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Context-specific Polycomb mechanisms in development

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Abstract

Polycomb group (PcG) proteins are critical chromatin regulators required for stable cell fate that maintain repression of lineage-inappropriate genes. Recent advances show that PcG proteins form distinct multi-protein complexes in different cellular environments, such as in early development, adult tissue maintenance, and cancer. This surprising compositional diversity provides the basis for mechanistic diversity. Understanding this complexity deepens and refines the principles of PcG complexes' recruitment, target gene repression, and inheritance of memory. We review how the core molecular mechanism of Polycomb complexes operates in diverse developmental settings. We propose that context-dependent changes in composition and mechanism are essential for the proper epigenetic regulation in development.

Introduction

Stable maintenance of acquired cell fate is the hallmark of cellular differentiation. Robust development, from making the correct number of digits to producing myriad brain cell types, relies on the memory of cell fate, especially on proper gene expression. To maintain active or inactive status of genes, eukaryotes employ chromatin and associated proteins to mark and sequester DNA elements. A key group of chromatin modifying complexes required for maintaining the repressed state in metazoans are formed by Polycomb group (PcG) proteins.

PcG genes were initially discovered in *Drosophila melanogaster* by homeotic transformation phenotypes, implicating them in cell fate determination[1,2]. Subsequent studies showed that PcG proteins are critical for preventing misexpression of *Hox* genes in inappropriate body segments[3–5]. Core PcG genes are conserved across many species from flowering plants to vertebrates, and they play similar roles in the maintenance of repression[6]. This Polycomb function is not only critical for proper embryonic development, but also for the regulation of adult stem cells. As such, PcG genes are often mutated or amplified in cancer.

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Competing interests

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Polycomb group proteins form stable multi-protein complexes to modify chromatin. Initial biochemical studies identified distinct Polycomb complexes called Polycomb Repressive Complex 1 (PRC1) and PRC2 with different molecular activities. Both PRC1 and PRC2 have subsequently been shown to encompass numerous related complexes with differing auxiliary subunits. PRC1 complexes catalyze mono-ubiquitylation of Lys119 residue of H2A (H2AK119Ub1)[7,8] and can compact chromatin[9,10]. PRC2 complexes catalyze mono- di- and tri-methylation of Lys27 residue of H3 (H3K27me1/2/3)[11–14]. The genome localization of PRC1 and PRC2 complexes frequently overlap at the promoters of developmentally important transcription factors that need to be repressed in a given cell type[15–18]. Cooperation between PRC1 and PRC2 activities can induce positive feedback of their recruitment and their biochemical activities[11,19–24], which is critical for the maintenance of the silent transcriptional state.

While the core functions of Polycomb complexes are conserved in diverse cell types and species, advances in the past decade have revealed surprisingly unique functions of distinct Polycomb complexes in different cellular contexts[25–27]. Both PRC1 and PRC2 form sub-complexes either by combinatorial assembly of paralogous components or by inclusion of accessory proteins. These distinct Polycomb complexes possess different molecular activities. Furthermore, the rapid advance of technologies now enables genome-wide chromatin analyses of these complexes in rare and specialized cell types, including adult stem cells and preimplantation embryos. With findings from these diverse contexts, there is a growing appreciation that different cell types might use different Polycomb complexes to meet their unique cellular needs. In this review, we will focus on how the behaviour of distinct Polycomb complexes in diverse contexts has expanded and refined our understanding of the Polycomb system and chromatin-mediated epigenetic regulation. More general principles on molecular mechanisms and developmental roles of PcG proteins can be found in other recent reviews[28,29].

Here, we first introduce distinct Polycomb complexes and their molecular functions. Then we will describe context-specific functions of Polycomb complexes, which are often achieved by formation of cell type-specific sub-complexes. We will examine how the genomic distribution of PcG is altered in special cell types and under certain perturbation conditions, and how this plasticity reveals the regulatory principles on the activity and targeting of PcG proteins. Finally, we will discuss how histone modifications and PcG proteins together maintain the memory of repression and stabilize cell fate.

Functions of Polycomb complexes

The composition and molecular function of cPRC1, ncPRC1 and PRC2

Three major Polycomb repressive complexes have been defined based on their distinct biochemical activities: canonical PRC1 (cPRC1), non-canonical PRC1 (ncPRC1, also known as variant PRC1, vPRC1), and PRC2. PRC2 and ncPRC1 are histone modifying enzyme complexes, while cPRC1 modifies chromatin structure and organization primarily through non-enzymatic means (FIG. 1, upper boxes).

PRC2 is composed of SUZ12, EED, RBBP4 (or paralog RBBP7) and EZH2 (or paralog EZH1) (FIG. 1a). It catalyzes mono-, di-, and tri-methylation of H3K27 using the EZH proteins' methyltransferase activity[11–14]. PRC2 is responsible for di-methylation of the broad intergenic genome[30,31], and tri-methylation of H3K27, for example on inactive and unmethylated CpG-rich promoters[32–35]. While H3K27me3 is strongly correlated with low gene expression[36], this histone mark alone is not sufficient for gene repression[37–39]. Proteins recruited to H3K27me3-enriched regions and the resulting physical changes in the chromatin structure are also required for stable gene silencing[9,40].

ncPRC1 is composed of RING1B (or paralog RING1A), PCGF1 (or paralog PCGF3/5/6, less likely PCGF2/4), and RYBP (or paralog YAF2) (FIG. 1b)[8,25,41,42]. The RING subunit[8,43] of ncPRC1 catalyzes mono-ubiquitylation of Lys119 of H2A at promoters[44,45] and across the genome[31,46]. RYBP, a specific component of ncPRC1, stimulates the ubiquitylation activity[47]. While PRC2 and cPRC1 are preferentially localized at inactive promoters, ncPRC1 is seen at both inactive (H3K27me3-high) and active (H2K27me3-low) promoters when assessed by RYBP, KDM2B, and RING1B binding patterns[48,49]. In mouse embryonic stem cells (mESCs) and developing skin, loss of ubiquitylation resulted in derepression of hundreds of Polycomb target genes[46,50–52]. However, a significant reduction of ubiquitylation by catalytic mutation of *Ring1b* in mice or *Sce* (*Ring1a/b* ortholog) in flies did not show patterning defects, indicating the maintenance of *Hox* gene repression[53,54]. Even fly larval cells replaced with histone H2A mutant that cannot be ubiquitylated did not derepress *Hox* genes[54]. Thus, the role for ubiquitylation in maintenance of a repressed state is likely context-dependent[55].

Contrary to PRC2 and ncPRC1, cPRC1 modifies chromatin primarily through non-enzymatic mechanisms. cPRC1 is composed of RING1A/B, PCGF2/4, CBX2 (or paralog CBX4/6/7/8), and PHC1 (or paralog PHC2/3) (FIG. 1c). CBX binding to RING1B is mutually exclusive with RYBP[56]. cPRC1 compacts nucleosome arrays and blocks chromatin remodeling by the mammalian SWItch/Sucrose NonFermentable (mSWI/SNF) complex (also called BAF) *in vitro* through its CBX subunit[9,10,40]. In addition, PHC proteins can bridge distant Polycomb bound sites through the oligomerization activity of its Sterile Alpha Motif (SAM) domain[57–60]. Both CBX and PHC proteins can phase separate with target chromatin *in vitro*[61–63]. These self-association properties might contribute to the formation of exclusive nuclear structures called Polycomb bodies, which have been proposed to be refractory to transcriptional activation[64,65]. Reflecting cPRC1's chromatin organization activity, PcG bound regions occupy less space than transcriptionally active regions[66], and distant PcG bound sites interact with each other[67,68]. Perturbing cPRC1 function by mutating CBX or PHC subunits resulted in derepression of Polycomb target genes in mESCs[69–71] or homeotic transformation of axial skeletons[57,69,72–74], suggesting that the structural function of cPRC1 is critical for maintenance of the repressed state.

Stabilization of repressed state by positive feedback of PcG complexes

One model that is largely consistent with the evidence on interplay between Polycomb complexes invokes hierarchical recruitment (FIG. 2)[75,76]. First, ncPRC1 is recruited to hypomethylated CpG-rich promoters through its KDM2B subunit[42,49,77] and ubiquitylates

H2A (FIG. 2a). PRC2 then recognizes the H2AK119Ub1 with its accessory component JARID2[23,78] and catalyzes tri-methylation of H3K27 (FIG. 2b). The EED subunit of PRC2 binds H3K27me3. This interaction further stimulates PRC2 activity, which promotes methylation of neighboring nucleosomes to spread H3K27me3 away from the initial nucleation site (FIG. 2c)[19,79]. Similar to H3K27me3 spreading, RYBP in ncPRC1 binds H2AK119Ub1, and this interaction stimulates ncPRC1 activity to spread H2AK119Ub1 to neighboring nucleosomes (FIG. 2c)[21,80]. The CBX subunit of cPRC1 then binds H3K27me3 through its chromodomain[11,22,24]. CBX and PHC subunits in cPRC1 further bridge and sequester Polycomb bound loci into Polycomb bodies[57,59–63]. They maintain a high local concentration of PcG complexes and a repressed state. PRC2 activity has been shown to be stimulated when the template contains densely packed nucleosomes[20], and ncPRC1 activity was stimulated when the template was compacted with H1[21], suggesting a compact local chromatin environment might strengthen the feedback loop in this proposed pathway (FIG. 2d)[57].

This model has been developed based upon functional analyses *in vitro*, structural analyses, and studies in cell culture done primarily with mESCs. It is not clear to what extent this model accurately depicts PcG function in every cell type. However, the overarching principle is that Polycomb complexes are capable of positive feedback interactions that might facilitate durable maintenance of repression at silenced genes across cell divisions. Thus, we will discuss findings from mESCs and diverse differentiated cells under the framework of this interplay of Polycomb complexes

Diverse Polycomb complexes

Advances over the past decade have identified diverse members of each of the three central Polycomb complexes in different cells, or even in a single cell type. The diversity of sub-complexes in each family is generated by distinct combinatorial assemblies of paralogs of core subunits and/or by inclusion of accessory proteins. How might this diversity impact PcG function?

Multiple cPRC1 complexes can be formed by combinatorial assembly of CBX2/4/6/7/8, PCGF2/4 or PHC1/2/3 proteins. In addition, sub-stoichiometric SCM (SCML1/2, SCMH1) proteins are also found in cPRC1 in different cell types (FIG. 1c). Different paralogs possess distinct molecular activities; for example, CBX4 has SUMOylation activity[81], and CBX7 has significantly lower chromatin compaction activity than other CBXs[10,82]. The genes encoding CBX2/4/8, which are normally expressed in differentiating cells, are found in tandem within a 70kb region of the same chromosome in most mammals. They all have analogous domain structures with similar compaction and phase separation properties suggesting that they might be the result of gene duplications during evolution[6,83]. However, CBX paralogs have diverged in their binding to H3K27me3 peptides[84], chromatin[85] and to mitotic chromosomes[86]. Among the CBX paralogs, only CBX2 has an AT-hook domain and can be targeted to AT-rich pericentromeres in mouse zygotes[87]. Another component of cPRC1, PHC also normally switches from PHC1 to PHC2 as cells differentiate[59,70]. As this component mediates long-range chromatin interactions[57,59] and is involved in phase

separation[62], it might contribute to changes in the cPRC1 functions that shape nuclear organization.

ncPRC1 is categorized by the presence of PCGF1/3/5/6 proteins and by specific accessory factors which are associated with each of the PCGF-specific ncPRC1s (FIG. 1b)[25]. Like CBXs in cPRC1, each PCGF paralog has different molecular characteristics. PCGF1/3/5/6 are more effective in relieving auto-inhibition of RING1B ubiquitylation activity than PCGF2/4 in cPRC1[43]. This difference contributes to the substantively stronger ubiquitylation activity of ncPRC1 compared to cPRC1. Furthermore, RING finger and WD40-associated ubiquitin-like (RAWUL) domains in PCGF2/4 can bind PHC proteins, whereas those of PCGF1/3 cannot[88]. In fact, sequences of RAWUL domains of PCGF1/3/5/6 are substantially different from PCGF2/4's, possibly conferring PHC-mediated oligomerization activity only to PCGF2/4 containing cPRC1[89]. Reflecting distinct nature of PCGF2/4, double KO of *Pcgf2/4* in developing skin resulted in ectopic formation of Merkel cells, while KO of *Pcgf1*, *Pcgf3/5*, or *Pcgf6* did not show noticeable defects[50]. PCGF3/5 are also functionally distinct from other PCGFs. When ectopically recruited to an artificial promoter, PCGF2/4/6 repressed while PCGF3/5 activated luciferase expression in a human cell line[90]. In addition, PCGF3/5 have longer residence times on the *Xist* RNA domains of the inactive X-chromosome and are required for ubiquitylation of the inactive X chromosome[91]. PCGF6 is also unique in that it is required to specifically repress genes involved in meiotic initiation in mESCs[92,93].

PRC2 can be divided into PRC2.1 (contains a PCL paralog and mutually exclusive EPOP or PALI) and PRC2.2 (contains AEBP2 and JARID2) (FIG. 1a) (recently reviewed by Yu et al.[94]). One major difference in these two PRC2 complexes is that accessory subunits provide different targeting mechanisms. PRC2.1 can be targeted to CpG-rich promoters by PCL subunit[95,96]. PRC2.2 can also be targeted to the CpG-rich promoters but by recognizing H2AK119Ub1 with the JARID2 subunit[23,78]. PRC2.1 and PRC2.2 act redundantly to deposit H3K27me3 at target genes in mESCs[97,98]. On the other hand, during neural progenitor cell (NPC) differentiation, PRC2.1 and PRC2.2 repress distinct sets of genes, suggesting context-dependent functions of PRC2 subcomplexes[99].

Differences in the composition of subcomplexes in each of the three families can therefore alter both functional characteristics and targeting of the complexes. Paralogs and accessory proteins of Polycomb complexes often show cell type-specific expression patterns (FIG. 3). Do these changes in function impact the cooperative interactions illustrated in the hierarchical model? How might they strengthen the fidelity of PcG function during differentiation processes? We summarize below studies that begin to address these issues, emphasizing that a full characterization of this area is essential to understand maintenance during development but is in its infancy.

Context-specific functions

Polycomb paralogs, overlapping or specific functions?

Paralogous components of Polycomb complexes have some functional redundancy so they can compensate for each other in certain settings. Many PCGF or CBX paralogs possess the

same molecular function of supporting ubiquitylation[43] or compacting chromatin[10], but they vary in efficiency. They are also frequently expressed in the same cell types, as has been shown in mESCs and NPCs by quantitative mass spectrometry[100]. When these paralogs are expressed in the same cell, the target gene binding patterns largely overlap. A significant fraction of H3K27me3 high-occupancy sites were co-bound by multiple PCGF paralogs in mESCs[46,90]. In addition, CBX6/7/8 and RING1A/B chromatin binding patterns largely overlapped by ChIP-seq from human fibroblasts[101].

These Polycomb complexes with different paralogous components are likely to have redundant functions, as simultaneous knockouts (KOs) of more than one paralog often exacerbate cellular and organismal phenotypes. While *Ring1b* KO mESCs can maintain the expression of genes associated with pluripotency, *Ring1a* and *Ring1b* double KO mESCs spontaneously differentiate and cannot be maintained as stem cells[102]. Depletion of both *Ezh1* and *Ezh2* showed stronger derepression of PcG target genes in mESCs[103] and stronger defects in hair follicle morphogenesis[104] than the *Ezh2* single KO. In characterization of genes encoding cPRC1 components, *Pcgf2* and *Pcgf4* double KO and *Phc1* and *Phc2* double KO mice showed more severe homeotic transformations of the axial skeletons than the single KOs[72,105]. ncPRC1s also have redundant functions. Combined KO of *Pcgf1/3/5 or Pcgf1/3/5/6* had stronger depletion of H2AK119Ub1 than the single *Pcgf* KOs in mESCs[46]. When a PcG protein is absent, sometimes expression of the other paralog is upregulated. RING1A was upregulated post-transcriptionally in *Ring1b* mutant mESCs[102,106] and in oocytes[107]. However, paralogous protein levels were not always upregulated by the loss of the other as in the examples of some PCGFs[46]. Often the amount of remaining paralog-containing Polycomb complexes is sufficient to provide compensatory function. Even different classes of Polycomb complexes can provide redundant functions, as combined KO of PRC1 and PRC2 exacerbates cellular and gene expression phenotypes[108–110].

Cell type-specific mutant phenotypes were the first indication that the Polycomb paralogs may have unique functions (FIG. 3a). Many viable Polycomb mutant mice show a homeotic transformation phenotype[57,69,72–75,92,105,111], which supports their conserved role in the maintenance of *Hox* gene repression in early development. However, in addition to the axial patterning defect, *Cbx2* KO mice showed a male-to-female sex reversal phenotype, suggesting CBX2 has a specialized role in the suppression of female pathway in the embryonic gonad[74,112]. Furthermore, *Pcgf3/5* double KO mice showed that only female embryos were severely degraded at mid-gestation with malformed placentas, while male embryos looked seemingly normal at the same stage[91]. This female-specific phenotype is likely due to failure of X-inactivation. Indeed, *Pcgf3/5* double KO mESCs showed defects in *Xist*-mediated chromosome-wide gene silencing by RNA-seq[91]. On the other hand, KO of accessory subunits of cPRC1, *Scmh1* or *Scml2* showed male-specific sterility[111,113,114].

Distinct cellular responses driven by different paralogous components were also shown by overexpression studies. PCGF4 was first isolated as an oncogene to drive B cell lymphomagenesis when overexpressed, even before it was identified as a part of Polycomb complexes[115,116]. Another cDNA overexpression screen isolated *CBX7* to block replicative senescence of human prostate epithelial cells[117]. CBX7, but not CBX2/4/8 drove proliferation and contributed to stem cell potential and leukemogenesis when overexpressed

in hematopoietic stem/progenitor cells[118]. CBX7 overexpression also maintained mESCs close to the pluripotent state without *Xist* expression[71]. These data suggest that CBX7's major function is to maintain an undifferentiated state, which is consistent with its high expression in mESCs[70,71]. Other CBX proteins can perform similar roles in different contexts. When CBX4/6/7/8 were each expressed in human primary keratinocytes, only CBX4 induced the cells to remain in a quiescent and undifferentiated state[119]. On the other hand, CBX8 was unique in its ability to drive MLL-AF9 fusion leukemogenesis or mammary tumorigenesis[120,121].

These loss-of-function and overexpression data suggest clear non-overlapping, context-specific functions of different Polycomb paralogous proteins. Distinct complexes containing different paralogs might have 1) different abilities in covalently modifying or altering chromatin structure, 2) possible underappreciated roles in direct gene regulation, 3) differential targeting to specific genes, or 4) a combination of these properties. The challenge will be to understand which of the changes listed above drive diverse phenotypic outcomes. The key conclusion is that mammalian PcG complexes are not only diverse, but that diversity is important for normal development.

Paralog switching and cell type-specific components

In line with cell type-specific paralog functions, there are many cases in which one paralog is replaced with another during cell fate transitions (FIG. 3a). One well studied example is the differentiation of mESCs to embryoid bodies, neural, or mesoderm progenitors. CBX7 is the dominant CBX component in mESCs, and it is replaced by CBX2/4/8 in embryoid bodies[70,71]. CBX7 is also replaced by CBX2 during the differentiation of mESC to early cardiac mesoderm[122]. Similarly, PHC1, the major component of cPRC1 in mESCs is replaced with PHC2 in NPCs[59,70]. PCGF proteins also transition from being PCGF1/6-dominant in mESCs to PCGF1/4-dominant in NPCs[100]. PRC2 can undergo EZH2 to EZH1 subunit switching in hematopoietic stem/progenitor to erythroid progenitor differentiation[123]. During skeletal muscle differentiation, both EZH1 and EZH2 are expressed in proliferative myoblasts, while EZH1 expression becomes dominant in post-mitotic myotubes[124]. In general, EZH2 expression is restricted to embryonic and proliferative tissues, whereas EZH1 expression is more ubiquitous, albeit in lower levels than EZH2 (FIG. 3b)[125,126]. Consistent with their expression pattern, EZH2 is required for embryonic development, whereas EZH1 is dispensable[104,127]. Similarly, RING1B but not RING1A is specifically required for embryogenesis[128,129]. Expression of mESC-specific paralogs, such as CBX7 that cannot compact chromatin[10,82], may contribute to the unique characteristics of pluripotency, when chromatin is hypothesized to be more fluid[130]. As mESCs differentiate into specific lineages, cPRC1 subunits such as compaction-capable CBXs are upregulated, and it has been proposed that this functional change consolidates gene expression changes. Knocking the domain responsible for chromatin compaction from CBX2 into CBX7 disrupted the ability of mESCs to differentiate properly[82], suggesting the importance of forming a cPRC1 complex with the appropriate characteristics.

mESCs are also characterized by the strong expression of many accessory components of PRC2 (FIG. 3a and 3b). mESCs have high expression of PCL2 (MTF2), which is

replaced by PCL3 (PHF19) during NPC differentiation[100]. JARID2 is also highly expressed in mESCs or in pluripotent embryonic carcinoma cells and downregulated during embryoid body or neuronal differentiation[131,132]. EPOP is another PRC2 component with high expression in mESCs and inner cell mass of mouse embryos[133]. In fact, mESCs are distinct in that they have much higher levels of core PRC2 components than differentiated cells[100,134]. It has been hypothesized that this higher expression of PRC2 components may be a characteristic of pluripotent cells, which may need higher levels of *de novo* PRC2 recruitment for their uncommitted state[100]. Alternatively, the fast cell cycle of mESCs may demand more PRC2 to restore H3K27me3 levels before next replication[100].

Cell type-specific subunits also confer unique properties to PcG complexes (FIG. 3a). Like PHC, the SCM proteins have SAM domains, which can form helical polymers *in vitro*, and are required for PcG target gene repression by bridging different PcG complexes[135,136]. SCMH1, a sub-stoichiometric component of mammalian cPRC1, showed testis-enriched expression, and is required for preventing mistargeting of cPRC1 to the XY-body (a male germ cell-specific X chromosome silenced compartment) in spermatocytes[111]. SCML2 is another testis-enriched component of PRC1 required for prevention of somatic gene expression in progenitor spermatogonia[113]. Interestingly, SCML2 is also involved in blocking H2AK119 ubiquitylation in the XY-body of differentiated spermatocytes[113,114]. The fly ortholog, Scm, is specifically upregulated in differentiating female germ cells (nurse cells) and is required for the formation of punctated H3K27me3 domains and fertility[137].

Cell type-specific components can reduce the repressive activity or even provide an opposing, gene activation role for PcG complexes. AUTS2 is undetectable in mESCs but is upregulated in motor neurons, and forms ncPRC1.3 and ncPRC1.5 with PCGF3/5, P300 and CK2[138,139] (FIG. 1b). AUTS2-ncPRC1.3 is required for neuronal gene activation, and a transcription factor, NRF1 is required for AUTS2-ncPRC1.3 chromatin targeting[139]. ncPRC1.3 in mESCs required a different transcription factor, USF1 for chromatin targeting, suggesting cell type-specific recruitment mechanisms[90]. In resting B lymphocytes, Aurora B kinase binds RING1B and inhibits H2AK119 ubiquitylation activity at active promoters. Both proteins are required for transcription of active genes[140]. EZHIP (also called CATACOMB[141]) is a placental mammal-specific accessory component of PRC2, which can inhibit PRC2 activity[141–143]. EZHIP expression is enriched in both male and female germ cells[143] and is also upregulated in posterior fossa type A (PFA) ependymoma[144]. Consistent with its gonad-specific expression, *Ezhip* KO females had a progressive decrease in the number of follicles and showed lower fertility[143].

Even without explicit subunit switching, cellular differentiation and the associated chromatin environment changes can demand different Polycomb functions. For example, in mouse neocortex development, ubiquitylation by ncPRC1 was required for gene repression only in the early neurogenic phase, but was dispensable in the late astrogenic phase. The oligomerization activity of PHC2 was instead required for repression of the same set of genes in the astrogenic phase[145].

In sum, paralogous Polycomb proteins clearly play redundant roles, providing a critical safeguarding mechanism for Polycomb-mediated gene silencing. At the same time, these

paralogs and accessory proteins possess unique molecular functions and are expressed in a cell type-specific manner, contributing to the diversified cellular behaviors in developing mammalian cells. The simplest hypothesis to explain the biological role for these changes in activity is that regulation of cell type-specific genes in distinct lineages has evolved to require specific functions that are only provided by certain PcG paralogs.

Atypical genomic distributions

Atypical H3K27me3 in early development and redistribution of PcG complexes

Dynamic changes in PcG function in different cellular contexts are further highlighted by atypical genomic distributions of PcG proteins and histone modifications early in development. Furthermore, in certain perturbation conditions, PcG proteins are redistributed from the usual sites to ectopic loci, suggesting correct allocation of limited amounts of PcG proteins is a key aspect of PcG regulation.

PcG proteins and their associated histone modifications are usually found at promoters and a subset of strong PcG enrichment sites, spread out across tens of kilobases. However, in mouse preimplantation embryos, the usual promoter H3K27me3 is depleted on both the maternal and paternal genome[146]. Before activation of zygotic genome transcription, developing fly, zebrafish, and human embryos also showed loss of H3K27me3 enrichment at promoters[147–149], indicating that the erasure and then re-establishment of promoter H3K27me3 is conserved across species. Additionally, in mouse oocytes and in preimplantation embryos, H3K27me3 is found in broad intergenic regions with little DNA methylation and transcription (FIG. 4a)[146]. The broad intergenic maternal H3K27me3-modified loci in mouse oocytes and early embryos form self-interacting domains that are dependent on PRC1 and not on the more commonly observed genome organizing protein cohesin[150]. H2AK119Ub1 also showed atypical broad distribution that foreshadows H3K27me3 pattern in mouse oocytes and early embryos[151,152]. Reflecting these events on intergenic regions, H3K27me3 and cPRC1 are enriched at paternal but not maternal pericentromeres in one-cell mouse zygotes, further underscoring plasticity of PcG functions in different regulatory contexts[87,153].

What is the utility of this atypical organization and how is it regulated? DNA hypomethylation is hypothesized to be one mechanism for the unusual Polycomb domain expansion in intergenic regions in oocytes and preimplantation embryos. DNA methylation antagonizes binding of a targeting component of ncPRC1.1, KDM2B, to the CpG-rich sequences (e.g. CpG islands)[42,49,154]. Furthermore, the degree of CpG methylation at CpG islands anti-correlates with H3K27me3 levels in human ES cells[155]. When mESCs or MEFs were severely hypomethylated by knocking out DNA methyltransferases, ectopic H3K27me3 domains appeared in previously CpG-methylated promoters and intergenic regions[155–157]. The ectopic accumulation of H3K27me3 was accompanied by the decrease of H3K27me3 in normal target sites[156,157], suggesting formation of ectopic H3K27me3 domains may dilute away Polycomb complexes from the normal targets (FIG. 4a). However, when PcG proteins are redistributed in developmentally regulated settings, such as in oocytes or in preimplantation embryos, this atypical localization is hypothesized to have a role for compartmentalizing the maternal genome for efficient repression of non-canonical

targets, such as transposons[150]. In addition, this maternally-inherited H3K27me3 was critical for repression of maternal alleles for a small group of genes including *Xist*, providing a DNA methylation-independent means of imprinting[158–161]. On the other hand, human embryos appear to lack H3K27me3-mediated imprinting, indicating divergent usage of Polycomb mechanisms even within mammalian species[149].

PcG complexes can also be redistributed following disruption of opposing chromatin modifying proteins. Proper levels of H2AK119Ub1 deposition are maintained by the balance between ubiquitin ligase and deubiquitylation activities. BAP1 is an evolutionarily conserved H2AK119Ub1 deubiquitylase, and loss of BAP1 in mESCs resulted in the increase of the genome-wide H2AK119Ub1 level[162,163]. However, some genes with strong Polycomb enrichment showed a decrease in PRC1(RING1B) and PRC2(SUZ12) occupancy with accompanying derepression of gene expression, potentially because PcG complexes were titrated away from high-occupancy targets when H2AK119Ub1 level increased genome-wide (FIG. 4b)[162,163]. mSWI/SNF complexes also contribute to concentrating Polycomb complexes at target loci. BRG1 is the enzymatic component of the mSWI/SNF chromatin remodeling complexes, which can counteract Polycomb repression[164–166]. Acute depletion of BRG1 in mESCs paradoxically resulted in derepression of many highly Polycomb-bound genes including *HoxA* and *HoxD* genes[167]. ChIP-seq revealed that PRC1(RING1B) and PRC2(SUZ12) were redistributed from high-occupancy sites to low-occupancy sites, resulting in the derepression of Polycomb high-occupancy genes (FIG. 4c)[167]. The effects of both BAP1 and BRG1 depletion underscore the importance of balancing the genomic distribution of the finite amount of PcG complexes to appropriate targets.

These examples of PcG redistribution suggest that PcG proteins' nuclear concentrations might be maintained at limiting levels to ensure proper regulation. In line with this, PcG genes show dosage sensitive phenotypes when mutated, and overexpression also disrupts PcG functions[168–170]. In addition, wild-type CBX2, a component of cPRC1, cannot be significantly overexpressed in mESCs, while a compaction and phase separation-deficient mutant CBX2 can[171]. Since concentration is an important factor in determining genomic distribution, overall enzymatic activity, and non-enzymatic functions such as condensation[172], one hypothesis is that regulation of PcG concentration might be critical to its role in genome organization.

Maintenance of Gene Repression

A defining feature of the Polycomb system is that PcG proteins are frequently required to maintain, rather than establish, gene silencing during differentiation. In both flies and mice, expression of *Hox* boundaries were initially correctly established, but PcG gene mutant embryos failed to maintain boundaries of *Hox* repression[3,105]. Recruitment of the PcG proteins is responsive to changes in gene expression, as exemplified by findings that PRC2 can be passively recruited to CpG-rich promoters by transcriptional inactivity[34,155,173,174]. Active recruitment of the PcG machinery also occurs. For example, ncPRCs can be recruited to chromatin through sequence-specific DNA binding proteins such as USF1 for ncPRC1.3 or MAX/MGA and E2F6 for ncPRC1.6 in mESCs[90,92,175]. However, findings from many

developmental transitions suggest that a key role for the PcG complexes is to stabilize acquired cell fates established by changes in gene expression. Among many examples, when H3K27me3 is depleted, embryoid bodies are more likely to adopt an earlier mESC-like state when cultured in mESC media[176], and pancreatic β-cells dedifferentiate to an immature state[177]. In addition, *Ring1a/b* double KO astrogenic neural progenitors aberrantly adopt an earlier neurogenic state[178], and *E(z)* (ortholog of *Ezh1/2*) mutant fly female germ cells cannot acquire oocyte fate and instead transdifferentiate to nurse-like cells[179].

Divergent roles of PcG complexes in the maintenance of repression

How might ncPRC1, PRC2 and cPRC1 be involved in maintenance? One straightforward model is that the hierarchical recruitment pathway elucidated in mESCs (FIG. 2) is recapitulated at each cell division in stably differentiated tissues, thereby recreating a PcG repressed pattern on the genomes of daughter cells. By this model, the interplay between these three complexes would remain when pluripotent cells become differentiated, however changes in function of components of these complexes (e.g., changes in CBX or PCGF proteins) would change the balance of activities in this cascade, impacting maintenance. Genetic analysis implies that ncPRC1, PRC2 and cPRC1 might all be required for maintenance although the extent of contribution differs between these three complexes in different cellular contexts. We summarize current data on the roles for each family of complexes and relate them to the hierarchical model. We emphasize the hypothetical nature of any model for maintenance at the current time as this is a developing area in whole organisms.

A role for PRC2 in maintenance is attractive because transmission of the H3K27me3 modification to daughter cells might ensure the transmission of the entire PcG machinery. However, loss of function analyses indicate that cellular division leads to a decrease in H3K27me3 by replicative dilution and ensuing gene derepression even in the presence of functional PRC2. For example, when *de novo* recruitment of PcG complexes was lost by excision of the Polycomb Response Element (PRE) in fly larvae, after several cell divisions H3K27me3 was depleted, and *Hox* genes were derepressed in wing discs[180,181]. In contrast, when proliferation of the PRE-excised cells was blocked by developmental arrest or drug treatment, H3K27me3 levels and gene repression were maintained[180,181]. The fact that H3K27me3 cannot be maintained without a PRE suggests that the mark alone is not sufficient for self-maintenance over replication and requires additional *de novo* recruitment mechanisms.

Replicative dilution of H3K27me3 has been observed in mammalian systems, although this has largely been studied in the context of PRC2 disruption or inhibition. In mouse intestinal stem cells, it took several cellular divisions over 21 days to completely lose H3K27me3 and derepress PcG target genes after deletion of PRC2[182]. Cellular proliferation was also required for derepression in cultured lymphoma cells after inhibition of PRC2 enzymatic activity, and blocking cell cycle significantly delayed derepression[182]. Importantly, in these studies, the derepression kinetics were distinct in different cell types and for different genes in the same cell type. Basal levels of activating transcription factors[180] or promoter H3K4me3 level were proposed to be the determinants of the derepression potential[183].

Numerous genetic studies, previously reviewed elsewhere[94], imply that PRC2 is necessary for maintenance, but the studies above suggest that PRC2 alone is not sufficient to generate memory and likely works with PRC1 family complexes.

The loss of memory can be stochastic. This observation came from studies on cPRC1 components and supports a key role for cPRC1 in faithful memory. The ability of cPRC1 to compact and phase separate increases the concentration of PcG components in the condensates (Polycomb bodies), which might help preserve memory by increasing local concentrations of PcG machinery to allow reformation of repressive structures across cell division. Fly cPRC1 remains bound to replicated chromatin *in vitro*[184], and mammalian cPRC1 component CBX2 associates with mitotic chromosomes in cells[86]; both of these may assist in the re-formation of condensates during cell division. During vernalization of plants, *FLC*, a repressor of flowering, is silenced by the Polycomb system. When a chromodomain protein LHP1, a putative functional ortholog of the mammalian CBX protein, was mutated, *FLC* became de-repressed, but sporadically in a few clusters of distinct cells in the roots (FIG. 5c)[185]. Even within a single cell, one allele could be derepressed while the other allele remained silent, suggesting the memory is stored in *cis*[185]. Mammalian cells also showed stochastic derepression by loss of cPRC1. When CBX7 was ectopically recruited and then released from a fluorescent reporter locus, mESCs showed a bimodal state of the reporter reactivation, with more than half of the cells maintaining full repression after 10–12 cell divisions[106]. Similar stochastic derepression was also observed in human cells with live-imaging studies that monitored reporter gene reactivation after the release of tethered PRC2[186]. In addition, certain PRC2 mutant flies showed small patches of posteriorly transformed cells, reminiscent of the sporadic *FLC* derepression seen in plants[187]. Even when PcG was functional, transgenic flies carrying PREs occasionally showed variegated silencing of associated reporters in eyes and wing discs[188,189]. This stochastic, all-or-nothing response of the maintenance of repression reflects the bistable nature of Polycomb-mediated gene silencing by competition between repressors (PcG) and activators (trithorax group) (FIG. 5c and 5d)[190,191]. Polycomb mutant flies and mice are known to have variable phenotypes[73,192–196]. In extreme cases, the same *Scmh1* mutant mice exhibit phenotypes ranging from infertile by loss of post-meiotic germ cells to fertile and indistinguishable from the wild type[111]. The stochastic nature of Polycomb-mediated memory provides one possible mechanism for the variable penetrance.

Not all Polycomb mutants show stochastic, bimodal pattern of target gene derepression. These observations imply that different PcG complexes may contribute to distinct aspects of maintenance. When RING1B was degraded in mESCs using a degron system, most of the cells showed uniform derepression of Polycomb target genes[37]. Unlike PRE or PRC2 mutants where derepression occurred over the course of multiple cell divisions, RING1B degradation resulted in almost immediate derepression, occurring two hours from the induction of degradation[37]. This might reflect the fact that RING1B is essential for formation of both ncPRC1 and cPRC1 and eliminating both complexes might generate a severe phenotype in a short period. Alternatively, the fast derepression may indicate that the role of ubiquitylation catalyzed by RING1B (mostly by ncPRC1) is essential for the direct repression of genes (FIG. 5b). Supporting ncPRC1 and H2AK119Ub1's role in the direct repression, inducible loss of H2AK119Ub1 by conditional catalytic

point mutations of RING1B resulted in rapid derepression of PcG target genes within 72 hours[52]. In addition, release of tethered RYBP (ncPRC1) from an ectopic fluorescent reporter locus showed uniform derepression of the reporter within 6 days[106]. Studies in flies also support divergent roles of PcG complexes in the maintenance of repression[197], while exact molecular functions of orthologs can be different between species[10,198]. When mutant clones were made in fly larval wing discs, all the clones showed fast, strong derepression in *Psc* (*Pcgf*) or *Ph* (*Phc*) mutant clones, while slower and variable derepression was observed in *Pc* (*Cbx*) or *E(z)* (*Ezh*) mutants[197].

Overall, the Polycomb system provides maintenance of gene silencing, which can be inherited across cell divisions. The varying kinetics and heterogenous cellular responses by disrupting different Polycomb complexes might reflect the two distinct core features of the Polycomb system, direct repression and memory/maintenance. Mutations that impact direct repression might be expected to show widespread levels of derepression while mutations that impact memory might be expected to show stochastic derepression. For example, if a memory function went from nearly 100% effective in wild-type cells to 90% effective in cPRC1 or PRC2 mutant cells, that decrease to 90% effectiveness would result in 50% of cells with derepression after 6–7 cell divisions and thus disrupt normal development. In contrast, components involved directly in repressing gene expression, such as ncPRC1, might be required for that repression on all cells. Uncoupling direct repression and memory remains a hypothesis that is difficult to test in animals as perturbation of one aspect of PcG complex influences other complexes. Furthermore, it is unlikely that each PcG complex's role is exclusive to one aspect of gene silencing; rather, it could be that cPRC1 and PRC2 have more pronounced roles in the 'stabilization' of silencing than ncPRC1. Nevertheless, it will be critical to investigate the distinct and occasionally stochastic nature of gene derepression by dysregulation of Polycomb components to better understand pathogenesis of diseases associated with Polycomb mutations.

Conclusions and future perspectives

In the past decade, many specialized Polycomb complexes have been identified, and their molecular functions have been characterized. What has emerged from recent work is a defined model for the interactions between the three major complexes central to full repression of gene expression in pluripotent cells. Studies also have uncovered that these complexes change components and modify their function as cells differentiate. These variations in composition and function can be lineage-specific. Understanding the divergent roles of these complexes in direct repression to the memory maintenance in differentiated cells is essential for understanding stabilization of cell fate.

With our expanded understanding of Polycomb mechanisms, there are new challenges and opportunities ahead. How do the mechanisms identified through biochemical or cell culture experiments play out in the development of complex animals? For example, does hierarchical recruitment of ncPRC1 to PRC2 to cPRC1 identified in mESCs happen in every cell type in every cell division? Consistent with the hierarchical recruitment model, ncPRC1.1-mediated H2AK119Ub1 was required for PRC2-mediated H3K27me3 during the differentiation of mESCs to embryoid bodies[199]. In addition, deposition of H2AK119Ub1

preceded H3K27me3 in mouse preimplantation embryos[151,152] and in zebrafish embryos before zygotic genome activation[200], and also in an inducible X-inactivation system[201]. However, in growing oocytes without cell divisions, there were minimal changes in H3K27me3 when H2AK119Ub1 was lost by *Pcgf1/6* dKO[152]. Human hematopoietic stem cells divide every several months, while zebrafish embryonic cells divide every 16 minutes, how do these dramatic differences in timing impact necessary mechanisms? Further studies, in many contexts, will be needed to investigate, generalize, and refine the models discussed here. Finally, little is known about the behaviour of Polycomb bodies in the context of whole animals. These Polycomb domains contain high local concentrations of Polycomb complexes. Which gene targets reside in these bodies? Do these separated structures add an additional dimension in self-reinforcing mechanism and consolidation of memory? Formation and dissolution of Polycomb bodies are developmentally controlled[137,202], and there will be many surprises as we further our understanding of their behaviour in their native contexts. In summary, analyses of the different requirements of diverse lineages in distinct settings will be required to understand the full scope of PcG mechanisms that are at play in complex organisms.

Technological advances will enable us to tackle these important questions. PcG complexes positively influence each other, thereby perturbing one component invariably impacts other PcG complexes as well. In addition, compensation by other paralogous proteins makes it difficult to observe the effects clearly in genetic loss-of-function studies. Acute depletion of target proteins using inducible genetic deletion or degron-based approach and evaluating immediate responses will be helpful to resolve these issues. Sequencing and microscopy-based single cell approaches in the whole animal will be helpful to identify critical cell populations and stages that require PcG complexes and to assess stochastic responses by disruption of Polycomb function. Many new low-input chromatin profiling methodologies will be crucial to assess genome-wide chromatin changes in those critical small populations. Importantly, simultaneous advances in the mechanistic understanding based on live-single molecule tracking, biochemistry and structural biology will be the foundation for these *in vivo* studies.

Biochemistry and genetics have been successfully integrated to advance our knowledge of the Polycomb system. With an even deeper understanding of the molecular mechanisms and by applying the principles to developing cells in the organisms, the Polycomb system will provide fruitful ground for future unexpected discoveries and will continue to serve as a paradigm of epigenetic regulation of cell fate.

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Glossary

Nucleosome array:

In vitro reconstituted chromatin template used to study biochemical properties of chromatin modifying proteins, which is made from DNA with nucleosome positioning sequences and linker mixed with histone octamers.

mSWI/SNF complex:
A protein complex that can destabilize histone-DNA interactions in an ATP-dependent manner. It can create accessibility to DNA and counteract Polycomb-mediated repression.

Phase separation:
A phenomenon where proteins transition to another phase with different physicochemical properties, often through multivalent interactions among themselves. Potentially one of the driving forces to form membranelles organelles and condensates in the cell.

Homeotic transformation:
A class of mutant phenotypes that a body segment transforms into another body segment usually by misregulation of *Hox* genes, such as fly *Antennapedia* mutant producing legs instead of antennae.

Paralog and ortholog:
From an ancestral gene, paralogs are derived by gene duplication events within the same species, whereas orthologs are derived by speciation events (therefore orthologs are present in different species). Paralogous proteins can retain similar functions, but they can also acquire distinct functions. The same is true for orthologs.

Preimplantation embryo:
Placental animal embryos from zygote to prior to implantation stages. First lineage specification between inner cell mass (gives rise to embryo proper) and trophectoderm (placenta) cells happens during this stage.

Pericentromere:
A region of chromosome adjacent to the centromere, composed of AT-rich satellite DNA tandem repeats, usually DNA-methylated and decorated with H3K9 methylation.

CpG islands:
A stretch of ~1kb DNA region in vertebrates with overrepresentation of CpG dinucleotides than the genome average. They are often a site of transcription initiation, and more than half of annotated gene promoters are CpG islands.

Polycomb Response Element:
Discrete regulatory DNA element that can nucleate Polycomb complexes recruitment and silencing in flies.

Vernalization:
A process of prolonged exposure to the cold that induces flowering in plants. Genetic screens to find genes required for vernalization uncovered a number of genes later identified to be part of plant Polycomb complexes.

Zygotic genome activation:

After fertilization, transcription is absent in the zygotic genome, therefore embryos develop with maternally provided transcripts. Zygotic genome activation happens as maternal mRNA decays, and genes are transcribed from the zygotic genome through the process called maternal-to-zygotic transition.

Trithorax group (trxG):
A group of chromatin regulators that maintains an active state of gene expression that includes mSWI/SNF complex. Genes encoding trxG proteins were originally discovered by a genetic suppression screen in flies to suppress the Polycomb mutant phenotype.

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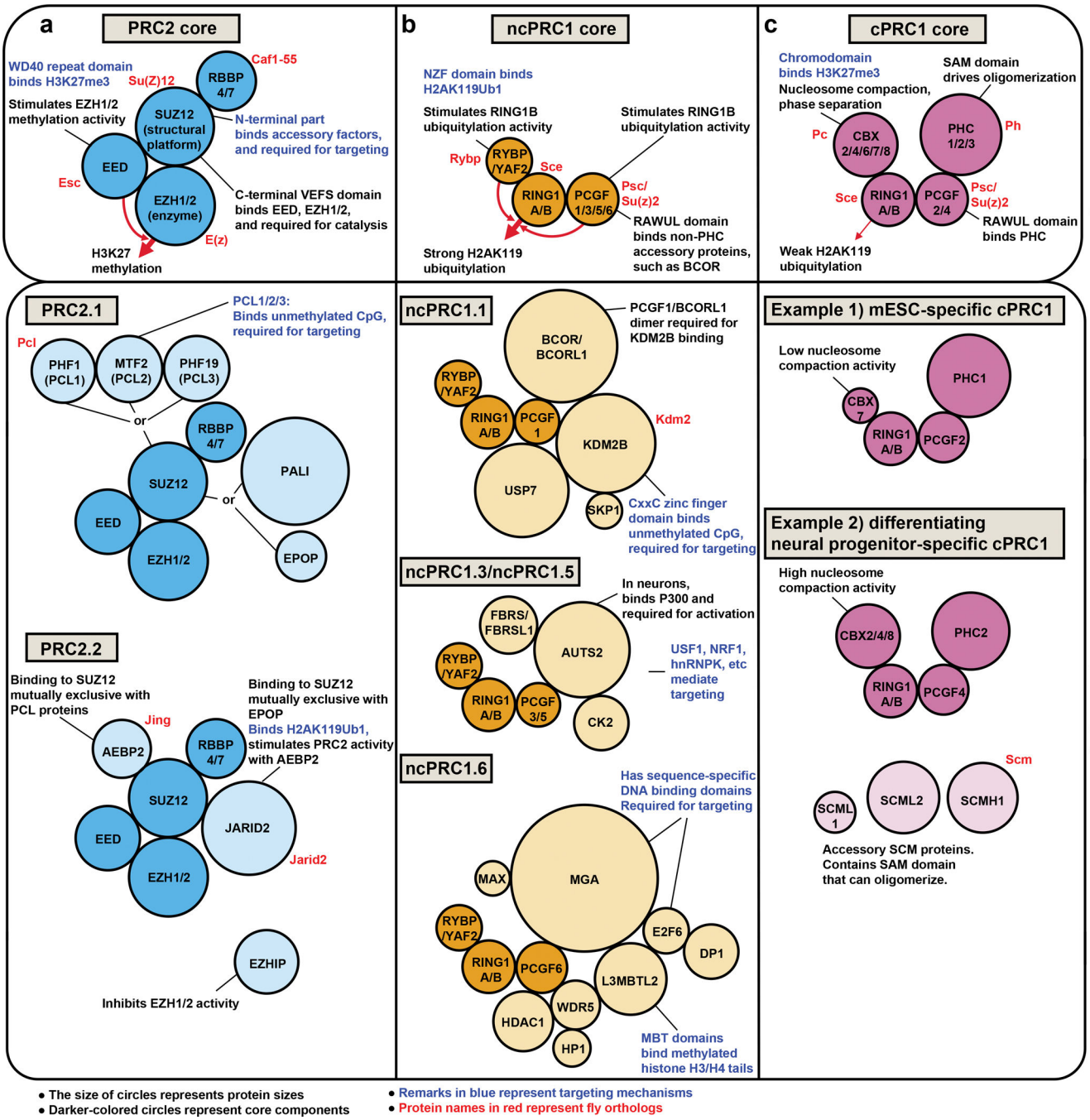

Figure 1 |. Composition and molecular functions of Polycomb complexes.

PRC2, ncPRC1 and cPRC1 have core components that form stable complexes with little variation between cell types (upper boxes) except when one paralog is switched with the other or accessory components are included (lower boxes). **a** | The core PRC2 complex can catalyze H3K27 methylation through the SET domain in EZH proteins. Sub-stoichiometric accessory proteins bind the N-terminal region of SUZ12. They can help PRC2 targeting. For example, PRC2.1 has PCL proteins that bind unmethylated CpG-rich DNA, and PRC2.2 has JARID2 that can bind H2AK119Ub1. Other accessory proteins such as PALI, EPOP

and EZHIP modulate PRC2 catalytic activity. **b, c** | Both cPRC1 and ncPRC1 have RING and PCGF proteins that dimerize through their respective RING domains[203,204]. RING1A/B has a RAWUL domain that binds either CBX proteins (for cPRC1), or RYBP/YAF2 (for ncPRC1). **b** | ncPRC1 can be defined by its specific PCGF paralog (for example, ncPRC1.1 contains PCGF1). In addition, each ncPRC1 has diverse accessory components that confer unique functions to the complexes. **c** | Cell type-specific cPRC1 complexes can be formed by combinatorial assembly of CBX, PHC and PCGF paralogs as shown in two examples of mESC and NPC-specific cPRC1s. In addition, SCM proteins can be incorporated in cPRC1 in a cell type-specific manner.

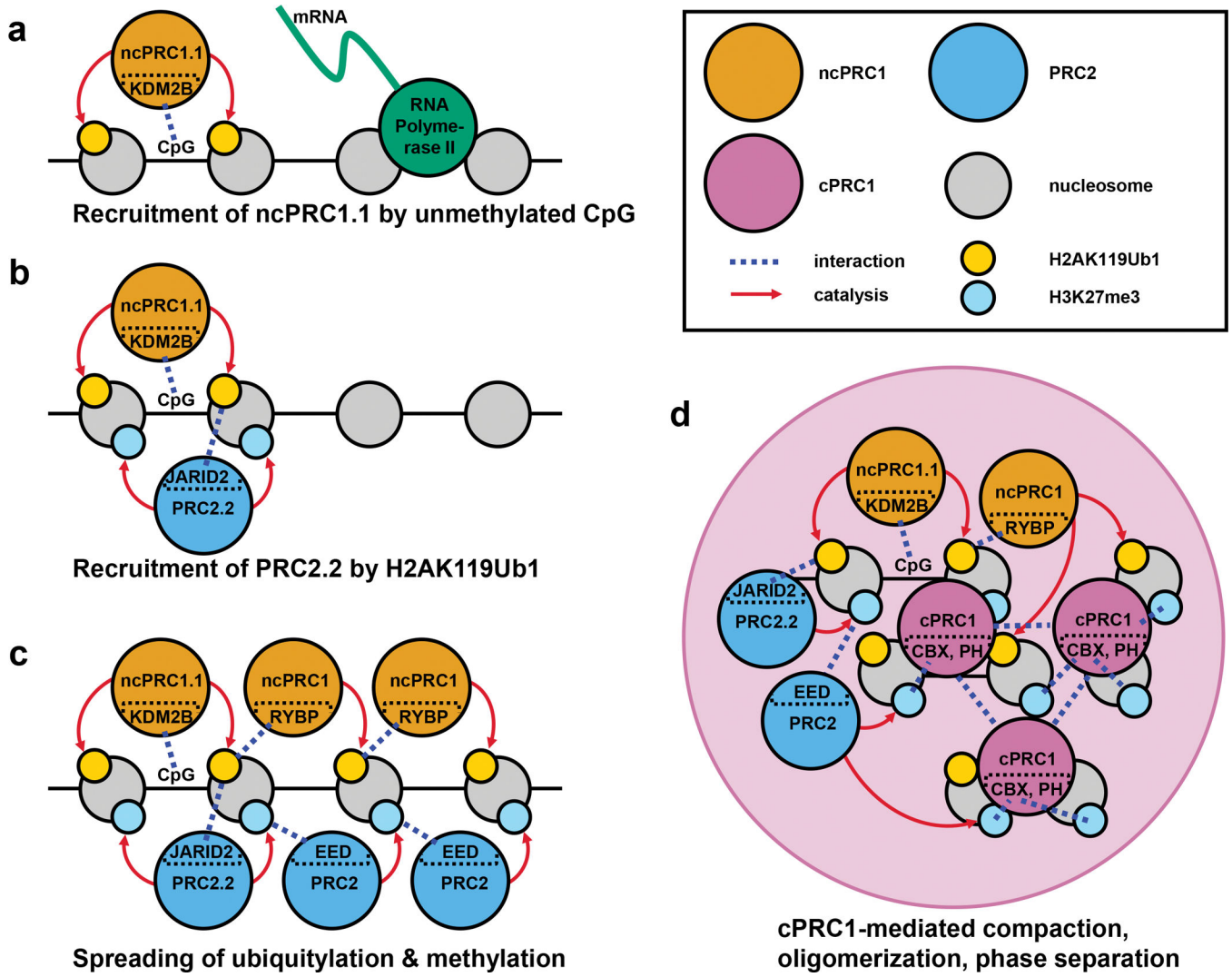

Figure 2 |. Formation of repressed domains by positive feedback of Polycomb complexes.

The process of Polycomb repressed domain formation by hierarchical recruitment of PcG complexes is represented. **a** | ncPRC1.1 can be targeted to hypomethylated CpG-rich promoters through its KDM2B subunit. KDM2B can be targeted to promoters regardless of its transcription status. Note that PRC2.1 can also be targeted to CpG-rich promoters through its PCL subunit, but that is not depicted here for simplicity and to emphasize the interplay between Polycomb complexes. **b** | PRC2.2 can be recruited to promoters by recognition of H2AK119Ub1 with its JARID2 subunit. Lack of transcription also contributes to PRC2 recruitment. **c** | H2AK119Ub1 and H3K27me3 modifications can spread beyond the initial recruitment site by 1) RYBP (ncPRC1) interaction with H2AK119Ub1, and 2) EED (PRC2) interaction with H3K27me3. **d** | The chromodomain in CBX proteins binds H3K27me3 and targets cPRC1 to the H3K27me3 enriched regions. CBX and PH subunits of cPRC1 compact nucleosome targets, bridge distant Polycomb bound regions, and phase separate to form Polycomb bodies. Modification of chromatin structure by cPRC1 can further help maintain high levels of H2AK119Ub1 and H3K27me3 as compacted and dense nucleosomes are better substrates for ncPRC1 and PRC2 enzymatic activities.

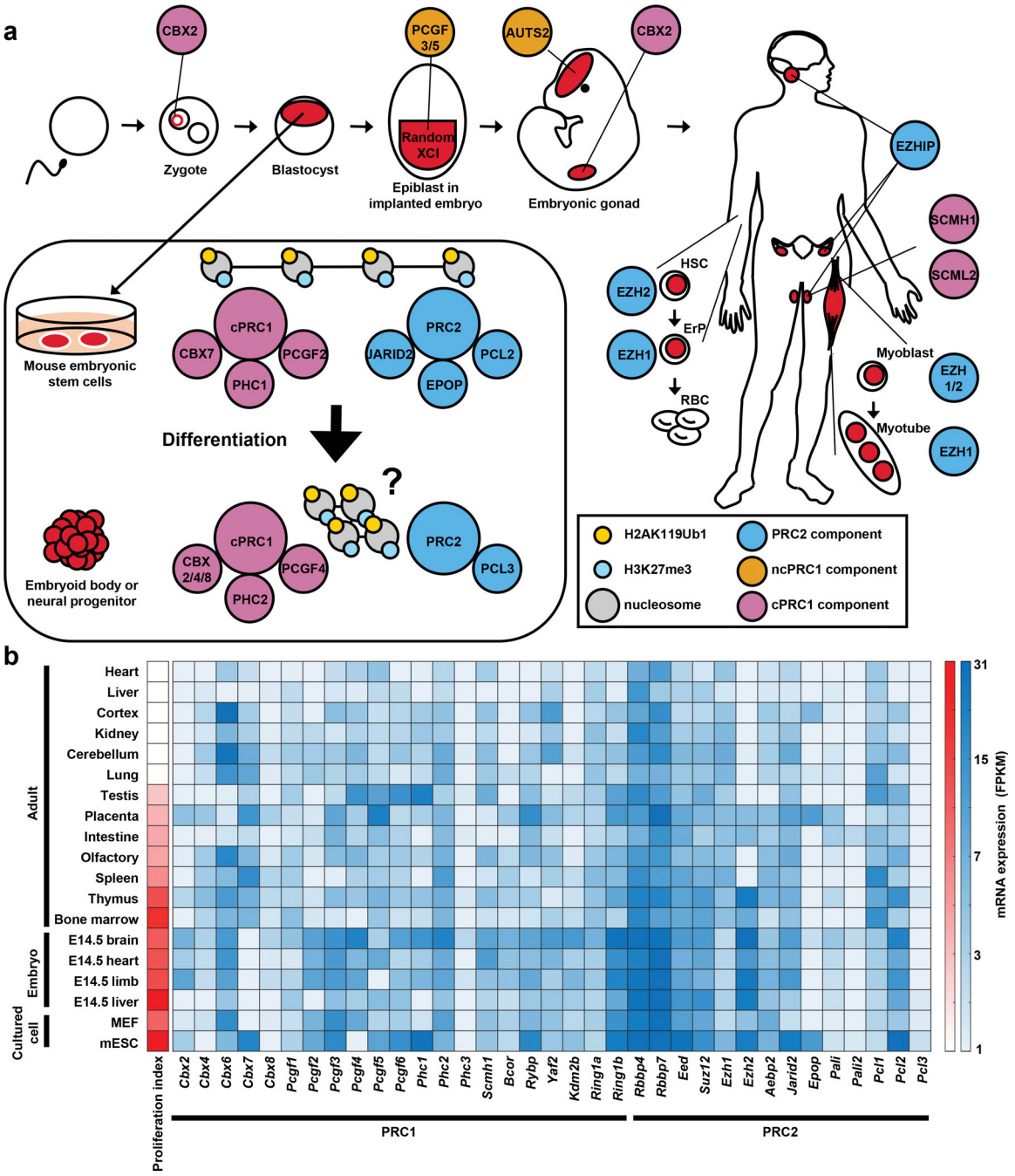

Figure 3 |. Examples of cell type-specific Polycomb complexes.

a | A cartoon describing mammalian development shows specific expression or phenotypes of PcG complex components in distinct cell types in embryogenesis or adult tissues. Inset shows paralog switching and accessory component incorporation in the transition from mESCs to differentiated cell types. **b** | Heat map showing mRNA expression of different PcG genes in the mouse embryo and adult tissues, as well as in cultured cell types, such as MEFs and mESCs. Note that core components of the complexes, such as *Ring1b*, *Eed*, show relatively uniform expression, while accessory components or components

with many paralogs exhibit more variable or tissue-specific expression. Proliferation index represents how proliferative the cells are in the tissues and is derived from median FPKM of 16 cell cycle genes, including *Mcm*s, *Cyclin*s, *Cdk*s. Data are from Gene Expression Omnibus GSE29278[205]. XCI, X-chromosome inactivation; HSC, hematopoietic stem cell; ErP, erythroid progenitor; E, embryonic day; MEF, mouse embryonic fibroblast; FPKM, fragments per kilobase million.

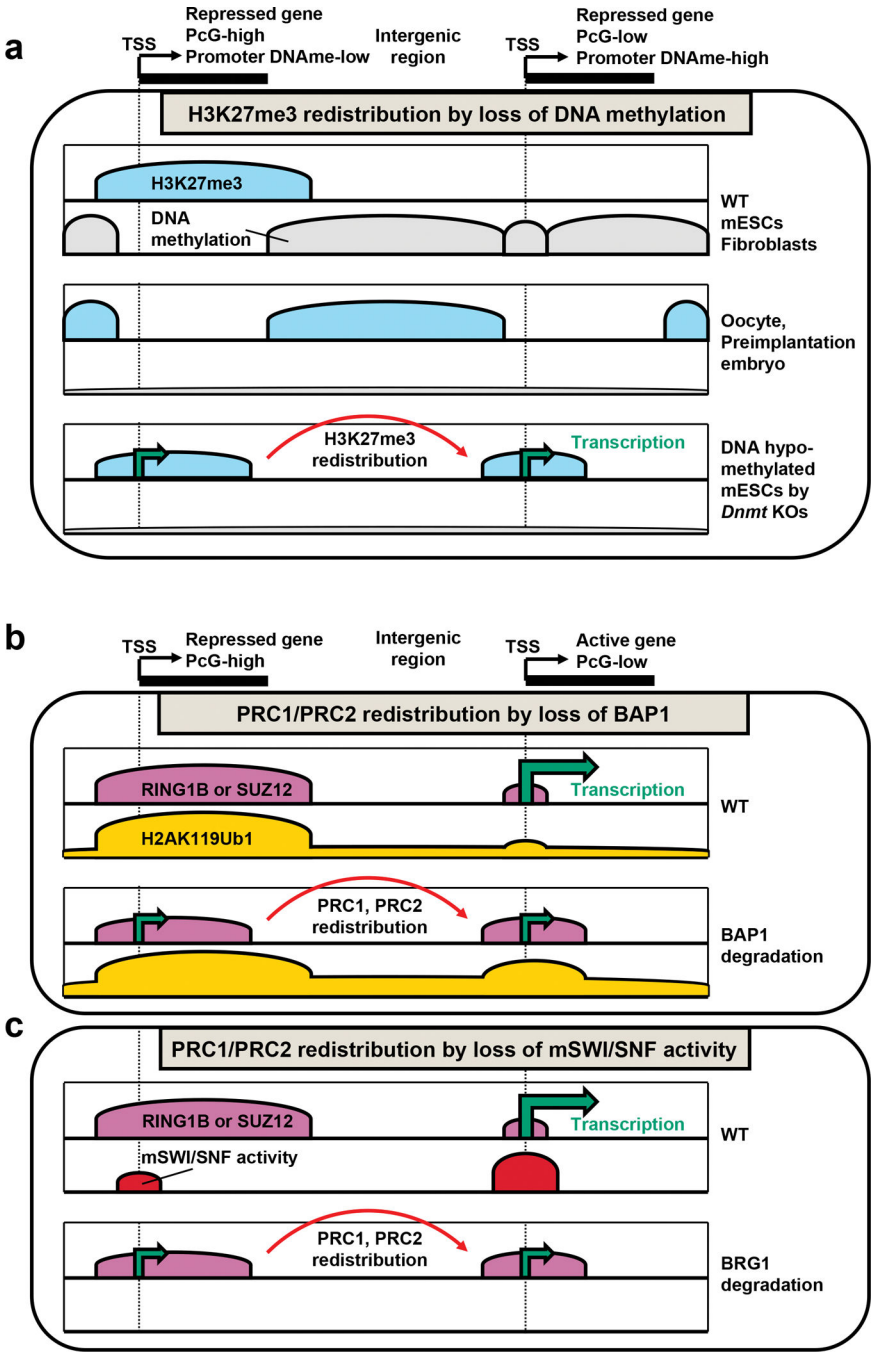

Figure 4 |. Redistribution of Polycomb complexes.

Schematic representation of PcG complexes and related DNA and histone modifications in a genome browser format to show the level and breadth of enrichment. **a** | Loss of DNA methylation: while one class of promoters (PcG-low, DNA-methyl high) gain H3K27me3 as they lose DNA methylation, another class of promoters (PcG-high, DNA-methyl low) show decrease in H3K27me3, suggesting potential redistribution of PRC2 from PcG-high to PcG-low promoters by global loss of DNA methylation. In oocytes and preimplantation embryos with developmentally regulated genome-wide DNA hypomethylation, H3K27me3

undergoes global remodelling to localize at intergenic regions. **b** | Loss of H2AK119Ub1 deubiquitylase BAP1: Global increase in H2AK119Ub1 level results in redistribution and decrease of PRC1(RING1B) and PRC2(SUZ12) from promoters normally have high levels of PcG complexes, accompanied by gene derepression. **c** | Loss of activity of the opposing chromatin modifying complex, mSWI/SNF: degradation of BRG1, the catalytic component of mSWI/SNF results in the increase of PRC1(RING1B) and PRC2(SUZ12) occupancy at usually PcG-low promoters, accompanied by the decrease of PRC1 and PRC2 from normally PcG-high promoters and gene derepression. TSS, transcription start site; DNAme, DNA methylation; Dnmt, DNA methyltransferase.

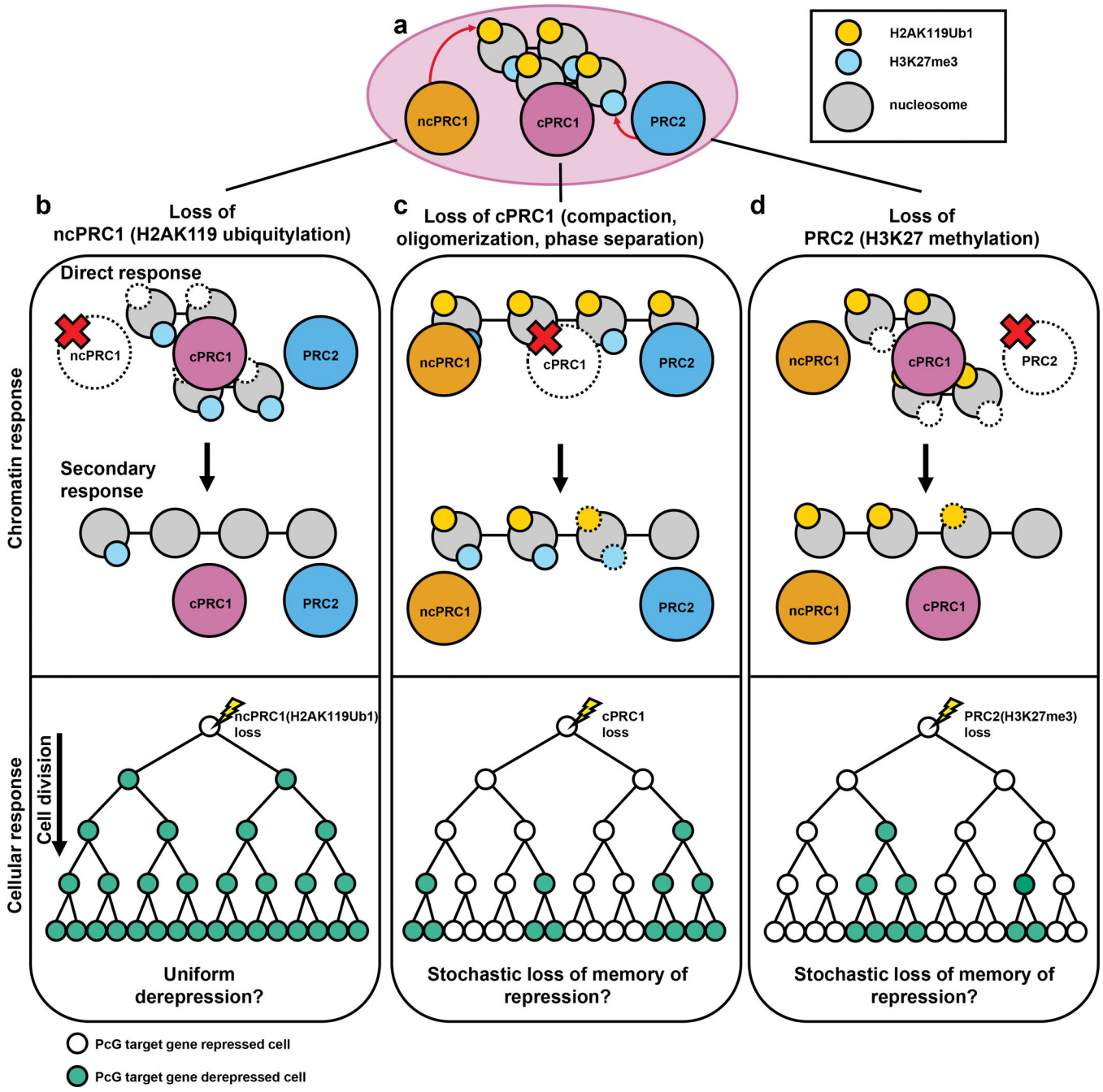

Figure 5 |. Hypothetical models on different roles of Polycomb complexes in the maintenance of gene repression.

a | A schematic of a repressed Polycomb domain with H2AK119Ub1 and H3K27me3 modifications. **b** | Chromatin response (upper): When ncPRC1 activity is disrupted, initially H2AK119Ub1 is lost. Because H2AK119Ub1 recruits PRC2.2, over time H3K27me3 deposition and in turn cPRC1 recruitment is decreased. Cellular response (lower): It is possible that ubiquitylation is involved in direct gene repression. Therefore, loss of H2AK119Ub1 may result in fast and uniform derepression of PcG target genes. **c** | Chromatin response: When cPRC1 activity is lost, chromatin compaction and long-range

interactions between PcG bound regions are disrupted. Because compacted and dense nucleosomes are better substrates for ncPRC1 and PRC2, loss of cPRC1 may eventually lead to decrease in H2AK119Ub1 and H3K27me3 levels over long term. Cellular response: Unlike ncPRC1, existing data are consistent with cPRC1 being involved in the memory of repression, resulting in slower and stochastic conversion to the “on” state of PcG target genes by loss of cPRC1 function. **d** | Chromatin response: When PRC2 activity is disrupted, H3K27me3 level is decreased at first, followed by the decrease in the cPRC1. Resulting disruption of Polycomb domain structure will negatively impact in reaching full H2AK119Ub1 level. Cellular response: Similar to cPRC1, PRC2 and H3K27me3 may also be involved in the memory of repression, resulting in slower and stochastic conversion of PcG targets to the “on” state by loss of PRC2 function.