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PRC1 Marks the Difference in Plant PcG Repression

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ABSTRACT From mammals to plants, the Polycomb Group (PcG) machinery plays a crucial role in maintaining the repression of genes that are not required in a specific differentiation status. However, the mechanism by which PcG machinery mediates gene repression is still largely unknown in plants. Compared to animals, few PcG proteins have been identified in plants, not only because just some of these proteins are clearly conserved to their animal counterparts, but also because some PcG functions are carried out by plant-specific proteins, most of them as yet uncharacterized. For a long time, the apparent lack of Polycomb Repressive Complex (PRC)1 components in plants was interpreted according to the idea that plants, as sessile organisms, do not need a long-term repression, as they must be able to respond rapidly to environmental signals; however, some PRC1 components have been recently identified, indicating that this may not be the case. Furthermore, new data regarding the recruitment of PcG complexes and maintenance of PcG repression in plants have revealed important differences to what has been reported so far. This review highlights recent progress in plant PcG function, focusing on the role of the putative PRC1 components.

Key words: Polycomb Group; epigenetic regulation; PRC1; gene repression; H2A monoubiquitination; chromatin compaction.

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INTRODUCTION

Polycomb Group (PcG) proteins were originally identified through genetic approaches in *Drosophila*. These proteins exist in multi-protein complexes that comprise specific chromatin-modifying activities. To date, five PcG complexes have been identified in *Drosophila*: Pho-repressive complex (PhoRC), Pc-repressive complex 2 (PRC2), PRC1, dRing-associated factors complex (dRAF), and Pc-repressive deubiquitinase complex (PR-DUB) (Scheuermann et al., 2012) (Figure 1). Three of these complexes contain activities directed to add covalent modifications on histones in nucleosomes: PRC2 contains a histone methyltransferase that trimethylates histone H3 at lysine 27 (H3-K27me3) (Czermin et al., 2002; Müller et al., 2002) and PRC1 and dRAF harbor an E3 ligase activity for the monoubiquitination of histone H2A (H2Aub) (Lagarou et al., 2008; Gutiérrez et al., 2012). On the other hand, PhoRC is involved in PcG recruitment through its DNA-binding component Pleiohomeotic (Pho) (Klymenko et al., 2006) and PR-DUB in H2A deubiquitination (Scheuermann et al., 2010). Similar complexes have been identified in vertebrates; however, in these organisms, the system tends to be more complex, since there are multiple paralogs for most subunits.

The two best-characterized complexes in animals are PRC2 and PRC1 (Lanzuolo and Orlando, 2012). *Drosophila* PRC2 contains four core components (Figure 1): Enhancer of zeste (E(z)), Extra sex combs (Esc), Suppressor of zeste 12 (Su(z)12), and the nucleosome-remodeling factor Nurf 55 (Czermin et al., 2002; Müller et al., 2002). In addition, a fraction of PRC2 is associated with the plant homeodomain (PHD) protein Polycomb-like (Pcl), which is needed to generate high levels of H3K27 trimethylation at PcG target genes (Nekrasov et al., 2007). In mammals, two related genes, EZH1 and EZH2, encode the homologs of E(z) (Margueron et al., 2008). Alternative translation start sites produce four different isoforms of the mammalian homolog of Esc, termed embryonic ectoderm development (EED) (Kuzmichev et al., 2005) and, finally, the mammalian homologs of Su(z)12 and Nurf 55 are SUZ12 and retinoblastoma-associated protein 46 and 48 (RbAp46/48), respectively (Kuzmichev et al., 2002). There are also three Pcl homologs, PCL1, PCL2, and PCL3 (Coulson et al., 1998; O'Connell et al., 2001; Sarma et al., 2008). On the other hand, *Drosophila* PRC1 contains Polycomb (Pc), Polyhomeotic (Ph), Posterior sex comb (Psc), and Sex combs extra (Sce, also known as dRing1) (Shao et al., 1999; Peterson et al., 2004) (Figure 1). Each of these proteins has multiple

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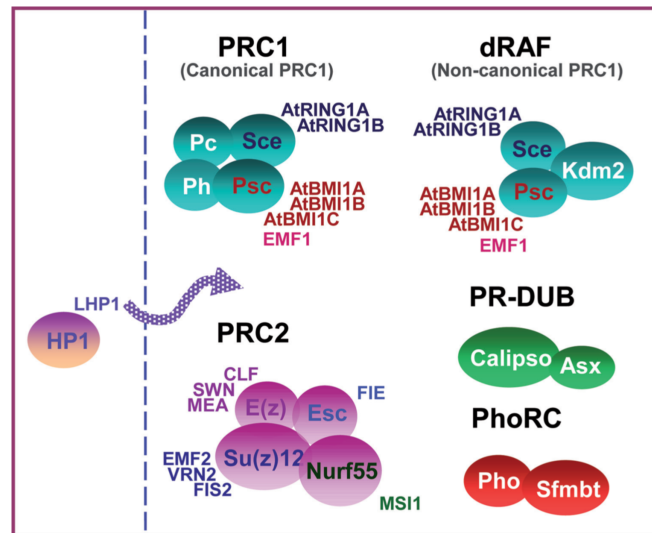

Figure 1. Schematic Representation of *Drosophila* PcG Complex Subunit Composition and the Homolog Subunits Found in *Arabidopsis*.

Several paralogs for some of the PRC1 and PRC2 subunits are present in *Arabidopsis*. Subunits named in white have not been identified in *Arabidopsis*. EMF1 is a plant-specific protein that displays the same sequence properties and effects on chromatin as the Psc C-terminal region. LHP1 is the plant homolog to HP1 that has been recruited to the PcG mechanism.

homologs in vertebrates (Levine et al., 2002). The Chromobox protein family (CBX2,4,6,7,8) carry out Pc function in mammals (Morey et al., 2012). Homologs of *Drosophila* Ph are PH1, PH2, and PH3 (Tonkin et al., 2002), and homologs of Sce are termed RING1A and RING1B (Schoorlemmer et al., 1997). The six homologs of Psc are collectively called PcG RING fingers (PcGFs, among them is BMI1) and constitute six major PRC1s (Gao et al., 2012).

Genetic and genome mapping analyses in *Drosophila* and mouse have demonstrated that PcG target loci are often co-occupied by PRC1 and PRC2 (Boyer et al., 2006; Schwartz et al., 2006; Ku et al., 2008). Co-occupancy was explained according to the so-called hierarchical model in which the recruitment of PRC1 is a consequence of the interaction of Pc with the H3K27me3 mark mediated by PRC2 (Cao et al., 2002; Fischle et al., 2003; Wang et al., 2004; Boyer et al., 2006). The idea has been further substantiated in studies demonstrating a direct link between H3K27me3 and PRC1 recruitment (Agger et al., 2007; Lee et al., 2007). Binding of PRC1 to target loci is believed to be central to establish a stable transcriptional repression through cell divisions. The potential mechanisms by which PRC1 mediates gene repression include H2A monoubiquitination to initiate a block to transcription, direct inhibition of the transcriptional machinery, and chromatin compaction (Francis et al., 2001; King et al., 2002; Francis et al., 2004; Lo and Francis, 2010). Nevertheless, although the hierarchical model is true for the recruitment of PRC1 at a subset of target loci, the number of examples in which PRC1/H2Aub targeting is independent of H3K27me3 has increased significantly in recent years, indicating that the hierarchical model is not the consensus mechanism.

Comparable PRC2s are present in plants (Figure 1). The combination of different paralogs has resulted in different PRC2s also in *Arabidopsis*. MEDEA (MEA) (Grossniklaus et al., 1998), CURLY LEAF (CLF) (Goodrich et al., 1997), and SWINGER (SWN) (Chanvivattana et al., 2004) are the EZH2 homologs; EMBRYONIC FLOWER2 (EMF2), FERTILISATION INDEPENDENT SEED2 (FIS2), and VERNALIZATION2 (VRN2) are the SU(Z)12 homologs (Luo et al., 1999; Gendall et al., 2001; Yoshida et al., 2001); FERTILIZATION INDEPENDENT ENDOSPERM (FIE) is the unique EED homolog (Ohad et al., 1999); and MULTICOPY SUPRESSOR OF IRA1–5 (MSI1–5) are the five RbAp46/48 homologs, but only MSI1 has been shown to be part of the PRC2s (Köhler et al., 2003; De Lucia et al., 2008; Derkacheva et al., 2013). Although the different PRC2s have discrete roles in controlling distinct aspects of plant development, they also regulate a common set of target genes at different stages of development (Makarevich et al., 2006). As in animals, several PHD proteins, such as VERNALIZATION INSENSITIVE3 (VIN3) (Sung and Amasino, 2004; Wood et al., 2006), VERNALIZATION5 (VRN5, also known as VIL1) (Sung et al., 2006; Greb et al., 2007), and VIN3-LIKE 2 (VIL2) (Kim and Sung, 2010), have been shown to co-purify with the VRN2-PRC2. These proteins greatly stimulate the H3K27 trimethyltransferase activity of VRN2-PRC2 in the nucleation region of the gene *FLOWERING LOCUS C* (*FLC*), which is the region where the repressive machinery accumulates in the cold during the vernalization process (De Lucia et al., 2008; Song et al., 2012; Kim and Sung, 2013).

It has long been known that PRC2-mediated H3K27me3 is indispensable for the repression mechanism in plants; however, the implication of a plant PRC1 with H2A monoubiquitin ligase activity has been questioned as detection of PRC1

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homologs and H2Aub remained elusive for a long time. Accordingly, H3K27me3 was thought to be sufficient for the repression mechanism in plants. The lack of PRC1 components was explained according to the idea that plant cells do not require a long-term stable repression, since they must be able to switch fate rapidly (Goodrich and Tweedie, 2002; Pien and Grossniklaus, 2007). However, subsequent data indicated that H3K27me3 by itself does not repress gene expression and that other factors must be implicated (Schatlowski et al., 2008).

The identification of plant-specific proteins that apparently carried out PRC1-like functions in the repression of several PRC2 target genes (Figure 1), such as EMF1 that is proposed to be the functional equivalent to *Drosophila* Psc (Calonje et al., 2008) and LIKE-HETEROCHROMATIN PROTEIN1 (LHP1) that co-localizes with H3K27me3 marks similarly to animal Pc (Turck et al., 2007; Zhang et al., 2007), and the identification of additional plant proteins involved in the repression of specific PcG targets, such as VRN1 that acts downstream of VRN2–PRC2 to maintain *FLC* repression (Levy et al., 2002; Mylne et al., 2006), led to the speculation about an alternative PcG mechanism in which the implication of plant-specific components and the absence of PRC1 homologs allowed a more flexible repression (Pien and Grossniklaus, 2007). However, the discovery of plant PRC1 RING finger homologs a few years ago suggested that the general mechanism of PcG protein function is well conserved between animals and plants, although individual players have been exchanged during evolution (Hennig and Derkacheva, 2009).

Surprisingly, recent results regarding the mechanism by which plants establish and maintain PcG repression challenge some previously accepted ideas. Here, I discuss these exciting results and provide a possible new interpretation for the PcG-mediated repression mechanism in plants.

IDENTIFICATION OF THE LONG SOUGTH PRC1 RING FINGER HOMOLOGS

Plant PRC1 RING finger proteins were identified by analyzing the domain structure of mammalian BMI1 and RING1 proteins. These proteins share a conserved RING finger domain at their N-terminal region and a C-terminal region that contains a new Ubiquitin-like domain named RAWUL (*R*ing-finger *A*nd *W*D40 associated *U*biquitin-*L*ike) (Sanchez-Pulido et al., 2008; Bezsonova et al., 2009). The combination of a RING finger domain at the N-terminus and a RAWUL domain at the C-terminus is a key feature to define the PRC1 RING finger protein family, since no other protein presents this domain architecture. Database search revealed the presence of a family of proteins in plants that conserved the PRC1 RING finger characteristic domain architecture (Sanchez-Pulido et al., 2008). In *Arabidopsis*, there are five PRC1 RING finger homologs, two of them similar to RING1 (AtRING1A and AtRING1B) and three to BMI1 (AtBMI1A, AtBMI1B, and AtBMI1C) (Sanchez-Pulido et al., 2008; Xu and Shen, 2008) (Figure 1).

Functional analyses of these genes revealed their crucial role in plant development. Double mutants for *atring1a/b* and *atbmi1a/b* displayed derepression of embryonic traits during vegetative development (Bratzel et al., 2010; Chen et al., 2010), a similar phenotype to the one displayed by severely compromised PRC2 mutants, such as *clf/swn*, *fie*, or *emf2/vrn2* mutants (Chanvivattana et al., 2004; Aichinger et al., 2009; Bouyer et al., 2011). Accordingly, several key regulatory genes involved in embryogenesis and stem cell activity were ectopically expressed in all of these mutants. The lack of phenotype in *atring1* or *atbmi1* single mutants indicated that AtRING1A and B and AtBMI1A and B redundantly regulate a common set of targets (Bratzel et al., 2010; Chen et al., 2010); however, whether each protein has in addition specific target loci is still unknown. On the other hand, the identical phenotype of *atring1a/b* and *atbmi1a/b* mutants indicated that the two subfamilies of proteins play a non-redundant function; furthermore, the *in vitro* and *in vivo* interaction between AtRING1 and AtBMI1 proteins indicated that they may act together as part of the same complex (Bratzel et al., 2010; Chen et al., 2010).

Contrary to *AtRING1A* and *B* and *AtBMI1A* and *B*, which are ubiquitously expressed, *AtBMI1C* is expressed in the endosperm, stamen, and root (Bratzel et al., 2012; Li et al., 2011). As *atbmi1a* and *atbmi1b* single mutant, *atbmi1c* did not show any apparent alteration. Interestingly, *AtBMI1C* is a maternally imprinted gene in the endosperm (Bratzel et al., 2012), like the PRC2 components MEA and FIS2 (Kinoshita et al., 1999; Jullien et al., 2006). The progeny of *atbmi1a–/–b–/–c+/–* plants showed 13%–14% of aborted seeds and 11%–12% of seedlings that displayed an enhanced phenotype compared to *atbmi1a/b* mutants, as *atbmi1a/b/c* triple mutants did not develop any true vegetative tissue and showed a stunted pickle-like primary root (Yang et al., 2013). Thus, the three AtBMI1 proteins are required during vegetative and seed development, acting redundantly in the tissue where they are co-expressed.

In vitro H2A ubiquitination analyses showed that the two AtRING1 and the three AtBMI1 proteins are active E3 ubiquitin ligases (Bratzel et al., 2010). The *in vivo* activity was verified for the AtBMI1 proteins (Bratzel et al., 2010; Li et al., 2011; Bratzel et al., 2012). A recent report showed that mutant plants in *AtBMI1A/B* displayed a remarkable global decrease of H2Aub, which was reflected in a decrease of this mark at the TSS region of specific targets, and that this modification was almost undetectable in *atbmi1a/b/c* mutants (Yang et al., 2013). In mammals, RING1B depletion abrogates most H2Aub, and RING1A or BMI1 depletion greatly reduces the levels of H2Aub (Cao et al., 2005); therefore, although recombinant BMI1 and RING1A are not active ubiquitin E3 ligases for H2A (Wang et al., 2004), they are required for the *in vivo* activity. It has been proposed that the role of BMI1 is to stabilize and to stimulate RING1B (Buchwald et al., 2006; Li et al., 2006).

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In *Arabidopsis*, recombinant AtRING1 and AtBMI1 proteins are active ubiquitin E3 ligases *in vitro* (Bratzel et al., 2010), and depletion of AtBMI1 activity abrogates most, if not all, H2Aub *in vivo* (Yang et al., 2013). The *in vivo* H2A monoubiquitination activity of AtRING1A/B has not been tested yet; however, the identical phenotype of *atbmi1* and *atring1* mutants (Bratzel et al., 2010; Chen et al., 2010) suggests that both subfamilies of proteins are equally required to establish H2Aub in *Arabidopsis*.

UNEXPECTED ROLE OF H2AUB IN *ARABIDOPSIS*

It has been widely accepted that PRC1 activity in animals was required to stabilize the repression of H3K27me3 marked loci (Levine et al., 2004; Schuettengruber and Cavalli, 2009; Margueron and Reinberg, 2011). Therefore, the identification of plant PRC1 RING finger components that mediated H2Aub suggested a similar role of this mark in plants. However, recent data indicated that this may not be the case, at least in the regulation of the seed maturation genes *ABSCISIC ACID INSENSITIVE3* (*ABI3*), *FUSCA3* (*FUS3*), *LEAFY COTYLEDON2* (*LEC2*), and *LEAFY COTYLEDON1* (*LEC1*) (Yang et al., 2013). Interestingly, ChIP assays using anti-H2A and anti-H3K27me3 antibodies showed that removing AtBMI1 proteins significantly decreased H3K27me3 marks at seed maturation genes, whereas removing CLF and SWN increased H2Aub (Yang et al., 2013). According to these results, H2A monoubiquitination is required for H3K27me3 marking of these genes but not the other way around. Phenotypic and flow cytometric analyses indicated that *clf28/swn7* and *fie* mutants, in which H3K27me3 deposition is abolished (Bouyer et al., 2011; Lafos et al., 2011), were able to switch to generative development after germination but then they experienced a progressive loss of cell differentiation (Bouyer et al., 2011; Yang et al., 2013). Conversely, *atbmi1a/b/c* mutants remained in an embryo maturating-like stage after germination, indicating that they did not undergo phase transition (Yang et al., 2013). Together, these results strongly suggest that AtBMI1-mediated H2Aub is required for the initial repression of seed maturation genes and that the role of H3K27me3 marking is to maintain the repression during vegetative development (Yang et al., 2013). How PRC2 is recruited to seed maturation genes after H2Aub marking it is not known, but CLF has been shown to physically interact with AtRING1A in yeast two-hybrid assays (Xu and Shen, 2008).

On the other hand, the VAL (*V*P1/*A*BI3-*L*IKE)1/2/3 proteins may participate in the recruitment AtBMI1-containing complexes to seed maturation genes. The VAL proteins are plant-specific B3-domain transcription factors that have an important role in repressing the seed maturation program after germination (Lotan et al., 1998; Tsuchiya et al., 2004; Suzuki et al., 2007). The B3 domain of these proteins is predicted to bind *RY/Sph* DNA elements (Suzuki et al., 1997; Ezcurra et al., 2000; Yamasaki et al., 2004; Suzuki et al., 2007). VAL proteins also contain three chromatin-related domains: a CW-like zinc finger that is a novel module recognizing different methylated states of lysine 4 on H3 (H3K4me) (Hoppmann et al., 2011), an EAR motif that is a plant-specific transcriptional repression domain (Kagale and Rozwadowski, 2011), and a PHD-like domain that binds to different H3 modifications (Sanchez and Zhou, 2011). It has been shown that the CW domain of VAL1 has *in vitro* preference for H3K4me3 (Hoppmann et al., 2011) and that the PHD-like domain of VAL1 is required to repress a subset of seed maturation genes (Veerappan et al., 2012). Intriguingly, *val1/2* mutants displayed exactly the same phenotype as *atbmi1a/b/c* mutants. Furthermore, VAL and AtBMI1 proteins strongly interacted *in vitro*, and *val1/2* mutants showed significantly reduced levels of H2Aub and H3K27me3 marks at seed maturation genes (Yang et al., 2013). According to these results, the VAL proteins may participate in the mechanism that recruits the PRC1 to seed maturation genes. Although direct binding of VAL to *RY/Sph*-elements has not been demonstrated, several pieces of evidence support the specific targeting of VAL proteins. For instance, co-infiltration of a *RY/Sph* reporter (which is *in vivo trans*-activated by ABI3 protein) with the *35S::ABI3* and the *35S::VAL1* effectors into *Nicotiana benthamiana* leaves showed that ABI3 transactivation of the *RY/Sph* reporter was significantly down-regulated by VAL1 (Guerriero et al., 2009). In addition, VAL1 was able to repress a sporamin minimal promoter reporter that contains a *RY/Sph* element when co-expressed in *Arabidopsis* protoplasts (Tsukagoshi et al., 2005). Finally, yeast one-hybrid assay using the B3 domain of VAL2 placed within the context of the FUS3 protein was able to bind the *RY/Sph* element (Guerriero et al., 2009). Yet, it is also possible that the interaction of VAL with other factors contributes for specific binding to target loci. In addition, specific histone modifications could act as docking sites for VAL binding through the CW or and PHD-like domain. In any case, the identical phenotypes of *val1/2* and *atbmi1a/b/c* mutants, and the loss of H2Aub marks at seed maturation in *val1/2* mutants support that the VAL proteins act upstream of AtBMI1 and play a role in AtBMI1 recruitment (Yang et al., 2013).

Interestingly, VAL1 has been also shown to interact with the histone deacetylase 19 (HDA19). Mutation in HDA19 resulted in ectopic expression of seed maturation genes in seedlings, which was associated with increased levels of gene activation marks, such as histone H3 acetylation, histone H4 acetylation, and H3K4me3, but decreased levels of H3K27me3 marks (Zhou et al., 2013), suggesting VAL as a link between histone deacetylation and PcG-mediated repression of seed maturation genes (Figure 2).

Apart from seed maturation genes, it has been shown that deposition of H2Aub and H3K27me3 marks at other PcG targets occurs independently. For instance, the absence of AtBMI1 activity did not lead to a loss of H3K27me3 marks

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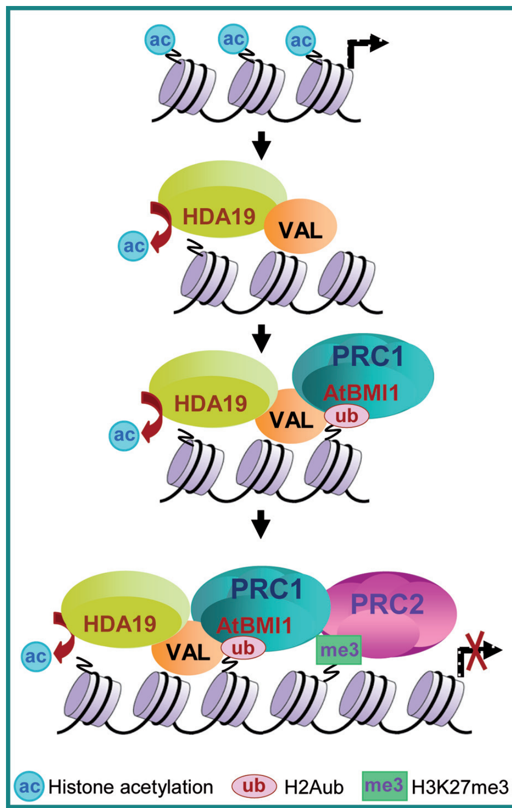

Figure 2. Possible Model for VAL Linking Histone Deacetylation and PcG-Mediated Repression of Seed Maturation Genes.

VAL proteins recruit HDA19 to actively expressed seed maturation genes to remove histone acetylation marks and PRC1 to incorporate H2Aub marks to initiate gene repression. Then, PRC2 is recruited to mediate H3K27me3, which maintains stable repression.

at the stem cell maintenance gene *WUSCHEL* (*WUS*) and vice versa (Yang et al., 2013). Moreover, the finding that global H2Aub levels were not significantly affected in *clf/swn* mutants (Yang et al., 2013) suggests that H2Aub targeting is mostly independent of H3K27me3 in *Arabidopsis*. Whether H2Aub marking of some target loci depends on the presence of H3K27me3 marks remains to be investigated. In any case, these results differ from the classic hierarchical model proposed for PcG repression in animals. However, an H3K27me3-independent targeting of H2Aub has been reported in animals, too, indicating that the independent recruitment of PcG complexes is conserved between plants and animals.

A few years ago, a novel PcG complex termed dRAF was found in *Drosophila*. The dRAF complex comprises Sce, Psc, and the histone demethylase dKdm2, but lacks Ph and Pc (Figure 1). dKdm2 greatly enhanced the H2A monoubiquitin ligase activity of Sce/Psc in addition to mediate the removal of the active H3K36me2 mark. Depletion of Sce or Psc caused a dramatic loss of H2Aub levels; however, knockdown of PRC1 subunits Pc or Ph had no effect on H2Aub. These observations indicated that dRAF is recruited to chromatin independently of H3K27me3 marks, and that dRAF, rather than the canonical PRC1, is responsible for the majority of H2A monoubiquitination (Lagarou et al., 2008). Similarly, non-canonical PRC1s have been recently discovered in mammals. The existence of six PCGF orthologs has given rise to different PRC1s. Only PCGF2/MEL18 and PCGF4/BMI1 are incorporated into canonical PRC1. In contrast, all six PCGF proteins can assemble into non-canonical PRC1 complexes lacking CBX (Pc homolog) and Ph. Non-canonical PRC1s are composed of RING1B–PCGF in combination with other chromatin regulators (Gao et al., 2012; Tavares et al., 2012). For instance, the mammalian dRAF equivalent contains Fbxl10, which is a mammalian Kdm2 homolog, RING1B, and the PCGF ortholog Nspc1. This complex is required for most H2Aub in embryonic stem cells (Wu et al., 2013). Therefore, given that a Pc/CBX subunit is an integral part of the hierarchical recruitment model, the absence of this subunit in non-canonical PRC1s indicates a different recruitment mechanism.

On the other hand, despite the implication of H2Aub marks in the PcG repression of the above-mentioned genes in *Arabidopsis*, there are other well-known PcG targets, such as *AGAMOUS* (*AG*) or *SHOOTMERISTEMLESS* (*STM*), in which the H2Aub marks were not detected (Yang et al., 2013). Apart from H2Aub, there are other potential mechanisms by which PRC1 mediates gene repression, such as direct inhibition of the transcriptional machinery and chromatin compaction. These activities have been extensively studied in Drosophila (Francis et al., 2001, 2004; Lo and Francis 2010) and may be also crucial for plant PRC1-mediated repression (as discussed in the next section). In any case, genome-wide localization of H2Aub marks in *Arabidopsis* will be required to determine the degree of overlap between H2Aub and H3K27me3 marks at PcG target genes.

EMF1, A VERSATILE PcG COMPONENT

EMF1 has been proposed to be a plant-specific PRC1 component (Calonje et al., 2008). *emf1* mutants and plants impaired in components of EMF2–PRC2 have similar phenotype. *emf1*, *emf2*, *clf/swn*, and *fie* mutants skip the vegetative phase and flower after germination as a consequence of derepression of flowering genes such as *AG*, *PISTILLATA* (*PI*), and *APETALA3* (*AP3*) (Sung et al., 1992; Yang et al., 1995; Kinoshita et al., 2001; Chanvivattana et al., 2004; Bouyer et al., 2011). Weak *emf1* mutants are *emf2*-like, while strong *emf1*-2 mutants have a more severe phenotype than *emf2* or *fie* regarding flowering time and flower development; however, *emf1-2* mutants do not develop embryo- or callus-like structures as *clf/swn*, *fie*, or mutant plants in the PRC1 RING finger components do; accordingly, the seed maturation genes are not dramatically misexpressed in *emf1-2* (Yang et al., 2013). On the other hand, *atbmi1* or *atring1* mutants do not display early flowering or deregulation of flower homeotic genes (Bratzel et al., 2010; Chen et al., 2010), suggesting different roles of PRC1 components in the regulation of different subsets of PRC2 targets.

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Genome-wide localization of EMF1 binding and H3K27me3 modification in wild-type and *emf1-2* plants revealed a considerable number of genes marked with H3K27me3 and occupied by EMF1. Among these genes, 44% showed reduced levels of H3K27me3 in *emf1-2*, unveiling genes that depend on EMF1 for their H3K27me3 marking and others that do not (Kim et al., 2012). Consistently with these data, it has been shown that the levels of H3K27me3 at *STM* and *AG* are drastically reduced in *emf1-2* mutants, but less so at *ABI3*, *WUS*, *LEC1*, and *FUS3* (Yang et al., 2013). Interestingly, *AG* and *STM* are not targets for H2Aub but they are strongly misexpressed in *emf1-2* mutants, whereas the other genes are H2Aub targets but not significantly deregulated in *emf1-2* (Yang et al., 2013); therefore, the repression of *STM* and *AG* and their levels of H3K27me3 are highly dependent on EMF1, while the repression of these known H2Aub targets and their levels of H3K27me3 are less dependent on EMF1.

In *Drosophila*, there are two distinct classes of PRC1-regulated genes. The repression of class I genes requires H2Aub, whereas the repression of class II depends on Psc non-covalent effects on chromatin structure (Lagarou et al., 2008; Gutiérrez et al., 2012). *Drosophila* Psc contains the conserved RING and RAWUL domains of BMI1 proteins (Beh et al., 2012); however, it also has a long C-terminal region (CTR) characterized as an intrinsically disordered region that exhibits low contiguous negative charge (Beh et al., 2012). This region is necessary and sufficient to mediate chromatin compaction, inhibition of chromatin remodeling, and repression of transcription (King et al., 2002; Francis et al., 2004). Interestingly, in mammals and plants, these activities are not linked to BMI1 proteins, which only contain the RING and RAWUL domains, but are carried out by a different protein that shares with Psc the sequence properties of its CTR (Beh et al., 2012). In plants, EMF1 has been shown to display the same sequence properties and effects on chromatin and transcription as Psc-CTR (Calonje et al., 2008; Beh et al., 2012). Therefore, similarly to *Drosophila*, it seems that there are two distinct classes of PRC1-mediated repression in *Arabidopsis*, the H2Aub-dependent and the EMF1-dependent repression, which is H2Aub-independent. Genome-wide localization of H2Aub and gene expression analyses in wild-type and mutant plants will determine whether this is a general rule.

Since EMF1 is required for the H3K27 trimethylation of H2Aub-independent targets, it might be possible that EMF1 creates an appropriate local chromatin conformation for H3K27me3 marking. In support of this, a recent report shows that local chromatin compaction precedes the establishment of H3K27me3 in mouse embryonic stem cells (Yuan et al., 2012). On the other hand, EMF1 might contribute to maintain appropriate H3K27me3 levels for the stable repression of H2Aub-marked genes; accordingly, *emf1-2* mutants show slightly reduced H3K27me3 levels at several H2Aub-marked genes, which leads to a mild misregulation of the genes. The finding that EMF1 *in vitro* interacts with the PRC1 RING finger proteins (Bratzel et al., 2010) and the PRC2 component MSI1 (Calonje et al., 2008) suggests that EMF1 connects PRC1 to PRC2 activities, although with a versatile role in the mechanism.

LHP1, A PcG COMPONENT WITH HP1 STRATEGIES

LHP1, also known as TERMINAL FLOWER2 (TFL2), has been proposed to be another plant-specific PRC1 component. LHP1 was identified as a homolog of animal HETEROCHROMATIN PROTEIN1 (HP1) (Gaudin et al., 2001). Similarly to HP1, LHP1 contains a chromodomain and a chromo shadow domain (Gaudin et al., 2001; Kotake et al., 2003). However, unlike HP1, LHP1 is usually localized in euchromatin and is needed for maintenance of gene repression in euchromatin but not in heterochromatin (Libault et al., 2005; Nakahigashi et al., 2005); accordingly, LHP1 is associated with genes marked with H3K27me3 but not with H3K9me *in vivo* (Turck et al., 2007; Zhang et al., 2007; Exner et al., 2009). Moreover, *lhp1* mutants shows misexpression of several PcG target genes, most of which are involved in flower promoting pathways (Kotake et al., 2003; Libault et al., 2005; Nakahigashi et al., 2005). Therefore, despite its sequence similarity to HP1, LHP1 seemed to display a similar role to *Drosophila* Pc, leading to proposition that LHP1 interacts with H3K27me3 marks and recruits a plant PRC1 for stable repression of PcG targets (Turck et al., 2007; Zhang et al., 2007).

Nevertheless, given the proposed role of LHP1 in PcG repression, the mild phenotype of *lhp1* mutants compared to other PRC1 mutants and the fact that only a subset of PcG targets are misexpressed in *lhp1* mutants have been always intriguing and suggested that other proteins may have a Pc-like role in *Arabidopsis*. However, genome-wide localization of H3K27me3 marks and LHP1 showed that around 90% of the regions marked with H3K27me3 are occupied by LHP1 (Turck et al., 2007; Zhang et al., 2007), indicating that the majority of PRC2 target loci are also targeted by LHP1.

Interestingly, recent data showed that LHP1 could be recruited to specific targets by interaction with different transcription factors, revealing a possible H3K27me3-independent recruitment of LHP1. For instance, SCARECROW (SCR) recruits LHP1 to *MAGPIE* (Cui and Benfey, 2009) and SHORT VEGETATIVE PHASE (SVP) to *SEP3*. Surprisingly, SVP-mediated recruitment of LHP1 is required for *SEP3* H3K27 trimethylation (Liu et al., 2009). In addition, although LHP1 has been previously shown to interact with the PRC1 components AtRING1A and AtRING1B (Xu and Shen, 2008), a recent report showed that LHP1 co-purifies with PRC2, directly interacts with MSI1, and co-exists with EMF2 in shared complex(es), indicating that LHP1 and PRC2 functions are closely integrated in *Arabidopsis* (Derkacheva et al., 2013). Furthermore, this report also showed that the levels of H3K27me3 at several PcG targets in dividing cells were significantly lower in *lhp1*

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mutants than in wild-type plants, suggesting that LHP1 assists the recruitment of PRC2 to target sites for re-establishing the levels of H3K27me3 after replication (Derkacheva et al., 2013). According to this model, LHP1 binds to H3K27me3 but also contributes to recruit PRC2 for H3K27me3 marking of newly incorporated histones (Figure 3), which is consistent with the high LHP1 expression in proliferating cells (Kotake et al., 2003; Baerenfaller et al., 2011) and the interaction of LHP1 with the POL2 alpha subunit of DNA polymerase epsilon in plants (also known as EARLY IN SHORT DAYS 7 (ESD7)) (del Olmo et al., 2010). A similar mechanism has been proposed for HP1-mediated re-establishment of H3K9me marks in yeast and animal (Bannister et al., 2001).

On the other hand, LHP1 has been shown to interact with LHP1-INTERACTING FACTOR2 (LIF2) that contains three RNA-recognition motifs (RRM) and belongs to the hnRNP large protein family. It has been proposed that LIF2 modulates LHP1 activity on a subset of LHP1 targets in responses to external cues (Latrasse et al., 2011), opening novel links between chromatin dynamics, RNA processing, and developmental plasticity; however, the mechanism by which LIF2 modulates LHP1 activity remains to be investigated. Interestingly, animal HP1alpha also interact with hnRNPs (Ameyar-Zazoua et al., 2009; Piacentini et al., 2009); thus, the interaction between HP1/LHP1 proteins and hnRNP proteins seems to be conserved.

In summary, these new data strongly suggest that LHP1 has a different role in the PcG mechanism than the one previously thought, and that it may deploy similar strategies to animal HP1, although in the context of PcG-mediated repression.

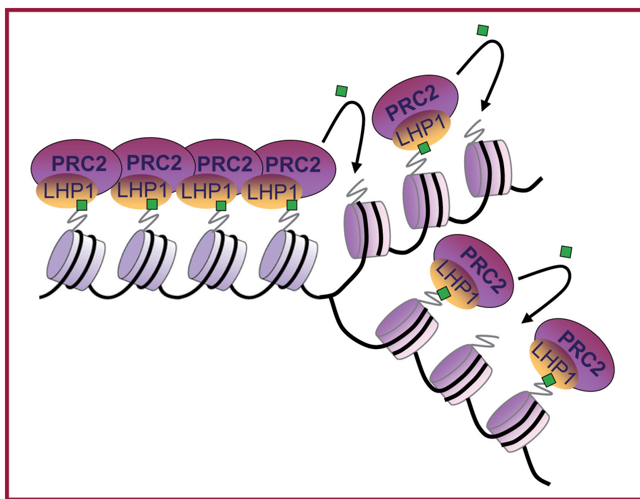

Figure 3. Model of LHP1 Function in Re-Establishing H3K27me3, according to Derkacheva et al. (2013).

H3K27me3 modifications are evenly distributed to the daughter strands during the replication process. LHP1 binds to nucleosomes that carry H3K27me3 marks and recruits PRC2 via interaction with MSI1, which H3K27 trimethylates newly incorporated histones. H3K27me3 is symbolized by green squares.

A NEW INTERPRETATION FOR PcG MECHANISM IN PLANTS

According to recent evidence, it seems that H2Aub in plants takes place independently of H3K27me3 and, furthermore, that either H2Aub marking or EMF1 activity is required for establishing H3K27me3 marks at some target loci, which places PRC1 activity upstream of PRC2 (Figure 4). Remarkably, a requirement of PRC1 activity for H3K27me3 marking has not been reported in animals so far, albeit a recent report showed that local chromatin compaction precedes the establishment of H3K27me3 in mouse embryonic stem cells (Yuan et al., 2012).

In animals, canonical PRC1s are recruited to target genes through the binding of Pc to H3K27me3 marks and mediate gene repression by compacting chromatin, which is consistent with the hierarchical model. Conversely, non-canonical PRC1s are recruited to target loci through a Pc-independent mechanism and mediate repression via H2Aub (Gao et al., 2012; Tavares et al., 2012; Wu et al., 2013), which may be comparable to *Arabidopsis* H2Aub-dependent repression. However, as plants lack Ph and Pc homologs, a non-canonical PRC1 and a Pc-independent recruitment seem to be used also for H2Aub-independent repression.

Unfortunately, the identity of PRC1 in plants has not been established yet, since the *in vivo* complex has not been purified; however, the fact that the repression of H2Aub-dependent targets is somehow released in *emf1-2* mutants (Yang et al., 2013) indicates that EMF1 is involved in the repression of both H2Aub-dependent and -independent genes. Hence, AtBMI1 and EMF1 functions seem to be tightly connected, which strongly suggests their participation in the same protein complex. On the other hand, the identical phenotype of *atbmi1* and *atring1* mutants (Bratzel et al., 2010; Chen et al., 2010) supports that AtBMI1 and AtRING1 proteins are part of the same complex. Whether the putative plant PRC1 contains other components it is still unknown, but it would not be surprising to find specific subunits involved in selecting the target and determining the type of repression. Some of these subunits could be considered as participants of PcG repression rather than core components. This might be the case of VRN1 that was proposed to display a PRC1-like function in the regulation of *FLC* (Mylne et al., 2006).

According to this possibility, the specific targeting of PRC1 to seed maturation genes in *Arabidopsis* requires the VAL B3-domain-containing transcription factors (Yang et al., 2013), suggesting that PRC1 is tethered to target genes via DNA-binding proteins (Figure 4). Interestingly, DNA-binding proteins have been also involved in the recruitment of mammalian non-canonical PRC1s and genome-wide target analyses revealed the existence of different recruitment programs that may depend on the subunit composition of the individual complexes (Gao et al., 2012). For instance, the new PRC1 component RING1/YY1-binding protein (RYBP) (Tavares et al.,

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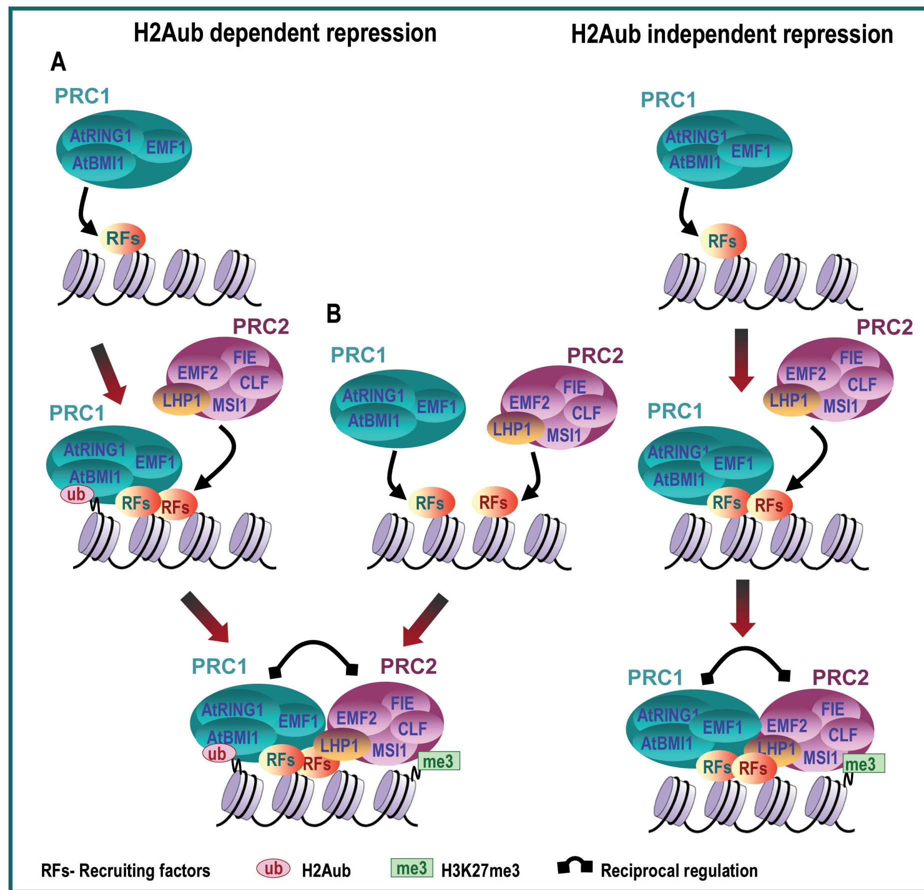

Figure 4. Different PcG Mechanisms in *Arabidopsis*.

Plant PRC1s lack Ph and Pc homologs; therefore, a non-canonical PRC1 and a Pc-independent recruitment operate in the different PcG mechanisms. There are two possible mechanisms. (1) an H2A-dependent repression in which PRC1 and PRC2 are tethered to chromatin via recruiting factors (RFs) to incorporate H2Aub and H3K27me3 marks, respectively. The recruitment of PRC2 may be dependent (a) or independent (b) of PRC1. (2) An H2A-independent repression in which PRC1 is recruited to chromatin and mediates chromatin remodeling and compaction. These activities favor the recruitment of PRC2 and the incorporation of H3K27me3 marks. In both types of repression, PRC1 and PRC2 have a reciprocal regulatory role to maintain an appropriate balance of histone marks.

2012) interacts with YY1, which is the mammalian homolog of the *Drosophila* PRC1/2 recruiting factor Pho (Klymenko et al., 2006; Simon and Kingston, 2009). It has been proposed that RYBP might serve to bridge PRC1 to YY1. In addition, the mammalian Kdm2 homolog Fbxl10 binds to CpG islands through its CXXC domain and recruits the PRC1 proteins RING1B and Nspc1 to DNA in embryonic stem cells (Wu et al., 2013). Moreover, several transcription factors have been involved in the recruitment of a subset of PRC1s (Gearhart et al., 2006; Trojer et al., 2011). Therefore, the use of DNA-binding proteins to tether PRC1s to target genes may be a conserved mechanism. In addition, there may be other mechanisms that operate to recruit PRC1s to target genes, such as interaction with previously established histone modifications and non-coding RNAs (ncRNAs). Interestingly, EMF1 *in vitro* binds to RNA (Calonje et al., 2008); hence, it would not be surprising to find that it participates in an ncRNA-mediated recruitment.

Although PRC1 activity seems to be required for PRC2s at a subset of targets, there are also examples in which PRC1 and PRC2 activities are independent (Yang et al., 2013) (Figure 4). The combination of different factors may mediate a nuanced repression repertoire to affect a context-dependent outcome. Nevertheless, in any case, the two activities co-regulate gene expression, suggesting interplay between PRC1 and PRC2 (Figure 4). Moreover, apparently, PRC1 and PRC2 have a reciprocal regulatory role to maintain an appropriate balance of histone marks. For instance, the lack of AtBMI1 proteins leads to increased levels of H3K27me3, even at H2Aub-independent targets. Similarly, the lack of CLF/SWN activities leads to increased levels of H2Aub (Yang et al., 2013). Therefore, PRC1 and PRC2 may either negatively regulate each other's activity or an unknown regulatory pathway aims to compensate the loss of one activity by stimulating the other. In support of their reciprocal regulatory role, it has been reported recently that PRC2-mediated H3K27me3

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is moderately inhibited by H2Aub on mononucleosomes carrying H2Aub (Whitcomb et al., 2012) and, in animals, one of the possible roles of the PcG H2A deubiquitinase complex PR–DUB is to confine H2Aub to specific regions (Scheuermann et al., 2012). It might be possible that, in *Arabidopsis*, the H2A deubiquitinase activity is directly or indirectly linked to PRC2.

Unlike plant PRC1, there is a considerable amount of information about plant PRC2s (Bemer and Grossniklaus, 2012). Recent biochemical data have confirmed the identity of the different complexes (Köhler et al., 2003; De Lucia et al., 2008; Derkacheva et al., 2013) and, surprisingly, the putative PRC1 component LHP1 has been shown to co-purify with PRC2 components (Derkacheva et al., 2013); also, LHP1 is apparently recruited to specific PcG targets via interaction with transcription factors in an H3K27me3-independent manner, and it is required to maintain H3K27me3 levels at some loci (Cui and Benfey, 2009; Liu et al., 2009; Derkacheva et al., 2013). All together, these results argue against the previously proposed role of LHP1 in recruiting PRC1 via H3K27me3 targeting. In animals, heterochromatin and PcG systems use similar factors and mechanisms for recruitment, such as DNA-binding proteins, non-coding RNAs, and interaction with histone modifications (Beisel and Paro, 2011). Interestingly, transcription factor-mediated deposition of HP1 has been reported in animals. This deposition establishes microenvironments of heterochromatin for the repression of gene transcription (Sripathy et al., 2006; Sdek et al., 2011). In addition, comparable to the Su(vAR)3–9–HP1–H3K9me3 interdependency in heterochromatin formation (Grewal and Elgin, 2002), recent evidence supports the model in which PRC2–LHP1–H3K27me3 interaction creates a self-reinforcing loop to ensure the propagation of the mark and its re-establishment after histone exchange or demethylation (Derkacheva et al., 2013). Therefore, LHP1 may participate in tethering PRC2 through interaction with DNA recruiting factors and/or H3K27me3, favoring the H3K27me3 marking of the target genes. Since LHP1 also interacts with several PRC1 components (Xu and Shen, 2008), it might be possible that this interaction connects PRC1 and PRC2 activities, as PcG targets in *Arabidopsis* are often co-regulated by PRC1 and PRC2.

CONCLUDING REMARKS

A picture is emerging in which the recruitment, establishment, and maintenance of PcG repression in plants show similarities but also important differences to animals. The lack of plant protein homologs to some of animal PcG components, the incorporation of plant-specific components, the acquisition of novel activities by chromatin factors, and the association of different proteins may have given rise to distinct mechanisms. Nevertheless, as in animals, PcG repression in plants seems to follow different rules depending on the target gene set. Whether the different PcG mechanisms exert different biological functions by regulating distinct subsets of targets in specific cell types, developmental stages, or in responses to external cues remains to be investigated. In any case, it seems that PRC1 has an important role in determining the type of repression. Further subunit characterization of different PRC1s, together with genome-wide analyses to define their specific targets *in vivo*, will be necessary to unravel the precise roles of the different PcG mechanisms in plants.

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