression contributes to chromosome number instability. *Cancer Res*. 2002;62:5168-77.
179. Chen YJ, Chen CM, Twu NF, *et al.* Overexpression of Aurora B is associated with poor prognosis in epithelial ovarian cancer patients. *Virchows Arch*. 2009;455:431-40.
180. Tanaka S, Arii S, Yasen M, *et al.* Aurora kinase B is a predictive factor for the aggressive recurrence

- of hepatocellular carcinoma after curative hepatectomy. *Br J Surg.* 2008;95:611-9.
181. van Attikum H, Gasser SM. The histone code at DNA breaks: a guide to repair? *Nat Rev Mol Cell Biol.* 2005;6:757-65.
 182. Srivastava N, Gochhait S, de Boer P, Bamezai RN. Role of H2AX in DNA damage response and human cancers. *Mutat Res.* 2009;681:180-8.
 183. Goldknopf IL, Taylor CW, Baum RM, *et al.* Isolation and characterization of protein A24, a "histone-like" non-histone chromosomal protein. *J Biol Chem.* 1975;250:7182-7.
 184. Shroff R, Arbel-Eden A, Pilch D, *et al.* Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. *Curr Biol.* 2004;14:1703-11.
 185. Burma S, Chen BP, Murphy M, Kurimasa A, Chen DJ. ATM phosphorylates histone H2AX in response to DNA double-strand breaks. *J Biol Chem.* 2001;276:42462-7.
 186. Chanoux RA, Yin B, Urtishak KA, *et al.* ATR and H2AX cooperate in maintaining genome stability under replication stress. *J Biol Chem.* 2009;284:5994-6003.
 187. Stucki M, Clapperton JA, Mohammad D, *et al.* MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. *Cell.* 2005;123:1213-26.
 188. Ward IM, Chen J. Histone H2AX is phosphorylated in an ATR-dependent manner in response to replicational stress. *J Biol Chem.* 2001;276:47759-62.
 189. Fernandez-Capetillo O, Chen HT, Celeste A, *et al.* DNA damage-induced G2-M checkpoint activation by histone H2AX and 53BP1. *Nat Cell Biol.* 2002;4:993-7.
 190. Manke IA, Lowery DM, Nguyen A, Yaffe MB. BRCT repeats as phosphopeptide-binding modules involved in protein targeting. *Science.* 2003;302:636-9.
 191. Schultz LB, Chehab NH, Malikzay A, Hazonetis TD. p53 binding protein 1 (53BP1) is an early participant in the cellular response to DNA double-strand breaks. *J Cell Biol.* 2000;151:1381-90.
 192. Wood JL, Singh N, Mer G, Chen J. MCPH1 functions in an H2AX-dependent but MDC1-independent pathway in response to DNA damage. *J Biol Chem.* 2007;282:35416-23.
 193. Motoyama N, Naka K. DNA damage tumor suppressor genes and genomic instability. *Curr Opin Genet Dev.* 2004;14:11-6.
 194. Stucki M. Histone H2A.X Tyr142 phosphorylation: a novel sWtICH for apoptosis? *DNA Repair (Amst).* 2009;8:873-6.
 195. Bassing CH, Chua KF, Sekiguchi J, *et al.* Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX. *Proc Natl Acad Sci U S A.* 2002;99:8173-8.
 196. Bassing CH, Suh H, Ferguson DO, *et al.* Histone H2AX: a dosage-dependent suppressor of oncogenic translocations and tumors. *Cell.* 2003;114:359-70.
 197. Fang Y, Tsao CC, Goodman BK, *et al.* ATR functions as a gene dosage-dependent tumor suppressor on a mismatch repair-deficient background. *EMBO J.* 2004;23:3164-74.
 198. Cheung WL, Ajiro K, Samejima K, *et al.* Apoptotic phosphorylation of histone H2B is mediated by mammalian sterile twenty kinase. *Cell.* 2003;113:507-17.
 199. Ura S, Masuyama N, Graves JD, Gotoh Y. Caspase cleavage of MST1 promotes nuclear translocation and chromatin condensation. *Proc Natl Acad Sci U S A.* 2001;98:10148-53.
 200. Ura S, Nishina H, Gotoh Y, Katada T. Activation of the c-Jun N-terminal kinase pathway by MST1 is essential and sufficient for the induction of chromatin condensation during apoptosis. *Mol Cell Biol.* 2007;27:5514-22.
 201. Hurd PJ, Bannister AJ, Halls K, *et al.* Phosphorylation of histone H3 Thr-45 is linked to apoptosis. *J Biol Chem.* 2009;284:16575-83.
 202. Di Lorenzo A, Bedford MT. Histone arginine methylation. *FEBS Lett.* 2011;585:2024-31.
 203. Mosammamparast N, Shi Y. Reversal of histone methylation: biochemical and molecular mechanisms of histone demethylases. *Annu Rev Biochem.* 2010;79:155-79.
 204. Wysocka J, Allis CD, Coonrod S. Histone arginine methylation and its dynamic regulation. *Front Biosci.* 2006;11:344-55.
 205. Weiss T, Hergeth S, Zeissler U, *et al.* Histone H1 variant-specific lysine methylation by G9a/KMT1C and Glp1/KMT1D. *Epigenetics Chromatin.* 2010;3:7.
 206. Shi X, Hong T, Walter KL, *et al.* ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. *Nature.* 2006;442:96-9.
 207. Li H, Ilin S, Wang W, *et al.* Molecular basis for site-specific read-out of histone H3K4me3 by the BPTF PHD finger of NURF. *Nature.* 2006;442:91-5.
 208. Lee J, Thompson JR, Botuyan MV, Mer G. Distinct binding modes specify the recognition of methylated histones H3K4 and H4K20 by JMJD2A-tudor. *Nat Struct Mol Biol.* 2008;15:109-11.
 209. Nielsen PR, Nietlispach D, Mott HR, *et al.* Structure of the HP1 chromodomain bound to histone H3 methylated at lysine 9. *Nature.* 2002;416:103-7.
 210. Xu C, Bian C, Yang W, *et al.* Binding of different histone marks differentially regulates the activity and specificity of polycomb repressive complex 2 (PRC2). *Proc Natl Acad Sci U S A.* 2010;107:19266-71.
 211. Couture JF, Collazo E, Trievel RC. Molecular recognition of histone H3 by the WD40 protein WDR5. *Nat Struct Mol Biol.* 2006;13:698-703.
 212. Bartke T, Vermeulen M, Xhemalce B, *et al.* Nucleosome-interacting proteins regulated by DNA and histone methylation. *Cell.* 2010;143:470-84.
 213. Pasini D, Bracken AP, Hansen JB, Capillo M, Helin K. The polycomb group protein Suz12 is required for embryonic stem cell differentiation. *Mol Cell Biol.* 2007;27:3769-79.
 214. Pasini D, Bracken AP, Jensen MR, Lazzarini Denchi E, Helin K. Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. *EMBO J.* 2004;23:4061-71.
 215. Kimura M, Koseki Y, Yamashita M, *et al.* Regulation of Th2 cell differentiation by mel-18, a mammalian polycomb group gene. *Immunity.* 2001;15:275-87.
 216. Torres-Padilla ME, Parfitt DE, Kouzarides T, Zernicka-Goetz M. Histone arginine methylation regulates pluripotency in the early mouse embryo. *Nature.* 2007;445:214-8.
 217. Schotta G, Sengupta R, Kubicek S, *et al.* A chromatin-wide transition to H4K20 monomethylation impairs genome integrity and programmed DNA rearrangements in the mouse. *Genes Dev.* 2008;22:2048-61.
 218. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. *Nature.* 2011;469:343-9.
 219. Kuzmichev A, Jenuwein T, Tempst P, Reinberg D. Different EZH2-containing complexes target methylation of histone H1 or nucleosomal histone H3. *Mol Cell.* 2004;14:183-93.
 220. Trojer P, Zhang J, Yonezawa M, *et al.* Dynamic histone H1 isotype 4 methylation and demethylation by histone lysine methyltransferase G9a/KMT1C and the Jumonji domain-containing JMJD2/KDM4 proteins. *J Biol Chem.* 2009;284:8395-405.
 221. Tang X, Milyavsky M, Shats I, *et al.* Activated p53 suppresses the histone methyltransferase EZH2 gene. *Oncogene.* 2004;23:5759-69.
 222. Bracken AP, Pasini D, Capra M, *et al.* EZH2 is downstream of the pRB-E2F pathway, essential for proliferation and amplified in cancer. *EMBO J.* 2003;22:5323-35.
 223. O'Carroll D, Erhardt S, Pagani M, *et al.* The polycomb-group gene *Ezh2* is required for early mouse development. *Mol Cell Biol.* 2001;21:4330-6.
 224. Lee TI, Jenner RG, Boyer LA, *et al.* Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell.* 2006;125:301-13.
 225. Boyer LA, Plath K, Zeitlinger J, *et al.* Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature.* 2006;441:349-53.
 226. Kleer CG, Cao Q, Varambally S, *et al.* EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc Natl Acad Sci U S A.* 2003;100:11606-11.
 227. Varambally S, Dhanasekaran SM, Zhou M, *et al.* The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature.* 2002;419:624-9.
 228. Sasaki M, Yamaguchi J, Itatsu K, Ikeda H, Nakanuma Y. Over-expression of polycomb group protein EZH2 relates to decreased expression of p16 INK4a in cholangiocarcinogenesis in hepatolithiasis. *J Pathol.* 2008;215:175-83.
 229. Alajez NM, Shi W, Hui AB, *et al.* Enhancer of Zeste homolog 2 (EZH2) is overexpressed in recurrent nasopharyngeal carcinoma and is regulated by miR-26a, miR-101, and miR-98. *Cell Death Dis.* 2010;1:e85.
 230. Karanikolas BD, Figueiredo ML, Wu L. Polycomb group protein enhancer of zeste 2 is an oncogene that promotes the neoplastic transformation of a benign prostatic epithelial cell line. *Mol Cancer Res.* 2009;7:1456-65.

231. Gonzalez ME, Li X, Toy K, *et al.* Downregulation of EZH2 decreases growth of estrogen receptor-negative invasive breast carcinoma and requires BRCA1. *Oncogene*. 2009;28:843-53.
232. Min J, Zaslavsky A, Fedele G, *et al.* An oncogene-tumor suppressor cascade drives metastatic prostate cancer by coordinately activating Ras and nuclear factor-kappaB. *Nat Med*. 2010;16:286-94.
233. Chen Y, Xie D, Yin Li W, *et al.* RNAi targeting EZH2 inhibits tumor growth and liver metastasis of pancreatic cancer in vivo. *Cancer Lett*. 2010;297:109-16.
234. Holland D, Hoppe-Seyler K, Schuller B, *et al.* Activation of the enhancer of zeste homologue 2 gene by the human papillomavirus E7 oncoprotein. *Cancer Res*. 2008;68:9964-72.
235. Fujii S, Ochiai A. Enhancer of zeste homolog 2 downregulates E-cadherin by mediating histone H3 methylation in gastric cancer cells. *Cancer Sci*. 2008;99:738-46.
236. Fujii S, Ito K, Ito Y, Ochiai A. Enhancer of zeste homologue 2 (EZH2) down-regulates RUNX3 by increasing histone H3 methylation. *J Biol Chem*. 2008;283:17324-32.
237. Yu J, Cao Q, Mehra R, *et al.* Integrative genomics analysis reveals silencing of beta-adrenergic signaling by polycomb in prostate cancer. *Cancer Cell*. 2007;12:419-31.
238. Yu J, Mani RS, Cao Q, *et al.* An integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate cancer progression. *Cancer Cell*. 2010;17:443-54.
239. Ernst T, Chase AJ, Score J, *et al.* Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet*. 2010;42:722-6.
240. Morin RD, Johnson NA, Severson TM, *et al.* Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet*. 2010;42:181-5.
241. Varambally S, Cao Q, Mani RS, *et al.* Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. *Science*. 2008;322:1695-9.
242. Zhang B, Liu XX, He JR, *et al.* Pathologically decreased miR-26a antagonizes apoptosis and facilitates carcinogenesis by targeting MTDH and EZH2 in breast cancer. *Carcinogenesis*. 2011;32:2-9.
243. Wei Y, Chen YH, Li LY, *et al.* CDK1-dependent phosphorylation of EZH2 suppresses methylation of H3K27 and promotes osteogenic differentiation of human mesenchymal stem cells. *Nat Cell Biol*. 2011;13:87-94.
244. Chen S, Bohrer LR, Rai AN, *et al.* Cyclin-dependent kinases regulate epigenetic gene silencing through phosphorylation of EZH2. *Nat Cell Biol*. 2010;12:1108-14.
245. Garcia-Cao M, O'Sullivan R, Peters AH, Jenwein T, Blasco MA. Epigenetic regulation of telomere length in mammalian cells by the Suv39h1 and Suv39h2 histone methyltransferases. *Nat Genet*. 2004;36:94-9.
246. Peters AH, O'Carroll D, Scherthan H, *et al.* Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell*. 2001;107:323-37.
247. Nielsen SJ, Schneider R, Bauer UM, *et al.* Rb targets histone H3 methylation and HP1 to promoters. *Nature*. 2001;412:561-5.
248. Li Z, Chen L, Kabra N, *et al.* Inhibition of SUV39H1 methyltransferase activity by DBC1. *J Biol Chem*. 2009;284:10361-6.
249. Tachibana M, Sugimoto K, Nozaki M, *et al.* G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes Dev*. 2002;16:1779-91.
250. Wu H, Chen X, Xiong J, *et al.* Histone methyltransferase G9a contributes to H3K27 methylation in vivo. *Cell Res*. 2011;21:365-7.
251. Chen MW, Hua KT, Kao HJ, *et al.* H3K9 histone methyltransferase G9a promotes lung cancer invasion and metastasis by silencing the cell adhesion molecule Ep-CAM. *Cancer Res*. 2010;70:7830-40.
252. Krivtsov AV, Armstrong SA. MLL translocations histone modifications and leukaemia stem-cell development. *Nat Rev Cancer*. 2007;7:823-33.
253. Yu BD, Hess JL, Horning SE, Brown GA, Korsmeyer SJ. Altered Hox expression and segmental identity in Mll-mutant mice. *Nature*. 1995;378:505-8.
254. Ernst P, Fisher JK, Avery W, *et al.* Definitive hematopoiesis requires the mixed-lineage leukemia gene. *Dev Cell*. 2004;6:437-43.
255. Hess JL. MLL: a histone methyltransferase disrupted in leukemia. *Trends Mol Med*. 2004;10:500-7.
256. Ayton PM, Cleary ML. Transformation of myeloid progenitors by MLL oncoproteins is dependent on Hoxa7 and Hoxa9. *Genes Dev*. 2003;17:2298-307.
257. Hamamoto R, Furukawa Y, Morita M, *et al.* SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells. *Nat Cell Biol*. 2004;6:731-40.
258. Hamamoto R, Silva FP, Tsuge M, *et al.* Enhanced SMYD3 expression is essential for the growth of breast cancer cells. *Cancer Sci*. 2006;97:113-8.
259. Silva FP, Hamamoto R, Kunizaki M, *et al.* Enhanced methyltransferase activity of SMYD3 by the cleavage of its N-terminal region in human cancer cells. *Oncogene*. 2008;27:2686-92.
260. Esteve PO, Chin HG, Benner J, *et al.* Regulation of DNMT1 stability through SET7-mediated lysine methylation in mammalian cells. *Proc Natl Acad Sci U S A*. 2009;106:5076-81.
261. Esteve PO, Chang Y, Samaranyake M, *et al.* A methylation and phosphorylation switch between an adjacent lysine and serine determines human DNMT1 stability. *Nat Struct Mol Biol*. 2011;18:42-8.
262. Huang J, Perez-Burgos L, Placek BJ, *et al.* Repression of p53 activity by Smyd2-mediated methylation. *Nature*. 2006;444:629-32.
263. Huang J, Sengupta R, Espejo AB, *et al.* p53 is regulated by the lysine demethylase LSD1. *Nature*. 2007;449:105-8.
264. Chuikov S, Kurash JK, Wilson JR, *et al.* Regulation of p53 activity through lysine methylation. *Nature*. 2004;432:353-60.
265. Scoumanne A, Chen X. Protein methylation: a new mechanism of p53 tumor suppressor regulation. *Histol Histopathol*. 2008;23:1143-9.
266. Huang J, Berger SL. The emerging field of dynamic lysine methylation of non-histone proteins. *Curr Opin Genet Dev*. 2008;18:152-8.
267. Pasqualucci L, Dominguez-Sola D, Chiarenza A, *et al.* Inactivating mutations of acetyltransferase genes in B-cell lymphoma. *Nature*. 2011;471:189-95.
268. Mack GS. To selectivity and beyond. *Nat Biotechnol*. 2010;28:1259-66.
269. Collins RE, Northrop JP, Horton JR, *et al.* The ankyrin repeats of G9a and GLP histone methyltransferases are mono- and dimethyl-lysine binding modules. *Nat Struct Mol Biol*. 2008;15:245-50.