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H3K27 methylation: a promiscuous repressive chromatin mark

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Abstract

Polycomb Repressive Complex 2 (PRC2) is a multiprotein complex that catalyzes the methylation of lysine 27 on histone H3 (H3K27me). This histone modification is a feature of facultative heterochromatin in many eukaryotes and maintains transcriptional repression established during early development. Understanding how PRC2 targets regions of the genome to be methylated remains poorly understood. Different cell types can show disparate patterns of H3K27me, and chromatin perturbations, such as loss of marks of constitutive heterochromatin, can cause redistribution of H3K27me, implying that DNA sequence, per se, is not sufficient to define the distribution of this mark. Emerging information supports the idea that the chromatin context – including histone modifications, DNA methylation, transcription, chromatin structure and organization within the nucleus – informs PRC2 target selection.

Introduction

Methylation of lysine 27 on histone H3 (H3K27me) by Polycomb Repressive Complex 2 (PRC2) is a hallmark of facultative heterochromatin in numerous organisms but, despite decades of research, it is not yet completely clear how this chromatin mark functions in gene repression and how it is controlled. Work in Drosophila first identified and characterized the Polycomb group (PcG) protein complexes as writers and readers of H3K27me and demonstrated that it maintains repression established early in development [1–3]. The SET domain of the PRC2 component EZH2 catalyzes H3K27 di-methylation and tri-methylation (H3K27me2/3) and requires both EED and SUZ12 to perform this function; these proteins are the core components of PRC2. H3K27me3 can be recognized by a chromodomain protein in the canonical Polycomb Repressive Complex 1 (PRC1) [4–6]. PRC1 is thought to help mediate transcriptional repression *via* H2AK119 monoubiquitylation (H2Aub1) and chromatin compaction [7–9].

PRC2 exists in some single celled eukaryotes, many fungi (although neither *S. cerevisae* nor *S. pombe*), plants and metazoans [10]. While plants contain a PRC1-like complex [11], true PRC1 homologs appear to be limited to metazoans [10]. The high degree of evolutionary conservation speaks to a critical role for PcG complexes; however, differences among

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species provide clues to general mechanisms of control and function of H3K27me. One major difference among organisms is the role of PRC1 in repression.

That H3K27me serves as a repressive mark even in organisms lacking PRC1 [12], implicates alternative repressive mechanisms downstream of H3K27me. This idea is also supported by findings showing that PRC1 does not localize to all H3K27me marked repressed targets [13] and that its enzymatic activity is not required for all repression [14]. Another intriguing difference among species is in the role of PRC2 in development. In some fungi such as *Neurospora crassa* and *Cryptococcus neoformans*, PRC2 and H3K27me are dispensable for normal growth and development [12,15]. Drosophila and higher eukaryotes are sensitive to disruption of PRC2 function, perhaps because lineage specification programs depend on the Polycomb system [16–20].

PcG proteins maintain gene silencing that is established during early development and is required for appropriate cell fate specification. The significance of H3K27me in maintaining appropriate long-term gene expression patterns is demonstrated by the range of mutations in PRC2 complex members, and its substrate (H3K27), in cancers. Both loss of function and change of function mutations in PRC2 have been reported in cancers, but a common outcome is an altered distribution of H3K27me, which perturbs differentiation [21]. Similarly, a heterozygous histone H3K27M mutation in only one of multiple genes encoding the histone H3 protein leads to glioblastoma [22]. H3K27M acts as a partially dominant negative mutation by binding to PRC2 with 22-fold higher affinity than to H3K27 [23]. This results in an overall reduction, but not complete absence, of H3K27me.

Despite the critical role of PRC2 in development and disease, some fundamental questions remain. The mechanisms controlling placement of H3K27me by PRC2, which will be the focus of this review, remain unclear. Direct targeting mechanisms have been proposed but likely only account for PRC2 recruitment in certain organisms or to certain loci. Partially defined DNA sequences called Polycomb Response Elements (PREs) recruit PRC2 in Drosophila but equivalent elements do not seem to target PRC2 in other organisms that have been examined [24]. Furthermore, even in Drosophila, PREs may not be directly required for transcriptional repression as deletion of four well-characterized strong Drosophila PREs had no effect on H3K27me-mediated silencing [25]. Sequence-specific transcription factors and noncoding RNAs can direct PRC2 to specific loci in mouse embryonic stem cells [26,27] and human cell lines [28,29], respectively, but again these do not seem to be prominent targeting strategies genome-wide. We favor a model similar to that proposed previously by others [30–33] suggesting that the local chromatin environment dictates H3K27me deposition. Our model considers the chromatin environment broadly and accounts for organisms that do not require PRC1 for PRC2 recruitment.

Features of constitutive heterochromatin prevent H3K27me deposition

In principle, the distribution of a histone mark, such as H3K27me, could be directly dictated by the sequence of associated DNA. In the case of this mark, however, this simple model is not highly compatible with recent findings. H3K27me regions greatly vary among different developmental stages, cell types, and in healthy versus diseased states of the same organism,

even in cases in which the primary sequence of the genome is invariant. This raises the possibility that changes in chromatin environment may be responsible for the observed plasticity. Some candidate variables include histone modifications, DNA methylation, transcriptional state and nucleosome occupancy. While outside the scope of this review, sub-nuclear location and higher-order chromatin structure which are known to be influenced by H3K27me [34–36], may also impact placement of H3K27me. Striking examples of the plasticity of H3K27me, and indications that 'chromatin environment' is important for the distribution of the mark were provided by recent observations of redistribution of H3K27me when features of constitutive heterochromatin were disrupted [15,37–39] (see Table 1).

The first indication that perturbation of constitutive heterochromatin impacts the distribution of H3K27me came over a decade ago in work with mouse embryonic stem cells (ESCs). Cytological observations showed that loss of H3K9me3 in pericentric heterochromatin in *Suv39h* double null cells was associated with new domains of H3K27me3 in patterns resembling those typical of H3K9me3 [37]. More recent studies with fungi revealed similar redistribution of H3K27me to centromeric regions. In *C. neoformans,* a chromodomain protein, associated with PRC2 in this organism, is required for appropriate H3K27me3 localization. When the chromodomain of this protein was mutated, H3K27me3 was lost from its normal location near the telomeres, and ectopic H3K27me3 appeared at the centromeres. The authors proposed that the chromodomain protein normally binds H3K27me3 to restrict this histone modification to its normal location, while in its absence the EED subunit of PRC2 binds to H3K9me2 at the centromere leading to methylation of H3K27 at that region [15]. Chromodomain proteins are not typically found in PRC2 complexes, but redistribution of H3K27me2/3 was also observed in *N. crassa*, which has a canonical PRC2 complex [12,38] and this was confirmed in another study [40]. When H3K9me3 was eliminated, H3K27me2/3 was reduced or lost at most of its normal locations and redistributed to constitutive heterochromatin, including centromeres. Moreover, elimination of HP1, normally bound to H3K9me3, caused equivalent redistribution of H3K27me2/3, without affecting H3K9me3, providing a rare example of cohabitation of these two marks on the same molecule [38]. These findings are consistent with the results with ESCs but contrast the situation in *C. neoformans*, where redistribution apparently depends on H3K9me. The findings with *N. crassa* are also consistent with experiments in mouse zygotes, where Hp1β prevents ectopic accumulation of H3K27me3 on maternal pericentric heterochromatin [39].

There are indications that another repressive mark, DNA methylation, can also, directly or indirectly, antagonize H3K27me. In a variety of cell types, loss of DNA methylation, caused by disruption of DNA methyltransferase genes or treatment with the demethylating agent 5-azacytidine, results in accumulation of H3K27me at regions previously marked by 5-methylcytosine [41–46]. These studies are consistent with data demonstrating that unmethylated CpG islands inserted at ectopic genomic locations can efficiently recruit H3K27me [47,48]. Neither H3K9me nor DNA methylation directly inhibits PRC2 activity *in vitro* as measured by histone methyltransferase assays [49,50] suggesting that these modifications, or proteins that recognize and bind to them, may prevent PRC2 recruitment or productive association with chromatin. Supporting this idea, DNA methylation prevents binding of factors, such as KDM2B and BEND3, that may be required for PcG recruitment

[51–54]. It is noteworthy that DNA methylation is not universally important for the normal distribution of H3K27me as elimination of DNA methylation in *N. crassa* has no effect on the distribution of H3K27me [38] and both *D. melanogaster* and *C. elegans* lack DNA methylation [55]. The impact of perturbing DNA methylation in higher organisms may reflect recognized effects of DNA methylation on H3K9me3 in these systems [38, 56,57].

Active histone marks antagonize H3K27me

Actively transcribed regions of the genome marked by H3K4me3 and H3K36me2/3 are generally distinct from those marked by H3K27me3. H3K4me3 and H3K27me3 are mutually exclusive at HOX genes in Drosophila embryos [58] and differentiated mammalian cells, but these modifications can coexist in “bivalent” domains in ESCs [59]. In *C. elegans* embryos and murine mesenchymal progenitor cells, loss of H3K36 methyltransferases results in methylation of H3K27 at previously H3K36me regions [60,61]. A mutation found in human chondroblastomas, H3.3K36M, which causes global loss of H3K36me2/3, also induces gains of H3K27me3 in regions that have lost H3K36me [61]. More broadly, quantitative mass spectrometry experiments demonstrated that H3K4me3 and H3K27me3[62] or H3K36me3 and H3K27me3 [63,64] rarely co-exist on the same H3 molecule in mouse or human cell lines. A mechanistic basis for this was demonstrated by finding that PRC2 catalytic activity is inhibited by H3K4me3 and H3K36me2/3 *in vitro* [50].

Histone acetylation is also associated with gene expression [65]. Because acetylation of H3K27 (H3K27ac) is not compatible with methylation of this residue, histone deacetylation is required to create a chromatin context permissive for PRC2 activity [66–69]. Together these findings support the idea that active chromatin regions are refractory to PRC2 activity, preventing H3K27me3-mediated repression of transcriptionally active genes.

H2Aub1 deposited by PRC1 recruits H3K27me

Histone modifications can render a chromatin environment permissive for H3K27me. The recent observation that H2Aub1, a mark catalyzed by a RING protein in PRC1, can recruit PRC2 and cause H3K27me3 has challenged the classical view of PcG recruitment in which H3K27me3 deposited by PRC2 serves as a binding platform for chromodomain proteins in the canonical PRC1 complex [49,70,71]. Tethering a variant PRC1 complex to a bacterial artificial chromosome containing human DNA provided evidence in mouse ESCs that H2Aub1 catalyzed by the tethered protein can recruit PRC2 and H3K27me3. A complementary study demonstrated that variant PRC1 can also recruit PRC2 and H3K27me3 to pericentric heterochromatin in mouse ESCs [49]. In addition to promoting PRC2 recruitment, H2Aub1 apparently stimulates its enzymatic activity [72].

Transcription inhibits H3K27me

In addition to responding, directly or indirectly, to histone modifications and DNA methylation, PRC2 can respond directly to transcription. The complex can bind RNA and there are indications that PRC2 is sensitive to the transcriptional state of promoters. Given

the role of PRC2 in transcriptional repression, it was surprising that RIP-seq studies, which detect RNAs associated with a protein of interest, suggested that PRC2 binds promiscuously to nascent RNAs at active promoters genome-wide [73,74]. This observation was reconciled by data demonstrating that RNA binding inhibits the catalytic activity of PRC2, perhaps accounting for the absence of H3K27me in regions producing RNA bound by PRC2. ChIP-seq experiments with human cell lines revealed chromatin-associated PRC2 enriched at promoters of repressed genes, the majority of which were marked by H3K27me3 [74]. Additional support for the idea that PRC2 responds to the transcriptional status of chromatin comes from studies demonstrating that chemical inhibition of RNA pol II-dependent transcription in mESCs is sufficient to recruit PRC2 and H3K27me3 to thousands of genes. Importantly, nearly all of the genes that gained H3K27me3 upon transcriptional repression are *bona fide* PRC2 target genes in differentiated tissues, although not in mESCs [32]. This observation was reiterated by finding that deletion of the genomic region containing the transcription start site led to loss of transcription, histone deacetylation and accumulation of H3K27me3 [75]. Taken together these data support a model in which PRC2 takes cues from the chromatin environment where it can sense transcription by interacting with nascent transcripts, which then prevent productive interaction with chromatin and modification of H3K27.

Conclusions

The findings summarized above are consistent with the idea that H3K27me catalyzed by PRC2 is directed by various inputs from the chromatin environment (Figure 1). There are at least three layers of regulation that contribute to PRC2 target selection: 1. Recruitment 2. Loading onto chromatin and 3. Regulation of catalytic activity. Some loci may utilize sequence-specific PcG targeting mechanisms using lncRNAs or transcription factor binding motifs, but many loci appear to lack any direct recruitment strategy. H3K27me targeting to these loci may rely on the ability of PRC2 to sense the chromatin environment. Once PRC2 is recruited to a given genomic location, it must productively associate with chromatin. This step likely requires the chromatin to be “accessible”, which might in part be dictated by lack of transcription and associated chromatin marks, as well as the absence of repressive epigenetic marks and proteins that recognize and bind to these modifications. Both high [76] and low nucleosome density [32] have been reported to promote H3K27me. Other factors such as chromosomal conformation and spatial location within the nucleus may also facilitate PRC2 access to chromatin. The final layer of regulation is the catalytic activity of PRC2. Signs of active transcription, in the form of RNA, H3K4me3, H3K36me2/3, and histone acetylation may prevent methylation of H3K27. While repressive marks, such as DNA methylation and H3K9me, do not directly inhibit PRC2 enzymatic activity, the effects of proteins that bind to these modifications have not been directly tested. Conversely the chromatin environment, specifically marks associated with PcG, H3K27me and H2Aub1, can stimulate PRC2 activity.

The ability of PRC2 to respond to a variety of local signals in chromatin is striking. Available information suggests PRC2 does not have reciprocal effects on other chromatin modifiers. For example, PRC2 has little effect on the distribution of DNA-me or H3K9me [38,45]. Similarly, while H3K36me2/3 inhibits PRC2 activity, H3K27me does not inhibit the

H3K36 methyltransferases HYBP [63] or NSD2 [50]. While the adaptability of PRC2 to different chromatin environments may preclude a unifying model for control of H3K27me, this remarkable plasticity may be crucial to its ability to maintain silencing of specific genes in different cell lineages.

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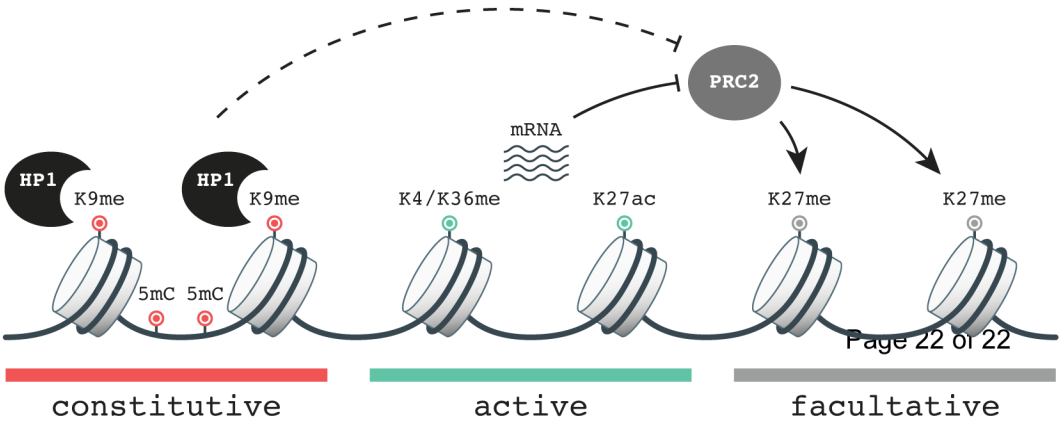

Figure 1. Model in which PRC2 responds to the chromatin environment to establish H3K27me domains

Repressive features of constitutive heterochromatin including DNA methylation (5mC), H3K9me (K9me), and HP1 binding influence methylation of H3K27 by PRC2. Conversely, histone modifications associated with transcription such as H3K4me (K4me), H3K36me (K36me), and H3K27ac (K27ac), as well as RNA can directly inhibit PRC2 catalytic activity to prevent H3K27me at regions of active gene expression. Genomic regions that do not contain features of active or repressed chromatin may be targeted by PRC2. Other properties of the chromatin environment such as nucleosome occupancy, chromosome conformation and location within the nucleus (not pictured) may also contribute to PRC2 target selection.

Table 1

Examples of alterations to chromatin that cause redistribution of H3K27me

Organism	Normal H3K27me distribution	Perturbation	H3K27me redistribution
A. thaliana	Promoters and gene bodies[77]	Loss of MET1 (DNA methyltransferase)	• Some normal H3K27me regions lost and replaced by H3K9me and DNA-me • H3K27me gained at constitutive heterochromatin and CG hypomethylated transposons [42]
C. neoformans	Sub-telomeres	Loss of Ccc1 (chromodomain protein in PRC2 complex)	• H3K27me3 lost at telomeres • H3K27me3 gained at centromeres [15]
N. crassa	Sub-telomeres and interspersed genic regions	**1** Loss of DIM-2 (DNA methyltransferase) **2** Loss of DIM-5 (H3K9 methyltransferase) or HP1	**1** Normal H3K27me2/3 **2** H3K27me lost or reduced at normal regions and gained at centromeres and other regions normally bound by HP1 [38,40]
C. elegans	Alternating H3K27me3 and H3K36me3 domains on autosomes	Loss of MES-4 (H3K36 methyltransferase)	• H3K27me3 reduced at some normally methylated genes • H3K27me3 gained at genes that lost H3K36me3 [60]
M. musculus (mesenchymal progenitor cells)	Promoters and gene bodies	H3.3K36M or depletion of Nsd1/2 and Setd2 (H3K36 methyltransferases)	• H3K27me3 retained at normal target genes • H3K27me3 gained at intergenic regions that lost H3K36me2/3 [61]
M. musculus (neural stem cells)	Promoters and gene bodies	DNMT3a KO (DNA methyltransferase)	• H3K27me3 retained at normal targets • H3K27me3 gained at genes that are DNA-me depleted and down- regulated [46]
M. musculus (embryonic fibroblasts)	Promoters and gene bodies	DNMT1$^{n/n}$ (DNA methyltransferase hypomorph)	• H3K27me lost from normally methylated promoters • H3K27me gained at CpGs that normally have high levels of DNA-me [44]