

Functional Crosstalk Between Lysine Methyltransferases on Histone Substrates: The Case of G9A/GLP and Polycomb Repressive Complex 2

Chiara Mozzetta,* Julien Pontis, and Slimane Ait-Si-Ali

Abstract

Significance: Methylation of histone H3 on lysine 9 and 27 (H3K9 and H3K27) are two epigenetic modifications that have been linked to several crucial biological processes, among which are transcriptional silencing and cell differentiation. **Recent Advances:** Deposition of these marks is catalyzed by H3K9 lysine methyltransferases (KMTs) and polycomb repressive complex 2, respectively. Increasing evidence is emerging in favor of a functional crosstalk between these two major KMT families. **Critical Issues:** Here, we review the current knowledge on the mechanisms of action and function of these enzymes, with particular emphasis on their interplay in the regulation of chromatin states and biological processes. We outline their crucial roles played in tissue homeostasis, by controlling the fate of embryonic and tissue-specific stem cells, highlighting how their deregulation is often linked to the emergence of a number of malignancies and neurological disorders. **Future Directions:** Histone methyltransferases are starting to be tested as drug targets. A new generation of highly selective chemical inhibitors is starting to emerge. These hold great promise for a rapid translation of targeting epigenetic drugs into clinical practice for a number of aggressive cancers and neurological disorders. *Antioxid. Redox Signal.* 22, 1365–1381.

Introduction

ALTHOUGH ALL THE cells within a multicellular organism contain the same genetic material, each tissue and cell type express only specific subsets of genes. How the same genetic information is translated into different cellular identities is a process mainly regulated at the epigenetic level. Epigenetic regulators and transcription factors (TFs) act to organize the genome into accessible or closed regions fine-tuning the proper transcriptional program in any given cell type. As such, epigenetic regulation is fundamental to maintain cell identity and the unique physical characteristics and biological functions of specific tissues and organs. Importantly, the epigenetic state of a cell is highly malleable, evolving in an ordered manner, during cellular differentiation

and development of an organism, in response to environmental changes.

Epigenetic processes are classically defined as those events or phenotypes that are not associated to changes in the deoxyribonucleic acid (DNA) sequence, but rather as heritable differences in the packaging of DNA and chromatin. The DNA of eukaryotic cells is finely organized within the nucleus, folded into nucleosomes, the fundamental units of chromatin. Nucleosomes comprise ~147 bp of DNA wrapped around a histone octamer of four highly evolutionary conserved core histone proteins—H2A, H2B, H3, and H4. The N-terminal histone tails protrude outside the nucleosome core and are subjected to several post-translational modifications (PTMs), such as phosphorylation, acetylation, methylation, ubiquitination, or sumoylation (150). These histone PTMs alter chromatin

Laboratoire Epigénétique et Destin Cellulaire, UMR7216, Centre National de la Recherche Scientifique CNRS, Université Paris Diderot, Sorbonne Paris Cité, Paris, France.

*Current affiliation: Institute of Cell Biology and Neurobiology (IBCN), National Research Council (CNR), Rome, Italy.

compaction and function, thus influencing a large number of nuclear processes. Indeed, histone PTMs can, for example, generate docking sites for, or influence the affinity of, chromatin-binding proteins with nucleosomes, therefore regulating the binding of chromatin-modifying complexes and influencing transcriptional status of the underlying DNA sequences (63). Histone PTMs (*i.e.*, lysine acetylation) can also directly alter histone–DNA interactions by neutralizing the positive charges of histone tails, thereby altering chromatin compaction. Therefore, an appropriate balance of different histone PTMs is necessary to finely regulate crucial nuclear and cellular functions.

One of the most studied histones' PTMs is lysine methylation. Lysines can be mono-, di-, or tri-methylated. Depending on the degree of methylation, the specific lysine residue that is methylated, and their localization within the gene and the genome, different methylation states have been associated with distinct nuclear functions and transcriptional outcomes (14).

Depending on the biological context, some methylation states may need to be stably maintained (*i.e.*, methylation involved in the stable repression of heterochromatin states), while others need to be more dynamic and amenable to change (*i.e.*, during cellular differentiation or when cells respond to environmental cues) (47).

Over the past decade, a number of major discoveries and technological advances have emphasized the biological importance of lysine-modifying enzymes and a plethora of histone lysine methyltransferases (KMTs) and demethylases have now been identified that mediate the addition or removal of methyl groups from different lysine residues on histones and non-histone substrates.

Histone lysine methylation can be found in both active and inactive regions of chromatin. In particular, methylation of histone H3 on lysine 4 or 36 (H3K4 and H3K36) is generally associated with transcriptionally active genes, while methylation of lysine 27 of the histone H3 (H3K27) and lysine 9 of the histone H3 (H3K9) are hallmarks of condensed chromatin at silent *loci*.

Deposition of H3K27 and H3K9 methylation is achieved by two major families of KMTs, Polycomb repressive complex 2 (PRC2) and H3K9 KMTs, respectively. These two histone lysine methylation machineries play key roles in several cellular and nuclear functions, such as cellular differentiation, stem cell pluripotency, reprogramming, and genome stability. Here we will review the current knowledge on the physiological functions and mechanisms of action of these two main epigenetic silencing pathways. We will discuss their role in biological processes such as stem cell pluripotency and differentiation, highlighting how their aberrant regulation might often lead to several malignancies and diseases.

“Histone” KMTs in Gene Silencing

H3K9 methyltransferases structure and enzymatic activity

Methylation of H3K9 is mainly involved in gene repression and heterochromatin formation and is achieved by different KMTs (63). The most studied thus far, has been Suv39h1. Suv39h1 not only plays major roles in the establishment of pericentromeric heterochromatin and genome stability (116, 117, 121), but it has also been involved in *de novo* gene silencing during differentiation (1, 75, 156). Accordingly,

Suv39h1 has been found to interact with *de novo* DNA methyltransferases (70), strongly suggesting that it participates in the establishment of facultative heterochromatin (116).

At the enzymatic level, Suv39h1 prefers mono- or dimethylated H3K9 as a primary substrate to deposit dimethylation (me2)- and trimethylation (me3) (116, 121), which suggests cooperation with a mono- or dimethyltransferase. Interestingly, a subset of Suv39h1 coexists in the same complex with the other H3K9 KMTs, G9a/KMT1C/EHMT2, GLP/KMT1D/EHMT1, and SETDB1/KMT1E, to functionally cooperate in the regulation of gene silencing (46, 158).

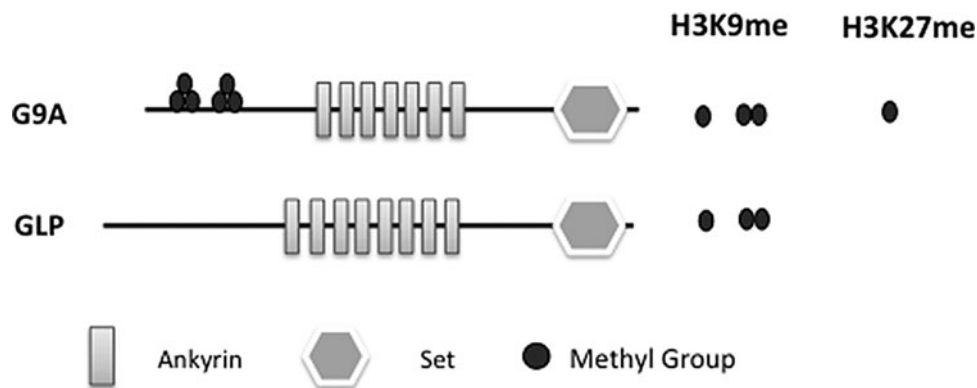
Similar to Suv39h1, SETDB1 has been implicated in H3K9me2/3 at euchromatic regions (33) and linked to both gene silencing (13, 73, 75, 122, 162) and pericentric heterochromatin (18, 79, 173).

Other important H3K9 KMTs, which are becoming increasingly studied given their emerging role in regulation of gene silencing in several progenitor cells, are G9a and GLP (137). G9a and GLP are mainly responsible for monomethylation (me1) and me2 of H3K9 in euchromatic regions (146, 148). They exist predominantly as a G9a–GLP heterodimeric complex, which appears to be the functional H3K9 mono- and di-methyltransferase *in vivo* (148). G9a and GLP contain nearly identical Su(var)3–9 family Su(var)3–9 Enhancer-of-zeste Trithorax (SET) methyltransferase domains, with which they bind and methylate H3K9me0/1, and ankyrin repeat domains that create a methyl-lysine-binding module that allows binding of H3K9 me1/me2 marks separately from their catalytic domains (30) (Fig. 1). Thus, G9a and GLP have distinct “reading” and “writing” functions and can “read” their own marks and this may account for a mechanism of nucleation and spreading of H3K9me2 marks along chromatin (29). *G9a/GLP* loss abolishes methylated H3K9 in euchromatic regions (123, 146), while H3K9 trimethylation (H3K9me3) seems unaffected and probably maintained by Suv39h1 and/or SETDB1 (116, 123). However, some reports suggest that G9a/GLP might also be involved in H3K9me3 deposition (37, 42, 46, 102, 160).

Despite G9a and GLP have been primarily described to methylate H3K9; other histone targets also have been reported. G9a/GLP can methylate both *in vitro* and *in vivo* the histone linker H1, thereby mediating chromatin compaction by providing a recognition surface for the chromatin-binding proteins heterochromatin protein 1 (HP1) and L3MBTL1 (154, 165). Moreover, G9a is required for the me1 of H3K56, which acts as a chromatin docking site for proliferating cell nuclear antigen, thus regulating DNA replication (174). Intriguingly, G9a and GLP have been reported to methylate both *in vitro* and *in vivo* H3K27 (111, 145, 169). It is noteworthy that H3K9 and H3K27 lysines are embedded within a similar peptide motif, namely Alanine-Arginine-Lysine-Serine (ARKS), making possible their modification(s) by common enzymes. Indeed, in *G9a* or *GLP* knock-out (KO) embryonic stem cells (ESCs), H3K27me1 levels decrease drastically (169). Interestingly, G9a-mediated H3K27 methylation has been suggested to increase PRC2 enzymatic activity at least *in vitro* (92).

Beyond histones, G9a/GLP have been shown to methylate a plethora of non-histone targets, including the tumor suppressor p53, SIRT1, Reptin, MyoD, Wiz, CDYL and several chromatin regulators (50, 67, 77, 88, 120). Moreover, G9a methylates itself and this modification mediates its interaction with HP1 and CDYL (127). Although the biological role

FIG. 1. G9a/GLP characterized domains. G9a and GLP consist of an N-terminal domain with auto-methylation site(s), the ankyrin repeats that recognize H3K9me1–2 established by its catalytic SET domain. G9a and GLP form heterodimers through their SET domains. me1, mono-methylation; me2, dimethylation; SET, Su(var)3–9 Enhancer-of-zeste Trithorax.



of these non-histone methylations are still not well understood, it is clear that G9a/GLP can regulate chromatin functions at different layers, by either direct modification of histones of chromatin-binding proteins, thus mediating recruitment and activity of chromatin complexes.

H3K27 methyltransferases, PRC2 complex, and enzymatic activity

Polycomb group (PcG) proteins form multimeric repressive complexes in most metazoan species. Classically, PcG complexes are divided into PRC1 and PRC2 (131).

In mammals, the core PRC2 complex is composed of four proteins: Suppressor-of-Zeste 12 (Suz12), embryonic ectoderm development (Eed), Retinoblastoma-Associated Protein 46/48,

the SET domain-containing proteins Enhancer-of-Zeste 2 (Ezh2/KMT6B), and 2 (Ezh2/KMT6A), which are the catalytic subunits of the complex methylating H3K27 (140) (Fig. 2). All the PRC2 core members are required for the formation of a catalytically active PRC2 complex (21, 107). However, several sub-stoichiometric components, Jarid2, Aebp2, Phf1, MTF2, and Phf19, have been shown to interact with PRC2, suggesting the existence of considerable interactions between PcG complexes and chromatin regulators outside the strict definition of the PcG. Although these “accessory” proteins do not seem to be essential for PRC2 basal KMT activity, they appear to fine-tune PRC2 activity and to modulate its recruitment (82, 140). Aebp2 is a Zinc finger protein that interacts with several components of PRC2, enhances its enzymatic activity (21), and co-localizes with PRC2 at some target genes (57). Pcl proteins, Pcl1/2/3

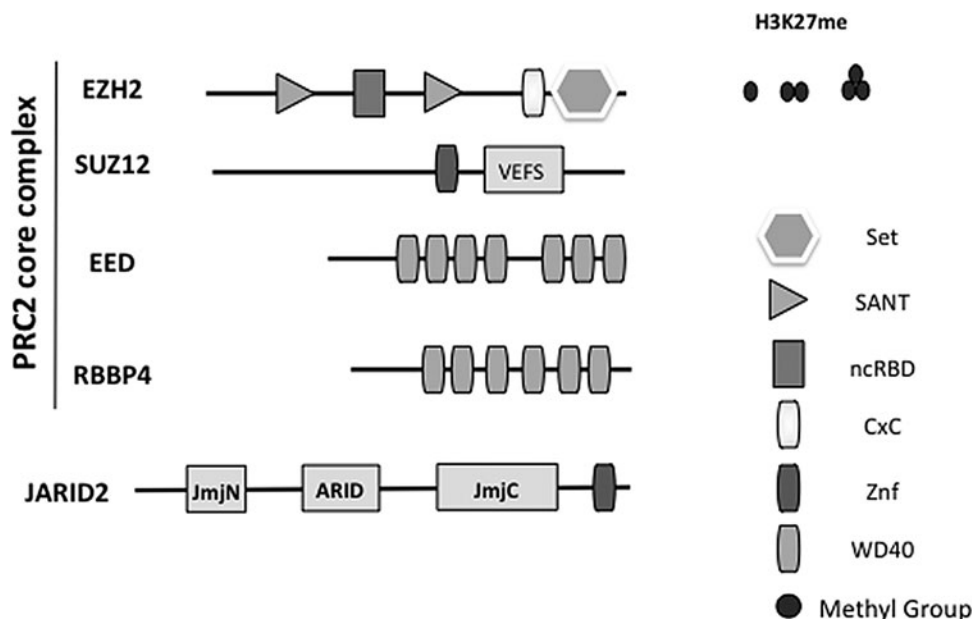


FIG. 2. PRC2 characterized domains. PRC2 core components: Ezh2 contains a catalytic SET domain; a CXC domain (cystein-rich domain) is a CpG-rich binding domain; ncRBD, non-coding RNA-binding domain; SANT domains, which are DNA/Protein-binding domains. Suz12 (Suz12-protein homolog) contains VEFS and Znf (C2H2-type zinc finger) domains, which are DNA/Protein-binding domains. Eed, RBBP4/7 (Retinoblastoma-binding protein 4/7 or RBAP48/46) contains WD40, short ~40 amino-acid motifs that mediate protein–protein interactions. JARID2 contains JmjN/C (for Jumonji), an inactive demethylase domain, and two DNA binding domains (ARID and Znf). DNA, deoxyribonucleic acid; Eed, embryonic ectoderm development; Ezh2, Enhancer-of-Zeste 2; PRC2, polycomb repressive complex 2; RbAp46/48, retinoblastoma-associated protein 46/48; RNA, ribonucleic acid; SANT, switching-defective protein 3 (Swi3), adaptor 2 (Ada2), nuclear receptor co-repressor (N-CoR), transcription factor (TFIIIB)

(Phf1, MTF2, and Phf19) interact with PRC2 through Ezh2 and Suz12 (7, 95) and genome-wide studies revealed that Pcl2 (72, 161) and Pcl3 (7, 17) are found at PRC2 target genes. Different functions have been attributed to Pcl proteins, from the regulation of PRC2 enzymatic activity (95, 128) to its targeting (7, 17, 19, 161).

Jarid2 has been also strongly linked to PRC2 activity and recruitment to chromatin. Jarid2 belongs to the Jumonji family of proteins that catalyze lysine demethylation; however, Jarid2 itself seems devoid of enzymatic activity. Different groups identified Jarid2 as a part of PRC2 complex and identified a large genome-wide overlap (66, 72, 108, 113, 134). Jarid2 has been shown to regulate PRC2 recruitment, but its function in regulating PRC2 enzymatic activity is still a matter of debate. H3K27me3 levels are only modestly affected by Jarid2 knockdown (66, 134), suggesting that Jarid2 might serve to fine-tune PRC2 activity by inhibiting H3K27me3 deposition (113, 134). However, further characterization of PRC2 enzymatic activity indicated that Jarid2 enhances PRC2 activity under defined biochemical conditions (72).

PRC2 has been generally recognized to be responsible for the deposition of me2 and me3 of H3K27 (H3K27me2/3) (106), while its contribution in mediating H3K27me1 is still a matter of debate (81, 135). Ezh1, the Ezh2 homolog, has been suggested to play such a role (135) but its activity toward H3K27 remains controversial (81). Although the evidence that H3K27me1 levels remain unaffected after PRC2 disruption *in vivo* (23) suggests the existence of a H3K27 monomethyltransferase other than a known PRC2 component (92), a recent report has directly implicated PRC2 in the genomic distribution of H3K27me1 (43). This work demonstrates that PRC2 controls all forms of H3K27 methylation, including H3K27me1, and shows that H3K27me2 is actually the main activity of PRC2 (43). Intriguingly, genomic localization of the different H3K27 methylations (mono- di- and tri-) seems to be mutually exclusive and regulates different genomic functions. While H3K27me3 is mostly associated to CpG-rich promoters (64, 85) and involved in cell-specific maintenance of epigenetic silencing (87), H3K27me2 has been rather suggested to exert a protective function by inhibiting the firing of non-cell type-specific enhancers (43). In contrast, H3K27me1 has been shown to accumulate, in a PRC2-dependent manner, in the bodies of actively transcribed genes (11, 43), thereby promoting gene transcription (43). Intriguingly, Ezh1 has been recently suggested to interact with ribonucleic acid (RNA) polymerase II and promote RNA elongation, thus inducing transcriptional activation of muscle-specific genes in differentiating myoblasts (90). Although the role of Ezh1 in depositing H3K27me1 has been controversial (81, 135), the fact that both Ezh1 and H3K27me1 localize on active regions leads one to speculate that PRC2-Ezh1 might effectively contribute to H3K27me1 deposition. However, their genome-wide colocalization has been never explored thus far and further work is needed to definitely address this question.

Targeting and Modus Operandi: The Case of PRC2 and G9a/GLP Cooperation

Chromatin targeting

How PRC2 and G9a/GLP are recruited to their target sites in mammalian cells is still poorly understood and a matter of intense debate in the field.

None of the PRC2 core members, neither G9a nor GLP, possesses DNA-binding properties, suggesting that these proteins have to rely on additional partners or mechanisms to be targeted to chromatin. These mechanisms comprise interactions with chromatin-binding molecules, sequence-specific TFs, and non-coding RNAs.

Recognition of specific DNA sequences has been well elucidated as a mechanism that recruits PcG proteins in *Drosophila*. Indeed, in *Drosophila*, PcG proteins bind to the so-called polycomb responsive elements (PREs), which contain consensus sites for several TFs (94, 124, 130, 152). Nonetheless, such a mechanism does not seem to be conserved in mammals and the finding of DNA-specific consensus sites targeted by PRC2 or G9a/GLP has been elusive.

Two recent reports have identified two specific DNA sequences that seem to function as PREs in mammals (141, 167). Sing *et al.* identified a murine PRE-like element that regulates the *MafB* gene during neural development, defining it as a critical 1.5 kb sequence element that is able to recruit PRC1, but not PRC2, in a transgenic cell assay (141). Woo *et al.* identified a 1.8 kb region of the human *HoxD* cluster that recruits both PRC1 and PRC2 and represses a reporter construct in mesenchymal tissues (167). Interestingly, in both works, the PRE regions contain YY1 motifs. YY1 is the mammalian homolog of Pho, whose role in PcG recruitment has been well documented in flies (124, 130, 152). However, YY1 does not seem to have a major role as a general PRC2 recruiting factor in mammals (64, 85, 159).

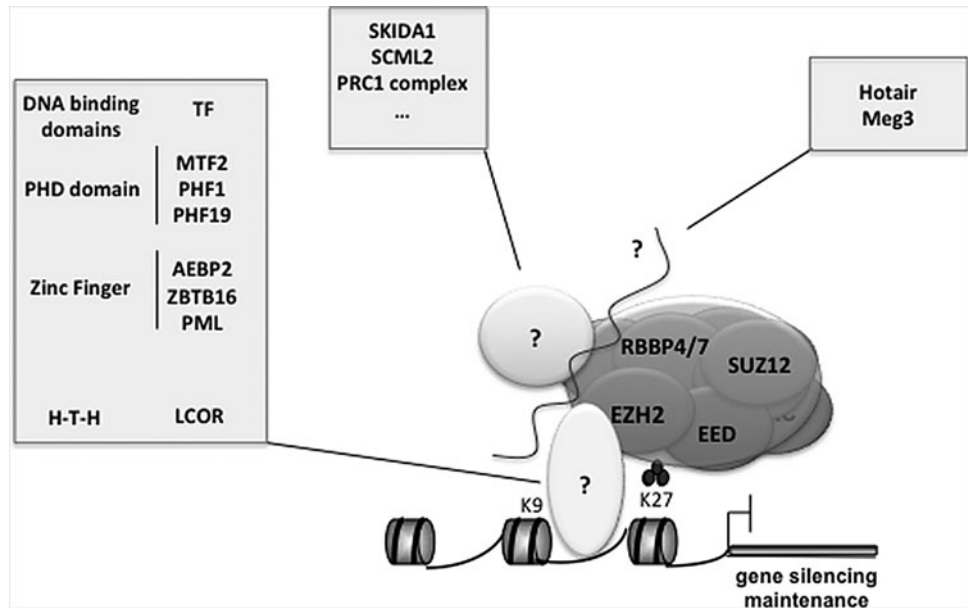
Despite these locus-specific evidences, PRC2 genome-wide localization has demonstrated an almost complete overlap between PRC2 target genes and CpG islands (64, 151) and it has been shown that GC-rich elements are sufficient to induce recruitment of PRC2 (80, 85), clearly implying CpG islands as preferential sites of PRC2 targeting. Likewise, G9a seems to co-localize with PRC2 preferentially at promoters and CpG islands (92). However, how are PRC2 and G9a/GLP recruited to these regions? Different, but not mutually exclusive, mechanisms have been proposed.

Accessory chromatin-binding proteins that co-purify with PRC2 and G9a/GLP complexes have been reported to regulate their chromatin targeting. Among all, Jarid2 has been claimed by several groups as one of the major PRC2 recruiting factors (72, 108, 113, 134). It has been also reported to interact with G9a and GLP (92, 138) and to overlap to some extent with G9a binding sites (92), thus implicating it as a potential common recruiting factor of PRC2 and G9a/GLP.

Sequence-specific TFs have been also proposed to mediate PRC2 (Fig. 3) or G9a/GLP (Fig. 4) recruitment to their target loci. Among others, SNAIL, REST, CDYL, and Msx1 (Fig. 5) have been shown to mediate both PRC2 (3, 31, 49, 164) and G9a (34, 35, 126, 163) chromatin targeting, suggesting that common recruiting mechanisms for PRC2 and G9a/GLP might be more common than expected. Given the high affinity of specific TFs for their binding sites and their cell-specific expression, TF-mediated PRC2 G9a/GLP recruitment might be a way to ensure proper PRC2-mediated gene silencing in a cell type-specific manner.

Finally, long non-coding RNAs (lncRNAs) are becoming appreciated as important regulators through which PRC2 and G9a/GLP are recruited to chromatin target sites. A paradigmatic example is represented by PRC2 and H3K27me3 recruitment on the inactive X mediated by the *Xist* lncRNA,

FIG. 3. Co-regulators, interaction/recruitment of PRC2. Here are listed the factors and non-coding RNAs that have been suggested to interact with and regulate PRC2 activity, including a recent study using BioTAP-XL to decipher the PRC2 complexes (2a).



which coats the X chromosome in *cis*, during X inactivation (118, 179). Likewise, the lncRNA *Hotair* has been reported to mediate PRC2 recruitment *in trans* to the *HOXD* cluster (125). The imprinted-monoallelically expressed lncRNA *Air* regulates G9a targeting, inducing the epigenetic allele-specific silencing of the *cis*-linked *Slc22a3*, *Slc22a2*, and *Igf2r* genes in mouse placenta (93). Interestingly, two different lncRNAs have been shown to bind G9a and PRC2, *Kcnqot1* (105) and *AS1DHR54* (76)

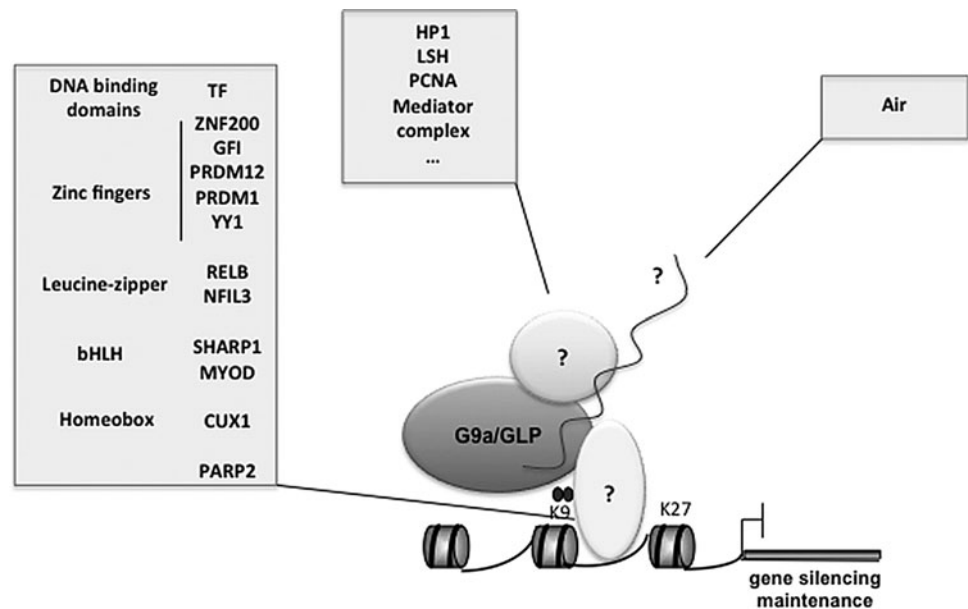
Despite a unifying view on PRC2/G9a/GLP recruitment being still missing, the evidences described earlier point toward a multi-step process, which might comprise a combination of the different mechanisms, thereby ensuring a tight and dynamic regulation of PRC2 and G9a/GLP chromatin targeting.

Mechanisms of gene regulation

Genome-wide analyses of different histone modifications suggest that both H3K27me3 and H3K9me2 mark are linked with transcriptional repression (11). PcG proteins and G9a/GLP mainly mediate gene silencing relying on several different mechanisms.

Both PRC2 and G9a/GLP have been found in complexes containing co-repressors (99, 136), which include H3K4 and H3K36 demethylases (17, 19, 24, 41, 109, 149), suggesting that they mediate gene silencing through a coordinated mechanism by which the concomitant removal of activating histone marks is needed for the deposition of repressive histone modifications, thereby inducing transcriptional repression. Beyond this, gene repression is thought to depend

FIG. 4. Co-regulators, interaction/recruitment of G9a. Here are listed the factors and non-coding RNAs that have been suggested to interact with and regulate G9a functions, including a recent study using BioTAP-XL to decipher the PRC2 complexes (2a).



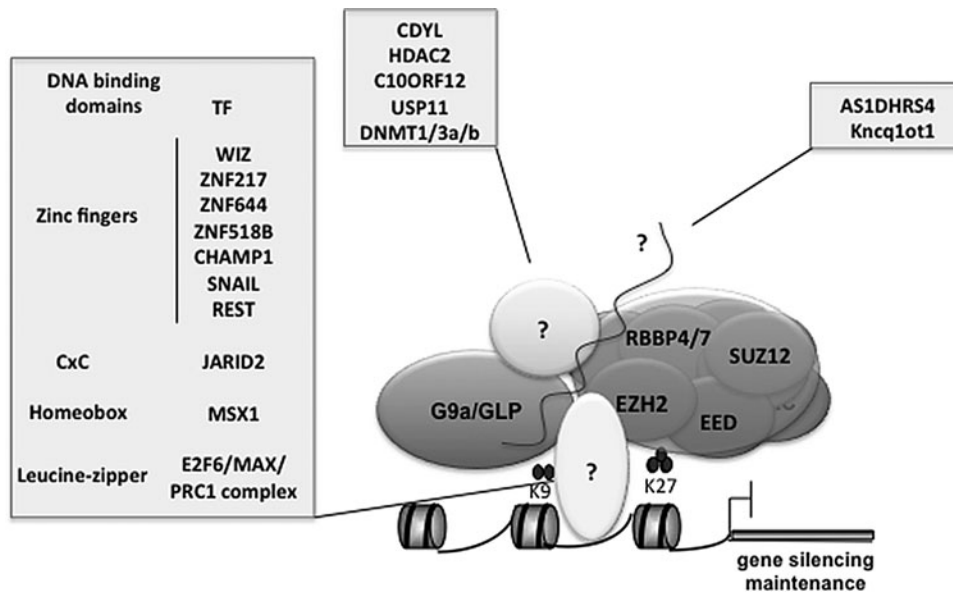


FIG. 5. Co-regulators, interaction/recruitment specificity of G9a and PRC2. G9a and PRC2 directly interact but are neither part of a unique complex nor share exclusively the same targets. So, one or several common factors could mediate this specificity of interaction and/or recruitment. This/these factor(s) could be DNA binding factors, co-factors, or non-coding RNAs. Here are listed the factors that have been suggested to interact with G9a and PRC2, including a recent study using BioTAP-XL to decipher the PRC2 complexes (2a).

on chromatin compaction and/or spatial segregation into silent nuclear domains.

Regarding Polycomb proteins, the general assumption is that PRC2-mediated H3K27me3 serves as a docking site for the subsequent recruitment of PRC1, which monoubiquitinates H2AK119 (H2AK119 monoubiquitination [ub1]) by the E3-ligase activity of the PRC1 subunit Ring1 (45, 128), thus inducing chromatin compaction and transcriptional repression (38). Indeed, compacted chromatin has reduced accessibility to TFs and adenosine triphosphate-dependent chromatin-remodeling machineries, such as SWI/SNF (9), thus preventing gene activation. In addition, it has been proposed that H2AK119ub1 at poised promoters represses RNA pol II activity (143) and that it restrains the eviction of the H2A-H2B dimers from nucleosomes that is necessary for transcription elongation (180). Moreover, PRC1-mediated H2AK119ub1 is required for efficient repression of PcG target genes (36).

Similarly, me2 or me3 of H3K9 creates a binding site for chromodomain-containing proteins of the HP1 family (8, 65), which is speculated to lead to gene repression *via* changes in higher-order chromatin structure. In accordance with this, G9a/GLP have been shown to also bind and methylate H1, further mediating chromatin compaction.

Chromatin compaction is often observed within nuclear foci termed PcG bodies (10), which represent discrete foci of silenced genes. Despite the fact that there is still no direct evidence linking these nuclear structures to H3K27me3, it is tempting to speculate that these structures might comprise large blocks of H3K27me3. Indeed, analyzing different H3K27me3 genome-wide datasets, large (around 43 kb) H3K27me3 domains (broad local enrichments) have been identified, which overlap with silent genes and intergenic regions (112).

Similarly, large and diffuse regions of H3K9me2 that cover approximately 4.9 Mb, and collectively represent around 40% of the genome, named Large Organized Chromatin K modifications, have been described (166). This evidence suggests that G9a/GLP-mediated H3K9me2 is in-

tegral to the establishment of these facultative heterochromatin domains, which are contained within larger regions of euchromatin.

Strikingly, these domains overlap more than 80% with nuclear lamina-associated domains (LADs) (48), which are large chromatin regions (0.1–10 Mb) that are highly methylated with H3K9me2 and H3K27me3 (48, 166) that establish contact with nuclear lamina (NL). The majority of genes located in LADs are transcriptionally inactive, indicating that the NL constitutes a repressive environment (48, 115) and suggesting that G9a/GLP-induced transcriptional silencing might be mediated by relocalization of chromatin targeted regions in spatially defined nuclear structures, such as NL. Indeed, in mouse ESCs, most of the G9a-repressed genes that are marked with H3K9me2 are localized to the nuclear periphery (172). Moreover, two recent papers causally link H3K9 methylation and chromatin tethering to NL (58, 153). These works uncovered evolutionary conserved mechanisms (in *Caenorhabditis Elegans* and mammals) through which H3K9 KMTs and H3K9 methylation promote the peripheral localization and silencing of chromatin regions (58, 153). Strikingly, G9a has been found as a regulator of NL contacts in human cells (58); however, fluorescence in situ hybridization experiments showed that the H3K9me2 mark seems not essential for the nuclear peripheral localization of specific chromatin loci because the G9a-repressed genes remain in the nuclear periphery in mouse ESCs after G9a inactivation and reduction in H3K9me2 levels (172). These apparent discrepancies might be due to species- or cell type-specific effects, to technological differences or compensatory effects mediated by another KMT, that is, GLP. Nonetheless, the evidence described earlier clearly suggests that H3K9 methylation is needed to create a repressive environment at the nuclear periphery and, thus, induce and maintain gene silencing. Moreover, the observation that LADs are particularly enriched in both H3K9me2 and H3K27me3 (48, 166) leads one to speculate that NL might be the preferred nuclear compartment where the functional interplay between PRC2 and G9a/GLP takes place.

A clue in favor of this hypothesis derives from studies performed in muscle cells where both PRC2 and H3K27me3 are recruited to the nuclear periphery by the TF Msx1(164) and G9a seems to control the Msx1-dependent redistribution of H3K27me3 genomic bound sites (163), thus suggesting that G9a/GLP-PRC2 might cooperate with tissue-specific TFs to modulate gene silencing by regulating the spatial segregation of the co-targeted regions.

Physiological Roles of PRC2 AND G9A/GLP

Role in ESCs pluripotency and differentiation

We and others have shown that SETDB1 (13, 175) and G9a (92) are involved in the epigenetic silencing of key developmentally regulated genes in ESCs that are known as PRC2 targets (15, 68, 104). The relative abundance of different epigenetic marks at precise lysine residues has recently emerged as a key strategy used by ESCs to fine-tune the expression of key genes involved in lineage commitment. In fact, in pluripotent ESCs, promoters of developmentally regulated genes are repressed but poised for activation by the concomitant presence of the silencing (H3K27me3) and activating (H3K4me3) histone marks (12, 86). In agreement with this, it has been shown that H3K9me3 marks the so-called *bivalent* genes (12, 86) in trophoblast stem cells, unraveling the existence of *trivalent* domains (H3K4me3/H3K27me3/H3K9me3) (2) and suggesting that H3K9 and H3K27 methylations could act in synergy to stabilize a repressed state in silent genes.

Both PRC2 and G9a/GLP have been shown to play critical roles in mouse development. KO of either *G9a/GLP* (146, 148) or PRC2 core members (16) results in severe defects during early embryonic development, thus suggesting that these chromatin silencers have critical functions in ESCs pluripotency and lineage differentiation.

The evidence that in the absence of PRC2 core members several lineage-specific genes are derepressed in pluripotent ESCs (15), along with the initial failure to establish *Ezh2* KO cells (98) have led to the conclusion that these proteins might be crucial to maintain ESCs pluripotency and self-renewal (15, 98). Nonetheless, ESCs lacking *Ezh2*, *Eed*, or *Suz12* can be generated and maintained in culture with self-renewal capacities similar to those of wild-type ESCs (23, 106, 135). Moreover, *Eed* KO cells can contribute to the development of all tissues in chimeric embryos, clearly indicating that PRC2 KO cells retain pluripotency (23) and that PRC2 activity seems dispensable for ESCs self-renewal. Interestingly, it has been recently shown that PRC2 members' expression levels are regulated by microRNAs (20), and this has been proposed as an additional, evolutionary conserved, mechanism through which ESCs regulate their stemness (178).

Similar to PRC2, G9a and GLP KO ESCs can also be generated and maintained *in vitro*. G9a and GLP ablation shows embryonic lethality at E9.5, when early lineage specification of pluripotent ESCs has already taken place, thereby suggesting that G9a/GLP are also dispensable for ESCs pluripotency and self-renewal (146, 148).

However, both G9a and PRC2 play a role in loss of pluripotency and differentiation of ESCs (Fig. 6), as both are required for proper silencing of ESCs pluripotency factors on differentiation (23, 42, 106, 135, 171).

Indeed, in mouse ESCs, inactivation of *Suz12*, *Jarid2*, or *Pcl2* was reported to be associated with an inefficient si-

lencing of the pluripotency factors *Nanog* and *Oct3/4* (66, 72, 161). Likewise, G9a and H3K9me2 have been implicated in silencing of *Nanog* and *Oct3/4* in differentiating ESCs (42, 171). In particular, it has been shown that G9a-mediated *Oct3/4* and *Nanog* repression is dependent on activation of protein kinase A, which regulates ESC differentiation in a timely manner by inducing pluripotency factor silencing (171). Furthermore, G9a itself is capable of causing *de novo* DNA methylation, independently of its methyltransferase activity, by recruiting DNA methyltransferases Dnmt3a and Dnmt3b and inducing irreversible silencing of *Oct3/4* in differentiated cell lineages (37).

Taken together, this evidence highlights that G9a/GLP and PRC2 are not required for ESCs self-renewal and pluripotency, act in a dynamic and regulated manner during post-implantation to induce direct inhibition of transcription and heterochromatinization of pluripotency factors, thus mediating proper differentiation programs during development.

Role in adult stem cell differentiation

Several reports in the past years have begun to shed light on the roles of PRC2 and G9a/GLP in the silencing of specific transcriptional programs in adult stem cells, unraveling a crucial role for these chromatin players as mediators of adult stem cell identity and differentiation.

Pioneering evidence demonstrated that beyond its known role in the control of ESCs lineage commitment, PRC2 was also involved in the regulation of tissue-specific stem cells (22, 40). Indeed, PRC2 acts by preventing the unscheduled expression of the structural genes required for epidermal differentiation, thereby regulating skin stem cell differentiation and regenerative capacity (39, 40).

Similarly, it has been recently shown that conditional inactivation of *Eed* leads to multiple and profound defects in hematopoiesis. *Eed* mutant hematopoietic stem cells (HSCs) fail to differentiate into mature blood cells, showing that PRC2 coordinates diverse pathways to ensure proper self-renewal and differentiation of HSCs in a developmental stage-specific manner (170). Interestingly, G9a/GLP have been also recently shown to regulate hematopoiesis (27). In particular, G9a/GLP-mediated H3K9me2 patterning is involved in critical steps during human hematopoietic stem and progenitor cells (HSPCs) lineage commitment and their inhibition leads to delayed differentiation and retention of the primitive HSPCs (27).

Skeletal muscle is another system where both PRC2 and G9a/GLP emerge as critical players in the regulation of muscle stem cell differentiation and regenerative capacity. Indeed, PRC2 deposits H3K27me3 in a coordinated fashion to ensure proper silencing of both muscle-specific genes in undifferentiated myoblasts (5, 22, 55, 144) and *Pax7*, a marker of undifferentiated muscle stem cells, on induction of muscle differentiation (91, 103). Moreover, it has been shown that PRC2 plays critical roles to preserve the transcriptional identity of skeletal muscle stem cells by repressing non-muscle cell lineage-specific genes (5, 54). Interestingly, recent works have also implicated G9a/GLP in MyoD target gene repression (77, 100). Particularly, GLP has been recently found in the PRDM16 complex (100), a well-known potent inhibitor of muscle differentiation (133), to mediate H3K9me2 deposition and silencing of muscle

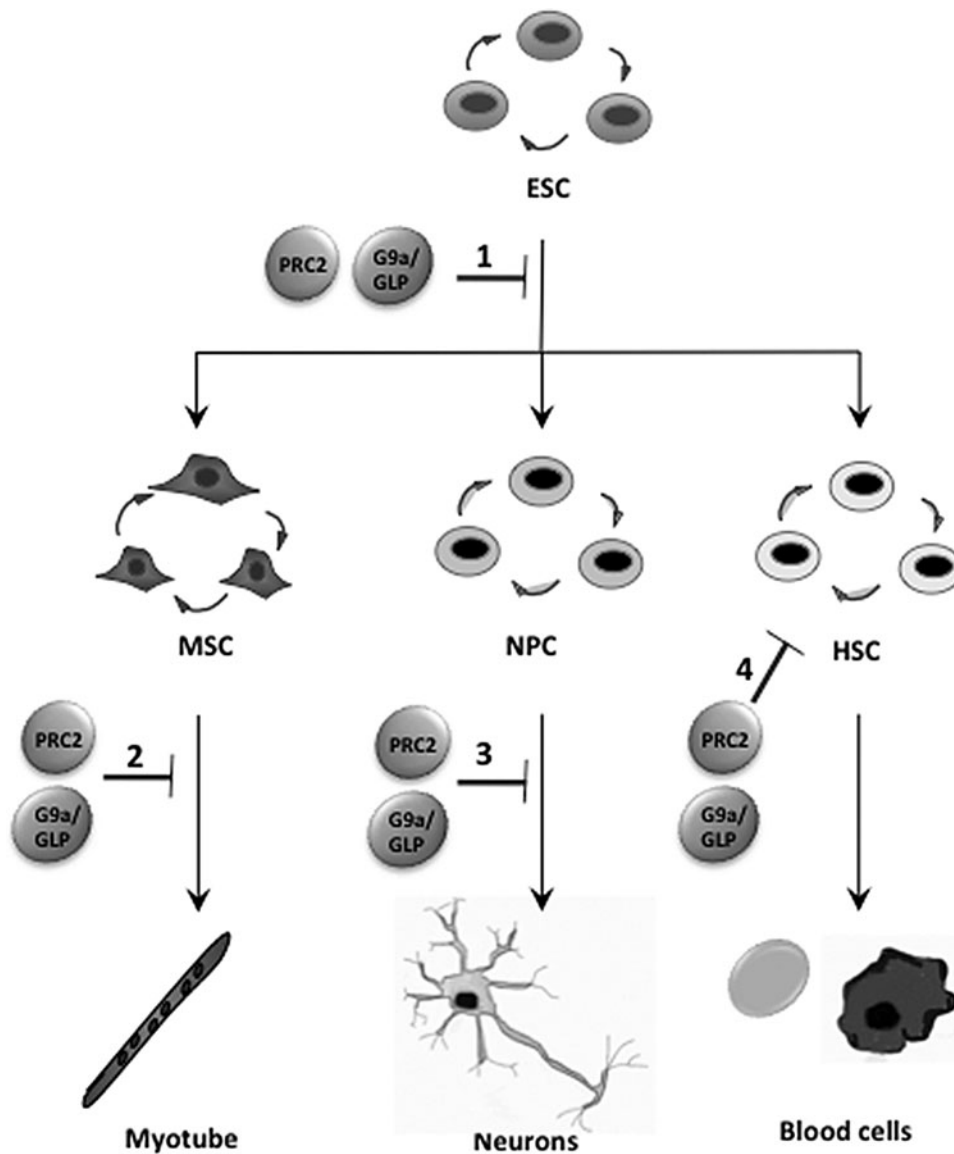


FIG. 6. G9a/GLP and PRC2 mediate regulation of pluripotency and differentiation. (1) PRC2 and G9a/GLP act in ESCs to maintain the silencing of lineage specific genes, as suggested by evidence that in absence of PRC2 core members or G9a/GLP induces embryonic development defects. (2) PRC2 and G9a/GLP are necessary to repress muscle differentiation genes in proliferating myoblasts, thus regulating the differentiation capacity of MSCs. (3) G9a/GLP and PRC2 are required for the maintenance of neuronal gene repression in NPCs and proper neuronal development. (4) G9a and PRC2 regulate HSCs self-renewal and differentiation timing. ESC, embryonic stem cell; HSC, hematopoietic stem cell; MSC, muscle stem cell; NPC, neuronal progenitor cell.

specific genes, thus favoring the adipogenic differentiation of a population of *Myf5*+ progenitors, known to be the common precursors of brown adipocytes and muscle cells (133), suggesting a previously unappreciated role for GLP in regulating muscle progenitors lineage choice. In line of this evidence, G9a has also been shown to mediate muscle gene repression, although through an alternative mechanism implying *MyoD* methylation (77). In fact, G9a seems to directly methylate *MyoD*, thus driving its recruitment onto *myogenin* promoter in proliferating myoblasts (77) and antagonizing the competing acetylation by p300/CBP-associated factor, an event required to facilitate recruitment of additional co-activators and to promote muscle gene transcription (119).

Finally, both PRC2 and G9a/GLP are emerging as important regulators of the reprogramming of somatic cells into induced pluripotent stem cells (iPSCs), though with different roles. Indeed, while PRC2 has been shown to facilitate the acquisition of induced pluripotency in different somatic cells (44, 101, 114, 176), G9a/GLP and H3K9 methylation have been instead suggested as a barrier of cellular reprogramming (25, 142). Probably, the requirement of PRC2 for an efficient

generation of iPSCs relies on its capacity to maintain the silencing of somatic cells transcriptional programs while being involved in repression of pluripotency genes; H3K9 methylation needs instead to be overcome for the reacquisition of pluripotency and an efficient cellular reprogramming (25).

H3K9/H3K27 Methylation, Aberrant Regulation in Disease

As described earlier, PRC2 and G9a/GLP mediate a number of regulatory mechanisms that are crucial in several cellular processes and their misregulation and genomic lesions may be critical determinants in several cancers and neurological diseases.

Cancer

Although cancer is fundamentally a genetic disease that is driven by irreversible genomic mutations that subsequently activate oncogenes and/or inactivate tumor suppressor genes, there is increasing evidence that many epigenetic regulatory

proteins are deregulated in cancer, and that histone marks are globally and locally altered within cancer epigenomes (53). Aberrant changes in the methylation pattern of either H3K9 or H3K27 have been linked with increased recurrence and poor survival of several malignancies (47). Whether these changes are causative or just a consequence of the disease has remained an open question for many years. However, the growing number of reports directly linking genetic mutations or aberrant expression of histone KMTs in a number of cancers rather suggest a causal role for aberrant histone methylation during tumorigenesis.

For instance, elevated expression of PRC2 subunits, in particular Ezh2, has been linked to various cancers, including prostate cancer (157), breast cancer (61), and lymphoma (110, 168). These findings have led to ascribe to Ezh2 a pro-oncogenic role, with misregulation of H3K27me3 levels likely leading to aberrant silencing of genes that are important to tumor growth and survival. However, inactivating mutations in *Ezh2* have been found in several hematopoietic malignancies (78, 84, 89, 96, 97, 139, 155, 177), raising the potential of a tumor suppressor function for the protein and highlighting that the role of Ezh2 in promoting or inhibiting tumorigenesis and/or maintenance is likely to be context dependent.

Interestingly, somatic mutations in H3K27 in the genes encoding for histone H3 have been observed in some glioma, and have been linked to reduced activity to PRC2, suggesting that beyond a causative role for oncogenic mutations in histone methyltransferases, mutations in lysine residues themselves might trigger tumorigenicity and might be important cancer targets (56, 71, 132, 168).

Similar to PRC2, elevated levels of G9a expression have been observed in many types of human cancers and associated with greater mortality in cancer patients, while G9a knockdown has been shown to inhibit the proliferation of cancer cell lines (26, 28, 50, 62). The evidence that in mouse models of acute myeloid leukemia, loss of G9a significantly delays disease progression and reduces leukemia stem cell frequency (69) provides further evidence of a tumorigenic role of elevated levels of G9a. Interestingly, G9a has been recently shown to sustain cancer cell survival and proliferation by transcriptional activation, through deposition of H3K9me1, of the serine-glycine biosynthetic pathway (32). G9a inactivation depletes serine and its downstream metabolites, triggering cell death with autophagy in cancer cell lines of different tissue origins. These findings identify a G9a-dependent epigenetic program in the control of cancer metabolism, providing a rationale for G9a inhibition as a therapeutic strategy for cancer (32).

Indeed, a growing number of (pre)-clinical trials with epigenetic drugs, targeting histone methyltransferases, are starting to investigate the potential clinical relevance of the use of small-molecule inhibitors as a possible therapeutic approach to treat malignancies (4).

Neuro-associated disorders

Regulation of lysine methylation is not just important in cancer, but has also emerged as a critical regulator of neurological function and disease. Various histone modifiers have been implicated in intellectual disability syndromes (52), supporting the concept that appropriate regulation of

histone modifications during nervous system development is essential for brain function.

Neuron-specific post-natal deficiency of G9a and GLP has been clearly linked to mental retardation and behavioral defects (129). A causal role of G9a/GLP in mental retardation in mice and humans suggests a key function for G9a/GLP-mediated H3K9 dimethylation in regulation of brain function through maintenance of the transcriptional homeostasis in adult neurons (129).

The behavioral changes triggered by G9a/GLP deficiency are similar to key symptoms of the human 9q34 mental retardation syndrome that is associated with the deletion or disruption of one copy of the *EHMT1/GLP* gene in 9q34.3 subtelomeric region (59, 60). The potential causal role of the *GLP* gene alterations in the human 9q34 mental retardation syndrome has been further underscored by the identification of various intragenic *GLP/EHMT1* mutations in patients with a mental retardation syndrome clinically indistinguishable from 9q34 deletion syndrome (60a, 63a). Mice that are heterozygous for *GLP* display features resembling autism (6), suggesting that GLP has a conserved role in regulating normal neural function. Interestingly, a critical role of G9a-mediated H3K9me2 in cocaine-induced structural and behavioral plasticity has been further reported (83). In fact, G9a downregulation increases the dendritic spine plasticity of nucleus accumbens neurons and enhances the preference for cocaine, thereby establishing a crucial role for histone methylation in the long-term actions of cocaine (83).

Recently, Ezh2-mediated H3K27 trimethylation has been shown to mediate neurodegeneration in Ataxia-Telangiectasia (A-T). A-T symptoms include a progressive neurodegeneration caused by Ataxia Telangiectasia mutated (ATM) protein deficiency, and Ezh2 has been identified as a new ATM kinase target. ATM-mediated phosphorylation of Ezh2 on Ser734 reduces protein stability. This study linked ATM deficiency to Ezh2 hyperactivity, thereby unraveling Ezh2 as a key factor in A-T neurodegeneration (74).

Conclusion

Given the rather recent links to disease of histone KMTs, these enzymes become heavily investigated as potential drug targets for the treatment of both cancers and neurological disease. Thanks to our increasing understanding on the mechanisms of action of these enzymes, of their biochemical features and biological roles, a new generation of highly selective chemical inhibitors is starting to emerge and promises to greatly improve the selectivity of epigenetic therapy. The ability to translate the lessons learned from epigenomic profiling, structural studies, and regulatory mechanisms to clinical studies holds great promise for a rapid translation of targeting epigenetic drugs into clinical practice for a number of aggressive cancers and neurological disorders.

Acknowledgments

Work in Ait-Si-Ali's group was supported by the Agence Nationale de la Recherche (H3K9-methylome and EPILIS grants), Association Française contre les Myopathies (AFM); Fondation ARC pour la Recherche sur le Cancer (Fondation ARC); Groupement des Entreprises Françaises pour la Lutte contre le Cancer (GEFLUC); Institut National du Cancer (INCa, grant 2012-1-PLBIO); CNRS; Université Paris

Diderot and the “Who Am I?” Laboratory of Excellence #ANR-11-LABX-0071 funded by the French Government through its “Investments for the Future” program operated by the ANR under grant #ANR-11-IDEX-0005-01. C.M. was recipient of an FRM and EMBO Long-Term fellowships, and J.P. was recipient of the Ministère de la Recherche PhD and Fondation ARC PhD fellowships.

References

- Ait-Si-Ali S, Guasconi V, Fritsch L, Yahi H, Sekhri R, Naguibneva I, Robin P, Cabon F, Poleskaya A, and Harel-Bellan A. A Suv39h-dependent mechanism for silencing S-phase genes in differentiating but not in cycling cells. *EMBO J* 23: 605–615, 2004.
- Alder O, Lavial F, Helness A, Brookes E, Pinho S, Chandrashekar A, Arnaud P, Pombo A, O'Neill L, and Azuara V. Ring1B and Suv39h1 delineate distinct chromatin states at bivalent genes during early mouse lineage commitment. *Development* 137: 2483–2492, 2010.
- Alekseyenko AA, Gorchakov AA, Kharchenko PV, Kuroda MI. Reciprocal interactions of human C10orf12 and C17orf96 with PRC2 revealed by BioTAP-XL cross-linking and affinity purification. *Proc Natl Acad Sci USA* 111: 2488–2493, 2014.
- Arnold P, Scholer A, Pachkov M, Balwierz PJ, Jorgensen H, Stadler MB, van Nimwegen E, and Schubeler D. Modeling of epigenome dynamics identifies transcription factors that mediate polycomb targeting. *Genome Res* 23: 60–73, 2013.
- Arrowsmith CH, Bountra C, Fish PV, Lee K, and Schapira M. Epigenetic protein families: a new frontier for drug discovery. *Nat Rev Drug Discov* 11: 384–400, 2012.
- Asp P, Blum R, Vethantham V, Parisi F, Micsinai M, Cheng J, Bowman C, Kluger Y, and Dynlacht BD. Genome-wide remodeling of the epigenetic landscape during myogenic differentiation. *Proc Natl Acad Sci U S A* 108: E149–E158, 2011.
- Balemans MC, Huibers MM, Eikelenboom NW, Kuipers AJ, van Summeren RC, Pijpers MM, Tachibana M, Shinkai Y, van Bokhoven H, and Van der Zee CE. Reduced exploration, increased anxiety, and altered social behavior: autistic-like features of euchromatin histone methyltransferase 1 heterozygous knockout mice. *Behav Brain Res* 208: 47–55, 2010.
- Ballare C, Lange M, Lapinaite A, Martin GM, Morey L, Pascual G, Liefke R, Simon B, Shi Y, Gozani O, Carmagno T, Benitah SA, and Di Croce L. Phf19 links methylated Lys36 of histone H3 to regulation of polycomb activity. *Nat Struct Mol Biol* 19: 1257–1265, 2012.
- Bannister AJ and Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res* 21: 381–395, 2011.
- Bantignies F and Cavalli G. Polycomb group proteins: repression in 3D. *Trends Genet* 27: 454–464, 2011.
- Bantignies F, Roure V, Comet I, Leblanc B, Schuetten-gruber B, Bonnet J, Tixier V, Mas A, and Cavalli G. Polycomb-dependent regulatory contacts between distant Hox loci in *Drosophila*. *Cell* 144: 214–226, 2011.
- Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, and Zhao K. High-resolution profiling of histone methylations in the human genome. *Cell* 129: 823–837, 2007.
- Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, and Lander ES. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* 125: 315–326, 2006.
- Bilodeau S, Kagey MH, Frampton GM, Rahl PB, and Young RA. SetDB1 contributes to repression of genes encoding developmental regulators and maintenance of ES cell state. *Genes Dev* 23: 2484–2489, 2009.
- Black JC, Van Rechem C, and Whetstine JR. Histone lysine methylation dynamics: establishment, regulation, and biological impact. *Mol Cell* 48: 491–507, 2012.
- Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK, Bell GW, Otte AP, Vidal M, Gifford DK, Young RA, and Jaenisch R. Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* 441: 349–353, 2006.
- Bracken AP and Helin K. Polycomb group proteins: navigators of lineage pathways led astray in cancer. *Nat Rev Cancer* 9: 773–784, 2009.
- Brien GL, Gambero G, O'Connell DJ, Jerman E, Turner SA, Egan CM, Dunne EJ, Jurgens MC, Wynne K, Piao L, Lohan AJ, Ferguson N, Shi X, Sinha KM, Loftus BJ, Cagney G, and Bracken AP. 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.
- Brower-Toland B, Riddle NC, Jiang H, Huisinga KL, and Elgin SC. Multiple SET methyltransferases are required to maintain normal heterochromatin domains in the genome of *Drosophila melanogaster*. *Genetics* 181: 1303–1319, 2009.
- Cai L, Rothbart SB, Lu R, Xu B, Chen WY, Tripathy A, Rockowitz S, Zheng D, Patel DJ, Allis CD, Strahl BD, Song J, and Wang GG. An H3K36 methylation-engaging Tudor motif of polycomb-like proteins mediates PRC2 complex targeting. *Mol Cell* 49: 571–582, 2013.
- Cao Q, Mani RS, Ateeq B, Dhanasekaran SM, Asangani IA, Prensner JR, Kim JH, Brenner JC, Jing X, Cao X, Wang R, Li Y, Dahiya A, Wang L, Pandhi M, Lonigro RJ, Wu YM, Tomlins SA, Palanisamy N, Qin Z, Yu J, Maher CA, Varambally S, and Chinnaiyan AM. Coordinated regulation of polycomb group complexes through microRNAs in cancer. *Cancer Cell* 20: 187–199, 2011.
- Cao R and Zhang Y. SUZ12 is required for both the histone methyltransferase activity and the silencing function of the EED-EZH2 complex. *Mol Cell* 15: 57–67, 2004.
- Caretti G, Di Padova M, Micales B, Lyons GE, and Sartorelli V. The polycomb Ezh2 methyltransferase regulates muscle gene expression and skeletal muscle differentiation. *Genes Dev* 18: 2627–2638, 2004.
- Chamberlain SJ, Yee D, and Magnuson T. Polycomb repressive complex 2 is dispensable for maintenance of embryonic stem cell pluripotency. *Stem Cells* 26: 1496–1505, 2008.
- Chaturvedi CP, Somasundaram B, Singh K, Carpenedo RL, Stanford WL, Dilworth FJ, and Brand M. Maintenance of gene silencing by the coordinate action of the H3K9 methyltransferase G9a/KMT1C and the H3K4 demethylase Jarid1a/KDM5A. *Proc Natl Acad Sci U S A* 109: 18845–18850, 2012.
- Chen J, Liu H, Liu J, Qi J, Wei B, Yang J, Liang H, Chen Y, Wu Y, Guo L, Zhu J, Zhao X, Peng T, Zhang Y, Chen

- S, Li X, Li D, Wang T, and Pei D. H3K9 methylation is a barrier during somatic cell reprogramming into iPSCs. *Nat Genet* 45: 34–42, 2013.
26. Chen M-W, Hua K-T, Kao H-J, Chi C-C, Wei L-H, Johansson G, Shiah S-G, Chen P-S, Jeng Y-M, Cheng T-Y, Lai T-C, Chang J-S, Jan Y-H, Chien M-H, Yang C-J, Huang M-S, Hsiao M, and Kuo M-L. H3K9 histone methyltransferase G9a promotes lung cancer invasion and metastasis by silencing the cell adhesion molecule Ep-CAM. *Cancer Res* 70: 7830–7840, 2010.
 27. Chen X, Skutt-Kakaria K, Davison J, Ou YL, Choi E, Malik P, Loeb K, Wood B, Georges G, Torok-Storb B, and Paddison PJ. G9a/GLP-dependent histone H3K9me2 patterning during human hematopoietic stem cell lineage commitment. *Genes Dev* 26: 2499–2511, 2012.
 28. Cho HS, Kelly JD, Hayami S, Toyokawa G, Takawa M, Yoshimatsu M, Tsunoda T, Field HI, Neal DE, Ponder BA, Nakamura Y, and Hamamoto R. Enhanced expression of EHMT2 is involved in the proliferation of cancer cells through negative regulation of SIAH1. *Neoplasia* 13: 676–684, 2011.
 29. Collins R and Cheng X. A case study in cross-talk: the histone lysine methyltransferases G9a and GLP. *Nucleic Acids Res* 38: 3503–3511, 2010.
 30. Collins RE, Northrop JP, Horton JR, Lee DY, Zhang X, Stallcup MR, and Cheng X. The ankyrin repeats of G9a and GLP histone methyltransferases are mono- and dimethyllysine binding modules. *Nat Struct Mol Biol* 15: 245–250, 2008.
 31. Dietrich N, Lerdrup M, Landt E, Agrawal-Singh S, Bak M, Tommerup N, Rappsilber J, Sodersten E, and Hansen K. REST-mediated recruitment of polycomb repressor complexes in mammalian cells. *PLoS Genet* 8: e1002494, 2012.
 32. Ding J, Li T, Wang X, Zhao E, Choi JH, Yang L, Zha Y, Dong Z, Huang S, Asara JM, Cui H, and Ding HF. The histone H3 methyltransferase G9A epigenetically activates the serine-glycine synthesis pathway to sustain cancer cell survival and proliferation. *Cell Metab* 18: 896–907, 2013.
 33. Dodge JE, Kang YK, Beppu H, Lei H, and Li E. Histone H3-K9 methyltransferase ESET is essential for early development. *Mol Cell Biol* 24: 2478–2486, 2004.
 34. Dong C, Wu Y, Yao J, Wang Y, Yu Y, Rychahou PG, Evers BM, and Zhou BP. G9a interacts with Snail and is critical for Snail-mediated E-cadherin repression in human breast cancer. *J Clin Invest* 122: 1469–1486, 2012.
 35. Dong C, Yuan T, Wu Y, Wang Y, Fan TW, Miriyala S, Lin Y, Yao J, Shi J, Kang T, Lorkiewicz P, St. Clair D, Hung MC, Evers BM, and Zhou BP. Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. *Cancer Cell* 23: 316–331, 2013.
 36. Endoh M, Endo TA, Endoh T, Isono K, Sharif J, Ohara O, Toyoda T, Ito T, Eskeland R, Bickmore WA, Vidal M, Bernstein BE, and Koseki H. Histone H2A mono-ubiquitination is a crucial step to mediate PRC1-dependent repression of developmental genes to maintain ES cell identity. *PLoS Genet* 8: e1002774, 2012.
 37. Epsztejn-Litman S, Feldman N, Abu-Remaileh M, Shufaro Y, Gerson A, Ueda J, Deplus R, Fuks F, Shinkai Y, Cedar H, and Bergman Y. *De novo* DNA methylation promoted by G9a prevents reprogramming of embryonically silenced genes. *Nat Struct Mol Biol* 15: 1176–1183, 2008.
 38. Eskeland R, Leeb M, Grimes GR, Kress C, Boyle S, Sproul D, Gilbert N, Fan Y, Skoultschi AI, Wutz A, and Bickmore WA. Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination. *Mol Cell* 38: 452–464, 2010.
 39. Ezhkova E, Lien WH, Stokes N, Pasolli HA, Silva JM, and Fuchs E. EZH1 and EZH2 cogovern histone H3K27 trimethylation and are essential for hair follicle homeostasis and wound repair. *Genes Dev* 25: 485–498, 2011.
 40. Ezhkova E, Pasolli HA, Parker JS, Stokes N, Su IH, Hannon G, Tarakhovskiy A, and Fuchs E. Ezh2 orchestrates gene expression for the stepwise differentiation of tissue-specific stem cells. *Cell* 136: 1122–1135, 2009.
 41. Fang R, Barbera AJ, Xu Y, Rutenberg M, Leonor T, Bi Q, Lan F, Mei P, Yuan GC, Lian C, Peng J, Cheng D, Sui G, Kaiser UB, Shi Y, and Shi YG. Human LSD2/KDM1b/AOF1 regulates gene transcription by modulating intragenic H3K4me2 methylation. *Mol Cell* 39: 222–233, 2010.
 42. Feldman N, Gerson A, Fang J, Li E, Zhang Y, Shinkai Y, Cedar H, and Bergman Y. G9a-mediated irreversible epigenetic inactivation of Oct-3/4 during early embryogenesis. *Nat Cell Biol* 8: 188–194, 2006.
 43. Ferrari KJ, Scelfo A, Jammula S, Cuomo A, Barozzi I, Stutzer A, Fischle W, Bonaldi T, and Pasini D. Polycomb-dependent H3K27me1 and H3K27me2 regulate active transcription and enhancer fidelity. *Mol Cell* 53: 49–62, 2014.
 44. Fragola G, Germain PL, Laise P, Cuomo A, Blasimme A, Gross F, Signaroldi E, Bucci G, Sommer C, Pruneri G, Mazzarol G, Bonaldi T, Mostoslavsky G, Casola S, and Testa G. Cell reprogramming requires silencing of a core subset of polycomb targets. *PLoS Genet* 9: e1003292, 2013.
 45. Francis NJ, Kingston RE, and Woodcock CL. Chromatin compaction by a polycomb group protein complex. *Science* 306: 1574–1577, 2004.
 46. Fritsch L, Robin P, Mathieu JR, Souidi M, Hinaux H, Rougeulle C, Harel-Bellan A, Ameyar-Zazoua M, and Ait-Si-Ali S. 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.
 47. Greer EL and Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat Rev Genet* 13: 343–357, 2012.
 48. Guelen L, Pagie L, Brasset E, Meuleman W, Faza MB, Talhout W, Eussen BH, de Klein A, Wessels L, de Laat W, and van Steensel B. Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. *Nature* 453: 948–951, 2008.
 49. Herranz N, Pasini D, Diaz VM, Franci C, Gutierrez A, Dave N, Escriva M, Hernandez-Munoz I, Di Croce L, Helin K, Garcia de Herreros A, and Peiro S. Polycomb complex 2 is required for E-cadherin repression by the Snail1 transcription factor. *Mol Cell Biol* 28: 4772–4781, 2008.
 50. Huang J, Dorsey J, Chuikov S, Perez-Burgos L, Zhang X, Jenuwein T, Reinberg D, and Berger SL. G9a and Glp methylate lysine 373 in the tumor suppressor p53. *J Biol Chem* 285: 9636–9641, 2010.
 51. This reference has been deleted.
 52. Iwase S and Shi Y. Histone and DNA modifications in mental retardation. *Prog Drug Res* 67: 147–173, 2011.

53. Jones PA and Baylín SB. The epigenomics of cancer. *Cell* 128: 683–692, 2007.
54. Juan AH, Derfoul A, Feng X, Ryall JG, Dell’Orso S, Pasut A, Zare H, Simone JM, Rudnicki MA, and Sartorelli V. Polycomb EZH2 controls self-renewal and safeguards the transcriptional identity of skeletal muscle stem cells. *Genes Dev* 25: 789–794, 2011.
55. Juan AH, Kumar RM, Marx JG, Young RA, and Sartorelli V. Mir-214-dependent regulation of the polycomb protein Ezh2 in skeletal muscle and embryonic stem cells. *Mol Cell* 36: 61–74, 2009.
56. Khuong-Quang DA, Buczkowicz P, Rakopoulos P, Liu XY, Fontebasso AM, Bouffet E, Bartels U, Albrecht S, Schwartzentruber J, Letourneau L, Bourgey M, Bourque G, Montpetit A, Bourret G, Lepage P, Fleming A, Lichter P, Kool M, von Deimling A, Sturm D, Korshunov A, Faury D, Jones DT, Majewski J, Pfister SM, Jabado N, and Hawkins C. K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol* 124: 439–447, 2012.
57. Kim H, Kang K, and Kim J. AEBP2 as a potential targeting protein for polycomb repression complex PRC2. *Nucleic Acids Res* 37: 2940–2950, 2009.
58. Kind J, Pagie L, Ortobozkoyun H, Boyle S, de Vries SS, Janssen H, Amendola M, Nolen LD, Bickmore WA, and van Steensel B. Single-cell dynamics of genome-nuclear lamina interactions. *Cell* 153: 178–192, 2013.
59. Kleefstra T, Brunner HG, Amiel J, Oudakker AR, Nillesen WM, Magee A, Genevieve D, Cormier-Daire V, van Esch H, Fryns JP, Hamel BC, Siskerman EA, de Vries BB, and van Bokhoven H. Loss-of-function mutations in euchromatin histone methyl transferase 1 (EHMT1) cause the 9q34 subtelomeric deletion syndrome. *Am J Hum Genet* 79: 370–377, 2006.
60. Kleefstra T, Smidt M, Banning MJ, Oudakker AR, Van Esch H, de Brouwer AP, Nillesen W, Siskerman EA, Hamel BC, de Bruijn D, Fryns JP, Yntema HG, Brunner HG, de Vries BB, and van Bokhoven H. Disruption of the gene euchromatin histone methyl transferase 1 (EHMT1) is associated with the 9q34 subtelomeric deletion syndrome. *J Med Genet* 42: 299–306, 2005.
- 60a. Kleefstra T, van Zelst-Stams WA, Nillesen WM, Cormier-Daire V, Houge G, Foulds N, van Dooren M, Willemsen MH, Pfundt R, Turner A, Wilson M, McGaughan J, Rauch A, Zenker M, Adam MP, Innes M, Davies C, López AG, Casalone R, Weber A, Brueton LA, Navarro AD, Braló MP, Venselaar H, Stegmann SP, Yntema HG, van Bokhoven H, Brunner HG. Further clinical and molecular delineation of the 9q subtelomeric deletion syndrome supports a major contributor of EHMT1 haploinsufficiency to the core phenotype. *J Med Genet* 46: 598–606, 2009.
61. Kleer CG, Cao Q, Varambally S, Shen R, Ota I, Tomlins SA, Ghosh D, Sewalt RG, Otte AP, Hayes DF, Sabel MS, Livant D, Weiss SJ, Rubin MA, and Chinnaiyan AM. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc Natl Acad Sci U S A* 100: 11606–11611, 2003.
62. Kondo Y, Shen L, Ahmed S, Boumber Y, Sekido Y, Haddad BR, and Issa JP. Downregulation of histone H3 lysine 9 methyltransferase G9a induces centrosome disruption and chromosome instability in cancer cells. *PLoS One* 3: e2037, 2008.
63. Kouzarides T. Chromatin modifications and their function. *Cell* 128: 693–705, 2007.
- 63a. Klamer JM, van Bokhoven H. Genetic and epigenetic defects in mental retardation. *Int J Biochem Cell Biol* 41: 96–107, 2009.
64. Ku M, Koche RP, Rheinbay E, Mendenhall EM, Endoh M, Mikkelsen TS, Presser A, Nusbaum C, Xie X, Chi AS, Adli M, Kasif S, Ptaszek LM, Cowan CA, Lander ES, Koseki H, and Bernstein BE. Genomewide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains. *PLoS Genet* 4: e1000242, 2008.
65. Lachner M, O’Carroll D, Rea S, Mechtler K, and Jenuwein T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* 410: 116–120, 2001.
66. Landeira D, Sauer S, Poot R, Dvorkina M, Mazzarella L, Jorgensen HF, Pereira CF, Leleu M, Piccolo FM, Spivakov M, Brookes E, Pombo A, Fisher C, Skarnes WC, Snoek T, Bezstarosti K, Demmers J, Klose RJ, Casanova M, Tavares L, Brockdorff N, Merckenschlager M, and Fisher AG. Jarid2 is a PRC2 component in embryonic stem cells required for multi-lineage differentiation and recruitment of PRC1 and RNA Polymerase II to developmental regulators. *Nat Cell Biol* 12: 618–624, 2010.
67. Lee JS, Kim Y, Kim IS, Kim B, Choi HJ, Lee JM, Shin HJ, Kim JH, Kim JY, Seo SB, Lee H, Binda O, Gozani O, Semenza GL, Kim M, Kim KI, Hwang D, and Baek SH. Negative regulation of hypoxic responses via induced Reptin methylation. *Mol Cell* 39: 71–85, 2010.
68. Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, Chevalier B, Johnstone SE, Cole MF, Isono K, Koseki H, Fuchikami T, Abe K, Murray HL, Zucker JP, Yuan B, Bell GW, Herbolsheimer E, Hannett NM, Sun K, Odom DT, Otte AP, Volkert TL, Bartel DP, Melton DA, Gifford DK, Jaenisch R, and Young RA. Control of developmental regulators by polycomb in human embryonic stem cells. *Cell* 125: 301–313, 2006.
69. Lehnertz B, Pabst C, Su L, Miller M, Liu F, Yi L, Zhang R, Kros J, Yung E, Kirschner J, Rosten P, Underhill TM, Jin J, Hebert J, Sauvageau G, Humphries RK, and Rossi FM. The methyltransferase G9a regulates HoxA9-dependent transcription in AML. *Genes Dev* 28: 317–327, 2014.
70. Lehnertz B, Ueda Y, Derijck AA, Braunschweig U, Perez-Burgos L, Kubicek S, Chen T, Li E, Jenuwein T, and Peters AH. Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. *Curr Biol* 13: 1192–1200, 2003.
71. Lewis PW, Muller MM, Koletsky MS, Cordero F, Lin S, Banaszynski LA, Garcia BA, Muir TW, Becher OJ, and Allis CD. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. *Science* 340: 857–861, 2013.
72. Li G, Margueron R, Ku M, Chambon P, Bernstein BE, and Reinberg D. Jarid2 and PRC2, partners in regulating gene expression. *Genes Dev* 24: 368–380, 2010.
73. Li H, Rauch T, Chen ZX, Szabo PE, Riggs AD, and Pfeifer GP. 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.
74. Li J, Hart RP, Mallimo EM, Swerdel MR, Kusnecov AW, and Herrup K. EZH2-mediated H3K27 trimethylation mediates neurodegeneration in ataxia-telangiectasia. *Nat Neurosci* 16: 1745–1753, 2013.

75. Li J, Lin Q, Yoon HG, Huang ZQ, Strahl BD, Allis CD, and Wong J. Involvement of histone methylation and phosphorylation in regulation of transcription by thyroid hormone receptor. *Mol Cell Biol* 22: 5688–5697, 2002.
76. Li Q, Su Z, Xu X, Liu G, Song X, Wang R, Sui X, Liu T, Chang X, and Huang D. AS1DHRS4, a head-to-head natural antisense transcript, silences the DHRS4 gene cluster in cis and trans. *Proc Natl Acad Sci U S A* 109: 14110–14115, 2012.
77. Ling BM, Bharathy N, Chung TK, Kok WK, Li S, Tan YH, Rao VK, Gopinadhan S, Sartorelli V, Walsh MJ, and Taneja R. Lysine methyltransferase G9a methylates the transcription factor MyoD and regulates skeletal muscle differentiation. *Proc Natl Acad Sci U S A* 109: 841–846, 2012.
78. Lohr JG, Stojanov P, Lawrence MS, Auclair D, Chapuy B, Sougnez C, Cruz-Gordillo P, Knoechel B, Asmann YW, Slager SL, Novak AJ, Dogan A, Ansell SM, Link BK, Zou L, Gould J, Saksena G, Stransky N, Rangel-Escareno C, Fernandez-Lopez JC, Hidalgo-Miranda A, Melendez-Zajgla J, Hernandez-Lemus E, Schwarz-Cruz y Celis A, Imaz-Rosshandler I, Ojesina AI, Jung J, Peadarallu CS, Lander ES, Habermann TM, Cerhan JR, Shipp MA, Getz G, and Golub TR. Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. *Proc Natl Acad Sci U S A* 109: 3879–3884, 2012.
79. Loyola A, Tagami H, Bonaldi T, Roche D, Quivy JP, Imhof A, Nakatani Y, Dent SYR, and Almouzni G. The HP1 α -CAF1-SetDB1-containing complex provides H3K9me1 for Suv39-mediated K9me3 in pericentric heterochromatin. *EMBO Rep* 10: 769–775, 2009.
80. Lynch MD, Smith AJ, De Gobbi M, Flenley M, Hughes JR, Vernimmen D, Ayyub H, Sharpe JA, Sloane-Stanley JA, Sutherland L, Meek S, Burdon T, Gibbons RJ, Garrick D, and Higgs DR. An interspecies analysis reveals a key role for unmethylated CpG dinucleotides in vertebrate polycomb complex recruitment. *EMBO J* 31: 317–329, 2012.
81. Margueron R, Li G, Sarma K, Blais A, Zavdil J, Woodcock CL, Dynlacht BD, and Reinberg D. Ezh1 and Ezh2 maintain repressive chromatin through different mechanisms. *Mol Cell* 32: 503–518, 2008.
82. Margueron R and Reinberg D. The polycomb complex PRC2 and its mark in life. *Nature* 469: 343–349, 2011.
83. Maze I, Covington HE, 3rd, Dietz DM, LaPlant Q, Renthall W, Russo SJ, Mechanic M, Mouzon E, Neve RL, Haggarty SJ, Ren Y, Sampath SC, Hurd YL, Greengard P, Tarakhovskiy A, Schaefer A, and Nestler EJ. Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science* 327: 213–216, 2010.
84. McCabe MT, Ott HM, Ganji G, Korenchuk S, Thompson C, Van Aller GS, Liu Y, Graves AP, Della Pietra A, 3rd, Diaz E, LaFrance LV, Mellinger M, Duquenne C, Tian X, Kruger RG, McHugh CF, Brandt M, Miller WH, Dhanak D, Verma SK, Tummino PJ, and Creasy CL. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature* 492: 108–112, 2012.
85. Mendenhall EM, Koche RP, Truong T, Zhou VW, Issac B, Chi AS, Ku M, and Bernstein BE. GC-rich sequence elements recruit PRC2 in mammalian ES cells. *PLoS Genet* 6: e1001244, 2010.
86. Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim TK, Koche RP, Lee W, Mendenhall E, O'Donovan A, Presser A, Russ C, Xie X, Meissner A, Wernig M, Jaenisch R, Nusbaum C, Lander ES, and Bernstein BE. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* 448: 553–560, 2007.
87. Mohn F, Weber M, Rebhan M, Roloff TC, Richter J, Stadler MB, Bibel M, and Schubeler D. Lineage-specific polycomb targets and *de novo* DNA methylation define restriction and potential of neuronal progenitors. *Mol Cell* 30: 755–766, 2008.
88. Moore KE, Carlson SM, Camp ND, Cheung P, James RG, Chua KF, Wolf-Yadlin A, and Gozani O. A general molecular affinity strategy for global detection and proteomic analysis of lysine methylation. *Mol Cell* 50: 444–456, 2013.
89. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, Paul JE, Boyle M, Woolcock BW, Kuchenbauer F, Yap D, Humphries RK, Griffith OL, Shah S, Zhu H, Kimbara M, Shashkin P, Charlot JF, Tcherpakov M, Corbett R, Tam A, Varhol R, Smailus D, Mokska M, Zhao Y, Delaney A, Qian H, Birol I, Schein J, Moore R, Holt R, Horsman DE, Connors JM, Jones S, Aparicio S, Hirst M, Gascoyne RD, and Marra MA. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet* 42: 181–185, 2010.
90. Mousavi K, Zare H, Wang AH, and Sartorelli V. Polycomb protein Ezh1 promotes RNA polymerase II elongation. *Mol Cell* 45: 255–262, 2012.
91. Mozzetta C, Consalvi S, Saccone V, Forcales SV, Puri PL, and Palacios D. Selective control of Pax7 expression by TNF-activated p38 α /polycomb repressive complex 2 (PRC2) signaling during muscle satellite cell differentiation. *Cell Cycle* 10: 191–198, 2011.
92. Mozzetta C, Pontis J, Fritsch L, Robin P, Portoso M, Proux C, Margueron R, and Ait-Si-Ali S. The histone H3 lysine 9 methyltransferases G9a and GLP regulate polycomb repressive complex 2-mediated gene silencing. *Mol Cell* 53: 277–289, 2014.
93. Nagano T, Mitchell JA, Sanz LA, Pauler FM, Ferguson-Smith AC, Feil R, and Fraser P. The air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. *Science* 322: 1717–1720, 2008.
94. Negre N, Hennein J, Sun LV, Lavrov S, Bellis M, White KP, and Cavalli G. Chromosomal distribution of PcG proteins during *Drosophila* development. *PLoS Biol* 4: e170, 2006.
95. Nekrasov M, Klymenko T, Fraterman S, Papp B, Oktaba K, Kocher T, Cohen A, Stunnenberg HG, Wilm M, and Muller J. Pcl-PRC2 is needed to generate high levels of H3-K27 trimethylation at polycomb target genes. *EMBO J* 26: 4078–4088, 2007.
96. Nikoloski G, Langemeijer SM, Kuiper RP, Knops R, Massop M, Tonnissen ER, van der Heijden A, Scheele TN, Vandenberghe P, de Witte T, van der Reijden BA, and Jansen JH. Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. *Nat Genet* 42: 665–667, 2010.
97. Ntziachristos P, Tsirigos A, Van Vlierberghe P, Nedjic J, Trimarchi T, Flaherty MS, Ferrer-Marco D, da Ros V, Tang Z, Siegle J, Asp P, Hadler M, Rigo I, De Keersmaecker K, Patel J, Huynh T, Utro F, Pogliano S, Samon JB, Paietta E, Racevskis J, Rowe JM, Rabadan R, Levine RL, Brown S, Pflumio F, Dominguez M, Ferrando A, and

- Aifantis I. Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. *Nat Med* 18: 298–301, 2012.
98. O'Carroll D, Erhardt S, Pagani M, Barton SC, Surani MA, and Jenuwein T. The polycomb-group gene *Ezh2* is required for early mouse development. *Mol Cell Biol* 21: 4330–4336, 2001.
 99. Ogawa H, Ishiguro K, Gaubatz S, Livingston DM, and Nakatani Y. A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. *Science* 296: 1132–1136, 2002.
 100. Ohno H, Shinoda K, Ohyama K, Sharp LZ, and Kajimura S. EHMT1 controls brown adipose cell fate and thermogenesis through the PRDM16 complex. *Nature* 504: 163–167, 2013.
 101. Onder TT, Kara N, Cherry A, Sinha AU, Zhu N, Bernt KM, Cahan P, Marcarci BO, Unternaehrer J, Gupta PB, Lander ES, Armstrong SA, and Daley GQ. Chromatin-modifying enzymes as modulators of reprogramming. *Nature* 483: 598–602, 2012.
 102. Osipovich O, Milley R, Meade A, Tachibana M, Shinkai Y, Krangel MS, and Oltz EM. Targeted inhibition of V(D)J recombination by a histone methyltransferase. *Nat Immunol* 5: 309–316, 2004.
 103. Palacios D, Mozzetta C, Consalvi S, Caretti G, Saccone V, Proserpio V, Marquez VE, Valente S, Mai A, Forcales SV, Sartorelli V, and Puri PL. TNF/p38alpha/polycomb signaling to Pax7 locus in satellite cells links inflammation to the epigenetic control of muscle regeneration. *Cell Stem Cell* 7: 455–469, 2010.
 104. Pan G, Tian S, Nie J, Yang C, Ruotti V, Wei H, Jonsdottir GA, Stewart R, and Thomson JA. Whole-genome analysis of histone H3 lysine 4 and lysine 27 methylation in human embryonic stem cells. *Cell Stem Cell* 1: 299–312, 2007.
 105. Pandey RR, Mondal T, Mohammad F, Enroth S, Redrup L, Komorowski J, Nagano T, Mancini-Dinardo D, and Kanduri C. *Kcnq1ot1* antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. *Mol Cell* 32: 232–246, 2008.
 106. Pasini D, Bracken AP, Hansen JB, Capillo M, and Helin K. The polycomb group protein *Suz12* is required for embryonic stem cell differentiation. *Mol Cell Biol* 27: 3769–3779, 2007.
 107. Pasini D, Bracken AP, Jensen MR, Lazzarini Denchi E, and Helin K. *Suz12* is essential for mouse development and for EZH2 histone methyltransferase activity. *EMBO J* 23: 4061–4071, 2004.
 108. Pasini D, Cloos PA, Walfridsson J, Olsson L, Bukowski JP, Johansen JV, Bak M, Tommerup N, Rappsilber J, and Helin K. *JARID2* regulates binding of the polycomb repressive complex 2 to target genes in ES cells. *Nature* 464: 306–310, 2010.
 109. Pasini D, Hansen KH, Christensen J, Agger K, Cloos PA, and Helin K. Coordinated regulation of transcriptional repression by the RBP2 H3K4 demethylase and polycomb-repressive complex 2. *Genes Dev* 22: 1345–1355, 2008.
 110. Pasqualucci L, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, Kasper LH, Lerach S, Tang H, Ma J, Rossi D, Chadburn A, Murty VV, Mullighan CG, Gaidano G, Rabadan R, Brindle PK, and Dalla-Favera R. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. *Nature* 471: 189–195, 2011.
 111. Patnaik D, Chin HG, Esteve PO, Benner J, Jacobsen SE, and Pradhan S. Substrate specificity and kinetic mechanism of mammalian G9a histone H3 methyltransferase. *J Biol Chem* 279: 53248–53258, 2004.
 112. Pauler FM, Sloane MA, Huang R, Regha K, Koerner MV, Tamir I, Sommer A, Aszodi A, Jenuwein T, and Barlow DP. 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.
 113. Peng JC, Valouev A, Swigut T, Zhang J, Zhao Y, Sidow A, and Wysocka J. *Jarid2/Jumonji* coordinates control of PRC2 enzymatic activity and target gene occupancy in pluripotent cells. *Cell* 139: 1290–1302, 2009.
 114. Pereira CF, Piccolo FM, Tsubouchi T, Sauer S, Ryan NK, Bruno L, Landeira D, Santos J, Banito A, Gil J, Koseki H, Merkenschlager M, and Fisher AG. ESCs require PRC2 to direct the successful reprogramming of differentiated cells toward pluripotency. *Cell Stem Cell* 6: 547–556, 2010.
 115. Peric-Hupkes D, Meuleman W, Pagie L, Bruggeman SW, Solovei I, Brugman W, Graf S, Flicek P, Kerkhoven RM, van Lohuizen M, Reinders M, Wessels L, and van Steensel B. Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. *Mol Cell* 38: 603–613, 2010.
 116. Peters AH, Kubicek S, Mechtler K, O'Sullivan RJ, Derijck AA, Perez-Burgos L, Kohlmaier A, Opravil S, Tachibana M, Shinkai Y, Martens JH, and Jenuwein T. Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. *Mol Cell* 12: 1577–1589, 2003.
 117. Peters AH, O'Carroll D, Scherthan H, Mechtler K, Sauer S, Schofer C, Weipoltshammer K, Pagani M, Lachner M, Kohlmaier A, Opravil S, Doyle M, Sibilia M, and Jenuwein T. Loss of the *Suv39h* histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell* 107: 323–337, 2001.
 118. Plath K, Fang J, Mlynarczyk-Evans SK, Cao R, Worringer KA, Wang H, de la Cruz CC, Otte AP, Panning B, and Zhang Y. Role of histone H3 lysine 27 methylation in X inactivation. *Science* 300: 131–135, 2003.
 119. Puri PL and Sartorelli V. Regulation of muscle regulatory factors by DNA-binding, interacting proteins, and post-transcriptional modifications. *J Cell Physiol* 185: 155–173, 2000.
 120. Rathert P, Dhayalan A, Murakami M, Zhang X, Tamas R, Jurkowska R, Komatsu Y, Shinkai Y, Cheng X, and Jeltsch A. Protein lysine methyltransferase G9a acts on non-histone targets. *Nat Chem Biol* 4: 344–346, 2008.
 121. Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M, Opravil S, Mechtler K, Ponting CP, Allis CD, and Jenuwein T. Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 406: 593–599, 2000.
 122. Regha K, Sloane MA, Huang R, Pauler FM, Warczok KE, Melikant B, Radolf M, Martens JH, Schotta G, Jenuwein T, and Barlow DP. Active and repressive chromatin are interspersed without spreading in an imprinted gene cluster in the mammalian genome. *Mol Cell* 27: 353–366, 2007.
 123. Rice JC, Briggs SD, Ueberheide B, Barber CM, Shabanowitz J, Hunt DF, Shinkai Y, and Allis CD. Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. *Mol Cell* 12: 1591–1598, 2003.
 124. Ringrose L, Rehmsmeier M, Dura JM, and Paro R. Genome-wide prediction of polycomb/trithorax response

- elements in *Drosophila melanogaster*. *Dev Cell* 5: 759–771, 2003.
125. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Bruggmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E, and Chang HY. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell* 129: 1311–1323, 2007.
 126. Ropra A, Qazi R, Schoenike B, Daley TJ, and Morrison JF. Localized domains of G9a-mediated histone methylation are required for silencing of neuronal genes. *Mol Cell* 14: 727–738, 2004.
 127. Sampath SC, Marazzi I, Yap KL, Krutchinsky AN, Mecklenbrauker I, Viale A, Rudensky E, Zhou MM, Chait BT, and Tarakhovskiy A. Methylation of a histone mimic within the histone methyltransferase G9a regulates protein complex assembly. *Mol Cell* 27: 596–608, 2007.
 128. Sarma K, Margueron R, Ivanov A, Pirrotta V, and Reinberg D. Ezh2 requires PHF1 to efficiently catalyze H3 lysine 27 trimethylation *in vivo*. *Mol Cell Biol* 28: 2718–2731, 2008.
 129. Schaefer A, Sampath SC, Intrator A, Min A, Gertler TS, Surmeier DJ, Tarakhovskiy A, and Greengard P. Control of cognition and adaptive behavior by the GLP/G9a epigenetic suppressor complex. *Neuron* 64: 678–691, 2009.
 130. Schwartz YB, Kahn TG, Nix DA, Li XY, Bourgon R, Biggin M, and Pirrotta V. Genome-wide analysis of polycomb targets in *Drosophila melanogaster*. *Nat Genet* 38: 700–705, 2006.
 131. Schwartz YB and Pirrotta V. Polycomb silencing mechanisms and the management of genomic programmes. *Nat Rev Genet* 8: 9–22, 2007.
 132. Schwartzenuber J, Korshunov A, Liu XY, Jones DT, Pfaff E, Jacob K, Sturm D, Fontebasso AM, Quang DA, Tonjes M, Hovestadt V, Albrecht S, Kool M, Nantel A, Konermann C, Lindroth A, Jager N, Rausch T, Ryzhova M, Korbel JO, Hielscher T, Hauser P, Garami M, Klekner A, Bogner L, Ebinger M, Schuhmann MU, Scheurlen W, Pekrun A, Fruhwald MC, Roggendorf W, Kramm C, Durken M, Atkinson J, Lepage P, Montpetit A, Zakrzewska M, Zakrzewski K, Liberski PP, Dong Z, Siegel P, Kulozik AE, Zapatka M, Guha A, Malkin D, Felsberg J, Reifemberger G, von Deimling A, Ichimura K, Collins VP, Witt H, Milde T, Witt O, Zhang C, Castelo-Branco P, Lichter P, Faury D, Tabori U, Plass C, Majewski J, Pfister SM, and Jabado N. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature* 482: 226–231, 2012.
 133. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scime A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, and Spiegelman BM. PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454: 961–967, 2008.
 134. Shen X, Kim W, Fujiwara Y, Simon MD, Liu Y, Mysliwiec MR, Yuan GC, Lee Y, and Orkin SH. Jumonji modulates polycomb activity and self-renewal versus differentiation of stem cells. *Cell* 139: 1303–1314, 2009.
 135. Shen X, Liu Y, Hsu YJ, Fujiwara Y, Kim J, Mao X, Yuan GC, and Orkin SH. 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.
 136. Shi Y, Sawada J, Sui G, Affar el B, Whetstone JR, Lan F, Ogawa H, Luke MP, Nakatani Y, and Shi Y. Coordinated histone modifications mediated by a CtBP co-repressor complex. *Nature* 422: 735–738, 2003.
 137. Shinkai Y and Tachibana M. H3K9 methyltransferase G9a and the related molecule GLP. *Genes Dev* 25: 781–788, 2011.
 138. Shirato H, Ogawa S, Nakajima K, Inagawa M, Kojima M, Tachibana M, Shinkai Y, and Takeuchi T. A jumonji (Jarid2) protein complex represses cyclin D1 expression by methylation of histone H3-K9. *J Biol Chem* 284: 733–739, 2009.
 139. Simon C, Chagraoui J, Kros J, Gendron P, Wilhelm B, Lemieux S, Boucher G, Chagnon P, Drouin S, Lambert R, Rondeau C, Bilodeau A, Lavallée S, Sauvageau M, Hébert J, and Sauvageau G. A key role for EZH2 and associated genes in mouse and human adult T-cell acute leukemia. *Genes Dev* 26: 651–656, 2012.
 140. Simon JA and Kingston RE. Occupying chromatin: polycomb mechanisms for getting to genomic targets, stopping transcriptional traffic, and staying put. *Mol Cell* 49: 808–824, 2013.
 141. Sing A, Pannell D, Karaiskakis A, Sturgeon K, Djabali M, Ellis J, Lipshitz HD, and Cordes SP. A vertebrate polycomb response element governs segmentation of the posterior hindbrain. *Cell* 138: 885–897, 2009.
 142. Sridharan R, Gonzales-Cope M, Chronis C, Bonora G, McKee R, Huang C, Patel S, Lopez D, Mishra N, Pellegrini M, Carey M, Garcia BA, and Plath K. Proteomic and genomic approaches reveal critical functions of H3K9 methylation and heterochromatin protein-1gamma in reprogramming to pluripotency. *Nat Cell Biol* 15: 872–882, 2013.
 143. Stock JK, Giadrossi S, Casanova M, Brookes E, Vidal M, Koseki H, Brockdorff N, Fisher AG, and Pombo A. Ring1-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in mouse ES cells. *Nat Cell Biol* 9: 1428–1435, 2007.
 144. Stojic L, Jasencakova Z, Prezioso C, Stutzer A, Bodega B, Pasini D, Klingberg R, Mozzetta C, Margueron R, Puri PL, Schwarzer D, Helin K, Fischle W, and Orlando V. Chromatin regulated interchange between polycomb repressive complex 2 (PRC2)-Ezh2 and PRC2-Ezh1 complexes controls myogenin activation in skeletal muscle cells. *Epigenetics Chromatin* 4: 16, 2011.
 145. Tachibana M, Sugimoto K, Fukushima T, and 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.
 146. Tachibana M, Sugimoto K, Nozaki M, Ueda J, Ohta T, Ohki M, Fukuda M, Takeda N, Niida H, Kato H, and Shinkai Y. 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.
 147. This reference has been deleted.
 148. Tachibana M, Ueda J, Fukuda M, Takeda N, Ohta T, Iwanari H, Sakihama T, Kodama T, Hamakubo T, and Shinkai Y. 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.
 149. Tahiliani M, Mei P, Fang R, Leonor T, Rutenberg M, Shimizu F, Li J, Rao A, and Shi Y. The histone H3K4 demethylase SMCX links REST target genes to X-linked mental retardation. *Nature* 447: 601–605, 2007.
 150. Tan M, Luo H, Lee S, Jin F, Yang JS, Montellier E, Buchou T, Cheng Z, Rousseaux S, Rajagopal N, Lu Z, Ye

- Z, Zhu Q, Wysocka J, Ye Y, Khochbin S, Ren B, and Zhao Y. Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. *Cell* 146: 1016–1028, 2011.
151. Tanay A, O'Donnell AH, Damelin M, and Bestor TH. Hyperconserved CpG domains underlie polycomb-binding sites. *Proc Natl Acad Sci U S A* 104: 5521–5526, 2007.
 152. Tolhuis B, de Wit E, Muijers I, Teunissen H, Talhout W, van Steensel B, and van Lohuizen M. Genome-wide profiling of PRC1 and PRC2 polycomb chromatin binding in *Drosophila melanogaster*. *Nat Genet* 38: 694–699, 2006.
 153. Towbin BD, Gonzalez-Aguilera C, Sack R, Gaidatzis D, Kalck V, Meister P, Askjaer P, and Gasser SM. Step-wise methylation of histone H3K9 positions heterochromatin at the nuclear periphery. *Cell* 150: 934–947, 2012.
 154. Trojer P, Zhang J, Yonezawa M, Schmidt A, Zheng H, Jenuwein T, and Reinberg D. 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.
 155. Ueda T, Sanada M, Matsui H, Yamasaki N, Honda ZI, Shih LY, Mori H, Inaba T, Ogawa S, and Honda H. EED mutants impair polycomb repressive complex 2 in myelodysplastic syndrome and related neoplasms. *Leukemia* 26: 2557–2560, 2012.
 156. Vandel L, Nicolas E, Vaute O, Ferreira R, Ait-Si-Ali S, and Trouche D. Transcriptional repression by the retinoblastoma protein through the recruitment of a histone methyltransferase. *Mol Cell Biol* 21: 6484–6494, 2001.
 157. Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, Ghosh D, Pienta KJ, Sewalt RG, Otte AP, Rubin MA, and Chinnaiyan AM. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* 419: 624–629, 2002.
 158. Vassen L, Fiolka K, and Moroy T. Gfi1b alters histone methylation at target gene promoters and sites of gamma-satellite containing heterochromatin. *EMBO J* 25: 2409–2419, 2006.
 159. Vella P, Barozzi I, Cuomo A, Bonaldi T, and Pasini D. Yin Yang 1 extends the Myc-related transcription factors network in embryonic stem cells. *Nucleic Acids Res* 40: 3403–3418, 2012.
 160. Wagschal A, Sutherland HG, Woodfine K, Henckel A, Chebli K, Schulz R, Oakey RJ, Bickmore WA, and Feil R. G9a histone methyltransferase contributes to imprinting in the mouse placenta. *Mol Cell Biol* 28: 1104–1113, 2008.
 161. Walker E, Chang WY, Hunkapiller J, Cagney G, Garcha K, Torchia J, Krogan NJ, Reiter JF, and Stanford WL. Polycomb-like 2 associates with PRC2 and regulates transcriptional networks during mouse embryonic stem cell self-renewal and differentiation. *Cell Stem Cell* 6: 153–166, 2010.
 162. Wang H, An W, Cao R, Xia L, Erdjument-Bromage H, Chatton B, Tempst P, Roeder RG, and Zhang Y. mAM facilitates conversion by ESET of dimethyl to trimethyl lysine 9 of histone H3 to cause transcriptional repression. *Mol Cell* 12: 475–487, 2003.
 163. Wang J and Abate-Shen C. The MSX1 homeoprotein recruits G9a methyltransferase to repressed target genes in myoblast cells. *PLoS One* 7: e37647, 2012.
 164. Wang J, Kumar RM, Biggs VJ, Lee H, Chen Y, Kagey MH, Young RA, and Abate-Shen C. The Msx1 homeoprotein recruits polycomb to the nuclear periphery during development. *Dev Cell* 21: 575–588, 2011.
 165. Weiss T, Hergeth S, Zeissler U, Izzo A, Tropberger P, Zee BM, Dunder M, Garcia BA, Daujat S, and Schneider R. Histone H1 variant-specific lysine methylation by G9a/KMT1C and Glp1/KMT1D. *Epigenetics Chromatin* 3: 7, 2010.
 166. Wen B, Wu H, Shinkai Y, Irizarry RA, and Feinberg AP. Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. *Nat Genet* 41: 246–250, 2009.
 167. Woo CJ, Kharchenko PV, Daheron L, Park PJ, and Kingston RE. A region of the human HOXD cluster that confers polycomb-group responsiveness. *Cell* 140: 99–110, 2010.
 168. Wu G, Broniscer A, McEachron TA, Lu C, Paugh BS, Becksfors J, Qu C, Ding L, Huether R, Parker M, Zhang J, Gajjar A, Dyer MA, Mullighan CG, Gilbertson RJ, Mardis ER, Wilson RK, Downing JR, Ellison DW, Zhang J, Baker SJ, and St. Jude Children's Research Hospital-Washington University Pediatric Cancer Genome Project. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat Genet* 44: 251–253, 2012.
 169. Wu H, Chen X, Xiong J, Li Y, Li H, Ding X, Liu S, Chen S, Gao S, and Zhu B. Histone methyltransferase G9a contributes to H3K27 methylation *in vivo*. *Cell Res* 21: 365–367, 2011.
 170. Xie H, Xu J, Hsu JH, Nguyen M, Fujiwara Y, Peng C, and Orkin SH. Polycomb repressive complex 2 regulates normal hematopoietic stem cell function in a developmental-stage-specific manner. *Cell Stem Cell* 14: 68–80, 2014.
 171. Yamamizu K, Fujihara M, Tachibana M, Katayama S, Takahashi A, Hara E, Imai H, Shinkai Y, and Yamashita JK. Protein kinase A determines timing of early differentiation through epigenetic regulation with G9a. *Cell Stem Cell* 10: 759–770, 2012.
 172. Yokochi T, Poduch K, Ryba T, Lu J, Hiratani I, Tachibana M, Shinkai Y, and Gilbert DM. G9a selectively represses a class of late-replicating genes at the nuclear periphery. *Proc Natl Acad Sci U S A* 106: 19363–19368, 2009.
 173. Yoon J, Lee KS, Park JS, Yu K, Paik SG, and Kang YK. dSETDB1 and SU(VAR)3–9 sequentially function during germline-stem cell differentiation in *Drosophila melanogaster*. *PLoS One* 3: e2234, 2008.
 174. Yu Y, Song C, Zhang Q, DiMaggio PA, Garcia BA, York A, Carey MF, and Grunstein M. Histone H3 lysine 56 methylation regulates DNA replication through its interaction with PCNA. *Mol Cell* 46: 7–17, 2012.
 175. Yuan P, Han J, Guo G, Orlov YL, Huss M, Loh YH, Yaw LP, Robson P, Lim B, and Ng HH. Eset partners with Oct4 to restrict extraembryonic trophoblast lineage potential in embryonic stem cells. *Genes Dev* 23: 2507–2520, 2009.
 176. Zdzienbilo D, Li X, Lin Q, Zenke M, Illich DJ, Becker M, and Muller AM. Pcgf6, a polycomb group protein, regulates mesodermal lineage differentiation in murine ES cells and functions in IPS reprogramming. *Stem Cells* 2014 [Epub ahead of print]; DOI: 10.1002/stem.1826.
 177. Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, Payne-Turner D, Easton J, Chen X, Wang J, Rusch M, Lu C, Chen SC, Wei L, Collins-Underwood JR, Ma J, Roberts

- KG, Pounds SB, Ulyanov A, Becksfort J, Gupta P, Hue-ther R, Kriwacki RW, Parker M, McGoldrick DJ, Zhao D, Alford D, Espy S, Bobba KC, Song G, Pei D, Cheng C, Roberts S, Barbato MI, Campana D, Coustan-Smith E, Shurtleff SA, Raimondi SC, Kleppe M, Cools J, Shimano KA, Hermiston ML, Doulatov S, Eppert K, Laurenti E, Notta F, Dick JE, Basso G, Hunger SP, Loh ML, Devidas M, Wood B, Winter S, Dunsmore KP, Fulton RS, Fulton LL, Hong X, Harris CC, Dooling DJ, Ochoa K, Johnson KJ, Obenauer JC, Evans WE, Pui CH, Naeve CW, Ley TJ, Mardis ER, Wilson RK, Downing JR, and Mullighan CG. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature* 481: 157–163, 2012.
178. Zhang Y, Teng F, Luo GZ, Wang M, Tong M, Zhao X, Wang L, Wang XJ, and Zhou Q. MicroRNA-323-3p regulates the activity of polycomb repressive complex 2 (PRC2) via targeting the mRNA of embryonic ectoderm development (Eed) gene in mouse embryonic stem cells. *J Biol Chem* 288: 23659–23665, 2013.
179. Zhao J, Sun BK, Erwin JA, Song JJ, and Lee JT. Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. *Science* 322: 750–756, 2008.
180. Zhou W, Zhu P, Wang J, Pascual G, Ohgi KA, Lozach J, Glass CK, and Rosenfeld MG. Histone H2A monoubiquitination represses transcription by inhibiting RNA polymerase II transcriptional elongation. *Mol Cell* 29: 69–80, 2008.

Address correspondence to:

Dr. Chiara Mozzetta

Laboratoire Epigénétique et Destin Cellulaire, UMR7216

Centre National de la Recherche Scientifique CNRS

Université Paris Diderot, Sorbonne Paris Cité

35 rue Hélène Brion

Paris 75013

France

E-mail: chiara.mozzetta@ibcn.cnr.it

Dr. Slimane Ait-Si-Ali

Laboratoire Epigénétique et Destin Cellulaire, UMR7216

Centre National de la Recherche Scientifique CNRS

Université Paris Diderot, Sorbonne Paris Cité

35 rue Hélène Brion

Paris 75013

France

E-mail: slimane.aitisial@univ-paris-diderot.fr

Date of first submission to ARS Central, September 8, 2014; date of final revised submission, October 27, 2014; date of acceptance, November 3, 2014.

Abbreviations Used

ARKS = Alanine-Arginine-Lysine-Serine
A-T = Ataxia-Telangiectasia
ATM = Ataxia Telangiectasia mutated
DNA = deoxyribonucleic acid
Eed = embryonic ectoderm development
ESC = embryonic stem cell
Ezh2 = Enhancer-of-Zeste 2
H3K27 = lysine 27 of the histone H3
H3K9 = lysine 9 of the histone H3
HP1 = heterochromatin protein 1
HSC = hematopoietic stem cell
HSPC = hematopoietic stem and progenitor cell
iPSCs = induced pluripotent stem cells
KMT = lysine methyltransferase
KO = knock-out
LAD = lamina-associated domain
lncRNA = long non-coding RNA
me1 = monomethylation
me2 = dimethylation
me3 = trimethylation
MSC = muscle stem cell
ncRBD = non-coding RNA-binding domain
NL = nuclear lamina
NPC = neuronal progenitor cell
PcG = polycomb group
PCNA = proliferating cell nuclear antigen
PCAF = p300/CBP-associated factor
PRC1 = polycomb repressive complex 1
PRC2 = polycomb repressive complex 2
PRE = polycomb responsive element
PTM = post-translational modification
RbAp46/48 = retinoblastoma-associated protein 46/48
RNA = ribonucleic acid
SANT = switching-defective protein 3 (Swi3), adaptor 2 (Ada2), nuclear receptor co-repressor (N-CoR), transcription factor (TFIIIB)
SET = Su(var)3-9 Enhancer-of-zeste Trithorax
Suz12 = suppressor-of-Zeste 12
TF = transcription factor
ub1 = monoubiquitination
VEFS = VRN2-EMF2-FIS2-SUZ12