Mechanisms guiding Polycomb activities during gene silencing in Arabidopsis thaliana

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Polycomb group (PcG) proteins act in an evolutionarily conserved epigenetic pathway that regulates chromatin structures in plants and animals, repressing many developmentally important genes by modifying histones. PcG proteins can form at least two multi-protein complexes: Polycomb Repressive Complexes 1 and 2 (PRC1 and PRC2, respectively). The functions of Arabidopsis thaliana PRCs have been characterized in multiple stages of development and have diverse roles in response to environmental stimuli. Recently, the mechanism that precisely regulates Arabidopsis PcG activity was extensively studied. In this review, we summarize recent discoveries in the regulations of PcG at the three different layers: the recruitment of PRCs to specific target loci, the polyubiquitination and degradation of PRC2, and the antagonism of PRC2 activity by the Trithorax group proteins. Current knowledge indicates that the powerful activity of the PcG pathway is strictly controlled for specific silencing of target genes during plant development and in response to environmental stimuli.

Keywords: Arabidopsis, epigenetics, histone modification, Polycomb group, Polycomb Repressive Complex, Trithorax group

INTRODUCTION TO POLYCOMB REPRESSIVE COMPLEXES IN Arabidopsis thaliana

Multicellular eukaryotic organisms develop from a single cell called a zygote, which goes through cell division and differentiation to develop into multiple tissues and organs. Precise control of gene expression under the guidance of developmental cues/signals or environmental stimuli is strictly regulated to ensure proper development of organisms. Plants and animals have evolved to have multiple methods to regulate gene expression, among which epigenetic regulation is essential for correct genome-wide gene expression profiles (Russo et al., 1996; Liu et al., 2011a).

Polycomb group (PcG) proteins, one of the evolutionarily conserved epigenetic pathways, have critical roles in plant and animal development via regulation of gene expression levels (Whitecotton et al., 2007; Molitor and Shen, 2013). In the model plant Arabidopsis thaliana, PcG proteins are incorporated into two multi-protein complexes: Polycomb Repressive Complexes 1 and 2 (PRC1 and PRC2, respectively), which both have functions in the epigenetic repression of gene expression via histone modifications (Schatlowski et al., 2008; Molitor and Shen, 2013). The major function of PRC2 is to tri-methylate lysine 27 on histone H3 (H3K27me3), while PRC1 recognizes the H3K27me3 marker and mono-ubiquinatates histone H2A (H2Aub; Schatlowski et al., 2010). Although the genome-wide identification of H3K27me3-modified loci revealed that this dynamic modification occurs during the shoot apical meristem (SAM)-to-leaf organ formation (Lafos et al., 2011), the embry-to-seedling phase transition (Bourier et al., 2013), in leaf-to-callus regeneration (He et al., 2012), and in the endosperm (Weinhofer et al., 2010). Therefore, PRC2-mediated H3K27me3 is precisely controlled during development.

PRC3 components have also been characterized in Arabidopsis. LIKE HETEROCHROMATIN PROTEIN1 (LHP1), also called TERMINAL FLOWER2 (TFL2), can recognize chromatin that is modified by H3K27me3, and its genome-wide binding sites show significant overlap with the H3K27me3 modification (Turck et al., 2007; Zhang et al., 2007b). There are two groups of RING-domain proteins in PRC1: two ATRX/RING proteins and three ATMM1 proteins (Xu and Shen, 2008; Bratzel et al., 2010; Chen et al., 2010). These RING-domain proteins function in the modification of H2Aub (Bratzel et al., 2010). Although the genome-wide identification of H2Aub loci has not been performed, it is possible that the role of PRC3 is also developmentally controlled (Kim et al., 2012).

RECRUITMENT OF PRCS TO SPECIFIC TARGET GENES

How PRCS specifically and dynamically recognize their targets during development and in different tissues or organs is a
key question for understanding the mechanism that controls the PcG pathway. Currently, there are two types of recruiters found in Arabidopsis: transcription factors and non-coding RNAs (ncRNAs).

Transcription factors usually bind to certain specific cis-elements, termed Polycomb response elements (PREs), and recruit PRCs to their specific targets via direct interaction with PRCs (Schwartz and Pirrotta, 2008). AGAMOUS (AG), a MADS-box transcription factor that is essential for establishing floral organ identity and termination of floral meristem (FM), represses WUSCHEL (WUS) expression in FM (Bowman et al., 1989; Lenhard et al., 2001; Lohmann et al., 2001). AG binds to the WUS locus at CAG boxes and then recruits PRC2-mediated H3K27me3 and LHP1 to repress WUS (Liu et al., 2011). Mutation in AG results in the decreased H3K27me3 level and loss of LHP1 binding at the WUS locus. In this case, CAG boxes may serve as the PRE in WUS expression. However, it is not clear whether AG directly or indirectly recruits PcG proteins.

BREVIPEDICELLUS (BP) and KNOTTED-LIKE FROM ARABIDOPSIS THALIANA2 (KNAT2) are members of the KNOX gene family and are expressed in the SAM but are silenced in leaves (Lincoln et al., 1994; Ori et al., 2000; Pauw et al., 2001). The MYB-domain transcription factor ASYMMETRIC LEAVES1 (AS1) and the LOB-domain transcription factor AS2 form a protein complex to repress BP and KNAT2 expression in leaves (Byrne et al., 2000; Ori et al., 2000; Semmarti et al., 2001; Iwakawa et al., 2002; Sun et al., 2002; Lin et al., 2003; Xu et al., 2003). The AS1-AS2 complex targets the cis-elements in the promoters of BP and KNAT2 (Guo et al., 2008; Liet al., 2012). Recently, it was shown that AS1-AS2 interacts with PRC2 to recruit it to the BP and KNAT2 loci (Lodha et al., 2013). Mutations in AS1, AS2, and the AS1-AS2 binding sites in BP and KNAT2 promoters all result in decreased H3K27me3 levels at BP and KNAT2 loci and ectopic expression of the two KNOX genes in leaves. In this case, the AS1-AS2 binding sites are likely to serve as PREs in PcG-mediated gene silencing.

Another possible PRE was found in the promoter of LEAFY COF2LEON2 (LEC2; Berger et al., 2011), a gene involved in embryo development, but which is silenced in the post-embryo stage (Stone et al., 2001). A cis-element, called Repressive LEC2 Element (RLE), was identified to recruit PRC2 to trimethylate the LEC2 locus (Berger et al., 2011). RLE is essential for LEC2 repression in the post-embryo stage. PRC2 is unable to trimethylate H3K27 at the LEC2 locus once RLE is mutated, leading to ectopic expression of LEC2 in the post-embryo stage. An RLE-driven reporter gene could be repressed, accompanied by H3K27me3 modification at the transgene locus, suggesting that RLE is necessary and sufficient to recruit PRC2 for histone modification and gene silencing. It seems important in the future to identify the transcription factor that binds RLE and is able to interact with PRC2.

An analysis of cis-regulatory elements in the promoter of FLOWERING LOCUS T (FT), a key gene that controls flowering time (Kardailsky et al., 1999; Kohaya et al., 1999), also indicated the existence of PRE within the promoter region (Adrian et al., 2010). However, the exact sequence that serves as the PRE and the transcription factor that binds the PRE of the FT promoter have not yet been identified.

The B3 domain proteins VP1/ABU1-LIKE1 (VAL1) and VAL2 are key regulators in the prevention of embryo traits in somatic tissues via repression of embryo-specific genes. The loss-of-function double mutant val1 val2 results in somatic embryogenesis, with ectopic expressions of embryo genes at the post-embryo stage (Suzuki et al., 2007). Somatic embryogenesis and ectopic expression of embryo genes were also observed in mutants corresponding to PRC1 components (Bratzel et al., 2010; Chen et al., 2010). A recent study showed that VAL proteins interact with PRC1 and recruit PRC1-mediated H2Aub to initiate repression of the embryonic genes after germination (Yang et al., 2013). The H2Aub modification at the embryo-gene loci is lost in val1 val2 and Arabidopsis AtAtm1b mutants. Therefore, VALs may serve as a recruiter for PRC1.

In Arabidopsis, two ncRNAs, COLD INDUCED LONG ANTI-SENSE INTRAGENIC RNA (COOLAIR) and COLD ASSISTED NONCODING RNA (CLUDAIR; Swiezewski et al., 2009; Heo and Sung, 2011; Ietswaart et al., 2012), regulate FLOWERING LOCUS C (FLC) expression. FLC is a flowering repressor that is essential for vernalization in response to cold treatment (Michaels and Amasino, 1999; Sheldon et al., 1999). COOLAIR is an antisense transcript that is transcribed in response to cold treatment (Swiezewski et al., 2009). COOLAIR is alternatively polyadenylated at the 3′ end, resulting in a proximal poly(A) site or a distal poly(A) site (Liu et al., 2010b). The proximal poly(A) site stimulates the activity of FLD, a homolog of the human LYSINE SPECIFIC DEMETHYLASE1 (LSD1; Sanda and Amasino, 1996; Liu et al., 2007), to reduce the H3K4me2 level at the FLC locus, leading to a transition from an active chromatin state to a repressive state (Liu et al., 2010b). The reduction of H3K4me2 might benefit the H3K27me3 modification; thus, COOLAIR acts as an indirect recruiter of PRC2. However, how FLD is activated using the proximal site of COOLAIR remains unknown.

CLUDAIR is a sense ncRNA that contains a 3′ cap, but no poly(A) tail (Heo and Sung, 2011). COUDAIR is induced by low temperature, and its transcription reaches a maximum level after 20 days of cold treatment, which is about 10 days later than COOLAIR. COULDAIR can directly interact with the CXC domain of the core PRC2 components CLF and SWN. In COULDAIR knockdown plants, CLF is not properly recruited to FLC, resulting in insufficient H3K27me3 modification at the FLC locus, consistent with the late flowering phenotype of these plants. Therefore, COULDAIR serves as a direct recruiter for PRC2 in Arabidopsis.

PRC2 DEGRADATION THROUGH POLYUBIQUITINATION

The Arabidopsis PRC2 is post-translationally regulated by the F-box protein UPWARD CURLED LEAF1 (UCL1; Jeong et al., 2011). UCL1 is a component of the SCF E3 ubiquitin ligase complex, which has a role in the degradation of proteins via polyubiquitination and the 26S proteasome pathway (Vierstra, 2003). Both activation tagging dominant mutant ucl1-D and plants over-expressing UCL1 under the control of the CaMV 35S promoter resulted in phenotypes that were similar to the clf mutant, with ectopic expression of some typical PRC2 targets, whose loci exhibit decreased H3K27me3 levels (Jeong et al., 2011). These results indicated that UCL1 inhibits PRC2 activity. Additionally, UCL1
directly interacts with CLF, but not with MEA, and overexpression of UCL1 causes a reduced CLF protein level. Therefore, UCL1 was thought to polyubiquitinate CLF and to degrade CLF through the 26S proteasome pathway.

Interestingly, UCL1 is expressed in the endosperm, where CLF and MEA transcripts are detectable. However, overexpression of CLF in the endosperm causes mosaic-like phenotypes, suggesting that the CLF protein level is strictly controlled in the endosperm. Jeong et al. suggested that UCL1 functions in the endosperm to specifically degrade CLF, and therefore to prevent CLF from competing with MEA during the formation of PRC2 (Jeong et al., 2011).

**PRC2 FUNCTIONS ARE ANTAGONIZED BY THE TRITHORAX GROUP PROTEINS**

Trithorax group (TrxG) proteins were first identified in Drosophila and function in antagonism of PcG (Schuettengruber et al., 2011). The first identified plant TrxG protein was *Arabidopsis* HOMOLOG OF TRITHORAX1 (ATX1), which encodes a SET-domain protein that specifically trimethylates H3K4 (Alvarez-Venegas et al., 2003; Pien et al., 2008; Saleh et al., 2010). Two other SET-domain proteins were shown to participate in genome-wide control of histone methylation. SDG8 is responsible for H3K36me2/3 (Zhao et al., 2005; Xu et al., 2008), and SDG2 acts for H3K4me2/3 (Berr et al., 2010; Guo et al., 2019). The histone modifications H3K4me2/3 and H3K36me2/3 function in activation of gene expression, showing the opposite role of PRC2-mediated H3K27me3 (Zhang et al., 2007a, 2009; Roudier et al., 2011). However, the molecular mechanism whereby H3K36me2/3 and H3K36me2/3 antagonize H3K27me3 is not clear. Recently, it was proposed that the chromatin remodeling pathway coordinates with the PcG pathway to regulate their downstream targets. Overall, these studies revealed that chromatin structure, which is organized by chromatin remodeling factors using energy from ATP hydrolysis, is essential for regulation of histone modification states.

**CONCLUSION AND PERSPECTIVES**

Controls for the PcG action at different levels are essential to ensure its specificity and activity (see the model in Figure 1). However, many questions still remain to be answered. First, although several candidates that recruit PRC1 and PRC2 to the target genes have been characterized, the common rule of the recruitment remains unclear. For example, how do these transcription factors bind PcG? If PcGs are recruited generally by transcription factors, common domains or motifs might exist in the PcG proteins and transcription factors, similar to the binding of the TOPOLESS corepressor and transcription factors (Caussier et al., 2012). On the other hand, if ncRNAs are the common factors that associate with PcG, the RNA-binding domain of PcG proteins may contain various ncRNAs. Detection of these ncRNAs might be helpful in studying the specificity of PcG. In addition, how the ncRNAs are transcribed in response to developmental or environmental cues is not clear. Recently, it was proposed that the chromatin structure and its modification status also affect the recruitment of PcGs to target loci in the “chromatin sampling” model (Klose et al., 2013), and it was also shown that PRC2 binding sites contain GAGA factor binding sequences (Deng et al., 2013). Second, the mechanism guiding the balance between PcG and TrxG activities in regulating developmental processes in plants is currently unknown. In animals, several transcription factors can regulate both PcG and TrxG activity, providing a dynamic and reversible epigenetic state (Schwartz and Pirrotta, 2008). Therefore, comparison of the regulation mechanisms of PcG and TrxG may be helpful to understand how a balance is established between PcG-mediated gene silencing and TrxG-mediated gene activation.

Third, some novel proteins were identified in plant PcG, for example, the CLF interacting protein BLESTER (BLL; Schatiolowski et al., 2010) and LHFI-INTERACTING FACTOR2 (LIF2; Latrasse et al., 2011). It will be of interest to test whether these proteins act as regulators of PcG. LIF2 is an RNA-binding protein, suggesting that PRC1 may also be subject to regulation from RNAs.

Finally, recent studies revealed that PRC1 and PRC2 physically interact in *Arabidopsis* (Derkacheva et al., 2013), indicating the possibility that the two PcG complexes have a crosstalk in silencing common targets. Further studies on mechanisms that regulate PcG
activity would be helpful to understand the epigenetic regulation of plant development.

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