CellPress

Trends in Plant Science

Review

PRC2 activity, recruitment, and silencing: a comparative perspective

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Polycomb repressive complex (PRC)-mediated gene silencing is vital for cell identity and development in both the plant and the animal kingdoms. It also modulates responses to stress. Two major protein complexes, PRC1 and PRC2, execute conserved nuclear functions in metazoans and plants through covalent modification of histones and by compacting chromatin. While a general requirement for Polycomb complexes in mitotically heritable gene repression in the context of chromatin is well established, recent studies have brought new insights into the regulation of Polycomb complex activity and recruitment. Here, we discuss these recent advances with emphasis on PRC2.

Highlights

Polycomb Repressive Complex 2 (PRC2) comprises core and accessory subunits in animals and plants.

Recent structural insights revealed mechanisms for mammalian PRC2 substrate specificity, and allosteric and competitive inhibition, some of which are likely conserved in plants.

Different recruitment modes for PRC2 in mammals as opposed to *Drosophila* and plants suggest that different constraints operate in organisms with larger genomes.

H3K27me3 deposited by PRC2 is interpreted by reader protein-containing complexes to repress transcription, compact local chromatin, and form long-range loops and Polycomb bodies

Polycomb complexes as blackout artists of the genome

Despite having the same genome, different cell types exist in multicellular eukaryotes that each have unique identities, due, in large part, to the fact that unique portions of the genome are accessible in each cell type. Genome accessibility is controlled at the level of chromatin, that is, by interactions between the genomic DNA and small basic proteins called histones in nucleosomes, as well as non-histone proteins [1]. On the one hand, the formation of nucleosomes and condensation of the chromatin into tertiary structures as well as incorporation of linker histone H1 enables the genomic DNA to fit into the nucleus, a ~10 000-fold compaction [2]. On the other hand, the organization of the genomic DNA into chromatin creates regions of the genome that are more accessible (such as the linker DNA not wrapped around the nucleosome), or less accessible (such as nucleosomal DNA). Certain genomic regions are constitutively inaccessible to promote genome integrity and to silence selfish DNA (such as transposons) in the gene-poor heterochromatin [3]. However, in the gene-rich euchromatin, condensed chromatin prevents access to vital genomic information. This limitation can be overcome by enzymatic machineries, such as chromatin-modifying and remodeling activities that can increase or decrease the accessibility of genomic DNA. Genome accessibility is modulated such that genomic information that is not needed or even detrimental at a given stage, in a given tissue or condition is 'shut off' [4]. In this manner, the same genome can give rise to different cell types. Thus, the identity of a cell relies, in large part, on the genetic programs not actualized, in much the same way as meaning is derived after hiding information in newspaper blackout poems (Figure 1A).

The major chromatin regulatory mechanism for silencing unnecessary or unwanted gene expression programs in euchromatin is Polycomb repression. This is carried out by two general classes of protein complexes, **PRC1** (see Glossary) and **PRC2**. Both can form unique variant complexes based on the subunits present and their activity [5,6]. Consistent with their critical roles, malfunction of Polycomb repression leads to homeotic developmental transformations in drosophila (*Drosophila melanogaster*), mammals, and plants (Figure 1B). A major tenet of Polycomb repression is its mitotic inheritance and, hence, 'memory' of the silent chromatin state. Several recent studies in animals and plants have shed light on the mechanism that enables memory of silencing [5,7,8]. PRC1 complexes are often subdivided into canonical PRC1, which is recruited by

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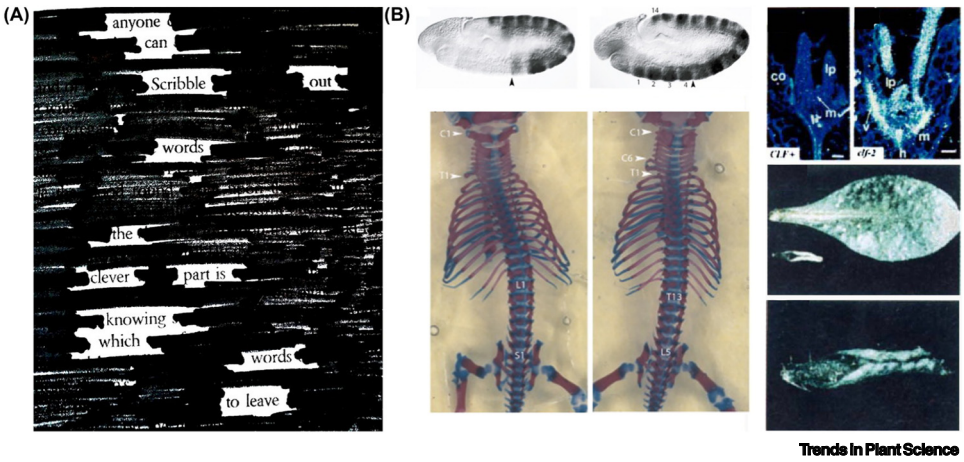

Figure 1. Polycomb: hiding away unnecessary or detrimental information. As in newspaper blackout poems by anonymous authors, where meaning arises from hiding words (A), in the context of cells, identity is aided by hiding unnecessary or detrimental genetic programs through Polycomb repression (B). Defects in Polycomb repression lead to homeotic defects in flies [top center: ectopic expression of the homeotic gene ULTRABITHORAX in *suz12* mutants (right) compared with the wildtype (left)], in mammals [bottom center: posterior transformations of the axial skeleton in mice deficient in *Cbx2* (right) compared with the wildtype (left)], or arabidopsis *(Arabidopsis thaliana)* [right-most images, top: ectopic expression of the floral homeotic gene *AGAMOUS* in seedling leaves in *clf* mutants (right) compared with the wildtype (left). Below: the small upward-curling leaves typical of *clf* mutants represent a partial phenocopy of carpels, floral organs patterned by *AGAMOUS*]. Modified from [118] (B); *clf* images from [119].

H3K27me3 readers and can compact nucleosomes, and noncanonical or variant PRC1, which is recruited to chromatin independently of H3K27me3 and can ubiquitylate histone H2A (H2AK119/121ub) [9,10]. Excellent recent reviews have addressed the roles and mechanisms of action of PRC1 [11,12]. Here, we mainly focus on PRC2, which is highly conserved even between different kingdoms of life and the catalytic role of which is mono-, di-, and tri-methylation of lysine 27 of histone H3 (H3K27me1/2/3) by the histone methyltransferase (HMT) or 'writer' EZH.

Polycomb repressive complex composition

To execute its diverse roles with precision, different PRC2 complexes with specific functions have evolved. Moreover, the activity and **recruitment** of PRC2 complexes is tightly regulated.

Core complex components

PRC2 comprises four core subunits (in humans: SUZ12, EED, EZH1/EZH2, and RBBP4/7), which form two functional 'lobes' [13]. The catalytic lobe comprises either of the two paralogous histone H3K27 methyltransferases (EZH1 or EZH2), EED (which binds H3K27me3), and the C-terminal VEFS domain of SUZ12 (a PRC2 scaffold protein) (Figure 2). This may be the ancestral PRC2 complex [14]. The targeting lobe of PRC2 is formed by the SUZ12 N terminus together with RBBP4/7 [13,15] (Figure 2). EZH2 is the major isoform in dividing cells, while EZH1 expression is lower overall but uniform [5]. Unlike EZH1-PRC2, EZH2-PRC2 requires an **accessory subunit** called JARID2 (see later for more details) for efficient nucleosome binding *in vitro* [16] and is strongly dependent on allosteric activation [17]. Accordingly, PRC2 relies on EZH1 in cells that do not express JARID2 [16]. EZH1-PRC2 displays higher chromatin affinity and may be able to compact nucleosomes *in vivo* and *in vitro* independent of its catalytic activity (discussed in [5]), perhaps due to its ability to simultaneously bind two nucleosomes [18].

In arabidopsis (*Arabidopsis thaliana*), two subunits of the PRC2 core complex (SUZ12 and EZH2) are encoded by multiple genes: SUZ12 (*FIS2*, *EMF2* and *VRN2*) and EZH2 (*MEA*, *CLF* and *SWN*).

Glossary

Accessory subunit: subunits present in a subpopulation of a given protein complex enabling it to bind specific substrates or modulating its activity.
Affinity purification followed by mass spectrometry (AP-MS): method for identification of protein interactors in which a protein of interest is purified under native conditions using antibodies or other enrichment methods to identify interactions with other proteins by MS.
Allosteric regulation: form of enzymatic activity regulation in which binding of regulatory molecules outside the catalytic center induces changes in enzyme conformation and activity.
Chromatin loop anchor: intramolecular contacts between two chromosomal sites that bring together distant chromosomal sites at the base of chromatin loops.
Chromatin loop: interphase chromatin structures formed when two distant chromosomal sites are brought together.
Competitive regulation: form of enzymatic activity regulation in which regulatory molecules compete with the substrate for access to the catalytic center of the enzyme.
CpG islands (CGIs): long stretches of low complexity sequences rich in cytosine-guanine dinucleotides; frequently found upstream of mammalian genes.
Cryogenic electron microscopy (cryo-EM): combination of highly sensitive electron microscopy with advanced computational methods that can resolve 3D structures of large protein complexes with resolution previously achieved only by X-ray crystallography.
Degron: short amino acid sequence recognized by ubiquitin ligases, which mark the degron-containing protein for degradation.
H3K27me3 nucleation: H3K27 methylation at the initial site of PRC2 recruitment.
H3K27me3 spreading: formation of larger domains of H3K27me3-marked chromatin.
Long non-coding (lnc)RNA: nonprotein-coding, functionally diverse RNAs longer than 200 bp.
Polycomb body: 3D chromatin structures formed by spatial concentration of distant PRC2 recruitment sites, visible by light

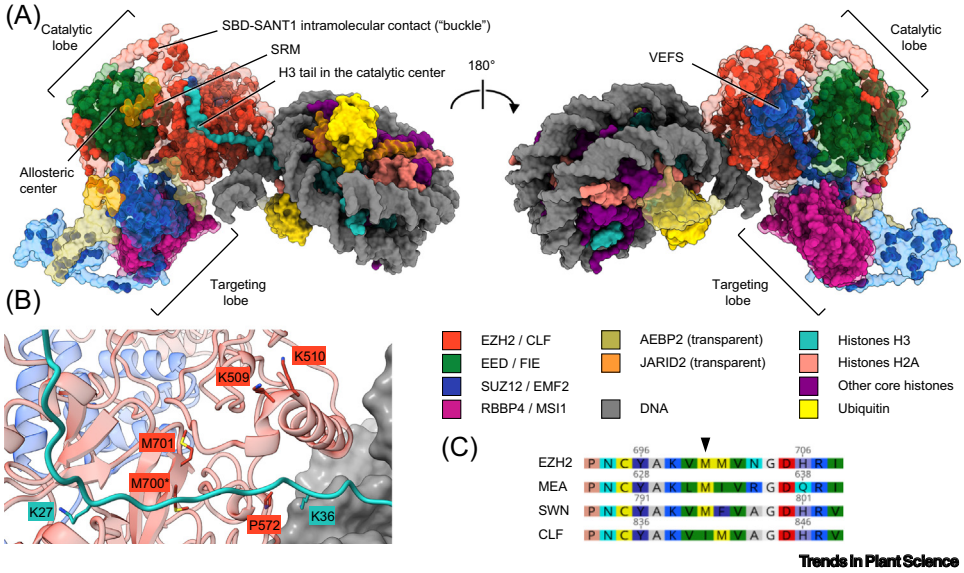

Figure 2. Residues conserved in plants map to functionally important regions of Polycomb repressive complex 2 (PRC2). (A) Two views of the PRC2 complex and its catalytic and targeting lobes. Residues conserved between human and arabidopsis *(Arabidopsis thaliana)* PRC2 core subunits mapped onto the structure of the complete human PRC2.2 interacting with a H2K119ub nucleosome [15] [Protein Data Bank (PDB): 6WKR]. Residues of EZH2, EED, SUZ12, and RBBP4 conserved in their arabidopsis homologs are displayed as atomic spheres. The remaining structure is overlayed as a color-matched transparent surface. The accessory subunits AEBP2 and JARID2 have no known homologs in arabidopsis and are displayed as transparent surfaces. (B) The interface between EZH2 (red, cartoon), the N-terminal tail of the substrate histone H3 (turquoise, cartoon), and nucleosomal DNA (gray, surface). Critical residues conserved in plants are displayed as atomic stick structures with heteroatoms N and S colored blue and yellow, respectively. Lack of M700 conservation in CLF is indicated by an asterisk. (C) Alignment of the amino acid sequence surrounding M700 in EZH2, MEA, SWN, and CLF. Images in panels (A) and (B) were generated with UCSF ChimeraX software [120].

There is one EED (*FIE*) and one RBBP4/7 (*MSI1*) subunit (Figure 2). The SUZ12 and EZH2 core subunits exhibit spatiotemporally restricted and inducible accumulation, generating PRC2 complexes with distinct and overlapping function [19]. The H3K27me3 methyltransferase MEA is specifically expressed in the female gametophyte and endosperm and forms a complex with FIS2 in these tissues [19]. Moreover, an F-box protein specifically degrades CLF in the endosperm, in which *CLF* and *MEA* RNA accumulation overlaps [20]. Finally, the hormone auxin removes sporophytic PRC2 activity, by a mechanism not yet understood, to allow formation of the seed coat [21]. Conversely, in inflorescences, FIE forms cytoplasmic complexes with MEA [22] (Figure 3A).

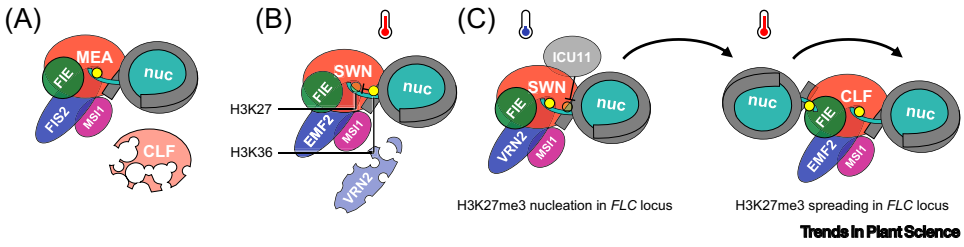

Figure 3. Regulation of Polycomb repressive complex 2 (PRC2) by subunit composition. (A) In the endosperm, the FIS2-PRC2 subunit MEA replaces CLF, which is targeted for degradation [20]. (B) In warm temperatures, PRC2 activity is blocked by H3K36me3 present at the active FLC locus. The intensities of the small yellow circles indicate unmethylated or methylated states of H3K27 and H3K36 [25,51]. (C) VRN2 is stabilized by cold (during vernalization) and may form an H3K36me3-insensitive complex with SWN for H3K27me3 nucleation at the *FLC* locus [25,50]. After a return to warm conditions, H3K27me3 is mediated by CLF, possibly aided by ICU11 demethylating H3K36me3 [8,33].

microscopy as speckles after fluorescent labeling of PRC2 proteins.

Polycomb domain: large chromosomal region of continuous H3K27me3-nucleosomal marking covering multiple genes.

Polycomb repressive complex 1 (PRC1): complex that promotes H2A ubiquitination or chromatin compaction for transcriptional gene silencing; often contains H3K27me3 reader subunits.

Polycomb repressive complex (PRC2): enzymatic protein complex that catalyzes H3K27 mono-, di-, and tri-methylation.

Polycomb response element (PRE): DNA regulatory element containing cognate motifs bound by a combination of TFs, which cooperatively recruit PRC2.

Quadruplex RNA: stable secondary structure formed by G-rich RNAs involved in specific interactions with proteins.

Recruitment: stable binding of an enzyme to specific genomic locations in the chromatin substrate, often aided by TFs or accessory subunits.

R-loop: RNA–DNA hybrids, in which one strand in a DNA duplex is replaced with RNA.

Telobox: short DNA sequence forming telomeric repeats; also found in low copy number in regulatory regions of genes.

Environmental cues and stress conditions also modulate the expression of core PRC2 components. UV-B light downregulates the expression of *MSI1* and *CLF*, resulting in delayed developmental phase transitions [23]. Pathogens or biotic stress hormones induce the expression of *MEA* in vegetative tissues, such as leaves, to blunt the defense response [24]. An oxygen-sensitive N-terminal **degron** leads to VRN2 protein accumulation in hypoxia and cold, increasing tolerance to stress [25] (Figure 3B,C). Regulation of PRC2 complex composition via controlled turnover of specific core subunits has not yet been observed in animals. Moreover, different activities have been described for the CLF and SWN PRC2 subunits. Although generally expressed at similar levels and in the same tissues of the sporophyte, *clf* mutants display more dramatic developmental phenotypes and effects on H3K27me3 deposition compared with *swn*; yet, phenotypic and H3K27me3 deposition defects are enhanced in the *clf swn* double mutant [26]. At some loci, including *FLC*, SWN appears to be responsible for nucleation/recruitment, while CLF aids in spreading of H3K27me3 (reviewed in [8]; Figure 3C). Differences in enzymatic activities and substrate preferences of the CLF or SWN PRC2 complexes are not yet well understood.

Accessory PRC2 subunits

Accessory subunits, defined as genetic modifiers of PRC2 that physically interact with core complex subunits, have been described for arabidopsis PRC2. Most of these phenocopy PRC2 subunit mutants when mutated, co-purify with core complex components based on **affinity purification followed by mass spectrometry (AP-MS)**, and reduce H3K27me3 accumulation/spread (Table 1). Several accessory proteins bind different histone modifications, including H3K27me2/3 through CHROMO (LHP1; reviewed in [27]) or BAH domains (SHL and EBS [28–30]); H3K4me2/3 via PHD domains (VIN3, VRN5, SHL, and EBS [29,31]) or H3 (not H3S28p) via the PWWP domain (PWO1 [32]). The H3K27me3 reader *LHP1* is a homolog of HETEROCHROMATIN PROTEIN1 in animals, VIN3 and VRN5 share the PHD domain and winged helix DNA contact domain with mammalian POLYCOMB LIKE (PCL) accessory complex components, while EMF1 shares features with subunits of the canonical PRC1 complexes in animals, which compact nucleosomes (reviewed in [8,27]). Two members of a novel family of putative Jumonji-type 2-oxoglutarate/Fe(II)-dependent dioxygenases, called ICU11 and CP2,

Table 1. Accessory PRC2 subunits in arabidopsis (*Arabidopsis thaliana*)[a,b]

Accessory subunit of PRC2	Core PRC2 subunit interaction determined by AP-MS		PRC2-loss phenotype	Reduced H3K27me3
	As bait (prey names)	As prey (bait names)		
LHP1, EBS	No data	MSI1 (LHP1 only) [105], CLF (EBS only) [33]	√ [28,29]	√ [28]
ICU11, CP2	CLF, SWN, FIE, MSI1 [33]	CLF [33,35], SWN [33]	√ [113]	√ [33,113]
EMF1	CLF, SWN, FIE [33]	CLF [33,35], SWN [33]	√ [27]	√ [114]
VIN3 or VRN5	SWN, FIE, MSI1 [115]	CLF, SWN [33], MSI1 (VRN5 only) [105]	No [116]	√ [116]
TRB1/2/3	No data	CLF [33,35], SWN [33]	√ [34]	√ [34]
ALP1/ALP2	CLF SWN, FIE, MSI1 [35,36]	CLF [33,35], SWN [33]	No: suppress *lhp* and *clf* [35,36]	No [36]
VAL1/2	None detected [70]	Not detected	√ [71]	√ [71]
PWO1	None detected [117]	Not detected	No: enhances *clf* [32]	No: reduced nucleosome [32]

[a]Putative accessory subunits were selected based on reported physical interaction with PRC2 and assessed using the following criteria: AP-MS-confirmed physical association with PRC2 core subunits (columns 2 and 3), genetic interaction with PRC2 subunit mutants (column 4), or reduced H3K27me3 in their absence (column 5).
[b]Symbols and shortcuts used: √, condition met; no data, this protein has not been used as a bait in any of the published AP-MS experiments; none detected, no PRC2 core subunits have been identified when this protein was used as an AP-MS bait (column 2); not detected, this protein has not been detected in published AP-MS experiments that used PRC2 core subunits as baits (column 3).

may enable the transition between H3K36me3-marked active and H3K27me3-marked inactive chromatin states [33]. Other accessory proteins include telomere repeat-binding factors (TRB1–3), which contribute to PRC2 recruitment and phenocopy strong PRC2 subunit mutants [34] as well as harbinger transposase-derived ALP1 and ALP2 proteins, which antagonize PRC2 activity [35,36].

In animals, two PRC2 complexes have been described based on the presence of alternative accessory subunits [37]. PRC2.1 contains one of the three PCL family proteins (PHF1, MTF2, or PHF19), as well as either EPOP or PALI1/2. PRC2.2 contains JARID2 and AEBP2. Subunits characteristic of either of the two complexes compete for binding to SUZ12, considered a PRC2 scaffold protein due to its direct contact with all other subunits [5]. The accessory subunits of the mammalian PRC2 have roles such as DNA binding (PCLs, AEBP2, and JARID2), complex stabilization (AEBP2 and JARID2), binding to histone post-translational modifications (PHF1/19 to H3K36me3; JARID2 and AEBP2 to H2AK119ub) and allosteric autoactivation of the complex (JARID2 and PALI1) [13,15,38]. PRC2.1 and PRC2.2 largely colocalize on chromosomes and maintain H3K27me3 in mouse embryonic stem cells (ESCs) both synergistically and independently [39]. While loss of either complex alone leads to partial displacement of the other and moderate loss of H3K27me3, simultaneous disruption of both complexes leads to complete loss of H3K27me3 over Polycomb targets [39]. Instead of covering different targets, PRC2.1 and PRC2.2 were proposed to function sequentially, with PCL proteins mediating initial PRC2.1 recruitment to unmethylated CG islands ('initiation phase'), followed by PRC2.2-dependent spreading of H3K27me3 ('amplification'), enhanced by the PRC2–PRC1 feedback loop involving reciprocal readers JARID2 (H2AK119ub) and CBX (H3K27me3) (discussed in [40]). Auxin-dependent degradation was used to reveal that, while PRC2.1 promotes H3K27me3 maintenance at target genes already repressed by Polycomb in ESCs, PRC2.2 silences new target genes during differentiation [41]. Biochemical data suggest that PRC2.1 and PRC2.2 can contain either EZH1 or EZH2 as the catalytic subunit [37].

Regulation of PRC2 activity

Post-translational modifications

Proteins forming the PRC2 complex in mammals are targets of many different post-translational modifications, which can affect their stability, interactions with other subunits of the complex, and the activity of the whole complex (reviewed in [42]). Essential PRC2 modifications involve automethylation of EZH2 at lysines 510, and 514, which enhance enzymatic conversion of H3K27me2 to H3K27me3 [43]. Interestingly this activity is sensitive to the cellular levels of the methyl group donor S-adenosyl-L-methionine -as well as competition from the H3 tails [44]. The automethylated loop region of EZH2 becomes structured upon nucleosome binding, forming an alpha helix that bridges EZH2 to nucleosomal DNA and the H3 tail [15]. The modified amino acids are conserved in CLF [15], suggesting conservation of this mechanism (Figure 2B).

Histone substrates and modifications

Different histone substrates can regulate the activity of PRC2 [5]. Not all H3 variants make equally good substrates for K27 methylation. H3.1 and H3.3 display similar levels of K27me3 in arabidopsis and neither of them is favored by the plant PRC2 *in vitro* [45]. However, H3.1 is a preferred substrate of the monomethylases ATXR5/6 [45], which are crucial for maintenance of H3K27 methylation through mitotic divisions [45,46]. Methylation of H3K27 by an enzyme other that PRC2 is unique to plants. The preference of ATRX5/6 for H3.1 over H3.3 is due to a single amino acid difference at position 31 of H3, where alanine promotes methylation of H3.1 and threonine blocks methylation of H3.3 [45]. Interestingly, the animal H3.3 variant also differs from the replicative H3.1 histone by a small hydrophilic residue (serine) instead of alanine at position

31. Phosphorylation of this residue activates transcription, while possibly also interfering with the recruitment of PRC2 by a PCL accessory subunit [47]. Evidence for H3.3T31 phosphorylation is lacking. Recently, a more specialized H3 variant, H3.10, has been found to be refractive to H3K27 methylation in arabidopsis. Accordingly, H3.10 is deployed in arabidopsis sperm cells to erase H3K27me3 [48]. Similarly, wounding-induced activation of the H3.15 variant lacking K in position 27 decreases H3K27me3 levels, promoting cell dedifferentiation [49]).

Histone modifications also impact PRC2 activity. H3K27ac cannot be methylated unless the acetyl group has been removed. In addition, H3K27ac, H3K4me3, and H3K36me2/3 strongly reduce di- and tri-methylation of H3K27 by core PRC2 complexes reconstituted with recombinant *Drosophila*, mouse, or arabidopsis homologs of EZH2, EED, and SUZ12 *in vitro* [50]. The mechanism of allosteric inhibition of PRC2 by H3K4me3 may involve loss of stable binding of the H3 tail to the active site of the complex, as indicated by a recent structure of the complete human PRC2.2 complex bound to an H3K4me3-modified nucleosome [15]. Inhibition of PRC2 by H3K36me3 is possibly explained by a compound effect of a steric clash with surrounding EZH2 residues and loss of electrostatic interaction between H3K36 and nucleosomal DNA [15]. Indeed, H3K36me3 and H3K27me3 generally do not colocalize on chromatin in mammals [13]. *In vitro*, human PRC2 can methylate H3K27me3 even when H3K36me3 is present on the same tail, albeit at a reduced efficiency and only when the AEBP2 and JARID2 subunits are present [15].

In arabidopsis, H3K36me3 and H3K27me3 rarely coexist on the same histone tail [51]. HMT activity of EMF2-PRC2 is inhibited on H3K4me3- or H3K36me3-containing nucleosomes, whereas that of the VRN2-PRC2 is not [50]. A gain-of-function mutant of *CLF* has been described in which P704 is replaced by S [52]. P704 of CLF aligns with P572 of EZH2 at the H3 interface. The proline to serine substitution may increase flexibility in this region and loss of CLF sensitivity to inhibition by H3K36me2/3. Indeed, the CLF_{P704S}-PRC2 complex does not require VRN2 for *FLC* silencing [52]. Loss of H3K36me3-dependent inhibition was recently engineered into human EZH2 by substation of M700 in the M700M701 dipeptide by either A or V [53]. P572 and M700 are located close to each other and to the axis connecting K27 and K36-binding pockets in the folded EZH2 (Figure 2B). Interestingly, the MM dipeptide of human EZH2 is replaced by MI in MEA, MF in SWN, and IM in CLF (Figure 2C).

Allosteric and competitive regulation of PRC2 catalytic activity

Allosteric activation of PRC2 is important for **H3K27me3 spreading** beyond the site of PRC2 recruitment and epigenetic memory of the H3K27 methylated chromatin state; however, the underlying molecular mechanism became clear only recently [13,54]. Activation of PRC2 by H3K27me3 requires cooperation between two of its subunits, EZH2, containing the catalytic center of the complex, and EED, containing the aromatic cage that binds the allosteric H3K27me3 on a second nucleosome. Crucial to this process is the Stimulation-Responsive Motif (SRM) of EZH2. SRM makes direct contacts with EED, H3K27me3, and the SET inserted (SET-I) domain of EZH2 [55]. Binding of H3K27me3 to EED forces SRM to transition from an unstructured state to an α-helical conformation, which induces downstream structural changes in the substrate-binding pocket of EZH2, leading to increased HMT activity [55]. Two distinct active conformations of the human PRC2 were revealed by **cryogenic electron microscopy (cryo-EM**) imaging of the human PRC2 complex with bound cofactors JARID2 and AEBP2 [56]. The EZH2 conformation differs between the two structures in the region of the SBD–SANT1 intramolecular contact referred to as a 'buckle' which closes a belt-like structure surrounding EED [55,56]. Another difference between the two structures is the configuration of the SRM domain, α-helical in one and disordered in the other, despite no significant difference in

the structure of the SET domain [56]. In agreement with this role, a single amino acid substitution within the SRM domain was found to be the likely cause of the difference in allosteric activation potential between EZH1 and EZH2 [17].

Allosteric activation of PRC2 is also a focal point of natural regulatory mechanisms, clinically important mutations, and potential therapeutic interventions [57]. PRC2 self-activates by trimethylating the JARID2 or PALI1 subunits of PRC2.2 and PRC2.1, respectively, which mimic the binding of H3K27me3 to EED [38,58]. Molecular mimicry can also be used to specifically block allosteric activation of PRC2 with synthetic compounds imitating amino acid residues of the SRM α-helix or blocking the H3K27me3 binding pocket of EED [59]. Finally, recent elegant studies showed that G-tract **quadruplex RNA** can allosterically inhibit PRC2 by binding to the crucial regulatory site formed by EZH2 and EED [60]. Inhibition by quadruplex RNA is overcome by H3K27me3 or JARID2-K116me3 [60].

In addition, a common oncomutation underlying glioblastomas, substitution of lysine 27 of H3.3 with methionine (H3K27M), acts as a strong competitive inhibitor, especially on the allosterically activated PRC2 [61]. An endogenous locus, termed EZHIP/Catacomb, expressed in germ cells in mice [62] (and in U2OS and DAOY cancer cell lines [63]), contains a short peptide that resembles H3K27M and likewise blocks PRC2 activity [63].

Recruitment of PRCs to Polycomb targets

Besides regulation of activity, how PRC2 'finds' the genomic regions that need to be silenced is tightly controlled. Recent data converge on the conclusion that *de novo* recruitment of PRC2 to 'nucleation sites' is frequently genetically encoded.

PRE mode of recruitment

First observed in drosophila, multiple transcription factors (TFs) jointly recruit Polycomb complexes to *cis* motifs found in small genomic regions (~600 bp in size) called **Polycomb response elements** (PREs) [64]. Loss of the *cis* motifs or *trans* factors involved causes a significant reduction of H3K27me3 levels and PcG protein binding in canonical **Polycomb domains** [65]. Recently, PREs were also shown to globally contribute to PRC2 recruitment in arabidopsis. Two key *cis* motifs and associated TFs were identified: a GA repeat bound by class I BPC TFs and the **telobox** (AAACCCTA) bound by a family of zinc finger TFs. Members of both TF families colocalize with PRC2 at thousands of loci and their loss of/reduction in function causes morphological and molecular phenotypes typical of Polycomb mutants, as well as widespread loss of PRC2 binding [66] (Figure 4A). Moreover, the combined *cis* motifs were necessary and sufficient for PRE activity [66]. Of note, GA repeats are also one of the functionally important motifs of PREs in drosophila [64]. The single telobox motif in arabidopsis PREs is recognized by a second class of proteins, telomere repeat-binding factors (TRBs), which recruit PRC2 and may represent PRC2 accessory proteins [33,34] (Figure 4A).

In addition, RY *cis* motifs (TGCATG) have also been linked to PRC2 recruitment during vernalization-triggered silencing of the flowering repressor *FLC* and silencing of the seed program during germination (reviewed in [8]; see also [67,68]). The RY motif is bound by the B3 TFs VAL1 and VAL2, also featuring an H3K27me3-specific PHD domain. VAL1 and VAL2 were proposed to interact with PRC2 core and accessory subunits [67,69]. Whether VAL1 and VAL2 are PRC2 accessory subunits is unclear, because published AP-MS data instead revealed copurification with subunits of PRC1 and histone deacetylase complexes [70]. Nevertheless, *val1 val2* double mutants phenocopy *clf swn* mutants and trigger a reduction in SWN occupancy at VAL/SWN co-occupied genomic regions [71]. Interestingly, the VAL1/VAL2 PRC2 targets are largely

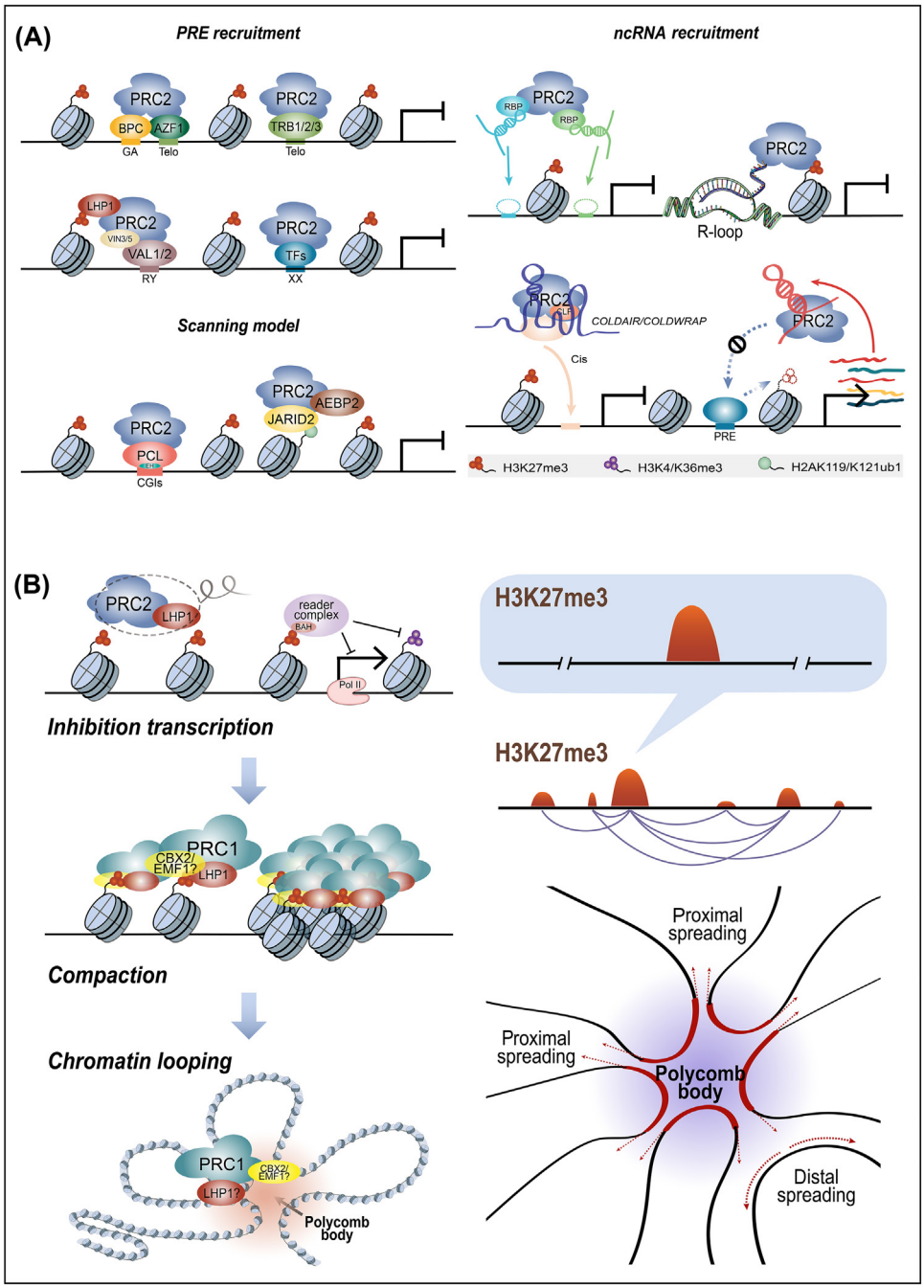

Figure 4. Recruitment of Polycomb repressive complex 2 (PRC2) to Polycomb targets and PRC2/H3K27me3-mediated silencing. (A) Top left: the Polycomb response element (PRE) and transcription factor-mediated recruitment of PRC2 first described in drosophila [64] is also found in arabidopsis *(Arabidopsis thaliana)*. GA repeats (the telobox in arabidopsis) are bound by transcription factors, which directly interact with, and recruit, PRC2 to targets [34,66]. Below: other motifs that are also likely to directly or indirectly contribute to PRC2 recruitment [71]. Bottom left: the chromatin-scanning mode of PRC2 recruitment in mammals. PRC2 associates with unmethylated CG-rich promoter proximal DNA via PCL and with H2AK119ub via the JARID2 accessory complex components. Additional interactions further stabilize the

(Figure legend continued at the bottom of the next page.)

independent of those of class I BPC and zinc finger TFs and those of TRB1–3 [71] (Figure 4A). Together, these three PRE recruitment modules account for approximately two-thirds of global H3K27me3 peaks [34,66,71].

The scanning mode of recruitment

In mammals, a 'scanning model' instead underlies PRC2 genome targeting to unmethylated promoter proximal **CpG islands (CGIs)** via core and accessory PRC2 components (reviewed in [5,54]). This mode differs from that described earlier in that no recurrent *cis* motifs or sequence-specific binding proteins are involved; instead, PRC2 residence time is enhanced by a combination of factors that includes presence of CG repeats or G/A tracks, DNA conformation, DNA methylation status, histone occupancy, or histone modification status (reviewed in [54]) (Figure 4A). Both core and PCL-containing PRC2 prefer binding to naked DNA, as often found at PREs and CGIs (reviewed in [13,54]). The N-terminal region of SUZ12 forms a PRC2 recruitment module together with accessory PRC2 complex subunits [13,56]. The PCL accessory components of PRC2.1 recognize unmethylated CpG repeats via their extended homologous (EH) region, which folds into a non-canonical winged-helix structure [72]. PCL-mediated PRC2 recruitment to unmethylated CGIs is enhanced in the context of reduced helical twist [73]. PCLs also enhance PRC2 residence time on nucleosomal templates [74].

That PREs and unmethylated CGIs are necessary and sufficient for recruitment of PRC2 is underscored by loss- and gain-of-function approaches. Deletion of these DNA elements led to loss of H3K27me3 specifically in dividing cells, suggesting a role in PRC2 recruitment and re-establishment of silencing after replication; while their ectopic insertion into the genome triggered PRC2 binding and silencing in flies, plants, and mammals ([34,66,75]; reviewed in [54]). It is tempting to speculate that the scanning mode of recruitment may be better suited to larger genomes, given that the PRE-TF mode has thus far been observed in organisms with very compact genomes.

Finally, H2AK119ub, deposited by noncanonical PRC1, promotes PRC2 recruitment via the JARID2 accessory subunit (as well as PRC2 activity via the AEBP accessory subunit) of PRC2.2 ([15,75]; reviewed in [76]). In a recent structure of human PRC2.2, JARID2 binds H2AK119ub and the acidic patch of the H2A-H2B histone dimer through its N-terminal ubiquitin-interacting motif (UIM) and a second motif rich in positively charged residues [15]. In arabidopsis, H2AK121ub marks the majority of PRC2 targets [77].

Role of ncRNA in recruitment?

While noncoding (nc)RNA may be linked to PRC2 recruitment in animals and plants, this is not always mediated via direct RNA/PRC2 interaction or of functional importance, as recently shown for the *XIST* and *HOTAIR* **long (l)ncRNAs** (reviewed in [78]). Yet, perturbation of the RNA-PRC2 interaction suggests a role for RNA in PRC2 targeting in human pluripotent stem cells [79]. PRC2 has a strong affinity for RNA and interactions with diverse RNAs likely modulate activity of this complex in as many diverse ways as protein–PRC2 interactions do. In particular, inactivation of PRC2 activity by RNA is becoming well established; this includes nascent RNA

complex on chromatin [5,54]. Right: the role of noncoding (nc)RNA in PRC2 recruitment and regulation (bottom). RNAs may indirectly [via RNA-binding proteins (RBPs)] recruit PRC2 and RNA/DNA hybrids (**R-loops**) target PRC2 to promoters [82], while the long (l)ncRNAs *COLDAIR* and *COLDWRAP* recruit PRC2 to *FLC* in *cis* [79,83]. Finally, RNA functions as a decoy to compete with PRC2 binding to DNA [121]. (B) Gene silencing mediated by PRCs/H3K27me3. H3K27me3 promotes silencing by recruiting reader proteins, such as LHP1 or BAH domain proteins, that inhibit transcription via inactivation of Pol II [28–30,90,92]. These readers and additional PRC1 components direct local chromatin compaction, formation of short- or long-range chromatin loops, as well as phase-separated Polycomb bodies [95,122].

from actively transcribed loci, as well as RNA competition with DNA binding of this complex ([60,80]; reviewed in [78]). Recently, promoter proximal RNA/DNA loops (R loops) were shown to tether PRC2 to promoter proximal sites and to regulate PRC2 activity [81,82] (Figure 4A).

In plants, some lncRNAs generated from the *FLC* locus have been implicated in recruitment of PRC2 (reviewed in [83]). The antisense *COOLAIR* lncRNA does not directly interact with PRC2 and is not required for initial PRC2 recruitment and **H3K27me3 nucleation** [84]. More recently, PRC2 was linked to *COOLAIR* via the intermediary RNA-binding protein FCA [85]. Two additional lncRNAs have been described at *FLC*: the intronic lncRNA *COLDAIR* and *COLDWRAP,* which originates 225 bp upstream of the *FLC* transcription start site. These lncRNA are induced by vernalization, and point mutations in each lead to reduced PRC2 occupancy and H3K27me3 during nucleation, suggesting a role in recruitment [86,87].

PRC2/H3K27me3-mediated silencing

Spreading of H3K27me3 and inheritance of silencing

After PRC2 recruitment to nucleation sites and initial deposition of H3K27me3, H3K27 methylation spreads to cover entire genes (arabidopsis) or larger Polycomb domains that include multiple genes (mammals), supported by the 'write and read' capability of PRC2 core subunits and accessory proteins and allosteric stimulation by dense nucleosome templates (reviewed in [5,7,14]). In both animals and plants, this requires histone turnover during replication [46,88], in agreement with the idea that replication-based parental histone dilution represents a window of opportunity for faithful inheritance of the chromatin state or for overturning it [7,89]. In plants, the H3K27me3 reader LHP1 has been linked to the spreading of H3H27me3 ([90]; reviewed in [7]; Figure 4B). Whether the BAH domain proteins EBS and SHL, which act in parallel with LHP1 [28–30], contribute to this process remains to be determined. Tethering revealed the CBX7 reader of the canonical PRC1 complex in mammals to contribute to inheritance of the silent state [91].

H3K27me3 readout and phase separation-mediated chromatin compaction

How PRC2-derived H3K27me3 directs transcriptional silencing is just beginning to be unraveled. Importantly, H3K27me3 recruits 'readers' or 'effectors', such as BAH domain-containing proteins in plants and animals and the canonical PRC1 complex in animals (reviewed in [14]). These effectors in turn engage in diverse activities that include blocking transcription, formation of local and long-range **chromatin loops**, as well as nuclear condensates that include small protein clusters and phase-separated **Polycomb bodies** (Figure 4B) [14]. Accumulating evidence suggests that some of these processes are linked.

For example, a complex containing a BAH and a PHD domain protein recruited to H3K27me3 in the context of unmodified H3K4 was described in plants that directs transcriptional repression via RNA polymerase dephosphorylation [92]. Likewise, a BAH H3K27me3 reader was identified in mammals (BAHCC1) that is required for gene silencing via recruitment of histone deacetylases and co-repressors [93]. In addition, H3K27me3 recruits Polycomb or CBX reader proteins in drosophila or mammals, respectively, that are part of the canonical PRC1 complex. The canonical PRC1 complex also contains Pleiohomeotic [Ph (drosophila)] and PHC1/2 (mammals). In drosophila, canonical PRC1 mediates gene repression via chromatin loops and long-range interaction that depend on protein clusters and liquid–liquid phase separation (LLPS) [94–97]. Of importance for this chromatin condensation is the STERILE ALPHA MOTIF SAM oligomerization domain of drosophila Ph [96,97]. In mammals, charged residues in the intrinsically disordered region of the H3K27me3 reader CBX2 have instead been linked to LLPS, as well as to chromatin compaction *in vitro* and the ability to silence critical target genes *in vivo* [98–100]. In addition, the SAM domain of Ph homolog PHC1/2 contributes to chromatin loop formation in ESCs in

mammals, where H3K27me3 demarcates anchors of chromatin loops [75,101,102]. It is not yet clear whether PHC1/2 can undergo LLPS in ESCs, in which CBX2 is not expressed.

Plants do not have homologs of Polycomb or Ph, but do form chromatin loops the anchors of which are enriched in H3K27me3 [103]. In arabidopsis, the H3K27me3 reader LHP1 is not only implicated in local loops that generate polycomb domains, but also links to both PRC1 and PRC2 [90,104,105]. Of note, the intrinsically disordered hinge region of LHP1, similar to that of the related H3K9me reader Heterochromatin Protein 1 in animals, is required for formation of subnuclear foci that resemble Polycomb bodies and arise via LLPS [106–109]. The PRC2 accessory factor EMF1 also links to PRC1 in arabidopsis [33,110] and shows structural and functional similarities with drosophila PSC, at least *in vitro* ([111]; reviewed in [27]; Figure 4B). A 500-amino acid region in the center of EMF1 is required for formation of Polycomb bodies [112]. More recently, EMF1 was implicated in forming a PRC1 complex together with the BAH domain H3K27me3 reader EBS [28].

Concluding remarks

In summary, the combined recent findings, despite notable differences, suggest remarkable functional and mechanistic conservation of PRC2 in animals and plants. Recent structural, genetic, and genomic investigations have increased our understanding of the regulation of PRC2 enzymatic activity and recruitment, and have begun to shed light on steps that lead from H3K27me3-marked domains to compacted and transcriptionally silent chromatin. However, many questions remain (see Outstanding questions). A key frontier is the identification of novel PRC2 reader complexes and their activities, as well as the role of Polycomb protein clusters and larger Polycomb bodies in alteration of chromatin topology and silencing. Likewise, how tissue and condition-specific PRC2 activity is triggered and leads to gene silencing are active areas of investigation.

Outstanding questions

What is the structure and subunit composition of different types of PRC2 holo-complexes in plants and do they have unique biochemical activities?

Are there canonical PRC1-like activities in plants and what proteins and complexes execute these activities?

What is the role of Polycomb complex components in regulating 3D chromatin structure, especially in plant species with larger genome, such as rice, maize, and soybean?

Does the mode of PRC2 recruitment in plant species with larger genomes differ from that of arabidopsis?

Acknowledgments

Work on Polycomb is funded by National Science Foundation grants MCB 1916431 and IOS PGRP 1953279 to D.W. and an NSFC- 31970529 grant to J.X.

Declaration of interests

None declared by authors.

References

1. Kornberg, R.D. and Lorch, Y. (1999) Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell* 98, 285–294
2. Luger, K. *et al.* (2012) New insights into nucleosome and chromatin structure: an ordered state or a disordered affair? *Nat. Rev. Mol. Cell Biol.* 13, 436–447
3. Elgin, S.C. and Reuter, G. (2013) Position-effect variegation, heterochromatin formation, and gene silencing in *Drosophila*. *Cold Spring Harb. Perspect. Biol.* 5, a017780
4. Simon, J.A. and Kingston, R.E. (2009) Mechanisms of polycomb gene silencing: knowns and unknowns. *Nat. Rev. Mol. Cell Biol.* 10, 697–708
5. Yu, J.R. *et al.* (2019) PRC2 is high maintenance. *Genes Dev.* 33, 903–935
6. Illingworth, R.S. (2019) Chromatin folding and nuclear architecture: PRC1 function in 3D. *Curr. Opin. Genet. Dev.* 55, 82–90
7. Hugues, A. *et al.* (2020) Mitotic inheritance of PRC2-mediated silencing: mechanistic insights and developmental perspectives. *Front. Plant Sci.* 11, 262
8. Costa, S. and Dean, C. (2019) Storing memories: the distinct phases of Polycomb-mediated silencing of *Arabidopsis* FLC. *Biochem. Soc. Trans.* 47, 1187–1196
9. Gao, Z. *et al.* (2012) PCGF homologs, CBX proteins, and RYBP define functionally distinct PRC1 family complexes. *Mol. Cell* 45, 344–356
10. Tavares, L. *et al.* (2012) RYBP-PRC1 complexes mediate H2A ubiquitylation at polycomb target sites independently of PRC2 and H3K27me3. *Cell* 148, 664–678
11. Piunti, A. and Shilatifard, A. (2021) The roles of Polycomb repressive complexes in mammalian development and cancer. *Nat. Rev. Mol. Cell Biol.* 22, 326–345
12. Wang, Q. and Shen, W.H. (2018) Chromatin modulation and gene regulation in plants: insight about PRC1 function. *Biochem. Soc. Trans.* 46, 957–966
13. Glancy, E. *et al.* (2020) Structural basis for PRC2 engagement with chromatin. *Curr. Opin. Struct. Biol.* 67, 135–144
14. Guo, Y. *et al.* (2021) Polycomb gene silencing mechanisms: PRC2 chromatin targeting, H3K27me3 'readout', and phase separation-based compaction. *Trends Genet.* Published online January 22, 2021. https://doi.org/10.1016/j.tig.2020.12.006
15. Kasinath, V. *et al.* (2021) JARID2 and AEBP2 regulate PRC2 in the presence of H2AK119ub1 and other histone modifications. *Science* 371, eabc3393

16. Son, J. *et al.* (2013) Nucleosome-binding activities within JARID2 and EZH1 regulate the function of PRC2 on chromatin. *Genes Dev.* 27, 2663–2677
17. Lee, C.H. *et al.* (2018) Distinct stimulatory mechanisms regulate the catalytic activity of polycomb repressive complex 2. *Mol. Cell* 70, 435–448
18. Grau, D. *et al.* (2021) Structures of monomeric and dimeric PRC2:EZH1 reveal flexible modules involved in chromatin compaction. *Nat. Commun.* 12, 1–12
19. Derkacheva, M. and Hennig, L. (2014) Variations on a theme: Polycomb group proteins in plants. *J. Exp. Bot.* 65, 2769–2784
20. Jeong, C.W. *et al.* (2011) An E3 ligase complex regulates SET-domain polycomb group protein activity in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* 108, 8036–8041
21. Figueiredo, D.D. *et al.* (2016) Auxin production in the endosperm drives seed coat development in *Arabidopsis*. *Elife* 5, e20542
22. Oliva, M. *et al.* (2016) FIE, a nuclear PRC2 protein, forms cytoplasmic complexes in *Arabidopsis thaliana*. *J. Exp. Bot.* 67, 6111–6123
23. Dotto, M. *et al.* (2018) UV-B radiation delays flowering time through changes in the PRC2 complex activity and miR156 levels in *Arabidopsis thaliana*. *Plant Cell Environ.* 41, 1394–1406
24. Roy, S. *et al.* (2018) The Polycomb-group repressor MEDEA attenuates pathogen defense. *Plant Physiol.* 177, 1728–1742
25. Gibbs, D.J. *et al.* (2018) Oxygen-dependent proteolysis regulates the stability of angiosperm polycomb repressive complex 2 subunit VERNALIZATION 2. *Nat. Commun.* 9, 1–11
26. Shu, J. *et al.* (2019) Genome-wide occupancy of histone H3K27 methyltransferases CURLY LEAF and SWINGER in *Arabidopsis* seedlings. *Plant Direct* 3, e00100
27. Merini, W. and Calonje, M. (2015) PRC1 is taking the lead in PcG repression. *Plant J.* 83, 110–120
28. Li, Z. *et al.* (2018) Polycomb-mediated gene silencing by the BAH-EMF1 complex in plants. *Nat. Genet.* 50, 1254–1261
29. Yang, Z. *et al.* (2018) EBS is a bivalent histone reader that regulates floral phase transition in Arabidopsis. *Nat. Genet.* 50, 1247–1253
30. Qian, S. *et al.* (2018) Dual recognition of H3K4me3 and H3K27me3 by a plant histone reader SHL. *Nat. Commun.* 9, 1–11
31. Kim, D.H. and Sung, S. (2013) Coordination of the vernalization response through a VIN3 and FLC gene family regulatory network in *Arabidopsis*. *Plant Cell* 25, 454–469
32. Hohenstatt, M.L. *et al.* (2018) PWWP-DOMAIN INTERACTOR OF POLYCOMBS1 Interacts with Polycomb-group proteins and histones and regulates *Arabidopsis* flowering and development. *Plant Cell* 30, 117–133
33. Bloomer, R.H. *et al.* (2020) The *Arabidopsis* epigenetic regulator ICU11 as an accessory protein of Polycomb Repressive Complex 2. *Proc. Natl. Acad. Sci. U. S. A.* 117, 16660–16666
34. Zhou, Y. *et al.* (2018) Telobox motifs recruit CLF/SWN-PRC2 for H3K27me3 deposition via TRB factors in Arabidopsis. *Nat. Genet.* 50, 638–644
35. Liang, S.C. *et al.* (2015) Kicking against the PRCs - a domesticated transposase antagonises silencing mediated by Polycomb group proteins and is an accessory component of Polycomb Repressive Complex 2. *PLoS Genet.* 11, e1005660
36. Velanis, C.N. *et al.* (2020) The domesticated transposase ALP2 mediates formation of a novel Polycomb protein complex by direct interaction with MSI1, a core subunit of Polycomb Repressive Complex 2 (PRC2). *PLoS Genet.* 16, e1008681
37. Hauri, S. *et al.* (2016) A high-density map for navigating the human Polycomb complexome. *Cell Rep.* 17, 583–595
38. Zhang, Q. *et al.* (2020) Convergent evolution between PALI1 and JARID2 for the allosteric activation of PRC2. *bioRxiv* Published online May 9., 2020. https://doi.org/10.1101/2020.05.28.122556
39. Healy, E. *et al.* (2019) PRC2.1 and PRC2.2 synergize to coordinate H3K27 trimethylation. *Mol. Cell* 76, 437–452
40. van Mierlo, G. *et al.* (2019) The complexity of PRC2 subcomplexes. *Trends Cell Biol.* 29, 660–671
41. Petracovici, A. and Bonasio, R. (2021) Distinct PRC2 subunits regulate maintenance and establishment of Polycomb repression during differentiation. *Mol. Cell* 81, 2625–2639
42. Yang, Y. and Li, G. (2020) Post-translational modifications of PRC2: signals directing its activity. *Epigenetics Chromatin* 13, 1–16
43. Lee, C.H. *et al.* (2019) Automethylation of PRC2 promotes H3K27 methylation and is impaired in H3K27M pediatric glioma. *Genes Dev.* 33, 1428–1440
44. Wang, X. *et al.* (2019) Regulation of histone methylation by automethylation of PRC2. *Genes Dev.* 33, 1416–1427
45. Jacob, Y. *et al.* (2014) Selective methylation of histone H3 variant H3.1 regulates heterochromatin replication. *Science* 343, 1249–1253
46. Jiang, D. and Berger, F. (2017) DNA replication-coupled histone modification maintains Polycomb gene silencing in plants. *Science* 357, 1146–1149
47. Armache, A. *et al.* (2020) Histone H3.3 phosphorylation amplifies stimulation-induced transcription. *Nature* 583, 852–857
48. Borg, M. *et al.* (2020) Targeted reprogramming of H3K27me3 resets epigenetic memory in plant paternal chromatin. *Nat. Cell Biol.* 22, 621–629
49. Yan, A. *et al.* (2020) The atypical histone variant H3.15 promotes callus formation in *Arabidopsis thaliana*. *Development* 147, dev184895
50. Schmitges, F.W. *et al.* (2011) Histone methylation by PRC2 is inhibited by active chromatin marks. *Mol. Cell* 42, 330–341
51. Yang, H. *et al.* (2014) Antagonistic roles for H3K36me3 and H3K27me3 in the cold-induced epigenetic switch at *Arabidopsis* FLC. *Curr. Biol.* 24, 1793–1797
52. Doyle, M.R. and Amasino, R.M. (2009) A single amino acid change in the enhancer of zeste ortholog CURLY LEAF results in vernalization-independent, rapid flowering in *Arabidopsis*. *Plant Physiol.* 151, 1688–1697
53. Jani, K.S. *et al.* (2019) Histone H3 tail binds a unique sensing pocket in EZH2 to activate the PRC2 methyltransferase. *Proc. Natl. Acad. Sci. U. S. A.* 116, 8295–8300
54. Laugesen, A. *et al.* (2019) Molecular mechanisms directing PRC2 recruitment and H3K27 methylation. *Mol. Cell* 74, 8–18
55. Jiao, L. and Liu, X. (2015) Structural basis of histone H3K27 trimethylation by an active polycomb repressive complex 2. *Science* 350, aac4383
56. Kasinath, V. *et al.* (2018) Structures of human PRC2 with its cofactors AEBP2 and JARID2. *Science* 359, 940–944
57. Chammas, P. *et al.* (2020) Engaging chromatin: PRC2 structure meets function. *Br. J. Cancer* 122, 315–328
58. Sanulli, S. *et al.* (2015) Jarid2 methylation via the PRC2 complex regulates H3K27me3 deposition during cell differentiation. *Mol. Cell* 57, 769–783
59. Lee, C.H. *et al.* (2018) Allosteric activation dictates PRC2 activity independent of its recruitment to chromatin. *Mol. Cell* 70, 422–434
60. Zhang, Q. *et al.* (2019) RNA exploits an exposed regulatory site to inhibit the enzymatic activity of PRC2. *Nat. Struct. Mol. Biol.* 26, 237–247
61. Jain, S.U. *et al.* (2020) H3 K27M and EZHIP impede H3K27-methylation spreading by inhibiting allosterically stimulated PRC2. *Mol. Cell* 80, 726–735
62. Ragazzini, R. *et al.* (2019) EZHIP constrains Polycomb Repressive Complex 2 activity in germ cells. *Nat. Commun.* 10, 1–18
63. Piunti, A. *et al.* (2019) CATACOMB: an endogenous inducible gene that antagonizes H3K27 methylation activity of Polycomb repressive complex 2 via an H3K27M-like mechanism. *Sci. Adv.* 5, eaax2887
64. Kassis, J.A. and Brown, J.L. (2013) Polycomb group response elements in *Drosophila* and vertebrates. *Adv. Genet.* 81, 83–118
65. Brown, J.L. *et al.* (2018) Global changes of H3K27me3 domains and Polycomb group protein distribution in the absence of recruiters Spps or Pho. *Proc. Natl. Acad. Sci. U. S. A.* 115, E1839–E1848
66. Xiao, J. *et al.* (2017) Cis and trans determinants of epigenetic silencing by Polycomb repressive complex 2 in *Arabidopsis*. *Nat. Genet.* 49, 1546–1552
67. Chen, N. *et al.* (2018) HSI2/VAL1 silences AGL15 to regulate the developmental transition from seed maturation to vegetative growth in *Arabidopsis*. *Plant Cell* 30, 600–619
68. Jing, Y. *et al.* (2019) The B3-domain transcription factor VAL1 regulates the floral transition by repressing FLOWERING LOCUS T. *Plant Physiol.* 181, 236–248

69. Yuan, W. *et al.* (2016) A cis cold memory element and a trans epigenome reader mediate Polycomb silencing of FLC by vernalization in *Arabidopsis*. *Nat. Genet.* 48, 1527–1534
70. Qüesta, J.I. *et al.* (2016) *Arabidopsis* transcriptional repressor VAL1 triggers Polycomb silencing at FLC during vernalization. *Science* 353, 485–488
71. Yuan, L. *et al.* (2021) The transcriptional repressors VAL1 and VAL2 recruit PRC2 for genome-wide Polycomb silencing in *Arabidopsis*. *Nucleic Acids Res.* 49, 98–113
72. Li, H. *et al.* (2017) Polycomb-like proteins link the PRC2 complex to CpG islands. *Nature* 549, 287–291
73. Perino, M. *et al.* (2018) MTF2 recruits Polycomb Repressive Complex 2 by helical-shape-selective DNA binding. *Nat. Genet.* 50, 1002–1010
74. Choi, J. *et al.* (2017) DNA binding by PHF1 prolongs PRC2 residence time on chromatin and thereby promotes H3K27 methylation. *Nat. Struct. Mol. Biol.* 24, 1039–1047
75. Oksuz, O. *et al.* (2018) Capturing the onset of PRC2-mediated repressive domain formation. *Mol. Cell* 70, 1149–1162
76. Barbour, H. *et al.* (2020) Polycomb group-mediated histone H2A monoubiquitination in epigenome regulation and nuclear processes. *Nat. Commun.* 11, 1–16
77. Zhou, Y. *et al.* (2017) H2A monoubiquitination in *Arabidopsis thaliana* is generally independent of LHP1 and PRC2 activity. *Genome Biol.* 18, 1–13
78. Almeida, M. *et al.* (2020) The many faces of Polycomb regulation by RNA. *Curr. Opin. Genet. Dev.* 61, 53–61
79. Long, Y. *et al.* (2020) RNA is essential for PRC2 chromatin occupancy and function in human pluripotent stem cells. *Nat. Genet.* 52, 931–938
80. Beltran, M. *et al.* (2019) G-tract RNA removes Polycomb repressive complex 2 from genes. *Nat. Struct. Mol. Biol.* 26, 899–909
81. Skourti-Stathaki, K. *et al.* (2019) R-Loops enhance polycomb repression at a subset of developmental regulator genes. *Mol. Cell* 73, 930–945
82. Alecki, C. *et al.* (2020) RNA-DNA strand exchange by the *Drosophila* Polycomb complex PRC2. *Nat. Commun.* 11, 1–14
83. Chen, L. *et al.* (2020) Long non-coding RNAs in plants: emerging modulators of gene activity in development and stress responses. *Planta* 252, 1–14
84. Csorba, T. *et al.* (2014) Antisense COOLAIR mediates the coordinated switching of chromatin states at FLC during vernalization. *Proc. Natl. Acad. Sci. U. S. A.* 111, 16160–16165
85. Tian, Y. *et al.* (2019) PRC2 recruitment and H3K27me3 deposition at *FLC* require FCA binding of *COOLAIR*. *Sci. Adv.* 5, eaau7246
86. Kim, D.H. *et al.* (2017) Modular function of long noncoding RNA, COLDAIR, in the vernalization response. *PLoS Genet.* 13, e1006939
87. Kim, D.H. and Sung, S. (2017) Vernalization-triggered intragenic chromatin loop formation by long noncoding RNAs. *Dev. Cell* 40, 302–312
88. Chory, E.J. *et al.* (2019) Nucleosome turnover regulates histone methylation patterns over the genome. *Mol. Cell* 73, 61–72
89. Stewart-Morgan, K.R. *et al.* (2020) Chromatin replication and epigenetic cell memory. *Nat. Cell Biol.* 22, 361–371
90. Veluchamy, A. *et al.* (2016) LHP1 Regulates H3K27me3 spreading and shapes the three-dimensional conformation of the *Arabidopsis* genome. *PLoS ONE* 11, e0158936
91. Moussa, H.F. *et al.* (2019) Canonical PRC1 controls sequence-independent propagation of Polycomb-mediated gene silencing. *Nat. Commun.* 10, 1–12
92. Zhang, Y.Z. *et al.* (2020) Coupling of H3K27me3 recognition with transcriptional repression through the BAH-PHD-CPL2 complex in *Arabidopsis*. *Nat. Commun.* 11, 1–16
93. Fan, H. *et al.* (2020) BAHCC1 binds H3K27me3 via a conserved BAH module to mediate gene silencing and oncogenesis. *Nat. Genet.* 52, 1384–1396
94. Cheutin, T. and Cavalli, G. (2018) Loss of PRC1 induces higher-order opening of Hox loci independently of transcription during *Drosophila* embryogenesis. *Nat. Commun.* 9, 1–11
95. Ogiyama, Y. *et al.* (2018) Polycomb-dependent chromatin looping contributes to gene silencing during *Drosophila* development. *Mol. Cell* 71, 73–88
96. Seif, E. *et al.* (2020) Phase separation by the polyhomeotic sterile alpha motif compartmentalizes Polycomb Group proteins and enhances their activity. *Nat. Commun.* 11, 1–19
97. Wani, A.H. *et al.* (2016) Chromatin topology is coupled to Polycomb group protein subnuclear organization. *Nat. Commun.* 7, 1–13
98. Grau, D.J. *et al.* (2011) Compaction of chromatin by diverse Polycomb group proteins requires localized regions of high charge. *Genes Dev.* 25, 2210–2221
99. Lau, M.S. *et al.* (2017) Mutation of a nucleosome compaction region disrupts Polycomb-mediated axial patterning. *Science* 355, 1081–1084
100. Plys, A.J. *et al.* (2019) Phase separation of Polycomb-repressive complex 1 is governed by a charged disordered region of CBX2. *Genes Dev.* 33, 799–813
101. Kundu, S. *et al.* (2017) Polycomb Repressive Complex 1 generates discrete compacted domains that change during differentiation. *Mol. Cell* 65, 432–446
102. Isono, K. *et al.* (2013) SAM domain polymerization links subnuclear clustering of PRC1 to gene silencing. *Dev. Cell* 26, 565–577
103. Liu, C. *et al.* (2016) Genome-wide analysis of chromatin packing in *Arabidopsis thaliana* at single-gene resolution. *Genome Res.* 26, 1057–1068
104. Xu, L. and Shen, W.H. (2008) Polycomb silencing of KNOX genes confines shoot stem cell niches in *Arabidopsis*. *Curr. Biol.* 18, 1966–1971
105. Derkacheva, M. *et al.* (2013) *Arabidopsis* MSI1 connects LHP1 to PRC2 complexes. *EMBO J.* 32, 2073–2085
106. Keenen, M.M. *et al.* (2021) HP1 proteins compact DNA into mechanically and positionally stable phase separated domains. *Elife* 10, e64563
107. Larson, A.G. *et al.* (2017) Liquid droplet formation by HP1α suggests a role for phase separation in heterochromatin. *Nature* 547, 236–240
108. Berry, S. *et al.* (2017) Disruption of an RNA-binding hinge region abolishes LHP1-mediated epigenetic repression. *Genes Dev.* 31, 2115–2120
109. Strom, A.R. *et al.* (2017) Phase separation drives heterochromatin domain formation. *Nature* 547, 241–245
110. Wang, Y. *et al.* (2014) Photoperiodic control of the floral transition through a distinct polycomb repressive complex. *Dev. Cell* 28, 727–736
111. Beh, L.Y. *et al.* (2012) A core subunit of Polycomb repressive complex 1 is broadly conserved in function but not primary sequence. *Proc. Natl. Acad. Sci. U. S. A.* 109, E1063–E1071
112. Calonje, M. *et al.* (2008) EMBRYONIC FLOWER1 participates in polycomb group-mediated AG gene silencing in *Arabidopsis*. *Plant Cell* 20, 277–291
113. Mateo-Bonmatí, E. *et al.* (2018) INCURVATA11 and CUPULIFORMIS2 are redundant genes that encode epigenetic machinery components in *Arabidopsis*. *Plant Cell* 30, 1596–1616
114. Kim, S.Y. *et al.* (2012) EMF1 and PRC2 cooperate to repress key regulators of *Arabidopsis* development. *PLoS Genet.* 8, e1002512
115. De Lucia, F. *et al.* (2008) A PHD-polycomb repressive complex 2 triggers the epigenetic silencing of FLC during vernalization. *Proc. Natl. Acad. Sci. U. S. A.* 105, 16831–16836
116. Greb, T. *et al.* (2007) The PHD finger protein VRN5 functions in the epigenetic silencing of *Arabidopsis* FLC. *Curr. Biol.* 17, 73–78
117. Mikulski, P. *et al.* (2019) The chromatin-associated protein PWO1 interacts with plant nuclear lamin-like components to regulate nuclear size. *Plant Cell* 31, 1141–1154
118. Whitcomb, S.J. *et al.* (2007) Polycomb Group proteins: an evolutionary perspective. *Trends Genet.* 23, 494–502
119. Goodrich, J. *et al.* (1997) A Polycomb-group gene regulates homeotic gene expression in *Arabidopsis*. *Nature* 386, 44–51
120. Goddard, T.D. *et al.* (2018) UCSF ChimeraX: Meeting modern challenges in visualization and analysis. *Protein Sci.* 27, 14–25
121. Davidovich, C. *et al.* (2013) Promiscuous RNA binding by Polycomb repressive complex 2. *Nat. Struct. Mol. Biol.* 20, 1250–1257
122. Tatavosian, R. *et al.* (2019) Nuclear condensates of the Polycomb protein chromobox 2 (CBX2) assemble through phase separation. *J. Biol. Chem.* 294, 1451–1463