A new world of Polycombs: unexpected partnerships and emerging functions

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Abstract | Polycomb group (PcG) proteins are epigenetic repressors that are essential for the transcriptional control of cell differentiation and development. PcG-mediated repression is associated with specific post-translational histone modifications and is thought to involve both biochemical and physical modulation of chromatin structure. Recent advances show that PcG complexes comprise a multiplicity of variants and are far more biochemically diverse than previously thought. The importance of these new PcG complexes for normal development and disease, their targeting mechanisms and their shifting roles in the course of differentiation are now the subject of investigation and the focus of this Review.

Homeotic genes
A set of related master transcription regulatory factors that regulate morphogenesis and tissue differentiation.

The classical view of the mechanisms of Polycomb group (PcG) proteins is based on genetic evidence from *Drosophila melanogaster* — genes were classified as belonging to the PcG on the basis of mutations that result in the derepression of *D. melanogaster* homeotic genes[1,2]. Complexes of PcG proteins are recruited to any given homeotic gene if that gene is transiently repressed by segmentation gene products, which are themselves governed by maternal positional cues. As a result, PcG complexes keep homeotic genes repressed in specific embryonic domains, and this repressed state is, in most cases, maintained for the rest of development[3].

Work in mammalian and fly systems over the past 10 years has changed our perspective of this PcG paradigm. High-throughput genomic techniques have shown that, in addition to homeotic genes, hundreds, and perhaps thousands, of other genes are also regulated by PcG proteins. Many of these target genes encode transcription factors or morphogens that control key developmental processes. PcG-mediated repression of many of these genes is dynamic and can vary during development and differentiation, although the repressed state tends to be maintained from one cell cycle to the next. Therefore, a major question is how PcG proteins provide both the flexibility and versatility that are needed for different developmental targets. Recent advances in the biochemical characterization of PcG complexes have revealed a range of new components, which lead to a large number of variant PcG complexes. In addition, analyses of cancer-associated mutations have revealed the role of both overexpression and underexpression of some PcG complexes in oncogenesis.

This Review attempts to summarize what has been learned about the varieties of PcG complexes, the range of roles that they might have on chromatin and non-chromatin targets, and the ways in which they may be recruited to their targets. As is often the case, the technically challenging functional studies of PcG complexes lag behind their biochemical characterization. Therefore, we suggest the reader to take some of the emerging new roles of PcG complexes with caution, as they have yet to stand the test of time in this rapidly developing research field. We first review the classical (or canonical) model for the structure and function of PcG complexes, and we then discuss various novel Polycomb repressive complex 1 (PRC1)-related complexes and their possible roles in flies and mammals. We then focus on variant PRC2 complexes, before moving on to the problem of recruitment and concluding with a discussion of new discoveries on the role of PcG complexes in disease. When mammalian results are not further specified, they refer to both mouse and human data.

PcG complexes — the canonical view

Genetic and biochemical experiments in flies and mammals converged to give a molecular picture of the basic PcG-mediated repressive mechanism. Two principal multiprotein Polycomb repressive complexes PRC1 and PRC2 are recruited to PcG-target genes and collaborate to effect transcriptional repression. In *D. melanogaster*,

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specific Polycomb response elements (PREs) have been identified at many of the target genes of PcG proteins; PREs are the binding sites to which PRC1 and PRC2 are recruited, often together with additional proteins that are thought to modulate repressive functions (BOX 1). Neither PRC1 nor PRC2 has DNA-binding components. Unlike most DNA-binding transcription factors, a key feature of PcG complexes in both flies and mammals is that, although they are present in all cells, whether they bind to a specific target gene depends on the prior history and the chromatin state of that gene.

Repressive functions of PcG complexes. The function of PcG complexes, which has been well demonstrated in plants, insects and vertebrates, is to suppress the expression of their target genes. How this is exactly accomplished is less clear, but it is most likely that both PRC1 and PRC2 have repressive activities[4]. It is generally

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Box 1 | The canonical Polycomb group complexes

PRC1

Polycomb repressive complex 1 (PRC1) has a core of four proteins[122–124]. In *Drosophila melanogaster*, these are Polycomb (Pc), which contains a chromodomain that binds to trimethylated histone H3 lysine 27 (H3K27me3); Polyhomeotic (Ph), which has two paralogues Polyhomeotic-proximal (Ph-p) and Polyhomeotic-distal (Ph-d); RING1, the product of *Sex combs extra* (*Sce*); and Posterior sex combs (Psc), or the closely related Suppressor of zeste 2 (Su(z)2) (see the figure). RING1 and Psc are structurally related and form a heterodimer, which promotes the E3 ubiquitin ligase activity of RING1 on histone H2A[125–127]. The RING1–Psc heterodimer is the framework on which the core PRC1 complex is assembled. More loosely associated with the core complex is Sex comb on midleg (Scm), a protein with two malignant brain tumour (MBT) repeats and a sterile α-motif (SAM) domain, through which it is thought to interact with Ph[122,128,129]. Representations of the core PRC1 and PRC2 complexes are shown in the figure. The areas of the circles that depict subunits of the *D. melanogaster* complexes reflect the relative sizes of the corresponding proteins. The dashed outline of the Scm subunit indicates its weak association with the PRC1 complex. The relative arrangement of the subunits reflects known direct associations.

Mammalian homologues have been discovered for each of the PRC1 proteins, and mammalian genomes have many alternative paralogues for each (TABLE 1). Thus, mammals have RING1 and RING2, although RING2 predominates. The two proteins seem to be interchangeable in at least some of the complexes, but this has not been systematically examined. There are at least three Ph homologues (Polyhomeotic-like protein 1 (PHC1), PHC2 and PHC3), five Pc homologues (chromobox protein homologue 2 (CBX2), CBX4, CBX6, CBX7 and CBX8), two Psc homologues (BMI1 and MEL18) and four other Polycomb group RING finger proteins (PCGFs)[15,19,20].

PRC2

PRC2 contains the Enhancer of zeste methyltransferase (E(z)) that monomethylates, dimethylates and trimethylates H3K27 (REFS 130–133). The methylation of H3K27 is essential for Polycomb group (PcG)-mediated repression and, in *D. melanogaster*, the replacement of wild-type histone H3 with a Lys27Arg variant mimics the loss of E(z)[134]. *D. melanogaster* E(z) is the core which binds to the WD40 domain of Extra sexcombs (Esc) (or of its close homologue Escl) and to Su(z)12, both of which are essential for PRC2 activity because they interact with both the target and the surrounding nucleosomes and receive inputs that regulate the methyltransferase activity[91–95]. The histone chaperone Caf1 binds to Su(z)12 and contributes to the activity of PRC2 (see the figure). Mammalian PRC2 complexes contain the direct homologues EZH2 (or, in some cases, EZH1), EED, SUZ12 and the Caf1 homologues histone-binding proteins RBBP4 and RBBP7. Although there is only one *EED* gene, alternative transcription start sites result in several products that may give rise to different functions[133]. An additional component, zinc-finger protein AEBP2 (the mammalian homologue of Jing in *D. melanogaster*), promotes the stability of the complex and the binding to at least a subset of target sites[135–137], but it is not essential for function.

Supporting components

Analyses of other *D. melanogaster* PcG genes showed that their products are not components of PRC1 and PRC2 but form distinct accessory complexes. It is becoming clear that the binding and/or the repressive activities of PcG complexes result from a multiplicity of fairly weak interactions that collectively constitute the robust repressive mechanism.

The Pho repressive complex (PhoRC) contains Pho (a DNA-binding protein that is homologous to the mammalian transcriptional repressor protein YY1) and SFMBT (Scm-like with four MBT domains protein)[138,139]. As a sequence-specific DNA-binding protein, Pho is thought to help the recruitment of PcG complexes to Polycomb response elements (PREs). The mammalian YY1 has long been thought to interact with PcG complexes, but genomic binding profiles show little overlap between YY1-binding sites and PcG proteins in mammalian genomes[139–141].

The Polycomb repressive deubiquitinase complex (PR-DUB) contains the Calypso ubiquitin carboxy-terminal hydrolase and Additional sex combs (Asx). It has a specific H2A deubiquitinase activity that is paradoxically required for PcG-mediated repression[142], which suggests that the appropriate regulation of ubiquitylation is essential for PcG-mediated repression. Mammalian homologues of these proteins exist, but their role in PcG-mediated repression has not been established.

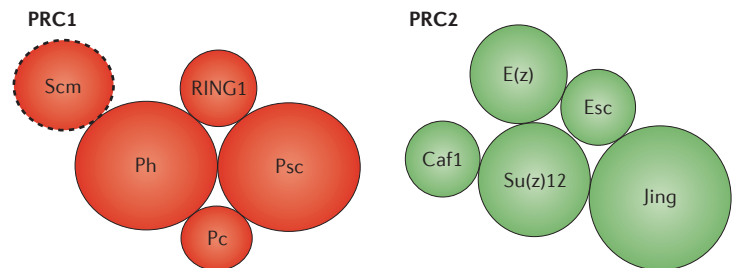For more comprehensive reviews of canonical PcG complexes and their action, see REFS 144,145.

Table 1 | **PRC1 and PRC2 core complex components in *Drosophila melanogaster* and humans**

Drosophila melanogaster subunits	Characteristic domains	Homologous subunits in humans
Polycomb repressive complex 1 (PRC1)		
E3 ubiquitin-protein ligase RING1 (also known as Sce)	RING	RING2 (also known as RING1B and RNF2) and RING1 (also known as RING1A and RNF1)
Posterior sex combs (Psc) and Suppressor of zeste 2 (Su(z)2)	RING	BMI1 (also known as PCGF4) and MEL18 (also known as PCGF2)
Polyhomeotic-proximal (Ph-p) and Polyhomeotic-distal (Ph-d)	Sterile α-motif (SAM) and zinc-finger	Polyhomeotic-like protein 1 (PHC1; also known as EDR1), PHC2 (also known as EDR2) and PHC3 (also known as EDR3)
Polycomb (Pc)	Chromodomain	Chromobox protein homologue 2 (CBX2), CBX4, CBX6, CBX7 and CBX8
Sex comb on midleg (Scm)	Malignant brain tumour (MBT), SAM and zinc-finger	Sex comb on midleg homologue 1 (SCMH1) and Sex comb on midleg-like protein 2 (SCML2)
Polycomb repressive complex 2 (PRC2)		
Enhancer of zeste (E(z))	SANT, CXC and SET (Su(var)3-9–Enhancer of zeste–Trithorax)	Enhancer of zeste homologue 2 (EZH2; also known as KMT6) and EZH1
Extra sex combs (Esc) and Extra sex combs-like (Escl)	WD40	EED
Suppressor of zeste 12 (Su(z)12)	Zinc-finger and VEFS (VRN2–EMF2–FIS2–Su(z)12) box	SUZ12
Chromatin assembly factor 1 subunit Caf1	WD40	Histone-binding protein RBBP4 (also known as RBAP48) and RBBP7 (also known as RBAP46)
Jing	Zinc-finger	Zinc-finger protein AEBP2

considered that the histone H2A ubiquitylation produced by the E3 ubiquitin-protein ligases RING1 or RING2 components of PRC1 interferes with transcription elongation by RNA polymerase II[5], but PcG-mediated repression has also been shown to prevent Pol II from forming the initiation complex[6]. It has also been claimed that PRC1 induces local chromatin condensation even in the absence of H2A ubiquitylation[7,8]. Repressive functions of PRC2 are less well characterized, but it is clear that histone H3 lysine 27 (H3K27) methylation by PRC2 prevents H3K27 acetylation, a modification that is associated with both the promoter and enhancer regions of active genes.

A genetic study of PRC1 functions in *D. melanogaster* showed that different PRC1-binding genes have different requirements. For some, repression requires all four core components of PRC1, whereas others are not affected by the absence of RING1 (the product of *Sex comb extra* (*Sce*); also known as dRING) or Polycomb (Pc) but are more dependent on the Polyhomeotic (Ph), Posterior sex combs (Psc) and Suppressor of zeste 2 (Su(z)2) components[9] (TABLE 1). These results, taken at face value, suggest four main conclusions. First, the repressive activity associated with PRC1 is far more heterogeneous than expected. Second, the canonical PRC1 complex, at least in *D. melanogaster*, can be partially disassembled without necessarily losing all repressive function. Third, repression does not always require H2A ubiquitylation. Fourth, the repression of some genes in the absence of the Pc component, which binds to trimethylated H3K27 (H3K27me3), suggests that H3K27me3 is not specifically required in these cases. It is clear that some *D. melanogaster* genes bind to PRC1 in the absence of PRC2 or H3K27me3 (REF. 10). This last conclusion

was also reached for mouse embryonic stem cells by comparing the genes that were derepressed by the knockout of the gene encoding the mouse RING2 protein with those that were derepressed by the knockout of embryonic ectoderm development (*Eed*) (hence the knockout of PRC2)[4].

Clearly, despite two decades of intensive studies, many gaps remain in our understanding of how PRC1 and PRC2 effect transcriptional repression. Such repression most probably involves multiple mechanisms that interfere with productive gene expression.

RING2 complexes

The RING2 protein (also known as RING1B and RNF2) is considered the heart of the PRC1-mediated repressive mechanism. In the past few years, the nature and functions of RING2-containing complexes have been discovered to be far more diverse with the exuberant expansion in our knowledge of the range of complexes that differ in the number and variety of components (FIG. 1). Whether all of these new complexes function as epigenetic repressors remains an open question.

KDM2-containing complexes. In both mammals and flies, the RING2 protein and its activity as an H2A E3 ubiquityl transferase are crucial for the repression of *HOX* genes. However, a surprising discovery was that much of this activity does not reside in the canonical PRC1 complex. It was first reported in *D. melanogaster* that a complex called dRING-associated factors (dRAF) — containing RING1, Psc, and the histone H3K36 demethylase Kdm2 — is in fact responsible for most of the H2A ubiquitylation[11]. Although the genomic distribution of dRAF is not available, a comparison of RING1,
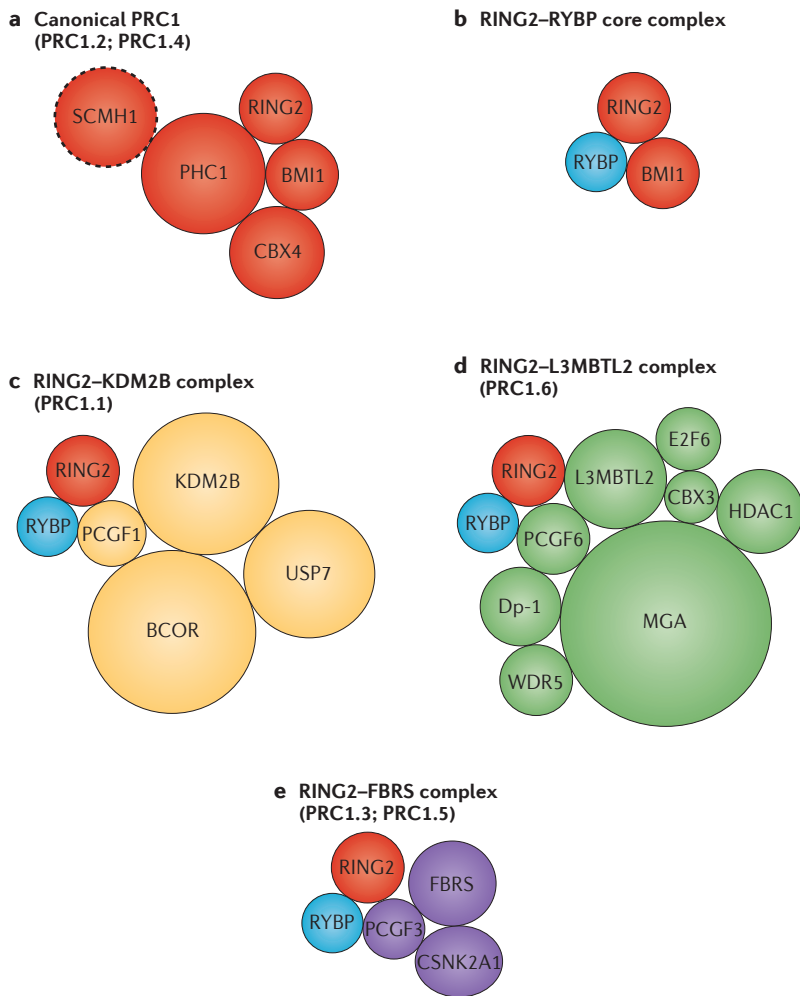
Figure 1 | **Mammalian RING2 complexes.** The assignment of different human proteins to complexes is primarily based on a biochemical purification study[15], but other reports[12,13] were also consulted. The areas of the circles reflect the relative sizes of the primary isoforms of their corresponding proteins as defined in the UniProt database. The subunits present in the canonical Polycomb repressive complex 1 (PRC1) are shown in red; names for variant complexes according to REF. 15 are shown in parentheses. **a** | A representative canonical PRC1 is shown. Some variants of this complex (such as PRC1.2 and PRC1.4) incorporate the related chromobox protein homologue (CBX) proteins and Polyhomeotic-like proteins (PHC), or MEL18 and E3 ubiquitin-protein ligase RING1, instead of BMI1 and RING2; see TABLE 1 for the full list of related proteins. The dashed outline of the Sex comb on midleg homologue 1 (SCMH1) subunit indicates its weak association with the PRC1 core components. **b** | Although the existence of RING2–RYBP (RING1 and YY1-binding protein; shown in blue) or its related RING1–RYBP (not shown) core components is strongly suggested by glycerol centrifugation analyses[15], it remains to be seen whether this entity exists *in vivo* or whether it is a product of partial dissociation during biochemical purification. For the complexes shown in parts **b**, **c** and **d**, alternative complexes in which the RYBP subunit is substituted by the closely related YY1-associated factor 2 (YAF2) protein have also been purified but are not represented here. **c** | The subunits that are specific to RING2–KDM2B (lysine-specific demethylase 2B) complexes are shown in yellow. Among these subunits, only BCL-6 co-repressor (BCOR) is known to have a related variant protein BCL-6 corepressor-like protein 1 (BCORL1). **d** | The subunits that are specific to the RING2–L3MBTL2 (lethal(3)malignant brain tumour-like protein 2) complex are shown in green. Among these subunits, only histone deacetylase 1 (HDAC1) is known to be substituted in some instances by HDAC2. **e** | Both Polycomb group RING finger protein 3 (PCGF3) and PCGF5 can be incorporated into RING2–FBRS (probable fibrosin-1) and its variant complexes; components that are unique to these complexes are shown in purple. CSNK2A1, casein kinase 2, α1 polypeptide; USP7, ubiquitin carboxy-terminal hydrolase 7; WDR5, WD repeat-containing protein 5.

Pc and Psc distributions indicates that few sites bind to both RING1 and Psc but not to Pc, which suggests that dRAF and PRC1 target the same genes.

In mammals, Kdm2 has two homologues, KDM2A and KDM2B. Both of these contain a zinc-finger-CxxC motif that binds to unmethylated CpG islands and removes the dimethylation or trimethylation mark of H3K36 that is widely distributed in mammalian chromatin[12–14]. Similarly to *D. melanogaster* Kdm2, mammalian KDM2B, but not KDM2A, forms a complex that includes RING2 and a Psc-related protein, Polycomb group RING finger protein 1 (PCGF1). The zinc-finger-CxxC motif of KDM2B targets this complex to a subset of unmethylated CpG islands that are bound by PRC1 and PRC2 (REFS 12–14), where it seems to be responsible for most of the H2AK119 ubiquitylation (H2AK119ub), at least in embryonic stem cells[13]. In addition, the mammalian analogue of dRAF incorporates either RYBP (RING1 and YY1-binding protein) or its close homologue YY1-associated factor 2 (YAF2) (see below), both of which greatly stimulate the ubiquityl ligase activity of the complex[15]. We may surmise that the *D. melanogaster* dRAF complex also contains the fly RYBP homologue.

Biological roles of mammalian RING2–KDM2B complexes. Complicating the function of the mammalian RING2–KDM2B complex is the fact that a large proportion of KDM2B probably has roles that are independent of RING2 and PCGF1, and binds to thousands of transcriptionally active unmethylated CpG-rich promoters[12–14]. A partial knockdown of KDM2B in mouse embryonic stem cells, in which both KDM2A and KDM2B are expressed[12], leads to subtle but distinct defects in differentiation[13,14]. Thus, KDM2B-depleted mouse embryonic stem cells can proliferate as well as control cells, but the resulting embryoid bodies are denser and lack central cavities[13]. In addition, the KDM2B-depleted embryonic stem cells fail to differentiate in a monolayer culture[13,14]. These defects resemble those caused by the knockdown of RYBP[16] and are accompanied by both the reduction of H2AK119ub levels and the derepression of some PcG-target genes[12,13]. Collectively, these observations suggest that, in embryonic stem cells, the RING2–KDM2B complex functions together with both PRC1 and PRC2 to repress genes that are important for development and differentiation. Consistent with this, the overexpression of KDM2B inhibits replicative senescence and immortalizes mouse embryonic fibroblasts[17], in which KDM2B (but not KDM2A), together with canonical PcG complexes, represses cyclin-dependent kinase inhibitor 2A (*Cdkn2a*)[18], which encodes two distinct proteins (ARF and INK4A (also known as p16)) that normally block cell cycle progression. Curiously, the histone demethylase activity of KDM2B seems to be dispensable for its function in mouse embryonic stem cells[14] but is required for *Cdkn2a* repression in immortalized embryonic fibroblasts[18], which indicates that the demethylation of H3K36 may be more important for repression in differentiated cells.

Box 2 | **Possible new molecular roles of variant PRC1 complexes**

Polycomb repressive complex 1 (PRC1) and its variant complexes that contain chromobox protein homologue 7 (CBX7) are predominant in embryonic stem cells and are required to maintain pluripotency. In some cases, complexes that contain other CBX variants may have specialized roles that are regulated by an interplay between the post-translational modification of CBX variants and binding to alternative non-coding RNAs (ncRNAs). In cultured cells, the Polycomb group (PcG) protein E3 SUMO-protein ligase CBX4 is methylated by the histone-lysine N-methyl transferase SUV39h at lysine 191 (REF. 143). This causes the binding of CBX4 to the ncRNA taurine upregulated 1 (*TUG1*), changes its chromodomain-binding preference from trimethylated histone H3 lysine 9 (H3K9me3) to H3K27me3 and represses its target genes. The demethylation of CBX4 by lysine-specific demethylase 4C (KDM4C) switches its association to a different ncRNA, metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*; also known as *NEAT2*), and its binding preference to H2A acetylated at K5 or K13, both of which are marks of transcriptional activity. This switch is accompanied by the nuclear relocation of the target genes with their associated CBX4 from the Polycomb foci, which is the location of CBX4 when they are repressed, to interchromatin granules, where transcriptional activity takes place. Furthermore, the unmethylated CBX4 sumoylates the growth regulator E2F1 that binds to growth-promoting genes which are subject to CBX4 regulation, a modification that seems to be necessary for their activation[143].

PRC1-related complexes and beyond

A spate of recent publications[12–16,19,20] has greatly expanded the range of RING2 complexes (or non-canonical PRC1) discovered in both humans and mice, and has placed the KDM2B complex in the framework of a much broader classification. RING1 (also known as RING1A and RNF1) can replace RING2 in at least some of these complexes but is much less abundant (FIG. 1). At least six alternatives are known for PCGF1, the heterodimeric partner of RING2. In addition to the well-known BMI1 (also known as PCGF4) and MEL18 (also known as PCGF2), PCGF1, PCGF3, PCGF5 and PCGF6 have also been found to associate with RING2. The RING2–PCGF heterodimer is catalytically competent as an E3 ubiquityl transferase and is the scaffold for the assembly of additional components[21–23]. The RING2–BMI1 or RING2–MEL18 dimers can further bind to one of five alternative chromobox protein homologue (CBX) components and to the remaining core subunits of the canonical PRC1 (BOX 1; FIG. 1).

The position occupied by CBX, together with the human homologues of Ph and Sex comb on midleg (Scm) components, can alternatively be occupied by RYBP or its close homologue YAF2 (REF. 15). Unlike CBX proteins, RYBP and YAF2 can form a complex with any RING2–PCGF combination (FIG. 1). RING2 complexes that contain RYBP or YAF2 have no chromodomain-containing CBX proteins, and their binding to chromatin sites is therefore thought to be independent of histone H3 methylation. The only exception from this rule is the RING2–L3MBTL2 (lethal(3)malignant brain tumour-like protein 2) class of complexes that harbour CBX3 (also known as HP1γ), the chromodomain of which recognizes both H3K9me2 and H3K9me3.

Biological functions of alternative PRC1 and RING2–RYBP complexes in mammals. The abundance of alternative PRC1 subunits greatly varies between different cell types[24–26]. Thus, CBX7 predominates in mouse

embryonic stem cells, in which it is needed to maintain pluripotency. The level of CBX7 sharply drops upon differentiation, concomitant with an increase in CBX2 and CBX8 (REF. 25). The level of CBX7 is controlled both at the transcriptional level — activated by the pluripotency factor OCT4 — and at the post-transcriptional level by microRNAs of the miR-125 and miR-181 families[25]. In turn, *Cbx2* and *Cbx8* genes are directly repressed by complexes that contain CBX7, which permits the coordinated switching between these variants. Similarly, BMI1 and MEL18 are, in some cases, exclusively present in different cell types. For example, fetal liver cells require BMI1 but not MEL18 (REF. 27). The dominant presence of one CBX or PCGF subunit in certain kinds of cells would, in principle, explain the lack of genetic redundancy and fit with the crucial role of CBX7 in maintaining pluripotency[24–25], as well as with both haematological and neurological defects observed in *Bmi1*-mutant mice[28]. It should be noted that, although we know something about the tissue specificity of some of the paralogous components, we currently have little or no information about the tissue-specific roles of alternative PRC1-related complexes, and it is clear that multiple PRC1 variants are generally present in the same cell.

An attractive hypothesis is that the PRC1 variants have intrinsically different biochemical properties that may be used for targeting different subsets of genes and/or for context-dependent repression (BOX 2). Consistent with this hypothesis, the overexpression of different CBX subunits has different effects on the haematopoietic lineage[26]. Thus, the overexpression of CBX7, but not of CBX2, CBX4 and CBX8, induces self-renewal in multipotent cells but not in more differentiated progenitors. Recent genomic experiments suggest that this is due to the repression of a small set of genes that are specifically regulated by CBX7 in haematopoietic stem and progenitor cells[26], but further studies are needed to confirm this.

These conclusions are put in a broader perspective when all possible RING2 or RING1 complexes are considered. Genomic profiling in human cells shows that the target genes of CBX-containing and RYBP-containing complexes are partially overlapping, which indicates that, although these alternative complexes may often function in parallel, they have independent recruiting mechanisms[15]. The binding sites of different PCGFs show different degrees of overlap. Thus, BMI1- and MEL18-binding sites are nearly identical and partially overlap with PCGF1-binding sites[12,13] in mouse embryonic stem cells. However, there is little overlap with PCGF6-binding sites[15], which is consistent with the idea that RING2–L3MBTL2 and its variant complexes are functionally distinct from other PcG complexes. The L3MBTL2 complexes are frequently found at genes that also bind to the cell cycle factors E2F6 and E2F4, and may co-purify with these proteins[29,30]. When different RING1 or RING2-containing complexes bind to the same gene, it is not known whether the binding of different complexes occurs simultaneously, alternatively, at different stages of the cell cycle, or whether the binding of one complex promotes or interferes with the binding

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CpG islands
Vertebrate genomic regions of the order of 1 kb that are rich in CpG dinucleotides; they often lack 5-methylcytosine and frequently correspond to promoter regions.

Embryoid bodies
Three-dimensional aggregates of pluripotent stem cells.
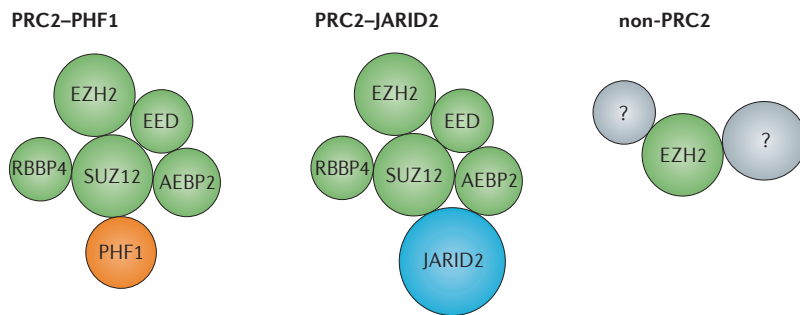
PRC2–PHF1

PRC2–JARID2

non-PRC2

Figure 2 | **Alternative enhancer of zeste complexes.** The complexes are depicted such that the areas of the circles reflect the relative sizes of the primary isoforms of their corresponding proteins, as defined in the UniProt database. The core Polycomb repressive complex 2 (PRC2), which is stabilized by zinc-finger protein AEBP2, are shown in green. Interchangeable components PHF1 (PHD finger protein 1) and JARID2 (Jumonji, ARID domain-containing protein 2) are shown in orange and blue, respectively. Although multiple laboratories have purified core complexes of PRC2, there remains a possibility that this complex is a result of the partial dissociation of larger complexes during biochemical purification. Recent reports[120,121] indicate that enhancer of zeste homologue 2 (EZH2) can methylate non-histone substrates independently of other PRC2 core subunits. Currently, we do not know whether this is done by EZH2 alone or, more probably, in complex with other proteins (shown in grey) that are yet to be identified. RBBP4, histone-binding protein RBBP4.

of another. Consistent with the variable presence of a CBX component, only a subset of target genes of the RING1 and RING2 complexes contains H3K27me3. By contrast, all variants of RING2 complexes studied are found at sites that are enriched for H2A ubiquitylation, although opinions differ on whether RYBP-containing complexes are more active in ubiquitylation[15,16]. The multiplicity of these parallel binding patterns is perplexing, but it may reflect stages in the process of recruitment or of gene silencing.

Variant PRC2 complexes

D. melanogaster and mammals both have their own assortment of variants of PRC2 core subunits (FIG. 2; TABLE 1). Their alternative use stems from differential expression of corresponding genes in specific tissues or at specific stages of development. For example, of the two mouse enhancer of zeste (*E(z)*) paralogues, the expression of enhancer of zeste homologue 2 (*Ezh2*; also known as *Kmt6*) predominates during early embryonic development and in embryonic stem cells[31]. Consistently, the loss of *Ezh2* causes early embryonic lethality[32]. At later stages of development, however, *Ezh1* is broadly expressed and is fully redundant with *Ezh2* in tissues such as the postnatal skin, in which the relationship between the two was carefully investigated by conditional knockout experiments[33]. Mice that lack *Ezh1* are phenotypically normal and fertile, which indicates that all vital EZH1 functions can be carried out by EZH2. The incorporation of a particular EED isoform results in the methylation of lysine 26 of a histone H1 isoform[34].

Several additional proteins often associate with the core components of PRC2 in a mutually exclusive manner in both mice and *D. melanogaster* (FIG. 2). Untangling the relative contribution of these extended variant PRC2 complexes to PcG-mediated repression is complicated by

the fact that, in addition to mediating extensive trimethylation of H3K27 at PcG-target genes, PRC2 is responsible for pervasive dimethylation of H3K27 throughout the transcriptionally inactive genome. H3K27me2 accounts for nearly 60% of all histone H3 in the genome and is probably accompanied by low levels of diffuse H3K27me3 which, when added up, may well account for much of the total genomic H3K27me3 (REFS 35–37). It is also possible that a basal level of H3K27me2 is a prerequisite for the timely onset of targeted PcG-mediated repression, thus connecting the two H3K27 methylation states.

PRC2–PCL complex function. A portion of PRC2 core proteins co-purifies with *D. melanogaster* Polycomblike (Pcl) or its mammalian orthologues PHD finger protein 1 (PHF1), PHF19 and MTF2 (REFS 38–44). Pcl is a 'classical' PcG protein, the loss of which results in the derepression of *HOX* genes in flies and enhances the effects caused by the partial loss of Pc[45]. Consistent with its direct role in PcG-mediated repression, Pcl binds to *D. melanogaster* PREs[39,46], and its mammalian homologues bind to PcG-target genes[42,43,47,48]. The loss of Pcl has little effect on global H3K27me2 levels[39] but, reportedly, causes a major loss of H3K27me3 at PcG-target genes[40,41,47], which is replaced by H3K27me2 (REF. 39). The incorporation of the mammalian Pcl homologue PHF1 subunit increases the efficiency of H3K27 trimethylation by PRC2 *in vitro*[40]. In addition, Pcl in flies (or PHF19 in mammals) may have a role in anchoring PRC2 at PcG-target genes[39,43,48]. Both the promotion of trimethylation and the binding of PRC2 depend on the Tudor domain of Pcl[43,48]. Interestingly, two recent studies have shown that, in mammalian homologues of Pcl, the Tudor domains specifically recognize H3K36me2 and H3K36me3 (REFS 42,43,49), which suggests that these proteins help to anchor PRC2 to partially active PcG-target genes and thereby allow their efficient re-silencing[42,43]. Curiously, although *D. melanogaster* Pcl has a similar effect on both PRC2 and H3K27 methylation as its mammalian counterparts, the Tudor domain of the fly Pcl has several amino acid differences that result in an atypical, incomplete aromatic cage[50] and therefore does not bind to H3K36 regardless of its methylation state[42,50]. Thus, some property of Pcl other than recognition of H3K36 methylation is likely to be more important for PcG-mediated repression.

JARID2-containing PRC2 complexes. A separate portion of mammalian PRC2 core components associates with Jumonji, ARID domain-containing protein 2 (JARID2; also known as JUMONJI)[51–55], and a similar complex exists in flies[56]. Unlike *Pcl*, *Jarid2* was not identified as a PcG gene in *D. melanogaster* genetic screens[2,45]. Although several groups have found that JARID2 forms a stable complex with the PRC2 core and promotes the binding of PRC2 to many PcG-target genes[51–55], its effect on both PRC2 function and gene repression remains controversial. Thus, two studies suggest that the incorporation of JARID2 reduces PRC2 catalytic activity and that the loss of JARID2 leads to higher H3K27me3 levels

Paralogues
Genes that are originated by a duplication event within the genome.

Orthologues
Genes in different species that are originated from a single gene of the last common ancestor.

at PcG-target genes[51,52], whereas two other studies report exactly the opposite[53,54]. The JARID2 JmjC domain, which is characteristic of histone demethylases of the JARID family, is probably catalytically inactive owing to crucial amino acid substitutions. Unfortunately, the phenotypes of a clean *Jarid2* deletion mutant have not been described in mice or in flies, but loss-of-function gene-trap alleles of murine *Jarid* (REF. 57) show late embryonic lethality and defects in both neural tube fusion and cardiovascular development[57,58]. These phenotypes are milder than the early embryonic lethality caused by the loss of PRC2 core subunits, which suggests that JARID2 is dispensable for some aspects of PRC2 function.

The role of the PRC2–JARID2 complex is not restricted to PcG-target genes. A recent study[59] shows that the murine PRC2–JARID2 complex methylates cardiac transcription factor GATA4 at lysine 299, which prevents its acetylation at the same position by the acetyltransferase p300 and impairs the ability of GATA4 to recruit p300 to its target genes. Importantly, PRC2-dependent repression of the GATA4-target gene *Myh6* (myosin heavy chain 6, cardiac muscle-α) is not accompanied by PRC2 binding or H3K27 trimethylation, which indicates that GATA4 is methylated outside a chromatin context. This and other evidence supports the existence of a free pool of PRC2–JARID2 complexes that may also have a role in the pervasive H3K27 dimethylation of the genome or even contribute to the cytoplasmic PRC2 fraction that is reported to play a part in signal transduction[60].

A different type of larger PRC2-related complexes were reported to contain NAD-dependent histone deacetylase Sir2 and the histone deacetylase sirtuin 1 (SIRT1) in *D. melanogaster* larvae and in human cancer cells, respectively[61,62]. Their role in PRC2 biology awaits investigation.

Targeting PcG-mediated repression

PREs in D. melanogaster. A crucial question for PcG mechanisms is how they are recruited to specific genes, as the selection of target genes ultimately determines the function of the particular PcG complex. Here, the outlook has also been changing. Functional studies in *D. melanogaster* had shown that PREs, specific DNA elements that are a few hundred base pairs long[63,64], were responsible for the recruitment of PcG complexes[3,65,66]. PREs can be tens of kilobases upstream or downstream of the target promoter, within introns or, in many cases, close to the transcription start site[67]. PREs are frequently enriched in consensus binding motifs for Pleiohomeotic (Pho), Trithorax-like (Trl; also known as GAF), Dorsal switch protein 1 (Dsp1) and other DNA-binding factors[64,68,69] that may cooperate in the recruitment of PRC1 and PRC2 (FIG. 3a). However, no single DNA-binding protein so far identified is capable of recruiting PcG complexes to PREs.

Genetics data, as well as genomic binding and gene expression data, concur that PRC1 and PRC2 generally function together to produce the repressed state at target genes. This is widely taken to imply that PRC2 is recruited first and methylates H3K27, and that PRC1

then follows by affinity for the H3K27me3 mark. However, it is clear that, in *D. melanogaster*, the regions methylated by PRC2 are broad domains, whereas the binding of PRC1 is much more localized at PRE sites[10,66]. Nevertheless, the effective interaction of PRC1 with promoter regions is likely to require H3K27me3 to mediate looping, particularly if the PRE is distant from the promoter. To what extent H3K27me3 helps to recruit PRC1 (and its variant complexes) in mammals is less clear, but not all H3K27me3 domains are also binding sites for PRC1 (REF. 4), and mutation of the CBX chromodomain is reported to have little effect on CBX distribution[70].

Recruitment to unmethylated CpG-rich DNA sequences. Most mammalian PcG-target genes bind to PcG complexes in close proximity to the transcription start site but over a broad region that does not suggest the presence of a specific recruiting sequence. Attempts to identify a mammalian PRE-like (PRE-L) element have mostly failed apart from two notable exceptions. A sequence element PRE-kr in the mouse *Kreisler* gene (also known as *Mafb*) recruits PRC1 well and PRC2 poorly[71]. A fragment from the human homeobox D (HOXD) cluster recruits PRC1 and PRC2 components and represses a reporter gene[72]. In a different approach, the analysis of bivalent domains (that is, domains containing both H3K27me3 and H3K4me3) in embryonic stem cells suggested that the domains that bind to both PRC1 and PRC2 corresponded well with CpG islands that lack both 5-methylcytosine and activator-binding sites[73]. Tests showed that GC-rich elements, even those derived from bacterial genomes, could indeed recruit PRC2 but not PRC1, the binding of which was identified by the presence of RING2 (REF. 74). Comparison across species and in either the presence or the absence of DNA methylation supports the idea that clusters of unmethylated CpGs that are unaccompanied by active transcription can recruit PcG complexes[75].

Certain proteins that contain a zinc-finger-CxxC DNA-binding domain bind preferentially to unmethylated CpG islands[76]. One such protein is CXXC finger protein 1 (CXXC1; also known as CFP1) — a component of the SET1 H3K4 methyltransferase complex — which accounts for the presence of H3K4me3 at CpG islands in embryonic stem cells. Two other CpG-binding proteins are the H3K36 demethylases KDM2A and KDM2B (REFS 12,13,77). As discussed above, KDM2B was found to be a component of a variant RING2 or RING1 complex and helps to recruit the complex to a subset of CpG islands (FIG. 3b). It remains unclear how to account for the binding to CpG islands of PRC2 or of other PRC1 variants in the observed distribution of PcG-mediated repression. JARID2 might help to recruit PRC2. A low initial level of binding to CpG islands could provide the opportunity for PcG complexes to colonize a large class of target genes and, when conditions are suitable, to establish a bivalent state or even a repressed state[12].

Certain mammalian DNA-binding transcriptional regulators have been reported to recruit PRC complexes to their binding sites. In mice, PRC1 and PRC2 colocalize with subsets of sites that bind to the neuronal
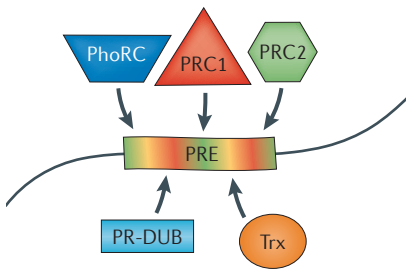
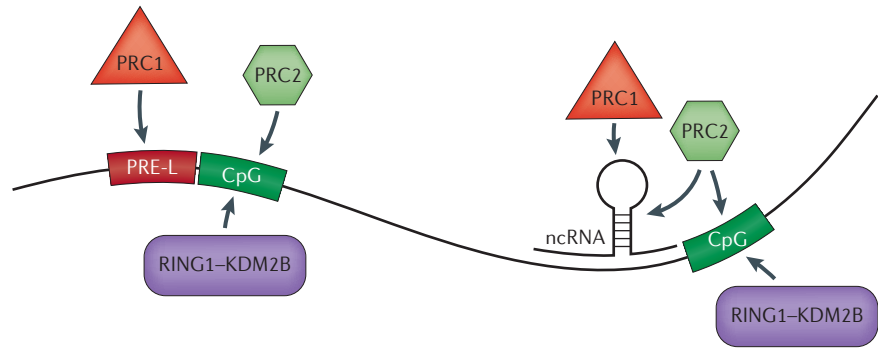**a** In *Drosophila melanogaster*   **b** In mammals

Figure 3 | **Targeting of Polycomb group complexes.** **a** | In *Drosophila melanogaster*, Polycomb response elements (PREs) mediate the recruitment of all known Polycomb group (PcG) complexes, including Pho repressive complex (PhoRC) and Polycomb repressive deubiquitinase complex (PR-DUB), which contribute to stabilizing the binding of Polycomb repressive complex 1 (PRC1) and PRC2. Although, with the exception of PhoRC, the precise DNA-binding determinants are not known, several are thought to contribute cooperatively. Note that PREs also recruit Trithorax (Trx), a histone methyltransferase that counteracts PcG-mediated repression, and such recruitment turns PREs into switchable memory elements. Shapes and colours of the complexes are coordinated to identify corresponding mammalian and fly homologues. **b** | The mammalian recruitment platform is probably modular. Experimental evidence indicates that the existence of PRE-like modules (PRE-L) is sufficient for the recruitment of PRC1 and that CpG-rich modules can recruit PRC2 and E3 ubiquitin-protein ligase RING1–lysine-specific demethylase 2B (KDM2B) complexes. In addition, non-coding RNAs (ncRNAs) may help to recruit PRC1 and PRC2, but it is not known how ncRNAs target specific chromatin regions. We envision that various combinations of the two modules and/or ncRNAs are used at different target genes and that appropriate interactions turn the weak recruitment of any individual component into a robust targeting mechanism. Whether mixed-lineage leukaemia 1 (MLL1) and MLL2, the mammalian counterparts of Trx, are also concomitantly recruited is unknown.

inhibitor REST or to repressors of the SNAIL family and depend on these factors to repress the genes that are associated with those sites[78,79].

Recruitment by non-coding RNA. An alternative, and apparently entirely independent, recruitment mechanism makes use of RNA molecules either as a scaffold to assemble complexes or as a targeting device. In several cases, compelling evidence has shown that non-coding RNAs (ncRNAs) bind to PcG complexes and that these RNAs are important for PcG-mediated regulation of some targets (FIG. 3b). The ncRNA HOX transcript antisense RNA (*HOTAIR*) from the human HOXC gene cluster binds to PRC2, as well as to the Co-REST complex that contains the H3K4 demethylase KDM1A (also known as LSD1), and recruits them both in *cis* to HOXC genes and in *trans* to HOXD genes[80,81]. When overexpressed, *HOTAIR* also recruits PRC2 to many other genomic sites, which are often developmentally regulated genes, but the basis for such targeting is unclear[82]. However, in mice, deletion of the *Hotair* gene has no effect on PcG complex binding or on transcriptional regulation, which indicates a divergent or redundant function[83]. The ncRNA-based recruitment of PRC2 is essential at various stages in the establishment of mammalian X chromosome inactivation. A sequence contained in three overlapping transcripts — *RepA*, inactive X-specific transcripts (*Xist*) and X (inactive)-specific transcript, opposite strand (*Tsix*) — at the X inactivation centre binds to PRC2 and initiates the process that eventually spreads its binding in *cis*, together with PRC1, over large parts of the inactive X chromosome[84]. The ncRNA

ANRIL from the human *CDKN2A–CDKN2B* (which encodes INK4B (also known as p15)) locus binds to a PRC1-related complex that contains CBX7 and, together with H3K27me3, recruits the complex to the locus to promote cell cycle progression[85].

The molecular details of the interactions of ncRNAs with either PRC1 or PRC2 are still unclear, but it is likely that they differ in different situations. The allele-specific recruitment, such as that involved in X inactivation or imprinted gene silencing, seems to be easier to understand if the PcG complexes bind to nascent ncRNA[86]. Less clear is the action in *trans*. In some cases, ncRNAs may recruit PcG complexes to homologous sequences; in other cases, ncRNAs may have a scaffolding function that brings together multiple chromatin regulators, but it is not known whether base pairing has a role in targeting. How pervasive the involvement of ncRNAs might be is currently unclear. Genome-wide screens for RNAs that bind to PcG complexes have been reported to yield thousands of RNA species[87,88]. It has also been claimed that, in mouse and human embryonic stem cells, short RNAs produced from the 5′ region of PcG-repressed genes bind to PRC2 and retain it to those genes, thus contributing to repression[89]. At this stage, it is probably unwise to assume that all RNA molecules that seem to associate with PcG complexes are in fact functionally involved in repression, but some of them clearly play a part.

Unrecruited activities. The most abundant product of PRC2 activity is not the H3K27me3 mark that is associated with PcG-mediated repression but H3K27me2, which is broadly distributed and accounts for 50–60%

of total nuclear histone H3 (REF. 35). The dimethylated state of H3K27 is ubiquitous and is depleted only at sites that contain H3K27me3 and at sites of transcriptional activity. The global dimethylation state must be attributed to a 'hit-and-run' activity of PRC2. It is accompanied by a low but measurable amount of trimethylation, the deposition of which is a much slower process. The role of this widespread methylation is debatable, but it is most likely to be important for the establishment of H3K27me3 domains and of other repressed chromatin domains[90]. Similarly, there are indications that a low global level of H2A ubiquitylation that is dependent on RING-containing complexes is also detectable. These low-level distributions might have little effect on their own, but they might be important as 'seeds' for the establishment of more targeted repressive activities if we imagine PcG-mediated repression to occur opportunistically as suggested for CpG islands[12].

Regulation of PcG complexes

Much evidence suggests that the activity of PcG complexes is modulated at various levels. Several remarkable features of the core components of PRC2 allow its activity to be modulated by inputs from surrounding chromatin. Briefly, the presence of H3K4me3, H3K36me2 or H3K36me3 decreases the catalytic activity of PRC2 (REFS 91–93), whereas high nucleosome density and the presence of H3K27me2 or H3K27me3 stimulate its catalytic activity[94,95], thus favouring the maintenance of the methylated state. PRC1 and its variant complexes also seem to be regulated. For example, the human CBX4 protein has been reported to function as an E3 SUMO transferase[96,97]. PRC1 components themselves are sumoylated through an interaction that is mediated by sterile α-motif (SAM) domains[98], and the sumoylation of CBX4 seems to be important for its recruitment to target genes or for the stabilization of the repressive complexes[99]. Sumoylation of human BMI1 by CBX4 has also been found to be involved in the recruitment of PRC1 to sites of DNA damage[100]. Phosphorylation of various PRC1 components has been reported, but its functions in modulating PRC1 activities are poorly understood. Phosphorylation of human BMI1 by MAP kinase-activated protein kinase 3 results in the dissociation of BMI1 from its binding sites[101], whereas phosphorylation of MEL18 does not preclude its binding to chromatin and, in fact, increases the ability of RING2–MEL18 complexes to ubiquitylate nucleosomal H2A[102].

PcG complexes and disease

PcG mechanisms modulate the expression of most genes that control differentiation, specify cell lineages in development and regulate morphogenesis. The loss of basic Polycomb functions results in early embryonic lethality[1,2,32,103,104]. Mutations in PcG proteins may alter the response of a PcG-target gene and result in disease. The two examples discussed below are remarkable because they illustrate both interesting functional properties of PRC2 (and its variant complexes) and the still puzzling fact that both hyperactivity and loss of activity of PRC2 can produce oncogenic disease.

Altered levels of PcG proteins have been linked to cancer. The best known example is BMI1 and its role in promoting B cell lymphomas[105]. The overexpression of BMI1 and a few other components of PRC1 was also found in other types of haematological neoplasms (reviewed in REFS 106,107), as well as in medulloblastoma[108] and non-small-cell lung cancer[109]. The oncogenic function of BMI1 and other PRC1 components has mainly been attributed to their repression of the *CDKN2A* locus (FIG. 4a), which, when expressed, restricts cell proliferation, but the inappropriate repression of other tumour suppressor genes may also be involved[110,111].

The overexpression of EZH2 and SUZ12 has been linked to haematological and other malignancies (reviewed in REF. 112). In addition, certain recurring mutations in the catalytic domain of EZH2 were found in some types of B cell lymphomas. These mutations alter the substrate preference and/or processivity, which leads to increased levels of total H3K27me3 in the nucleus[113,114]. A specific small-molecule inhibitor of PRC2 catalytic activity arrests proliferation of these cancer cells, which shows the causal role of the mutations and provides hope that, one day, such inhibitors may be used as a treatment for patients with 'activating' mutations in EZH2 (REFS 115,116).

Although the implication of PRC1 components in cancer is associated with their overexpression, surprisingly, the deletion of *Ezh2* in mice was found to cause high frequency of spontaneous γδT cell acute lymphoblastic leukaemia[117]. Added to this, two recent studies have shown that missense Lys27Met mutations in genes that encode the human histones H3.3 and H3.1 inhibit the genome-wide histone methyltransferase activity of PRC2 and occur frequently in paediatric brain cancers of diffuse intrinsic pontine glioma type[118,119].

The apparent tumour suppressor role of PRC2 components but not of PRC1 components is unusual and indicates that functions of EZH2 and PRC2 outside the canonical PcG mechanism may be involved. Supporting this notion are the two recent studies of the role of EZH2 in castration-resistant prostate cancer and breast cancer cells. In one study[120], castration-resistant prostate cancer cells overexpressed EZH2, which was hyperphosphorylated at serine 21, probably owing to increased levels of activated AKT kinase. Phosphorylated EZH2 associates with the androgen receptor, binds to its target genes and stimulates its transcriptional activity. Strikingly, this is not accompanied by the binding of other PRC2 core subunits or by increased H3K27me3 levels, but it does require the methyltransferase activity of EZH2, which directly or indirectly causes methylation of the androgen receptor. Taken together, these observations indicate that the phosphorylation of EZH2 can switch its function from a PcG repressor to a transcriptional co-activator through a PRC2-independent methylation of a non-histone protein (FIG. 4b). In a second study[121], EZH2 monomethylated nuclear receptor RORα at lysine 38 independently of other PRC2 subunits. Methylated RORα is specifically recognized by DCAF1 (DDB1 and CUL4-associated factor 1), which targets it for
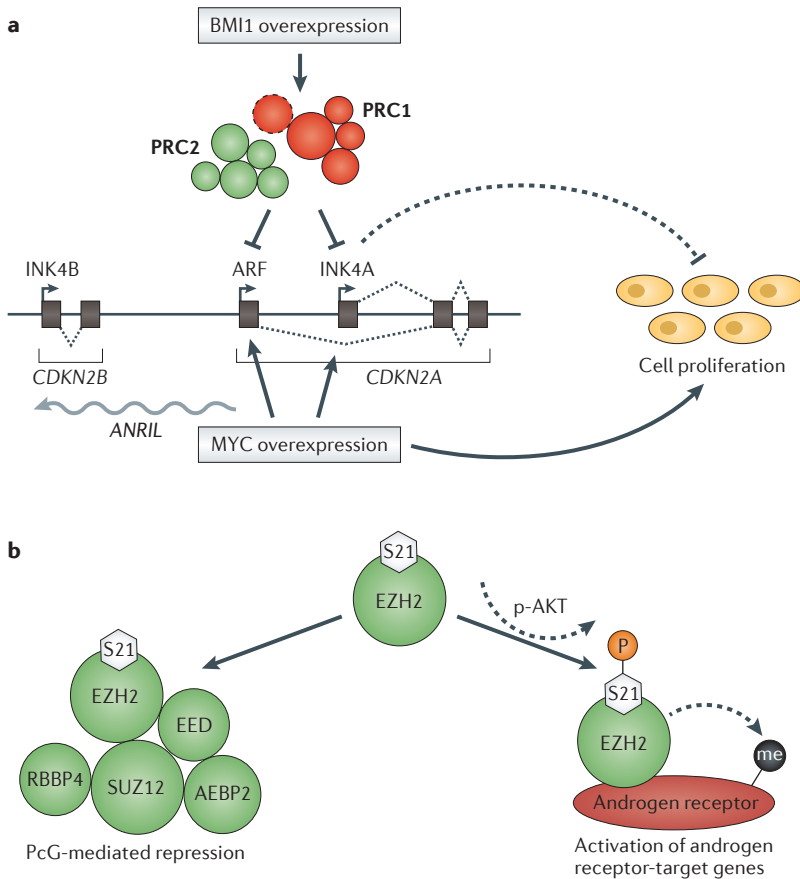
Figure 4 | The roles of Polycomb group proteins in cancer. a | Overexpressed BMI1 and MYC cooperate in driving the proliferation of blood cancer cells. High levels of MYC increase cell proliferation, but they also activate the expression of ARF and INK4A (alternatively spliced isoforms encoded by the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) locus), both of which trigger cellular senescence and counteract proliferation. When BMI1 is overexpressed together with MYC, it drives the recruitment of both Polycomb repressive complex 1 (PRC1; red circles) and PRC2 (green circles) to the *CDKN2A* locus, which leads to the repression of these genes and, consequently, to uncontrolled cell proliferation[106]. The genes encoding INK4B (also known as p15; encoded by *CDKN2B*), ARF and INK4A are contained in a short ~35 kb-stretch of the human genome. Whereas *CDKN2B* has a physically distinct open reading frame, ARF and INK4A have different promoters but share the last two exons (black rectangles). Although the last two exons are common to both ARF and INK4A, the proteins are encoded by alternative open reading frames and bear no similarity. There is no evidence of interplay between MYC and Polycomb group (PcG) proteins in the regulation of *CDKN2B*, but all three genes of the *CDKN2B–CDKN2A* locus are targeted by PcG proteins in some cells. The non-coding RNA *ANRIL* (also known as CDKN2B antisense RNA 1; wavy arrow) is involved in the targeting of PcG proteins to *CDKN2B*. **b |** In castration-resistant prostate cancer cells, high levels of enhancer of zeste homologue 2 (EZH2) and activated AKT kinase (p-AKT) lead to the phosphorylation (P) of a proportion of EZH2 at serine 21 (white hexagon)[118]. Unphosphorylated EZH2 is incorporated into PRC2 (and its variant complexes) and participates in the repression of PcG-target genes, whereas phosphorylated EZH2 binds to androgen receptor, which leads to androgen receptor methylation (me) and the stimulation of transcriptional activity of androgen receptor-target genes. Dashed arrows indicate the enzymatic actions of p-AKT and EZH2. AEBP2, zinc-finger protein AEBP2; RBBP4, histone-binding protein RBBP4.

degradation. In breast cancer cells, the levels of EZH2 and RORα are inversely correlated, and either the overexpression of RORα or the knockdown of DCAF1 reduces proliferation of MCF7 cells. These reports suggest that the well-known correlation between EZH2 overexpression and tumour aggressiveness is partly due to methylation-dependent degradation of tumour suppressor proteins such as RORα[121]. To conclude, the emerging evidence indicates that high levels of EZH2 can methylate proteins other than histones independently of other PRC2 components. It remains to be seen whether PRC2-independent E(Z) activity also has a role in untransformed cells and whether this requires the cellular E(Z) pool to exceed the levels of other PRC2 components.

Conclusions

Recent advances in the biochemical characterization of mammalian RING2, RING1 and E(Z) complexes, and in the genome-wide mapping of the binding sites of these complexes have revealed an unexpected diversity. Some of these complexes are clearly involved in PcG-mediated repression, whereas the function of others remains to be determined. The biochemical studies should now be followed by in-depth genetic and genomic experiments to probe the functional roles of each of the RING2, RING1 and E(Z) complexes, to investigate the poorly understood importance of the numerous variants, as well as to understand how their functions differ or complement one another and their differential role in different tissues or processes. In this case, the *D. melanogaster* model is likely to be a useful starting point owing to the lower redundancy of the PcG protein family, the smaller genome size and the availability of genetic tools.

Important questions to be tackled concern the functional role of the H2AK119ub mark and how it contributes to transcriptional repression. We need to learn more about the role of the pervasive H3K27 dimethylation of the transcriptionally inactive genome. Equally exciting is the idea of non-histone substrates that E(Z) homologues may methylate independently of other PRC2 core subunits. Several reports have suggested that certain forms of PcG proteins have a surprising role in activating transcription. Finally, but no less importantly, we need to understand the timing of the alternative RING2 and RING1 or PRC2 complexes at a given gene during the cell cycle and their relationships. We might then finally be in a position to chart a dynamic picture of PcG-mediated regulation, in which the turnover of both PcG complexes and histone marks yield epigenetically stable transcriptional repression, and to relate this picture to the roles of some PcG members beyond their classical repressive function.

1. Jürgens, G. A group of genes controlling the spatial expression of the bithorax complex in *Drosophila*. *Nature* **316**, 153–155 (1985).
2. Gaytán de Ayala Alonso, A. *et al.* Genetic screen identifies novel Polycomb group genes in *Drosophila*. *Genetics* **176**, 2099–2108 (2007).
3. Poux, S., Kostic, C. & Pirrotta, V. Hunchback-independent silencing of late Ubx enhancers by a Polycomb group response element. *EMBO J.* **15**, 4713–4722 (1996).
4. Leeb, M. *et al.* Polycomb complexes act redundantly to repress genomic repeats and genes. *Genes Dev.* **24**, 265–276 (2010).
5. Zhou, W. *et al.* Histone H2A monoubiquitination represses transcription by inhibiting RNA polymerase II transcriptional elongation. *Mol. Cell* **29**, 69–80 (2008).
6. Dellino, G. I. *et al.* Polycomb silencing blocks transcription initiation. *Mol. Cell* **13**, 887–893 (2004).

7. Grau, D. J. *et al.* Compaction of chromatin by diverse Polycomb group proteins requires localized regions of high charge. *Genes Dev.* **25**, 2210–2221 (2011).

8. Eskeland, R. *et al.* Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination. *Mol. Cell* **38**, 452–464 (2010).

9. Gutiérrez, L. *et al.* The role of the histone H2A ubiquitinase Sce in Polycomb repression. *Development* **139**, 117–127 (2012).

10. Schwartz, Y. B. *et al.* Genome-wide analysis of Polycomb targets in *Drosophila melanogaster*. *Nature Genet.* **38**, 700–705 (2006).

11. Lagarou, A. *et al.* dKDM2 couples histone H2A ubiquitylation to histone H3 demethylation during Polycomb group silencing. *Genes Dev.* **22**, 2799–2810 (2008).
This study gives the first evidence of alternative protein complexes that contain core PRC1 components.

12. Farcas, A. M. *et al.* KDM2B links the Polycomb repressive complex 1 (PRC1) to recognition of CpG islands. *eLife Sciences* **1**, e00205 (2012).

13. Wu, X., Johansen, J. V. & Helin, K. Fbxl10/Kdm2b recruits Polycomb repressive complex 1 to CpG islands and regulates H2A ubiquitylation. *Mol. Cell.* **49**, 1134–1146 (2013).

14. He, J. *et al.* Kdm2b maintains murine embryonic stem cell status by recruiting PRC1 complex to CpG islands of developmental genes. *Nature Cell Biol.* **15**, 373–384 (2013).
References 12–14 shows that the zinc-finger-CxxC DNA-binding domain of KDM2B recruits a variant RING2 or RING1 complex to unmethylated CpG, thereby contributing to PcG-mediated repression in mouse embryonic stem cells.

15. Gao, Z. *et al.* PCGF homologs, CBX proteins, and RYBP define functionally distinct PRC1 family complexes. *Mol. Cell* **45**, 344–356 (2012).
This key paper provides a comprehensive description of variant RING1 and RING2 protein complexes.

16. Tavares, L. *et al.* RYBP–PRC1 complexes mediate H2A ubiquitylation at Polycomb target sites independently of PRC2 and H3K27me3. *Cell* **148**, 664–678 (2012).

17. Pfau, R. *et al.* Members of a family of JmjC domain-containing oncoproteins immortalize embryonic fibroblasts via a JmjC domain-dependent process. *Proc. Natl Acad. Sci. USA* **105**, 1907–1912 (2008).

18. Tzatsos, A., Pfau, R., Kampranis, S. C. & Tsichlis, P. N. Ndy1/KDM2B immortalizes mouse embryonic fibroblasts by repressing the *Ink4a/Arf* locus. *Proc. Natl Acad. Sci. USA* **106**, 2641–2646 (2009).

19. Wang, R. *et al.* Polycomb group targeting through different binding partners of RING1B C-terminal domain. *Structure* **18**, 966–975 (2010).

20. Vandamme, J., Völkel, P., Rosnoblet, C., Le Faou, P. & Angrand, P.-O. Interaction proteomics analysis of Polycomb proteins defines distinct PRC1 complexes in mammalian cells. *Mol. Cell. Proteomics* **10**, M110.002642 (2011).

21. Cao, R., Tsukada, Y.-I. & Zhang, Y. Role of Bmi-1 and Ring1A in H2A ubiquitylation and Hox gene silencing. *Mol. Cell* **20**, 845–854 (2005).

22. Wei, J., Zhai, L., Xu, J. & Wang, H. Role of Bmi1 in H2A ubiquitylation and hox gene silencing. *J. Biol. Chem.* **281**, 22537–22544 (2006).

23. Wu, X. *et al.* Cooperation between EZH2, NSPc1-mediated histone H2A ubiquitination and Dnmt1 in HOX gene silencing. *Nucleic Acids Res.* **36**, 3590–3599 (2008).

24. Morey, L. *et al.* Nonoverlapping functions of the Polycomb group Cbx family of proteins in embryonic stem cells. *Cell Stem Cell.* **10**, 47–62 (2012).

25. O'Loghlen, A. *et al.* MicroRNA regulation of Cbx7 mediates a switch of Polycomb orthologs during ESC differentiation. *Cell Stem Cell.* 2012 **10**, 33–46 (2012).

26. Klauke, K. *et al.* Polycomb Cbx family members mediate the balance between haematopoietic stem cell self-renewal and differentiation. *Nature Cell Biol.* **15**, 353–362 (2013).

27. Iwama, A. *et al.* Enhanced self-renewal of hematopoietic stem cells mediated by the Polycomb gene product Bmi-1. *Immunity.* **21**, 843–851 (2004).

28. van der Lugt, N. M. *et al.* Posterior transformation, neurological abnormalities, and severe hematopoietic defects in mice with a targeted deletion of the bmi-1 proto-oncogene. *Genes Dev.* **8**, 757–769 (1994).

29. Trojer, P. *et al.* L3MBTL2 protein acts in concert with PcG protein-mediated monoubiquitination of H2A to establish a repressive chromatin structure. *Mol. Cell* **42**, 438–450 (2011).

30. Ogawa, H., Ishiguro, K., Gaubatz, S., Livingston, D. M. & Nakatani, Y. A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G$_0$ cells. *Science* **296**, 1132–1136 (2002).

31. Shen, X. *et al.* EZH1 mediates methylation on histone H3 lysine 27 and complements EZH2 in maintaining stem cell identity and executing pluripotency. *Mol. Cell* **32**, 491–502 (2008).

32. O'Carroll, D. *et al.* The Polycomb-group gene Ezh2 is required for early mouse development. *Mol. Cell. Biol.* **21**, 4330–4336 (2001).

33. Ezhkova, E. *et al.* EZH1 and EZH2 cogovern histone H3K27 trimethylation and are essential for hair follicle homeostasis and wound repair. *Genes Dev.* **25**, 485–498 (2011).

34. Kuzmichev, A., Jenuwein, T., Tempst, P. & Reinberg, D. Different Ezh2-containing complexes target methylation of histone H1 or nucleosomal histone H3. *Mol. Cell* **14**, 183–193 (2004).

35. Peters, A. H. F. M. *et al.* Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. *Mol. Cell* **12**, 1577–1589 (2003).

36. Jung, H. R., Pasini, D., Helin, K. & Jensen, O. N. Quantitative mass spectrometry of histones H3.2 and H3.3 in Suz12-deficient mouse embryonic stem cells reveals distinct, dynamic post-translational modifications at lys-27 and lys-36. *Mol. Cell. Proteomics* **9**, 838–850 (2010).

37. Voigt, P. *et al.* Asymmetrically modified nucleosomes. *Cell* **151**, 181–193 (2012).

38. Tie, F., Prasad-Sinha, J., Birve, A., Rasmuson-Lestander, Å. & Harte, P. J. A 1-megadalton ESC/E(Z) complex from *Drosophila* that contains Polycomblike and RPD3. *Mol. Cell. Biol.* **23**, 3352–3362 (2003).

39. Nekrasov, M. *et al.* Pcl–PRC2 is needed to generate high levels of H3-K27 trimethylation at Polycomb target genes. *EMBO J.* **26**, 4078–4088 (2007).

40. Sarma, K., Margueron, R., Ivanov, A., Pirrotta, V. & Reinberg, D. Ezh2 requires PHF1 to efficiently catalyze H3 lysine 27 trimethylation *in vivo. Mol. Cell. Biol.* **28**, 2718–2731 (2008).

41. Cao, R. *et al.* Role of hPHF1 in H3K27 methylation and Hox gene silencing. *Mol. Cell. Biol.* **28**, 1862–1872 (2008).

42. Ballaré, C. *et al.* Phf19 links methylated Lys36 of histone H3 to regulation of Polycomb activity. *Nature Struct. Mol. Biol.* **19**, 1257–1265 (2012).

43. Cai, L. *et al.* An H3K36 methylation-engaging Tudor motif of Polycomb-like proteins mediates PRC2 complex targeting. *Mol. Cell* **49**, 571–582 (2013).

44. Savla, U., Benes, J., Zhang, J. & Jones, R. S. Recruitment of *Drosophila* Polycomb-group proteins by Polycomblike, a component of a novel protein complex in larvae. *Development* **135**, 813–817 (2008).
References 39–44 combine the current knowledge of the features and roles of PRC2–PCL complexes.

45. Duncan, I. M. Polycomblike: a gene that appears to be required for the normal expression of the Bithorax and Antennapedia gene complexes of *Drosophila melanogaster. Genetics* **102**, 49–70 (1982).

46. Papp, B. & Muller, J. Histone trimethylation and the maintenance of transcriptional ON and OFF states by trxG and PcG proteins. *Genes Dev.* **20**, 2041–2054 (2006).

47. Walker, E. *et al.* Polycomb-like 2 associates with PRC2 and regulates rranscriptional networks during mouse embryonic stem cell self-renewal and differentiation. *Cell Stem Cell.* **6**, 153–166 (2010).

48. Hunkapiller, J. *et al.* Polycomb-Like 3 promotes Polycomb repressive complex 2 binding to CpG Islands and embryonic stem cell self-renewal. *PLoS Genet.* **8**, e1002576 (2012).

49. Musselman, C. A. *et al.* Molecular basis for H3K36me3 recognition by the Tudor domain of PHF1. *Nature Struct. Mol. Biol.* **19**, 1266–1272 (2012).

50. Friberg, A., Oddone, A., Klymenko, T., Müller, J. & Sattler, M. Structure of an atypical Tudor domain in the *Drosophila* Polycomblike protein. *Protein Sci.* **19**, 1906–1916 (2010).

51. Shen, X. *et al.* Jumonji modulates Polycomb activity and self-renewal versus differentiation of stem cells. *Cell* **139**, 1303–1314 (2009).

52. Peng, J. C. *et al.* Jarid2/Jumonji coordinates control of PRC2 enzymatic activity and target gene occupancy in pluripotent cells. *Cell* **139**, 1290–1302 (2009).

53. Pasini, D. *et al.* JARID2 regulates binding of the Polycomb repressive complex 2 to target genes in ES cells. *Nature* **464**, 306–310 (2010).

54. Li, G. *et al.* Jarid2 and PRC2, partners in regulating gene expression. *Genes Dev.* **24**, 368–380 (2010).

55. Landeira, D. *et al.* Jarid2 is a PRC2 component in embryonic stem cells required for multi-lineage differentiation and recruitment of PRC1 and RNA Polymerase II to developmental regulators. *Nature Cell Biol.* **12**, 618–624 (2010).

56. Herz, H.-M. *et al.* Polycomb repressive complex 2-dependent and -independent functions of Jarid2 in transcriptional regulation in *Drosophila. Mol. Cell. Biol.* **32**, 1683–1693 (2012).
References 51–56 outline the properties of PRC2–JARID2 complexes.

57. Takeuchi, T. *et al.* Gene trap capture of a novel mouse gene, Jumonji, required for neural tube formation. *Genes Dev.* **9**, 1211–1222 (1995).

58. Lee, Y. *et al.* Jumonji, a nuclear protein that is necessary for normal heart development. *Circ. Res.* **86**, 932–938 (2000).

59. He, A. *et al.* PRC2 directly methylates GATA4 and represses its transcriptional activity. *Genes Dev.* **26**, 37–42 (2012).

60. Su, I.-h. *et al.* Polycomb group protein Ezh2 controls actin polymerization and cell signaling. *Cell* **121**, 425–436 (2005).

61. Furuyama, T., Banerjee, R., Breen, T. R. & Harte, P. J. SIR2 is required for Polycomb silencing and is associated with an E(z) histone methyltransferase complex. *Curr. Biol.* **14**, 1812–1821 (2004).

62. Kuzmichev, A. *et al.* Composition and histone substrates of Polycomb repressive group complexes change during cellular differentiation. *Proc. Natl Acad. Sci. USA* **102**, 1859–1864 (2005).

63. Chan, C.-S., Rastelli, L. & Pirrotta, V. A Polycomb response element in the *Ubx* gene that determines an epigenetically inherited state of repression. *EMBO J.* **13**, 2553–2564 (1994).

64. Müller, J. & Kassis, J. A. Polycomb response elements and targeting of Polycomb group proteins in *Drosophila. Curr. Opin. Genet. Dev.* **16**, 476–484 (2006).

65. Horard, B., Tatout, C., Poux, S. & Pirrotta, V. Structure of a Polycomb response element and *in vitro* binding of Polycomb group complexes containing GAGA factor. *Mol. Cell. Biol.* **20**, 3187–3197 (2000).

66. Kahn, T. G., Schwartz, Y. B., Dellino, G. I. & Pirrotta, V. Polycomb complexes and the propagation of the methylation mark at the *Drosophila Ubx* gene. *J. Biol. Chem.* **281**, 29064–29075 (2006).

67. Kharchenko, P. V. *et al.* Comprehensive analysis of the chromatin landscape in *Drosophila melanogaster*. *Nature* **471**, 480–485 (2011).

68. Hodgson, J. W., Argiropoulos, B. & Brock, H. W. Site-specific recognition of a 70-base-pair element containing d(GA)(n) repeats mediates bithoraxoid Polycomb group response element-dependent silencing. *Mol. Cell. Biol.* **21**, 4528–4543 (2001).

69. Dejardin, J. *et al.* Recruitment of *Drosophila* Polycomb group proteins to chromatin by DSP1. *Nature* **434**, 533–538 (2005).

70. Ren, X., Vincenz, C. & Kerppola, T. K. Changes in the distributions and dynamics of Polycomb repressive complexes during embryonic stem cell differentiation. *Mol. Cell. Biol.* **28**, 2884–2895 (2008).

71. Sing, A. *et al.* A vertebrate Polycomb response element governs segmentation of the posterior hindbrain. *Cell* **138**, 885–897 (2009).
This paper is the first report of the mammalian PRE-like element. In contrast to *D. melanogaster* PREs, this element can recruit PRC1 but not PRC2.

72. Woo, C. J., Kharchenko, P. V., Daheron, L., Park, P. J. & Kingston, R. E. A region of the human HOXD cluster that confers Polycomb-group responsiveness. *Cell* **140**, 99–110 (2010).

73. Ku, M. *et al.* Genomewide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains. *PLoS Genetics* **4**, e1000242 (2008).

74. Mendenhall, E. M. *et al.* GC-rich sequence elements recruit PRC2 in mammalian ES cells. *PLoS Genet.* **6**, e1001244 (2010).

75. Lynch, M. D. *et al.* An interspecies analysis reveals a key role for unmethylated CpG dinucleotides in vertebrate Polycomb complex recruitment. *EMBO J.* **31**, 317–329 (2012).

76. Thomson, J. P. *et al.* CpG islands influence chromatin structure via the CpG-binding protein Cfp1. *Nature* **464**, 1082–1086 (2010).

REVIEWS

77. Blackledge, N. P. *et al.* CpG islands recruit a histone H3 lysine 36 demethylase. *Mol. Cell* **38**, 179–190 (2010).
78. Dietrich, N. *et al.* REST-mediated recruitment of Polycomb repressor complexes in mammalian cells. *PLoS Genet.* **8**, e1002494 (2012).
79. Arnold, P. *et al.* Modeling of epigenome dynamics identifies transcription factors that mediate Polycomb targeting. *Genome Res.* **23**, 60–73 (2013).
80. Rinn, J. L. *et al.* Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell* **129**, 1311–1323 (2007).
81. Tsai, M.-C. *et al.* Long noncoding RNA as modular scaffold of histone modification complexes. *Science* **329**, 689–693 (2010).
82. Gupta, R. A. *et al.* Long non-coding RNA *HOTAIR* reprograms chromatin state to promote cancer metastasis. *Nature* **464**, 1071–1076 (2010).
83. Schorderet, P. & Duboule, D. Structural and functional differences in the long non-coding RNA *Hotair* in mouse and human. *PLoS Genet.* **7**, e1002071 (2011).
84. Zhao, J., Sun, B. K., Erwin, J. A., Song, J.-J. & Lee, J. T. Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. *Science* **322**, 750–756 (2008).
85. Yap, K. L. *et al.* Molecular interplay of the noncoding RNA *ANRIL* and methylated histone H3 lysine 27 by Polycomb CBX7 in transcriptional silencing of *INK4a*. *Mol. Cell* **38**, 662–667 (2010).
86. Lee, J. T. Epigenetic regulation by long noncoding RNAs. *Science* **338**, 1435–1439 (2012).
87. Khalil, A. M. *et al.* Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc. Natl Acad. Sci. USA* **106**, 11667–11672 (2009).
88. Zhao, J. *et al.* Genome-wide identification of Polycomb-associated RNAs by RIP–seq. *Mol. Cell* **40**, 939–953 (2010).
89. Kanhere, A. *et al.* Short RNAs are transcribed from repressed Polycomb target genes and Interact with Polycomb repressive complex-2. *Mol. Cell* **38**, 675–688 (2010).
90. Ebert, A. *et al.* Su(var) genes regulate the balance between euchromatin and heterochromatin in *Drosophila*. *Genes Dev.* **18**, 2973–2983 (2004).
91. Ketel, C. S. *et al.* Subunit contributions to histone methyltransferase activities of fly and worm Polycomb group complexes. *Mol. Cell. Biol.* **25**, 6857–6868 (2005).
92. Schmitges, F. W. *et al.* Histone methylation by PRC2 is inhibited by active chromatin marks. *Mol. Cell* **42**, 330–341 (2011).
93. Yuan, W. *et al.* H3K36 methylation antagonizes PRC2-mediated H3K27 methylation. *J. Biol. Chem.* **286**, 7983–7989 (2011).
94. Yuan, W. *et al.* Dense chromatin activates Polycomb repressive complex 2 to regulate H3 lysine 27 methylation. *Science* **337**, 971–975 (2012).
95. Margueron, R. *et al.* Role of the Polycomb protein EED in the propagation of repressive histone marks. *Nature* **461**, 762–767 (2009).
   References 92–95 report the effects of pre-existing H3K27, H3K36 and H3K4 methylation marks and of nucleosome density on the catalytic activity of PRC2.
96. Kagey, M. H., Melhuish, T. A. & Wotton, D. The Polycomb protein Pc2 is a SUMO E3. *Cell* **113**, 127–137 (2003).
97. Kagey, M. H., Melhuish, T. A., Powers, S. E. & Wotton, D. Multiple activities contribute to Pc2 E3 function. *EMBO J.* **24**, 108–119 (2005).
98. Zhang, H. *et al.* SUMO modification is required for *in vivo* Hox gene regulation by the *Caenorhabditis elegans* Polycomb group protein SOP-2. *Nature Genet.* **36**, 507–511 (2004).
99. Kang, X. *et al.* SUMO-specific protease 2 is essential for suppression of Polycomb group protein-mediated gene silencing during embryonic development. *Mol. Cell* **38**, 191–201 (2010).
100. Ismail, I. H. *et al.* CBX4-mediated SUMO modification regulates BMI1 recruitment at sites of DNA damage. *Nucleic Acids Res.* **40**, 5497–5510 (2012).
101. Voncken, J. W. *et al.* MAPKAP Kinase 3pK phosphorylates and regulates chromatin association of the Polycomb group protein Bmi1. *J. Biol. Chem.* **280**, 5178–5187 (2005).

102. Elderkin, S. *et al.* A phosphorylated form of Mel-18 targets the Ring1B histone H2A ubiquitin ligase to chromatin. *Mol. Cell* **28**, 107–120 (2007).
103. Schumacher, A., Faust, C. & Magnuson, T. Positional cloning of a global regulator of anterior-posterior patterning in mice. *Nature* **383**, 250–253 (1996).
104. Voncken, J. W. *et al.* Rnf2 (Ring1b) deficiency causes gastrulation arrest and cell cycle inhibition. *Proc. Natl Acad. Sci. USA* **100**, 2468–2473 (2003).
105. van Lohuizen, M. *et al.* Identification of cooperating oncogenes in Eμ-myc transgenic mice by provirus tagging. *Cell.* **65**, 737–752 (1991).
106. Sparmann, A. & van Lohuizen, M. Polycomb silencers control cell fate, development and cancer. *Nature Rev. Cancer* **6**, 846–856 (2006).
107. Radulovic, V., de Haan, G. & Klauke, K. Polycomb-group proteins in hematopoietic stem cell regulation and hematopoietic neoplasms. *Leukemia* **27**, 523–533 (2013).
108. Leung, C. *et al.* Bmi1 is essential for cerebellar development and is overexpressed in human medulloblastomas. *Nature* **428**, 337–341 (2004).
109. Vonlanthen, S. *et al.* The bmi-1 oncoprotein is differentially expressed in non-small cell lung cancer and correlates with *INK4A–ARF* locus expression. *Br. J. Cancer* **84**, 1372–1376 (2001).
110. Bruggeman, S. W. *et al.* Bmi1 controls tumor development in an Ink4a/Arf-independent manner in a mouse model for glioma. *Cancer Cell* **12**, 328–341 (2007).
111. Gargiulo, G. *et al. In vivo* RNAi screen for bmi1 targets identifies TGF-β/BMP–ER stress pathways as key regulators of neural- and malignant glioma-stem cell homeostasis. *Cancer Cell* **23**, 660–676 (2013).
112. Simon, J. A. & Lange, C. A. Roles of the Ezh2 histone methyltransferase in cancer epigenetics. *Mut. Res.* **647**, 21–29 (2008).
113. Sneeringer, C. J. *et al.* Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. *Proc. Natl Acad. Sci. USA* 2010 **107**, 20980–20985 (2010).
114. McCabe, M. T. *et al.* Mutation of A677 in histone methyltransferase EZH2 in human B-cell lymphoma promotes hypertrimethylation of histone H3 on lysine 27 (H3K27). *Proc. Natl Acad. Sci. USA* **109**, 2989–2994 (2012).
115. McCabe, M. T. *et al.* EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature* **492**, 108–112 (2012).
116. Knutson, S. K. *et al.* A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. *Nature Chem. Biol.* **8**, 890–896 (2012).
   References 115 and 116 show that treatment with small-molecule inhibitors is a promising therapeutic strategy for lymphomas that have catalytically hyperactive variants of EZH2.
117. Simon, C. *et al.* A key role for EZH2 and associated genes in mouse and human adult T-cell acute leukemia. *Genes Dev.* **26**, 651–656 (2012).
118. Chan, K. M. *et al.* The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. *Genes Dev.* **27**, 985–990 (2013).
119. Lewis, P. W. *et al.* Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. *Science* **340**, 857–861 (2013).
120. Xu, K. *et al.* EZH2 oncogenic activity in castration-resistant prostate cancer cells is Polycomb-independent. *Science* **338**, 1465–1469 (2012).
   This paper reports a surprising PRC2-independent role of EZH2 in the proliferation of castration-resistant prostate cancer cells.
121. Lee, J. M. *et al.* EZH2 generates a methyl degron that is recognized by the DCAF1/DDB1/CUL4 E3-ubiquitin ligase complex. *Mol. Cell* **48**, 572–586 (2012).
122. Shao, Z. *et al.* Stabilization of chromatin structure by PRC1, a Polycomb complex. *Cell* **98**, 37–46 (1999).
123. Francis, N. J., Saurin, A. J., Shao, Z. & Kingston, R. E. Reconstitution of a functional core Polycomb repressive complex. *Mol. Cell* **8**, 545–556 (2001).
124. Saurin, A. J., Shao, Z., Erdjument-Bromage, H., Tempst, P. & Kingston, R. E. A *Drosophila* Polycomb group complex includes Zeste and dTAFII proteins. *Nature* **412**, 655–660 (2001).
125. Buchwald, G. *et al.* Structure and E3-ligase activity of the Ring–Ring complex of Polycomb proteins Bmi1 and Ring1b. *EMBO J.* **25**, 2465–2474 (2006).

126. de Napoles, M. *et al.* Polycomb group proteins Ring1A/B link ubiquitylation of histone H2A to heritable gene silencing and X inactivation. *Dev. Cell* **7**, 663–676 (2004).
127. Wang, H. *et al.* Role of histone H2A ubiquitination in Polycomb silencing. *Nature* **431**, 873–878 (2004).
128. Bornemann, D., Miller, E. & Simon, J. The *Drosophila* Polycomb group gene *Sex comb on midleg* (*Scm*) encodes a zinc finger protein with similarity to polyhomeotic protein. *Development* **122**, 1621–1630 (1996).
129. Peterson, A. J. *et al.* A domain shared by the Polycomb group proteins Scm and Ph mediates heterotypic and homotypic interactions. *Mol. Cell. Biol.* **17**, 6683–6692 (1997).
130. Cao, R. *et al.* Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* **298**, 1039–1043 (2002).
131. Czermin, B. *et al. Drosophila* Enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell* **111**, 185–196 (2002).
132. Müller, J. *et al.* Histone methyltransferase activity of a *Drosophila* Polycomb group repressor complex. *Cell* **111**, 197–208 (2002).
133. Kuzmichev, A., Nishioka, K., Erdjument-Bromage, H., Tempst, P. & Reinberg, D. Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of zeste protein. *Genes Dev.* **22**, 2893–2905 (2002).
134. Pengelly, A. R., Copur, Ö., Jäckle, H., Herzig, A. & Müller, J. A histone mutant reproduces the phenotype caused by loss of histone-modifying factor Polycomb. *Science* **339**, 698–699 (2013).
135. Cao, R. & Zhang, Y. SUZ12 is required for both the histone methyltransferase activity and the silencing function of the EED–EZH2 complex. *Mol. Cell* **15**, 57–67 (2004).
136. Kim, H., Kang, K. & Kim, J. AEBP2 as a potential targeting protein for Polycomb repression complex PRC2. *Nucl. Acids Res.* **37**, 2940–2950 (2009).
137. Ciferri, C. *et al.* Molecular architecture of human Polycomb repre ssive complex 2. *eLife* **1**, e00005 (2012).
138. Klymenko, T. *et al.* A Polycomb group protein complex with sequence-specific DNA-binding and selective methyl-lysine-binding activities. *Genes Dev.* **20**, 1110–1122 (2006).
139. Oktaba, K. *et al.* Dynamic regulation by Polycomb group protein complexes controls pattern formation and the cell cycle in *Drosophila*. *Develop. Cell* **15**, 877–889 (2008).
140. Mendenhall, E. M. *et al.* GC-rich sequence elements recruit PRC2 in mammalian ES cells. *PLoS Genet.* **6**, e1001244 (2010).
   This study shows that GC-rich elements that lack transcription activator-binding sites can autonomously recruit mammalian PRC2, even when these elements are derived from bacterial genomes.
141. Vella, P., Barozzi, I., Cuomo, A., Bonaldi, T. & Pasini, D. Yin Yang 1 extends the Myc-related transcription factors network in embryonic stem cells. *Nucleic Acids Res.* **40**, 3403–3418 (2012).
142. Scheuermann, J. C. *et al.* Histone H2A deubiquitinase activity of the Polycomb repressive complex PR–DUB. *Nature* **465**, 243–247 (2010).
143. Yang, L. *et al.* ncRNA- and Pc2 methylation-dependent gene relocation between nuclear structures mediates gene activation programs. *Cell* **147**, 773–788 (2011).
144. Schuettengruber, B., Chourrout, D., Vervoort, M., Leblanc, B. & Cavalli, G. Genome regulation by Polycomb and Trithorax proteins. *Cell* **128**, 735–745 (2007).
145. Schwartz, Y. B. and Pirrotta, V. Polycomb silencing mechanisms and the management of genomic programmes. *Nature Rev. Genet.* **8**, 9–22 (2007).