POST-TRANSLATIONAL MODIFICATIONS

Sound of silence: the properties and functions of repressive Lys methyltransferases

Chiara Mozzetta[1,2], Ekaterina Boyarchuk[1], Julien Pontis[1] and Slimane Ait-Si-Ali[1]

Abstract | The methylation of histone Lys residues by Lys methyltransferases (KMTs) regulates chromatin organization and either activates or represses gene expression, depending on the residue that is targeted. KMTs are emerging as key components in several cellular processes, and their deregulation is often associated with pathogenesis. Here, we review the current knowledge on the main KMTs that are associated with gene silencing: namely, those responsible for methylating histone H3 Lys 9 (H3K9), H3K27 and H4K20. We discuss their biochemical properties and the various mechanisms by which they are targeted to the chromatin and regulate gene expression, as well as new data on the interplay between them and other chromatin modifiers.

Writer
An enzyme that catalyses the addition of chemical modifications to DNA or histones.

Eraser
An enzyme that catalyses the removal of chemical modifications from DNA or histones.

[1]*Centre National de la Recherche Scientifique (CNRS), Université Paris Diderot, Sorbonne Paris Cité, Epigenetics and Cell Fate, UMR7216 CNRS, Paris, France.*
[2]*Present address: National Research Council (CNR), Institute of Cell Biology and Neurobiology (IBCN), 00143 Rome, Italy.*
Correspondence to S.A.-S.-A. and C.M.
emails: slimane.aitsiali@univ-paris-diderot.fr; chiara.mozzetta@ibcn.cnr.it
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Eukaryotic DNA is wrapped around histone octamers that consist of two copies of each of the histone proteins H2A, H2B, H3 and H4 to form nucleosomes, which are the basic units of chromatin. Post-translational modifications (PTMs) of histones control the various degrees of chromatin condensation, thereby mediating the accessibility of DNA to the transcriptional machinery. Thus, histone PTMs have crucial roles in modifying nuclear organization, chromatin structure and gene expression, thereby ensuring the epigenetic heritability of defined cellular states over generations. These epigenetic mechanisms are pivotal during embryonic development and cellular differentiation, as well as in adaptive responses to environmental cues. Increasing evidence points to the occurrence of epigenetic abnormalities in complex non-Mendelian diseases such as cancer, diabetes mellitus, asthma, epilepsy and neuropsychiatric disorders. Thus, understanding of epigenetic mechanisms and modifications holds promise for the development of therapeutic interventions[1].

Recent genome-wide studies have begun to shed light on the global distribution of specific PTMs, highlighting the interplay between regulation of chromatin structure and genome function. In particular, Lys methylation is emerging as a dynamic PTM. Lys methylation is a mark of both transcriptionally active and inactive chromatin, depending on the residue that is methylated, its degree of methylation (mono-, di-, or trimethylation; referred to as me1, me2 or me3, respectively) and the position of the methylated nucleosome within the gene and the genome. The human genome encodes approximately 66 methyltransferases, comprising both SET-domain Lys methyltransferases (KMTs; see below) and 7 β-strand enzymes that methylate different Lys residues[2], which are the writer enzymes of the genomic methylation code, and 20 Lys demethylases (KDMs), which function as the eraser enzymes of the methylation code[3]. Among the various chromatin modifiers, KMTs are emerging as pivotal, with key roles in regulating several crucial cellular processes, such as determination of cell fate in response to environmental cues[4]. Each of the KMTs identified so far in mammals has a high degree of specificity towards a particular Lys residue within histone (FIG. 1) and non-histone substrates (see Supplementary information S1, S2 (box, table)).

In this Review, we focus on describing the main KMTs that are involved in gene silencing: namely, those that methylate histone H3 Lys 9 (H3K9), H3K27 and H4K20 (KMTs that are involved in gene activation are discussed in BOX 1). We summarize their biochemical properties and biological functions, and discuss their recruitment to chromatin and how they mediate transcriptional silencing, with an emphasis on the functional crosstalk between them.

Properties of KMTs

All KMTs, with the exception of DOT1-like protein (DOT1L; also known as KMT4) (BOX 2), belong to a large family of proteins that are characterized by the presence of the conserved SET domain, which is

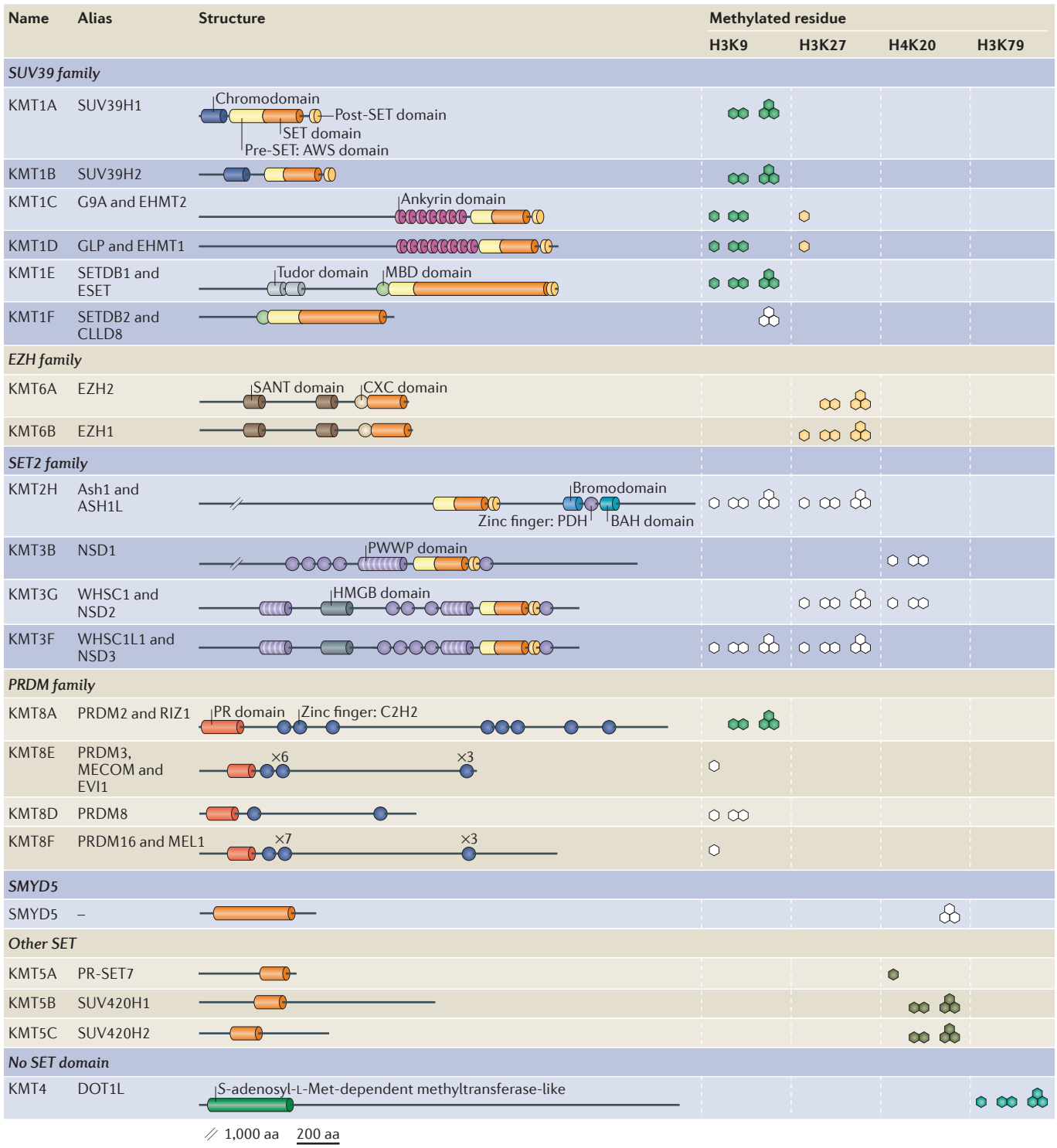


Figure 1 | **The domain composition and substrate specificities of the different repressive Lys methyltransferases.** Lys methyltransferases (KMTs) are divided into six families on the basis of the structural features of their SET domain[6]. For each KMT, alternative names and corresponding domain structures are shown. Domain structure predictions are based on the InterPro database. Domains are grouped on the basis of their putative functions. Also shown are the histone substrate specificities of the different KMTs, with the number of icons corresponding to the degree of Lys methylation. The icons are in white if only one publication describes the catalytic activity or if substrate specificity is still controversial. Ash1, Absent, small or homeotic discs 1; ASH1L, Ash1-like protein; AWS, associated with SET; BAH, bromo-adjacent homology; chromodomain, chromatin organization modifier domain; CXC, Tesmin/TSO1-like CXC domain; DOT1L; DOT1-like protein; ESET, ERG-associated protein with SET domain; EZH, enhancer of zeste homologue; GLP, G9A-like protein 1; H3K, histone H3 Lys; HMGB, high mobility group box; MBD, methyl-CpG-binding domain; NSD, nuclear receptor-binding SET domain-containing protein; PHD, plant homeodomain; PRDM, PR domain-containing; PR-SET7, PR/SET domain-containing protein 7; PWWP, Pro-Trp-Trp-Pro motif; SANT, SWI3, ADA2, N-CoR and TFIIIB DNA-binding domains; SETDB, SET domain bifurcated; SMYD, SET and MYND domain-containing.

Box 1 | **Lys methyltransferases associated with gene activation**

Methylations of histone H3 Lys 4 (H3K4), H3K36 and H3K79 are generally associated with transcriptional activation, although the role of H3K79 methylation in the regulation of gene expression is still controversial (BOX 2). Currently, many Lys methyltransferases (KMTs) that establish these marks have been shown to act through different mechanisms to directly activate transcription (for reviews on H3K4 and H3K36 methylation, see REF. 163 and REF. 164, respectively). KMTs that are responsible for H3K4 and H3K36 methylation, and that were originally described in yeast, can directly interact with RNA polymerase II (Pol II) during the formation of the transcription pre-initiation complex and/or during elongation[163]. The first H3K4 methyltransferase to be identified, SET domain-containing 1 (Set1) in yeast, can establish the three levels of methylation (mono-, di- and trimethylation)[165]. Several mammalian orthologues of Set1, such as the mixed-lineage leukaemia protein 1 (MLL1), MLL2, MLL3, MLL4, MLL5, SET1A and SET1B, are likely to be non-redundant[165]. More recently, other mammalian H3K4 methyltransferases have been described, such as SET and MYND domain-containing protein 1 (SMYD1), SMYD2, SMYD3, PR/SET domain-containing protein 7 (PR-SET7), PR domain-containing protein 6 (PRDM6) and PRDM9 (for a review, see REF. 7).

All three degrees of H3K4 methylation are associated with transcriptional activation, although their different genomic localization suggests that they have slightly different functions. H3K4 monomethylation (H3K4me1), which is most likely to be established by MLL3 and MLL4 (REF. 166), is found at enhancers, whereas H3K4me3 is strongly enriched at the promoter regions of active genes, showing a strong correlation with the level of transcription, the presence of Pol II and histone acetylation[126]. Furthermore, H3K4me3 at transcription start sites is important for the initiation of transcription, as it facilitates the recruitment of general transcription factors[167] and of the chromatin remodelling complex nucleosome remodelling factor that mediates elongation[168].

One of the most-studied H3K36 methyltransferases, SET2, catalyses all three degrees of methylation and was shown to associate with elongating Pol II and to deposit the trimethyl group onto H3K36 during transcription elongation[169]. Finally, even though their target specificity seems to depend on the nature of the substrate[170], nuclear receptor-binding SET domain-containing protein 1 (NSD1), NSD2 and NSD3 were shown to mono- and dimethylate H3K36 (REF. 171), suggesting that they have a role in activating gene expression, similar to SET2.

named after the three *Drosophila melanogaster* proteins in which it was first identified: Suppressor of variegation 3-9 (Su(var)3-9), Enhancer of zeste (E(z)) and the homeobox gene regulator Trithorax (Trx)[5]. The SET domain carries out the catalytic activity of the enzymes on ε-amino groups of Lys residues. Depending on their biochemical properties and the presence of cofactors, SET domain-containing proteins are able to mono-, di- or trimethylate Lys substrates by using *S*-adenosyl-L-Met as a methyl group donor. On the basis of sequence homology in and around the catalytic SET domain, SET-containing KMTs can be divided into six subfamilies: the SUV39 family of Su(var)3-9 homologues; the E(z) homologue (EZH) family; the SET-domain containing 1 (SET1) family of activating KMTs and the SET2 family; and the PR domain-containing (PRDM) and SET and MYND domain-containing (SMYD) families[6] (FIG. 1). The features of the SET domain often reflect its specificity for histone substrates (reviewed in REF. 7). Most of the SET-containing KMTs have at least one additional module (FIG. 1), which confers the ability to recognize various PTMs, including epigenetic marks. Such domains facilitate the coupling of writer properties with PTM reader properties, which allows KMTs to concomitantly recognize certain histone PTMs and catalyse methylation[8], indicating the existence of protein crosstalk mechanisms for the establishment and/or propagation of different histone marks (FIG. 2a).

H3K9 methyltransferases. H3K9 methyltransferases include the SUV39 subfamily members histone-Lys *N*-methyltransferase SUV39H1 (also known as KMT1A), SUV39H2, SET domain bifurcated 1 (SETDB1; also known as KMT1E), SETDB2 (also known as KMT1F), protein G9A (also known as EHMT2) and G9A-like protein 1 (GLP; also known as EHMT1), and four members of the PRDM family: PRDM2, PRDM3, PRDM8 and PRDM16 (FIG. 1). The members of the SUV39 subfamily (which is named after the first characterized mammalian KMT, SUV39H1, a homologue of *D. melanogaster* Su(var)3-9) are defined by the presence of two Cys-rich modules: the pre-SET and post-SET domains[9]. These domains bind to three zinc ions that are necessary for the catalytic activity of the SET domain: in particular, for maintaining the structural stability of the pre-SET domain and for forming part of the active site at the post-SET domain. SETDB1 and SETDB2 possess a unique SET domain that is interrupted by an insertion of hundreds of amino acids[10,11] and is conserved across various organisms, including mammals, worms and flies. The function of this interposed sequence is unknown, although it might be involved in regulation of the catalytic activity of the enzymes.

In addition, SUV39 subfamily members contain unique domains that are involved in the recognition of epigenetic marks, including methylated H3K9. This gives the H3K9 methyltransferases the ability both to write and to read the marks they establish, a mechanism that accounts for the nucleation and spreading of H3K9 methylation along chromatin (FIG. 2a). SUV39H1 and SUV39H2 have a chromatin-organization modifier domain (chromodomain), which is a module that confers the capacity to target methylated Lys. Chromodomain integrity is crucial for the function of cryptic loci regulator 4 (Clr4; the yeast orthologue of SUV39H) in the fission yeast *Schizosaccharomyces pombe*[12], and for pericentromeric heterochromatin localization of mouse SUV39H1 (REF. 13). SETDB1 and SETDB2 possess a putative methyl-CpG-binding domain. Although it is unknown whether this domain can bind to methylated DNA, the presence of DNA-interacting Arg residues suggests that it is functional. SETDB1 also has two consecutive Tudor domains, which are known to bind to both methylated Arg and Lys. Structural studies have shown that two adjacent Tudor domains of SETDB1 participate in binding to methylated Lys, suggesting that they might recognize methylated Lys but not Arg.

G9A and GLP have ankyrin repeats, which mediate protein–protein interactions. Interestingly, these ankyrin domains were shown to possess a methyl-Lys-binding module that allows binding to H3K9me1 and H3K9me2 marks, independently of SET domains[14]. Binding to H3K9me2 by GLP through its ankyrin domains stimulates its activity on the neighbouring nucleosomes and is required for the establishment of H3K9 methylation *in vivo*[15].

In addition to combining the reader and writer properties in the same protein, spreading of the marks could be assisted by adaptor proteins, which function

Reader
An effector protein that binds only to histones or DNA that have certain chemical modifications.

Pre-SET
The region at the amino-terminal side of the SET domain of most Lys methyltransferases, which stabilizes the SET domain.

Post-SET
Part of the active site of most Lys methyltransferases. It is located at the carboxy-terminal side of the SET domain and participates in binding to histone tails and interactions with cofactors.

Pericentromeric heterochromatin
Repeats of constitutive heterochromatin that flank the central kinetochore-forming region of the centromere and that are necessary for proper centromere function.

Box 2 | **The case of the Lys H3K79 methyltransferase DOT1-like protein**

DOT1-like protein (DOT1L) is the only Lys methyltransferase that lacks a SET domain; instead, it has a domain similar to that of Gly *N*-methylase[172]. It is so far the only enzyme that is known to methylate Lys79 of histone H3 (H3K79) (FIG. 1), which is a mark that was controversially associated with both active and repressed genes. Recent analyses in yeast, flies, mice and humans have shown that H3K79 methylation is mainly a mark of active transcription[172]. DOT1L establishes the three methylation levels (mono-, di- and trimethylation) in a non-processive manner[173,174]. Disruption of the *Dot1l* gene in mice resulted in complete loss of H3K79 methylation and revealed the essential role of DOT1L in embryonic development, haematopoiesis and cardiac function[175]. *In vitro* examination of DOT1L substrate specificity showed that it has no capacity for methylating peptides or free histones but only methylates nucleosomal substrates. A stretch of positively charged residues at the carboxy-terminal part of human DOT1L is crucial for its nucleosome-binding capacity and therefore for its enzymatic activity (reviewed in REF. 172). DOT1L-deficient cells show severe loss of H3K79 methylation but also show reduced levels of dimethylated H3K9 (H3K9me2) and H4K20me3 at centromeres and telomeres[175]. These changes result in aneuploidy, telomere elongation and proliferation defects, thus highlighting DOT1L-mediated methylation of H3K79 as crucial for heterochromatin formation and chromosome integrity. Additionally, H3K79 methylation was implicated in cell cycle regulation and in the DNA damage response (reviewed in REF. 172).

as readers that recruit writers, as in the case of heterochromatin protein 1 (HP1), which is a H3K9me2 and H3K9me3 reader that recruits the H3K9me3 writer SUV39H1 (FIG. 2a) (see below).

Each H3K9 methyltransferase has different affinities to the various degrees of methylation of its substrate residue (or residues) (FIG. 1). SUV39H1 is able to methylate unmethylated H3K9 (REF. 9) but prefers binding to H3K9me1 (REF. 16) to establish the H3K9me2 and H3K9me3 marks, whereas its methyltransferase activity is inhibited by H3K9me2 *in vitro*[9,16]. By contrast, SETDB1 is able to mono, di- and trimethylate unmethylated H3K9 *in vitro*[10,16–18], aided by the ATPase-activating transcription factor 7-interacting protein 1 (ATF7IP), which is necessary for the conversion of H3K9me2 to H3K9me3 (REF. 18). G9A[19] and GLP[20] target unmethylated H3K9 to catalyse mono-, di- and trimethylation, at least *in vitro*[21,22]. *In vivo*, it has been more challenging to assign a specific function (or functions) to each KMT, partially owing to potential redundancy and functional cooperation between KMTs[23–25]. Nevertheless, SUV39H1 and SUV39H2 are strongly enriched at the heterochromatin[9,13,26], and their loss decreases H3K9me3 levels genome-wide[27], with a particular loss at constitutive heterochromatin[27,28] and facultative heterochromatin (such as the inactive X chromosome)[29], leading to transcription activation. Notably, SUV39H1 is also involved in *de novo* gene silencing during differentiation[30,31]. By contrast, loss of SETDB1 in mice is associated with a substantial reduction of H3K9me3 at euchromatic regions[10] but also at pericentromeric heterochromatin. The reduction at pericentromeric heterochromatin is potentially a result of the function of SETDB1 as a H3K9 monomethyltransferase on free, non-nucleosomal H3, which is integrated into the chromatin during DNA replication and provides a substrate for SUV39H1 and SUV39H2 (REFS 16,23). Notably, H3K9 methyltransferases of the PRDM family were also shown to function as H3K9 monomethyltransferases of free H3 (REF. 25) (see below).

G9A and GLP were mainly found to mono- and dimethylate H3K9 in euchromatic regions[32]. Although G9A and GLP can independently exert their catalytic activity and form homodimeric and heterodimeric complexes through their SET domains, the heterodimeric G9A–GLP complex was described as the only functional H3K9 mono- and dimethyltransferase *in vivo*[32,33]. Indeed, the levels of H3K9me1 and H3K9me2 in euchromatic regions were severely reduced by knockout of the genes encoding G9A and/or GLP[33,34], whereas H3K9me3 levels seemed unaffected, probably because they were maintained by SUV39H1 and/or SETDB1 (REFS 29,34). However, many reports suggest that G9A and GLP might also be involved in the deposition of H3K9me3 (REFS 24,35). Other histone targets were also reported for G9A and GLP, such as the histone linker H1 (REF. 19). G9A-dependent methylation of H1 isotype 4 (H1.4) at Lys26 provides a recognition surface for the chromatin-binding proteins HP1 and lethal(3)malignant brain tumour-like protein 1 (L3MBTL1) and promotes subsequent H1 deposition, thus inducing chromatin compaction by reducing nucleosome spacing[36]. In addition, G9A directly monomethylates H3K56 *in vitro* and is required for H3K56 monomethylation *in vivo*, thereby creating a chromatin-docking site for proliferating cell nuclear antigen (PCNA) and regulating DNA replication[37]. Finally, G9A and GLP have been implicated in the monomethylation of H3K27 (see below).

The PRDM methyltransferase family comprises 16 members (only 4 of which are H3K9 methyltransferases), which are defined by the presence of a conserved amino-terminal catalytic domain called PR (PRDI–BF1–RIZ1 homologous), which is 20–30% identical to the SET domain, in addition to DNA-binding zinc-finger repeats (FIG. 1). This implies a dual role for PRDM proteins: as bona fide transcription factors (owing to their zinc-finger repeats) and as histone-modifying enzymes[38]. However, intrinsic methyltransferase activity was reported only for some members, including PRDM2, PRDM3, PRDM6, PRDM8, PRDM9 and PRDM16. Nevertheless, nearly all PRDM proteins were shown to regulate chromatin functions through recruitment of chromatin-modifying enzymes[38,39].

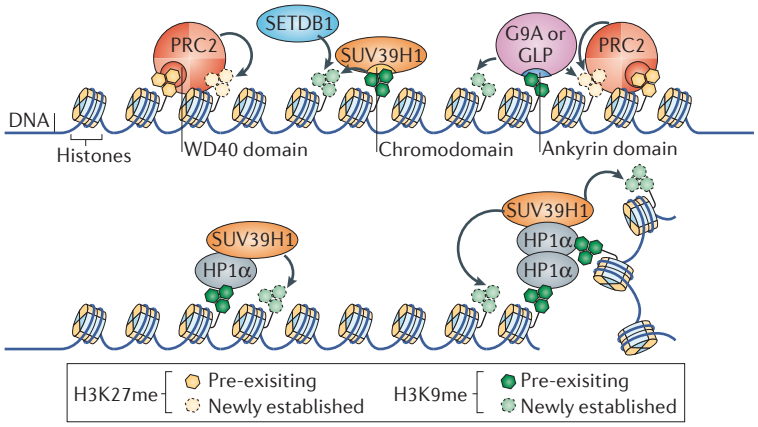
Different histone methylation activities have been ascribed to the various catalytically active PRDM proteins. Whereas PRDM6 and PRDM9 were implicated in the methylation of H4K20 and H3K4 (REF. 38), respectively, H3K9 methylation was reported for PRDM2, PRDM8 (REF. 38), PRDM3 and PRDM16 (REF. 25). PRDM3 and PRDM16 were recently characterized as cytoplasmic H3K9 monomethyltransferases of free H3 histones[25]. This suggests that PRDM3 and PRDM16 initiate heterochromatin formation by establishing a cytoplasmic pool of H3K9me1, which is converted to H3K9me3 in the nucleus by the SUV39H enzymes after it has been incorporated into nucleosomes. A similar role was ascribed to SETDB1, which can also be localized in the cytoplasm[16,23,25]. The functional relevance of this localization is still unclear, but it suggests a close collaboration and/or synergism between the SUV39 and PRDM subfamilies. In fact, depletion of both PRDM3

Tudor domains
Conserved structural motifs of approximately 60 amino acids, which were originally found in the *Drosophila melanogaster* Tudor protein. They bind to methylated Lys and Arg residues.

Constitutive heterochromatin
Highly compact chromatin at regions such as centromeres and telomeres, which are mostly transcriptionally inactive in all cell types.

Facultative heterochromatin
A dynamic subtype of heterochromatin that is formed from euchromatin in a cell type-dependent manner to consolidate the repression of genes.

a



b

Histone marks	Transcription	Genomic regions
H3K4me1 H3K4me2 H3K4me3 H3K9me1 H3K27me1 H3K36me1 H3K36me3 H4K20me1	Active	• Promoters • Enhancers • Gene bodies
H3K4me3 H3K27me3 H3K9me3	Poised	• Promoters (for example, of developmental genes)
H3K27me3	Repressed	• Promoters • Enhancers • CpG-rich regions
H3K9me3	Repressed	• Promoters • Enhancers
H3K9me2 H3K27me3	Repressed	• Promoters (for example, of neuronal genes) • X-inactivation centre
H3K9me2 H3K9me3 H3K20me3	Repressed	• Repeats (for example, pericentromeric and LTR)

Figure 2 | **Interdependency in the establishment and propagation of the different repressive histone Lys methylation marks. a** | The top panel shows that Lys methyltransferases recognize their own substrates when methylated, and this feature gives the histone H3 Lys 9 (H3K9) methyltransferases the ability both to 'write' and to 'read' the marks that they establish, which is a mechanism that was proposed to account for the process of nucleation and spreading of H3K9 methylation (H3K9me) and H3K27me along chromatin during and after DNA replication. The WD40 domains of the Polycomb repressive complex 2 (PRC2) subunit embryonic ectoderm development (EED) recognize and bind to pre-existing H3K27 trimethylation (H3K27me3) marks, which promotes the *de novo* methylation of the neighbouring nucleosome by the PRC2 catalytic subunit enhancer of zeste homologue 2 (EZH2). Similarly, the chromatin organization modifier domain (chromodomain) of SUV39H1 recognizes pre-existing H3K9me3; the spreading of this mark is accomplished by the cooperative action of SET domain bifurcated 1 (SETDB1), which monomethylates H3K9 and thereby provides the substrate for SUV39H1 to establish H3K9me3. G9A and G9A-like protein 1 (GLP) can bind to pre-existing H3K9me2 through their ankyrin repeats, to spread the H3K9me2 mark to the neighbouring nucleosome and/or to establish the H3K27me1 mark, which is recognized by PRC2 and used as a substrate to establish H3K27me3. The bottom panel shows that epigenetic mark reader proteins can also participate in the spreading of marks by recruiting the writers of the same mark. Heterochromatin protein 1α (HP1α), which is a reader of H3K9me2 and H3K9me3, forms a stable complex with SUV39H1, thereby providing a means for the spreading and maintenance of H3K9me3 at the heterochromatin. Additionally, the ability of HP1α to form homo-oligomers and to modulate higher-order chromatin structures aids in the spreading of H3K9me3. **b** | Histone Lys methylation marks, their genomic locations and their usual effects on transcription are shown in the table. LTR, long terminal repeat.

and PRDM16 in mice leads to the dispersion of heterochromatin and disruption of the nuclear lamina, suggesting that PRDM-mediated H3K9 monomethylation is important for mammalian heterochromatin integrity[25]. A study in the nematode *Caenorhabditis elegans* supports the importance of H3K9 methylation in maintaining heterochromatin interactions with the nuclear lamina; the depletion of MET-2, which is the *C. elegans* homologue of SETDB1, and SET-25, which is a functional orthologue of SUV39H, resulted in detachment of chromosomes from the nuclear periphery[40].

H3K27 methyltransferases. Two main H3K27 methyltransferases have been described so far in mammals: EZH1 (also known as KMT6B) and EZH2 (also known as KMT6A). These are the catalytic subunits of the mammalian Polycomb repressive complex 2 (PRC2), which is responsible for H3K27 di- and trimethylation[41] (FIG. 1). Although both EZH1 and EZH2 contain a SET domain, they exert their methyltransferase activity only when in complex with the other three PRC2 core subunits: suppressor of zeste 12 homologue (SUZ12), embryonic ectoderm development (EED), and retinoblastoma-associated protein 46 (RBAP46; also known as RBBP7) or RBAP48 (also known as RBBP4). The resolution of the three-dimensional structure of the human PRC2 provided key insights into the functional interactions among the core subunits[42], revealing that protein interactions within the PRC2 modulate the trajectory of the post-SET and 'inserted within SET' (I-SET) domains of EZH2 in favour of a catalytically competent conformation. Indeed, EZH2 is involved in an intricate network of three-dimensional interactions with EED and SUZ12, through both its SET domain and its two SWI3, ADA2, N-CoR and TFIIIB (SANT) DNA-binding domains[42]. Thus, the SANT domains of EZH2 may couple the binding of methylated H3K27 to EED[43] to the methyltransferase activity of EZH2 (REF. 42). Consistent with this, previous work has shown that EED is able to bind to H3K27me3 through its WD40 domain, thus enhancing PRC2-mediated methylation of H3K27 in nucleosomes[43]. This process was proposed as the mechanism by which H3K27me3 propagates to newly incorporated histones in neighbouring nucleosomes during DNA replication[43] (FIG. 2a). Intriguingly, it was also recently suggested that binding of PRC2 to histone marks that are associated with active transcription (H3K4me3, H3K36me2 and H3K36me3) inhibits its activity if these marks are placed on the same histone tail that contains the targeted H3K27 (REF. 44). This indicates that PRC2 can integrate signals from inhibitory as well as activating PTMs to regulate its activity according to the surrounding chromatin modifications[44]. It is likely that PRC2 is capable of self-inhibition once in the boundary of active genes that are marked by H3K4me3, H3K36me2 and H3K36me3, ensuring that H3K27 di- and trimethylation is limited to repressed genes[42,44].

Whereas the role of PRC2 in mediating di- and trimethylation of H3K27 is well appreciated, its contribution to catalysing monomethylation is still unclear. The finding that H3K27me1 levels remain unaffected by PRC2 loss *in vivo*[45] has led to the assumption that another KMT

catalyses the monomethylation of H3K27. However, a recent report has directly implicated PRC2 in the genomic accumulation of H3K27me1 (REF. 46) in the bodies of actively transcribed genes[46] (FIG. 2b), and EZH1-containing PRC2 (PRC2–EZH1) was reported to be involved in the deposition of H3K27me1 (REF. 47). Although the role of EZH1 in catalysing the monomethylation of H3K27 has been controversial[48], the co-localization of both EZH1 and H3K27me1 to active genes suggests that PRC2–EZH1 might indeed contribute to H3K27me1 deposition. Nonetheless, a contribution by other KMTs cannot be excluded. Indeed, G9A and GLP were reported to methylate H3K27 both *in vitro* and *in vivo*[19,49]. Accordingly, H3K27me1 levels decrease drastically in *G9A*- or *GLP*-knockout embryonic stem (ES) cells[49], and it was recently suggested that G9A-mediated H3K27 methylation increases the enzymatic activity of PRC2 *in vitro*[50].

H4K20 methyltransferases. Five SET-containing H4K20 methyltransferases have been characterized: PR/SET domain-containing protein 7 (PR-SET7; also known as SETD8 and KMT5A)[51], Su(var) 4-20 homologue 1 (SUV420H1; also known as KMT5B) and SUV420H2 (also known as KMT5C)[52,53], nuclear receptor-binding SET domain-containing protein 1 (NSD1; also known as KMT3B)[54] and Absent, small or homeotic discs 1 (Ash1; in humans, this is known as ASHL1 and KMT2H)[55] (FIG. 1). One study has suggested that SMYD5 is able to establish H4K20me3 (REF. 56). PR-SET7 monomethylates H4K20 through a Tyr residue in its active site, which forms hydrogen bonds with the monomethylated Lys residues to inhibit further addition of methyl groups. Loss of PR-SET7 in mice decreases global H4K20 monomethylation, but also di- and trimethylation[57], confirming that H4K20 di- and trimethyltransferases use H4K20me1 as a substrate. SUV420H1 and SUV420H2 generate H4K20me2 and H4K20me3 *in vivo* and co-localize with HP1 proteins in heterochromatin[58].

Genomic targeting and modes of action

How the different KMTs are (co)-recruited to target sites in mammals is a matter of debate. No KMTs except the PRDM family members possess DNA-binding properties, so they have to rely on DNA-binding partners and various mechanisms to be targeted to the chromatin. In this section, we mainly discuss how H3K9 and H3K27 methyltransferases are (co)-recruited to (and potentially interact at) their genomic target loci by interacting with sequence-specific transcription factors, other chromatin-binding proteins and non-coding RNAs (ncRNAs) (FIG. 3).

Transcription factor-mediated recruitment. In *D. melanogaster,* PRC2 recruitment is well described and involves the recognition of Polycomb responsive elements (PREs) by the transcription factor Pleiohomeotic (Pho; the orthologue of mammalian YY1). In mammals, several sequence-specific transcription factors, such as zinc-finger protein SNAI1, RE1-silencing transcription factor and homeobox protein MSX1, mediate chromatin targeting of both PRC2 and H3K9 methyltransferases (FIG. 3a) (for a review, see REF. 59). Indeed, PRC2, and G9A and GLP have been found to interact physically[50,60] and functionally[50], and it is plausible that they are co-targeted to common genomic loci by a shared recruiting factor[59]. Using proteomic approaches, a recent study supported the existence of such interactions by showing that PRC2, and G9A and GLP share several partners; these include the novel zinc-finger proteins (ZFPs) ZNF518A and ZNF518B, which mediate the structural association between PRC2 and G9A and GLP and probably their genomic targeting[60] (FIG. 3a).

A set of transcription factors was found to target particular H3K9 methyltransferases, such as transcription factor E2F6, within the GLP complex, to silence proliferation genes in quiescent cells[20]. G9A and GLP were recently shown to be preferentially targeted to promoters by the ZFPs widely interspaced zinc-finger-containing protein (WIZ) and ZNF664 (REF. 61). Originally discovered through its interaction with transcriptional regulator ERG[17], SETDB1 was also shown to interact with the pluripotency transcription factors POU class 5 homeobox 1 (POU5F1)[62] and ZNF274 (REF. 63). Similarly, targeting of SUV39H1 to euchromatic loci is mediated by its association with transcription factors such as RUNT-related transcription factor 1 (RUNX1), RUNX3 (REFS 64,65) and E2F1 (REF. 66), whereas paired box proteins PAX3, PAX9 and zinc-finger E-box-binding homeobox 1 might instead account for its targeting to heterochromatin[67]. Thus, transcription factor-dependent recruitment of KMTs could lead to the silencing of not only euchromatic loci but potentially also of constitutive heterochromatin loci such as endogenous retroviruses (ERVs).

Recruitment by other chromatin-binding proteins. Chromatin-binding proteins that interact with KMTs can regulate their genomic targeting. Jumonji/ARID domain-containing protein 2 (JARID2; also known as Jumonji) was suggested as the major PRC2-recruiting factor[68,69]. Indeed, the carboxy-terminal half of JARID2 contains some conserved domains, such as AT-rich interaction domain (ARID) and zinc-finger domains, which could mediate its binding to DNA, especially to CpG islands[68]. The genome-wide distribution of JARID2 in ES cells overlaps almost entirely with that of PRC2, and its knockdown leads to a substantial decrease in binding of PRC2 to chromatin[68,69]. JARID2 also interacts with G9A and GLP[50], and its binding overlaps to some extent with G9A-binding sites[50], suggesting that it could co-recruit PRC2, G9A and GLP (FIG. 3b). Similarly, chromodomain Y-like protein (CDYL), which has been described as a potential reader of both H3K9me2 and H3K27me3 (REF. 70), mediates the recruitment of both PRC2 and G9A, again pointing to co-recruitment of PRC2 and H3K9 methyltransferases as a mechanism of gene silencing[59] (FIG. 3b). Additionally, the PRC2-associated Polycomb-like protein 1 (PCL1; also known as PHF1), PCL2 (also known as MTF2) and PCL3 (also known as PHF19) are required for recruiting PRC2 in ES cells. The Tudor domains of the PCL proteins recognize nucleosomes marked by H3K36me3 and mediate the recruitment of both PRC2 and H3K36me3 demethylases, which leads to the

Zinc-finger proteins
(ZFPs). DNA-binding proteins that contain the zinc-finger motif and recognize DNA in a sequence-specific manner. They are often found in complex with Lys methyltransferases and are likely to mediate their recruitment to specific loci.

Endogenous retroviruses
(ERVs). Relics of retroviruses that successfully colonized metazoan genomes. They constitute approximately 8% of the human genome and transpose through an RNA intermediate.

CpG islands
Genomic regions with high cytosine and guanine dinucleotide levels found in many mammalian promoters, where they are normally hypomethylated to allow gene expression.

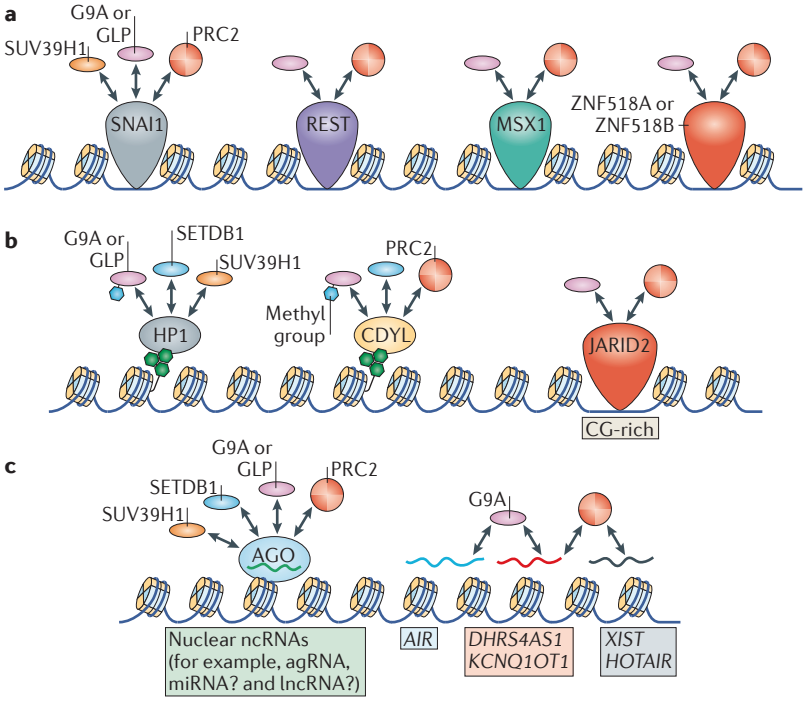


Figure 3 | **Recruitment and crosstalk of Lys methyltransferase at target regions.** **a** | Sequence-specific transcription factors, such as RE1-silencing transcription factor (REST), zinc-finger protein SNAI1, homeobox protein MSX1, zinc-finger protein 518A (ZNF518A) and ZNF518B interact with and recruit Polycomb repressive complex 2 (PRC2) and histone H3 Lys 9 (H3K9) methyltransferases such as G9A, G9A-like protein 1 (GLP) and SUV39H1, thus possibly mediating the genomic (co)-targeting of different Lys methyltransferases. **b** | The chromatin-binding proteins chromodomain Y-like protein (CDYL), heterochromatin protein 1 (HP1) and Jumonji/ARID domain-containing protein 2 (JARID2) interact with and probably mediate the recruitment of both PRC2 and H3K9 methyltransferases to their genomic targets. Lys residues on G9A and GLP can also be methylated and are involved in mediating protein–protein interactions. **c** | Argonaute (AGO)-associated nuclear non-coding RNAs (ncRNAs) and two long ncRNAs (lncRNAs), DHRS4 antisense RNA 1 (*DHRS4AS1*) and KCNQ1 overlapping transcript 1 (*KCNQ1OT1*), mediate the genomic targeting of PRC2 and/or H3K9 methyltransferases. G9A cooperates with the ncRNA acute insulin response (*AIR*) in the placenta to promote the dimethylation of H3K9 and gene repression, whereas PRC2 interacts with the ncRNAs X inactive-specific transcript (*XIST*) and HOX transcript antisense RNA (*HOTAIR*). agRNA, antigene RNAs; miRNA, microRNA; SETDB1, SET domain bifurcated 1.

concomitant deposition of H3K27me3 and removal of H3K36me3 from active genes, resulting in gene silencing and spreading of epigenetic silencing signatures[71,72]. However, some chromatin-binding factors bind only to particular KMTs; for example, adipocyte enhancer-binding protein 2 (AEBP2) was found to interact with PRC2 and, given its ability to bind to DNA, is thought to bring PRC2 to its target sites. Consistent with this, AEBP2 and PRC2 were shown to co-localize genome-wide[73]; however, it is not clear whether AEBP2 has an important role in mediating PRC2 recruitment.

H3K9 methyltransferases interact with many chromatin-binding complexes, which facilitate their chromatin targeting. For instance, SUV39 subfamily members interact with the transcription co-repressors tripartite motif-containing protein 28 (TRIM28; also known as TIF1β and KAP1)[10], methyl-CpG-binding domain protein 1 (MBD1)[74], ATF7IP[18], paired amphipathic helix protein SIN3A and SIN3B[75]; with DNA (cytosine-5)-methyltransferase 3A (DNMT3A) and DNMT3B[76,77]; and with NAD-dependent protein deacetylase sirtuin 1 (REF. 78). These proteins probably mediate targeting of the H3K9 methyltranferases to both euchromatin and heterochromatin loci. The H3K9me2 and H3K9me3 reader HP1α is likely to mediate the recruitment of H3K9 KMTs to both heterochromatic and euchromatic loci[13,23,79] (FIG. 3b). In particular, SUV39H1 catalyses the trimethylation of H3K9, to which HP1α binds[80] and SUV39H1 is recruited, which leads to the spreading of the H3K9me3 mark and heterochromatin formation (FIG. 2a). The same interaction might be important for the initial targeting of SUV39H1 to pericentromeric heterochromatin — for example, during its establishment in early development — as it has been suggested that SUMO-modified HP1α, which has an affinity for major satellite RNA, could direct SUV39H1 to unmarked pericentromeres[81]. Importantly, binding of SUV39H1, SUV39H2 and HP1 to methylated H3K9 at pericentromeric heterochromatin is additionally stabilized by their interactions with other heterochromatin components such as methyl-CpG-binding protein 2, MBD1 and sentrin-specific protease 7 (REFS 82,83), highlighting the importance of keeping multiple interactions between various chromatin-binding proteins in the generation of heterochromatin nucleation sites. Notably, recent findings show that, in *S. pombe*, binding of the HP1 orthologue Swi6 to methylated H3K9 leads to oligomerization of Swi6 on neighbouring nucleosomes; the Swi6 oligomers provide a platform for the recruitment of other heterochromatin components, and this results in heterochromatin spreading[84,85].

ncRNA-mediated recruitment. Long ncRNAs (lncRNAs) and short ncRNAs are important regulators of the recruitment of KMTs to chromatin. The recruitment of PRC2 and subsequent establishment of the H3K27me3 mark on the inactive X chromosome is mediated by the lncRNA X inactive-specific transcript (*XIST*), which coats the chromosome during inactivation of the X chromosome in female cells[86,87]. The lncRNA HOX transcript antisense RNA (*HOTAIR*) mediates PRC2 recruitment in *trans* to the *HOXD* gene cluster, thus ensuring the proper temporal and spatial expression of HOX genes during development[88]. The imprinted lncRNA acute insulin response (*AIR*) regulates targeting of G9A to induce the allele-specific imprinting in *cis* of the solute carrier family 22 (organic cation transporter) member 2 (*Slc22a2*), *Slc22a3* and insulin-like growth factor 2 receptor genes in the mouse placenta[89] (FIG. 3c). Interestingly, two lncRNAs can bind to both G9A and PRC2: KCNQ1 overlapping transcript 1 (*Kcnq1ot1*) and DHRS4 antisense RNA 1 (*DHRS4AS1*). *Kcnq1ot1* controls the imprinting and silencing of the potassium voltage-gated channel, subfamily Q (KCNQ) gene cluster by recruiting both PRC2 and G9A[90]. *DHRS4AS1* silences the DHRS4 gene cluster by physically interacting with G9A and EZH2 and targeting them to the promoters of the downstream *DHRS4L2* and *DHRS4L1* genes, where they deposit the repressive marks H3K9me2 and H3K27me3, respectively[91] (FIG. 3c).

Major satellite RNA
RNA that is transcribed from major satellite DNA repeats found at pericentromeric regions in mice.

In human breast cancer cells, SETDB1 is recruited to the promoter of the androgen receptor gene by a member of the Argonaute family, Argonaute protein 2 (AGO2)[92]. SETDB1 was found to interact with AGO2 in the nucleus and to regulate AGO2-mediated transcriptional gene silencing. AGO2-mediated recruitment of SETDB1 depends on small antigene RNAs (agRNAs) that are complementary to the promoter of the androgen receptor gene. AGO2–agRNA-mediated recruitment was also suggested for EZH2 (REF. 92). Furthermore, G9A and SUV39H1 were also recently identified as part of an AGO2 chromatin-associated complex that is involved in promoting pre-mRNA splicing by methylating H3K9 to slow down the transcription machinery and thus facilitate splicing[93] (FIG. 4a). Finally, AGO-dependent recruitment of G9A and GLP to mRNA 3′ untranslated regions was implicated in termination of transcription[94]. A group of lncRNAs were also shown to guide SUV420H2 to specific genomic loci in growth-arrested cells to establish a more compact chromatin structure[95], suggesting that ncRNA-mediated targeting might be a common mechanism of silencing by KMTs.

Despite our still-limited understanding of the recruitment of KMTs to their target loci, the evidence points towards this being a multistep process, which might comprise a combination of the mechanisms described above. Importantly, the growing number of reports of the co-targeting of different KMTs to the same loci suggests the requirement for a specific chromatin environment to promote and stabilize the formation of a repressed chromatin state.

Gene silencing

In this section, we describe the mechanisms by which KMTs induce or maintain gene repression, highlighting their interplay with other chromatin-modifying enzymes (FIG. 4).

Gene silencing by H3K9 methylation. H3K9 methylation is generally associated with transcriptional silencing and heterochromatin formation, which ensures stable repression and genomic integrity. Gene repression by H3K9 methyltransferases usually correlates with increased H3K9 methylation both on promoters and across large genomic regions (detailed below), thus depending on both local chromatin compaction and localization at the nuclear periphery. Whereas SUV39H1 and SUV39H2 binding is enriched at constitutive heterochromatin[27,29], SETDB1 (REF. 10), G9A and GLP[32] are responsible for H3K9 methylation at the euchromatin and facultative heterochromatin, and are particularly involved in dynamic transcription repression. Importantly, H3K9 methyltransferase-mediated transcription repression occurs as part of the concerted action of different enzymes, including DNA methyltransferases[76,77,96], and histone deacetylases[75,97] and H3K4 demethylases[98–100], which concomitantly deposit repressing histone marks and remove activating marks, respectively (FIG. 4b). For example, the euchromatic mark H3K4me3 prevents H3K9 methylation[101], suggesting that H3K9 methyltransferases might need to collaborate with H3K4 demethylases at actively transcribed chromatin[98–100] to initiate the formation of the repressive epigenetic state.

H3K9 methyltransferases have been suggested to be chromatin compaction gatekeepers, owing to their capacity to interact with and recruit the H1 linker histone and the chromodomain-containing HP1 proteins[13,102]. H1 positioning affects chromatin compaction, as it prevents nucleosome sliding[103]. Methylation of H1.4 at Lys26 by G9A was suggested to create a binding site for HP1, promoting chromatin condensation[102]. Furthermore, G9A and GLP are capable of automethylation, which can be recognized by HP1 and mediates its recruitment to chromatin and binding to G9A- and GLP-catalysed H3K9me2 marks[79]. As HP1 has been mainly implicated in the recruitment of SUV39H1 (REF. 13) and other repressing factors to constitutive heterochromatin, G9A- and GLP-mediated H3K9 methylation might also be required for several mutually reinforcing silencing mechanisms at both euchromatic and facultative heterochromatic regions (FIG. 4b). Similarly, SETDB1-mediated stepwise methylation was suggested to be required for the maintenance of H3K9me3 at pericentromeres during DNA replication[16,23]. Intriguingly, in addition to facilitating H3K9 stepwise methylation[16,23,24], SUV39H1 and SETDB1 collaborate in the regulation of rDNA transcription, although this occurs by different mechanisms. Whereas SUV39H1 is targeted to rDNA genes (together with sirtuin 1, as part of the energy-dependent nucleolar silencing complex) to methylate H3K9 (REF. 78), SETDB1 negatively regulates rDNA transcription by methylating one of the major nucleolar transcription factors, upstream-binding factor 1 (UBF1; also known as nucleolar transcription factor 1)[104].

Comprehensive genome-wide analyses highlighted the importance of H3K9 methyltransferases in silencing several DNA repeats and transposable elements (retrotransposons) (FIG. 4b). In contrast to differentiated cells, in which the silencing of transcription from DNA repeats mostly depends on DNA methylation, transposon repression in mouse ES cells relies mostly on histone Lys methylation[105]. SETDB1 is necessary for silencing of class I and class II ERVs in ES cells and during germline development[106,107]. SUV39H1 and SUV39H2, together with SETDB1, also bind to retrotransposons in ES cells, in particular at long interspersed nuclear elements (LINEs) and ERVs, inducing their H3K9me3-mediated silencing[108]. Interestingly, SUV39H1 and SUV39H2 collaborate with SETDB1 to prevent the transposition of intact ERVs by expanding the repressive H3K9me3 domain into neighbouring regions[108], whereas the subset of class I and class II ERVs might be silenced instead by collaboration between SETDB1 and the H3K4 demethylase Lys-specific demethylase 1 (LSD1; also known as KDM1A) (reviewed in REF. 105). In turn, LSD1 (but not SETDB1) is particularly involved in silencing of class III ERVs[109]. Intriguingly, both SETDB1 and LSD1 bind to the transcriptional co-repressor TRIM28, which is suggested to target them to their specific ERV classes; in turn, TRIM28 might be targeted to the DNA by sequence-specific DNA-binding Krüppel-associated box (KRAB)-containing ZFPs, which recognize specific retroviral sequence elements[106,109,110]. However, it is

Argonaute
Family of proteins that are essential components of RNA-induced silencing complexes, which, in conjugation with short interfering RNAs, target genes for silencing.

Transcriptional gene silencing
Silencing that is achieved by the formation of repressive chromatin modifications (DNA methylation and/or histone modifications) at a locus, through the targeting of non-coding RNA.

Antigene RNAs
(agRNAs). Small double-stranded RNAs that target gene promoters and mostly inhibit transcription by interacting with Argonaute proteins.

Krüppel-associated box
(KRAB). A domain found in a subgroup of zinc-finger proteins that mediates protein–protein interactions and represses transcription by recruiting co-repressors.

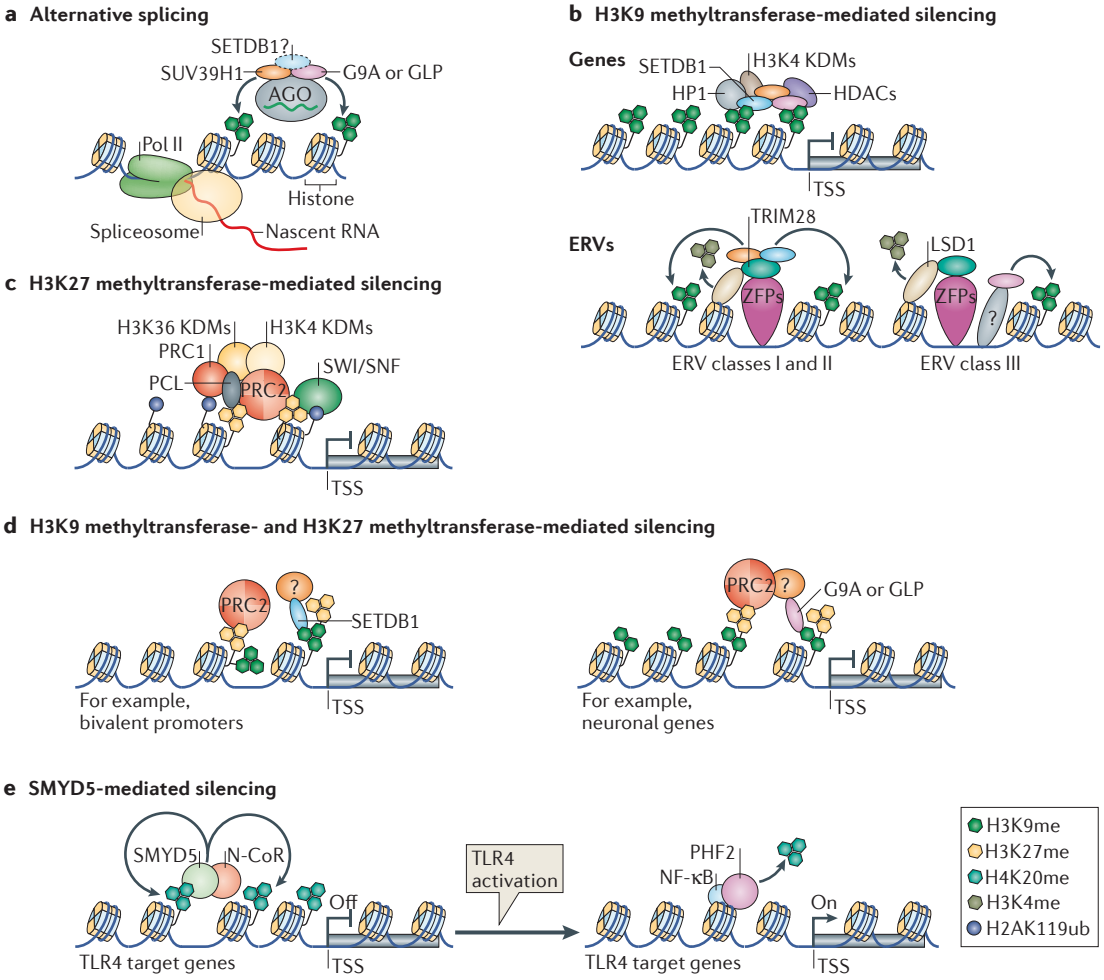


Figure 4 | **Crosstalk between repressive Lys methyltransferases and other chromatin modifiers in the regulation of gene expression.** **a** | Regulation of alternative splicing by histone H3 Lys 9 (H3K9) methyltransferases. RNA polymerase II (Pol II) elongates rapidly over constant exons (not shown); however, at alternatively spliced exons, argonaute 1 (AGO1) and AGO2 are recruited to the chromatin by non-coding RNAs and co-recruit SUV39H1, G9A and possibly SET domain bifurcated 1 (SETDB1), which leads to the deposition of H3K9 trimethylation (H3K9me3) marks and the subsequent recruitment of the H3K9me3 reader heterochromatin protein 1γ (HP1γ). As a result, a repressive chromatin environment is established, which slows down Pol II, thereby facilitating the recruitment of the spliceosome and thus the modulation of splicing. **b** | Transcription silencing by H3K9 methyltransferases. In gene silencing (top panel), H3K9 methyltransferases cooperate with each other to establish the repressive H3K9me3 mark. In addition, they are co-recruited to the chromatin, probably by interacting with HP1, together with histone deacetylases (HDACs) and H3K4 Lys demethylases (KDMs), to ensure gene silencing. Silencing of endogenous retroviruses (ERVs) is shown in the bottom panel. In mouse embryonic stem cells, zinc-finger protein (ZFP)-mediated recruitment of tripartite motif-containing protein 28 (TRIM28) results in the co-targeting of SETDB1 and the H3K4 demethylase Lys-specific demethylase 1 (LSD1) to class I and class II ERVs, which leads to the removal of the active mark H3K4me3 and the deposition of H3K9me3, and thus to gene silencing. The deposition of H3K9me3 is additionally ensured by the recruitment of SUV39H1, probably also through TRIM28. By contrast, at class III ERVs, G9A is recruited to establish H3K9me3 deposition, although the mechanism of its recruitment is not clear. **c** | Transcriptional silencing by Polycomb repressive complex 2 (PRC2). The co-recruitment of PRC2 and PRC1 results in the trimethylation of H3K27 and the ubiquitylation of H2A Lys 119 (H2AK119ub). This is accompanied by targeting of H3K36 demethylases (probably by Polycomb-like (PCL) proteins), H3K4 demethylases and the SWI/SNF chromatin remodelling complex. **d** | Transcriptional silencing by cooperation between PRC2 and H3K9 methyltransferases. PRC2 and H3K9 methyltransferases can be co-recruited to some promoters. In embryonic stem cells, SETDB1 and PRC2 are present at bivalent promoters (marked by H3K9me3, H3K27me3 and H3K4me3) (left panel); similarly, G9A, G9A-like protein (GLP) and PRC2 (and, correspondingly, the H3K9me2 and H3K27me3 marks) are present at the promoters of differentiation-promoting genes (particularly neuronal genes) (right panel). The co-recruitment of PRC2 and H3K9 methyltransferases is likely to be accompanied by targeting of additional chromatin modifiers; however, the composition of such repressive mega-complexes is still unknown. **e** | SET and MYND domain-containing protein 5 (SMYD5)-mediated silencing. A subset of Toll-like receptor 4 (TLR4)-pathway target genes in macrophages is silenced by the deposition of the H4K20me3 mark by SMYD5 in cooperation with nuclear receptor co-repressor 1 (N-CoR1) complexes. Following TLR4 pathway activation, H4K20me3 is erased by nuclear factor-κB (NF-κB)-dependent recruitment of the H4K20 demethylase PHF2.
TSS, transcription start site.

still unclear how TRIM28 specifically mediates targeting of LSD1 but not SETDB1 (or vice versa) to a particular class of ERVs. Additionally, whereas SETDB1 is required for the maintenance of silencing of exogenous retroviruses in ES cells, G9A is instead required for its initial establishment[111]. Similarly, G9A binds to ERVs in ES cells[112], and its genomic localization is modestly enriched at retroviral long terminal repeats (LTRs)[50]. Furthermore, G9A-mediated H3K9me2 is important for repressing *LINE1* non-LTR retrotransposons in the germ line, independent of DNA methylation[113].

Finally, a genome-wide study[114] revealed the existence of large and diffuse regions of H3K9me2 modification that cover up to 4.9 Mb, termed large organized chromatin K modifications (LOCKs), which are contained within larger regions of euchromatin. LOCKs are conserved between mice and humans and increase in size following cellular differentiation[114]. Although this has been disputed[115], LOCKs were suggested to lock certain chromatin domains and prevent the expression of the genes contained in those domains in differentiated cells, as many of the genes that are contained in LOCKs are developmentally regulated[114]. More than 80% of LOCKs in differentiated tissues overlap with nuclear lamin B1-associated domains (LADs)[116], the lamina attachment of which also slightly increases during differentiation[117]. As most of the genes located in LADs are transcriptionally inactive, H3K9me2 could have a role in docking LOCKs at the nuclear periphery. Accordingly, recent studies performed in *C. elegans* and in mammals causally linked H3K9 methylation and tethering of chromatin to the nuclear lamina[25,40,118], highlighting it as an evolutionarily conserved mechanism that promotes peripheral localization and silencing of chromatin. Additionally, G9A regulates chromatin contacts with the nuclear lamina in human cells[118], and most of the G9A-repressed genes, which are marked with H3K9me2 in ES cells, are localized to the nuclear periphery[119]. Together, these data suggest that the methylation of H3K9 may be an important determinant of higher-order chromosome structure and nuclear architecture, by promoting the association of transcriptionally inactive genomic regions with the nuclear periphery.

Gene silencing by PRC2. PRC2 is mainly responsible for the maintenance rather than the establishment of transcriptional silencing. Like other KMTs, PRC2 interacts with other protein complexes to repress genes through chromatin compaction[120]. Such co-repressors include H3K4 and H3K36 demethylases[72,121,122], strongly indicating that gene silencing involves the coupling of the removal of transcription activating marks and the deposition of repressive H3K27 methylation marks (FIG. 4c). The different methylation degrees of H3K27 are mutually exclusive and are found at different genomic locations. Although other KMTs were ascribed the deposition of H3K27me1 (REFS 41,123), PRC2 was reported to control all H3K27 methylation levels, with dimethylation being its main activity[46]. The H3K27me2 mark seems to have specific roles such as preventing H3K27 acetylation, which is thought to be antagonistic to PRC2-mediated silencing and is enriched in the absence of PRC2 (REF. 124). Accordingly, H3K27me2 inhibits the activity of non-cell-type-specific enhancers in different cell lineages to ensure proper cell fate decisions[46].

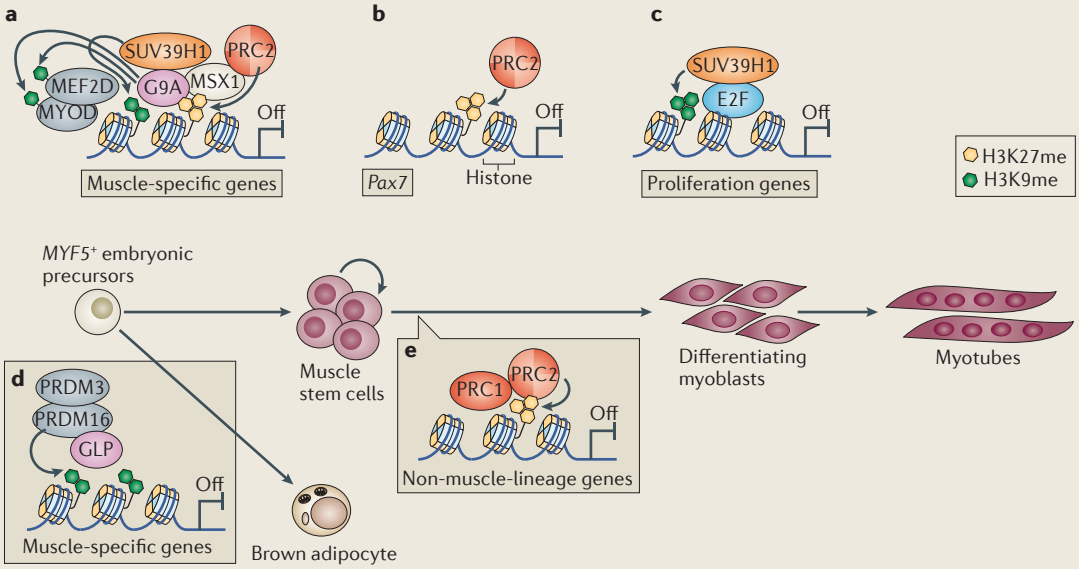
The H3K27me1 mark regulates transcription by a poorly understood mechanism. H3K27me1 tends to be associated with constitutive heterochromatin[29]; however, it is also enriched in the bodies of actively transcribed genes[46,125,126]. Whether PRC2-mediated deposition of H3K27me1 is linked to active transcription or is merely a product of demethylation of H3K27me2 and H3K27me3 (by the KDMs ubiquitously transcribed X chromosome tetratricopeptide repeat protein (also known as KDM6A) or Jumonji domain-containing 3 (JMJD3; also known as KDM6B)) is still controversial[41]. However, a role for PRC2-dependent H3K27 monomethylation in transcription activation was proposed, as SETD2-dependent deposition of H3K36me3, which is a mark present on actively transcribed regions, coexists with H3K27me1 and modulates the ratio between H3K27me1 and H3K27me2 (REF. 46). Consistent with this, EZH1, which is a putative H3K27 monomethyltransferase[47], interacts with RNA polymerase II (Pol II) and promotes transcription elongation[127].

The H3K27me3 mark correlates with gene silencing[126], and the distinct genomic locations of H3K27me3 and the activating histone mark H3K36me3 (REF. 128), together with evidence that PRC2 activity is inhibited by H3K36me3 (REF. 44), support this idea. In ES cells, promoters of developmental genes are marked by both H3K27me3 and H3K4me3 (REF. 129). Such bivalent domains poise differentiation genes for activation or stable repression following the removal, or a further gain, of H3K27me3, respectively[130,131]. Bivalent domains gain or lose H3K27me3 following differentiation of ES cells, and this correlates with the progressive restriction of differentiation potential[132]. It has been argued that bivalent domains might represent an artefact of working with heterogeneous cell populations[133], thus questioning their existence *in vivo*; however, mass spectrometry[134] and sequential chromatin immunoprecipitation experiments[135] have demonstrated that H3K4me3 and H3K27me3 can coexist on the same histone tail or genomic locus. Mechanistically, the presence of PRC2 at bivalent promoters interferes with transcription by keeping Pol II in a paused state[136].

It is now assumed that PRC2-mediated H3K27 trimethylation forms a docking site for the subsequent recruitment of PRC1, which monoubiquitylates H2AK119 (H2AK119ub1)[137], thereby promoting chromatin compaction[138] and reducing the accessibility of the DNA to transcription factors and ATP-dependent chromatin remodellers such as SWI/SNF proteins[139] (FIG. 4c). Recently, EED has been suggested as the crucial component that recruits canonical PRC1 (that is, PRC1 complexes containing the reader chromobox protein homologues (CBXs)) to H3K27 trimethylated loci, which leads to the displacement of PRC2, ubiquitylation of H2AK119 and subsequent gene silencing[140]. However, emerging evidence indicates that non-canonical PRC1

Long terminal repeats (LTRs). Repeats at both ends of retrotransposons that allow transcription and polyadenylation of retrotransposon mRNAs and are required for retroviral insertion into the genome.

Box 3 | **Roles of Lys methyltransferases in the differentiation of muscle stem cells**



Lys methyltransferases (KMTs) maintain heritable cell- and tissue-specific transcriptional programmes, thereby specifying and enforcing cell identity. As such, they have crucial roles in regulation of pluripotency and differentiation of embryonic stem cells, and are emerging as mediators of cell reprogramming (for a review, see REF. 176) and as key regulators of lineage allocation and differentiation in adult stem cells. In adult muscle stem cells and during myogenic differentiation, repressive KMTs coordinate the correct spatiotemporal silencing of muscle-specific genes and alternative transcriptional programmes (see the figure). In particular, Polycomb repressive complex 2 (PRC2), in concert with PRC1, preserves the transcriptional identity of muscle stem cells by repressing alternative transcriptional programmes[177] (see the figure, part **e**) and muscle-specific genes in undifferentiated myoblasts[178] (see the figure, part **a**) and, conversely, maintains the identity of differentiated myotubes by repressing muscle stem cell stemness genes such as paired box 7 (*Pax7*) (see the figure, part **b**)[179]. Interestingly, homeobox protein MSX1-dependent recruitment of PRC2 to muscle-specific genes (see the figure, part **a**) at the nuclear periphery was proposed to ensure their transcriptional silencing in undifferentiated myoblasts[180]. Among the histone H3 Lys 9 (H3K9) methyltransferases, SUV39H1 has been shown to mediate the permanent silencing of both proliferation-associated genes (such as E2F target genes) in differentiated myotubes (see the figure, part **c**)[30] and differentiation genes in undifferentiated myoblasts (see the figure, part **a**)[181]. More recently, G9A has been shown to directly methylate myoblast determination protein (MYOD)[182] and myocyte-specific enhancer factor 2D (MEF2D)[183] in undifferentiated, proliferating myoblasts, thereby inhibiting their activity, which is required to induce muscle gene expression (see the figure, part **a**). Furthermore, G9A directly interacts with basic helix–loop–helix family member E41 (bHLHE41; also known as SHARP1), which is a transcription factor and a potent repressor of skeletal muscle differentiation, and enhances its ability to transcriptionally repress MYOD target genes[184]. Intriguingly, MSX1 was found to mediate G9A-dependent deposition of H3K9 dimethylation marks (H3K9me2) at muscle-specific regulatory regions[185], suggesting a possible functional interplay with PRC2 in ensuring stable transcriptional repression (FIG. 3a). Moreover, in embryonic precursors of both brown adipocytes and muscle cells (*MYF5*$^+$ cells; see the figure), G9A-like protein 1 (GLP) has recently been shown to cooperate with PR domain-containing protein 16 (PRDM16)[186] and PRDM3 (REF. 187) to mediate H3K9me2 deposition and the silencing of muscle-specific genes (see the figure, part **d**), thereby favouring differentiation into brown adipose tissue. Finally, SUV420H1 was also reported to induce muscle terminal differentiation by contributing to the epigenetic repression of the myogenic inhibitor EP300-interacting inhibitor of differentiation 3 (REF. 188).

(that is, containing variant proteins other than CBXs) binding actually precedes PRC2 binding and induces its recruitment[141]. Indeed, H2AK119 ubiquitylation that is mediated by variant Polycomb group (PCG) RING finger proteins in complex with PRC1 is able to induce H3K27 trimethylation by recruiting PRC2 to unmethylated CpG islands through the PRC1 subunit KDM2B[141], which contains a zinc-finger CXXC domain that can recognize non-methylated DNA[142].

Polycomb-targeted genomic regions form nuclear foci that are enriched in PCG proteins, called PCG foci (for a review, see REF. 143), which represent discrete nuclear compartments of silenced genes. Although there is still no direct evidence in mammals that links H3K27 trimethylation to PCG foci, H3K27me3-enriched domains of about 43 kb, termed broad local enrichments (BLOCs), were found to overlap with silent genes in mouse embryonic fibroblasts[144] and in human fibroblasts and ES cells[145]. Interestingly, H3K27me3 domains in human ES cells are smaller and contain developmental and neural-specific promoters, and it was suggested that they expand during differentiation to silence specific genes[145]. However, no evidence exists so far that BLOCs form distinct nuclear compartments such as PCG foci. Beyond these genomic domains, CpG islands are preferential sites for

binding of PRC2 (REFS 146,147), and indeed H3K27me3 is mostly associated with CpG island-containing promoters[146,148] (FIG. 3b). However, binding of PRC2 at CpG islands seems to be a consequence, not a cause, of gene silencing, as experimental inhibition of transcription is sufficient to induce the ectopic recruitment of PRC2 without the requirement for additional factors[149]. This finding supports the emerging notion that the general function of PRC2 in mammals is not to initiate gene repression but to maintain gene silencing.

Finally, as mentioned above, PRC2 has been recently shown to cooperate with H3K9 KMTs in maintaining the silencing of differentiation genes in ES cells[50,150] (FIG. 4d). This evidence further emphasizes that different repressive KMTs might indeed cooperate to maintain stable transcriptional silencing.

Regulation of silencing and replication by H4K20 KMTs. Methylation at H4K20 is conserved during evolution, and the abundance of its three states varies between species, tissues and cellular states such as differentiation and cell cycle progression (reviewed in REF. 151). Methylated H4K20 is recognized by various readers, including the PCG protein L3MBTL, the DNA damage response protein tumour suppressor p53-binding protein 1 (TP53BP1), the histone demethylase JMJD2A (also known as KDM4A) and nuclear transcription factor Y (reviewed in REF. 151). The different methylation states of H4K20 have different effects on gene expression. It is difficult to establish whether H4K20me1 is a stable mark or an intermediate substrate that precedes H4K20me2 and H4K20me3. Nevertheless, H4K20me1 is associated both with active transcription, by regulating H4K16 acetylation and release of Pol II promoter-proximal pausing[152], and with silencing of genomic regions, including the inactive X chromosome[153–155]. The role of H4K20me2 in regulation of transcription is not well understood, but its co-localization with H4K20me3, which is mainly linked to gene silencing[156,157] and heterochromatin formation, suggests that it has a role in transcription repression. In agreement with this, knockout or inhibition of SUV420H, which catalyses the di- and trimethylation of H4K20, was shown to induce derepression of genes[58,158].

H4K20me3 is enriched at constitutive heterochromatin[52], telomeres[159] and centromeres[160], and also mediates gene silencing at euchromatic regions. In particular, SMYD5-mediated deposition of H4K20me3 was linked to the silencing of a subset of target genes of the Toll-like receptor 4 (TLR4) pathway in macrophages, mediated by the association of SMYD5 with nuclear receptor co-repressor 1 complexes. Following activation of the TLR4 pathway, H4K20me3 is erased at the promoters of these genes by nuclear factor-κB-dependent recruitment of the H4K20 demethylase PHF2 (REF. 56) (FIG. 4e).

Interestingly, H4K20 methylation is strongly associated with DNA replication. The H4K20 monomethyltransferase PR-SET7 regulates cell cycle progression[161], and its expression increases during S phase[162], in which it associates with chromatin. Ectopic targeting of PR-SET7 induces H4K20 monomethylation and promotes the formation of pre-replication complexes, indicating that H4K20 monomethylation has a role in defining the localization of origins of replication[161].

Conclusion

The involvement of KMTs in the regulation of gene expression, genome integrity, control of stem cell plasticity and differentiation (BOX 3) highlights their key roles in the maintenance of tissue homeostasis. Thus, it is not surprising that an increasing number of reports indicate that dysregulation of KMTs and their associated epigenetic modifications and effectors is often linked to pathological conditions such as malignancies and neurological diseases (see Supplementary information S3 (box)). Given the reversible nature of epigenetic signatures, there is an increasing interest in developing pharmacological approaches to target KMTs and KDMs with the aim of reversing pathological epigenetic states. Several inhibitors are currently being tested in preclinical studies. However, it is currently unknown whether the pathological effects that are associated with the aberrant action of KMTs are mediated only through the methylation of histones or also through the methylation of non-histone substrates. A better understanding of the biochemical features of KMTs and their mechanisms of action and biological roles should be instrumental in devising potent epigenetics-based therapies.

1. Greer, E. L. & Shi, Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat. Rev. Genet.* **13**, 343–357 (2012).
2. Clarke, S. G. Protein methylation at the surface and buried deep: thinking outside the histone box. *Trends Biochem. Sci.* **38**, 243–252 (2013).
3. Hojfeldt, J. W., Agger, K. & Helin, K. Histone lysine demethylases as targets for anticancer therapy. *Nat. Rev. Drug Discov.* **12**, 917–930 (2013).
4. Badeaux, A. I. & Shi, Y. Emerging roles for chromatin as a signal integration and storage platform. *Nat. Rev. Mol. Cell Biol.* **14**, 211–224 (2013).
5. Jenuwein, T., Laible, G., Dorn, R. & Reuter, G. SET domain proteins modulate chromatin domains in eu- and heterochromatin. *Cell. Mol. Life Sci.* **54**, 80–93 (1998).
6. Völkel, P. & Angrand, P.-O. The control of histone lysine methylation in epigenetic regulation. *Biochimie* **89**, 1–20 (2007).
7. Herz, H.-M., Garruss, A. & Shilatifard, A. SET for life: biochemical activities and biological functions of SET domain-containing proteins. *Trends Biochem. Sci.* **38**, 621–639 (2013).
8. Ruthenburg, A. J., Li, H., Patel, D. J. & Allis, C. D. Multivalent engagement of chromatin modifications by linked binding modules. *Nat. Rev. Mol. Cell Biol.* **8**, 983–994 (2007).
9. Rea, S. *et al.* Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* **406**, 593–599 (2000).
10. Schultz, D. C., Ayyanathan, K., Negorev, D., Maul, G. G. & Rauscher, F. J. SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. *Genes Dev.* **16**, 919–932 (2002).
11. Falandry, C. *et al.* CLLD8/KMT1F is a lysine methyltransferase that is important for chromosome segregation. *J. Biol. Chem.* **285**, 20234–20241 (2010).
12. Ivanova, A. V., Bonaduce, M. J., Ivanov, S. V. & Klar, A. J. The chromo and SET domains of the Clr4 protein are essential for silencing in fission yeast. *Nat. Genet.* **19**, 192–195 (1998).
13. Melcher, M. *et al.* Structure-function analysis of SUV39H1 reveals a dominant role in heterochromatin organization, chromosome segregation, and mitotic progression. *Mol. Cell. Biol.* **20**, 3728–3741 (2000).
14. Collins, R. E. *et al.* The ankyrin repeats of G9a and GLP histone methyltransferases are mono- and dimethyllysine binding modules. *Nat. Struct. Mol. Biol.* **15**, 245–250 (2008).
15. Liu, N. *et al.* Recognition of H3K9 methylation by GLP is required for efficient establishment of H3K9 methylation, rapid target gene repression, and mouse viability. *Genes Dev.* **29**, 379–393 (2015).
16. Loyola, A., Bonaldi, T., Roche, D., Imhof, A. & Almouzni, G. PTMs on H3 variants before chromatin assembly potentiate their final epigenetic state. *Mol. Cell* **24**, 309–316 (2006).
17. Yang, L. *et al.* Molecular cloning of ESET, a novel histone H3-specific methyltransferase that interacts with ERG transcription factor. *Oncogene* **21**, 148–152 (2002).
18. Wang, H. *et al.* mAM facilitates conversion by ESET of dimethyl to trimethyl lysine 9 of histone H3 to cause transcriptional repression. *Mol. Cell* **12**, 475–487 (2003).

19. Tachibana, M., Sugimoto, K., Fukushima, T. & Shinkai, Y. Set domain-containing protein, G9a, is a novel lysine-preferring mammalian histone methyltransferase with hyperactivity and specific selectivity to lysines 9 and 27 of histone H3. *J. Biol. Chem.* **276**, 25309–25317 (2001).
20. Ogawa, H., Ishiguro, K., Gaubatz, S., Livingston, D. M. & Nakatani, Y. A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G_0 cells. *Science* **296**, 1132–1136 (2002).
21. Collins, R. E. *et al. In vitro* and *in vivo* analyses of a Phe/Tyr switch controlling product specificity of histone lysine methyltransferases. *J. Biol. Chem.* **280**, 5563–5570 (2005).
22. Kubicek, S. *et al.* Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. *Mol. Cell* **25**, 473–481 (2007).
23. Loyola, A. *et al.* The HP1α–CAF1–SetDB1-containing complex provides H3K9me1 for Suv39-mediated K9me3 in pericentric heterochromatin. *EMBO Rep.* **10**, 769–775 (2009).
24. Fritsch, L. *et al.* A subset of the histone H3 lysine 9 methyltransferases Suv39h1, G9a, GLP, and SETDB1 participate in a multimeric complex. *Mol. Cell* **37**, 46–56 (2010).
This paper provides the first evidence that the main H3K9 KMTs can be part of protein complexes and that they functionally cooperate to regulate gene silencing.
25. Pinheiro, I. *et al.* Prdm3 and Prdm16 are H3K9me1 methyltransferases required for mammalian heterochromatin integrity. *Cell* **150**, 948–960 (2012).
This work identifies PRDM3 and PRDM16 as KMTs that establish H3K9me1 and initiate the formation of mammalian heterochromatin.
26. O'Carroll, D. *et al.* Isolation and characterization of *Suv39h2*, a second histone H3 methyltransferase gene that displays testis-specific expression. *Mol. Cell. Biol.* **20**, 9423–9433 (2000).
27. Peters, A. H. F. M. *et al.* Loss of the *Suv39h* histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell* **107**, 323–337 (2001).
This paper describes the severe phenotype of *Suv39h*-knockout mice, highlighting the crucial role of SUV39H1 in the regulation of genome stability.
28. García-Cao, M., O'Sullivan, R., Peters, A. H. F. M., Jenuwein, T. & Blasco, M. A. Epigenetic regulation of telomere length in mammalian cells by the Suv39h1 and Suv39h2 histone methyltransferases. *Nat. Genet.* **36**, 94–99 (2004).
29. Peters, A. H. *et al.* Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. *Mol. Cell* **12**, 1577–1589 (2003).
30. Ait-Si-Ali, S. *et al.* A Suv39h-dependent mechanism for silencing S-phase genes in differentiating but not in cycling cells. *EMBO J.* **23**, 605–615 (2004).
31. Allan, R. S. *et al.* An epigenetic silencing pathway controlling T helper 2 cell lineage commitment. *Nature* **487**, 249–253 (2012).
32. Tachibana, M. *et al.* Histone methyltransferases G9a and GLP form heteromeric complexes and are both crucial for methylation of euchromatin at H3-K9. *Genes Dev.* **19**, 815–826 (2005).
This work provides evidence that G9A and GLP function mainly as heterodimers to establish mono- and dimethylation of H3K9 at euchromatic regions.
33. Tachibana, M. *et al.* G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes Dev.* **16**, 1779–1791 (2002).
34. Rice, J. C. *et al.* Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. *Mol. Cell* **12**, 1591–1598 (2003).
35. Epsztejn-Litman, S. *et al. De novo* DNA methylation promoted by G9a prevents reprogramming of embryonically silenced genes. *Nat. Struct. Mol. Biol.* **15**, 1176–1183 (2008).
36. Trojer, P. *et al.* Dynamic histone H1 isotype 4 methylation and demethylation by histone lysine methyltransferase G9a/KMT1C and the Jumonji domain-containing JMJD2/KDM4 proteins. *J. Biol. Chem.* **284**, 8395–8405 (2009).
37. Yu, Y. *et al.* Histone H3 lysine 56 methylation regulates DNA replication through its interaction with PCNA. *Mol. Cell* **46**, 7–17 (2012).
38. Fumasoni, I. *et al.* Family expansion and gene rearrangements contributed to the functional specialization of PRDM genes in vertebrates. *BMC Evol. Biol.* **7**, 187 (2007).
39. Hohenauer, T. & Moore, A. W. The Prdm family: expanding roles in stem cells and development. *Development* **139**, 2267–2282 (2012).
40. Towbin, B. D. *et al.* Step-wise methylation of histone H3K9 positions heterochromatin at the nuclear periphery. *Cell* **150**, 934–947 (2012).
This elegant paper demonstrates that H3K9 KMTs mediate the anchoring and repression of heterochromatin at the nuclear periphery.
41. Margueron, R. & Reinberg, D. The Polycomb complex PRC2 and its mark in life. *Nature* **469**, 343–349 (2011).
42. Ciferri, C. *et al.* Molecular architecture of human polycomb repressive complex 2. *eLife* **1**, e00005 (2012).
This is the first three-dimensional electron microscopy structure of the human PRC2 complex, which provided key information on the interactions between the core components of the complex.
43. Margueron, R. *et al.* Role of the polycomb protein EED in the propagation of repressive histone marks. *Nature* **461**, 762–767 (2009).
44. Schmitges, F. W. *et al.* Histone methylation by PRC2 is inhibited by active chromatin marks. *Mol. Cell* **42**, 330–341 (2011).
45. Chamberlain, S. J., Yee, D. & Magnuson, T. Polycomb repressive complex 2 is dispensable for maintenance of embryonic stem cell pluripotency. *Stem Cells* **26**, 1496–1505 (2008).
46. Ferrari, K. J. *et al.* Polycomb-dependent H3K27me1 and H3K27me2 regulate active transcription and enhancer fidelity. *Mol. Cell* **53**, 49–62 (2014).
47. Shen, X. *et al.* EZH1 mediates methylation on histone H3 lysine 27 and complements EZH2 in maintaining stem cell identity and executing pluripotency. *Mol. Cell* **32**, 491–502 (2008).
48. Margueron, R. *et al.* Ezh1 and Ezh2 maintain repressive chromatin through different mechanisms. *Mol. Cell* **32**, 503–518 (2008).
49. Wu, H. *et al.* Histone methyltransferase G9a contributes to H3K27 methylation *in vivo*. *Cell Res.* **21**, 365–367 (2011).
50. Mozzetta, C. *et al.* The histone H3 lysine 9 methyltransferases G9a and GLP regulate polycomb repressive complex 2-mediated gene silencing. *Mol. Cell* **53**, 277–289 (2014).
This paper demonstrates that G9A and GLP interact and functionally cooperate with PRC2 to maintain the silencing of developmental genes.
51. Nishioka, K. *et al.* PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. *Mol. Cell* **9**, 1201–1213 (2002).
52. Schotta, G. *et al.* A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. *Genes Dev.* **18**, 1251–1262 (2004).
53. Pannetier, M. *et al.* PR-SET7 and SUV4-20H regulate H4 lysine-20 methylation at imprinting control regions in the mouse. *EMBO Rep.* **9**, 998–1005 (2008).
54. Huang, N. *et al.* Two distinct nuclear receptor interaction domains in NSD1, a novel SET protein that exhibits characteristics of both corepressors and coactivators. *EMBO J.* **17**, 3398–3412 (1998).
55. Beisel, C., Imhof, A., Greene, J., Kremmer, E. & Sauer, F. Histone methylation by the *Drosophila* epigenetic transcriptional regulator Ash1. *Nature* **419**, 857–862 (2002).
56. Stender, J. D. *et al.* Control of proinflammatory gene programs by regulated trimethylation and demethylation of histone H4K20. *Mol. Cell* **48**, 28–38 (2012).
57. Oda, H. *et al.* Monomethylation of histone H4-lysine 20 is involved in chromosome structure and stability and is essential for mouse development. *Mol. Cell. Biol.* **29**, 2278–2295 (2009).
58. Schotta, G. *et al.* A chromatin-wide transition to H4K20 monomethylation impairs genome integrity and programmed DNA rearrangements in the mouse. *Genes Dev.* **22**, 2048–2061 (2008).
59. Mozzetta, C., Pontis, J. & Ait-Si-Ali, S. Functional crosstalk between lysine methyltransferases on histone substrates: the case of G9A/GLP and polycomb repressive complex 2. *Antioxid. Redox Signal.* **22**, 1365–1381 (2015).
60. Maier, V. K. *et al.* Functional proteomic analysis of repressive histone methyltransferase complexes PRC2 and G9A reveals ZNF518B as a G9A regulator. *Mol. Cell. Proteomics* http://dx.doi.org/10.1074/mcp.M114.044586 (2015).
61. Bian, C., Chen, Q. & Yu, X. The zinc finger proteins ZNF644 and WIZ regulate the G9a/GLP complex for gene repression. *eLife* **4**, e05606 (2015).
62. Yuan, P. *et al.* Eset partners with Oct4 to restrict extraembryonic trophoblast lineage potential in embryonic stem cells. *Genes Dev.* **23**, 2507–2520 (2009).
This work describes how transcriptional repression at pericentromeric repeats is mediated by transcription factors that are essential for the integrity of heterochromatin.
63. Frietze, S., O'Geen, H., Blahnik, K. R., Jin, V. X. & Farnham, P. J. ZNF274 recruits the histone methyltransferase SETDB1 to the 3′ ends of ZNF genes. *PLoS ONE* **5**, e15082 (2010).
64. Reed-Inderbitzin, E. *et al.* RUNX1 associates with histone deacetylases and SUV39H1 to repress transcription. *Oncogene* **25**, 5777–5786 (2006).
65. Chakraborty, S., Sinha, K. K., Senyuk, V. & Nucifora, G. SUV39H1 interacts with AML1 and abrogates AML1 transactivity. AML1 is methylated *in vivo*. *Oncogene* **22**, 5229–5237 (2003).
66. Nielsen, S. J. *et al.* Rb targets histone H3 methylation and HP1 to promoters. *Nature* **412**, 561–565 (2001).
67. Bulut-Karslioglu, A. *et al.* A transcription factor-based mechanism for mouse heterochromatin formation. *Nat. Struct. Mol. Biol.* **19**, 1023–1030 (2012).
68. Peng, J. C. *et al.* Jarid2/Jumonji coordinates control of PRC2 enzymatic activity and target gene occupancy in pluripotent cells. *Cell* **139**, 1290–1302 (2009).
69. Pasini, D. *et al.* JARID2 regulates binding of the Polycomb repressive complex 2 to target genes in ES cells. *Nature* **464**, 306–310 (2010).
70. Fischle, W., Franz, H., Jacobs, S. A., Allis, C. D. & Khorasanizadeh, S. Specificity of the chromodomain Y chromosome family of chromodomains for lysine-methylated ARK(S/T) motifs. *J. Biol. Chem.* **283**, 19626–19635 (2008).
71. Ballare, C. *et al.* Phf19 links methylated Lys36 of histone H3 to regulation of Polycomb activity. *Nat. Struct. Mol. Biol.* **19**, 1257–1265 (2012).
72. Brien, G. L. *et al.* Polycomb PHF19 binds H3K36me3 and recruits PRC2 and demethylase NO66 to embryonic stem cell genes during differentiation. *Nat. Struct. Mol. Biol.* **19**, 1273–1281 (2012).
73. Kim, H., Kang, K. & Kim, J. AEBP2 as a potential targeting protein for Polycomb repression complex PRC2. *Nucleic Acids Res.* **37**, 2940–2950 (2009).
74. Fujita, N. *et al.* Methyl-CpG binding domain 1 (MBD1) interacts with the Suv39h1-HP1 heterochromatic complex for DNA methylation-based transcriptional repression. *J. Biol. Chem.* **278**, 24132–24138 (2003).
75. Yang, L. *et al.* An ERG (ets-related gene)-associated histone methyltransferase interacts with histone deacetylases 1/2 and transcription co-repressors mSin3A/B. *Biochem. J.* **369**, 651–657 (2003).
76. Li, H. *et al.* The histone methyltransferase SETDB1 and the DNA methyltransferase DNMT3A interact directly and localize to promoters silenced in cancer cells. *J. Biol. Chem.* **281**, 19489–19500 (2006).
77. Lehnertz, B. *et al.* Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. *Curr. Biol.* **13**, 1192–1200 (2003).
78. Murayama, A. *et al.* Epigenetic control of rDNA loci in response to intracellular energy status. *Cell* **133**, 627–639 (2008).
79. Sampath, S. C. *et al.* Methylation of a histone mimic within the histone methyltransferase G9a regulates protein complex assembly. *Mol. Cell* **27**, 596–608 (2007).
This work demonstrates how G9A automethylation as well as phosphorylation modulate its interaction with HP1 *in vivo*.
80. Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. & Jenuwein, T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* **410**, 116–120 (2001).
81. Maison, C. *et al.* SUMOylation promotes *de novo* targeting of HP1α to pericentric heterochromatin. *Nat. Genet.* **43**, 220–227 (2011).
82. Muller-Ott, K. *et al.* Specificity, propagation, and memory of pericentric heterochromatin. *Mol. Syst. Biol.* **10**, 746 (2014).
83. Romeo, K. *et al.* The SENP7 SUMO-protease presents a module of two HP1 interaction motifs that locks HP1 protein at pericentric heterochromatin. *Cell Rep.* **10**, 771–782 (2015).
84. Canzio, D. *et al.* A conformational switch in HP1 releases auto-inhibition to drive heterochromatin assembly. *Nature* **496**, 377–381 (2013).

85. Canzio, D. *et al.* Chromodomain-mediated oligomerization of HP1 suggests a nucleosome-bridging mechanism for heterochromatin assembly. *Mol. Cell* **41**, 67–81 (2011).
86. Zhao, J., Sun, B. K., Erwin, J. A., Song, J. J. & Lee, J. T. Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. *Science* **322**, 750–756 (2008).
87. McHugh, C. A. *et al.* The *Xist* lncRNA interacts directly with SHARP to silence transcription through HDAC3. *Nature* **521**, 232–236 (2015).
88. Rinn, J. L. *et al.* Functional demarcation of active and silent chromatin domains in human *HOX* loci by noncoding RNAs. *Cell* **129**, 1311–1323 (2007).
89. Nagano, T. *et al.* The *Air* noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. *Science* **322**, 1717–1720 (2008).
90. Pandey, R. R. *et al. Kcnq1ot1* antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. *Mol. Cell* **32**, 232–246 (2008).
91. Li, Q. *et al.* AS1DHRS4, a head-to-head natural antisense transcript, silences the *DHRS4* gene cluster in *cis* and *trans*. *Proc. Natl Acad. Sci. USA* **109**, 14110–14115 (2012).
92. Cho, S., Park, J. S. & Kang, Y.-K. AGO2 and SETDB1 cooperate in promoter-targeted transcriptional silencing of the androgen receptor gene. *Nucleic Acids Res.* **42**, 13545–13556 (2014).
93. Ameyar-Zazoua, M. *et al.* Argonaute proteins couple chromatin silencing to alternative splicing. *Nat. Struct. Mol. Biol.* **19**, 998–1004 (2012).
94. Skourti-Stathaki, K., Kamieniarz-Gdula, K. & Proudfoot, N. J. R-loops induce repressive chromatin marks over mammalian gene terminators. *Nature* **516**, 436–439 (2014).
This work shows that there is functional crosstalk between G9A and proteins of the RNA interference machinery to mediate termination of transcription.
95. Bierhoff, H. *et al.* Quiescence-induced LncRNAs trigger H4K20 trimethylation and transcriptional silencing. *Mol. Cell* **54**, 675–682 (2014).
96. Esteve, P. O. *et al.* Direct interaction between DNMT1 and G9a coordinates DNA and histone methylation during replication. *Genes Dev.* **20**, 3089–3103 (2006).
97. Czermin, B. *et al.* Physical and functional association of SU(VAR)3-9 and HDAC1 in *Drosophila*. *EMBO Rep.* **2**, 915–919 (2001).
98. Chaturvedi, C. P. *et al.* Maintenance of gene silencing by the coordinate action of the H3K9 methyltransferase G9a/KMT1C and the H3K4 demethylase Jarid1a/KDM5A. *Proc. Natl Acad. Sci. USA* **109**, 18845–18850 (2012).
99. Fang, R. *et al.* Human LSD2/KDM1b/AOF1 regulates gene transcription by modulating intragenic H3K4me2 methylation. *Mol. Cell* **39**, 222–233 (2010).
100. Tahiliani, M. *et al.* The histone H3K4 demethylase SMCX links REST target genes to X-linked mental retardation. *Nature* **447**, 601–605 (2007).
101. Binda, O. *et al.* Trimethylation of histone H3 lysine 4 impairs methylation of histone H3 lysine 9: regulation of lysine methyltransferases by physical interaction with their substrates. *Epigenetics* **5**, 767–775 (2010).
102. Daujat, S., Zeissler, U., Waldmann, T., Happel, N. & Schneider, R. HP1 binds specifically to Lys26-methylated histone H1.4, whereas simultaneous Ser27 phosphorylation blocks HP1 binding. *J. Biol. Chem.* **280**, 38090–38095 (2005).
103. Happel, N. & Doenecke, D. Histone H1 and its isoforms: contribution to chromatin structure and function. *Gene* **431**, 1–12 (2009).
104. Hwang, Y. J. *et al.* ESET methylates UBF at K232/254 and regulates nucleolar heterochromatin plasticity and rDNA transcription. *Nucleic Acids Res.* **42**, 1628–1643 (2014).
105. Leung, D. C. & Lorincz, M. C. Silencing of endogenous retroviruses: when and why do histone marks predominate? *Trends Biochem. Sci.* **37**, 127–133 (2012).
106. Matsui, T. *et al.* Proviral silencing in embryonic stem cells requires the histone methyltransferase ESET. *Nature* **464**, 927–931 (2010).
107. Liu, S. *et al. Setdb1* is required for germline development and silencing of H3K9me3-marked endogenous retroviruses in primordial germ cells. *Genes Dev.* **28**, 2041–2055 (2014).
108. Bulut-Karslioglu, A. *et al.* Suv39h-dependent H3K9me3 marks intact retrotransposons and silences LINE elements in mouse embryonic stem cells. *Mol. Cell* **55**, 277–290 (2014).
109. Macfarlan, T. S. *et al.* Endogenous retroviruses and neighboring genes are coordinately repressed by LSD1/KDM1A. *Genes Dev.* **25**, 594–607 (2011).
110. Rowe, H. M. *et al. De novo* DNA methylation of endogenous retroviruses is shaped by KRAB-ZFPs/KAP1 and ESET. *Development* **140**, 519–529 (2013).
111. Leung, D. C. *et al.* Lysine methyltransferase G9a is required for *de novo* DNA methylation and the establishment, but not the maintenance, of proviral silencing. *Proc. Natl Acad. Sci. USA* **108**, 5718–5723 (2011).
112. Maksakova, I. A. *et al.* Distinct roles of KAP1, HP1 and G9a/GLP in silencing of the two-cell-specific retrotransposon MERVL in mouse ES cells. *Epigenetics Chromatin* **6**, 15 (2013).
113. Di Giacomo, M., Comazzetto, S., Sampath, S. C., Sampath, S. C. & O'Carroll, D. G9a co-suppresses LINE1 elements in spermatogonia. *Epigenetics Chromatin* **7**, 24 (2014).
114. Wen, B., Wu, H., Shinkai, Y., Irizarry, R. A. & Feinberg, A. P. Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. *Nat. Genet.* **41**, 246–250 (2009).
115. Filion, G. J. & van Steensel, B. Reassessing the abundance of H3K9me2 chromatin domains in embryonic stem cells. *Nat. Genet.* **42**, 4 (2010).
116. Guelen, L. *et al.* Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. *Nature* **453**, 948–951 (2008).
117. Peric-Hupkes, D. *et al.* Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. *Mol. Cell* **38**, 603–613 (2010).
118. Kind, J. *et al.* Single-cell dynamics of genome-nuclear lamina interactions. *Cell* **153**, 178–192 (2013).
In this work, G9A is identified as a regulator of chromatin anchoring to the nuclear lamina in mammalian cells.
119. Yokochi, T. *et al.* G9a selectively represses a class of late-replicating genes at the nuclear periphery. *Proc. Natl Acad. Sci. USA* **106**, 19363–19368 (2009).
120. Di Croce, L. & Helin, K. Transcriptional regulation by Polycomb group proteins. *Nat. Struct. Mol. Biol.* **20**, 1147–1155 (2013).
121. Pasini, D. *et al.* Coordinated regulation of transcriptional repression by the RBP2 H3K4 demethylase and Polycomb-repressive complex 2. *Genes Dev.* **22**, 1345–1355 (2008).
122. Cai, L. *et al.* An H3K36 methylation-engaging Tudor motif of polycomb-like proteins mediates PRC2 complex targeting. *Mol. Cell* **49**, 571–582 (2013).
123. Xu, Y. *et al.* Genome-wide regulation of 5hmC, 5mC, and gene expression by Tet1 hydroxylase in mouse embryonic stem cells. *Mol. Cell* **42**, 451–464 (2011).
124. Tie, F. *et al.* CBP-mediated acetylation of histone H3 lysine 27 antagonizes *Drosophila* Polycomb silencing. *Development* **136**, 3131–3141 (2009).
125. Cui, K. *et al.* Chromatin signatures in multipotent human hematopoietic stem cells indicate the fate of bivalent genes during differentiation. *Cell Stem Cell* **4**, 80–93 (2009).
126. Barski, A. *et al.* High-resolution profiling of histone methylations in the human genome. *Cell* **129**, 823–837 (2007).
127. Mousavi, K., Zare, H., Wang, A. H. & Sartorelli, V. Polycomb protein Ezh1 promotes RNA polymerase II elongation. *Mol. Cell* **45**, 255–262 (2012).
128. Mikkelsen, T. S. *et al.* Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* **448**, 553–560 (2007).
129. Bernstein, B. E. *et al.* A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315–326 (2006).
130. Boyer, L. A. *et al.* Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* **441**, 349–353 (2006).
References 129 and 130 show that, in ES cells, genes encoding developmental regulators are poised for expression by H3K4me3 and H3K27me3 marks at their promoters.
131. Lee, T. I. *et al.* Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell* **125**, 301–313 (2006).
132. Mohn, F. *et al.* Lineage-specific polycomb targets and *de novo* DNA methylation define restriction and potential of neuronal progenitors. *Mol. Cell* **30**, 755–766 (2008).
133. Marks, H. *et al.* The transcriptional and epigenomic foundations of ground state pluripotency. *Cell* **149**, 590–604 (2012).
134. Voigt, P. *et al.* Asymmetrically modified nucleosomes. *Cell* **151**, 181–193 (2012).
This work proves that activating and repressing marks can coexist on the same nucleosome but on opposite histone tails, in so-called asymmetrically modified nucleosomes.
135. Alder, O. *et al.* Ring1B and Suv39h1 delineate distinct chromatin states at bivalent genes during early mouse lineage commitment. *Development* **137**, 2483–2492 (2010).
136. Brookes, E. *et al.* Polycomb associates genome-wide with a specific RNA polymerase II variant, and regulates metabolic genes in ESCs. *Cell Stem Cell* **10**, 157–170 (2012).
137. Sarma, K., Margueron, R., Ivanov, A., Pirrotta, V. & Reinberg, D. Ezh2 requires PHF1 to efficiently catalyze H3 lysine 27 trimethylation *in vivo*. *Mol. Cell. Biol.* **28**, 2718–2731 (2008).
138. Eskeland, R. *et al.* Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination. *Mol. Cell* **38**, 452–464 (2010).
139. Bantignies, F. & Cavalli, G. Polycomb group proteins: repression in 3D. *Trends Genet.* **27**, 454–464 (2011).
140. Cao, Q. *et al.* The central role of EED in the orchestration of polycomb group complexes. *Nat. Commun.* **5**, 3127 (2014).
141. Blackledge, N. P. *et al.* Variant PRC1 complex-dependent H2A ubiquitylation drives PRC2 recruitment and polycomb domain formation. *Cell* **157**, 1445–1459 (2014).
This work challenges the notion that PRC2 precedes PRC1 in chromatin targeting and compaction, showing instead that non-canonical PRC1-dependent H2AK119 ubiquitylation leads to PRC2 recruitment and H3K27me3 deposition.
142. Farcas, A. M. *et al.* KDM2B links the Polycomb repressive complex 1 (PRC1) to recognition of CpG islands. *eLife* **1**, e00205 (2012).
143. Cheutin, T. & Cavalli, G. Polycomb silencing: from linear chromatin domains to 3D chromosome folding. *Curr. Opin. Genet. Dev.* **25**, 30–37 (2014).
144. Pauler, F. M. *et al.* H3K27me3 forms BLOCs over silent genes and intergenic regions and specifies a histone banding pattern on a mouse autosomal chromosome. *Genome Res.* **19**, 221–233 (2009).
145. Hawkins, R. D. *et al.* Distinct epigenomic landscapes of pluripotent and lineage-committed human cells. *Cell Stem Cell* **6**, 479–491 (2010).
146. Mendenhall, E. M. *et al.* GC-rich sequence elements recruit PRC2 in mammalian ES cells. *PLoS Genet.* **6**, e1001244 (2010).
147. Lynch, M. D. *et al.* An interspecies analysis reveals a key role for unmethylated CpG dinucleotides in vertebrate Polycomb complex recruitment. *EMBO J.* **31**, 317–329 (2012).
148. Ku, M. *et al.* Genomewide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains. *PLoS Genet.* **4**, e1000242 (2008).
149. Riising, E. M. *et al.* Gene silencing triggers polycomb repressive complex 2 recruitment to CpG islands genome wide. *Mol. Cell* **55**, 347–360 (2014).
150. Bilodeau, S., Kagey, M. H., Frampton, G. M., Rahl, P. B. & Young, R. A. SetDB1 contributes to repression of genes encoding developmental regulators and maintenance of ES cell state. *Genes Dev.* **23**, 2484–2489 (2009).
151. Balakrishnan, L. & Milavetz, B. Decoding the histone H4 lysine 20 methylation mark. *Crit. Rev. Biochem. Mol. Biol.* **45**, 440–452 (2010).
152. Kapoor-Vazirani, P. & Vertino, P. M. A dual role for the histone methyltransferase PR-SET7/SETD8 and histone H4 lysine 20 monomethylation in the local regulation of RNA polymerase II pausing. *J. Biol. Chem.* **289**, 7425–7437 (2014).
153. Karachentsev, D., Sarma, K., Reinberg, D. & Steward, R. PR-Set7-dependent methylation of histone H4 Lys 20 functions in repression of gene expression and is essential for mitosis. *Genes Dev.* **19**, 431–435 (2005).
154. Kalakonda, N. *et al.* Histone H4 lysine 20 monomethylation promotes transcriptional repression by L3MBTL1. *Oncogene* **27**, 4293–4304 (2008).
155. Kohlmaier, A. *et al.* A chromosomal memory triggered by *Xist* regulates histone methylation in X inactivation. *PLoS Biol.* **2**, E171 (2004).

156. Regha, K. *et al.* Active and repressive chromatin are interspersed without spreading in an imprinted gene cluster in the mammalian genome. *Mol. Cell* **27**, 353–366 (2007).
157. Henckel, A. *et al.* Histone methylation is mechanistically linked to DNA methylation at imprinting control regions in mammals. *Hum. Mol. Genet.* **18**, 3375–3383 (2009).
158. Miranda, T. B. *et al.* DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation. *Mol. Cancer Ther.* **8**, 1579–1588 (2009).
159. Benetti, R., Garcia-Cao, M. & Blasco, M. A. Telomere length regulates the epigenetic status of mammalian telomeres and subtelomeres. *Nat. Genet.* **39**, 243–250 (2007).
160. Probst, A. V., Santos, F., Reik, W., Almouzni, G. & Dean, W. Structural differences in centromeric heterochromatin are spatially reconciled on fertilisation in the mouse zygote. *Chromosoma* **116**, 403–415 (2007).
161. Tardat, M. *et al.* The histone H4 Lys 20 methyltransferase PR-Set7 regulates replication origins in mammalian cells. *Nat. Cell Biol.* **12**, 1086–1093 (2010).
162. Rice, J. C. *et al.* Mitotic-specific methylation of histone H4 Lys 20 follows increased PR-Set7 expression and its localization to mitotic chromosomes. *Genes Dev.* **16**, 2225–2230 (2002).
163. Eissenberg, J. C. & Shilatifard, A. Histone H3 lysine 4 (H3K4) methylation in development and differentiation. *Dev. Biol.* **339**, 240–249 (2010).
164. Wagner, E. J. & Carpenter, P. B. Understanding the language of Lys36 methylation at histone H3. *Nat. Rev. Mol. Cell Biol.* **13**, 115–126 (2012).
165. Shilatifard, A. The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis. *Annu. Rev. Biochem.* **81**, 65–95 (2012).
166. Hu, G. *et al.* H2A.Z facilitates access of active and repressive complexes to chromatin in embryonic stem cell self-renewal and differentiation. *Cell Stem Cell* **12**, 180–192 (2013).
167. Vermeulen, M. *et al.* Selective anchoring of TFIID to nucleosomes by trimethylation of histone H3 lysine 4. *Cell* **131**, 58–69 (2007).
168. Wysocka, J. *et al.* A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature* **442**, 86–90 (2006).
169. Sun, X.-J. *et al.* Identification and characterization of a novel human histone H3 lysine 36-specific methyltransferase. *J. Biol. Chem.* **280**, 35261–35271 (2005).
170. Li, Y. *et al.* The target of the NSD family of histone lysine methyltransferases depends on the nature of the substrate. *J. Biol. Chem.* **284**, 34283–34295 (2009).
171. Rayasam, G. V. *et al.* NSD1 is essential for early post-implantation development and has a catalytically active SET domain. *EMBO J.* **22**, 3153–3163 (2003).
172. Nguyen, A. T. & Zhang, Y. The diverse functions of Dot1 and H3K79 methylation. *Genes Dev.* **25**, 1345–1358 (2011).
173. Min, J., Feng, Q., Li, Z., Zhang, Y. & Xu, R. M. Structure of the catalytic domain of human DOT1L, a non-SET domain nucleosomal histone methyltransferase. *Cell* **112**, 711–723 (2003).
174. Frederiks, F. *et al.* Nonprocessive methylation by Dot1 leads to functional redundancy of histone H3K79 methylation states. *Nat. Struct. Mol. Biol.* **15**, 550–557 (2008).
175. Jones, B. *et al.* The histone H3K79 methyltransferase Dot1L is essential for mammalian development and heterochromatin structure. *PLoS Genet.* **4**, e1000190 (2008).
176. Orkin, S. H. & Hochedlinger, K. Chromatin connections to pluripotency and cellular reprogramming. *Cell* **145**, 835–850 (2011).
177. Juan, A. H. *et al.* Polycomb EZH2 controls self-renewal and safeguards the transcriptional identity of skeletal muscle stem cells. *Genes Dev.* **25**, 789–794 (2011).
178. Caretti, G., Di Padova, M., Micales, B., Lyons, G. E. & Sartorelli, V. The Polycomb Ezh2 methyltransferase regulates muscle gene expression and skeletal muscle differentiation. *Genes Dev.* **18**, 2627–2638 (2004).
179. Palacios, D. *et al.* TNF/p38α/polycomb signaling to *Pax7* locus in satellite cells links inflammation to the epigenetic control of muscle regeneration. *Cell Stem Cell* **7**, 455–469 (2010).
180. Wang, J. *et al.* The Msx1 homeoprotein recruits Polycomb to the nuclear periphery during development. *Dev. Cell* **21**, 575–588 (2011).
181. Mal, A. K. Histone methyltransferase Suv39h1 represses MyoD-stimulated myogenic differentiation. *EMBO J.* **25**, 3323–3334 (2006).
182. Ling, B. M. T. *et al.* Lysine methyltransferase G9a methylates the transcription factor MyoD and regulates skeletal muscle differentiation. *Proc. Natl Acad. Sci. USA* **109**, 841–846 (2012).
183. Choi, J. *et al.* Modulation of lysine methylation in myocyte enhancer factor 2 during skeletal muscle cell differentiation. *Nucleic Acids Res.* **42**, 224–234 (2014).
184. Ling, B. M. *et al.* G9a mediates Sharp-1-dependent inhibition of skeletal muscle differentiation. *Mol. Biol. Cell* **23**, 4778–4785 (2012).
185. Wang, J. & Abate-Shen, C. The MSX1 homeoprotein recruits G9a methyltransferase to repressed target genes in myoblast cells. *PLoS ONE* **7**, e37647 (2012).
186. Ohno, H., Shinoda, K., Ohyama, K., Sharp, L. Z. & Kajimura, S. EHMT1 controls brown adipose cell fate and thermogenesis through the PRDM16 complex. *Nature* **504**, 163–167 (2013).
187. Harms, M. J. *et al.* Prdm16 is required for the maintenance of brown adipocyte identity and function in adult mice. *Cell. Metab.* **19**, 593–604 (2014).
188. Neguembor, M. V. *et al.* FSHD muscular dystrophy region gene 1 binds Suv4-20h1 histone methyltransferase and impairs myogenesis. *J. Mol. Cell. Biol.* **5**, 294–307 (2013).

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Competing interests statement

The authors declare no competing interests.

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