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REVIEW PAPER

Variations on a theme: Polycomb group proteins in plants

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Abstract

Polycomb group (PcG) proteins evolved early in evolution, probably in the common ancestor of animals and plants. In some unicellular organisms, such as *Chlamydomonas* and *Tetrahymena*, PcG proteins silence genes in heterochromatin, suggesting an ancestral function in genome defence. In angiosperms, the PcG system controls many developmental transitions. A PcG function in the vernalization response evolved especially in Brassicaceaea. Thus, the role of PcG proteins has changed during evolution to match novel needs. Recent studies identified many proteins associated with plant PcG protein complexes. Possible functions of these interactions are discussed here. We highlight recent findings about recruitment of PcG proteins in plants in comparison with animal system. Through the new data, a picture emerges in which PcG protein complexes do not function in sequential linear pathways but as dynamically interacting networks allowing stabilizing feedback loops. We discuss how the interplay between different PcG protein complexes can enable establishment, maintenance, and epigenetic inheritance of H3K27me3.

Key words: Chromatin, epigenetics, gene silencing, plants, Polycomb group proteins.

Introduction

Polycomb group (PcG) proteins control gene expression and enable developmental programmes in plants and animals. PcG proteins establish a silenced state of genes that is maintained through multiple mitotic divisions. However, PcG gene silencing can be modulated by developmental signals and therefore represents a dynamic and rapidly responding system of gene silencing. In animals, PcG proteins are involved in both cell differentiation and maintenance of stem cells (for review, see Sawarkar and Paro, 2010; Aloia *et al.*, 2013). Moreover, PcG proteins are needed for the self-renewal of cancer stem cells, and overexpression of PcG proteins is often associated with cancer, metastasis, and therapy resistance (Crea *et al.*, 2012). In mammals, PcG proteins play also a key role in X chromosome inactivation (for review, see Brockdorff, 2011). In plants, loss of PcG proteins leads to cell dedifferentiation and formation of callus-like structures, demonstrating a key role of PcG proteins in plant development and maintenance of cellular identity (Chanvivattana *et al.*, 2004). However, in contrast to animals, plant PcG proteins are not required for embryo body formation (Bouyer *et al.*, 2011). In *Arabidopsis*, PcG proteins can directly repress target genes or indirectly promote gene expression through repression of microRNA genes (Lafos *et al.*, 2011). Furthermore, PcG proteins function in regulation of genomic imprinting and the vernalization response (for reviews, see Jiang and Köhler, 2012; Song *et al.*, 2012).

First identified in *Drosophila* as regulators of *Hox* gene expression (Lewis, 1978), PcG proteins are found in many organisms and represent a conserved system of long-term gene inactivation. They are accompanied by a group of antagonists, the TRITHORAX GROUP (Trx) proteins, which are involved in gene activation (for review, see Schuettengruber and Cavalli, 2009). PcG proteins form large complexes, several of which are conserved in evolution. One of the key complexes acting in

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gene inactivation is POLYCOMB REPRESSIVE COMPLEX 2 (PRC2), which in *Drosophila* is composed of the four subunits Enhancer of Zeste [E(z)], Suppressor of Zeste [Su(z)12], Extra sex combs (Esc), and p55 (Czermin *et al.*, 2002; Müller *et al.*, 2002). In animals, PRC2 catalyses trimethylation of histone H3 on lysine 27 (H3K27me3). H3K27me3 may assist to recruit POLYCOMB REPRESSIVE COMPLEX 1 (PRC1) to target chromatin, which is then mono-ubiquitylated on lysine 119 of histone H2A (H2AK119ub) in mammals (lysine 118 in *Drosophila*) (for review, see Simon and Kingston, 2013). While PRC1 was initially considered to act strictly downstream of PRC2, recent data challenge this classical view of hierarchical recruitment of PcG complexes. PRC1 can be recruited to some targets in the absence of H3K27me3 (Tavares *et al.*, 2012), and presence of PRC2 and H3K27me3 at some genes is not followed by the recruitment of PRC1 (for reviews, see Lanzuolo and Orlando, 2012; Simon and Kingston, 2013). In *Drosophila*, H2AK118ub is required for the repression of only a subset of PcG protein targets (Gutierrez *et al.*, 2012). Furthermore, a complex involved in H2AK118 deubiquitylation is required for the repression of some PcG protein targets, suggesting that a balance between ubiquitylation and deubiquitylation of H2AK118 is needed for stable gene silencing (Scheuermann *et al.*, 2010).

The mechanism of transcriptional repression by PcG proteins is still enigmatic and is under active investigation. The H3K27me3 and H2AK118ub marks are thought not to change chromatin structure substantially on their own but to facilitate recruitment of other complexes, which might modulate chromatin structure or interfere with the transcription machinery. However, in *Drosophila*, the PRC1 complex does condense chromatin (Francis *et al.*, 2004), and chromatin at silent PcG target genes is more compact and less accessible than at active genes in animals and plants (Bell *et al.*, 2010; Grau *et al.*, 2011; Shu *et al.*, 2012). Moreover, compacted chromatin was shown to promote PRC2 activity, creating a positive feedback loop in the mechanism of PcG gene silencing (Yuan *et al.*, 2012). Thus, reducing DNA accessibility could be one of the mechanisms involved in gene silencing by PcG proteins. On the other hand, PcG proteins were suggested to block initiation and/or elongation of transcription (King *et al.*, 2002; Dellino *et al.*, 2004; Stock *et al.*, 2007; Chopra *et al.*, 2011; Enderle *et al.*, 2011; Chen *et al.*, 2012; Lehmann *et al.*, 2012). PcG proteins do not prevent binding of basic transcription factors (TBPs) to target genes (Lehmann *et al.*, 2012), potentially allowing rapid reactivation of PcG protein targets in particular developmental conditions, thus ensuring a dynamic and tight regulation of the PcG system.

Another challenge in understanding PcG protein function is the identification of mechanisms that recruit PcG proteins to the target genes. In *Drosophila*, PcG proteins are recruited to POLYCOMB RESPONSE ELEMENTs (PREs), which are characterized by a pattern of different sequence motifs recognized by various sequence-specific DNA-binding proteins (for review, see Beisel and Paro, 2011). For instance, the Pleiohomeotic Repressive complex (PhoRc), containing the sequence-specific DNA-binding protein PLEIOHOMEOTIC (PHO), plays a key role in PRC2 and PRC1 recruitment in *Drosophila* (for review, see Lanzuolo and Orlando, 2012). In mammals, the situation seems to be more complex and recruitment can be by several mechanisms. Computational analyses of sequences of PcG recruitment sites failed to identify any common DNA sequence motifs involved in PcG targeting. However, recently, several gene-specific PREs have been identified (for review, see Beisel and Paro, 2011). Another mechanism of PcG recruitment in mammals involves long non-coding RNAs (ncRNAs), acting *in cis*. The best-known example is X chromosome inactivation. Moreover, PcG proteins can be recruited by short ncRNAs acting *in cis* or by long intergenic ncRNAs acting *in trans* (for review, see Beisel and Paro, 2011). In mammals, sequences enriched in CpG nucleotides (CpG islands) overlap genome wide with H3K27me3. Nonmethylated CpG islands devoid of activators are suggested to recruit PcG proteins (for review, see Simon and Kingston, 2013). Similar to the situation in animals, diverse mechanisms may recruit PcG proteins in plants.

This review discusses the current understanding of gene silencing by PcG proteins in plants. We contrast the conserved and plant-specific features of the PcG system to discuss composition, function, and evolution of PRC1 and PRC2 complexes in plants and the mechanism of epigenetic inheritance in plant PcG protein function.

Evolutionary origins of the PcG system

The PcG system is an ancient gene-silencing machinery that exists not only in multicellular plants and animals but also in unicellular organisms such as *Tetrahymena thermofila.* This suggests that the PcG system of gene silencing evolved early in evolution, probably in the common ancestor of all eukaryotes (Liu *et al.*, 2007; Shaver *et al.*, 2010). Consequently, the absence of the PcG system from budding and fission yeast is most likely due to secondary gene loss (Liu *et al.*, 2007). Because the unicellular green algae *Chlamydomonas reinhardtii* lacks homologues of Su(z)12 and *Tetrahymena* lacks homologues of Esc and Su(z)12, the simplest PRC2 complex in unicellular organisms may contain only homologues of E(z) and p55 (Shaver *et al.*, 2010). Notably, Su(z)12 homologues, which are essential for fly, mammalian and *Arabidopsis* PRC2 function, are absent from nematodes (Ketel *et al.*, 2005). During evolution of the plant lineage, homologues for subunits of *Drosophila* PRC2 underwent multiple duplications, forming small gene families (Table 1). *Arabidopsis* has a single Esc homologue [FERTILIZATION INDEPENDENT ENDOSPERM (FIE)], three E(z) homologues [CURLY LEAF (CLF), SWINGER (SWN), and MEDEA (MEA)], three Su(z)12 homologues [EMBRYONIC FLOWER2 (EMF2), VERNALIZATION2 (VRN2), and FERTILIZATION INDEPENDENT SEED2 (FIS2)], and five p55 homologues [MULTICOPY SUPRESSOR OF IRA 1–5 (MSI1–5)] (Goodrich *et al.*, 1997; Grossniklaus *et al.*, 1998; Kiyosue *et al.*, 1999; Chanvivattana *et al.*, 2004; Hennig *et al.*, 2005). As evident from the variable copy number of homologues, diversification of PRC2 subunits occurred only recently in evolution, mostly even after the split of monocotyledonous and dicotyledonous plants. PRC1 subunits are much less conserved (Calonje and Sung, 2006; Sanchez-Pulido

Table 1. *Homologues of* Drosophila *PRC2 subunits in plants*

Values are the number of homologues. E(z), Enhancer of Zeste; Su(z)12, Suppressor of Zeste; Esc, Extra sex combs.

Species	*Drosophila* PRC2 subunits				References
	E(z)	Esc	Su(z)12	p55	
Arabidopsis	3	1	3	5	Ach *et al.*, 1997; Goodrich *et al.*, 1997; Grossniklaus *et al.*, 1998; Kiyosue *et al.*, 1999; Chanvivattana *et al.*, 2004; Katz *et al.*, 2004; Hennig *et al.*, 2005
Rice	2	2	2	3	Springer *et al.*, 2002; Hennig *et al.*, 2005; Luo *et al.*, 2009; Nallamilli *et al.*, 2013
Maize	3	2	2	3	Danilevskaya *et al.*, 2003; Hennig *et al.*, 2005; Haun *et al.*, 2007; Spillane *et al.*, 2007; Luo *et al.*, 2009
Moss (*Physcomytrella patens*)	1	1	3	2	Hennig and Derkacheva, 2009; Mosquna *et al.*, 2009; Okano *et al.*, 2009
Green alga (*Chlamydomonas reinhardtii*)	1	1	-	1	Hennig and Derkacheva, 2009; Shaver *et al.*, 2010

et al., 2008), suggesting that PRC2 was coupled independently to additional chromatin modifiers.

Genetic data suggest that not only PRC2 gene number but also biological function changed during development. The *Tetrahymena* E(z) homologue establishes H3K27me3 in heterochromatin in an RNA-interference-dependent manner, and H3K27me3 is needed for subsequent establishment of H3K9me3 at the same loci (Liu *et al.*, 2007). The *Chlamydomonas* E(z) homologue silences retrotransposons and transgenes (Shaver *et al.*, 2010). Thus, we hypothesize that the ancestral function of the PcG system was in defence responses against genomic parasites such as transposable elements. Only later, the PcG system may have been coopted for lineage specific functions such as developmental regulation in multicellular eukaryotes. This view is supported by findings in *Arabidopsis* that in the endosperm, a tissue with reduced DNA methylation, or in mutants for the DNA methyltransferase MET1, H3K27me3 is redistributed to heterochromatic sequences possibly resembling a reversion to a more ancient state (Weinhofer *et al.*, 2010; Deleris *et al.*, 2012).

In multicellular plants, PcG protein functions are mainly documented in developmental control. In the moss *Physcomytrella patens*, homologues of CLF or FIE repress heterochronic activation of sporophytic developmental programmes in gametophytes (Mosquna *et al.*, 2009; Okano *et al.*, 2009). In *Arabidopsis*, FIS2, MEA, FIE, and MSI1 repress heterochronic activation of sporophytic programmes in the female gametophyte (for review, see Köhler *et al.*, 2012). The function of PcG proteins in gene inactivation is not needed in developing *Arabidopsis* embryos but is indispensable during the embryo to seedling developmental transition (Bouyer *et al.*, 2011). Another well-documented phase change under PcG protein control is the transition to flowering, which is controlled by CLF, EMF2, and VRN2 in *Arabidopsis* and by an EMF2 homologue in rice (Yang *et al.* 1995; Chandler *et al.* 1996; Goodrich *et al.* 1997; Luo *et al.*, 2009). Vernalization in *Arabidopsis* is another well-characterized developmental process under control by PcG proteins (Zografos and Sung, 2012), but the PcG function in regulation of vernalization response evolved especially in Brassicaceaea, consistent with the absence of *VRN2* genes in other species (Luo *et al.*, 2009).

Similar to metazoan PRC2, plant PRC2 trimethylates H3K27 at target genes (Makarevich *et al.*, 2006; Schubert *et al.*, 2006; Jiang *et al.*, 2008; Bouyer *et al.*, 2011; Lafos *et al.*, 2011; Schmitges *et al.*, 2011; Derkacheva *et al.*, 2013). Unlike the situation in mouse and flies, however, H3K27me3 domains in plants are short and usually do not extend beyond single genes (for review, see Hennig and Derkacheva, 2009). Notably, lack of PcG proteins or H3K27me3 is not sufficient for upregulation of all genes that carry H3K27me3, suggesting that the presence of tissue-specific activators is necessary to trigger transcription (Takada and Goto, 2003; Farrona *et al.*, 2011; Derkacheva *et al.*, 2013). Thus, only genes with the potential to be expressed in a given tissue will be upregulated in plant PcG mutants. Genomic profiling has also revealed that about a quarter of all *Arabidopsis* genes is marked by H3K27me3, suggesting that PRC2 controls not only master regulators of development (Zhang *et al.*, 2007*a*; Oh *et al.*, 2008; Lafos *et al.*, 2011). More work is needed to uncover potential roles of PcG proteins in other physiological processes. To summarize, PcG gene silencing represents a conservative mechanism of gene inactivation, which is present already in unicellular organisms. In multicellular organisms, PcG proteins evolved as repressors of key developmental transitions.

Diversity of plant PRC2-like complexes

In contrast to *Drosophila*, which has one core PRC2 complex, at least three different PRC2 complexes are known to function at various developmental stages in *Arabidopsis*. The EMF complex regulates vegetative plant development and transition to flowering (Yoshida *et al.*, 2001; Schonrock *et al.*, 2006; Jiang *et al.*, 2008; Derkacheva *et al.*, 2013). The VRN complex regulates the vernalization response: i.e. the increased competence to flower after prolonged cold (Gendall *et al.*, 2001; Wood *et al.*, 2006; De Lucia *et al.*, 2008; Derkacheva *et al.*, 2013). The FIS complex regulates female gametophyte and seed development, preventing initiation of endosperm and seed development in the absence of fertilization (Spillane *et al.*, 2000; Yadegari *et al.*, 2000; Köhler *et al.*, 2003*a*; Wang *et al.*, 2006).

CLF and SWN are known to function in both the EMF and the VRN complex (Fig. 1). *CLF* and *SWN* are partially redundant but the strong developmental phenotype of *clf* and

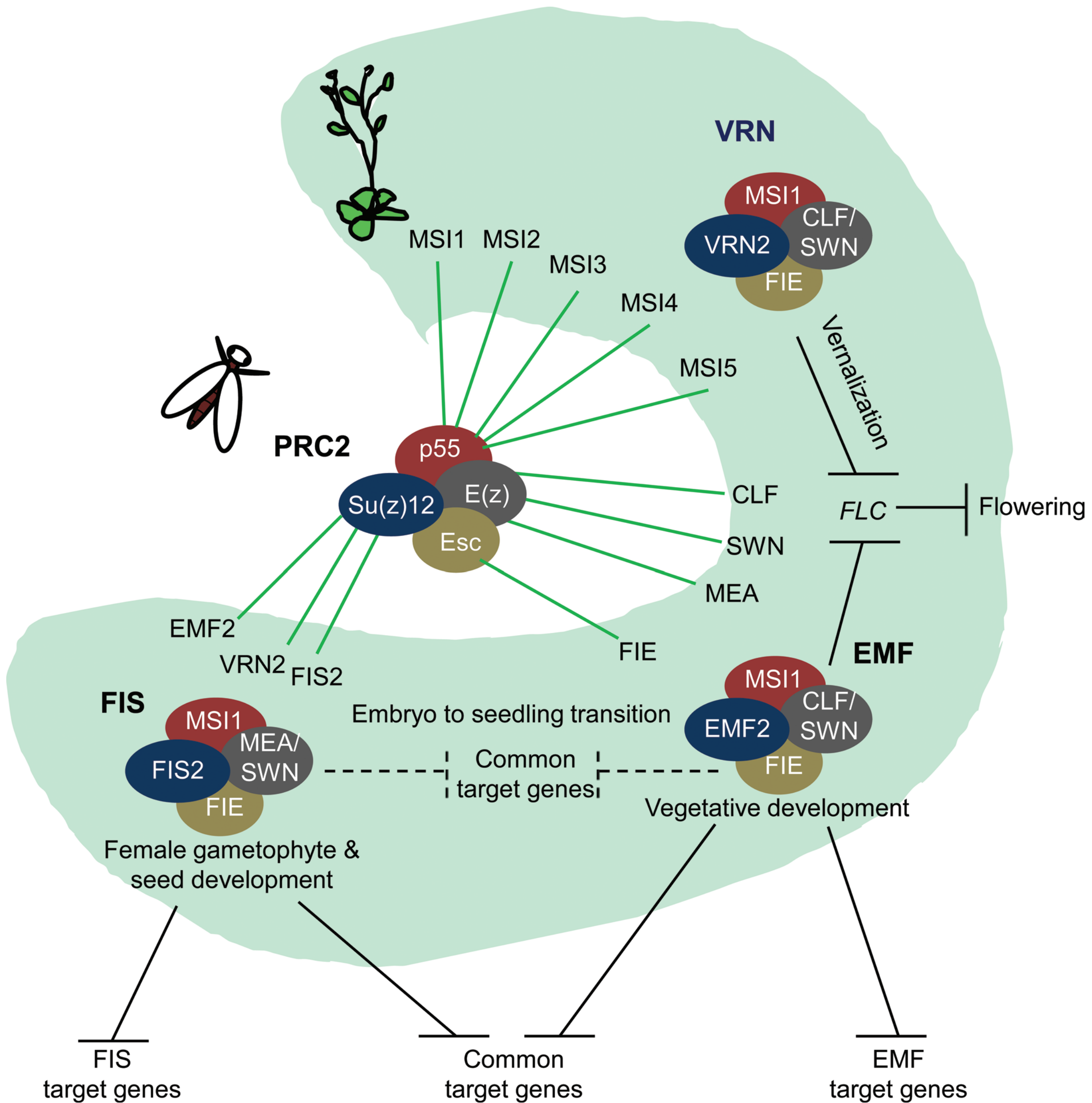

Fig. 1. *Arabidopsis* homologues of *Drosophila* POLYCOMB REPRESSIVE COMPLEX2 (PRC2) subunits form three PRC2-like complexes: EMBRYONIC FLOWER (EMF), VERNALIZATION (VRN), and FERTILIZATION INDEPENDENT SEED (FIS). These complexes have acquired specialized functions in plant development. Various PRC2-like complexes can regulate the same subset of Polycomb group targets at different developmental stages.

the absence of any obvious defects in *swn* has led to the idea that CLF is the most important E(z) homologue in the sporophyte (Chanvivattana *et al.*, 2004; Jiang *et al.*, 2008). SWN and MEA fail to complement *clf* mutants, suggesting unique molecular functions of CLF (Chanvivattana *et al.*, 2004). Notably, transcriptome and proteome data reveal that SWN is more abundant than CLF (Hruz *et al.*, 2008; Baerenfaller *et al.*, 2011) and is the main E(z) homologue purifying with the EMF and VRN complexes (De Lucia *et al.*, 2008; Derkacheva *et al.*, 2013). Currently, the reason for the discrepancy between genetic and biochemical data is not known. It is possible that CLF but not SWN is easily lost during PRC2 purification. CLF has been shown to be regulated at the protein level (Jeong *et al.*, 2011), so it might associate only with the active PRC2 complex and be unstable when PRC2 activity is low, while SWN is always stably bound. Alternatively, higher histone methyltransferase activity of CLF could compensate its lower abundance. Finally, it is possible that CLF has also PRC2-independent functions. Clearly, more work on the molecular functions of CLF and SWN is needed. MEA is the main histone methyltransferase functioning in the FIS complex *in vivo* (Köhler *et al.*, 2003*b*; Makarevich *et al.*, 2006). SWN interacts with FIS2 *in vitro* and in yeast two-hybrid assays. The *mea* mutant phenotype is strongly enhanced by *swn*, suggesting that SWN can partly compensate the lack of MEA in the FIS complex (Wang *et al.*, 2006).

The three homologues of Su(z)12—EMF2, VRN2, and FIS2—are most divergent and bestow partially specialized functions on the corresponding PRC2 complexes. Loss of EMF2 causes plants to skip the vegetative phase of development and flower directly from the germinated embryo stage (Yang *et al.*, 1995). Thus, EMF2 is an indispensable subunit of the EMF complex, and VRN2 fails to complement loss of EMF2. In turn, VRN2 is essential for the function of the VRN complex in the vernalization response (Gendall *et al.*, 2001; De Lucia *et al.*, 2008). Nevertheless, EMF and VRN complexes are both involved in *FLC* repression (Gendall *et al.*, 2001; Jiang *et al.*, 2008), and EMF2 and VRN2 both repress seed coat formation in unfertilized ovules (Roszak and Köhler, 2011) (Fig. 1). *FIS2* is an imprinted gene that is maternally expressed in the central cell of the female gametophyte and in the endosperm (Luo *et al.*, 2000; Wang *et al.*, 2006). FIS2 is an indispensable subunit of the FIS complex, and EMF2 and VRN2 cannot substitute it (Chanvivattana *et al.*, 2004; Roszak and Köhler, 2011). Nevertheless, the FIS and the EMF complex share target genes, which they repress during gametogenesis and early seed development and during sporophytic development, respectively (Makarevich *et al.*, 2006) (Fig. 1).

FIE and MSI1 are both essential subunits of all three PRC2 complexes in *Arabidopsis* (Hennig *et al.*, 2003; Köhler *et al.*, 2003*a*; De Lucia *et al.*, 2008; Derkacheva *et al.*, 2013) (Fig. 1). In contrast to *Drosophila* p55, which is not essential for *in vitro* enzymatic activity of PRC2, and *Neurospora crassa* p55, which is dispensable for most H3K27me3 *in vivo*, *Arabidopsis* MSI1 is needed for wild-type levels of H3K27me3 at EMF target genes and is essential for the repression of PcG targets (Schmitges *et al.*, 2011; Derkacheva *et al.*, 2013; Jamieson *et al.*, 2013). Moreover, MSI2–5 do not act redundantly with MSI1 in gene silencing by PcG proteins.

Taken together, the homologues of PRC2 subunits in *Arabidopsis* acquired some functional specialization; however, different PRC2 complexes often share subsets of target genes, regulating them at different developmental stages. All PRC2 complexes share the FIE and MSI1 subunits. EMF2, VRN2, and FIS2 are functionally most divergent (Chen *et al.*, 2009) and are possibly involved in specific interactions with other proteins and targeting of PRC2. Similarly, CLF, SWN, and MEA are not fully redundant, suggesting specific molecular interactions of these proteins.

Modifying PRC2 function: PRC2-associated proteins

In *Drosophila*, the Polycomb-like protein associates with the core PRC2 complex forming Pcl–PRC2. Lack of Pcl–PRC2 does not affect H3K27 mono- and dimethylation, but dramatically reduces trimethylation levels, leading to derepression of PcG targets (Nekrasov *et al.*, 2007). Similar, a mammalian Pcl–PRC2 complex is responsible for trimethylation of H3K27 and contains a homologue of Pcl, the PHD finger protein PHF1 (Cao *et al.*, 2008; Sarma *et al.*, 2008). PRC2 complexes in *Arabidopsis* can also associate with PHD finger proteins. The VRN complex together with VERNALIZATION INSENSITIVE 3 (VIN3), VRN5, and VIN3-like1 (VEL1) forms the VRN–PHD complex (De Lucia *et al.*, 2008) (Fig. 2). VRN–PHD is required for high H3K27me3 levels at *FLC* chromatin, spreading of H3K27me3 over the *FLC* locus and for the vernalization response (Fig. 1). Recent data suggest that VRN5 and VEL1 function mainly with the VRN and not with the EMF complex (Derkacheva *et al.*, 2013). Although purification of the EMF complex did not reveal any associated PHD-finger proteins (Derkacheva *et al.*, 2013), it is possible that these proteins escaped detection by mass spectrometry (Lubec and Afjehi-Sadat, 2007). Notably, the rice PHD finger protein VIN3-like2 (OsVIL2) interacts with one of the EMF2 homologues, EMF2b (Yang *et al.*, 2013). OsVIL2 is required for the repression and wild-type levels of H3K27me3 at *FUSCA3-LIKE 1* (*OsLFL1*) (Fig. 2). Thus, the association of PHD-finger proteins with PRC2 complexes seems to be conserved in evolution but may be required for the trimethylation catalytic activity of PRC2 only at certain loci or for particular combinations of PRC2 subunit.

Recently, several proteins have been identified that interact with CLF. The plant-specific protein BLISTER (BLI) is required for the repression of a subset of PcG targets (Schatlowski *et al.*, 2010) (Fig. 2). BLI is not needed for the establishment of wild-type H3K27me3 levels at PcG targets demonstrating that it does not affect the catalytic activity of PRC2. Double mutants of *bli* with *clf* or *lhp1* show some synergistic effects, suggesting that these proteins function in the same pathway. However, *bli* mutant plants exhibit developmental phenotypes not associated with known PcG functions, and *bli clf* and *bli lhp1* double mutants show additive effects, demonstrating that BLI also acts independently of PRC2 (Schatlowski *et al.*, 2010). Thus, the mechanism of BLI function in transcriptional repression by PcG proteins still has to be uncovered. TBP-Associated Factor TAF13 was reported to interact with SWN and MEA, and the *taf13* mutation caused seed defects, including embryo arrest and overproliferation of the chalazal endosperm similar to *fis* mutants (Lindner *et al.*, 2013). Another protein found to bind CLF is the F-box protein UPWARD CURLY LEAF1 (UCL1) (Jeong *et al.*, 2011). UCL1 overexpression reduces CLF levels and H3K27me3 at PcG target genes, demonstrating that UCL1 negatively regulates CLF. Moreover, UCL1 interacts *in vivo* with components of an E3 ligase complex, suggesting that it promotes CLF degradation through the ubiquitin-26S proteasome pathway (Jeong *et al.*, 2011). Another study demonstrated that CLF associates with the cullin ring ubiquitin ligase CUL4–DDB1 and MSI4 in the same complex (Pazhouhandeh *et al.*, 2011). Lack of MSI4 or CUL4–DDB1 complex increases *FLC* expression and decreases H3K27me3 levels at *FLC* and *FT* chromatin (Fig. 2). Moreover, CUL4 and MSI4 are recruited to the *FLC* locus. Because MSI1 is an indispensable subunit of the EMF, VRN, and FIS complexes (Derkacheva *et al.*, 2013) and because the MSI4 sequence is greatly diverged from other p55 homologues in plants and animals (Hennig *et al.*, 2005), we propose that MSI1 functions as a core subunit of the EMF complex and

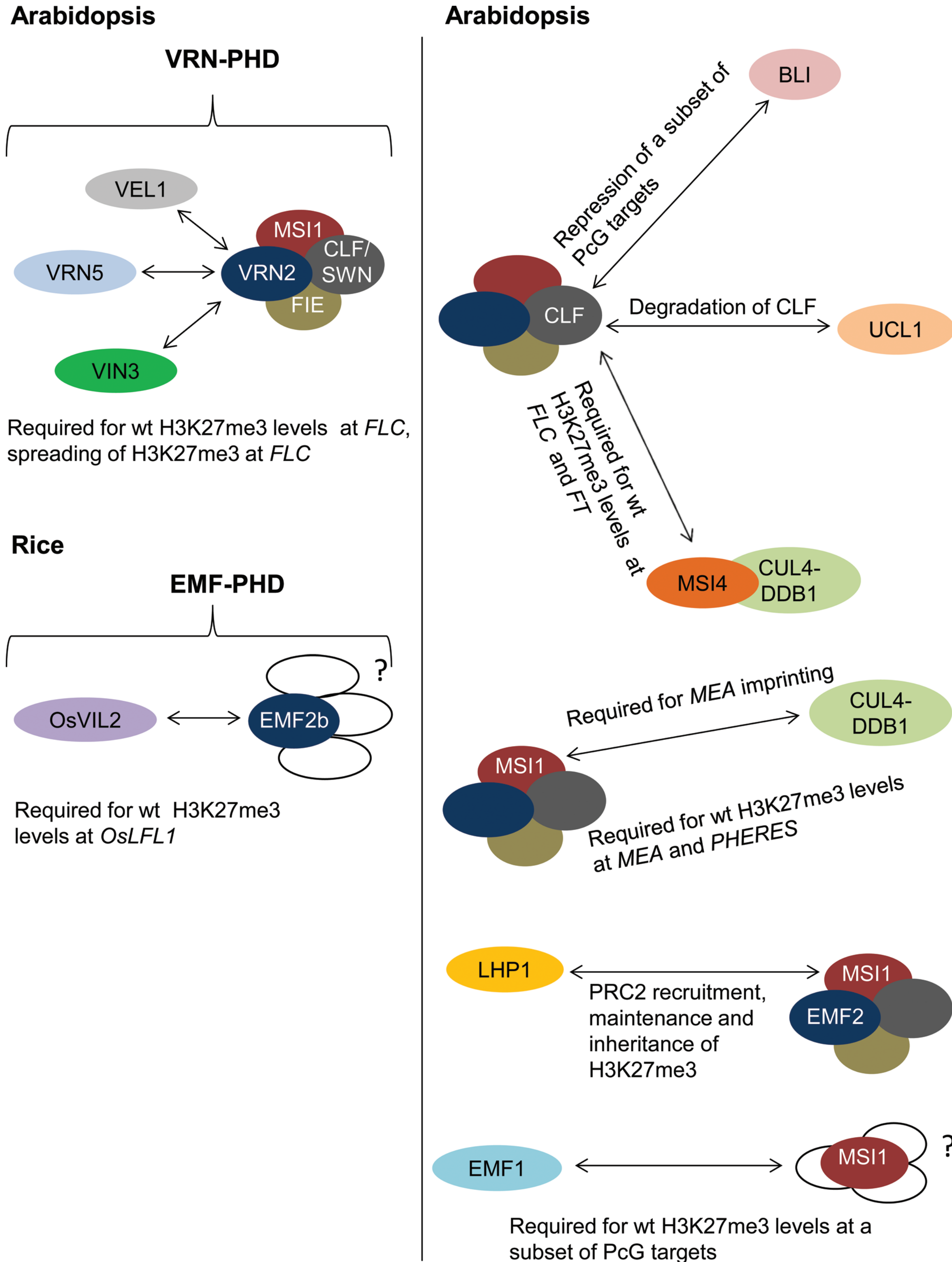

Fig. 2. Proteins interacting with POLYCOMB REPRESSIVE COMPLEX2 subunits in *Arabidopsis* and rice. Named proteins in the complex are responsible for the interaction, and known functions of the interactions are stated.

that MSI4 associates with this complex to repress specific target genes either as part of a histone deacetylase complex or a CUL4–DDB1 complex (Ausin *et al.*, 2004; Gu *et al.*, 2011; Pazhouhandeh *et al.*, 2011). The molecular function of CUL4–DDB1 in transcriptional repression by PcG proteins is not clear. Interestingly, in human cells, CUL4–DDB1^{DDB2} monoubiquitylates histone H2A when DNA is damaged (Kapetanaki *et al.*, 2006). Considering that CUL4 is recruited to *FLC* chromatin, it could therefore be required for ubiquitylation of histones or chromatin-associated proteins. In addition to binding MSI4, CUL4–DDB1 associates with MSI1 and is required for wild-type H3K27me3 levels at the FIS

complex target genes *MEA* and *PHERES1* (Dumbliauskas *et al.*, 2011). Moreover, *cul4* mutants exhibit autonomous endosperm development in the absence of fertilization resembling *fis* class mutants (Dumbliauskas *et al.*, 2011). Thus, CUL4–DDB1 is involved in the regulation of subset of PcG protein target genes. Future experiments need to identify PcG protein target genes that are bound by CUL4–DDB1 and how CUL4–DDB1 affects gene silencing by PcG proteins.

EMBRYONIC FLOWER1 (EMF1), a plant-specific protein with no homology to known PcG proteins, regulates vegetative plant development in a manner similar to PcG proteins (Aubert *et al.*, 2001). The *emf1* mutant phenotype resembles that of *emf2* (Sung *et al.*, 1992; Yang *et al.*, 1995). EMF1 interacts with MSI1 *in vitro* (Calonje *et al.*, 2008), suggesting that it could be associated with PRC2 or one of the other complexes that contain MSI1 (for review, see Hennig *et al.*, 2005) (Fig. 1). Thus, EMF1 association with PRC2 still awaits confirmation *in vivo*. Recently, EMF1 was shown to be recruited to genes with high levels of H3K27me3 and to be required for normal H3K27me3 levels at a subset of such genes (Kim *et al.*, 2012). However, EMF1 is also present at a subset of genes that are not PcG protein targets, revealing a function independentlt of the PcG system.

Recently, LIKE HETEROCHROMATIN PROTEIN1 (LHP1) was found to interact directly with MSI1 and to associate into the same complex with MSI1 and EMF2 *in vivo* (Derkacheva *et al.*, 2013). The consequences of LHP1 interaction with the EMF complex will be discussed later. Additional proteins have been reported to affect PcG function but are not known to interact with PcG proteins and because of space constraints are not discussed here. To summarize, recent studies found many PcG-associated proteins that affect to a different extend function of PcG system. These proteins might represent a system of regulation of PcG function, modifying recruitment, stability or activity of PcG proteins. Future studies will reveal in more detail the mechanism of function of PcG-associated proteins and the output of these interactions.

PRC1 in *Arabidopsis*: a matter of definition

Originally, PRC1 was identified in *Drosophila* as a core complex containing four main subunits: POLYCOMB (PC), POLYHOMEOTIC (PH), POSTERIOR SEX COMBS (PSC), and RING (Francis *et al.*, 2001; Mohd-Sarip *et al.*, 2002). PC binds to H3K27me3 (Fischle *et al.*, 2003) and RING catalyses H2AK118ub (de Napoles *et al.*, 2004; Wang *et al.*, 2004). PRC1 is known to compact chromatin, inhibit chromatin remodelling, and repress transcription *in vitro* (King *et al.*, 2002; Francis *et al.*, 2004). A C-terminal region of PSC is required for these effects (Francis *et al.*, 2001, 2004; King *et al.*, 2005; Beh *et al.*, 2012). Later, another complex called RING ASSOCIATED FACTORS (dRAF) was also shown to catalyse H2AK118ub in *Drosophila* (Lagarou *et al.*, 2008). This complex shares the RING and PSC subunits with PRC1 and in addition includes histone lysine demethylase dKDM2. Thus, this complex couples H3K36me2 demethylation—i.e. the removal of an active mark from chromatin—and H2A118 ubiquitylation. In mammals, homologues of *Drosophila* PRC1 subunits are encoded by small gene families (for review, see Lanzuolo and Orlando, 2012). Specific combinations of these subunits give rise to various PRC1 complexes with distinct localization and function (for review, see Luis *et al.*, 2012). Recently, a new PRC1 complex was discovered that contains KDM2B and binds to unmethylated CpG islands (Farcas *et al.*, 2012; Wu *et al.*, 2013). This complex promotes H2A119 ubiquitylation and lacks any homologues of PC. Thus, targeting of this complex seems to be entirely independent of H3K27me3.

In plants, the absence of clear homologues of the animal main PRC1 subunits made it difficult to identify a PRC1-like plant complex. LHP1, a homologue of metazoan HETEROCHROMATIN PROTEIN1 (HP1) that is also known as TERMINAL FLOWER 2, binds to H3K27me3 *in vitro* and colocalizes with H3K27me3 genome-wide *in vivo* (Gaudin *et al.*, 2001; Turck *et al.*, 2007; Zhang *et al.*, 2007*b*). Interestingly, in *Drosophila*, PC binding is restricted to PREs but H3K27me3 spreads over large domains including transcribed and regulatory regions, suggesting that H3K27me3 is not sufficient to recruit PC *in vivo* (Schwartz *et al.*, 2006). In plants, H3K27me3 domains are restricted to transcribed regions of the genes and do not occupy large genome regions. In contrast to PC, LHP1-binding sites largely coincide with H3K27me3 domains (Turck *et al.*, 2007; Zhang *et al.*, 2007*b*). Furthermore, LHP1 is needed for the repression of PcG target genes, and recent analyses have shown that the same subset of genes is regulated by LHP1 and CLF (Kotake *et al.*, 2003; Libault *et al.*, 2005; Exner *et al.*, 2009; Derkacheva *et al.*, 2013). The specific recognition of H3K27me3 is required for LHP1 function (Exner *et al.*, 2009). Together, LHP1 was suggested to fulfil in plants a role similar to animal PC.

Two homologues of mammalian RING1A/B—AtRING1A and AtRING1B—and three homologues of BMI1—AtBMI1A, AtBMI1B, and AtBMI1C—were identified in *Arabidopsis* based on sequence similarity and organization of protein domains (Sanchez-Pulido *et al.*, 2008). The AtRING1A/B and AtBMI1A/B proteins mediate H2A monoubiquitylation (H2AK121ub) *in vitro*, and AtBMI1A/B are mainly responsible for H2AK121ub in seedlings *in vivo* (Bratzel *et al.*, 2010). In yeast two-hybrid assays, RING1A binds to RING1B, and both proteins have a redundant function in the repression of class I KNOX genes (Xu and Shen, 2008). RING1A also interacts with CLF in the yeast two-hybrid assay and *in vitro* (Xu and Shen, 2008). Similar, AtBMI1A and AtBMI1B function redundantly in repression of embryonic and stem cell regulators (Bratzel *et al.*, 2010; Yang *et al.*, 2013). Interestingly, AtBMI1A/B ubiquitylate the DREB2A protein in response to water stress and thus promote its degradation by the 26S proteasome (Qin *et al.*, 2008), suggesting that AtBMI1A/B could also regulate certain PcG proteins by targeting them for degradation. AtBMI1C is expressed in the endosperm, root, and stamen, and it acts redundantly with AtBMI1A/B when it is coexpressed in the same tissue (Li *et al.*, 2011; Bratzel *et al.*, 2012). AtBMI1C interacts with AtRING1A/B and is involved in regulation of

flowering time (Bratzel *et al.*, 2010; Li *et al.*, 2011; Yang *et al.*, 2013). AtRING1A/B and AtBMI1A/B interact *in vitro* with both LHP1 and EMF1 (Xu and Shen, 2008; Bratzel *et al.*, 2010). In the *emf1* mutant, H2Aub is strongly decreased, supporting the view that EMF1 could function together with RING proteins *in vivo* (Bratzel *et al.*, 2010). Similar to *Drosophila*, where H2AK118ub is required for the repression of only a subset of PcG targets, RING proteins and H2Aub in *Arabidopsis* are also needed for the repression of only a subset of PcG targets (Xu and Shen, 2008; Bratzel *et al.*, 2010; Gutierrez *et al.*, 2012; Yang *et al.*, 2013). It is therefore of great interest to establish genome-wide profiles of H2Aub to test the overlap with H3K27me3. In *Drosophila*, most of PcG targets lack H2A118ub, suggesting that other mechanisms are involved in repression of these genes (Gutierrez *et al.*, 2012). In animals, PRC1 has the ability to compact chromatin. In *Drosophila*, PSC is responsible for chromatin compaction, while in mammals the homologues of PC have this function (for review, see Simon and Kingston, 2013). Compacted chromatin can stimulate PRC2 H3K27 trimethylation activity, creating a positive feedback and stimulating recruitment of PC containing PRC1, which can in turn promote chromatin compaction (Yuan *et al.*, 2012). Chromatin compaction at PcG targets does neither require H2Aub nor the function of RING proteins, suggesting that independent mechanisms are involved in gene silencing by PcG proteins. In *Arabidopsis*, EMF1 was suggested to fulfil the function of the C-terminal PSC domain, promoting chromatin compaction and inhibiting chromatin remodelling (Beh *et al.*, 2012).

What, then, is a PRC1-like complex in plants: a complex that binds H3K27me3, a complex that promotes H2A ubiquitylation, or a complex that is involved in chromatin compaction? In *Drosophila*, only the complex that binds to H3K27me3 and catalyses H2AK118 ubiquitylation is called PRC1. In mammals, the PRC1 definition embraces also the complex that does not have H3K27me3-binding activity. Because metazoan RAWUL domains appear to be restricted to proteins in PRC1 complexes, we propose to consider only complexes with RAWUL-domain proteins as PRC1 related in plants. We argue that the substantial differences in subunit conservation of plant PcG protein complexes should be reflected in terminology, and we propose the terms PRC2-like and PRC1-related complexes. It will be important to establish the *in vivo* subunit composition of the RING1A/B and AtBMI1A/B PRC1-like complexes and their functions. It will also be important to purify EMF1 and LHP1 complexes and establish their relation to the PRC1-related and PRC2-like complexes.

Mechanisms of PcG protein recruitment

Similar to *Drosophila* PREs, several DNA sequences were found in *Arabidopsis* that are able to recruit PRC2. Plant PcG-recruiting sequences are in the proximity or even inside the gene body (Sieburth and Meyerowitz, 1997; Schubert *et al.*, 2006; Berger *et al.*, 2011; Helliwell *et al.*, 2011). Such position of plant PREs reflects in general the organization of the *Arabidopsis* genome, which lacks long intergenic regions. A PRE-like sequence from *AGAMOUS* (*AG*) fused to the *β-GLUCURONIDASE* (*GUS*) sequence is sufficient for establishing and spreading of H3K27me3 into the *GUS* sequence in a CLF-dependent manner (Schubert *et al.*, 2006). Similarly, a PRE-like element in the promoter of *LEAFY COTELYDON2* (*LEC2*) is required for *LEC2* inactivation and promotes deposition of H3K27me3 and repression of transcription at reporter genes (Berger *et al.*, 2011). Dissection of the *FLC* sequence to identify elements involved in regulation of silencing revealed distinct sequences that are needed for initial *FLC* repression and for the maintenance of silencing after vernalization (Sheldon *et al.*, 2002; Buzas *et al.*, 2011; Helliwell *et al.*, 2011). When inserted into the genome, PRE-like sequences that are needed for maintenance of *FLC* repression trigger H3K27me3 deposition at neighboring loci and silence a fused transgene during the vernalization response (Finnegan *et al.*, 2004; Sheldon *et al.*, 2009; Buzas *et al.*, 2011).

In *Drosophila*, PcG proteins are recruited to PREs by different transcription factors (for review, see Beisel and Paro, 2011). In *Arabidopsis*, the EMF complex is recruited to *BREVIPEDICELLUS* (*BP*) and *KNAT2* chromatin by interaction with the MYB-type transcription factors ASYMMETRIC LEAVES1 (AS1) and AS2 (Lodha *et al.*, 2013). Moreover, a promoter region containing AS1/AS2-binding sites fused to *35S:GFP-GUS* triggers H3K27me3 modification in *GFP-GUS* chromatin. This is the first example of PRC2 recruitment by transcription factors to a PRE-like element in plants. In *Drosophila*, GAGA factors (GAF), which bind $(GA)_n$ sites, are among the transcription factors implicated in PcG recruitment (Ringrose *et al.*, 2003). Recently, genome-wide profiling of FIE-binding sites in *Arabidopsis* showed strong enrichment for GAGA-binding sites, suggesting that $(GA)_n$-binding proteins can play a role in recruitment of PcG proteins in plants as well (Deng *et al.*, 2013). Plants do not have homologues of *Drosophila* GAF but a group of BASIC PENTACYSTEINE (BPC) transcription factors that bind $(GA)_n$ sites evolved independently in plants (Meister *et al.*, 2002; Sangwan *et al.*, 2002). This is similar to mammals, where $(GA)_n$ sites are bound by proteins that are distinct from the insect GAF proteins (Ringrose and Paro, 2007). Thus, it is possible that GA-repeat sequences were independently targeted by newly evolving transcription factors during the evolution of mammals, plants and insects. Intriguingly, BPCs bind to some PcG target genes such as *SEEDSTICK*, *SEP3*, and *AG* and are required for their repression (Kooiker *et al.*, 2005; Simonini *et al.*, 2012). It remains to be tested whether BPC binding contributes to PcG protein recruitment. In contrast to *Drosophila*, where PcG proteins bind mainly in the promoter regions, which are depleted of H3K27me3, FIE-binding sites localize inside the coding sequences and overlap with H3K27me3. These data correlate with the localization of some plant PREs inside the coding sequences of the genes. Together, plant gene-specific PREs are consistent with the canonical function of *Drosophila* PREs (Table 2).

Recruitment via interaction with transcription factors has also been proposed for LHP1: this protein interacts with the

Table 2. *PRC2 recruitment to the target genes in animals and plants*

Gene	Mammals[a]	*Drosophila*[b]	*Arabidopsis*
PREs	+	+	+[c]
lncRNA *in cis*	+		+[d]
Short ncRNA *in cis*	+		
Long transgenic ncRNA *in cis*	+		
CpG islands	+	–	–

[a]For a review, see Simon and Kingston, 2013; Lanzuolo and Orlando, 2012.
[b]For a review, see Schuettengruber and Cavalli, 2009; Ringrose and Paro, 2007.
[c]Berger *et al.*, 2011; Buzas *et al.*, 2011; Helliwell *et al.*, 2011; Sheldon *et al.*, 2009; Schubert *et al.*, 2006; Finnegan *et al.*, 2004; Sieburth and Meyerowitz, 1997.
[d]Heo and Sung, 2011.

transcription factor SCARECROW (SCR) and is recruited to the *SCR* target gene *MAGPIE* (Cui and Benfey, 2009). LHP1 also interacts with CYCLOPHILIN AtCYP71, and AtCYP71 is required for LHP1 targeting (Li *et al.*, 2011). In *cyp71* mutant plants, H3K27me3 levels are reduced at a subset of PcG targets, suggesting that AtCYP71 also affects PRC2 function. It is not clear why LHP1 targeting is affected in *cyp71* plants: it could be because of reduced H3K27me3 levels or because of the disrupted LHP1–AtCYP71 interaction. Furthermore, LHP1 interacts with transcription factor SHORT VEGETATIVE PHASE (SVP), and LHP1 recruitment to *SEPALATA3* (*SEP3*) largely depends on this interaction (Liu *et al.*, 2009). Recently, LHP1 was shown to interact with the MSI1 and EMF2 subunits of the EMF complex, revealing an LHP1–PRC2 interplay at the protein level (Derkacheva *et al.*, 2013). Thus, LHP1 could be first recruited to some target genes via interaction with transcription factors and afterwards recruit PRC2 (Fig. 3A). In accordance with this hypothesis, the level of H3K27me3 is highly reduced at the *SEP3* locus in *lhp1* plants, suggesting that LHP1 is required for the PRC2 recruitment to *SEP3* (Liu *et al.*, 2009).

Another mechanism of PcG recruitment to plant target genes involves long ncRNAs (lncRNAs). CLF can be recruited to *FLC* by COLDASSISTED INTRONIC NONCODING RNA (COLDAIR), which results from sense transcription within the large second intron of the *FLC* locus (Heo and Sung, 2011). This lncRNA associates with CLF and SWN *in vitro* and with CLF *in vivo*. Similarly, in mammals, homologues of E(z) bind lncRNA *in vitro* (for review, see Simon and Kingston, 2013). Lack of COLDAIR reduces the enrichment of CLF at *FLC* chromatin. Thus, PcG recruitment by lncRNAs to target genes emerges as an evolutionary conserved mechanism (Table 2). Interestingly, LHP1 also interacts with the RNA-binding protein LHP1 INTERACTING FACTOR2 (LIF2) (Latrasse *et al.*, 2011). However, the consequences of this interaction are not clear, as LIF2 may antagonize or assist LHP1 function. LHP1 targeting to *FLC* does not require LIF2. However, it is still tempting to speculate that PRC2 and LHP1 targeting to some other PcG targets could depend on LIF2 bound to specific RNAs. Future studies will have to reveal the functional details of the LHP1–LIF2 interaction.

Does PRC1 take the lead?

In animals, PRC1 can be recruited to the target genes via interaction with transcription factors (for review, see Simon and Kingston, 2013). AtBMI1A/B/C interact with the VAL (VP1/ABI3-LIKE) 1 transcription factor and act in the same pathway to repress the seed maturation programme after germination (Yang *et al.*, 2013). Surprisingly, H2Aub levels at seed maturation genes are not affected in *clf swn* double mutant, suggesting that PRC2 function is not required for the recruitment and function of AtBMI1A/B/C proteins. Deposition of H3K27me3 at a subset of seed maturation genes depends on AtBMI1A/B/C and VAL1/2 function (Yang *et al.*, 2013). These data challenge the classical view of hierarchical recruitment of PcG protein complexes. For the seed maturation genes, PRC1-related AtBMI1A/B/C is considered to be recruited before PRC2 (Fig. 3B). In *val1/2* mutants, the level of H2Aub is strongly reduced, suggesting that either VAL1/2 proteins function together with AtBMI1A/B/C in a PRC1-related complex or they are required for the recruitment of AtBMI1A/B/C. Taken into account that LHP1 interacts with the EMF complex, it will be of high interest to check whether recruitment of PRC2-like complexes to these genes depends on LHP1. Interestingly, only a subset of LHP1 targets is marked by H2Aub, demonstrating that targeting of LHP1 and AtBMI1 complexes is distinct and that recruitment of LHP1 is not sufficient to promote deposition of H2Aub. Thus, LHP1 can function together or independently of RING proteins, supporting the hypothesis that diverse complexes with PRC1-like functions exist in plants.

To conclude, plant PRC2-dependent H3K27me3 can serve as landing pad for other PcG protein complexes but plant PRC1-related complexes can also be recruited independently of PRC2. In addition, H2Aub, a hallmark of metazoan PRC1 activity, may even serve to recruit plant PRC2-like complexes. Thus, instead of sequentially recruited protein complexes acting in a linear pathway, the plant PcG system should be viewed as a network of dynamically interacting and cofunctioning protein complexes.

Maintaining H3K27me3: new rules, old players

During interphase, H3K27me3 could be diluted due to histone turnover or active histone demethylation. In *Arabidopsis*, RELATIVE OF EARLY FLOWERING6 (REF6, also called Jumonji-domain-containing protein 12) demethylates H3K27me2/3 (Lu *et al.*, 2011). REF6 mutations lead to increased H3K27me3 levels and decreased expression of many genes, demonstrating that active demethylation is required for wild-type gene expression levels (Lu *et al.*, 2011). Overexpression of REF6 produces similar phenotypes to PcG mutants, suggesting that a balance between methylation and demethylation is important.

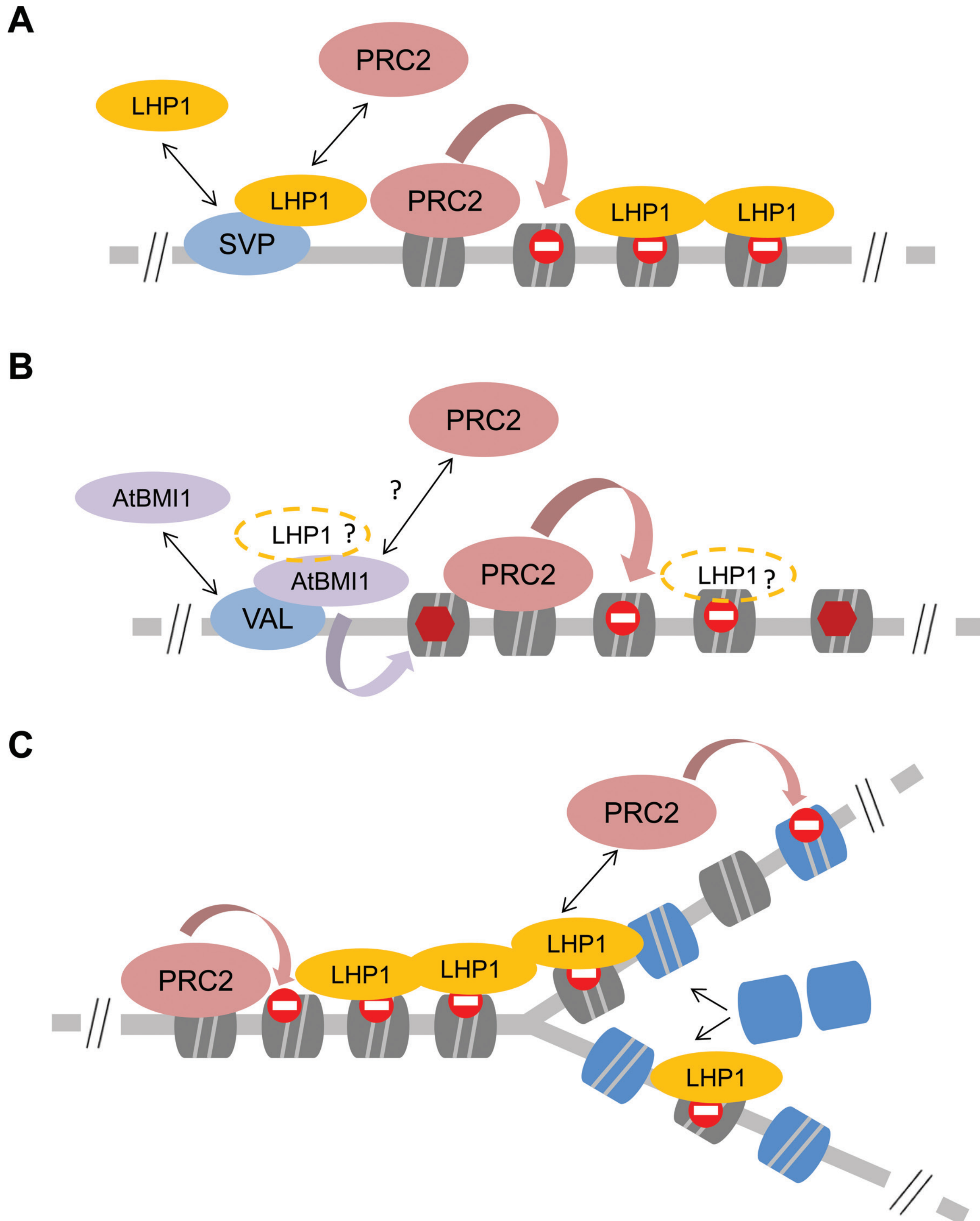

Fig. 3. Models of functional interactions of *Arabidopsis* Polycomb group proteins at different target genes. (A) Recruitment of LHP1 by transcription factors could direct PRC2 to target genes to promote H3K27me3, which assist to retain LHP1 in place. (B) AtBMI1 proteins could be recruited to target genes by transcription factors and then, via LHP1-dependent or -independent ways, recruit PRC2. AtBMI1 proteins catalyse H2Aub at the target locus and PRC2 catalyses H3K27me3; it is not clear whether H2Aub or H3K27me3 precede the other mark, or whether both are inserted simultaneously. (C) During DNA replication, newly synthesized nucleosomes are incorporated into chromatin leading to the dilution of epigenetic marks; LHP1 can bind H3K27me3 at the pre-existing nucleosomes (grey) and recruit PRC2 to catalyse H3K27me3 at newly incorporated nucleosomes (blue) ensuring epigenetic inheritance during mitosis; stop-signal symbols represent H3K27me3; red hexagons represent H2Aub.

Another critical step in PcG silencing is an epigenetic inheritance during DNA replication. In mammals, an ESC homologue can bind to H3K27me3, suggesting a positive feedback loop and self-recruitment mechanism for PRC2 (Margueron *et al.*, 2009). In *Drosophila*, *in vitro* studies showed that PRC1 associates with chromatin during DNA replication (Francis

et al., 2009; Lo *et al.*, 2012). In *Arabidopsis*, recent evidence of LHP1 association with the EMF complex suggested that LHP1 could rerecruit PRC2 to target genes to reestablish prereplication H3K27me3 levels after mitosis (Derkacheva *et al.*, 2013) (Fig. 3C). In accordance with this hypothesis, LHP1 is highly expressed in dividing cells and interacts with the catalytic subunit of DNA polymerase epsilon, EARLY IN SHORT DAYS7 (ESD7) (Kotake *et al.*, 2003; del Olmo *et al.*, 2010; Baerenfaller *et al.*, 2011). Controversial data exist about LHP1 interaction with the catalytic subunit of DNA polymerase α, INCURVATA2 (ICU2) (Barrero *et al.*, 2007; Hyun *et al.*, 2013). Previously, *in vitro* GST-pulldown experiments showed a direct LHP1–ICU2 binding but subsequently this could not be confirmed by yeast two-hybrid or bimolecular fluorescence complementation assays. Nevertheless, lack of ICU2 leads to defects in mitotic maintenance of vernalization memory while initial CLF recruitment and deposition of H3K27me3 at *FLC* during vernalization are not changed (Hyun *et al.*, 2013). In *icu2* mutants, maintenance of CLF and H3K27me3 at *FLC* chromatin is affected, leading to mosaic *FLC* derepression after vernalization (Hyun *et al.*, 2013). Importantly, LHP1 recruitment to *FT* and *AG* is severely affected in *icu2* plants, suggesting that LHP1 recruitment to *FLC* could be affected as well. Moreover, *FLC* is initially silenced during vernalization in *lhp1* plants but derepressed upon return to warm conditions, demonstrating that LHP1 is required for the maintenance of *FLC* repression through mitotic divisions (Mylne *et al.*, 2006; Sung *et al.*, 2006). Thus, lack of LHP1 at *FLC* could affect PRC2 rerecruitment and lead to failure in H3K27me3 maintenance. Strikingly, H3K27me3 levels were strongly reduced in highly dividing cells of *lhp1* mutant, supporting the idea that LHP1 is required for the inheritance of wild-type H3K27me3 levels (Derkacheva *et al.*, 2013). Taken together, these data suggest that the LHP1–PRC2 interaction plays a key role in inheritance of H3K27me3 during mitotic divisions. It remains to be tested whether direct binding of PRC2-like complexes to H3K27me3 via ESC homologues is conserved in plants. It is possible that multiple recruitment and self-recruitment mechanisms establish robustness for gene repression by PcG proteins.

It seems that propagation of H3K27me3 marks differs between species. In *Drosophila*, PHO, E(z), and PC are recruited to their target genes in early S-phase, transiently increasing H3K27me3 levels, which are probably diluted via incorporation of newly synthesized nucleosomes during replication in the later S-phase (Lanzuolo *et al.*, 2011). In human HeLa cells, methylation levels of histone H3 drop significantly during S phase and only later recover to the initial levels (Xu *et al.*, 2012). Association of *Arabidopsis* LHP1 with ICU2 and ESD7 suggests that LHP1 may be present at chromatin during replication. It will be exciting to test the dynamics of the LHP1–PRC2 interaction in relation to the cell cycle.

Conclusion

Recent data has improved the understanding of composition and function of PcG complexes in plants. In *Arabidopsis*, three PRC2-like complexes have been described with well-defined functions that reveal specialization and cooperation in targeting overlapping sets of genes at different developmental stages. The PcG system function may have evolved from defence against genomic parasites in unicellular organisms to stable maintenance of gene inactivation during development. Many plant proteins that associate with PcG proteins have recently been identified, indicating an extensive network of crosstalk with other cellular processes.

Several key mechanisms of PcG protein recruitment have been demonstrated to function in parallel in *Arabidopsis*. The interaction between LHP1 and PRC2 provides means for the maintenance and inheritance of H3K27me3. It becomes also clear that PcG protein complexes do not act sequentially in a linear pathway but form a network of dynamic interactions. Importantly, such networks seem to contain multiple feedback loops that can add stability and switch-like behaviour to the PcG system.

Many questions still await answers. What are the non-shared functions of CLF and SWN? Do PhD–PRC2 complexes have functions outside of vernalization? How much does H2Aub overlap with H3K27me3? How many PRC1-related complexes exist, and what composition and which function do they have? Future studies are needed to test whether PcG recruitment via short ncRNAs exists in plants and to reveal more transcription factors, DNA sequences, and lncRNAs involved in PcG recruitment. Finally, more details are needed to understand how silencing by plant PcG proteins can be inherited through mitosis to become epigenetic *sensu stricto*.

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