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The Polycomb Complex PRC2 and its Mark in Life

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The term *Polycomb* (Pc) initially referred to a *Drosophila* mutant that displayed improper body segmentation[1]. It was suggested that *Pc* encodes a negative regulator of those genes required for segmentation, the homeotic genes[2]. The Polycomb Group (PcG) now defines a set of genes whose mutations result in phenotypes similar to those of *Polycomb*. The critical role of PcG proteins during development is highlighted by the early embryonic lethality observed upon deletion of genes encoding some of these proteins (*Eed*, *Ezh2*, *Suz12*, *Ring1b*) in mice. The antagonistic activities of the PcG and the trithorax families of proteins culminate in the maintenance, throughout development and adulthood, of the appropriate patterns of homeotic gene expression in a spatially defined manner[3]. PcG proteins are found in several families of multiproteic complexes, including the Polycomb Repressive Complexes, PRC1 and PRC2 (Fig. 1). Two other PcG complexes were characterized in *Drosophila*, PhoRC and Pr-DUB, and their components have orthologs in mammals; however, the conservation of their functions has not yet been addressed[4-6].

Polycomb-mediated gene silencing is thought to rely mostly on regulation of chromatin structure, in part through post-translational modification (PTM) of histones. Hence, the PRC2 complex is responsible for the methylation (di- and tri-) of lysine 27 of histone H3 (H3K27me2/3)[3,6] via its enzymatic subunits Ezh1 and Ezh2, whereas the PRC1 complex mono-ubiquitylates lysine 119 of histone H2A (H2AK119ub) via the ubiquitin ligases Ring1a or Ring1b (Fig. 1). In addition, some PRC1 complexes can regulate gene expression by compacting chromatin in a manner independent of enzymatic activity[7]. The PRC1 component Pc (CBX in mammals), binds specifically to the product of PRC2 catalysis, H3K27me3, leading to the hypothesis that PRC1 functions downstream of PRC2. Although this logical premise is still cited in the literature, its operational status is equivocal as there are genes targeted by PRC2 that lack H2AK119ub[8] and genes targeted by PRC1 in the absence of PRC2[9,10]. Notwithstanding, PRC2 and PRC1 are often both required to maintain gene repression.

Due to the pivotal role of PRC2 in the coordination of PcG protein function, the still partial characterization of PRC1 and PRC1-like complexes in mammals, and the existence of up to date reviews on PRC1[3,6], we will focus this review primarily on mammalian PRC2. After considering PRC2 in terms of evolution, we next review the newly appreciated, variable composition of PRC2 and describe the function of its catalytic product and its localization. Finally, we discuss the biological roles of PRC2 and propose a model for its recruitment primarily mediated by non-coding RNA (ncRNA).

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Evolution of PRC2

The core PRC2 complex comprises four components: Ezh1/2, Suz12, Eed and RbAp46/48. The composition of PRC1 complexes exhibits more variability with only two core components being common (Fig. 1)[6,11]. PRC2 is well conserved throughout evolution and its presence in various unicellular eukaryotes led to the suggestion that it could have existed in the last common unicellular ancestor, although it was lost at times during evolution as exemplified by the cases of *S. Pombe* or *S. Cerevisae*[12]. Interestingly, components of PRC2, in contrast to those of PRC1, underwent little duplication in mammals, with vertebrates containing two copies of enhancer of Zeste, Ezh1 and Ezh2[12]. *Drosophila* has two copies of the Eed homolog, Esc and Esc-like. While ESC and ESC-like are interchangeable[13], the same might not be true for Ezh1 and Ezh2. Ezh1 and Ezh2 exhibit different expression patterns, with Ezh1 being present in dividing and differentiated cells and Ezh2 only in actively dividing cells. Also, PRC2 complexes containing Ezh1 (PRC2-Ezh1) in lieu of Ezh2 display low methyltransferase activity relative to PRC2-Ezh2[14]. These results suggest that it is PRC2-Ezh2, which establishes cellular H3K27me2/3 levels through its Ezh2-mediated methyltransferase activity and PRC2-Ezh1 restores H3K27me2/3 that could have been lost upon histone exchange or through demethylase activity. Moreover, PRC2-Ezh1 and –Ezh2 exhibit distinct chromatin binding properties, as illustrated by the specific chromatin compaction property of PRC2-Ezh1[14].

In contrast to mammals, PRC2 evolved towards a greater complexity in plants with species such *A Thaliana* having up to 12 homologs of PRC2 components[15]. A homolog of the mammalian and *S. Pombe* HP1 (heterochromatin protein 1) that binds to H3K9me3, also exists in plants and is denoted as LHP1. LHP1 binds to H3K27me3 and interacts with the Ring1 homologs AtRING1a/b, suggesting the existence of a PRC1-like complex in plants, although apparently with a function distinct from that in mammals and *Drosophila* given that H2AK119 monoubiquitylation is not detected in *A Thaliana*[15].

While Ezh1 and Ezh2 target the same genes and are expected to contribute to the same silencing pathway[16], the plant PRC2 complexes were reported to have distinct functions[15]. Based on all these criteria, we speculate that PRC2 actually evolved from a function that was partially redundant with gene silencing through the H3K9me3 pathway, gaining a more specific role as multicellular organisms acquired specific cell lineages.

PRC2 comprises more than 4 components

The first purifications of PRC2 led to the identification of the four components that are required for its enzymatic activity *in vitro.* Recently, it was shown that PRC2 comprises three additional polypeptides (Fig. 1) – AEBP2, Pcls and Jarid2 - the function of which will be described below. Of note, other proteins transiently interact with PRC2 (*i.e.* DNMTs, HDAC1, Sirt1); however, their impact on the function of PRC2 is currently unclear, and as such, they will not be discussed further here.

AEBP2 is a Zinc finger protein that was identified as part of the PRC2 complex. AEBP2 interacts with several components of PRC2, to enhance its enzymatic activity[17], and co-localizes with PRC2 at some target genes[18]. AEBP2 was postulated to bind DNA with an apparently relaxed specificity[18].

Pcl1/2/3 (PHF1/MTF2/Pcl3) are the three orthologues of *Drosophila* Polycomb-like (Pcl). They share the same protein motifs: one tudor domain, two PHD domains, a PCL extended domain, and a C-terminal domain tail[19] (Fig. 1). Pcls interact with PRC2 through Ezh2 and to some extent with Suz12 and the histone chaperones RbAp46/48[20]. Genome-wide studies revealed that Pcl2 co-occupied PRC2 target genes[21,22]. Various functions have been

attributed to Pcls, from the regulation of PRC2 enzymatic activity[20,23] to the recruitment of PRC2[21,24]. Mammalian Pcls are expressed in a tissue specific manner[21], and this redundancy might explain apparent discrepancies between studies. The phenotype associated with a Pcl mutant in *Drosophila* and *Xenopus*, and the interaction between Pcls and PRC2 and their co-localization, point to the Pcls having a critical role in PRC2 functioning. Understanding the underlying molecular mechanisms will probably require a detailed understanding of how Pcls interact with chromatin.

Jarid2 is the founding member of the Jumonji family of proteins that catalyses demethylation of histone proteins, yet Jarid2 itself lacks the key residues necessary for cofactor binding and is devoid of enzymatic activity. Its deletion results in severe defects in cardiovascular and liver development[25]. The C-terminal half of Jarid2 contains some conserved domains such the ARID domain (a potential DNA binding domain), the JmjC and JmjN domains, and a Zinc finger (Fig. 1).. Jarid2 was identified as a PRC2 component, and biochemical studies demonstrated its interaction with Ezh2[22,26-29]. Genome-wide studies revealed a large overlap between PRC2 and Jarid2 target genes[22,26-29] and Jarid2 and PRC2 recruitment appears partially interdependent. Surprisingly, while PRC2 recruitment is impaired upon Jarid2 inactivation, H3K27me3 levels are only modestly affected[27,29]. This observation led to the conclusion that Jarid2 is an inhibitor of PRC2 enzymatic activity[26,27]. However, further characterization of PRC2 enzymatic activity indicated that Jarid2 enhances PRC2 activity under defined biochemical conditions ([22] and Jinsook Son, R.M. and D.R., unpublished data). In addition, Jarid2 is able to bind DNA with a slight bias towards GC rich sequences[22]. This observation correlates with the reported sequence composition of PRC2 target genes[8] and is consistent with a potential function for Jarid2 in PRC2 recruitment.

Studies of these three recently identified PRC2 components discussed above have given rise to apparent discrepancies. Considering that these factors are not strictly required for PRC2 enzymatic activity *in vitro*, it is perhaps not surprising that their inactivation would exhibit milder consequences relative to inactivation of a core PRC2 component. Nonetheless, these factors are necessary for optimum PRC2 activity, and the regulation of PRC2 recruitment and its enzymatic activity are tightly connected. We propose that PRC2 functions as a holoenzyme (Fig. 1), with the additive contribution of each of its components being required for proper activity.

H3K27 methylation

It remains to be seen if the composition of PRC2 is modified as a consequence of, or during the processes of tumorigenesis, development, and/or maintenance of adult tissue specificity. Thus far, despite the different proteins found associated with the core complex, its integrity remains intact such that all PRC2 complexes containing either Ezh1 or Ezh2 catalyze H3K27 methylation. Lysine can be mono-, di-, or trimethylated, with each of these levels of methylation likely being functionally distinct. Methylation of H3K27 is processive (H3K27me3 results from monomethylation of H3K27me2) and H3K27me3 is a stable mark[30]. Methylated H3K27 is very abundant, with roughly 50% of the H3 histone being di-methylated, 15% tri-methylated, and 15% mono-methylated in embryonic stem (ES) cells[31]. Although the Pc component of PRC1 binds to H3K27me2 and -me3 through its chromodomain *in vitro*, it seems specific for H3K27me3 *in vivo,* and H3K27me2 appears to be of limited importance for maintenance of gene repression[23]. We previously hypothesized that H3K27me2 is an important intermediary PRC2 product, as it not only constitutes the substrate for subsequent H3K27me3 formation, but might also prevent H3K27 from being acetylated. Acetylated H3K27 is proposed to be antagonistic to PcG-mediated silencing and is enriched in the absence of PRC2[32].

With the exception of a viral protein, PRC2 is the only enzymatic activity found thus far that di- and trimethylates H3K27. These methyl marks are associated with facultative heterochromatin, a subdivision of heterochromatin that is subjected to specific developmental regulation[33]. The monomethylated version of H3K27 is associated with constitutive heterochromatin, but its enrichment through the gene body is correlated with actively transcribed genes[34]. Exactly how H3K27me1 arises is still a controversial issue. In plants, two enzymes, ATXR5/6, that are distinct from PRC2 and not conserved in mammals, monomethylate H3K27[35]. Yet in mammals, H3K27me1 is still detected in cells bearing non-functional PRC2 [10,36]. We speculate that in mammals, H3K27me1 is placed by an enzymatic activity distinct from that of PRC2 and that the presence of H3K27me1 in actively transcribed genes could arise from demethylation of H3K27me2/3 by the demethylases UTX or JMJD3[37]. Whether these demethylases can function on H3K27me1 *in vivo* is currently an open question.

In general, histone PTMs regulate biological processes either by altering chromatin structure (*i.e.* by loosening DNA/histone interaction) or by contributing to the recruitment of additional regulatory factors. Thus far, H3K27me3 has been implicated only in the latter mechanism of action suggesting that additional factors such as PRC1 are required to maintain gene repression. Yet, H3K27me3 might also indirectly regulate transcription by sterically preventing proteins from binding to chromatin. Enrichment of H3K27me3 correlates with gene silencing [38], and this observation is supported by the finding that H3K27me3 and H3K36me3, a mark that is linked to transcription elongation, exhibit distinct localizations[39]. Yet, RNA polymerase II (RNA PolII) that is phosphorylated at Ser-5 of its CTD is present at a substantial fraction of H3K27me3 enriched promoters[40] and low levels of transcripts are detected[41], leading to the suggestion that RNA PolII could be paused at PcG targeted genes[40]. Indeed, a number of PcG-regulated genes in *Drosophila* and mammals can recruit the RNA PolII transcription complex to their respective promoters and engage in early transcription, yet these polymerases encounter an early block to elongation. A recent report suggests that short transcripts that are generated upon transcription and remain bound to a paused RNA PolII could recruit PRC2[42]. If this report is confirmed, then it suggests that PRC2 and H3K27me3 can affect gene expression by controlling an engaged RNA PolII during promoter escape or elongation, rather than by regulating the initiation phase of transcription. A likely possibility is that PRC2 can repress transcription by different mechanisms and this may be gene specific.

Genome-wide localization of PRC2 and H3K27me3

A flurry of publications reported the genome-wide localization of H3K27me3 in various cell lines and organisms, with some divergent results depending on the methodology employed and the model analyzed. A conservative estimation is that PRC2 targets represent at least 10% of the genes in Embryonic Stem (ES) cells[43]. PRC2 specifically resides at, and targets for H3K27me3 deposition, the Hox genes as well as numerous genes encoding other developmental regulators[44-46]. Interestingly, in human cancer cells, the PRC2 component Suz12 is primarily enriched at promoters of genes encoding glycoprotein and immunoglobulin-like proteins[47]. Additional studies are required to determine whether this is a consequence of the genetic and epigenetic alterations of cancer cells or whether it is a reflection of the cancer cell origin.

In *Drosophila*, domains enriched in H3K27me3 were found to cover large regions of the genome, usually exceeding 10 kb[48,49]. In mammals, two different types of binding patterns have been reported for PRC2 or H3K27me3: some very large domains of more than 100 kb such as those containing the *Hox* loci and some smaller domains covering a few kb[41,45,47,50]**.** At promoters, H3K27me3 enrichment appears to be centered around the

transcription start site (TSS), but with a lower intensity over the TSS itself (Fig. 2)[41,51]. Some H3K27me3 is found at intergenic regions[34,41], and H3K27me3 is enriched in subtelomeric regions[52] and in long-terminal repeat retrotransposons[53].

To understand how PRC2 can maintain specific gene expression patterns, the overall chromatin structure in addition to H3K27me3 patterns should be considered[54]. This issue has generated a great deal of attention in the context of ES cell differentiation (Fig. 2). ES cells are characterized by a more open and flexible chromatin organization and by an overall higher rate of transcription, which is thought to be important for pluripotency[55]. Surprisingly, the H3K4me3 mark, often associated with active transcription, was present at most if not all PRC2 targeted genes in ES cells, forming the so-called "Bivalent Domain"[39,41,43,51,56]. While this pattern was initially believed to be ES cell-specific[56], bivalent domains have also been found in differentiated somatic cells, albeit at a lower frequency[39,43]; they were also found in Zebrafish[57] but are rarely detected in *Drosophila*[58]. Another histone species with seemingly disparate functionality that co-localizes with PRC2 is the histone variant H2Az, which is usually associated with active genes (Fig. 2). Indeed, PRC2 and H2Az co-localize in undifferentiated ES cells and their recruitment is interdependent[59]. The apparent contradiction in the presence of either H3K4me3 or H2Az with H3K27me3 at promoters of silent genes in ES cells might reflect the necessary plasticity of these cells, but might also result in partial leakiness of gene silencing. That PRC2 and H2Az co-localize is consistent with the levels of DNA methylation being low at PcG target genes in ES cells[60,61], given the evolutionary conserved exclusivity exhibited by H2Az and DNA methylation[62]. Upon differentiation of ES cells, a significant fraction of bivalent domains that lose H3K4me3 and H2Az do gain DNA methylation[43,60,61]. Interestingly, genes enriched in both H3K27me3 and H3K9me3, another mark associated with gene repression, are more abundant in human fetal lung fibroblasts (IMR90), relative to human ES cells[50]. In this same study, the authors noticed that H3K27me3 domains are more extended in IMR90 cells or $CD4^+$ T cells, relative to ES cells and that H3K27me3 domain expansion correlates with more efficient transcriptional silencing[50]. Altogether these results suggest that somatic cells reinforce gene silencing by increasing the length of H3K27me3 domains and, for a fraction of PRC2 targeted genes, through complementary silencing pathways (H3K27me3 together with H3K9me3 or DNA methylation). Not surprisingly, some pluripotency factors whose expression could be deleterious in differentiated cells, are silenced in this redundant fashion[50].

PRC2 recruitment

Exactly how mammalian PRC2 is recruited to chromatin is currently not clear. In *Drosophila*, DNA sequences called Polycomb Response Elements (PRE) are targets for PcG protein recruitment when inserted at exogenous locus[3,6]. Genetic experiments led to the identification of DNA binding proteins that are required for PcG binding; however, genome-wide analysis revealed that any one of these *trans*-acting factors overlap only partially with PcG target genes. Instead, it is thought that a combination of these factors might be responsible for the recruitment of PcG proteins.

In mammals, PRC2 occupies chromatin enriched in CpG, but these sequences alone do not indicate a consensus response element[8]. Recently, two publications identified a mammalian PRE based on PcG complex recruitment in *Drosophila*[9,63]. Both reports suggested an important role for YY1, the mammalian ortholog of the *Drosophila* PRE DNA binding protein PHO, as previously proposed[64]. RYBP, a protein interacting with both YY1 and PRC1, was shown to be required for PRC1 and PRC2 recruitment[63]. Yet genome-wide analysis in mammals did not reveal a clear overlap between YY1 and PcG target genes[65]. Moreover, PRC2 is under-represented at YY1 response elements[8]. Hence, to date, there is

no strong evidence for the involvement of transcription factors in the recruitment of PRC2 in mammals.

On the other hand, long ncRNAs are becoming appreciated as important participants in PRC2 function. In mammals, the process of X-chromosome inactivation initiates with the expression of a 17 kb nc RNA, Xist, which coats the X chromosome in *cis.* Xist RNA coating leads to a dramatic alteration of chromatin structure characterized by a progressive heterochromatinization. The inactive X chromosome becomes methylated on H3K27 in a Xist-dependent manner[66]. The two long stem-loop structures formed by the A repeats present 5′ in the Xist RNA interact with PRC2 *in vitro*[67,68], although additional regions of Xist are clearly involved as a Xist transcript deleted for the A repeats can still recruit PRC2 to the Xist RNA coated X chromosome[69]. Similarly, the long ncRNA Kcnq1ot1 can mediate PRC2 spreading *in cis,* thereby maintaining the imprinted expression of the Kcnq1 domain[70]. Long ncRNA could also promote PRC2 binding *in trans* as shown for HOTAIR[71,72], an RNA whose expression from the HOXC locus is associated with repression of 40 kb of the HOXD locus. Such mechanisms could be common to a large fraction of long ncRNAs[73]. In light of these results, ncRNA seems a strong candidate for PRC2 recruitment.

Considering this information, we propose a model in which the sum of relatively weak interactions or low energy steps that are established by each of the PRC2 holoenzyme components, would function together to attain the energy necessary to recruit PRC2 (Fig. 3). This model predicts up to four steps, not necessarily consecutive, that result in the successful recruitment of PRC2: 1) Interaction of Jarid2 and AEBP2 with DNA[18,22], 2) Interaction of the histone chaperones RbAp46/48 with histones H3 or H4 [74], 3) Interaction of Eed with the product of PRC2 catalysis, H3K27me3[75] and Pcls with a currently unknown histone mark, and 4) Interaction of PRC2 components with long ncRNA. The resultant binding specificity could then be modulated by the variation in the composition of the PRC2 holoenzyme and PTMs of its components. Indeed, Ezh2 was reported to be phosphorylated at threonine 350[76,77], a modification that modulates PRC2 recruitment[76]. Consistent with the hypothesis that ncRNA will be a major player in cell-specific recruitment of PRC2, phosphorylation of Ezh2-T350 enhances its binding to ncRNA[77]. The large pool of long ncRNA may function, in part, to direct the complex to defined target genes. This targeting may not necessarily entail linear base pairing with target sequences, but instead the tertiary structure of the RNA may be key to specific target gene recognition. In this regard, the global contribution of HOTAIR to PRC2 targeting indicates that ncRNA may also regulate overall PRC2 binding properties to chromatin, either directly or by bridging it to other factors[72]. Hence, ncRNA could regulate the affinity of PRC2 to chromatin in a manner similar to the recently described case of the PRC1 component CBX7[78]. the chromodomain of CBX7 was reported to bind both H3K27me3 and the ncRNA ANRIL, and binding to one ligand can modulate the affinity for the other *in vitro*.

It is not yet clear whether the initial recruitment of PRC2 to a defined gene and the maintenance of its recruitment involve the same mechanisms. Indeed, the PRC2 component Eed can bind H3K27me3 and PRC2 enzymatic activity is stimulated by the presence of H3K27me3, thus generating a positive feedback loop[75]. The importance of this mechanism is illustrated by the phenotype of *Drosophila* expressing point mutants of Eed that prevent its binding to H3K27me3 without altering PRC2 complex formation; this phenotype includes a global reduction in H3K27me2/3. Furthermore, given that some PcG proteins seem to stay bound to chromatin during replication[79] and that the same applies for PRC2 components during mitosis[80], PRC2 occupancy of chromatin may not necessitate its active recruitment to defined chromatin loci, in all cases.

PRC2 pluripotency and differentiation

Two straightforward models could potentially explain the maintenance of stem cell pluripotency in the context of PRC2-mediated gene repression. Either pluripotency is lost upon the expression of developmental regulators that promote differentiation or it is lost when the expression of factors requisite for pluripotency are silenced (Fig. 4A). The first hypothesis is in keeping with the role of PRC2 in maintaining the repression of numerous developmental regulators in ES cells. This led to the suggestion that PRC2 is required for maintenance of pluripotency[45]. However, later studies reported that ES cells in which a PRC2 component is inactivated could be kept undifferentiated. This finding draws our attention to the second model that posits the requisite repression of pluripotency-specific factors[16,36,81]. Indeed, in mouse ES cells, inactivation of Suz12, Jarid2 or Pcl2, was reported to be associated with an inefficient silencing of the pluripotency factors, Nanog and Oct4 (Fig. 4A)[21,22,29]. Furthermore, inactivation of mes-2, a homolog of Ezh2, extends the plasticity phase during *C. elegans* embryonic development[82]. This observation probably results from the failure of the mes-2 mutant to repress genes that should only be expressed during a defined window of time in early development. Altogether, it appears that the sustained expression of pluripotency factors overtakes the aberrant expression of developmental regulators in PRC2-deficient ES cells.

In contrast, when ES cells are induced to differentiate, mis-regulation of developmental programs become more apparent. Hence, although Eed$^{-/-}$ ES cells were unimpaired in their ability to contribute to all tissue lineages in chimeric embryos[81], Suz12$^{-/-}$ ES cells fail to form a proper endodermal layer[36] and, Ezh2$^{-/-}$ or Eed$^{-/-}$ ES cells display a severe defect in mesoendodermal lineage commitment[16]. This phenotype is not restricted to deletion of PRC2 core components as impaired differentiation was also reported in Jarid2$^{-/-}$ and Pcl2 knock-down ES cells[27-29]. Of note, in contrast to the mild phenotype that results from PRC2 deletion in ES cells, PRC1 inactivation (Ring1A/B double knockout) leads to a proliferation defect and ES cells cannot be maintained[83]. Furthermore, deletion of the PRC1 component Ring1b in the context of Eed$^{-/-}$ ES cells worsens the differentiation defects[53]. These results indicate that PRC1 is not just a downstream effector of PRC2, but instead has distinct functions and its recruitment is at least partially PRC2-independent.

Based on the example of ES cell differentiation, we would expect that PRC2 inactivation would in general prevent lineage commitment and terminal differentiation. While PRC2 defects do prevent adipogenesis and lymphopoiesis[84,85], PRC2 inactivation also promotes differentiation during myogenesis and epidermis formation[86,87] (Fig. 4B). During B cell maturation, Ezh2 is required for V_HJ558 gene rearrangement and, in its absence the transition from pro-B to pre-B cells is altered[84]. In the case of epidermis, inactivation of PRC2 leads to upregulation of epidermal genes mediated by the transcription factor AP1. Those genes are normally expressed at the late stage of differentiation [87]. Considering that upon PRC2 inactivation, only a small subset of its target genes are re-activated, it is likely that individual functions encoded by these targeted genes dictate the global consequences on cell differentiation.

PRC2 and cancer

The expression of PRC2 components is upregulated in various diseases such as melanoma, lymphoma, breast and prostate cancer. Ezh2 has been reported to be a marker of the aggressive stages of prostate and breast malignancies[88,89], and its overexpression promotes neoplastic transformation of normal prostatic cells[90] and hyperplasia in breast epithelium[89,91]. The expression of PRC2 components, with the exception of the Ezh1 homologue, is regulated by the pRB-E2F pathway and therefore is associated with cell

proliferation[14,92]. In addition, several miRNAs control Ezh2 expression, the deregulation of which could contribute to Ezh2 overexpression in cancer. Deletion of PRC2 components in somatic cells led to a dramatic reduction in cell proliferation[88,92], an effect that was linked to the PRC2-dependent regulation of the Ink4aA-Ink4B locus[87,93]. Given these findings, Ezh2 was proposed to function as an oncogene[92]. In contrast, recurrent somatic mutations resulting in reduced Ezh2 enzymatic activity occur in subtypes of lymphoma[94] and myeloid disorder[95,96] indicating that lowering PRC2 activity might also be associated with deregulated proliferation. Furthermore, inactivation of Ezh2 does not inhibit cell proliferation of all model cell lines for prostate cancer[97].

To understand the role of PRC2 in tumor progression, it might be more beneficial to determine whether PRC2 is required for the de-differentiation of somatic cells or for the epithelial to mesenchymal transition, rather than modulating Ezh2 levels to gauge its function as a tumor suppressor versus oncoprotein in a defined cell context. Indeed, the apparent outcome in the latter case is likely dependent on the genetic and epigenetic alterations that initiate cellular transformation. Interestingly, PRC2 seems to be required for the acquisition of pluripotency as $Eed^{-/-}$ and $Suz12^{-/-}$ ES cells fail to induce the reprogramming of B-lymphocytes in a heterokaryon assay[98]. If similar mechanisms operate during the reprogramming of somatic cells and during tumor progression, we would expect Ezh2 inhibition to be a good approach towards preventing the transition to advanced stages of cancer. Yet, if the carcinogenic process initiates from cancer stem cells, it will be critical to attain a better understanding of how PRC2 modulates proliferation and, in particular why PRC2 deletion inhibits the proliferation of some somatic cells, but not of ES cells.

Concluding remarks

The progress made in understanding the role of PcG proteins, and especially PRC2, have underscored their versatility. Not only is PRC2 involved in the regulation of a broad array of biological processes, but it also establishes regulatory cues that are stable and propagated throughout development. Yet these cues can be subject to adjustment at each step of differentiation or in response to external stimuli. With such a pivotal role in maintaining the repression of different sets of genes depending on the cell type and the developmental stage, PRC2 must be targeted to chromatin in a coordinated and intricate process, the steps of which may entail specific DNA sequence(s), ncRNAs/, and the chromatin structure associated with its target genes. However this model relies on hypotheses that require validation. Such validation entails clarification of how ncRNA can recognize defined genomic locations and the exact mechanism by which Jarid2 or Pcl proteins contribute to PRC2 recruitment.

Although it is now clearly established that several components of PRC2 are mis-regulated in disease, their involvement has been well defined yet. Mouse models that allow genetic manipulations, in conjunction with direct comparisons at the genome-wide level of normal versus pathogenic tissues in defined genetic backgrounds, may provide solid resources for pinpointing the parameters of PRC2-dictated processes.

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Synopsis

Polycomb Group (PcG) proteins maintain the pattern of gene expression of different cells set early during development by regulating chromatin structure. Two main PcG complexes exist in mammals. The Polycomb Repressive Complex 1 (PRC1) compacts chromatin and catalyzes monoubiquitylation of histone H2A, and PRC2 can also contribute to chromatin compaction and catalyzes methylation of histone H3 at Lys27. We focus here on PRC2, which is involved in various processes, including differentiation, cell identity and proliferation, and stem cell plasticity. Recent studies of PRC2 have expanded our perspectives on its function and regulation, and uncovered a new role for non-coding RNA in its recruitment to target genes.

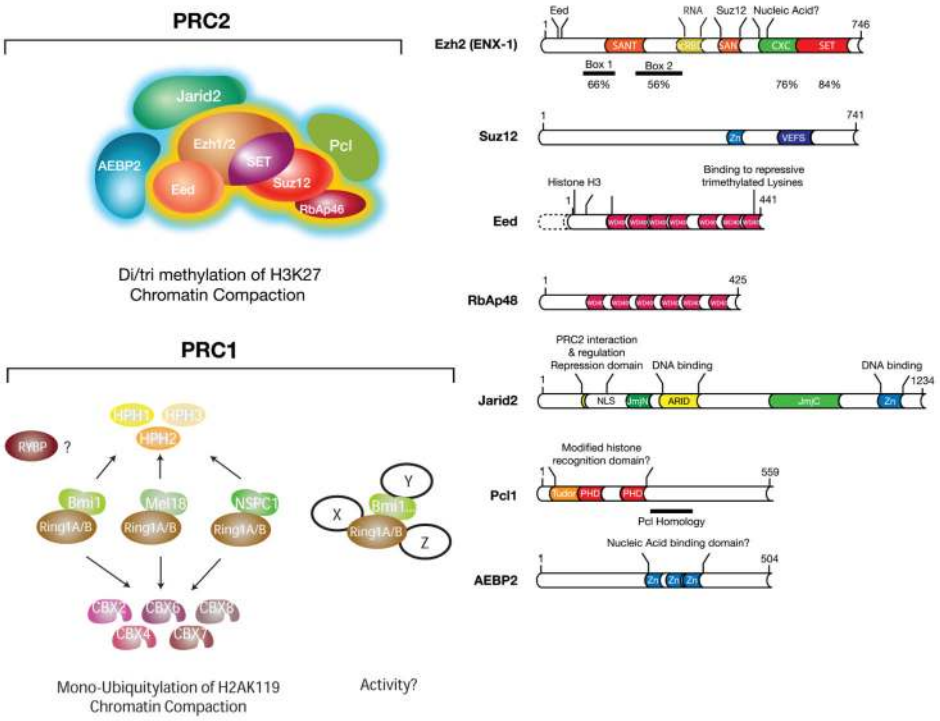

Figure 1.

Schematic representation of Polycomb complex PRC1/2. Left) Diagrams representing the composition of PRC2 and PRC1 are shown. In the case of PRC1, the left diagrams correspond to the classical PRC1 complexes, while the right one corresponds to the so-called PRC1-like complexes. Due to their homology with *Drosophila* Psc protein, we assumed that Bmi-1-, Mel18- and NSPC1-containing PRC1 complex could compact chromatin. The CBXs "pocket" shape represent the chromodomain that specifically recognized H3K9/27me3. HPH1/2/3 are the abbreviation for Human Polyhomeotic Homolog 1/2/3. X, Y and Z represent various proteins such as SCMH1/2, FBXL10, E2F6, JARID1d that could contribute to the formation of PRC1-like complexes whose exact composition is still enigmatic. B) Characterized domains with potential functions are indicated for each PRC2 component. Note that domains based on evolutionary conservation are underscored below. For Ezh2, the numbers indicate the percent homology between mouse and *Drosophila* homologs.

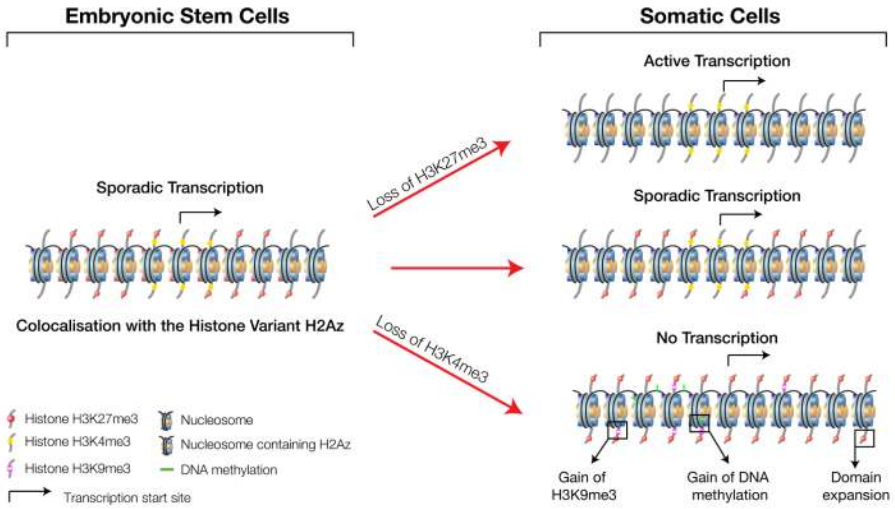

Figure 2.

Chromatin properties at PRC2 target genes in ES cells and differentiated cells. Schematic representation of chromatin at PcG target genes as a function of ES cell differentiation. In ES cells, most PcG targets are methylated on both H3K4 and H3K27 and colocalize with the histone variant H2Az. During differentiation, H2Az is removed, and some bivalent domains are resolved. For example, genes that are actively transcribed lose H3K27me3. A significant proportion of PcG targets that retain H3K27me3 but lose H3K4me3 are targeted by other silencing pathways such as DNA methylation or H3K9 trimethylation.

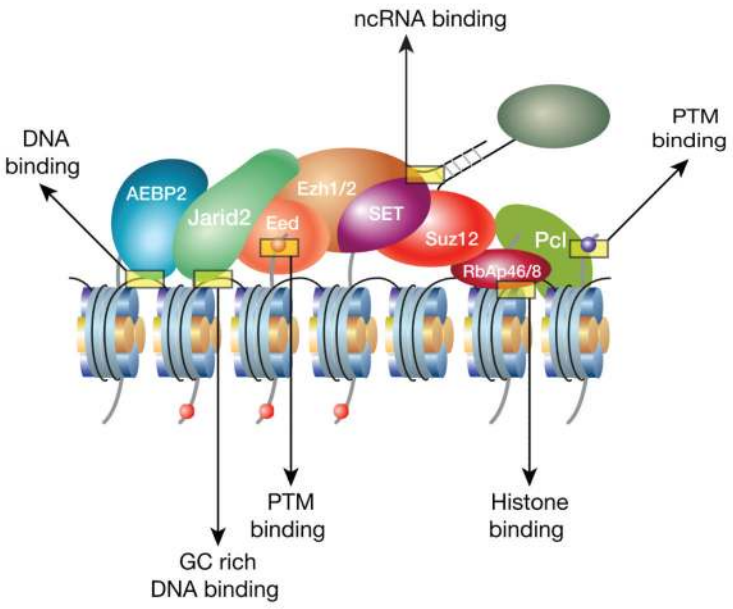

Figure 3.

Multiple interactions of PRC2 with chromatin. Schematic representation of PRC2 holoenzyme at chromatin. Putative interactions with either DNA or histones that could explain PRC2 recruitment are highlighted.

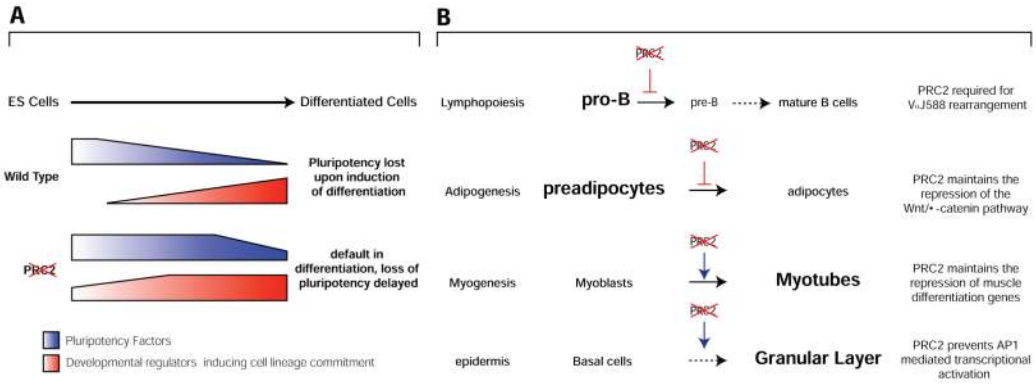

Figure 4.

PRC2 mediated regulation of pluripotency and differentiation. A) Comparison of expression levels of pluripotency factors and factors that induce cell commitment during ES cell differentiation in wild type and PRC2 impaired ES cells. B) Consequences to the outcome of cell differentiation upon PRC2 inactivation.