Targeting Polycomb systems to regulate gene expression: modifications to a complex story

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Abstract | Polycomb group proteins are transcriptional repressors that are essential for normal gene regulation during development. Recent studies suggest that Polycomb repressive complexes (PRCs) recognize and are recruited to their genomic target sites through a range of different mechanisms, which involve transcription factors, CpG island elements and non-coding RNAs. Together with the realization that the interplay between PRC1 and PRC2 is more intricate than was previously appreciated, this has increased our understanding of the vertebrate Polycomb system at the molecular level.

A remarkable feature of multicellular organisms is their capacity to create functionally unique cell types from an essentially invariant genome sequence that is shared by all cells in the organism. This diversity relies on the capacity of individual cell types to initiate and then maintain specific gene expression patterns during development. To achieve this, cellular signalling events are thought to regulate the activity of cell type-specific DNA-binding transcription factors that function as master regulators of gene expression networks. Interestingly, however, genetic screens for factors involved in the regulation of gene expression and cell fate specification have also identified additional genes that seem to affect these processes through chromatin structure and histone modification. This is exemplified by the Polycomb group genes, which were first identified in *Drosophila melanogaster* as regulators of Hox gene expression and normal developmental body plan specification[1]. Subsequently, orthologous genes were identified in vertebrate species, in which they also encode transcriptional repressors. Vertebrate Polycomb group proteins are essential for normal gene regulation during embryonic development and are perturbed in a wide range of human cancers (reviewed in REF. 2).

Since the initial identification of Polycomb group genes, an immense amount of biochemical work has focused on understanding how these chromatin-associated factors function. This has led to the discovery that Polycomb group proteins usually belong to one of two multi-subunit protein complexes: Polycomb repressive complex 1 (PRC1), which adds a ubiquityl moiety to histone H2A at Lys119 (H2AK119ub1); and PRC2, which catalyses the addition of one to three methyl groups to histone H3 at Lys27, leading to H3K27me1, H3K27me2 and H3K27me3 (BOX 1) (reviewed in REF. 3).

PRC1 and PRC2 usually co-occupy target sites in the genome, at which their combined activities create Polycomb chromatin domains consisting of the Polycomb group proteins themselves, H2AK119ub1, and H3K27me3. How vertebrate Polycomb protein complexes are recruited to chromatin, how different PRC1 and PRC2 complexes function together *in situ* and how Polycomb chromatin domains actually repress transcription still remain poorly understood. Several excellent recent review articles have described the general features and functions of Polycomb systems in different phyla[3–7]. In this Progress article, we focus on exciting new advances that have begun to shed light on the molecular mechanisms that underpin the recruitment of Polycomb group protein complexes to target sites, the formation of Polycomb chromatin domains and the functional relevance that this has for gene regulation in vertebrates.

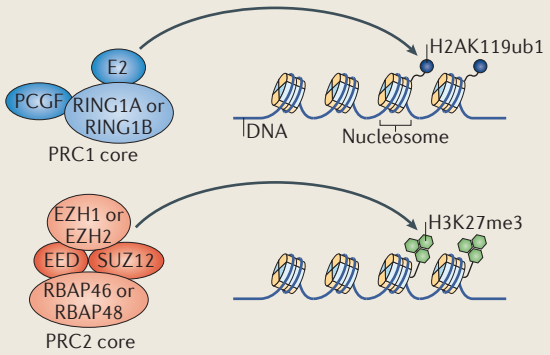
Recruitment of Polycomb complexes

In the *D. melanogaster* genome, Polycomb responsive elements (PREs) function as recruitment sites for PRCs. DNA-binding transcription factors are thought to play an important part in bringing Polycomb protein complexes to these sites (reviewed in REF. 4). However, attempts to define vertebrate PREs have proven largely unsuccessful, and emerging evidence supports the idea that vertebrate Polycomb complexes are directed to DNA by both locus-specific and more generalized targeting mechanisms.

Locus-specific targeting. Inspired by observations in *D. melanogaster*, it has been proposed that vertebrate transcription factors might recruit PRCs to target sites in chromatin (FIG. 1a). However, there are only a limited number of cases in which site-specific DNA-binding transcription factors have been identified in unbiased biochemical isolations of Polycomb complexes. Examples include E2F, MAX gene-associated protein (MGA) and MAX, which were found to interact with PRC1 (REFS 8,9). Thus, it has been brought into question whether interactions with transcription factors broadly underpin targeting. Candidate-based approaches have identified additional DNA-binding factors, including RE1-silencing transcription factor (REST), zinc-finger protein SNAI1 (also known as SNAIL) and RUNT-related transcription factor 1 (RUNX1), that interact with PRC1 or PRC2; however, these factors seem to contribute to Polycomb protein recruitment only in specific instances[10–14]. More recently, detailed proteomic profiling of Polycomb protein complexes has identified novel interactors, including proteins that contain zinc-finger domains (ZNF518A and ZNF518B[15]), that are often associated with DNA-binding activity. Whether these newly identified proteins contribute to Polycomb complex targeting remains to be determined.

In addition to transcription factors, it has been suggested that long non-coding RNAs (lncRNAs) could recruit Polycomb complexes to specific loci; however, the generality of this as a targeting mechanism remains a topic of debate (reviewed in REF. 16). This idea originated from the observation

Box 1 | **Core PRCs and their chromatin-modifying activities**



Polycomb repressive complex 1 (PRC1) and PRC2 comprise core protein components that are necessary for their respective enzymatic activities (see the figure). PRC1 is a histone H2A Lys 119 (H2AK119) ubiquitin ligase. In PRC1, two functionally equivalent yet mutually exclusive subunits, RING1A and RING1B, function as ubiquitin E3 ligases that guide the transfer of a ubiquityl moiety onto H2AK119 in chromatin (H2AK119ub1). RING1A or RING1B dimerize with a Polycomb group RING-finger (PCGF) subunit (of which there are six, PCGF1 to PCGF6, in vertebrates). The PCGF components are required for the core enzymatic activity of the complex and also define how the core complex interacts with auxiliary complex components to regulate its targeting to chromatin and its enzymatic activity.

PRC2 is a H3K27 methyltransferase. The methyltransferase activity of PRC2 resides in the SET domain of two mutually exclusive proteins, EZH1 and EZH2. Alone, these proteins have little enzymatic activity *in vitro*, but when bound to EED, SUZ12, and RBAP46 or RBAP48, they form an active methyltransferase complex that is specific towards chromatin substrates. EED, SUZ12, and RBAP46 or RBAP48 contribute to the stability and structural integrity of PRC2, and they also regulate its enzymatic activity and support its targeting to chromatin, either directly or through interactions with auxiliary complex components. Despite detailed characterization of the core PRC1 and PRC2 protein components, it remains unclear whether these complexes are stable entities inside cells or whether they also display some capacity to assemble in a dynamic fashion on chromatin. H3K27me3, H3K27 trimethylation.

that the X inactive specific transcript (*Xist*) lncRNA is required for the localization of PRC2 (REFS 17–20) to the inactivated X chromosome during mammalian dosage compensation, in a manner that relies on the sub-stoichiometric PRC2 component JARID2 (also known as Jumonji)[21]. Recent studies aimed at understanding the relationship between PRC2 and *Xist* have suggested that the interaction between *Xist* and the chromatin-remodelling protein transcriptional regulator ATRX induces conformational changes within the *Xist* RNA, which favours specific and direct interaction with PRC2 (REF. 22) (FIG. 1a). However, it has also been proposed that the relationship between *Xist* and PRC2 may be indirect. In support of an indirect interaction, super-resolution imaging studies have indicated that PRC2 is not intimately associated with *Xist* RNA on a *Xist*-inactivated chromosome[23], and the *Xist*-binding protein SHARP (also known as SPEN) was required to recruit PRC2 to *Xist*-coated chromosomes[24]. Nevertheless, there is additional evidence in favour of direct roles for lncRNAs in the recruitment of PRCs to chromatin. For example, PRCs are recruited to the KCNQ1 overlapping transcript 1 (*Kcnq1ot1*) locus on a paternally imprinted region of mouse chromosome 7 that transcribes lncRNAs[25]. Moreover, the lncRNA HOX transcript antisense RNA (*HOTAIR*), which is expressed from the *HOXC* locus on human chromosome 12, seems to function in *trans* to target PRC2 to the *HOXD* locus on chromosome 2 (REF. 26).

Although it is clear that in some specific cases, transcription factors and lncRNAs contribute to locus-specific recruitment of PRCs, it seems unlikely that these targeting mechanisms are sufficient to create the widespread, and often tissue-specific, Polycomb complex binding patterns that are observed *in vivo*.

Generic targeting. Important recent discoveries have shown that PRCs bind to regulatory sites across the genome through generic chromatin-binding activities, with local chromatin features dictating residency and function at these sites.

In vertebrate genomes, the most universal and striking feature of Polycomb-occupied sites is the presence of a CpG island (CGI). CGIs are approximately 1–2 kb regions of CpG-rich DNA that lack DNA methylation and that typically encompass gene promoters[27,28]. The striking correlation between Polycomb protein occupancy and CGIs, together with the observation that artificial DNA sequences with high CpG content are sufficient to nucleate Polycomb group proteins *in vivo*[29], has led to suggestions that CGIs may have a direct role in Polycomb protein recruitment. Attempts to define a molecular link between Polycomb complexes and CGIs have largely focused on Lys-specific demethylase 2B (KDM2B), which stably associates with PRC1. KDM2B encodes a zinc-finger–CXXC DNA-binding domain that specifically recognizes non-methylated CpG dinucleotides, allowing KDM2B to bind to CGIs genome-wide[30–32]. Recent studies have demonstrated that KDM2B contributes to PRC1 occupancy at CGIs (FIG. 1b). However, somewhat paradoxically, high-level PRC1 enrichment is only achieved at the most repressed sites, despite KDM2B residing at all CGIs. This suggests that the recruitment of PRC1 to CGIs by KDM2B, or the repressive activity of PRC1 at CGIs, is regulated by additional mechanisms that permit the establishment of Polycomb chromatin domains. Furthermore, KDM2B-independent mechanisms must also function at some CGIs to recruit or stabilize PRC1 binding, as the removal of KDM2B does not lead to a complete loss of PRC1 at all CGI target sites[30–33]. Interestingly, it has been reported that the PRC2 component JARID2 preferentially recognizes GC-rich DNA[34]. JARID2 could thus provide a complementary mechanism for targeting PRC2 to CGIs independently of KDM2B. Together, these observations suggest that Polycomb complexes can be recruited to all CGIs, although other mechanisms determine whether this results in the formation of stable Polycomb chromatin domains.

Although lncRNA-mediated targeting has been proposed as a locus-specific recruitment mechanism for Polycomb complexes, it has recently been reported that PRC2 binds to RNA promiscuously, with little sequence specificity[35,36]. This has led to several new suggestions as to how PRC2–RNA interactions may contribute to the generic recruitment or functionality of Polycomb complexes at gene regulatory elements and genes. At repressed genes, binding of PRC2 to short abortive RNA transcripts may help to retain Polycomb complexes and stabilize transcriptional repression during aberrant transcriptional initiation events[37,38]. Alternatively, nascent transcripts produced from active genes may interact with PRC2 and provide a 'decoy' to block stable interactions between PRC2 and chromatin, thereby protecting the transcribed gene from repressive Polycomb activity. This latter idea has received some support from the observation that interactions with nascent transcripts can constrain the

histone methyltransferase activity of PRC2 (REFS 39,40). An alternative possibility is that PRC2 uses interactions with nascent transcripts as a generalized targeting mechanism to increase residency around gene promoters (FIG. 1b), and that other counteracting signals, including H3K4 and H3K36 methylation, prevent the formation of stable Polycomb domains at active genes[41]. These emerging links between ncRNAs and PRC2 enzymatic activity seem to be evolutionarily conserved, as regulated bidirectional ncRNA expression from a *D. melanogaster* PRE acts as a switch to alter PRC2 activity and regulate gene expression[42]. Interestingly, vertebrate Polycomb target sites often exhibit bidirectional transcription, so it will be important to understand whether similar switch-like mechanisms are involved in regulating vertebrate PRC2 activity.

In addition to generic DNA- and RNA-binding activities, recent evidence suggests that certain PRCs recognize chromatin modifications placed by other histone-modifying systems that are broadly associated with genes and regulatory elements. For example, the sub-stoichiometric PRC2 subunits Polycomb-like 1 (PCL1; also known as PHF1), PCL2 and PCL3 encode TUDOR domains that recognize the H3K36me3 modification[43–46]. H3K36me3 is typically associated with actively transcribed gene bodies and, during cellular differentiation, PCL proteins were proposed to facilitate spreading of PRC2 into these regions (FIG. 1b). In addition, links have been identified between PRC2 and the H3K9 methylation systems. Most notably, biochemical interactions were identified between PRC2 and the H3K9 methyltransferases G9A and G9A-like protein 1 (GLP; also known as EHMT1), and loss of G9A resulted in impaired PRC2 recruitment at a subset of target sites[15,47].

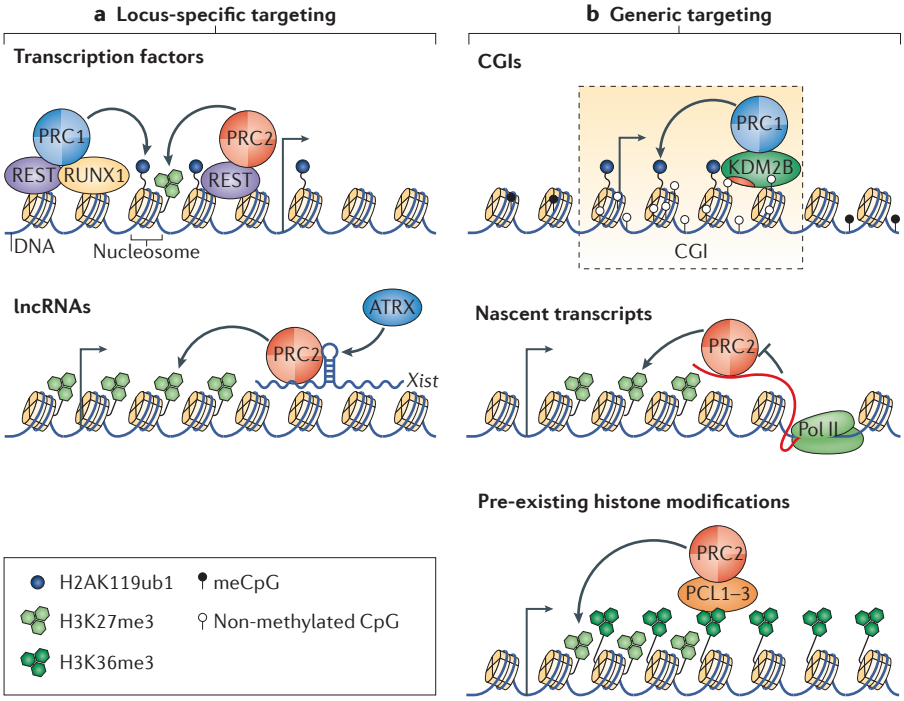


Figure 1 | **Getting Polycomb repressive complexes (PRCs) to chromatin. a** | PRC1 and PRC2 can associate with DNA-binding transcription factors (top left), such as RUNT-related transcription factor 1 (RUNX1) and RE1-silencing transcription factor (REST), which guide these complexes to chromatin. Similarly, interactions with long non-coding RNAs (lncRNAs) such as X inactive specific transcript (*Xist*) function in chromosome- and locus-specific targeting of Polycomb complexes (bottom left). The chromatin-remodelling protein ATRX may remodel the structure of *Xist* to achieve interaction with PRC2. Following recruitment to chromatin, PRC1 catalyses ubiquitylation of histone 2A Lys 119 (H2AK119ub1) and PRC2 catalyses trimethylation of H3K27 (H3K27me3), as indicated by rounded arrows. The square arrows indicate transcription start sites. **b** | A variant PRC1 complex contains the Lys-specific demethylase 2B (KDM2B) protein. KDM2B has a zinc-finger CXXC DNA-binding domain (red area) that specifically recognizes non-methylated CpG dinucleotides. This allows KDM2B to bind at CpG islands (CGIs) genome-wide and contributes to PRC1 occupancy at these elements (top right). PRC2 interacts with nascent RNA polymerase II (Pol II) transcripts at 5′ ends of genes, which may provide a mechanism to maintain repression at silent genes following stochastic transcription initiation events. Alternatively, at active genes, interaction of PRC2 with nascent RNA may constrain the catalytic activity of PRC2 and protect against Polycomb-mediated repression (middle right). A subset of PRC2 complexes contain Polycomb-like (PCL) proteins that bind to H3K36me3, which is a modification associated with active transcription. This may enable PRC2 to bind at, or spread into, previously transcribed regions, catalysing H3K27me3 at these regions. meCpG, methylated CpG dinucleotide.

Interplay between PRCs

Outlined above are simple examples of how PRC1 or PRC2 are individually recruited to target sites by locus-specific or generic targeting mechanisms. However, after their recruitment to chromatin, the functions of PRC1 and PRC2 are intimately related, as the enzymatic activity of each complex influences the occupancy of the other on chromatin and the full establishment of Polycomb chromatin domains.

The prevailing hierarchical model. PRC1 and PRC2 typically co-localize at target sites throughout the genome. This has largely been attributed to a mechanism discovered over a decade ago in *D. melanogaster*. This mechanism posits that *de novo* recruitment of PRC2 to target sites catalyses H3K27me3, which is subsequently recognized by a chromobox (CBX)-containing protein in PRC1, leading to H2AK119ub1 placement and Polycomb chromatin domain formation[48,49] (FIG. 2a). This pathway is generally referred to as the 'hierarchical' recruitment mechanism and places PRC1 recruitment and activity downstream of PRC2 function. On the basis of the conservation of CBX proteins, and on the evidence that PRC1 binding to chromatin is sensitive to loss of PRC2 (REF. 50), the hierarchical recruitment mechanism was widely adopted to explain Polycomb chromatin domain formation in vertebrates. However, detailed studies of the relationship between PRC1 and PRC2 in vertebrates indicate that other mechanisms are involved in the formation of Polycomb domains. For example, deletion of PRC2 components in mouse embryonic stem cells led to a reduction, but not the loss, of PRC1 proteins at target sites and had little effect on global levels of H2AK119ub1 (REF. 51). These observations suggested that the relationship between PRC1 and PRC2 is more complex than was originally envisaged.

A new twist in the hierarchy: PRC1 recruits PRC2. Our understanding of Polycomb systems has recently evolved, with the

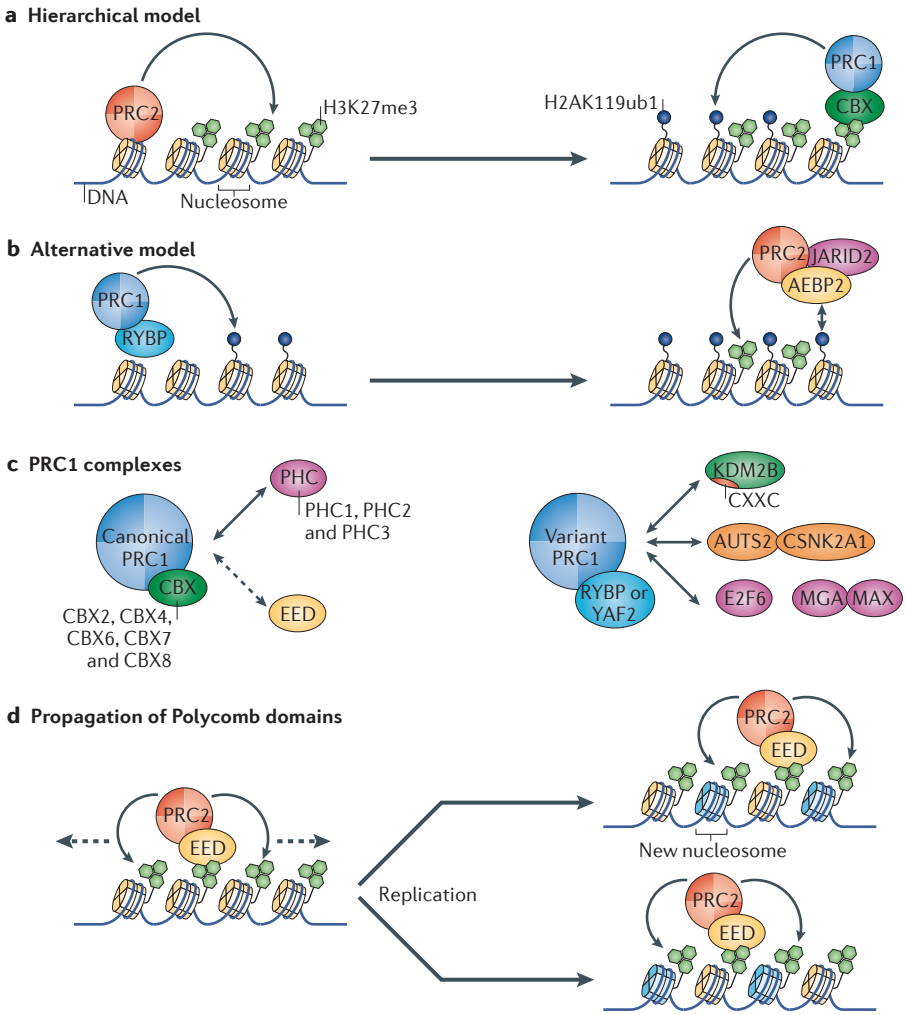


Figure 2 | **Beyond simple recruitment to more complex interactions.** **a** | In the 'hierarchical' model of Polycomb complex function, Polycomb repressive complex 2 (PRC2) first binds to chromatin and places H3 Lys27 trimethylation (H3K27me3). It is then proposed that H3K27me3 is recognized by chromobox (CBX) proteins, which are a subunit of canonical PRC1 complexes, thereby allowing PRC1 to bind to and monoubiquitylate H2AK119 (H2AK119ub1). **b** | In the 'alternative' model for Polycomb recruitment, the initial event is the binding of variant PRC1 complexes (which contain RYBP (RING1 and YY1-binding protein) instead of CBX) to chromatin. PRC1 then catalyses the formation of H2AK119ub1, independently of PRC2 activity and H3K27me3. The H2AK119ub1 modification then promotes the recruitment of PRC2, possibly through direct recognition of H2AK119ub1 by the AEBP2–JARID2–PRC2 complex and placement of the H3K27me3 mark. **c** | PRC1 complexes functionally segregate into 'canonical' complexes that contain CBX (CBX2, CBX4, CBX6, CBX7 and CBX8) and Polyhomeotic (PHC1, PHC 2 and PHC3) proteins, and a series of 'variant' complexes that contain RYBP (or the closely related YY1-associated factor 2 (YAF2) protein), and interact with proteins that are unique to individual variant PRC1 complexes (note that only selected proteins are shown here). Under some conditions, the PRC2 subunit EED may interact with a CBX-containing canonical PRC1 complex (dashed arrow). **d** | Within the core PRC2 complex, the EED subunit is able to recognize H3K27me3 through its WD40-repeat domain. This interaction potentially recruits PRC2 to sites of pre-existing H3K27me3, as well as stimulating the enzymatic activity of PRC2. The EED–H3K27me3 interaction may facilitate the spreading of H3K27me3 domains (left panel, dashed arrows) or the copying of H3K27me3 onto newly incorporated histones (right panel, blue nucleosomes) during DNA replication, thereby stably propagating H3K27me3 domains in actively dividing cells. AUTS2, autism susceptibility gene 2; CSNK2A1, casein kinase 2 subunit-α1; KDM2B, Lys-specific demethylase 2B; MGA, MAX gene-associated protein.

surprising finding that PRC1 can recruit PRC2 to chromatin through a mechanism that involves recognition of H2AK119ub1 (FIG. 2b). This alternative pathway was discovered using a cell-based system in which PRC1 was artificially recruited to a region of the genome that is devoid of genes and that is not normally occupied by Polycomb proteins. Strikingly, *de novo* PRC1 binding to this site and monoubiquitylation of H2AK119 resulted in the subsequent recruitment of PRC2 and H3K27me3 deposition, creating a new Polycomb chromatin domain[52]. A similar outcome was observed when PRC1 was artificially recruited to pericentric regions of the genome[53].

This newly discovered recruitment mechanism seems to play a part in Polycomb chromatin domain formation at natural target sites, as perturbation of PRC1 and loss of H2AK119ub1 caused a substantial reduction in PRC2 binding and H3K27me3 across the genome. The precise mechanism by which PRC1-dependent H2AK119ub1 underpins *de novo* Polycomb chromatin domain formation remains to be elucidated. However, *in vitro* studies have identified a PRC2 complex containing the auxiliary proteins AEBP2 and JARID2 that preferentially binds to and stimulates catalysis of H3K27me3 on chromatin containing H2AK119ub1 (REF. 54), suggesting that this complex may provide the molecular link between PRC1 activity on chromatin and recruitment of PRC2 and H3K27me3. Additional unexpected connections between the two Polycomb complexes include the finding that PRC2 can interact directly with an alternative H2AK119ub1 E3 ligase, TRIM37, which is highly expressed in breast cancer cells carrying the 17q23 amplification[55]. Furthermore, EED, which is a core PRC2 subunit, has been reported to directly interact with PRC1 (REF. 56) (FIG. 2c). Together, these observations indicate the potential for more functional overlap between PRC1 and PRC2 than had previously been appreciated.

Alternative roles for canonical PRC1. The observation that PRC1 can promote the recruitment of PRC2 to target sites on chromatin has placed a new focus on understanding how PRC1 complexes contribute to Polycomb chromatin domain formation and function. Systematic biochemical purifications have revealed that PRC1 in vertebrates can be separated into 'canonical' PRC1 complexes, which have CBX proteins and presumably function as part of the hierarchical recruitment pathway; and the less well-studied 'variant' PRC1 complexes, which lack CBX proteins and contain either RYBP (RING1 and YY1-binding protein) or YY1-associated factor 2 (YAF2)[8] (FIG. 2c). Importantly, RYBP and YAF2 lack the capacity to bind to H3K27me3 and can be recruited to chromatin in cells lacking functional PRC2, suggesting that variant PRC1 complexes must be recruited to chromatin

by PRC2-independent mechanisms[51]. Furthermore, individual canonical and variant PRC1 complexes contain distinct protein subunits that are likely to contribute to target site recognition or catalysis[8].

Examining the function of individual PRC1 complexes *in vivo* has revealed that variant PRC1 complexes are proficient at catalysing H2AK119ub1 on chromatin, whereas canonical complexes catalyse little of this modification[52]. This is consistent with observations that RYBP-containing PRC1 variant complexes have enhanced H2AK119ub1 catalysis *in vitro*[8]. As a consequence of their restricted catalytic activity *in vivo*, canonical PRC1 complexes seem to have limited capacity to recruit PRC2 and to form Polycomb chromatin domains. One exception to this generality was recently reported: a CBX2-containing canonical PRC1 complex seems to have a very specific role in atypical Polycomb chromatin domain formation by directly recognizing pericentromeric heterochromatin during early mouse development[57].

Nevertheless, if canonical PRC1 complexes are usually limited in their capacity to deposit H2AK119ub1 *in vivo*, why are they recruited to Polycomb chromatin domains at all? A hint as to possible functions came with the recent demonstration that a stable component of canonical PRC1 complexes, Polyhomeotic-like protein 2 (PHC2), can auto-polymerize through its sterile-alpha motif (SAM) domain, leading to chromatin compaction and gene silencing[58–60]. This is consistent with previous reports detailing a ubiquitin ligase-independent role for PRC1 in chromatin compaction[61]. Interestingly, inhibition of PHC2 polymerization resulted in loss of canonical PRC1 binding to chromatin only at sites marked with H3K27me3, suggesting that both H3K27me3–CBX interactions and SAM-domain polymerization may have important structural roles in creating and translating repressive chromatin structures on chromatin[59].

Propagation of Polycomb domains. Biochemical studies have demonstrated that PRCs can bind the histone modifications that they themselves place. For example, the EED subunit of the PRC2 core complex binds to H3K27me3 through its WD40 repeat, and this interaction seems to stimulate the catalytic activity of PRC2, to form an activity-based feedback loop[62,63]. It has been proposed that this could promote the spreading of H3K27me3 along chromatin and ensure the propagation of H3K27me3 on newly replicated chromatin[62] (FIG. 2d).

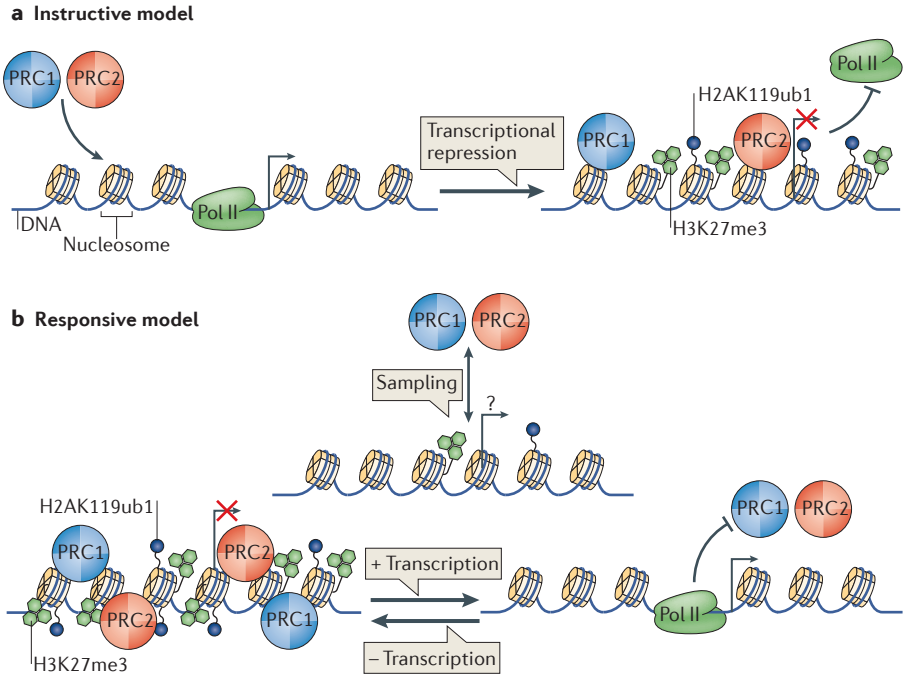


Figure 3 | **Polycomb systems and gene regulation.** **a** | An instructive model for Polycomb complex-mediated silencing would posit that newly recruited Polycomb complexes lead to Polycomb chromatin domain formation, which then directs repression of transcription by RNA polymerase II (Pol II) at the associated gene. **b** | A responsive model would posit that Polycomb complexes constantly 'sample' chromatin at regulatory elements through generic targeting modalities, in order to respond to the transcriptional state of the associated gene. Transcriptional cessation would lead to the subsequent establishment of Polycomb chromatin domains that protect against low-level or stochastic reactivation signals. However, in response to active transcription, the presence of Pol II or other features of transcriptional initiation would block establishment of Polycomb domains. H3K27me3, histone 3 Lys27 trimethylation; H2AK119ub1, histone 3 Lys119 monoubiquitylation; PRC, Polycomb repressive complex.

On the basis of the finding that PRC1, PRC2 and their chromatin-modifying activities are more intimately linked than was previously appreciated[52–54], it seems plausible that this robust series of PRC-dependent feedback mechanisms could underpin both the spreading and the maintenance of Polycomb chromatin domains once they are initially established. It is tempting to speculate that this would contribute not only to epigenetic maintenance of Polycomb chromatin domains but also to rigidly maintaining gene expression states during cell division and development. A detailed understanding of the epigenetic nature of Polycomb chromatin domains is an interesting and evolving area of Polycomb biology.

Polycomb systems and gene regulation

It is often suggested that vertebrate Polycomb group proteins are recruited to target sites to actively drive transcriptional repression (FIG. 3a). However, recent evidence suggests that this may not be the central modality connecting Polycomb chromatin domains and gene repression. Firstly, kinetic analysis of gene expression in a cell culture model of RAS-induced transformation showed that cessation of transcription preceded H3K27me3 acquisition[64]. Secondly, experiments in mouse embryonic stem cells demonstrated that small-molecule inhibitors that block transcription caused recruitment of PRC2 and H3K27me3 to previously active genes[65]. In light of these conceptually important findings, one interesting possibility is that Polycomb systems may exploit generic targeting activities to constantly interface with or 'sample' gene regulatory elements and respond to the transcriptional state of individual genes. Within the context of this model, features of active transcription — for example, the presence of RNA polymerase II (Pol II) or transcription-associated histone modifications — would decrease the residency time or the catalytic activity of Polycomb complexes at transcribed genes, meaning that full Polycomb chromatin domain establishment would only occur at sites at which transcriptional silencing has already been achieved (FIG. 3b) (reviewed in REF. 66).

What would be the purpose of establishing Polycomb chromatin domains at already silenced genes? One possibility is that following transcriptional silencing, Polycomb systems form repressive chromatin domains to limit the potential for stochastic reactivation events in inappropriate tissues. This general concept is consistent with observations in *D. melanogaster,* in which Polycomb group genes are important for the maintenance, but not the establishment, of gene expression programmes[67]. Interestingly, in pluripotent cells, Polycomb-occupied gene promoters also typically have low levels of the transcriptionally permissive histone modification H3K4me3, which is placed by Trithorax group proteins[68,69]. This modification is thought to result from the capacity of the Trithorax system to generically sample regulatory elements in a manner analogous to Polycomb proteins. Given that Polycomb and Trithorax are two opposing chromatin-based gene regulatory systems, these constant sampling activities at gene regulatory elements might form the basis for a chromatin-encoded bistable switch that helps to regulate the transition between, and the stable maintenance of, chromatin states that are either permissive or repressive to transcription (discussed in detail in REFS 4,27,66,70).

Although Polycomb chromatin domains are predominantly found at silenced genes, recent evidence has implicated Polycomb proteins and their chromatin modifications in active transcription and other gene regulatory functions. This is the case for a variant PRC1 complex containing the autism susceptibility gene 2 (AUTS2) protein, which recruits casein kinase 2 subunit-α (CSNK2A) to counteract PRC1 ubiquitin ligase activity and repression[71]. Similarly, in erythroid lineages, a PRC2 complex containing an alternative catalytic core associated with regions of the genome that are characterized by chromatin modifications indicative of active transcription (H3K4me3 and H3K27 acetylation (H3K27ac)) and promoted gene expression[72]. Furthermore, it was recently demonstrated that PRC2-dependent H3K27 monomethylation is enriched in the bodies of transcribed genes, whereas the H3K27 dimethyl state covers much of the genome, including non-genic locations, possibly as a mechanism to block aberrant activation of enhancer elements[73]. These interesting recent observations highlight our incomplete understanding of the precise mechanisms by which Polycomb systems regulate gene expression, and elucidating such mechanisms remains a key challenge.

Conclusion and future directions

A complex series of interactions between PRC1 and PRC2, chromatin, and transcription are essential for normal Polycomb chromatin domain formation and function. Although transcription factor and lncRNA-based mechanisms contribute to Polycomb complex targeting to specific chromatin regions, recent evidence suggests that Polycomb protein complexes also constantly interact with regulatory elements throughout the genome and establish repressive Polycomb chromatin domains in a manner that is often responsive to the transcriptional states of associated genes. These emerging principles prompt alternative ways of thinking about the vertebrate Polycomb system, and further experiments will be required to fully understand the molecular mechanisms underlying PRC function. Importantly, there is little evidence that the chromatin modifications placed by the Polycomb systems directly repress transcription, suggesting that components of the Polycomb protein complexes, such as PHC proteins, may induce transcriptional inhibition by directly modifying chromatin structure.

Understanding the precise molecular mechanisms by which Polycomb protein complexes repress gene expression remains a central and outstanding question in the field. A cryo-electron microscopy structure of the PRC2 holocomplex[74] and the recently solved atomic structure of the PRC1 core complex bound to a nucleosome[75] have provided exciting new insights into the interactions that occur within Polycomb complexes and also with their chromatin substrates, at high resolution. These and other new discoveries will contribute to our understanding of how Polycomb systems regulate gene expression programmes during normal multicellular development. They will also pave the way for new approaches to therapeutic interventions in human diseases, including cancer, in which Polycomb systems are frequently perturbed.

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Competing interests statement

The authors declare no competing interests.