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The control of histone lysine methylation in epigenetic regulation

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Abstract

Histone lysine methylation plays a fundamental role in chromatin organization and function. This epigenetic mark is involved in many biological processes such as heterochromatin formation, chromosome X inactivation, genomic imprinting and transcriptional regulation. Here, we review recent advances in how histone lysine methylation participates in these biological events, and the enzymes that control histone lysine methylation and demethylation.

Keywords: Histone lysine methylation; Demethylation; SET domain; JmjC domain; Epigenetics

1. Introduction

1.1. Histone lysine methylation and the ''Histone Code Hypothesis''

Eukaryotic DNA is packaged within the nucleus through its association with histone proteins, forming the fundamental repeating unit of chromatin, the nucleosome. The nucleosome consists of 146 bp of DNA wrapped around a histone core octamer composed of two each of H2A, H2B, H3, and H4 [1]. The histone proteins are each composed of a globular domain and unstructured N- and C-terminal tails. A striking feature of the histones is that they are subjected to a number of post-translational covalent modifications such as acetylation, phosphorylation, methylation, ubiquitinylation and sumoylation [2] (Fig. 1A).

Some modifications, including acetylation and phosphorylation, are reversible and dynamic, and are often associated with inducible regulation of individual genes. In contrast, histone methylation appears to be more stable and seems to be involved in the cellular memory of the transcriptional status by fixing the chromatin organization in a heritable manner. It has been demonstrated that histone tails play a role in the folding process of the chromatin [3]. The organization of the chromatin could then have implications on chromatin-template processes such as transcription, DNA repair, or replication, assuming that the accessibility of DNA to proteins that mediate these processes will be affected by chromatin condensation that results from histone tail modifications. Moreover, the covalent modifications that occur at multiple and specific sites on the histones generate a tremendous diversity in terms of histones/nucleosomes. It has been proposed that different combinations of histone modifications/nucleosome types may result in distinct outcomes in terms of chromatin-dependent functions such as gene expression. This idea was formally proposed as the ''Histone Code Hypothesis'' [4–6]. According to this hypothesis, histones act as signalling platforms, integrating upstream signalling pathways to elicit appropriate nuclear responses such as transcriptional activation or repression, depending on the modification status.

Both lysine and arginine residues from histones H3 and H4 can be methylated by enzymes that belong to three different protein families. The PRMT protein family uses arginines as a substrate (for reviews see Refs. [7,8]), while the SET domain-containing protein family [2,9,10] and the Dot1/DOT1L proteins are specific for lysines. As arginines can be either mono- or di-methylated, lysines can be mono-, di- or tri-methylated [11]. Here, we focus on histone lysine methylation

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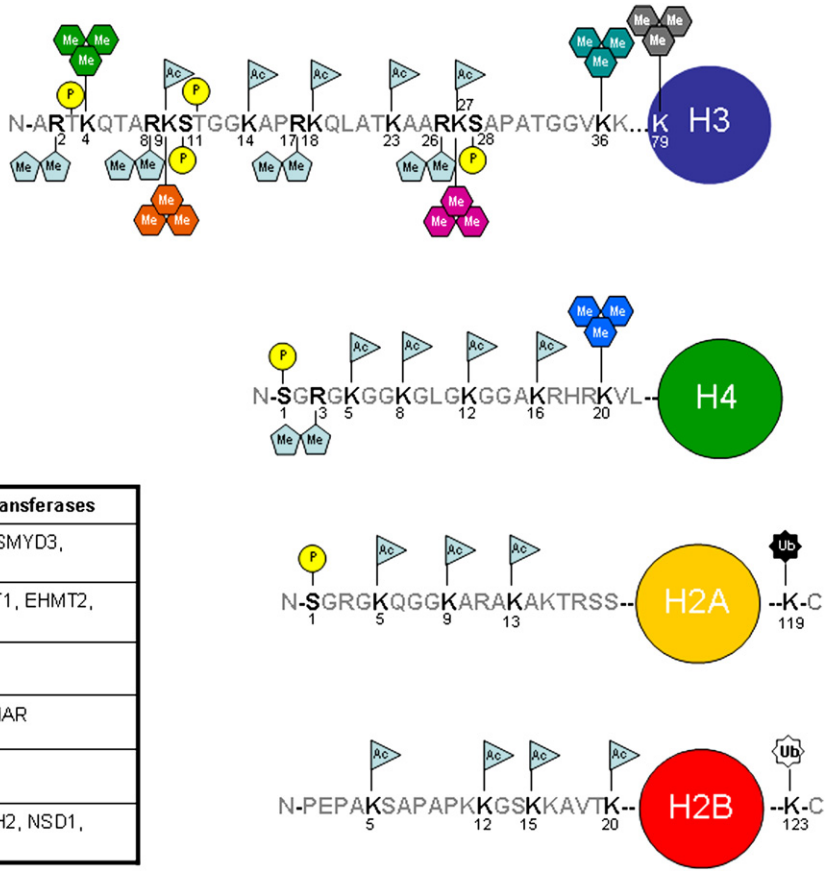
A



B

Substrate	Histone lysine methyltransferases
H3K4	SET9, SET1, MLL, ASH1L, SMYD3, PRDM9, SETMAR
H3K9	SUV39H1, SUV39H2, EHMT1, EHMT2, SETDB1, PRDM2, ASH1L
H3K27	EZH2, EHMT2
H3K36	NSD1, SETD2/HYPB, SETMAR
H3K79	DOT1L
H4K20	SET8, SUV420H1, SUV420H2, NSD1, ASH1L

Fig. 1. Histone modifications. (A) The modifications on human histones include methylation (Me) on arginine and lysine residues, acetylation (Ac) on lysine residues, phosphorylation (P) on serine and threonine residues and ubiquitination (Ub) on lysine residues. (B) The enzymes responsible for methylation of human histone lysine residues are listed according to their target sites. Histone lysine methyltransferases (HKMTs) are very specific but redundant in several cases.

because – in contrast to histone arginine methylation which is mainly linked to transcriptional activation – this modification could be associated to either activation or repression of the transcription, depending on the site and status (mono-, di-, or tri-) of the methylation of histone lysine residues. Chromatin-immunoprecipitation (ChIP) experiments have shown that expressed genes are methylated at lysine 4 (H3K4), lysine 36 (H3K36) and lysine 79 (H3K79) of histone H3 [12–14] whereas methylation at lysine 9 (H3K9) and lysine 27 (H3K27) of histone H3 and methylation at lysine 20 of histone H4 (H4K20) are epigenetic marks of a repressed chromatin state [15–18].

Moreover, in contrast to other histone modifying enzymes, histone lysine methyltransferases are enzymes devoted to methylation of highly specific lysine residues (Fig. 1B). In addition, *Drosophila* genetics led to the emergence of the concept that histone lysine methylation is involved in the maintenance of different types of active and repressed chromatin and therefore plays a role in propagating chromatin states as an epigenetic memory.

1.2. Lysine methylation and the memory of the transcriptional status

In *Drosophila*, the homeobox (HOX) genes, a highly conserved class of regulators, determine the positions of structures along the anterior–posterior axis and mutations in *HOX* genes transform one body segment into the identity of another. *HOX* gene expression is maintained throughout development in the absence of the transient factors having initiated their expression. This ‘‘cellular memory’’ [19] of the expression status of the *HOX* genes depends on two groups of genes that act antagonistically. The Polycomb group (PcG) genes encode proteins that maintain the *HOX* genes silent in the tissues where they should not be expressed while the Trithorax group (TrxG) genes are required to maintain the expression of specific *HOX* genes in the proper cells. Thus, the PcG and TrxG proteins appear to form the molecular basis of the cellular memory (for a review see Ref. [20]). Molecular analysis showed that many of the proteins encoded by the *PcG* and *TrxG* genes act in large complexes and modify the local properties of chromatin to maintain transcriptional repression (PcG) or activation (TrxG) of their target genes.

The target genes of the PcG and TrxG proteins carry *cis*-regulatory elements that enable both groups of proteins to bind, and to maintain the status of transcriptional activity of the gene over cell generations. These elements have dual functions, as PcG and TrxG response elements, and have been termed PREs (PcG response elements [21]), PRE/TREs (Polycomb/trithorax response elements [22,23]), or CMMs (cellular memory modules [24]).

PcG proteins, that mediate the repressed state of the cellular memory, include the ESC/E(Z) complex that methylates both lysines 9 and 27 on the histone H3 tail [15,25–27]. The histone methyltransferase activity of E(Z) is required for correct repression of *HOX* genes in larval imaginal discs [27].

The TrxG is composed of a heterogeneous set of proteins required for the active state, and includes the histone methyltransferases TRX and ASH1. The active state of the cellular memory is also accompanied by methylation of histone H3 on lysines 4, 9 and 36 by ASH1 [28] and H3K4 methylation by TRX [29]. These active epigenetic marks on chromatin at the PRE and its cognate promoter conspire to maintain transcription and to keep chromatin in a fluid state. Interestingly, the TRX and ASH1 histone methyltransferase are required throughout the development to counteract the dominant effect of PcG proteins [30].

Thus, histone methyltransferases, and lysine methylations as epigenetic marks, are involved in the cellular memory of the transcriptional status.

2. Histone lysine methyltransferases

2.1. Printing methylation on histone lysines

With the exception of Dot1/DOT1L, histone lysine (K) methyltransferases (HKMTs) contain a SET domain of about 130 amino acids. The SET domain was originally identified as a shared domain in three *Drosophila* proteins involved in epigenetic processes: the suppressor of position-effect variegation [*Su(var)3-9*]; an enhancer of the eye colour mutant *zeste* which belongs to the PcG proteins [*E(Z)*]; and the homeobox gene regulator *trithorax* [*TRX*] [31]. Mammalian homologues of *Drosophila* Su(var)3–9, Suv39h1 and Suv39h2, were the first HKMTs characterized. They specifically methylate histone H3 at lysine 9 (H3K9) and their HKMT activity maps to the SET domain [32]. So far, about 40 SET domain-containing HKMTs or potential HKMTs that methylate lysines 4, 9, 27, or 36 of histone H3 and lysine 20 of histone H4 have been identified (Figs. 1B and 2). The HKMTs can be classified into several different families according to sequence similarities within their SET domain and within the adjacent sequences, as well as based on other structural features such as the presence of other defined protein domains [9,33–35] (Fig. 2).

2.1.1. The SET1 family

The SET1 family of proteins includes the TRX homologues MLL (also referred as HRX) and MLL4 (TRX2, Mll2), the related proteins MLL2 and MLL3 (ALR and ALR-like), and the two proteins highly similar in their SET domain to the yeast Set1 protein, SET1 and SET1L. A defined structural characteristic of this family is a SET domain at the carboxyl terminus of the protein, followed by a Post-SET region. This Post-SET motif contains three conserved cysteine residues that are essential for HKMT activity [36]. The 3D structure of the SET domain of the *Neurospora grassa* DIM-5 HKMT reveals that these three cysteines from the Post-SET region tetrahedrally coordinate a zinc ion together with another cysteine residue within the SET domain [37].

SET1 HKMTs, like the mammalian MLL, the *Drosophila* TRX and the yeast Set1 proteins have the capacity to specifically methylate lysine 4 of histone H3 (H3K4), generating an epigenetic mark for active euchromatin. However, they might also play the functional role of transcriptional co-activators [20,29,38–40] and could interact with other transcriptional co-activators such as CREBBP [41,42] or with the elongating RNA polymerase II [13,29,43,44]. This suggests that the SET1 family of HKMTs might act on transcriptional activation through different mechanisms.

2.1.2. The SUV39 family

The SET domain of proteins from the SUV39 family is positioned at the carboxy terminus of the protein and is flanked by Pre-SET and Post-SET regions. These two additional domains are required for the histone methyltransferase activity of the SET domain from the SUV family [32]. The Pre-SET domain contains nine invariant cysteines that are grouped in two segments of five and four cysteines separated by various numbers of amino acid. The nine cysteine residues coordinate three zinc ions to form an equilateral triangular cluster [36,37,45].

The HKMTs from the SUV39 family specifically methylate H3K9 [32,46–48], but EHMT2 (G9a/BAT8) methylates lysine 27 of histone H3 (H3K27) in vitro, in addition to H3K9 [47]. Two proteins of the SUV39 family, SETDB1 and SETDB2 have an expanded SET domain that results in the large insertion of amino acids (about 300 residues in SETDB1 and 170 in SETDB2) within the SET domain between two conserved blocks of homology. However, this insertion does affect neither enzymatic activity nor selectivity of the SET domain, and SETDB1 has been shown to specifically methylate H3K9 [49].

Interestingly, some members of the SUV39 family possess protein domains that recognize other epigenetic marks; SUV39H1 and SUV39H2 harbour a chromodomain [50] that may recognize other methylated lysines in histones while SETDB1 and SETDB2 contain an MBD domain, a structure that is potentially capable of interacting with methylated DNA [51]. Finally, the Tudor domain [52,53] present in SETDB1 may potentially recognize histone methyl-lysines.

Within the SUV39 family, SETMAR/Metnase possesses unique features. In contrast to the other members, its SET domain is not located at the carboxy terminus of the protein and shows different substrate specificity. The SETMAR SET domain specifically methylates lysine 36 of histone H3 (H3K36) and to a lesser extent lysine 4 of histone H3 (H3K4) [54].

2.1.3. The SET2 family

The SET2 family contains the three highly related proteins NSD1, NSD2 and NSD3 [55], the homolog of the *Drosophila* TrxG protein ash1, ASH1L [56], the huntingtin interacting protein SETD2/HYPB for which the function is unknown, and another uncharacterized protein SET2L (Q6ZW69). All

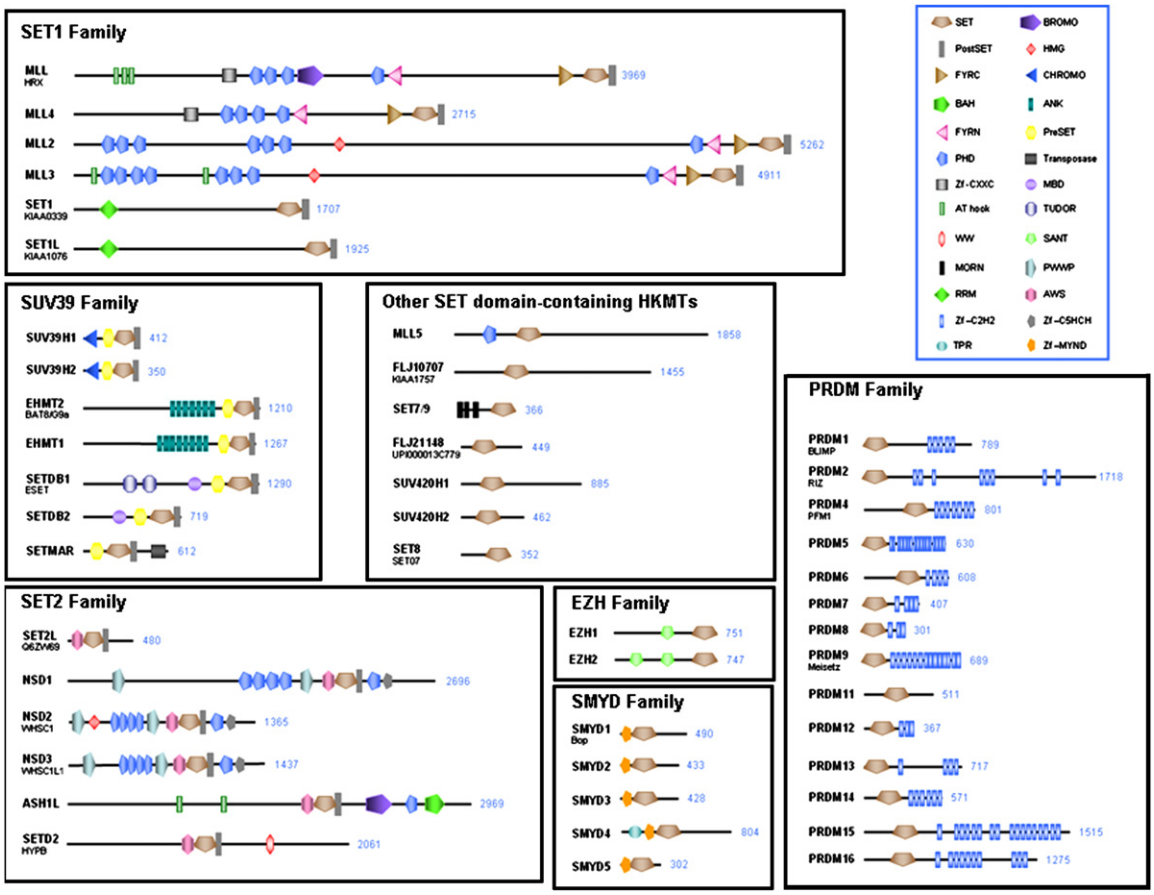


Fig. 2. Structural features of the SET domain-containing histone lysine methyltransferases grouped into seven families. (i) The SET1 family is characterized by the presence of a SET domain followed by a Post-SET motif at the C-terminus of the protein. (ii) The SUV39 family includes HKMTs harbouring a SET domain flanked by Pre-SET and Post-SET motifs. (iii) The SET domain of the HKMTs from the SET2 family is flanked by AWS and Post-SET motifs. (iv) HKMTs from the EZH family harbour the SET domain at the C-terminus of the protein as well as SANT motifs. (v) The members of the SMYD family contain a MYND-type zinc finger preceding the SET domain. (vi) The proteins of the PRDM family possess striking amino acid changes in a highly conserved motif (NHSCxPN, where x is a non-conserved amino acid) within the SET domain. However, two of the members have been shown to have an HKMT activity specific for H3K4 (PRDM9) or H3K9 (PRDM2). Moreover, most of the members contain a number of C2H2-type zinc fingers. (vii) A group of SET domain-containing proteins falls in none of these families. The different protein domains were identified using the SMART program (http://smart.embl-heidelberg.de/). A key to the various domains is shown at the upper right.

SET2 family members possess the SET domain, flanked by AWS and Post-SET motifs, in the central region of the protein. The AWS domain contains 7–9 cysteines and might play a similar role as the Pre-SET domain in the SUV39 family.

SETD2/HYPB specifically methylates H3K36 [57], but in several cases, the histone lysine methyltransferase activity of SET2 family members is not restricted to a single residue: NSD1 methylates H3K36 and lysine 20 of histone H4 (H4K20) [58], whereas ASH1 methylates H3K4, H3K9 and H4K20 [28].

Like the SET1 family members, proteins of the SET2 group are relatively large multi-domain proteins and are likely to play a role as epigenetic factors through their HKMT activity, as well as playing a role of transcriptional co-factors. Indeed, NSD1 was initially identified as a protein that interacts with nuclear hormone receptors and having both transcriptional activation and repression domains [59], whereas the bromodomain from ASH1 interacts with CREBBP [60], and SETD2/HYPB associates with hyperphosphorylated elongating RNA polymerase II [57].

2.1.4. The EZH family

The EZH family contains two related members, homologous to the PcG protein *Enhancer of zeste* [E(Z)]. Together with ESC and SU(Z)12, E(Z) is part of the Polycomb repressor complex 2 (PRC2) that methylates histone H3 at lysines 27 and 9 [25,27]. Additionally, the human EZH2 methylates histone H1 at lysine 26; an activity which is important for the transcriptional repression effect mediated by EZH2 [61].

The EZH family members have no Post-SET motif, however, the region located N-terminally to the SET domain contains about 15 cysteine residues that might play a similar role as the Pre-SET or AWS domains for the SUV39 or SET2 family members.

2.1.5. The SMYD family

The SMYD family forms a group of five related proteins, harbouring a MYND-type zinc finger [62]. The SMYD3 SET domain methylates specifically H3K4 while the MYND finger directly binds to specific DNA sequences [63]. Because SMYD3 also contacts the RNA polymerase II, this protein

might modulate transcription by two independent mechanisms: through a chromatin structure modification via the histone lysine methylation and at the level of transcriptional elongation by the recruitment of RNA polymerase II, as some members of the SET1 and SET2 families also do.

2.1.6. The PRDM family

The PRDM family is a vast family of homogeneous proteins having similar features. These proteins harbour toward the amino terminus a PR domain having 20–30% of sequence identity to the SET domain but being structurally related to it. This PR domain is considered as a subclass of SET domains [64,65]. The PR/SET domain of PRDM proteins possesses striking sequence changes in a highly conserved motif (NHSCxPN) which, when mutated in other HKMTs, abolishes catalytic activity [32]. Moreover the intrinsic HKMT activity of PRDM proteins is still controversial. Indeed, for several PRDM proteins, the histone methyltransferase activity has not been demonstrated [66]. However, in at least two cases, the PR/SET domain has been reported to process a catalytic activity: the PRDM2/RIZ1 PR/SET domain is associated to a histone H3K9-specific methyltransferase activity [67], whereas Meisetz, the mouse homologue of PRDM9 tri-methylates H3K4 [68]. Other members of the PRDM family, like PRDM1/BLIMP1 and PRDM6/PRISM, were shown to interact with the H3K9-specific methyltransferase EHMT2/G9a [66,69].

As another structural feature, the PRDM family members (with the exception of PRDM11) possess a number of Kruppel-type zinc fingers (C2H2 zinc fingers), suggesting that they directly bind specific DNA sequences. Interestingly, PRDM proteins are often associated with gene repression, tumour suppression and carcinogenesis (reviewed in Refs. [70,71]) and these properties rely on an intact PR/SET domain [72–74].

2.1.7. Other SET domain-containing proteins

A number of HKMTs have a SET domain not preceded by a cysteine-rich region and not followed by a Post-SET domain. This heterogeneous class of proteins includes SUV420H1 and SUV420H2 that specifically di- and tri-methylate H4K20, SET7/9, an H3K4 methyltransferase, SET8/PR-SET7, that monomethylates H4K20, as well as MLL5 and two uncharacterized proteins (FLJ10707 and FLJ21148) for which the HKMT activity has not been defined.

Four SET7/9 3D structures have been determined in different configurations showing that the lysine channel is formed by packing an α-helix onto the active site. In the SUV39 family, a metal centre determined by the Post-SET motif is used for the formation of this lysine channel. Thus, the comparison of the different 3D structures reveals a remarkable example of convergent evolution of the SET domain structure [75–78].

2.1.8. Specificity of HKMTs and a tyrosine/phenylalanine switch that controls product specificity

Despite recent advances in SET domain 3D structure, little is known about what determines their specificity. In particular the SET7/9 activity is not only limited to histones but also methylates the tumour suppressor p53 or TAF10 [79,80]. It is not excluded that like SET7/9, other HKMTs could methylate non-histone substrates.

In addition, HKMTs differ both in their histone lysine specificity and in their product specificity for the number of methyl groups they transfer: lysines may be mono-, di-, or tri-methylated. Moreover, the degree of methylation appears to be critical in terms of signalling properties and chromatin structure outcome [12,25,81]. The two SET domains for which the 3D structure has been established have different lysine and product specificities: the human SET7/9 protein generates exclusively histone H3 monomethyl-lysine 4 (H3K4me1) [37,78], while the *N. grassa* DIM-5 protein forms histone H3 trimethyl-lysine 9 (H3K9me3) [37,81]. The different product specificities, monomethyl-lysine versus trimethyl-lysine, may have a structural explanation: the lysine-binding channel of SET7/9 sterically excludes the lysine side chain with methyl group(s). Comparison of the active sites of the SET7/9 and DIM-5 SET domains defines a single amino acid (Y305 of SET7/9 and F281 of DIM-5) that occupies a structurally similar position in both enzymes and could be responsible for the product specificity. Although the two residues are not aligned at the primary sequence level, the edge of the F281 phenyl ring in DIM-5 points to the same position as the Y305 hydroxyl in SET7/9, both in close proximity to the terminal ε-amino group of a target lysine. Consequently, Zhang et al. [37] proposed that the Y305 hydroxyl in SET7/9 may be the source of steric hindrance limiting methylation. Indeed, a SET7/9 Y305F mutant generates H3K4me2 instead of H3K4me1, while a DIM-5 F281Y mutant produces H3K9me1/2 instead of H3K9me3 [37]. An equivalent change in EHMT2/G9a (F1205Y) confers to the enzyme the ability to produce H3K9me1 instead of H3K9me2/3 without affecting the catalytic activity [82]. Sequence alignment including all HKMTs with known product specificity suggests that the tyrosine/phenylalanine switch rule may be generalized [35].

2.1.9. Non-SET HKMTs

DOT1L is the human homolog of Dot1, a protein originally identified as a disruptor of telomeric silencing in *Saccharomyces cerevisiae* [83]. This evolutionary conserved protein is a histone methyltransferase that methylates lysine 79 of histone H3 (H3K79) in the core domain [84–87]. Unlike other HKMTs, Dot1/DOT1L does not contain a SET domain, and it specifically acts on nucleosomal histones. The resolution of the human DOT1L 3D structure has revealed that a flexible positively charged region at the C-terminus of the catalytic domain is critical for nucleosome binding and enzymatic activity [88].

3. Reading the methylated marks

Acetylation of histone lysine residues antagonizes the folding of chromatin in vitro [89,90]. As acetylation neutralizes the positive charge of lysines, this modification could operate on chromatin organization through an electrostatic mechanism. In contrast, methylation of lysine residues does not alter

the charge of the lysines, excluding a direct implication of histone lysine methylation on chromatin folding via an electrostatic mechanism effect.

Even if a structural impact on nucleosomal organization cannot be excluded, specific histone modifications, including lysine methylation, might generate docking sites for specific proteins that mediate downstream effects and chromatin organization [4–6]. Whereas the bromodomain recognizes acetylated lysines [91–93], several different protein domains were shown to be involved in methyl-lysine recognition: the chromodomain [50,94–98], the WD40 repeat [99,100], the Tudor domain [52,53,98], the MBT domain [98,102] and the PHD finger [103–106]. Proteins that contain chromodomains, WD40 repeats, Tudor domains, MBT domains or PHD fingers are recruited at specific methylated lysines and this interaction might be responsible for the specific biological outcomes, associated to precise methylation events.

The association between the chromodomain and methyl-lysine residues to promote downstream molecular events has been studied in several different models. For instance, H3K9 methylation recruits the heterochromatic proteins HP1 through the interaction between the chromodomain of HP1 and the methyl-lysine 9 residue in histone H3 (H3K9me) [95,96,107–109]. Then, it is hypothesized that once HP1 is recruited to heterochromatin, the process of heterochromatin spreading occurs through self-association of HP1 with other HP1 molecules. In addition, HP1 might also recruit additional histone H3 lysine 9 methyltransferase activities (SUV39H1/2), through their chromoshadow domain.

The H3K9me recognition is a feature of all the three isoforms of HP1 (HP1α/CBX5, HP1β/CBX1 and HP1γ/CBX3), however, not all isoforms are similarly targeted to heterochromatin, as HP1γ shows localization to euchromatin sites, too [110–112].

Similarly to HP1 proteins, the chromodomain-containing protein Polycomb (PC) binds specifically to H3K27me residues [15,26,27,113]. The targeting of the PC-associated protein complex to H3K27 epigenetic marks further modifies histone tails and represses gene promoters under the control of the PREs in *Drosophila*.

The chromodomain-containing protein Chd1 is a component of SAGA, a histone acetyltransferase (HAT) complex that regulates transcription of a large number of genes in *S. cerevisiae* [114]. The two chromodomains of Chd1 cooperate to target H3K4me [115] and the interaction seems to favour the recruitment of SAGA and its associated HAT activity to gene promoters [97].

Another identified H3K4me-binding protein is WDR5 a component of the HKMT MLL-associated complex [99,100,116]. In contrast to Chd1, WDR5 interacts with H3K4 through another type of methyl-lysine binding motif, the WD40-repeat domain. In addition, WDR5 preferentially binds to H3K4me2 and is required to maintain the global H3K4me3 levels, probably through the recruitment of the H3K4me3 activity of MLL to these sites. A similar situation is observed in yeast where Swd3, a WD40 repeat-containing protein homologous to WDR5, is a constituent of the H3K4 methyltransferase Set1 complex [117]. Then, it seems that maintenance of active chromatin occurs through a positive feedback loop, as the maintenance of heterochromatin is controlled by the association between SUV39H1 and HP1 proteins.

Finally, the PHD fingers of BPTF, a component of the ATP-dependent remodelling complex NURF, and of the tumour suppressor protein ING2 were also shown to target H3K4me marks [103,105]. In contrast to the WD40-repeat domain from WDR5, the BPTF and ING2 PHD fingers preferentially bind to H3K4me3 marks [104,106]. In these contexts, H3K4me3 recognition by PHD fingers would link this methylation mark to chromatin remodelling or active gene repression, respectively.

4. Lysine methylation at H3K9, H3K27 and H4K20 and transcriptional silencing

Among the different post-translational modifications, methylations at H3K9, H3K27 and H4K20 are, so far, the major covalent histone modifications that correlate with gene repression at the chromosomal level.

4.1. Constitutive heterochromatin

Heterochromatin was initially defined as chromosomal regions that remain condensed throughout the cell cycle and are associated to specific domain such as centromeres, telomeres, and pericentric regions that are satellite-repeat-rich [118]. More recently, a definition of heterochromatin in molecular terms has emerged. In a number of species and in a variety of cases, the SUV39 family of H3K9 HKMTs has been shown to be the major determinant of constitutive heterochromatin [119,120]. These HKMTs establish H3K9me2 and H3K9me3 methylation [16,121], and the subsequent selective binding of HP1 proteins to these epigenetic marks targets H4K20me3 and DNA methylations [18,96,157] (Fig. 3A). Trimethylation at H4K20 is accomplished by two SET domain-containing HKMTs that localize to pericentric heterochromatin, SUV420H1 and SUV420H2. Furthermore, mutations in the *Drosophila* homolog *Su(var)4-20* impair heterochromatin formation in fly [18].

In contrast to H4K20 methylation which seems to be restricted to metazoans, the initial part of the pathway that involves H3K9 methylation, and leads to heterochromatin formation is evolutionary conserved. For example, in the fission yeast *Schizosaccharomyces pombe*, H3K9me2 is catalyzed by the SUV39H1 homolog Clr4 [107]. Clr4-mediated H3K9 methylation recruits the *S. pombe* homolog of the mammalian HP1, Swi6 [95,96,107], and both Clr4 and Swi6 are required for heterochromatin formation [122].

Furthermore, the discovery that RNA interference (RNAi) plays a role in heterochromatin formation in *S. pombe* sheds light on the mechanism by which Clr4 and H3K9 methylation are targeted to heterochromatin [123,124] (Fig. 3B). It is thought that heterochromatin is initiated at specific regions as the result of bidirectional transcription of repeated

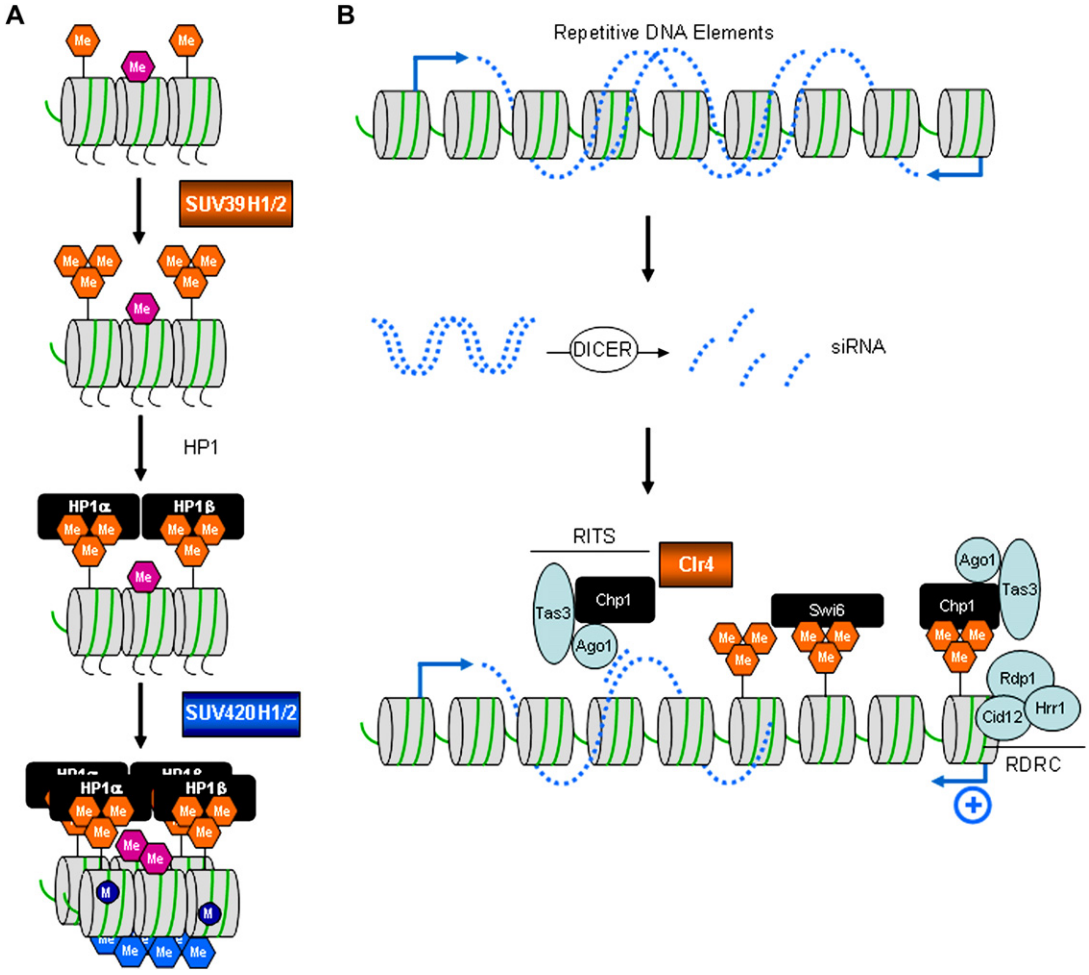


Fig. 3. Heterochromatin formation. (A) Model for sequential H3K9me3 and H4K20me3 methylation at constitutive heterochromatin in mammals. In a first step, the SUV39H1/2 HKMTs are targeted to repeat-rich sequences at pericentric heterochromatin. Because pericentric chromatin is also enriched in H3K27me1, and the SUV39H1/2 enzymes preferentially use H3K9me1 substrates, this initial step requires the activity of other H3K27 and H3K9 monomethylases. The SUV39H1/2 HKMTs produces the H3K9me3 epigenetic marks that will generate docking sites for the chromodomain-containing proteins HP1α and HP1β. Targeted HP1 molecules further recruit other SUV39H1/2 HKMTs that will favour the spreading of H3K9me3 methylations on one hand, and recruit the SUV420H1/2 HKMTs which in turn will tri-methylate H4K20 on the other hand. Since SUV420H1/2 di- and tri-methylate H4K20, the heterochromatin formation requires the activity of an additional H4K20 monomethylase. Consequently, constitutive heterochromatin formation depends on the interplay of several different HKMTs in addition to SUV39H1/2 and SUV420H1/2 (adapted from Ref. [18]). The initial targeting of SUV39H1/2 to heterochromatin presumably involves components of the siRNA machinery. (B) Heterochromatin formation and siRNA machinery in *Schizosaccharomyces pombe*. Heterochromatin formation is initiated at specific location where bidirectional transcription of repeated sequences occurs. The double-strand RNAs are converted into small interfering RNAs (siRNAs) by the Dicer enzyme and loaded onto the RITS (RNA-induced initiation of transcriptional gene silencing) complex. The RITS complex is subsequently targeted to heterochromatin formation sites, presumably through base-pairing interactions between siRNAs and the nascent transcripts. Then, RITS recruits Clr4 that will methylate histone lysine residues at H3K9. These H3K9me residues will serve as docking sites for (i) Swi6, the yeast homolog of the mammalian HP1 protein, involved in heterochromatin spreading and (ii) the RITS complex itself via direct interaction with the chromodomain of its component Chp1. RITS will then recruit the RDRC (RNA-dependent RNA polymerase) complex that functions in the production of additional double-strand RNAs that feed into the siRNA machinery (adapted from Ref. [10]). Hexagons represent lysine methylation states following the colour code used in Fig. 1A (orange, H3K9; purple, H3K27; blue, H4K20). DNA methylation is shown as dark blue circles.

sequences by RNA polymerase II. The double-strand RNAs are converted into small interfering RNAs (siRNAs) by the Dicer enzyme and then loaded onto the RITS (RNA-induced initiation of transcriptional gene silencing) complex, which consists of the proteins Chp1, Ago1 and Tas3. The siRNAs most likely target the RITS complex to specific sites through base-pairing interactions between siRNAs and nascent transcripts associated to repetitive DNA sequences [124]. The targeted RITS complex recruits Clr4. H3K9 methylation occurs, leading to the subsequent recruitment of Swi6. The RITS component protein Chp1 has a chromodomain that interacts also with methylated H3K9. This H3K9me–Chp1 interaction might further stabilize the targeted recruitment of RITS at H3K9 methylated heterochromatin. In addition, the RITS complex favours the recruitment of another protein complex, having an RNA-directed RNA polymerase activity [125]. This complex (RDRC) enhances the production of additional double-strand RNAs, from the repeated DNA sequences. These double-strand RNAs are subsequently processed by the Dicer machinery into siRNAs. Thus, heterochromatin formation is controlled by a positive feedback loop [126,127].

The contribution of the RNAi pathway in mammalian heterochromatin formation has not yet been confirmed but a similar mechanism may function and repression at the satellite repeats might involve the coupling of RNA production and repressive histone methylation.

4.2. Transcriptional silencing and PcG repression

In addition to H3K9 and H4K20 methylations, the methylation at H3K27 is associated to transcriptional silencing and gene repression. At the centre of H3K27 methylation are the PcG proteins that mediate the repressed state of epigenetic memory.

PcG-mediated gene repression and histone methylation converged when it was shown that the Polycomb repressor complex 2 (PRC2 or EZH2 complex) catalyses H3K27me [15,25,27,128]. Studies at the *Drosophila* homeobox gene *Ubx* led to a stepwise silencing model that involves the recruitment of PcG proteins [129] (Fig. 4). In a first step, the DNA-binding protein *Pleiohomeotic* (Pho) and *Pleiohomeotic-like* (PhoL) bind to specific DNA sequences known as Polycomb response elements (PRE) located upstream of the *Ubx* gene,

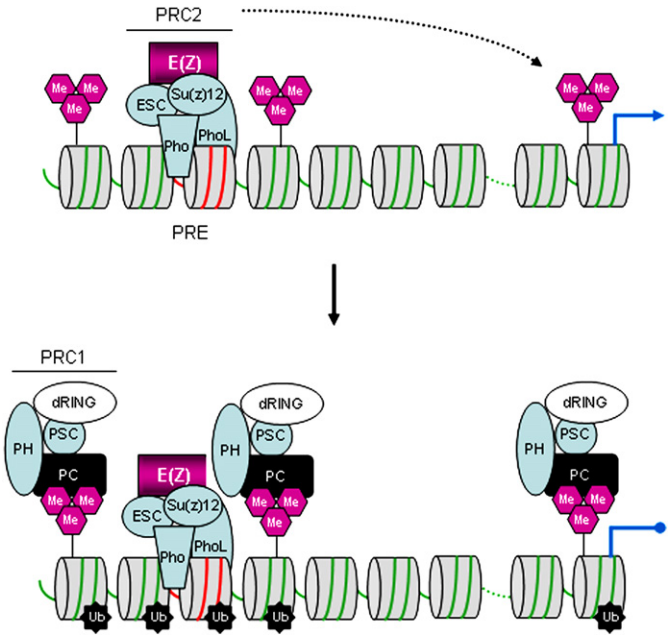


Fig. 4. H3K27 methylation and polycomb silencing in *Drosophila*. The polycomb repressor complex 2 (PRC2) is recruited at PREs via the DNA-binding protein Pleiohomeotic (Pho) and Pleiohomeotic-like (PhoL). A component of PRC2, the HKMT E(Z) methylates H3K27 residues around the PRE, but also at the promoters controlled by this PRE (dashed arrow), forming docking sites for the chromodomain-containing protein polycomb (PC) which is part of the polycomb repressor complex 1 (PRC1). The E3 ligase activity contributed by the PRC1 component dRING leads to H2AK119 mono-ubiquitination. This H2AK119ub1 mark presumably causes transcriptional silencing. Polycomb silencing is also associated to H3K9me3 marks not depicted in the picture. The PcG machineries are active and regulate gene expression in mammals. However, it is not clear how the mammalian homolog of the PRC2 complex (the EZH2 protein complex) is targeted to genomic regions since PRE elements have not been identified in vertebrates. Hexagons represent lysine methylation and start ubiquitination following the colour code used in Fig. 1A (purple, H3K27; black, H2AK119).

and target the PRC2 complex to the PRE. The PRC2 complex is composed at least of the HKMT *Enhancer of zeste* [E(Z)], *Extra sex combs* (ESC) and *Suppressor of Zeste-12* [Su(z)12]. The recruitment of PRC2 to the PREs leads to the establishment of H3K27me marks that then serve as binding sites for the chromodomain-containing *Polycomb* protein (PC) that are part of the Polycomb repressor complex 1 (PRC1) complex [50,94,130]. The PRC1 complex composed of the core proteins PC, *Polyhomeotic* (PH), *Posterior sex combs* (PSC) and the E3 ligase dRING, is primarily responsible for repression activity.

Even though the basic mechanisms of PcG regulation have been most extensively elucidated in *Drosophila*, these proteins play an equally important role in mammalian development, cellular proliferation and tumorigenesis [131]. No PRE elements have been yet identified in vertebrates, but some target genes are known, such as the *Hox* gene clusters [132]. Recently, the mammalian PRC1-like complex was purified to homogeneity and found to mono-ubiquitinate histone H2A within the nucleosome at lysine 119 (H2AK119ub1; [133]). Then, in the PcG-mediated silencing of mammalian *Hox* genes, H3K27 methylation recruits an E3 ligase activity that ubiquitinates H2AK119 [134]. However, the mechanism by which H2A ubiquitination contributes to gene silencing is not known. One possibility is that H2AK119ub1, like other histone modifications, allows the binding of other proteins that prevent gene expression and mediate gene silencing. Alternatively, H2AK119ub1 might directly interfere with transcription.

4.3. Genomic imprinting

Imprinting is a developmental gene regulation mechanism characterized by monoallelic silencing of either maternally or paternally inherited genes. About 80 transcriptional units have been reported to be imprinted in mammals [135]. Most of these transcriptional units correspond to genes having key functions in regulating placental and foetal growth, although others are important for metabolism or have functions in behaviour or social interactions. Imprinted genes are clustered and their allelic expression is under the control of specific DNA regions, the imprinted control regions (ICRs), which are differentially methylated during oogenesis or spermatogenesis [136]. The role of HKMTs in genomic imprinting is suggested by the allele-specific histone methylation at imprinted genes [137]. Imprinted repressed genes possess H3K9me2 and H3K27me3 marks. Moreover, a deficiency for *Eed*, the mouse homolog of ESC, leads to loss of imprinting at a subset of genes indicating that PcG-mediated repression is involved in genomic imprinting [138].

Recent studies on the mouse distal chromosome 7 indicate that histone methylation would maintain imprinted gene repression independently of DNA methylation [139,140] (Fig. 5). At loci on mouse chromosome 7, the *Kcnq1ot1* non-coding RNA (ncRNA) is transcribed from the paternal imprinting control region (ICR), which is required for transcriptional gene repression in *cis*. In contrast, the maternal ICR is

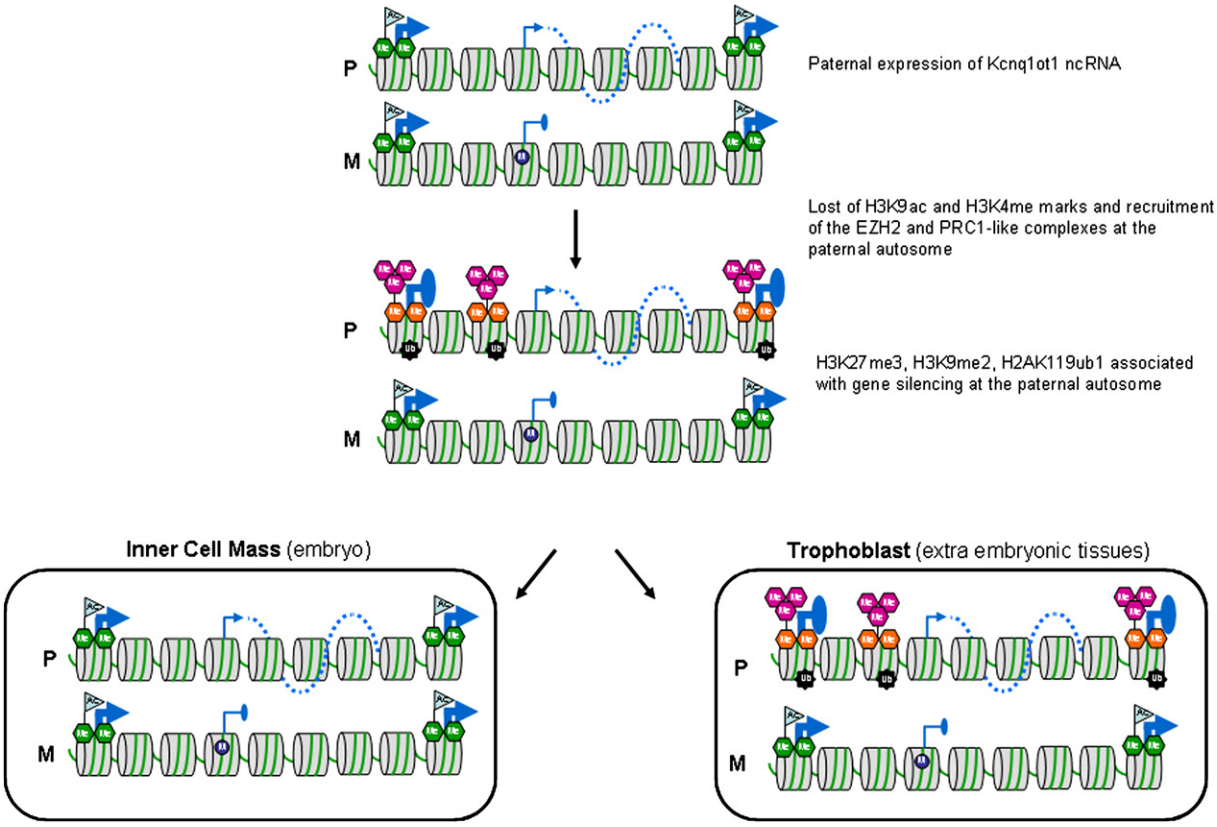


Fig. 5. Model for genomic imprinting at the mouse distal chromosome 7. At early zygotic stages, the *Kcnq1ot1* ncRNA (dashed blue line) is expressed from the paternal autosome while its maternal counterpart is not expressed due to DNA methylation of the maternal copy in the germ line (dark blue circle). It is not known whether the *Kcnq1ot1* ncRNA coats the area it inactivates on the paternal autosome. However, the *Kcnq1ot1* gene is required for targeting the EZH2 complex and subsequent H3K27me3 and H3K9me2 methylation at the imprinted region. The following targeting of the PRC1-like protein complex to H3K27me3 leads to H2AK119ub1 which might cause gene silencing of the paternal copies although the molecular mechanism is not known. Latter, the genomic imprinting is maintained in the placenta but lost in the embryonic tissues. Hexagons represent lysine methylation states following the colour code used in Fig. 1A (orange, H3K9; purple, H3K27; green, H3K4). Acetylation and H2AK119ub119ub1 marks are also shown.

methylated and silent. It is proposed that *Kcnq1ot1* ncRNAs target the PcG repressive system at loci distal to the paternal ICR: the EZH2 complex is recruited and H3K27 methylation occurs at imprinted genes. Then, the subsequent recruitment of the PRC1-like complex would ubiquitinate H2AK119 and silent gene expression, as for PcG-mediated repression of *Hox* genes.

4.4. X inactivation

In addition to homeobox gene silencing and genomic imprinting, PcG proteins have been implicated in female X-chromosome inactivation [141–143].

Importantly, the process of X inactivation can be divided into two distinct classes; imprinted X inactivation and random X inactivation. Early in development, imprinted X inactivation takes place and the paternal X chromosome becomes silent in all the cells of the embryo [144,145]. The paternal X chromosome remains inactivated in the cells that contribute to extra-embryonic tissues, but X inactivation is reversed in the cells that form the embryo proper. Then, X inactivation is re-established randomly on either the paternal or maternal X chromosome.

Xist, a non-coding RNA that is expressed from the X chromosome that is destined to become silent, coats the complete inactive X chromosome (Xi) and coincides with hypomethylation at H3K4. *Xist* coating is followed by the loading of EZH2 protein complex and subsequent H3K9me2 and H3K27me3 hypermethylation and H2AK119 ubiquitination (Fig. 6A). In this context, the mammalian homologues of the *Drosophila* dRING, RING1A and RING1B are responsible for H2AK119ub1 at the Xi in embryonic and extra-embryonic cells [146,147].

In both imprinted and random X inactivation, the recruitment of the EZH2 complex that methylates H3K27 and the PRC1-like complex that ubiquitinates H2AK119 is transient and required only during the initiation of the inactivation [146,148–150]. At later stages, H3K27me3 levels are reduced, macroH2A, a variant of histone H2A, is incorporated into the nucleosomes of the Xi, and DNA methylation occurs at the promoters of Xi-linked genes (Fig. 6B).

Thus, the process of X inactivation initiation shares mechanistic similarities with genomic imprinting based on ncRNAs and PcG protein silencing. In this context, the recent observations showing that transcription through the PREs is required for reversal of PcG-mediated *HOX* gene silencing in

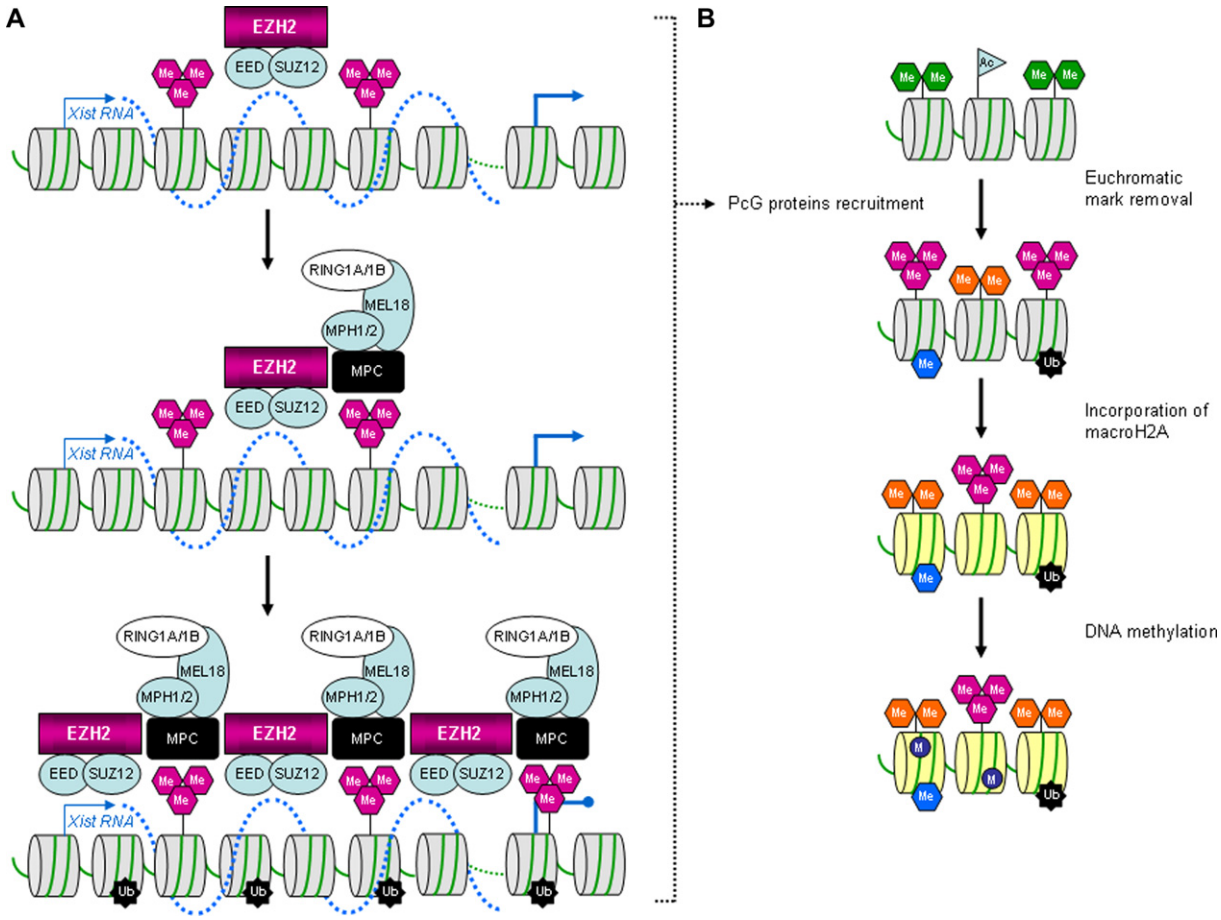


Fig. 6. H3K27 methylation and initiation of X-chromosome inactivation. (A) *Xist* RNA (dashed blue line) is transcribed at the X-inactivation centre, spreads outwards and coats the inactivated X chromosome. *Xist* RNA recruits the EZH2 protein complex and its H3K27 methyltransferase associated activity presumably through direct interactions. H3K27me3 marks are docking sites for chromodomain-containing proteins homologous to PC and associated with the E3 ligase activities RING1A/1B, within a PRC1-like protein complex. H2AK119 is subsequently ubiquitinated. The molecular mechanism by which H3K27me3 and H2AK119ub1 contributes to silencing is not known. (B) X-chromosome inactivation is associated to numerous epigenetic modifications at the chromatin. One of the earliest event following *Xist* RNA inactivation is the lost of euchromatic histone marks (histone lysine acetylation, H3K4me). This could happen through HDAC and HDM actions or histone H3 exchanges. LSD1, an H3K4me1/2-specific demethylase associated to HDAC1/2 within the BRAF complex, is a candidate for this HDM activity. At this same time window, several histone modifications occur at the *Xist* RNA-coated chromosome. The mechanisms involved in H3K9me2 and H4K20me1 methylations are not clearly identified. However, the EZH2 protein complex is responsible for the appearance of H3K27me3 marks and the PRC1-like complex for the H2AK119ub1 mark. At later stages of development, the EZH2 and PRC1-like complexes are excluded from the inactivated X-chromosome and H3K27me3 levels are reduced. MacroH2A is then incorporated into nucleosomes and DNA methylation occurs at the gene promoters of the inactivated X chromosome. Histone modifications are represented according to the colour code used in Fig. 1A. DNA methylation is shown as dark blue circles.

Drosophila, suggest that ncRNAs could have a general role in PcG protein function [151–153].

4.5. Histone and DNA methylation in silencing

DNA methylation at cytosine bases is another epigenetic modification involved in gene silencing, genomic imprinting or X-chromosome inactivation [154]. A number of studies have demonstrated that histone methylation at H3K9 is a prerequisite for DNA methylation [155–158]. H3K9me is linked to DNA methylation through HP1 proteins that might recruit DNA methyltransferases via protein–protein interactions [157]. In addition, it has been recently demonstrated that the EZH2 complex interacts with DNA methyltransferases and that the H3K27-specific HKMT EZH2 is required for DNA methylation at EZH2-target promoters [159]. Similarly, SETDB1, an H3K9-specific HKMT physically associates with the DNA methyltransferase DNMT3A [160].

Although histone methylation controls DNA methylation, the reverse relationship has also been documented. For instance, it has been observed that H3K9 methylation is dramatically reduced in DNA methyltransferase-deficient cells [161]. The connection between DNA methylation and histone methylation gained further support from the study of the methyl-CpG-binding proteins MBD1 and MeCP2. Both proteins associate with HKMT activities and might target histone methylation to genomic regions harbouring DNA methylated marks [162,163]. Moreover, SETDB1 and SETDB2 contain a methyl-CpG-binding domain (MBD), which might contribute to the recruitment of the two HKMTs to a methylated or hemi-methylated DNA region. This provides a putative mechanism for maintaining the recruitment of HKMTs to specific DNA regions.

These observations collectively link H3K9/H3K27 methylation and DNA methylation to gene silencing, and support the hypothesis that histone and DNA methylation cooperate to establish and maintain long-term states of transcriptional regulation.

5. Lysine methylation and active transcription

Histone lysine methylation is also involved in transcriptional activation, but in that case, the main methylation sites include H3K4, H3K36 and H3K79 [12–14]. Moreover, histone 3 lysine methylation at K4, K36 and K79 seems to be directly linked to the transcriptional process and enzymes responsible for H3K4 and H3K36 methylation physically associate with RNA polymerase II (RNAPII) during elongation [13,43,164–166]. In addition, H2B mono-ubiquitination at lysine 123 (H2BK123ub1) involving the conjugating enzyme system Rad6/Bre1, is required for methylation at H3K4 and H3K79, but not H3K36 [167–169].

The current model (Fig. 7) deduced from studies in *S. cerevisiae* is that gene specific trans-activators recruit an ubiquitination complex composed of Rad6 and Bre1 to gene promoters [170–172]. This complex is then loaded onto RNAPII and subsequently carried along the transcribed region of the gene [173]. Rad6/Bre1-mediated ubiquitination at H2BK123 facilitates the methylation at H3K4 by the HKMT Set1, possibly via opening the nucleosome structure [174]. Set1 recruitment itself involves the Paf1 elongation complex and coincides with phosphorylation at serine 5 of the C-terminal domain (CTD) of RNAPII. Similarly, the H3K79 HKMT Dot1 may also be recruited to the transcribed regions by the elongating RNAPII [175].

At some point during elongation, the CTD of RNAPII becomes phosphorylated at serine 2. Concomitant with this event, Set1 dissociates from RNAPII and Set2-mediated H3K36 methylation occurs. Thus, H3K4me seems to be a mark of early transcription events, whereas H3K36me marks efficient elongation.

In parallel to transcription elongation, the histone acetyltransferase (HAT) complex SAGA is recruited through specific interactions between its chromodomain-containing subunit Chd1 and H3K4me marks. Histone acetylation by the SAGA complex leads to transcriptional activation on one hand. In this context H3K4me3 could play the role of a "memory" of previous transcriptional activity [169]. On the other hand, Ubp8, a component of the SAGA complex is a deubiquitination factor erasing the H2BK123ub1 mark [170]. This deubiquitination step seems to be required for activation of the SAGA-regulated genes.

Although, the current model of lysine methylation coupled to transcription has been mainly derived from work in *S. cerevisiae*, similar methylation patterns are observed in higher eukaryotes [14]. Like the yeast Set1 HKMT, the SET domain of MLL has an H3K4-specific methyltransferase activity stimulated in presence of acetylated H3 peptide [38,176]. In addition, the characterization of protein complexes associated to MLL and MLL4 (Mll2) shows similarities to the Set1 protein

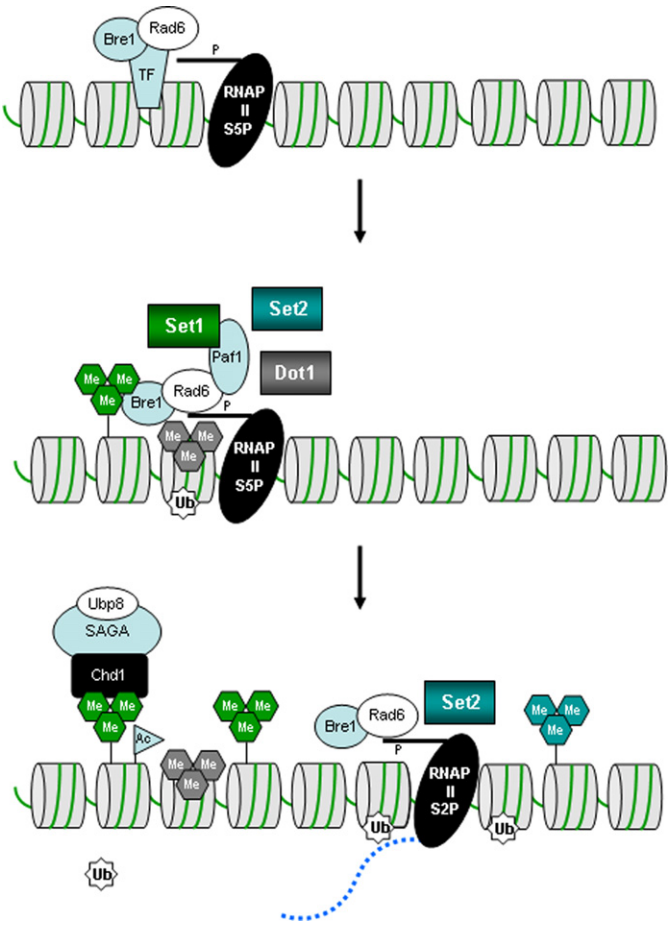


Fig. 7. H3K4, H3K36, H3K79 methylations and active transcription. According to the model developed from studies in *S. cerevisiae*, one role of trans-acting factors (TF) is to recruit the ubiquitin conjugating system Rad6/Bre1, which is subsequently loaded onto RNA polymerase II (RNPII) at the gene promoter. Mono-ubiquitination at H2BK123 favours the recruitment of the HKMTs Set1, Set2 and possibly the non-SET domain histone methyltransferase Dot1. Set1 recruitment itself involves the Paf1 elongation complex and coincides with phosphorylation at serine 5 of the C-terminal domain (CTD) of RNPII. During elongation, the CTD of RNPII becomes phosphorylated at serine 2 and Set1 dissociates from RNPII whereas Set2 methylates the transcribed region at H3K36. After the first round of transcription, the gene is marked by H3K4, H3K36 and H3K79 methylations. H3K4me3 marks serve as docking sites for the chromodomain-containing protein Chd1 which is a component of the SAGA histone acetyltransferase complex. SAGA actetylates histone tails on one hand, and its ubiquitin specific protease component Ubp8 removes the ubiquitin at H2BK123. These two events (acetylation and deubiquitination) promote transcriptional activation (adapted from Ref. [10]). Histone modifications are represented according to the colour code used in Fig. 1A.

complex [116,177,178]. Furthermore, like the yeast Set1 protein complex, the MLL-containing complex is associated with serine 5-phosphorylated RNAPII, and is recruited to *Hoxc6* and *Hoxc8* genes. Thus, the compositional and functional conservation between Set1 and MLL protein complexes emphasizes that H3K4me is required for proper regulation of gene expression. In contrast, very little is known about H3K36 methylation mediated by the SET2 family members in mammals.

6. Histone lysine demethylation

Early studies measuring the turnover of methylated histones suggested that histone methylation was stable and irreversible since the half-life of methylated histones was very similar to the half-life of the histones themselves [179]. However, in certain situations, histone methylation has been shown to be reversible and highly dynamic. For example, the H3K27me3 associated with the inactive X chromosome in trophoblast stem cells disappears during differentiation [148,149], whereas H3K9 methylation at some inducible inflammatory genes is removed upon activation and restored after transcriptional repression [180]. In absence of described demethylating activity – although envisaged [11] – at least two mechanisms were proposed to explain the turnover of methyl groups on histones. The first model involved histone tail clipping [181], while the second involved replacement of methylated histones with variant histones [182]. Finally, the view that histone methylation is irreversible was definitively dismantled with the identification of several enzymes that remove the methyl groups.

6.1. PADI4 and the demethylimination

The peptidylarginine deiminase 4 (PADI4/PAD4) was the first enzyme capable of turning over a methylated histone. PADI4 converts monomethyl-arginine from histones H3 and H4 into citrulline by demethylimination [183,184]. Since demethylimination does not generate an arginine but a citrulline and because PADI4 also acts on unmethylated arginines, PADI4 is not a strict histone demethylase but might create a new and unusual histone tail mark that might itself act as a recognition signal.

6.2. LSD1 and the amine oxidation

Shortly after the identification of PADI4, Shi and colleagues (2004) identified and characterized the first histone lysine demethylase, LSD1 (lysine-specific demethylase 1, also known as BHC110/AOF2 [185]). Recombinant LSD1 specifically demethylates H3K4me1 and H3K4me2 by amine oxidation, but acts poorly on nucleosomal histone H3 [186]. Biochemical characterization of the LSD1-associated proteins revealed that CoREST (co-repressor element silencing transcription) and BHC80 are required for LSD1 activity on nucleosomal histone H3 in vivo at defined promoters [187,188]. Moreover, LSD1 can also associate with the androgen receptor. This interaction not only confers a ligand-dependent targeting to, and activation of, androgen-responsive genes, but also alters the substrate specificity of the enzyme that can in this context, demethylate H3K9me1 and H3K9me2 [189]. The mechanism underlying the switch in substrate specificity (H3K4me1/2 versus H3K9me1/2) of LSD1 upon association with different proteins remains to be determined.

LSD1 is a flavin-dependent amine that is able to specifically demethylate mono- and di-methyl-lysines. The reaction involves the oxidation of an amine via the oxidative cleavage of the α-CH bond of the substrate to form an imine intermediate with concomitant reduction of the flavin cofactor. The imine intermediate is hydrolysed through a non-enzymatic process to produce a carbinolamine. This group is unstable and degrades to release formaldehyde and regenerates the unmethylated lysine residue [186]. Because the formation of an imine intermediate requires a protonated lysine, trimethyl-lysines cannot be used as substrates for amine oxidases like LSD1.

6.3. JmjC domain and hydroxylation of the methyl groups

In the search for an alternative class of enzymes that might be dedicated to the reversal of trimethylation, Trewick et al. [190] proposed that proteins that contain JmjC domains could play that role. It was hypothesized that some JmjC domain-containing proteins could be 2-oxoglutarate-Fe(II)-dependent dioxygenases that remove methyl groups from lysine residues based on a similar mechanism by which the DNA repair demethylase AlkB hydroxylates the methyl groups of certain forms of DNA methylation damage. Very recently, this model was confirmed with the characterization of the first JmjC domain-containing histone demethylase (HDM), JHDM1A/FBXL11 [191]. JHDM1 specifically demethylates H3K36me2 and H3K36me1 using α-ketoglutarate and Fe(II) as co-factors. Moreover the JmjC domain of JHDM1A is required for its demethylase activity. Even more recently, other JmjC domain-containing HDMs were identified and characterized: JHDM2A (JMJD1A) specifically demethylates H3K9me2 and H3K9me1 [192] whereas the JMJD2 protein family (JMJD2A, B and C) specifically acts on H3K9me2/3 and H3K36me2/3 residues [193].

The human genome contains about 30 JmjC domain-containing proteins that might be organized in several families (Fig. 8). Some of the JmjC domain-containing proteins are likely to target none histone proteins as substrate, such as the HIF1AN/FIH asparagine hydroxylase or the transmembrane phosphatidylserine receptor PTDSR. However, the presence of DNA-binding and/or chromatin-associated motifs such as zinc fingers, BRIGHT domain, PHD finger, or Tudor domain in many other JmjC domain-containing proteins confirms that a number of them might regulate chromatin organization. Based on JmjC domain homology and protein domain organization, we propose that the JmjC domain-containing histone demethylases (HDMs) might be organized in six distinct families (Fig. 8).

The JHDM1 family consists of the two related proteins JHDM1B/FBXL10 and JHDM1A/FBXL11 for which HDM activity specific for H3K36me2 and H3K36me1 has been proven [192].

The JHDM2 family comprises three related proteins, JMJD1A/JHDM2A, JMJD1B/JHDM2B and JMJD1C/JHDM2C/TRIP8. Among them, JMJD1A/JHDM2A was shown to harbour a specific HDM activity. Based on JmjC domain homology, we propose that the human hairless (HR) protein which is mutated in individuals with *alopecia*

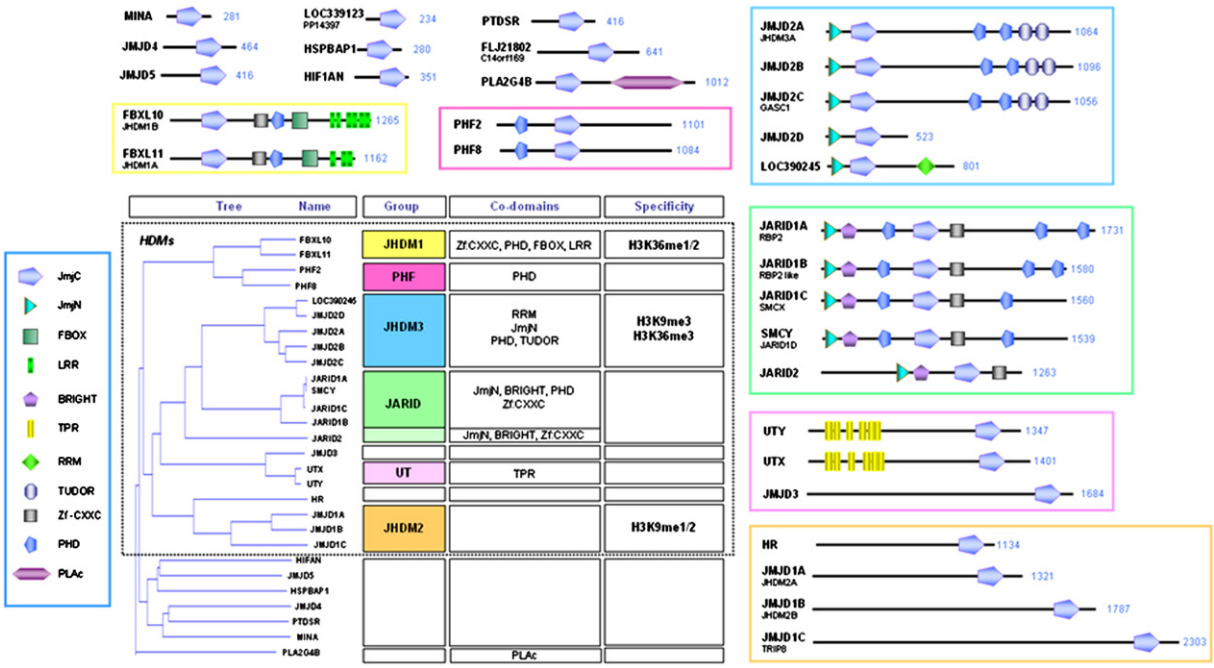


Fig. 8. Structural features of the JmjC domain-containing histone lysine demethylases. A dendogram showing the relationship between the human JmjC domain-containing proteins. The comparison is based on the homology within the JmjC domain using the Clustal W program. Putative or effective histone demethylases are boxed, and grouped into six families based on their homology within the JmjC domain and the associated protein domains. (i) FBXL10 and FBXL11 belong to the JHDM1 family; (ii) the PHF family comprises two PHD finger-containing proteins, PHF2 and PHF8; (iii) the JHDM3 family includes the four related proteins JMJD2A/JHDM3A, JMJD2B, JMJD2C/GASC1 and JMJD2D. The predicted protein LOC390245 is an additional member of the JHDM3 family; (iv) the JARID family contains four related proteins, JARID1A/RBP2, JARID1B/RBP2-like, JARID1C/SMCX, and SMCY/JARID1D, as well as the more distant protein JARID2/JUMONJI; (v) the UT family contains two proteins UTX and UTY with similar modular architecture. Based on JmjC domain homology, we propose that the protein JMJD3 belongs to this group; (vi) the JHDM2 family includes the proteins JMJD1A, JMJD1B and JMJD1C. Based on JmjC domain homology, we propose that the protein hairless (HR) also belongs to this family. The different protein domains were identified using the SMART program (http://smart.embl-heidelberg.de/). A key to the various domains is shown at the left.

universalis or *atrichia* with papular lesions, belongs to the same JmjC domain-containing HDM family. Moreover, HR acts as a transcriptional co-repressor for thyroid receptor [194] and it is striking to note that other JHDM2 protein family members also associate with nuclear hormone receptors. JHDM2A/JMJD1A associates with the androgen receptor in a ligand-dependent manner [192], whereas JHDM2C/JMJD1C/TRIP8 also interacts with the thyroid receptor and the retinoic X receptor α (RXRα) in a ligand-dependent fashion [195].

The JHMD3 family is composed of the four related proteins JMJD2A/JHDM3A, JMJD2B, JMJD2C/GASC1 and JMJD2D, as well as the predicted protein encoded by the gene LOC390245. JMJD2A/JHDM3A has been shown to act as a transcriptional repressor, in part through the recruitment of histone deacetylases (HDACs) and the co-repressor N-CoR [196,197]. However, JMJD2A also acts as an HDM specifically demethylating H3K9me3/2 and H3K36me3/2 [193,198]. In addition, other members of the JMJD2 family show HDM activities with slightly different specificities, at least in vitro: JMJD2B acts on H3K9me3 only, while JMJD2C/GASC1 demethylates H3K9me3 and H3K36me3 and the substrates of JMJD2D are the H3K9me3/2 residues [193,199,200].

The JARID family contains four related proteins, JARID1A/RBP2, JARID1B/RBP2-like, JARID1C/SMCX and SMCY/JARID1D, as well as the more distant protein JARID2/JUMONJI. None of them has been associated with an HDM activity, but they all possess DNA-binding and/or chromatin-associated domains. JARID1A/RBP2 is a co-activator of nuclear receptor enhancing receptor-mediated transcription [201], whereas JARID1B/PLU-1 [202] and JARID2/JUMONJI [203,204] act as transcriptional repressors. Interestingly, the *little imaginal discs* (LID) protein, the *Drosophila* homolog of JARID1, has been identified in a screen for new TrxG proteins, reinforcing the role of this family of proteins in chromatin regulating processes [205].

The PHF family contains PHF8 which is associated to the Siderus X-linked mental retardation syndrome [206], and a related protein, PHF2. Both proteins harbour a PHD finger suggesting a role at the chromatin level.

Finally, the last group of putative JmjC domain-containing HDMs is the UT family. This group is composed of two similar proteins, UTX and UTY. These proteins contain tetratricopeptide repeats (TPR), a motif involved in protein–protein interactions, as well as several putative nuclear localization signals. Moreover, both proteins seem to be involved in transcriptional repression [207]. Based on JmjC domain homology,

the JMJD3 protein might also fall in this family in spite of the absence of TPR motifs.

The JmjC domain-containing HDMs characterized so far, including JHDM1A/FBXL11, JHDM2A/JMJD1A and the JHDM3/JMJD2 family members, exhibit striking substrate specificity. They show selectivity for the histone lysine residues as well as for the methylation state. This situation is analogous to the selectivity observed for SET domain-containing HKMTs. Similar to SET domain-containing HKMTs, the JmjC domain-containing proteins are organized in sub-families harbouring a number of common chromatin-binding domains. We speculate that SET domain-containing HKMTs and JmjC domain-containing HDMs act together to tightly regulate the levels of histone methylation in cells. This methylation/demethylation balance more than histone methylation alone might then be responsible for the control of the structural organization of chromatin and all the subsequent cellular events which are associated with. Consistent with this proposal is the finding of the association of two JmjC domain-containing putative HDMs, Lid2 and Ecm5, together with the H3K4 histone methyltransferase Set1 in *S. pombe* protein complexes [208]. Moreover, JMJD2A/JHDM3A is a JmjC domain-containing HDM showing substrate specificity for H3K9me2/3 and H3K36me2/3 and possessing two Tudor domains that recognize H3K4me and H3K20me2/3 marks [98,101]. Then, it is tempting to speculate that the JMJD2A is targeted to chromatin regions enriched for particular methylated histones. Even if the physiological relevance of such a scenario remains to be established, the Tudor domains of JMJD2 family members reinforce the possible coordinated relationship between the marks imposed by the SET domain-containing HKMTs and the action of the JmjC domain-containing HDMs.

At present, only a restricted number of residues are antagonized by HDMs; LSD1 demethylates H3K4me1/2, while JmjC domain-containing HDMs act on H3K9me1/2/3 and H3K36me1/2. It is possible that other putative JmjC domain-containing HDMs will target other histone lysine residues, but the number of putative JmjC domain-containing HDM families (Fig. 8) suggests that several other HDMs, different from LSD1 and the JmjC domain-containing demethylases, remain to be discovered. Alternatively, as proposed recently [209], some histone marks like H3K27me and H4K20me1 could be used to transmit epigenetic information and therefore may have limited reversibility.

7. Conclusion

Six years ago, the biological significance of histone lysine methylation was unknown. Since then, and after the first identification of an HKMT activity [32], the field has tremendously flourished with the characterization of numerous HKMTs that participate in diverse biological processes. We cannot exclude that histone methylation could have a structural influence on nucleosomal structure but it is known that there are proteins that bind specifically to defined methylated lysine residues. Significantly, this interaction is sometimes modulated by modifications on adjacent histone residues. In addition, in many cases specific histone lysine methylations or particular combinations of post-translational modifications on histone tails strongly correlate with a defined chromatin organization and functional outcome. Thus, numerous evidences show that histone lysine methylation provides specific signals that are recognized by specific proteins that, in turn, modulate gene expression and other chromatin-associated functions.

The purification and characterization of a number of protein complexes associated with chromatin (see for instance Refs. [26,61,116,177,178,210,211]), in combination with the development of antibodies that are specific for defined histone methylation states, and technologies such as the chromatin-immunoprecipitation coupled with DNA microarrays (ChIP on chip), advanced fluorescence light microscopy in living cells or novel unbiased activity-based biochemical assays, have given great progress in linking the various histone modification patterns to biological processes. However, in spite of the power of these approaches – defined as *chromatinomics* – and the benefits from genetic studies in model organisms such as yeast and *Drosophila*, the molecular mechanisms by which histone methylation controls the different physiological processes are mostly unresolved.

Replicating cells maintain their identity from one cell generation to the next, implying that the pattern of gene expression is maintained through DNA replication. *Drosophila* genetics has demonstrated the implication of histone methylation in the cellular memory. However, we still do not comprehend how histone methylation marks are transmitted throughout the cell cycle, and how the balance between histone methylation and demethylation controls the epigenetic maintenance of histone lysine marks. Another challenge is the understanding of the role of histone methylation in various human diseases, particularly in cancer. While many HKMTs are linked to cancer, a more precise description of the molecular events implying HKMTs in tumorigenesis would permit to define new targets for therapeutic treatments.

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