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Dynamic regulation of *Polycomb* group activity during plant development

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Polycomb group (PcG) complexes play important roles in phase transitions and cell fate determination in plants and animals, by epigenetically repressing sets of genes that promote either proliferation or differentiation. The continuous differentiation of new organs in plants, such as leaves or flowers, requires a highly dynamic PcG function, which can be induced, modulated, or repressed when necessary. In this review, we discuss the recent advance in understanding PcG function in plants and focus on the diverse molecular mechanisms that have been described to regulate and counteract PcG activity in *Arabidopsis*.

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Introduction

Polycomb group (PcG) proteins are major regulators of gene expression in both plants and animals. The highly conserved and well-characterized *Polycomb* Repressive Complex 2 (PRC2) represses gene expression in an epigenetic manner by catalyzing the trimethylation of histone H3 at lysine 27 (H3K27me3). Both in plants and animals, the complex consists of four core members, which together are sufficient to generate the H3K27me3 mark, associated with repressive chromatin, *in vitro* [1]. The *Drosophila* PRC2 complex contains the core subunits Enhancer of zeste [E(z)], a histone methyltransferase, Suppressor of zeste 12 [Su(z)12], a Zinc finger protein, and the WD40 domain proteins Extra sex combs (Esc) and Nucleosome remodeling factor 55 (Nurf55). While in *Drosophila* all but one subunit are encoded by a single gene [1,2], most of the *Arabidopsis* PRC2 core subunits are encoded by small gene families. *MEDEA* (*MEA*), *CURLY LEAF* (*CLF*) and *SWINGER* (*SWN*) are homologs of *E(z)*, *FERTILIZATION INDEPENDENT SEED2* (*FIS2*), *VERNALIZATION2* (*VRN2*) and *EMBRYONIC FLOWER2* (*EMF2*) are homologs of *Su(z)12*, while *MULTICOPY SUPPRESSOR OF IRA1-5* (*MSI1-5*) are the five homologs of *Nurf55*. In contrast to *Drosophila*, where *Esc* and *Esc-like* share this function [2], the *Esc* homolog *FERTILIZATION INDEPENDENT ENDOSPERM* (*FIE*) is single copy in the *Arabidopsis* genome [1,3,4]. PRC2 complexes of distinct flavor can be formed by combining these different subunits. The complexes EMF-PRC2, VRN-PRC2 and FIS-PRC2 have been confirmed *in planta* and have both overlapping and independent functions [5].

A second PcG complex, which only to some extent is conserved in plants and animals, is the *Polycomb* Repressive Complex 1 (PRC1). The core *Drosophila* PRC1 complex consists of the proteins Polycomb (Pc), Posterior sex combs (Psc), Polyhomeotic (Ph) and dRING1, and binds to the H3K27me3 histone mark generated by PRC2 [6]. PRC1 catalyses the monoubiquitination of histone H2A at lysine 119 (H2AK119ub), thereby compacting the chromatin further and stabilizing the repressed state [7]. Recently, RING-finger homologs able to catalyze H2AK119 monoubiquitination have been identified in plants, as well as other proteins with a PRC1-like function [4,8•]. The function of the PRC1-like proteins in plants is further discussed below.

In both the animal and plant kingdoms, PcG complexes play important roles in phase transitions during development, cell fate determination and cellular differentiation, by repressing sets of genes that regulate either proliferation or differentiation. In contrast to animals, where the entire body plan is formed during embryogenesis, plants differentiate organs, such as leaves, flowers or lateral roots, throughout their life span and maintain the ability to initiate new pools of stem cells. This requires highly dynamic PcG function, and the ability to induce, modulate, or repress PcG in response to developmental or environmental signals. In this review, we discuss the recent advance in our understanding of PcG function in plants and focus on the various molecular mechanisms that have been found to regulate and counteract PcG activity in *Arabidopsis*.

PRC2 plays a role in cell fate transitions throughout plant development

The three PRC2 complexes in *Arabidopsis*, FIS-PRC2, EMF-PRC2 and VRN-PRC2, play important roles during plant development. The first complex identified, FIS-PRC2, consisting of MEA, FIS2, FIE and MSI1, has a

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specific role in female gametophyte and seed development. While *FIE* and *MSI1* are broadly expressed and serve as subunits of all three PRC2 complexes, *MEA* and *FIS2* are exclusively maternally expressed in the female gametophyte and developing seed. FIS-PRC2 prevents endosperm formation in the absence of fertilization and represses endosperm and embryo proliferation after fertilization [3,9–12]. EMF-PRC2, consisting of SWN/CLF, EMF2, FIE and MSI1, and VRN-PRC2, containing SWN/CLF, VRN2, FIE and MSI1, both control aspects of sporophytic development. While EMF-PRC2 represses important floral regulators, such as *FLOWERING LOCUS T* (*FT*), *AGAMOUS* (*AG*) and *APETALA3* (*AP3*), the VRN-PRC2 complex promotes flowering after vernalization by silencing *FLOWERING LOCUS C* (*FLC*) [13,14]. However, *EMF2* and *VRN2* also have redundant functions, and the *emf2 vrn2* double mutant, like the *clf swn* double mutant, produces mainly undifferentiated cells [13], indicating that both PRC2 complexes have a major role in ensuring differentiation and repressing stem cell genes.

Indeed, recent publications disclosed additional functions for PRC2 complexes in the promotion of cellular differentiation. Detailed analyses of *clf* primary roots showed that these are larger than in the wild type. This appeared to be caused by the upregulation of the meristem identity genes *WUSCHEL RELATED HOMEOBOX5* (*WOX5*), *AGAMOUS-LIKE21* (*AGL21*) and *AGL42* in *clf* mutants, resulting in increased meristem activity [15]. A different study uncovered the importance of *CLF* for floral meristem determinacy. To terminate the floral meristem, the stem cell maintenance gene *WUSCHEL* (*WUS*) becomes repressed at flower stage 6 by AG. In the *ag* mutant, *WUS* expression is prolonged, resulting in an increased meristem size and additional whirls with floral organs. The *clf* mutation enhanced this phenotype, indicating that *CLF* is important for a timely termination of the floral meristem. Both in *ag* and *clf/swn* mutants, H3K27me3 levels were reduced at the *WUS* locus and *AG* and *CLF* were found to act in the same genetic pathway. AG probably plays an active role in recruiting the PcG complex to the *WUS* locus, since H3K27me3 levels increase throughout the *WUS* locus 2 hours after induction of a glucocorticoid-inducible *35S:AG-GR* transgene in the *ag* mutant background [16].

While EMF-PRC2 and VRN-PRC2 are required for normal sporophytic development, the FIS-PRC2 complex plays a role in female gametophyte and seed development. Seed development requires the coordinated development of the two fertilization products, embryo and endosperm, with the sporophytic integuments that develop into the seed coat. Interestingly, the interplay between the maternally derived integuments and the female gametophyte was found to require the action of both EMF/VRN-PRC2 and FIS-PRC2 upon fertilization [17••]. Seed coat development from the integuments occurs in *msi1* and *fie* seeds that develop in the absence of fertilization, but not in autonomous *mea* and *fis2* seeds. However, their development could be induced in autonomous *mea* and *fis2* seeds by introducing these mutations into the *vrn2*, *emf2* or *swn* mutant backgrounds, indicating that seed coat development is actively repressed by the sporophytic EMF-PRC2 and VRN-PRC2 complexes before fertilization. Release of this repression depends on the sexually produced endosperm, and the MADS box gene *AGL62*, which is itself repressed by FIS-PRC2 during seed development [17••,18,19].

Mutants of the *fis*-class are maternal effect embryo lethal and homozygous mutants cannot be obtained. This complicates the functional analysis of FIE and MSI1, which are part of several PRC2 complexes. However, in a recent report, this problem could be circumvented by fertilizing *fie/FIE* plants with pollen deficient for *FIE* and *CYCLIN-DEPENDENT KINASE1;A* [20]. Homozygous *fie* mutant seeds, like those of *clf swn* double mutants [13,20,21], showed delayed germination and displayed a progressive loss of cell differentiation after germination, eventually resulting in callus growth. A similar de-differentiation phenotype had previously been described for a weak *fie* allele [13,20,21]. These data show that PRC2 also plays an essential role in the embryo-to-seedling phase transition.

H3K27me3 deposition in the *Arabidopsis* genome is abundant and dynamic

The increasing use of high-throughput techniques over the past decade has contributed significantly to the understanding of the importance of H3K27me3 for gene repression in *Arabidopsis*. About 17% of the Arabidopsis genes were reported to be marked with H3K27me3, and these marks were, unlike in animals, largely restricted to individual genes [22]. This percentage was found to increase to 28% if both meristematic and differentiated tissues were taken into account [23]. A distinct proportion of H3K27me3 target genes was also found to be specific for either the shoot apical meristem, differentiated leaf cells, roots, or the endosperm [23–25], showing that the H3K27me3 deposition is dynamic. However, all these studies used the H3K27me3 antibody instead of PRC2-subunit-specific antibodies to identify PRC2 targets. The specificity of this antibody has been questioned, since it was found to cross-react also with H3K27me1 and, to a lesser extent, with H3K27me2 [20]. In addition, it is not clear whether CLF, SWN and MEA are the only methyltransferases that can deposit H3K27me3 marks. Nonetheless, more than 75% of the genes lost their H3K27me3 mark in the *fie* mutant, indicating that the majority of the loci identified with an H3K27me3 antibody is indeed a target of one of the PRC2 complexes [20].

The functional analyses of mutants affecting PRC2 and the high-throughput studies have unraveled the

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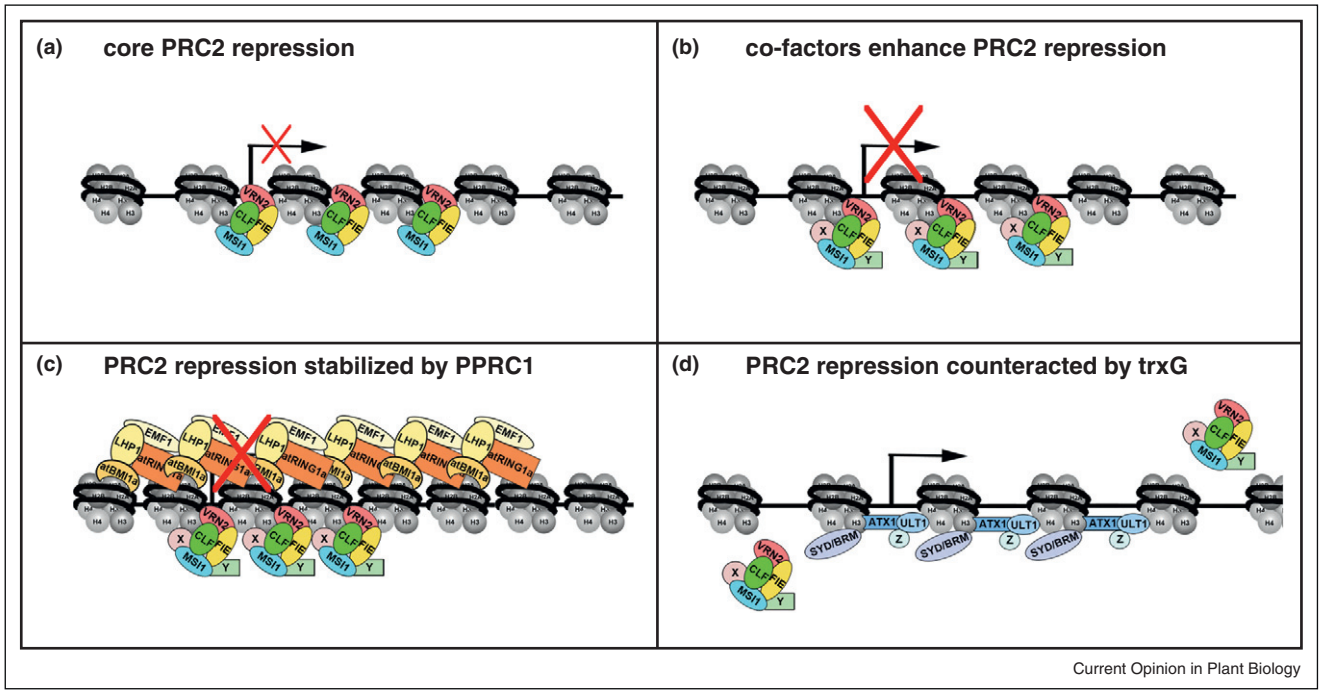
Figure 1



General mechanisms regulating the degree of PRC2 repression. **(a)** The core PRC2 complex (either VRN-PRC2, EMF-PRC2 or FIS-PRC2) represses target gene expression, but often does not abolish it completely. **(b)** Co-factors enhance the efficiency of PRC2, resulting in complete loss of target gene expression. **(c)** PRC2 repression can be stabilized by PPRC1, which further compacts the chromatin through H2Aub. This possibly allows target gene inhibition over long developmental time periods in a specific cell lineage. The composition of the PPRC1 complex(es) is still unclear; the figure shows a putative complex consisting of LHP1, EMF1, and the RING-finger homologs AtRING1a and AtBMI1a. **(d)** PRC2 repression can be released or counteracted by the action of trxG proteins. The figure depicts the putative release of PcG repression through the trxG protein SYD or BRM, which may remove the H3K27me3 mark, and the subsequent action of the ATX1–ULT1 complex, which deposits the H3K4me3 mark.

important roles that PRC2 complexes have in the promotion of differentiation throughout plant development. To allow the PcG proteins to play such essential roles, it is important that their activity is dynamically and tightly regulated. In the following paragraphs, we will discuss the recent advances in understanding how PRC2 repression can be stabilized, counteracted, or enhanced throughout the plant (summarized in Figure 1) or in a cell-type-specific manner (summarized in Figure 2).

PRC2 repression can be stabilized by PRC1-like complexes

In animals, the PRC1 complex is required to stabilize the silenced state of H3K27me3 marked loci through the monoubiquitination of H2A [1,6,26]. The existence of a similar PRC1 complex in plants is disputed, since only homologs of the RING-finger proteins RING1A/1B and Psc/BMI have been identified in *Arabidopsis*. However, double mutants for these homologs, *Atring1a|Atring1a* and *Atbmi1a|1b*, all displayed phenotypes similar to those in PRC2 mutants, and upregulation of genes marked by H3K27me3 [8•,27]. In addition, AtBMI1A/1B and the fifth RING-finger homolog, AtBMI1C, were shown to mediate H2A monoubiquitination *in planta* [8•,28]. Whether AtBMI1C, which was reported to be imprinted in the endosperm, also plays a role in H3K27me3 stabilization has not yet been elucidated [29]. The *Arabidopsis* RING-finger homologs have been shown to interact with each other, with the chromodomain protein LIKE HETEROCHROMATIN PROTEIN1 (LHP1), and the plant-specific protein EMF1. *lhp1* and *emf1* mutants show very early flowering, similar to *emf2* mutants, and an increased expression of H3K27me3 marked genes without loss of the H3K27me3 mark [30,31,32•]. In conclusion, plants also possess a PCR1-like complex (Plant PRC1 or PPRC1), which contains both plant-specific subunits and homologs of animal PRC1 proteins. Only a subset of PRC2 targets seems to be stabilized by PPRC1 however, and *AG* for example, is not upregulated in the *Atring1a|Atring1a* or *Atbmi1a|1b* double mutant [8•,33]. Likewise, in *Drosophila*, H2A monoubiquitination is only required for the repression of a subset of PRC2 target genes [7].

PcG and *trithorax* group proteins function antagonistically

Both in animals and plants, there are proteins that can counteract PcG action to release genes from H3K27me3-mediated repression. These PcG antagonists

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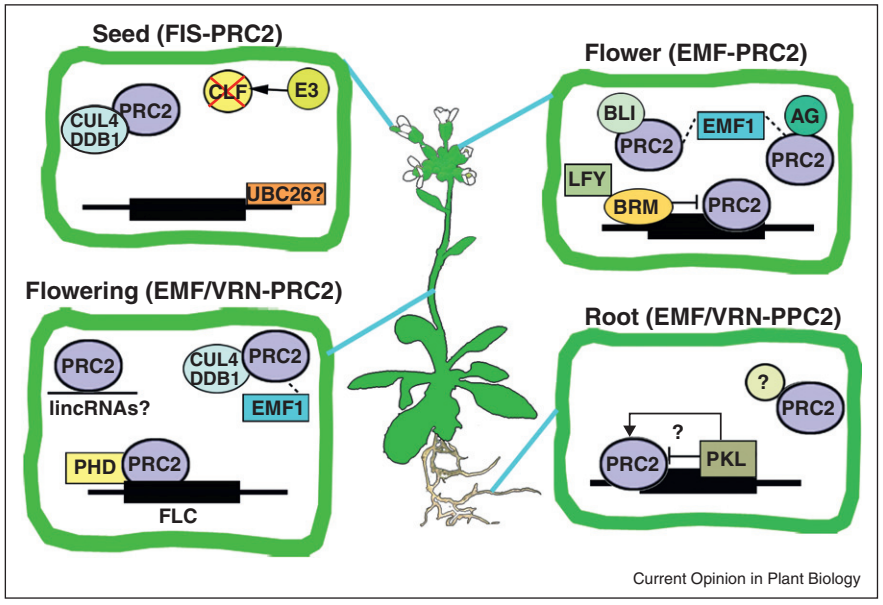
Figure 2



Cell-type specific regulation of PRC2 repression. Temporal and spatial control of PRC2 repression is achieved through association of the core PRC2 complex with cell-type specific co-factors. These co-factors can either recruit PRC2 to target loci or enhance the activity of PRC2 in a cell-type specific manner. The figure summarizes the current knowledge about co-factors that play a role in H3K27me3 deposition in different cell-types. Single proteins or protein complexes that were reported to associate with PRC2 are depicted as colored boxes or circles. Uncertain interactions are indicated with a question mark. The black box illustrates a PRC2 target gene.

are collectively referred to as *trithorax* group (trxG) proteins [4]. The first trxG protein identified in *Arabidopsis*, the histone methyltransferase ATX1, can trimethylate histone 3 at lysine 4 (H3K4me3) similar to its animal homologs. ATX1 is required to activate the floral homeotic genes that are repressed by CLF, probably in the context of EMF-PRC2 [34,35] and, together with ATX2, to activate the floral repressor *FLC*, a target of VRN-PRC2 [36]. Recently, it was revealed that ATX1 plays two distinct roles in transcriptional activation. First, ATX1 recruits the TATA binding protein (TBP) and RNA Polymerase II (Pol II) to the target gene promoter. Subsequently, ATX1 is recruited by a phosphorylated form of Pol II to the transcribed region, where it places the H3K4me3 marks, associated with active chromatin [37]. The SAND-domain DNA binding protein ULTRAPETALA1 (ULT1) was identified as a second trxG protein in *Arabidopsis*. Like ATX1, ULT1 activates the floral homeotic genes, and the *ult1* mutant can completely rescue the *clf* phenotype. It is probable that ULT1 acts in a complex with ATX1, because H3K4me3 deposition on the *AG* locus is also affected in the *ult1* mutant and ULT1 and ATX1 can interact *in vitro* [38].

The chromatin remodeling CHD3 protein PICKLE (PKL) was found as another important antagonist of PcG function. The *pkl* mutant could partly suppress the *clf* phenotype, and PKL affects H3K27me3 deposition. Interestingly, PKL and its homolog PICKLE RELATED2 (PKR2) were also found to be responsible for PcG activation in the roots, resulting in an indirect derepression of PcG targets, such as the embryonic regulators *LEAFY COTYLEDONS1* (*LEC1*) and *FUSCA3* (*FUS3*), in *pkl pkr2* roots [39]. However, a recent study reports H3K27me3 reduction in *pkl pkr2* germinating seeds without altered PcG gene expression, suggesting that PKL can also directly promote H3K27me3 mediated repression [40]. Thus, the role of PKL in the activation or repression of H3K27me3 marked genes might depend on the specific developmental or cellular context and its exact function will have to be elucidated in future studies. Recently, two other proteins with trxG activity have been identified. The SWI2/SNF2 chromatin remodeling ATPases SPLAYED (SYD) and BRAHMA (BRM) are recruited by LEAFY (LFY) and SEPALLATA 3 (SEP3) to the regulatory regions of *AP3* and *AG*, where they activate their expression at the right stage of flower development. This activation is accompanied by a reduction in H3K27me3 and an increase in H3K4me3. SYD and BRM possibly eject one or more nucleosomes to remove H3K27me3, and allow ATX1 and ULT1 access to the chromatin to deposit H3K4me3 [41]. Thus, trxG proteins promote the activation of PRC2 target genes in a stage-specific and cell type-specific manner.

The efficiency and specificity of PRC2 depends on higher order complex formation

In animals, the core PRC2 complex has only limited enzymatic activity *in vivo* and associates with various

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other factors that enhance the activity of the complex. The association of these factors can be transient or tissue-specific, thus allowing a dynamic increase or decrease in PRC2 activity [1]. There is increasing evidence that plant PRC2s depend in a similar way on the association with other proteins. Two recent reports reveal the significance of the CUL4-DDB1 E3 ubiquitin ligase complex for PRC2 activity in *Arabidopsis* [42••,43••]. CUL4-DDB1 was found to physically associate with the PRC2 subunit MSI1 to regulate the deposition of H3K27me3 in the female gametophyte and seed, where a lack of CUL4 activity leads to loss of imprinting at the *MEA* locus that is, partly, regulated by PRC2 [42••]. In addition, CUL4-DDB1 was reported to associate with PRC2 via MSI4 to control the transition to flowering. Silencing of *CUL4* induces early flowering and loss of H3K27me3 from both *FLC* and *FT* [43••]. However, loss of H3K27me3 in *cul4* is not as drastic as in *msi4*, and a *CUL4* knockdown has no global effect on H3K27me3 levels in *Arabidopsis*, suggesting that the association of CUL4-DDB1 with PRC2 is tissue-specific and not required for H3K27 trimethylation, but enhancing the efficiency of PRC2.

In addition to being required for PPRC1-catalyzed H2A ubiquitination, EMF1 was recently found to interact with MSI1 and to contribute to H3K27me3 deposition at a subset of PRC2 targets [32•]. Two groups of EMF1 targets were defined based on their dependency on EMF1 for H3K27 trimethylation. Group I genes required EMF1 for the deposition of H3K27me3, indicating that EMF1 acts before or as a member of PRC2 at these loci. Group II genes were marked with H3K27me3, but did not depend on EMF1 for this mark, suggesting that the repression of these genes is regulated via the PPRC1-function of EMF1. It is not yet clear how EMF1 participates in each PcG complex. A few other factors have been proposed to associate with PRC2 and to affect the catalysis of H3K27me3. These include AtUBP26, which probably deubiquitinates H2B at certain PRC2 target loci in the seed to enable trimethylation of H3K27 [44], and the plant-specific protein BLISTER (BLI), which interacts with CLF and represses a subset of PcG target genes [45].

However, the best-studied higher order complex is the PHD-PRC2 complex, required for the efficient and specific expression of *FLC* upon vernalization. A recent review comprehensively describes the coordinated silencing of *FLC* by PcG proteins [46], and we therefore discuss it only briefly here. The H3K27me3 mark is constitutively present at the *FLC* locus and does not increase upon vernalization. Instead, the increased repression of *FLC* depends on the PHD proteins VIN3, which is induced after prolonged cold, and VRN5, which only associates with PRC2 after cold. Only the complete PHD-PRC2 complex can silence *FLC* in an efficient and stable way [47].

Recruitment of PRC2 to specific target loci

To induce silencing of genes in particular cell-types only, PcG complexes have been found to be recruited to certain target loci by tissue-specific proteins or long noncoding RNAs (ncRNAs). The role of the latter in recruiting PRC1 has first been reported for X-chromosome inactivation in the mouse [48], but several long ncRNAs have subsequently been identified in mammals and *Drosophila* to also recruit PRC2 in *cis* or *trans* [1,49,50]. An interaction between PRC2 and ncRNAs has also been postulated in plants [51], but only recently were such RNAs identified at a PRC2 target gene, possibly recruiting PRC2 to the *FLC* locus (reviewed in [46]). Expression of the long noncoding RNAs COOLAIR [52] and COLDAIR [53] was found to correlate with *FLC* silencing during vernalization, and COLDAIR was reported to physically associate with CLF. However, it was recently shown that COOLAIR is not essential for the vernalization-induced silencing of *FLC* [54•]. Moreover, COLDAIR transcripts are difficult to detect and *FLC* transgenes without the COLDAIR promoter still respond to cold [55], such that the functional relationship between these ncRNAs and *FLC* silencing is unclear. Whether long ncRNAs are important for PRC2 recruitment in plants therefore still awaits confirmation. The identification of specific PRC2 recruitment proteins in *Arabidopsis* is so far restricted to AG, which was found to recruit PRC2 specifically in flower stage 6 to the *WUS* locus, thus repressing *WUS* and terminating the floral meristem (see above [16]). However, in animals, a number of recruitment proteins have been identified for different targets [26], and more are probably to be found in plants as well.

Conclusion

It is evident that PcG proteins play essential roles in phase transitions, cell fate determination and differentiation. A dynamic regulation of PcG activity is crucial for plant development, which is reflected by the many different mechanisms that evolved to secure a tight spatial and temporal control of PRC2 activity. The majority of these mechanisms appear to depend on interacting or counteracting proteins, although strict control of PcG gene expression, as reported for MEA [56], or post-translational cell-specific degradation of PRC2 subunits, as reported for CLF [57], also contribute to PRC2 specificity. In addition, activation of PRC2 target genes probably largely depends on cell type-specific activators, since only a fraction of PRC2 targets became upregulated in *fie* mutant seedlings [20]. Although the function of PRC2 in the regulation of cell proliferation and differentiation is conserved throughout plants [58], fine-tuning is probably achieved through the various mechanisms described above, which may be lineage-specific. The recent development of an efficient targeted technique to isolate protein complexes in plants may accelerate the identification of PRC2-associated co-factors in the future [59].

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- • of special interest
- •• of outstanding interest

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In this work, the function of EMF1 is dissected into a PRC2-like and a PRC1-like function. Transcriptome analysis combined with H3K27me3 profiling resulted in the identification of a group of EMF1 targets that were dependent on EMF1 for H3K27me3, and a group of targets that was marked with H3K27me3, but did not depend on EMF1 for this mark. The data suggest that EMF1 plays a dual role in H3K27me3 mediated repression of target genes.

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