BRIEFINGS IN FUNCTIONAL GENOMICS. VOL 9. NO 6. 429–443 doi:10.1093/bfgp/elq024

Chatting histone modifications in mammals

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Abstract

Eukaryotic chromatin can be highly dynamic and can continuously exchange between an open transcriptionally active conformation and a compacted silenced one. Post-translational modifications of histones have a pivotal role in regulating chromatin states, thus influencing all chromatin dependent processes. Methylation is currently one of the best characterized histone modification and occurs on arginine and lysine residues. Histone methylation can regulate other modifications (e.g. acetylation, phosphorylation and ubiquitination) in order to define a precise functional chromatin environment. In this review we focus on histone methylation and demethylation, as well as on the enzymes responsible for setting these marks. In particular we are describing novel concepts on the interdependence of histone modifications marks and discussing the molecular mechanisms governing this cross-talks.

Keywords: *histone modifications; histone methylation; cross-talk; epigenetic; chromatin*

INTRODUCTION

In mammals the genomic information is organized into chromatin. The structural and functional unit of chromatin is the nucleosome, that consists of an octamer of the core histones H2A, H2B, H3 and H4 around which 147 bp of DNA are wrapped [1]. In addition, the linker histone H1 binds the DNA entering and exiting the nucleosome and protects the linker DNA, further compacting chromatin. Chromatin is not a static structure, but in order to allow vital cellular processes to occur, it needs to be dynamically modulated. Three main mechanisms have been proposed to regulate chromatin compaction and decompaction. First, chromatin remodeling complexes use the energy liberated from ATP hydrolysis to actively move and reposition nucleosomes along the DNA [2]. Second, histone variants are incorporated at specific locations where they define a precise chromatin state [3] and third, covalent modifications of histones or DNA can be key to regulation of chromatin structure and all DNA dependent processes [4, 5].

So far the best studied histone modifications are located within the flexible N-terminal tail of the core histones. With the recent improvement of the sensitivity of mass-spectrometrical techniques, new, previously uncharacterized modifications have been identified *in vivo* both in the tails and in the core domain of histones [6–10]. However, for many histone modifications their functional role is not yet fully understood.

HOW DO HISTONE MODIFICATIONS WORK?

There are two main mechanisms explaining the impact of histone modifications on chromatin functions.

The first is the disruption of contacts between adjacent nucleosomes or between histones and DNA e.g. by charge changes. The best example for this is histone lysine acetylation. Due to its capacity to neutralize the positive charge of lysines, histone acetylation can weaken the affinity between histone

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and DNA, thus creating a more accessible and open chromatin state [11]. In line with these findings, the development of a strategy to produce recombinant nucleosomes fully modified at a specific site showed that histone H4 lysine 16 (H4K16) acetylation inhibits the formation of the condensed 30 nm fiber and the establishment of higher order of chromatin structure [12].

The second mechanism to regulate chromatin dynamics is the recruitment of specific binding proteins by histone marks. According to the so called 'histone code' hypothesis [13, 14], single histone modifications or combinations of modifications can be recognized by effector proteins or protein complexes that read these marks, convert them into specific functional chromatin states and regulate downstream responses. It is now well established that chromo-like domains (chromo, MBT, Tudor) and non-related plant homeo domains (PHD) specifically bind methylated lysines, whereas acetylation is specifically recognized by bromodomains and phosphorylation by 14-3-3 proteins [15–17].

Interestingly, the modification specific binding modules and the catalytic domains setting these marks can often be found within the same protein or protein complex, suggesting that pattern of histone modifications can be 'read and written' by the same interacting factor(s). For example the histone methyltransferases G9a and its interaction partner Glp1 bind H3K9me1/me2 via their ankyrin repeats and methylate neighboring histones on H3K9 via a distinct catalytic domain [18]. This product-binding capacity of G9a/Glp1 illustrates a general 'feed forward loop' mechanism how cells can maintain and propagate histone modifications and functionally defined chromatin states [19].

While modifications of the histone tails might regulate nucleosome function by affecting the binding of effector proteins, modifications within the histone fold domain are more probable to directly regulate nucleosome structure [20, 21].

LYSINE METHYLATION AND DEMETHYLATION

Methylation can occur at different aminoacid residues such as lysine, arginine and histidine. Methylation of lysines and arginines has been extensively studied and implicated in multiple cellular processes [4]. Histone methylation is so far the most complex modification, since its function depends on the precise methylation site and the degree of modification. Lysine residues can be mono-, di- or tri-methylated, whereas arginines can be mono- or di-methylated. In addition arginines can be symmetrically or asymmetrically di-methyleted. Therefore we will focus here on histone methylation and in particular on its complex cross-talk with other modifications.

Site and state-specific lysine methylation of histones is catalyzed by a group of lysine methyltransferases (KMT) containing the evolutionarily conserved SET domain [Su(var), enhancer of zeste, Tritorax] (Table 1). They have been sub-grouped into seven main families, named according to their founding member: SUV39, SET1, SET2, EZ, RIZ, SMYD and SUV4-20 [22]. In addition few orphan members have been identified: SET7/9 and SET8 (also known as PRSET7). Proteins within the same family share high similarity within the SET domain as well as in the surrounding sequences [22]. To date the only identified non-SET domain-containing lysine KMTase is DOT1, specific for H3K79 methylation in the core region of H3 [23, 24].

So far methylatiopn of five residues within the N-terminal tail (H3K4, H3K9, H3K27, H3K36 and H4K20) of histones H3 and H4, and of two residues in the globular domain (H3K64 and H3K79) of histone H3 have been functionally characterized. In addition, the linker histone H1 can also be methylated at H1.4K26. In general, H3K9, H3K27 H3K64 H4K20 and H1.4K26 methylation have been implicated in transcriptional silencing [25] whereas, H3K4, H3K36 and H3K79 methylation are associated with transcriptionally active regions [25]. However, depending on the methylation states and the genomic location the same modification might have different functional outcomes.

H3K9 methylation is involved in euchromatic gene silencing as well as in heterocromatin formation [26, 27]. H3K27 methylation has an important role in the repression of HOX genes during development and in X chromosome inactivation and imprinting [28–30]. More recently H3K64me3 has been shown by our lab to be enriched at pericentric heterochromatin and to be associated with repeat sequences and transcriptionally inactive genomic regions [31].

In the case of H4K20 each methylation state is implicated in different biological processes. H4K20me1 peaks in M phase and is involved in cell-cycle progression and chromosome condensation [32–34]. Outside of mitosis H4K20me1 is a

Table 1: Histone methylase and demethylase, their target sites and their chromatin function

	KMT	Human nomenclature	Modification sites	Implicated in
SET domain containing histone lysine methylases	SUV39 family			
	SUV39H1	KMT1A	H3K9me2/me3	Transcriptional silencing Heterochromatin formation
	SUV39H2	KMT1B	H3K9me2/me3	Transcriptional silencing Heterochromatin formation
	G9a	KMT1C	H3K9me1/me2	Transcriptional silencing
	GLP1 (euHMT)	KMT1D	H3K9me1/me2	Transcriptional silencing
	ESET (SETDB1)	KMT1E	H3K9me2/me3	Transcriptional silencing of euchromatic genes
	CLLD8 (SETDB2)	KMT1F	H3K9me2/me3	Transcriptional silencing Chromosome condensation and segregation
	SET1 family			
	MLL1 (HRX, ALL1)	KMT2A	H3K4me1/me2	Transcriptional activation
	MLL2 (ARL)	KMT2B	H3K4me1/me2/me3	Transcriptional activation
	MLL3	KMT2C	H3K4me1/me2/me3	Transcriptional activation
	MLL4 (HRX2)	KMT2D	H3K4me1/me2/me3	Transcriptional activation
	MLL5	KMT2E	H3K4me3	Transcriptional activation
	SET1 (ASH2)	KMT2	H3K4me1/me2/me3	Transcriptional activation
	SET2 family			
	WHSC1 (NSD2)		H3K4me2	Transcriptional suppressor of IL-5
			H3K27me2	Transcriptional activation during development
			H3K36me2	Wolf-Hirschhorn syndrome 1
			H4K20me2	
	WHSC1L1 (NSD3)		H3K4me3	Transcriptional repression
			H3K27me3	
	NSD1	KMT3B	H3K36me2	Transcriptional silencing
			H4K20me2	Transcriptional activation Acute non lymphoblastic leukemia (ANLL)
	SET2 (HYPB)	KMT3A	H3K36me3	Transcription elongation Repressor of intergenic transcription
	ASH1	KMT2H	H3K4me3	Transcriptional activation
	RIZ family			
	RIZ (PRDM2)	KMT8	H3K9 specificity not known	Transcriptional regulation of ER genes during neuronal differentiation
	BLIMP1 (PRDM1)		No enzymatic activity	Transcriptional repression
	SMYD family			
	SMYD1	KMT3D	H3K4me1/me2/me3	Transcriptional silencing
	SMYD2	KMT3C	H3K36me1/me2/me3	Transcriptional regulation
	SMYD3	KMT3E	H3K4me2/me3	Transcriptional activation
	EZ family			
	EZH1	KMT6B	H3K27me1/me2/me3	HOX genes silencing
	EZH2	KMT6A	H3K27me1/me2/me3	HOX gene silencing

(continued)

Table I: Continued

	KMT	Human nomenclature	Modification sites	Implicated in
	SUV4-20 family			
	SUV4-20HI	KMT5B	H4K20me2/me3	Transcriptional silencing, DNA repair
	SUV4-20H2	KMT5C	H4K20me2/me3	Transcriptional silencing, DNA repair
	Others			
	SET7/9	KMT7	H3K4mel/me2	Transcriptional activation
	SET8 (PR-SET7)	KMT5A	H4K20mel	Transcriptional activation
NON SET domain-containing KMTs	DOT family			
	DOTI	KMT4	H3K79mel/me2/me3	Transcriptional activation
Histone lysine demethylases	LSD family			
	LSDI	KDMI	H3K4mel/me2 H3K9mel/me2	transcriptional repression (with Corest) Transcriptional activation (with AR)
	LSD2	KDM2	H3K4mel/me2	Transcriptional repression
	JMJC family			
	JHDMIa/b	KDM2A/B	H3K4mel/me2/me3 H3K36me2/me3	Suppression of spontaneous mutation (C. elegans) heterochromatin maintenance at the mating type locus (S. cerevisiae)
	JHDM2	KDM3A	H3K9mel/me2	Transcriptional activation AR-dependent genes
	JHDM3/JMJD2	KDM4	H2K9me2/me3 H3K36me2/me3	Transcriptional activation and repression
	JMJD2a	KDM4A	H3K9mel/me2/me3 H3K36me2/me3	Transcriptional regulation
	JMJD2b/c	KDM4B/C	H3K9mel/me2/me3	AR-dependent gene activation
	JMJD3		H3K27me2/me3	
	JARIDa/b/c/d	KDM5A/B/C/D	H3K4mel/me2/me3	NR-dependent gene activation HOX gene activation Cardiac gene repression
	PHF2	JHDMIE	H3K9mel	rDNA genes activation
	PHF8	JHDMIF	H3K9mel/me2	rDNA genes activation
	UTX/UTY (KDM6A)	KDM6A	H3K27me2/me3	HOX gene activation
Histone arginine methylases	PRMT family			
	PRMTI	HRMTIL2	H4R3	Transcriptional activation
	PRMT2	HRMTILI	No enzymatic activity	Co-activator in ER-dependent transcription
	PRMT3	HRMTIL3	Ribosomal proteins	Ribosome biosynthesis
	PRMT4 (CARMI)	HRMTIL4	H3RI7, H3R26	Transcriptional activation of NR-regulated genes Epigenetic reprogramming in embryos
	PRMT5	HRMTIL5	H4R3	Transcriptional repression
			H3R8	Transcriptional activation of myogenic genes
			H2AR3	Epigenetic reprogramming of PGC
	PRMT6	HRMTIL6	H3R2 H2AR3	Transcriptional repression
	PRMT7	HRMTIL7	H2AR3 H4R3	Transcriptional regulation of imprinted genes
	PRMT8	HRMTIL8	H4 (specificity not known)	Unknown
	PRMT9 (isoform 4)	HRMTIL9	H2AR3, H4R3	Unknown
	PRMTI0, PRMTII	HRMTILI0 HRMTILII	Not determined	Unknown
Histone arginine demethylases	PADI family			
	PADI4		H3R2me2 H3R8me3 H3RI7me2 H3R26me2 H4R3me2	Transcriptional repression and activation
	JMJD6		H3R2me2 H4R3me2	Transcriptional regulation during differentiation

mark for active transcription [35]. H4K20me2 has a role in DNA repair [36] and H4K20me3 is enriched in heterochromatin and is implicated in heterochromatin maintenance and telomere stability [37, 38].

H3K4 methylation occurs in mammals in several distinct genomic distributions. Strong enrichments of H3K4me3 are found at transcription start sites (TSS) of active genes whereas H3K4me2 is present across the body of genes, where they contribute to transcriptional initiation and mRNA processing respectively [10, 35, 39]. H3K4me1 peaks instead at the 3′ end of active genes both in yeast and mammals [40, 41]. Targeting of H3K4 methylation to these sites can occur via the interaction of H3K4 specific KMTs with the active, phosphorylated form of RNA Pol II, providing a direct link with transcription [42]. Interestingly large domains of H3K4 methylation covering both genic and intergenic regions are evident at specific locations such as the HOX genes cluster. A deeper analysis of the HOXA and HOXB loci identified in these regions multiple promoters generating non-coding RNA [43]. These intergenic transcripts have been shown to enhance gene accessibility [44, 45] and in the case of the HOX cluster they might contribute to its tightly controlled pattern of expression during differentiation.

H3K36 methylation is coupled to the process of active transcriptional elongation and it is enriched towards the 3′ end of target genes [46]. However, when present within protein-coding regions it prevents inappropriate transcriptional initiation of intragenic sequences [47].

The function of H3K79 methylation in transcription is still somehow contradictory. In flies and in mammals H3K79 methylation has been suggested to act as an active mark [48, 49]. However, in a parallel study, Barski *et al.* [50] showed by using the Solexa sequencing that H3K79me1 is enriched at active genes, whereas H3K79me3 is present at transcriptionally repressed genes in human cells.

Modifications of histones H2A and H2B have been less studied [51]. However, deletion of the N terminal tail of H2A or H2B in yeast showed that they affect the expression of a large number of genes and that have then an important role in gene regulation. Recently by Solexa sequencing of the human genome, H2BK5 methylation has been mapped to active genes, suggesting a possible link with transcription [50].

In addition to the core histones, the linker histone H1 is methylated at H1.4K26. This mark is implicated in heterochromatin formation and transcriptional repression by Ezh2 [52, 53].

Although lysine methylation has been considered for a long time as an irreversible modification, it is now well established that like acetylation and phosphorylation also histone methylation can be a dynamic modification. To date two groups of histone lysine demethylases (KDM) have been identified. The first group of amine oxidase-domain containing enzymes is represented by LSD1 (also known as AOF2) and LSD2 (also known as AOF1). LSD1 demethylates H3K4me1/me2 *in vitro* and *in vivo* [54] and its activity on nucleosomes substrates requires the transcriptional co-repressor CoREST [55]. LSD1 is also recruited to androgen receptor (AR)-regulated genes where it acts as activator. It has been shown that interaction of LSD1 activity with AR redirects LSD1 enzymatic activity towards H3K9me1/me2 [56]. Moreover, LSD is also stimulated by HDAC1 (histone deacetylase 1) revealing a functional interconnection between histone demethylation and deacetylation [57]. LSD2 has been recently identified and shown to be specific for H3K4me1/me2 [58]. As opposed to LSD1, LSD2 does not form a biochemically stable complex with the co-repressor protein CoREST. Furthermore, LSD2 contains a CW-type zinc finger motif with potential zinc-binding sites that are not present in LSD1 [58].

The second group of KMTs is represented by the Jumonji domain-containing proteins (jmjC), the members of this group are Fe(II) and 2-oxoglutarate (2OG) dependent oxygenases [59]. Unlike LSD1 that can remove only mono and dimethyl groups from lysine residues, jmjC enzymes are able to revert all three histone lysine methylation states [60, 61]. Based on the presence of additional domains beside the jmjC domain, JmjC histone demethylases (JHDM) enzymes have been classified into seven evolutionary conserved subgroups (JHDM1, PHF2/PHF8, JARID, JHDM3/JMJD2, UTX/UTY, JHDM2 and JmjC domain only). Although for many of these proteins the enzymatic activity as histone demethylases still needs to be proven, generally the substrate specificity for the known JHDMs rely on both the JmjC domain and additional domains within each enzyme. For the most histone methylation marks demethylase have been identified, however so far no demethylase

enzymes have been found for H3K64 and H3K79 methylation [60].

ARGININE METHYLATION AND DEMETHYLATION

Whereas lysine methylation has been intensively globally mapped in multiple organisms, we currently know much less about the distribution and functions of arginine methylation. Arginine methylation is catalyzed by a family of enzymes called protein arginine methyltransferases (PRMTs). All members are able to monomethylate arginine, but according to the type of dimethylation they are classified into two classes: type I (PRMT1, 3, 4, 6, 8) asymmetrically dimethylate arginine; and type II (PRMT5, 7, 9) symmetrically dimethylate arginine [62]. The PRMT family members are ubiquitously expressed and evolutionary conserved from yeast to human. To date, 11 mammalian PRMTs have been identified. They share a conserved catalytic domain, but differ in their cellular localization as well as in their substrate specificity. PRMT2, PRMT10 and PRMT11 enzymatic activity has not yet been demonstrated [63]. Interaction of PRMTs with different cofactors has also been proposed to modulate their enzymatic activities and specificity at defined loci [63].

H3R2, H3R8 H3R17 H3R26, H4R3 H2AR3 are known PRMT targets *in vivo* [63] (Table 1). Like different lysine methylation states, symmetric or asymmetric methylation of the same arginine can mark distinct chromatin regions. However, in contrast to lysine methylation the functional role of arginine methylation in chromatin structure and transcription has been so far underexplored. Moreover, general levels of arginine methylation seemed to be lower compared to lysine methylation, indicative of a more restricted function in gene regulation rather than a general structural role in chromatin formation.

When asymmetrically methylated by PRMT1, H4R3me2 acts as a transcription activating mark of several ER regulated genes [64, 65] and is essential *in vivo* for the establishment and maintenance of open chromatin domains marked by H3 and H4 acetylation [66]. However, when symmetrically dimethylated by PRMT5, H4R3me2 is instead involved in transcriptional silencing, as expected since PRMT5 is also a subunit of the repressive MB2/NURD histone deacetylating complex [67]. In the case of the beta-globin locus transcriptional silencing by PRMT5 is achieved via the recruitment of DNMT3A and subsequent DNA methylation of the promoter region [68]. In agreement with a potential connection between arginine methylation and DNA methylation, H3R8me2 and H4R3me2 have been shown to regulate rDNA promoter activity in a DNA methylation-dependent manner [69]. Unlike other symmetrically methylated arginines, H3R8me2 catalyzed by PRMT5 can also act as an activating mark by cooperating with the SWI/SNF chromatin-remodeling complex in the regulation of genes involved in myogenic differentiation [70]. Moreover, the level of H2A/H4R3me2, another PRMT5 site, are dynamically modulated during epigenetic reprogramming of primordial germ cells (PGCs), pointing at an important function for this modification in the mouse germ cell lineage [71].

H3R17me2 and H3R26me2 are methylated by PRMT4 (also known as CARM1) and are implicated in nuclear receptor (NR)-mediated activation of transcription and the regulation of pluripotent genes during the early mouse development [72, 73].

H3R2me2 is mainly catalyzed by PRMT6. This modification is enriched at heterochromatin and silent euchromatin and depleted at the TSS of active promoters, thus acting as a repressive mark [74, 75]. H3R2me2 can also be targeted by CARM1 although to a lesser extent and it cooperates with histone acetylation in the activation of NR regulated genes [72].

Like other modifications involved in chromatin-regulated processes also arginine methylation needs to be reverted in order to maintain a functional equilibrium within the cells. However, very little is known about arginine demethylases. To date two proteins have been implicated in this mechanism: PADI4 and JMJD6. PADI4 is the only member of the protein arginine deiminase I (PADI) family with nuclear localization where it catalyses deimination of monomethylarginines to citrullines. PADI4 recognizes arginine residues surrounded by unstructured amino acid sequences and has therefore a broad range of substrate specificity. Monomethylarginines on both H3 (R2, R8, R17 and R26) and H4 (R3) are PADI4 targets [76]. However, this enzyme fails to meet the requirements for a true arginine demethylase since it converts the arginine to a citrulline.

One recent publication showed that JMJD6, a member of the JMJ lysine demethylases, can specifically demethylate H3R2 and H4R3 me1/me2 both

in vitro and *in vivo* [77], however there were so far no follow ups to this discovery.

HISTONE MODIFICATIONS CROSS-TALK

It is now well established that there is an intense cross-talk between histone modifications to drive distinct downstream functions. Cross regulation can occur in different flavors: on the one hand, one modification can promote/block the addition of another modification. On the other hand, one modification can stimulate/block the removal of another modification. Moreover, the cross-talk can occur on the same histone (cross-talk *in cis*; Figure 1A), between histones within the same nucleosome (cross-talk *in trans*; Figure 1B) or across nucleosomes (nucleosome cross-talk). An increasing number of histone modifying complexes are found to contain more than one distinct enzymatic activities. These enzymes can act in concert to determine the functional status of chromatin by coordinating multiple histone modifications (Figure 1C). For each of these scenarios multiple examples have been described and we will discuss some of the most exiting ones below. Cross-talks also have major implications for our understanding and interpretation of genome-wide mapping data of histone modifications. These approaches usually profile the average presence of histone modifications within a cell population at certain genomic regions. However, they do not provide any information about which marks co-occur at a given nucleosome and about the molecular mechanisms leading to the deposition of these marks on chromatin.

Only combining our knowledge of how histone modifications influence each other and cross-talk with the high-resolution maps obtained by high throughput sequencing will allow us to answer fundamental biological questions and to make further progresses in deciphering the multiple roles of histone modifications in DNA-dependent processes.

THE METHYL-PHOSPHO SWITCH

One of the first examples for cross-regulation of histone modifications *in cis* is between H3K9 methylation and the neighboring H3S10 phosphorylation [78]. H3S10 phosphorylation is required for chromosome condensation and segregation during mitosis [79]. H3K9me3 can be specifically bound by the chromodomain of heterochromatin protein 1 (HP1) and has a pivotal role in heterochromatin formation and propagation of pericentric heterochromatin [80, 81]. However, in mitosis HP1 is released from condensed chromatin despite the persistence of its recruiting mark H3K9me3 [82, 83]. To explain this apparent contradiction the methyl-phospho switch model has been proposed. It suggests that H3S10 phosphorylation displaces HP1 from chromatin by inhibiting its binding to the adjacent H3K9me3. Moreover, the loss of HP1 from chromatin during mitosis occurs in concomitance with an increase in H3S10 phosphorylation levels and prior to the loading of condensins to chromatin [78]. Interestingly, low levels of H3S10 phosphorylation have been detected also in interphase where H3S10 phosphorylation is found at the promoter of immediate early (IE) responsive genes. Like in mitosis also in interphase removal of HP1 from chromatin depends on H3S10 phosphorylation and is a prerequisite for transcriptional activation [84]. In support of an interdependence between these two marks, H3S10 phosphorylation levels are significantly increased in Suv39h double null MEFs cells [85].

This methyl-phospho switch model is not limited to directly neighboring residues. Recently two non-adjacent sites within H3 have been found to modulate each other, providing an additional intriguing example of methyl-phospho cross-talk (Figure 2). H3T6 phosphorylation by PKCβI is a novel mark for transcriptional activation of several AR (androgen-receptor)-regulated genes. It can block H3K4 demethylation by the demethylases LSD1 (specific for H3K4me1/me2) and JARID1B (specific for H3K4me2/me3) and it redirects their enzymatic activity towards H3K9 methylation [86]. In support of this model, PKCβI co-localizes with AR and LSD1 at target gene promoters and phosphorylates H3T6 upon androgen receptor activation. Depletion of PKCβI by RNAi abrogates H3T6 phosphorylation, enhances H3K4 demethylation by LSD1 and as a consequence AR dependent transcription is inhibited. While H3T6 phosphorylation blocks LSD1 and JARID1B activities, H3T11 phosphorylation by PRK1 acts in a different way by increasing the activity of LSD1 (H3K9me1/me2) and JMJD2C (H3K9me2/me3) for H3K9 methylation [87] (Figure 2). In agreement with a role for H3T11 phosphorylation in transcriptional activation

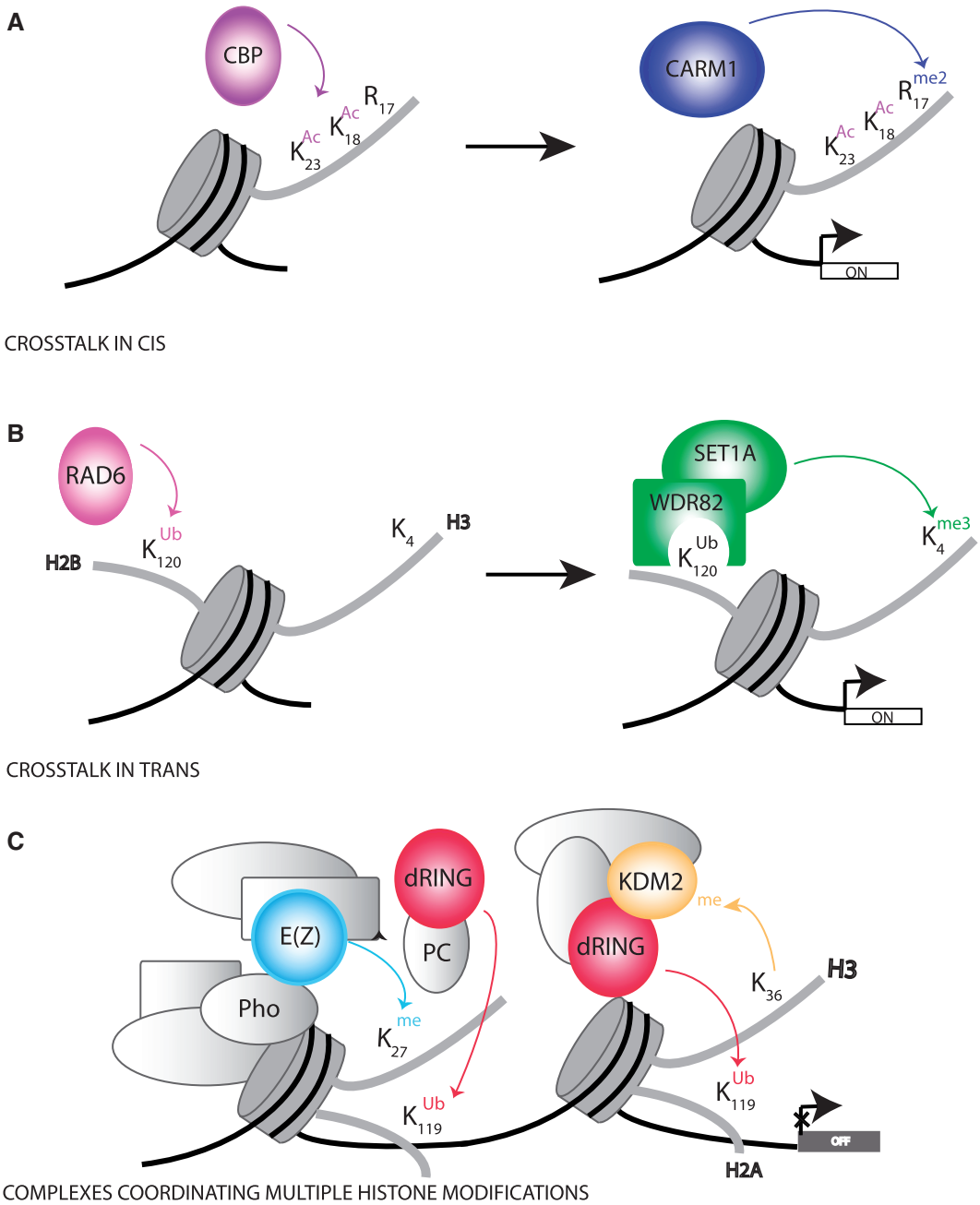

Figure 1: Mechanisms of cross-talk between histone modifications. (**A**) Cross-talk in *cis*; acetylation of H3K18 and H3K23 by CBP can promote the methylation of H3R17 by the methyltransferase CARM1, resulting in activation of estrogene-responsive genes [90]. (**B**) Cross-talk in *trans*; H2BK120 ubiquitination by RAD6 is recognized by the WDR82 subunit of the SETIA/B COMPASS complex and it is a prerequisite for efficient H3K4 methylation by SETIA/B and transcriptional activation of target genes in mammals. (**C**) Multifunctional histone modifications complexes, simplified model of PRC complex function; in Drosophila the Polycomb repressive complexes PRC1, PRC2 and PhoRC are recruited to chromatin in a hierarchical manner and they coordinate distinct histone modifications. The Pho subunit of PhoRC complex binds specific PRE (Polycomb responsive element) elements in the DNA. The PRC2 complex is recruited to this PRE via interactions between the Pho protein and E(Z) (drosophila homolog of human EZH2), the methyltarnsferase subunit of the PRC2 complex. E(Z), methylates H3K27 forming a binding site for PC, a subunit of the PRC1 complex. dRING, the E3 ligase within the PRC2 complex can mediate ubiquitination of H2AK119. dRING is also a subunit of the dRAF (dRING associated factors), an additonal Polycomb complex in *Drosophila*. dRAF contains the histone demethylase KDM2, which coordinates removal of H3K36me3 with stimulation of H2AK119 ubiquitination by dRING. dRAF cooperates with PRC1 in gene silecing by Polycomb complexes. Additionally non-coding RNAs had recently been implicated in the trageting of Polycomb complexes (not shown).

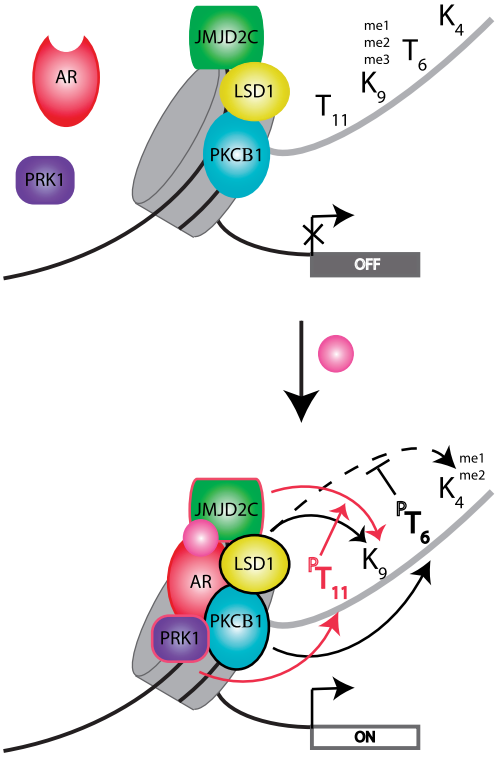

Figure 2: Cross-talk between H3 methylation and phosphorylation in AR-dependent transcriptional regulation. Top panel: in the absence of ligand, the androgen-receptor (AR) is present in the cytoplasma and AR-regulated genes are silenced by the presence of H3K9 methylation. PKCβI, LSDI and JMJD2C are already present on chromatin. Bottom panel: association of ligand-activated AR with PRKI leads to activation of PKCβI. Phosphorylation of H3T6 by activated PKCβI prevents LSDI from demethylating H3K4me2/mel (dashed black arrow) but not H3K9me2/mel and AR-dependent genes get activated. In addition, PRKI phosphorylates H3TII. This mark enhances JMJD2C demethylating activity for H3K9me3 further contributing to transcriptional activation. The PKCβI mediated pathway is indicated by black arrows. The PRKI mediated pathway is indicated by red arrows.

of AR dependent genes it has been shown that their expression is reduced upon PRK1 depletion, due to impairments in H3K9me1/me2 demethylation and in the establishment of H3K9 and K14 acetylation. Moreover, DNA damage induction in cells lacking H3T11 phosphorylation correlates with reduced binding of the histone acetyltransferase GCN5 and reduced H3K9 acetylation at cyclin B1 and cdk1 promoters [88], further supporting an active transcriptional role for this mark.

The discovery of novel methylation sites in close proximity to phosphorylated residues suggests that the methyl-phospho switch mechanism can modulate the binding of regulator proteins to multiple histone methylation marks. One additional example is the interaction of HP1 with H1.4K26me, blocked by the phosphorylation of the adjacent S27 [52]. Additionally, H3K27 is followed by S28 and H3K4 is preceded by T3. All these residues are modified *in vivo* by either methylation (H3K27 and H3K4) or phosphorylation (H3S27 and H3T3). In light of the numerous data pointing at a cross-talk between phosphorylation and methylation, it is tempting to speculate that also the recruitment of binding complexes to H3K27me3 and to H3K4me3 might undergo methyl-phospho switch regulation.

CROSS-TALK BETWEEN HISTONE METHYLATION AND ACETYLATION/DEACETYLATION

Although a methyl-phospho switch for arginine methylation has not been described so far, several examples of cross-talk between arginine methylation and histone acetylation have been documented. H4R3me2 by PRMT1 has been associated with transcriptional activation [89]. However, very little is known about the molecular mechanisms employed. One intriguing possibility is that H4R3me2 defines chromatin domains poised for transcription by regulating histone acetylation at specific target promoters. In agreement with this, H4R3me2 by PRMT1 activates transcription of the MMTV promoter by facilitating H4 acetylation by p300 [90]. RNAi of PRMT1 in erythroid cells, results in the loss of H4R3me2 as well as of H3 and H4 acetylation at the beta-globin locus, further supporting the previous *in vitro* data [66].

Like PRMT1, CARM1 has also been implicated as co-activator in the transcription of nuclear receptor (NR)-regulated genes [91]. CARM1 methylates H4R17 *in vivo* after CBP recruitment and sequential acetylation of H3K18 and H3K23 at the estrogen stimulated pS2 promoter. Interestingly H3K14 acetylation, another target site of p300/CBP does not induce CARM1 activity, providing a support for the specificity of this cross-talk [92].

While PRMT1 and CARM1 cooperate with histone acetylation to activate transcription, PRMT5 has been shown to act as a transcriptional co-repressor via modulating histone deacetylation

levels at specific loci [69]. One example of such a repressive cross-talk is the silencing of ST7 and NM23 tumor suppressor genes by PRMT5. Expression of these genes is reduced in cell line over-expressing PRMT5 and their silencing correlates with an increase in H3R8me2 levels and concomitant loss of H3K9 acetylation at their promoters [93].

HETEROCHROMATIN FORMATION: A CONSERVED MECHANISM

Transcriptionally repressed chromatin is required at telomeric and subtelomeric regions to stabilize chromosome ends and to ensure correct chromosome segregation in mitosis [94]. Due to its vital function, the molecular basis leading to the formation of heterochromatin have been conserved throughout evolution together with the employment of even more specialized mechanisms according to the genome complexity.

In mammals heterochromatin formation requires several sequential steps and a precise cross-talk between histone deacetylation and methylation. First, SIRT1 specifically deacetylates H4K16ac and H3K9ac at the promoter region. Second, it interacts and deacetylates H1.4K26ac establishing a repressive chromatin environment. Finally, chromatin compaction is achieved by further reduction of active marks both at the promoter and at the coding region and by the increase of repressive marks as H3K9me3 and H4K20me3 [38, 95, 96].

Similar players are involved in heterochromatin gene silencing both in worms and yeast. In *Caenorhabditis elegans* an interplay between SIR-2.1, histone H1 (HIS24) and MES-2 (ortholog of enhancer of zeste methyltransferase) has been recently shown to occur at subtelomeric regions in order to maintain constant level of H3K27 methylation [97]. In *Saccharomyces cerevisiae*, H3K79me3 is involved in telomeric silencing [98].

CROSS-TALK BETWEEN METHYLATED RESIDUES

PHD fingers are known to specifically recognize methylated lysines [99] and to regulate transcription via interacting with co-activators and co-repressors or by recruiting additional enzymatic activities. Recently, a new family of PHD finger proteins has been found to contain an additional jmjC demethylation module and to be directly responsible for both histone methylation and demethylation, providing a new example of cross-talk among methylation marks [100].

A member of this family is PHD finger 8 (PHF8) protein. PHF8 demethylates H3K9me1/me2 both *in vitro* and *in vivo*. Its PHD finger domain recognizes specifically H3K4me3. This interaction is fundamental to stimulate PHF8 demethylase activity and to induce the expression of rDNA genes [101]. Interestingly, a point mutation within the jmjC domain of PFH8 that abolishes its demethylase activity and transcriptional activation has been associated to X-linked mental retardation, linking histone modification cross-regulation and rDNA transcription to neuronal disease [102, 103]. Since many jmjC proteins contain PHD or Tudor domains, it seems that modulation or recruitment of demethylation activities by a pre-existing modified residue might be a common mechanism for this new class of enzymes to propagate histone modification states [104]. In support of this idea the mechanism of action described for PHF8 is conserved by another member of the same family, the jmjC containing protein PHF2 [105].

An additional example of cross-talk between histone methylation marks is the one between H3R2me2 and H3K4me3 [74, 75, 106]. By profiling the presence of several histone marks at the promoters of 151 human genes, Guccione *et al.* [74] found a counter-correlation between H3R2me2 and H3K4me3. The presence of H3K4me3 at gene promoters is positively correlating with messenger RNA levels, whereas H3R2me2 is not. However, H3R2me2 can be enriched within the body of genes regardless of their transcriptional status. Further analysis allowed to get insights into the molecular mechanisms that governs this negative cross-talk and showed that H3R2 methylation prevents the recruitment of WDR5, one of the subunits of the ASH2/MLL complex, responsible in human for H3K4 methylation. In line with this, genomic regions containing H3K4me3 cannot be methylated at H3R2 by PRMT6. The discovery that WDR5 is absent from regions enriched in H3R2me2 and that PRMT6 depletion affects both H3R2 and H3K4 methylation levels provide additional evidences for cross-talk *in vivo* between these two methylation sites. This negative cross-talk is highly specific for the H3R2me2, as it has shown that H3R2me1 has distinct functional characteristics and

it correlates with active transcription [106]. However, it is still not understood how PRMT6 is recruited to chromatin and how H3R2me2 clearance occurs when a gene is activated. The involvement of additional proteins, the action of a not yet identified arginine demethylase as well as histone replacement are all valid possibilities.

Although the molecular basis of many cross-talks between methylation sites have not been identified yet, recent findings support a model in which the coordinated removal of repressive marks and the deposition of activating marks are important for the stringent regulation of transcription during cellular differentiation [61].

CROSS-TALK BETWEEN LYSINE METHYLATION AND UBIQUITINATION

Although less studied, ubiquitination of lysines can occur within histones and regulate other modifications in different chromatin dependent processes.

So far a lot of attention has been given to the cross-talk between H2B ubiquitination and/or H3K4 and H3K79 methylation, and the molecular mechanisms governing this process have been identified and found to be conserved from yeast to human. H2BK120 monoubiquitination is catalyzed by the mammalian RAD6/BREI complex [107] and is a mark linked to transcriptional elongation [108, 109]. Most importantly, it is required for efficient methylation of H3K4me3 and H3K79me2/me3 both in yeast and mammals [110, 111]. It has been shown that Swd2, one of the subunits of the Set1C/COMPASS complex, responsible in yeast for H3K4 methylation, plays a crucial role in translating the H2B ubiquitination signal into H3 methylation [112]. In mammals, several Set1C/COMPASS-like complexes exist and WDR82, the human homolog of Swd2 has been recently identified as a specific subunit of the SET1A/SET1B complexes. WDR82 depletion in several human cell types leads to a drastic reduction in H3K4me3 levels whereas H3K4me1/me2 are unaffected [111]. Moreover, WDR82 loss affects the stability of the entire SET1A complex and SET1A levels also drops upon RNAi. Finally, WDR82 targeting to chromatin is strictly dependent on the monoubiquitination status of H2B, providing a direct link between H2BK120 ubiquitination and H3K4 methylation. Interestingly, Swd2 interact *in vitro* and *in vivo* with Dot1 and partial loss of Swd2 results in a significant reduction of H3K79me3 levels in yeast [111]. One intriguing possibility is that the cross-talk between H2BK120ub and H3K79me in mammals also depends on Swd2/WDR82. However this model requires further studies.

While H2B ubiquitination is linked to active transcription, H2A ubiquitination is considered as a repressive mark. H2AK119 ubiquitination is an abundant modification present in most eukaryotes with the exception of *S. cerevisiae and S. pombe*. RING1B, a subunit of the PRC1 polycomb complex is the E3 ubiquitin ligase catalyzing H2AK119 ubiquitination [113]. While H2BK120ub controls H3K4 and H3K79 methylation, H3K27me3 set by the PRC2 complex is a prerequisite for H2AK119ub, indicative of a role in HOX gene silencing in mammals [114, 115]. In line with these results, depletion of UTX, the specific H3K27me3 demethylase, results in increased occupancy of PRC1 at the promoter of target HOX genes and concomitant enhancement of H2AK119 ubiquitination [116]. The function of H2AK119ub in transcriptional repression has been reinforced by the finding that H2A deubiquitination by USP21 positively modulates H3K4 methylation at the promoter of several genes induced during liver regeneration [117]. Additionally, in *Drosophila* H2AK119 ubiquitination is stimulated by H3K36 demethylation catalyzed by dRING associated factors (dRAF), an alternative Polycomb repressive complex involved in transcriptional silencing [117]. dRAF shares with the PRC1 complex both dRING and PSC subunits, but it specifically contains dKDM2, a specific H3K36me2 demethylase. dKDM2 plays a pivotal role in this dynamic *trans*-histone mechanism by directly coupling H3K36me2 demethylation with stimulation of H2AK119 ubiquitination by dRING. Since H3K36me2 is a mark for transcriptional elongation it has been proposed that dRAF mediated silencing acts via blocking RNA Pol II progression through chromatin [118].

CONCLUDING REMARKS

In an era when rapidly advancing tools are available to perform high throughput genomic screenings, the knowledge about histone modifications interdependence and cross-regulation is crucial, in particular for a comprehensive analysis of histone genomic data in the context of chromatin functions.

Moreover, considering the enormous number of histone modifications that had been discovered, their broad combinatorial potential, their cross-talk, as well as the precise and fascinating network built up by the enzymes regulating their chromatin deposition and/or removal, it is logical to assume that the simplistic view of one modification equals one readout equals one specific function needs to be updated, if not reconsidered. So far, we focused on the cross-talk between two modifications, however there is no reason to exclude cross-talk between multiple modifications. We also know still very little about the *trans*-nucleosome cross-talks contributing to the establishment and maintenance of chromatin domains. Soon it will not be possible to depict these cross-talks in simple models or tables as in this review and the complete picture of the interdependence of histone modifications will be even more complex than originally predicted by the 'histone code' hypothesis. This does not account for a lack in specificity, but rather increasing layers of complexity could have been evolved by higher eukaryotes to further control the functional outputs of combination of modifications. Finally, we also need to keep in mind that not only histones are post-translationally modified, but that also the enzymes setting the marks are subjected to the same modifications. The cross-talk and the intense 'chat' between post-translation modifications and histone modifying enzymes as well as histone modifications and their readers is just starting to be appreciated and will be for sure one of the main focus of researchers in the field for the future.

Key Points

- Histone post-translational modifications regulate chromatin dynamics.
- Lysine and arginine methylation within histones have distinct functions in chromatin dependent processes.
- Combinations of different modifications are recognized by specific effector proteins and increase the epigenetic information.
- Histone modification can cross-talk *in cis* and *in trans*.

Acknowledgements
We thank E. Metzger for helpful discussions.

FUNDING
Max Planck Society; the Deutsche Forshungsgemeinschaft (DFG, SFB 746); the European Union (EU, the Epigenome); European Research Council (ERC) starting grant.

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