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Dynamics of histone H3 lysine 27 trimethylation in Plant Development

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Abstract

The development of multicellular organisms is governed in part by temporally and spatially controlled gene expression. DNA methylation, covalent modifications of histones and the use of histone variants are the major epigenetic mechanisms governing gene expression in plant development. In this review, we zoom in onto histone H3 lysine 27 trimethylation (H3K27me₃), a repressive mark that plays a crucial role in the dynamic regulation of gene expression in plant development, to discuss recent advances as well as outstanding questions in the deposition, recognition, and removal of the mark and the impacts of these molecular processes on plant development.

Introduction

An angiosperm features a predominant sporophytic form that undergoes several major developmental transitions to culminate in gametogenesis and fertilization to produce zygotes of the next generation. A zygote undergoes embryogenesis, at the end of which the shoot and root apical meristems are established and subsequently produce all the structures of the sporophyte. Mitosis and cell fate specification are key cellular events in the development of the sporophyte and meiosis occurs during gametogenesis to generate gametes. Cell fate specification not only entails temporally and spatially restricted gene expression brought about by transcription factors and posttranscriptional regulators such as microRNAs but also the Polycomb group (PcG) proteins that impart memory of the cell fate decisions through mitosis.

The PcG genes were originally identified from genetic screens as regulators of homeotic (*Hox*) gene expression in *Drosophila* [1,2]. In loss-of-function PcG mutants, the patterns of *Hox* gene expression are correctly established, but the genes become de-repressed in cells in which they are supposed to be repressed, indicating that PcG represses target gene expression and serves as a cellular memory through mitosis. Later studies revealed that PcG introduces histone H3 lysine 27 trimethylation (H3K27me₃), a repressive chromatin mark, onto target loci (reviewed in [3]). As the vital roles of PcG are further explored in various organisms including mammals, it has become evident that PcG not only imparts developmental memory of a repressed state of gene expression through mitosis but also

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participates actively in patterning development as a mechanism of regulating gene expression. For example, PcG is essential for the differentiation of embryonic stem (ES) cells into multiple lineages in mammals [4–6]. Chromatin immunoprecipitation-microarray analyses (ChIP-on-Chip) using anti-H3K27me3 antibodies show that genes acting in all major developmental processes are PcG targets in *Drosophila* [7,8]. Genome-wide mapping of PcG targets in murine and human cell lines confirms that PcG targets are enriched in developmental regulators [4,9,10].

The plant PcG

The *Drosophila* PcG is composed of several sub-complexes (reviewed in [3]): Polycomb repressive complex1 (PRC1), Polycomb repressive complex2 (PRC2), pleiohomeotic repressive complex (PhoRC), and a newly identified module named PR-DUB [11]. PRC2 is composed of four subunits, among which Enhancer of *zeste* (E(Z)) is a SET domain protein that possesses H3K27 methyltransferase activity [12,13]. PRC1 is thought to recognize the H3K27me3 mark to confer stable transcriptional repression. The chromodomain-containing PRC1 subunit, Polycomb (Pc), binds H3K27me3. Among the other three *Drosophila* PRC1 subunits are two RING finger proteins Posterior sex combs (Psc) and dRING, which are orthologs of mammalian PRC1 subunits BMI1 and RING1A/RING1B, respectively. Mammalian BMI1, RING1A and RING1B form an E3 ubiquitin ligase complex that causes the mono-ubiquitination of histone H2A lysine 119, which confers stable repression of target loci [14,15]. PR-DUB, on the other hand, has ubiquitin hydrolase activity that removes H2A mono-ubiquitination [11]. PhoRC has two subunits, one of which contains a DNA-binding domain.

The four *Drosophila* PRC2 subunits have conserved counterparts in plants (reviewed in [16–19]) (Figure 1A). The three paralogous genes *CURLY LEAF* (*CLF*), *SWINGER* (*SWN*), and *MEDEA* are orthologs of *Drosophila* E(Z). *MEDEA* appears to function only in gametophyte and seed development. *CLF* and *SWN* are broadly expressed and partially redundant in vegetative and reproductive development. The *SU(Z)12* subunit of *Drosophila* PRC2 also has three orthologs in plants, *VERNALIZATION2* (*VRN2*), *FERTILIZATION INDEPENDENT SEEDS2* (*FIS2*), and *EMBRYONIC FLOWER2* (*EMF2*). The ortholog of the *Drosophila* PRC2 subunit ESC is a single-copy gene *FERTILIZATION INDEPENDENT ENDOSPERM* (*FIE*). The *Drosophila* PRC2 subunit p55 has five orthologs in *Arabidopsis*, among which *MULTICOPY SUPPRESSOR OF IRA1* (*MSI1*) is essential for multiple aspects of vegetative and reproductive development. Genetic and molecular studies led to the proposal that at least three forms of PRC2 exist in plants: FIS2, VRN2 and EMF2, reflecting different *SU(Z)12* paralogs, and each complex acts at a specific developmental phase in *Arabidopsis*. The FIS2 complex mainly controls seed development; the VRN2 complex acts in vernalization response, a response to prolonged cold treatment that induces flowering; and the EMF2 complex is active in most vegetative and reproductive developmental processes. The FIS2 complex, which contains MEA/SWN, FIS2, FIE, and MSI1, and the VRN2 complex (Figure 1A), which contains CLF/SWN, VRN2, FIE, and MSI1, have been biochemically confirmed [20–22].

Drosophila or mammalian PRC1 subunits have no obvious orthologs in plants, but a functional counterpart of PRC1 has been identified in *Arabidopsis*. LIKE HETEROCHROMATIN PROTEIN1 (LHP1), also known as TERMINAL FLOWER2 (TFL2), binds H3K27me3 and is co-localized with H3K27me3 marks throughout the genome [23,24]. *lhp1/tfl2* mutants show similar phenotypes as other PcG mutants, such as curled leaves, early flowering, and defective vernalization responses [25–27]. Because H3K27me3 marks still remain in *lhp1/tfl2* mutants [23], it is thought that LHP1/TFL2 acts downstream of PRC2 as a PRC1-like protein. Molecular genetic evidence also supports a

PRC1-like function for two non-sequence-specific DNA binding proteins, EMF1 [28,29] and VRN1 [30–33], but their molecular functions in PcG-mediated silencing remain to be uncovered. Recent bioinformatics and functional studies have identified functional homologs to the *Drosophila* and mammalian PRC1 RING finger proteins [34–36]. AtRING1A and AtRING1B, homologs of mammalian RING1A and RING1B, interact with LHP1 and repress PcG targets *in vivo* [36]. AtBMI1A and AtBMI1B, two other *Arabidopsis* RING finger proteins, are more closely related to mammalian BMI1. They interact with LHP1 and EMF1 and repress PcG targets *in vivo* [34]. In summary, the *Arabidopsis* PRC1 complex probably consists of LHP1, AtRING1A/AtRING1B, AtBMI1A/AtBMI1B, and EMF1 (and possibly VRN1) (Figure 1B).

There are no obvious homologs of PhoRC genes in *Arabidopsis*. Potential *Arabidopsis* homologs of the newly identified PR-DUB complex will be discussed in a later section.

The developmental functions of the plant PcG are evident from the multitude of developmental defects exhibited by mutants in various PcG subunit genes [37–42]. Mutants in *EMF2* skip vegetative development and proceed to flowering soon after germination and display ectopic expression of floral homeotic genes in non-floral tissues [42]. A mutant in *CLF* shows ectopic activation of floral homeotic genes in leaves and exhibits pleiotropic phenotypes such as leaf curling, early flowering, and reduced stature [37]. A double mutant in *CLF* and its paralog *SWN* is deficient in pattern formation after germination such that the seedling develops into callus-like tissues [43]. Mutations in *MEDEA* and *FIS2* disrupt endosperm development in the seed [38,40]. These mutant phenotypes indicate that PcG, as a repressor of gene expression, plays a crucial role in all major stages of plant development. Consistent with this notion, genome-wide H3K27me3 profiling from 10-day-old seedlings identified ~4000 genes as PcG targets, among which genes involved in transcriptional regulation are enriched [23,24].

Deposition and maintenance of H3K27me3

Although biochemical evidence is lacking, molecular genetic evidence strongly supports the SET domain-containing E(Z) homologs CLF, SWN, and MEA as the methyltransferases that deposit H3K27me3 at target genes. The *clf-81* allele with an amino acid substitution at a highly conserved residue in the SET domain resembles null *clf* mutants in morphology [32,44]. H3K27me3 is completely lost at PcG target genes such as *AGAMOUS* (*AG*) or *SHOOT MERISTEMLESS* (*STM*) in either a *clf* single mutant or a *clf swn* double mutant [32]. Furthermore, CLF is found at the chromatin of PcG target genes [32].

While PRC2 deposits H3K27me3 at PcG targets *in vivo*, how PRC2 is recruited to specific target genes in a developmentally regulated manner is largely unknown in plants. The recruitment of PcG to targets is best understood in *Drosophila*. The *Hox* loci, which are targets of PcG, contain Polycomb responsive elements (PREs), which are necessary and sufficient for PcG recruitment (reviewed in [3]). PREs are sequences of hundreds of basepairs composed of short consensus binding sites for a number of DNA binding proteins. PhoRC is the only PcG subcomplex with DNA binding capability and is one, but not the only, recruiter for the other PcG subcomplexes. A number of transcription factors have also been implicated in the recruitment of PcG to targets in *Drosophila*. In plants, homologs of PhoRC are not readily recognizable and no PREs have been clearly defined. But persistent efforts to study PRC2 recruitment have begun to implicate DNA sequences, chromatin-related proteins, and non-coding RNAs in the establishment and/or maintenance of H3K27me3 at targets.

Studies with *AG* and *FLOWERING LOCUS C* (*FLC*), two best characterized PcG targets that play crucial roles in reproductive organ development and flowering, respectively, show

that DNA sequences in these genes are important for the establishment or maintenance of H3K27 and imply that PRC2 can spread from an initial entry site to methylate histones at adjacent sequences. A portion of the *AG* genomic region (starting at the 3' end of the upstream gene and extending into the third exon of *AG*) confers responsiveness to *CLF* to the *GUS* reporter gene in terms of its expression [45]. Strong enrichment of H3K27me3 was found at both the *AG* and *GUS* sequences of the transgene in wild type but not in *clf* mutants [32]. Since *GUS* is not an endogenous PcG target, these results suggest that the *AG* sequences recruit PRC2 to the transgene and that PRC2 is able to spread from its site of entry to nearby *GUS* sequences. Similarly, *FLC* sequences can confer PRC2-dependent H3K27me3 deposition at nearby *NPTII* and *GUS* genes [46,47], which are not endogenous PcG targets. Both the promoter and intron 1 of *FLC* are required for the initial establishment of H3K27me3 [46]. These studies with *AG* and *FLC* have not identified a small (hundreds of bp) region sufficient for PRC2 recruitment, although a small region (289bp; named vernalization response element or VRE) in intron 1 of *FLC* is required for the maintenance of H3K27me3 after it is induced by vernalization [27]. Although DNA sequences are clearly important in PRC2 recruitment, these studies have not pinpointed elements that may be bound by transcription factors or PhoRC-like proteins analogous to PREs in *Drosophila*, and leave open alternative mechanisms such as chromatin modification or noncoding RNA production at these sequences playing a role in PRC2 recruitment. In fact, a recent study uncovered the existence of a noncoding RNA (COLDAIR) whose 5' end lies within the VRE in *FLC* and revealed a role of this noncoding RNA in the recruitment of PRC2 to *FLC* [48].

The possibility that other histone marks could play a role in PRC2 recruitment has been suggested by studies on *FLC* and its paralogs *MADS AFFECTING FLOWERING* (*MAF*) 1 to 5. A family of Plant Homeodomain (PHD) Finger proteins consisting of VERNALIZATION INSENSITIVE 3 (VIN3), VERNALIZATION5 (VRN5)/VIN3-LIKE1 (VIL1), and three other VIL/VERNALIZATION LIKE (VEL) proteins is required for the repression of *FLC* or the *MAF* genes. In *vin3* or *vrn5* single mutants, the vernalization-triggered H3K27me3 deposition at *FLC* is impaired [33,49,50]. In fact, VIN3 was found to associate with the VRN2 PRC2 complex *in vivo* [22]. The VIN3-like proteins VIL1/VRN5 and VIL2/VEL1 participate in the repression of *MAF1* and *MAF5* triggered by environmental conditions such as vernalization or photoperiod, and are required for the establishment and/or maintenance of H3K27me3 at *MAF1* or *MAF5* chromatin [49–51]. VIL2 associates with CLF *in vivo* and binds dimethylated histone H3 lysine 9 (H3K9me2) *in vitro* [51]. This implies a crosstalk between H3K9me2 and H3K27me3.

Four recent studies show that a Jumonji C (JmjC) domain protein Jarid2 associates with PRC2, promotes the recruitment of PRC2 to targets, but inhibits the activities of the H3K27 methyltransferase activity of PRC2 in mammalian embryonic stem cells [52–55]. The binding of this protein to PRC2 requires a linear sequence motif GSGFP. The *Arabidopsis* genome contains at least four Jarid2 homologs (At4g20400, At1g08620, At1g63490, and At2g38950) containing the linear GSGFP motif. It remains to be determined whether these are PRC2-associated proteins and whether they influence PRC2 recruitment to targets.

Noncoding transcripts have been increasingly found to play a role in PcG targeting in animals. For example, the noncoding transcripts *HOTAIR* and *Xist* are associated with PRC2 and are required for PcG-mediated silencing of target loci [56,57]. Many long intergenic noncoding transcripts have been found associated with PcG in human cell culture, although the functions of the RNAs still await further investigation [58]. A recent study found short (50 to 200 nucleotides) RNAs transcribed from the 5' ends of thousands of PcG target genes in human T cells and embryonic stem cells and showed that PRC2 binds these RNAs through their short stem-loop features [59]. Recent studies have identified noncoding RNAs

associated with the PcG target *FLC* in *Arabidopsis* and demonstrated that PcG recruitment by noncoding RNAs is a conserved mechanism in plants and animals [48,60]. Vernalization induces two groups of noncoding RNAs at the *FLC* locus, COOLAIR, a group of antisense RNAs originating from the 3' end of the gene, and COLDAIR, a group of sense RNAs whose transcription initiation sites map within the VRE within the first intron. The induction of COOLAIR is the earliest known molecular response to vernalization, but the role of COOLAIR in *FLC* repression is currently unknown. COLDAIR, which is induced by cold with a slower kinetics than that of COOLAIR, binds CLF both *in vitro* and *in vivo*. RNAi-mediated knockdown of COLDAIR impairs cold-induced CLF occupancy and H3K27me3 enrichment at *FLC*, stable *FLC* repression, and early flowering. Therefore, COLDAIR recruits PRC2 to *FLC* (Figure 1A). Although this is the only example of noncoding RNA-mediated PRC2 recruitment to targets in plants, noncoding RNA-mediated PRC2 recruitment may come to be realized as a general mechanism.

Recognition of H3K27me3

As discussed above, the chromodomain protein LHP1/TFL2 is likely the functional counterpart of *Drosophila* Pc, a subunit of PRC1 that binds H3K27me3. Although LHP1/TFL2 binds H3K27me3 *in vitro* [23], its *in vivo* recognition of H3K27me3 may require additional factors. For example, the MADS-box transcription factor SHORT VEGETATIVE PHASE1 physically interacts with LHP1/TFL2 and helps recruit LHP1/TFL2 to the PcG target *SEPALLATA3* [61] (Figure 1B). A recent study showed that the antisense RNA *ANRIL* from the PcG target *INK4b/ARF* locus in humans associates with PRC1 and that the *ANRIL*-PRC1 interaction is crucial for PcG-mediated silencing of *INK4b/ARF* [62]. It remains to be determined whether noncoding RNAs play a role in the recruitment of LHP1/TFL2 to PcG targets in plants.

How the recognition of H3K27me3 leads to compaction of chromatin and transcriptional gene silencing is little understood in plants. In *Drosophila* and mammals, PcG-mediated silencing requires histone H2A ubiquitination at lysine 119 by the E3 ubiquitin ligase comprised of RING finger proteins RING and BMI [14,15,63]. *Arabidopsis* homologs of these proteins have been established as PRC1 subunits [34,36]. The studies by Bratzel et al. demonstrated that the *Arabidopsis* PRC1 also exhibits H2A monoubiquitination activity [34]. Recombinant AtRING1A, AtRING1B, AtBMI1A, and AtBMI1B proteins were able to monoubiquitinate histone H2A.1 (one of 13 H2A isoforms in *Arabidopsis*) *in vitro*. The levels of monoubiquitinated H2A.1 *in vivo* were drastically reduced in the *atbmi1a atbmib* double mutant. Levels of H2A.1 monoubiquitination were also reduced in an *emf1* mutant. Whether the H2A.1 monoubiquitination activity of PRC1 is essential for gene repression remains to be determined.

Intriguingly, the newly identified PcG subcomplex in *Drosophila* and humans, PR-DUB, has ubiquitin hydrolase activity that removes H2A mono-ubiquitination and is also required for PcG-mediated silencing, suggesting that the dynamics of H2A ubiquitination somehow influences silencing [11]. The BAP1 protein in PR-DUB contains the ubiquitin C-terminal hydrolase (UCH) domain, which shows approximately 40% sequence identities to that of three *Arabidopsis* proteins, UCH1, 2, and 3 [64]. It remains to be determined whether these *Arabidopsis* proteins have PcG-related roles.

Removal of H3K27me3

It is long known that PcG-mediated transcriptional gene silencing can be overcome in response to endogenous or exogenous cues. For example, the floral homeotic MADS-box genes such as *AG* and *APETALA3* that specify floral organ identities are kept silent in leaves by the PcG [37], but are later activated in the proper cells in the floral meristem. Although

unknown, the re-activation of PcG target genes later in development probably entails the removal of H3K27me₃, which could be through active demethylation or passive dilution upon cell division.

In humans, JmjC domain proteins Hs KDM6A/UTX and Hs KDM6B/JMJD3 demethylate H3K27me₃ and H3K27me₂ [65]. Although 21 JmjC proteins are found in *Arabidopsis*, none of them fall in the same clade as the human H3K27me_{2/3} demethylases [66]. It is possible that a JmjC protein from a different clade has acquired H3K27me₃ demethylase activity in plants. In addition to removal of the H3K27me₃ mark, PRC2 itself may also need to be displaced from PcG target genes that are to be activated. A recent study in mammalian cells found that phosphorylation of the nearby serine on histone H3 (H3 S28) to generate the H3K27me₃S28p double mark accompanies the displacement of PRC2 and the activation of PcG target genes upon developmental or environmental cues [67].

Although the mechanisms of H3K27me₃ removal in plants are unknown, a recent study [68] implicated H3K27me₃ removal as a means to achieve temporal control in gene activation by transcription factors. The study shows that the PcG target gene *KNUCKLES* (*KNU*) is a direct target of the transcription factor AG. While AG expression commences at stage 3 of flower development, the expression of *KNU* is not activated until stage 6. By controlling the timing of AG activation, it was found that a large reduction in H3K27me₃ levels at *KNU* preceded *KNU* expression, suggesting that H3K27me₃ contributes to the delay in *KNU* activation. Such a delay is functionally important in flower development, as precocious activation of *KNU* would lead to the lack of a complete set of floral organs due to its activity in repressing floral stem cells. Interestingly, it was also found that the reduction in H3K27me₃ levels at *KNU* depended on AG activity, suggesting that transcription factors contribute to the removal of H3K27me₃ at specific PcG target genes.

The PcG-mediated repression of *FLC* triggered by vernalization is mitotically stable but is re-set upon meiosis. The mechanisms that re-set H3K27me₃ marks at meiosis or fertilization are largely unknown. A recent study that examined the dynamic expression patterns of histone H3 variants in *Arabidopsis* provides a glimpse into a potential resetting mechanism [69]. This study shows that among the 15 *HISTONE THREE RELATED* (*HTR*) genes encoded by the *Arabidopsis* genome, only a few of the variants are expressed in male and female gametes. Furthermore, a few hours after fertilization, the male and female H3 variants are removed from the zygote chromatin and *de novo* synthesis of H3 variants restores the somatic H3 composition in the embryo. Therefore, it is conceivable that the somatic H3K27me₃ marks are discarded together with their resident histone variants during gametogenesis and early embryogenesis.

Conclusion

PcG-mediated gene silencing is a common mechanism employed by plants and animals to precisely control the expression of a large number of genes in development. With the identification of PcG targets at the genomic scale and the in-depth studies on a few PcG targets in plants, the crucial role of the PcG in plant development is highly appreciated. But the mechanisms of action of the PcG in plants are still poorly understood and this impedes the progress towards a full understanding of plant development. Major questions that await further experimentation include: 1) How is PRC2 recruited to specific target genes and what governs the extent of spreading of PRC2 from its site of entry? 2) How is PRC1 recruited to targets and how does PRC1 lead to transcriptional gene silencing? 3) How is the H3K27me₃ mark removed, and if a demethylase exists, how is the demethylase recruited to specific targets in a temporally or spatially controlled manner?

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Figure 1.

A diagram of the compositions of PcG and mechanisms of its recruitment to targets. (A) Composition of a PRC2 complex and its recruitment to the *FLC* gene by a noncoding RNA. The VRN2 complex is composed of CLF and/or its paralog SWN, FIE, MSI and VRN2. A vernalization response element (VER) in the first intron of the PcG target *FLC* serves as a promoter for the transcription of a noncoding RNA (COLDAIR), which binds CLF and recruits PRC2 to *FLC*. Pol II is probably the polymerase that produces COLDAIR. By analogy to findings from animal systems, CLF may recognize short stem-loop features within COLDAIR. (B) Composition of a PRC1-like complex and its recruitment to the *SEPALLATA3* (*SEP3*) gene. A plant PRC1 complex likely consists of TFL2, AtRING1A/AtRING1B, AtBMI1A/AtBMI1B, and EMF1. The transcription factor SVP binds to its specific recognition site known as a CArG box in *SEP3* and recruits TFL2 to *SEP3*.