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Epigenetic control by plant Polycomb proteins: new perspectives and emerging roles in stress response

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Abstract: In eukaryotes, cell-fate determination, differentiation and developmental programs require precise spatial and temporal control of gene expression. Polycomb group (PcG) proteins are key transcriptional regulators in these mechanisms. Research over the past decade has demonstrated the significance of the Polycomb system in establishing silent chromatin states and perpetuating them through cell divisions. PcG mediated gene silencing is a fundamental and conserved mechanism employed by both plants and animals. In plants these proteins maintain cell identity and imprinting and control important developmental transitions, hence having agronomical impacts.

In this chapter, we discuss the impact of Polycomb regulation in controlling epigenetic states in plants. We highlight recent reports about the role of long non-coding RNAs as PcG cofactors and the involvement of PcG complexes in stress responses. Indeed, how plants respond to environmental stresses by modifying expression of the genome is a challenge in plant biology, and unexplored links between stress response and PcG regulation are promising.

Key words: Polycomb group (PcG) proteins, Polycomb responsive element (PRE), Polycomb repressive complex (PRC), chromatin, long non-coding RNAs, histone modification, histone marks.

3.1 Introduction

Polycomb group (PcG) proteins are key chromatin regulators present in both plants and animals. They work as multi-protein complexes, cooperatively establish silent chromatin states and are involved in the transmission of epigenetic states. Despite the recent progress in both animal and plant fields, PcG mechanisms of action are still poorly understood, impeding the progress towards a full understanding of eukaryote development. How these key chromatin complexes are recruited to specific target genes still remains one of the major questions. Two important achievements in the field have recently had a profound impact on the understanding of the mechanism of action of the Polycomb system and the regulation of PcG target genes: (i) the discovery of non-coding RNAs (ncRNAs) interacting with PcG proteins (Spitale et al., 2011; Wang and Chang, 2011) and (ii) the interplay between nuclear organization and PcG mediated gene regulation (Bantignies and Cavalli, 2011).

In the plant model *Arabidopsis thaliana*, PcG complexes have been extensively characterized in relation to plant development (for reviews see Butenko and Ohad, 2011; Holec and Berger, 2012; Köhler and Hennig, 2010), but they have only recently been reported to play a role in perenniality and stress responses. Here, we focus on these questions and we will integrate the current knowledge in the field at the interface with plant responses under stress conditions.

3.2 Conserved multi-protein complexes with histone post-translational modifying activities

Studies in *Drosophila melanogaster* described at least three main types of complexes with different functions which serve as reference types in both animal and plant species: Polycomb repressive complex 1 (PRC1), PRC2, and the Pleiohomeotic repressive complex (Pho-RC) (reviewed in Margueron and Reinberg, 2011; Schuettengruber and Cavalli, 2009; Simon and Kingston, 2009). Pho-RC contains Pleiohomeotic (Pho), a sequence-specific DNA-binding protein, and the Smc-related protein containing four MBT domains (Sfmbt). This complex remains to be described in plants. While no enzymatic activity has been associated with the Pho-RC complex, the two PRC complexes have histone tail

post-translational modifying activities. PRC2 catalyses the trimethylation of the histone H3 lysine 27 residue (H3K27me3) at target loci, while PRC1 can recognize H3K27me3 and establish H2A histone monoubiquitination. Thus, PRC1 functions downstream of PRC2 to maintain the repressive state and mediate gene expression control in various biological processes. However, besides the current hierarchical model, which posits two sequential steps by PRC2 and PRC1, respectively, recent studies in animals suggest that H3K27me3 is not always sufficient or required as a docking site for PRC1 recruitment, and that PRC1 may act in a PRC2-independent manner (Gao et al., 2012; Simon and Kingston, 2009; Sing et al., 2009; Tavares et al., 2012). Besides its catalytic activity, PRC1 also has chromatin compaction activity which can be independent of monoubiquitination; however both activities impact gene expression regulation (Endoh et al., 2012; Eskeland et al., 2010).

A PRC2-type complex is composed of four subunits, among which Enhancer of Zeste (E(Z)) is a SET (Su(var)-E(z)-Thrithorax) domain protein that possesses H3K27 histone methyltransferase (HMT) activity. The E(Z) protein on its own has a null or low activity and requires non-catalytic subunits. The other subunits are Suppressor of Zeste 12 (Su(Z)12), the nucleosome remodelling p55 factor and Extra sex combs (Esc/EED) (Figure 3.1).

Su(Z)12 is a protein containing a C2H2 zinc-finger domain and VEFS, a conserved domain among plant and drosophila homologs (VRN2-EMF2-FIS2-Su(Z)12). The p55/NURF55/CAF1 protein and Esc are two WD 40 proteins, the former being involved in interaction with

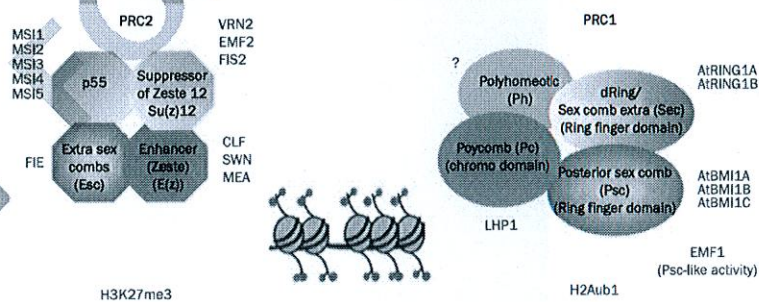


Figure 3.1 Core components of the PRC complexes in *Drosophila* and *A. thaliana*. PRC2 deposits the H3K27me3 silent epigenetic mark whereas H2A monoubiquitination is achieved by PRC1

nucleosomes and the latter able to modulate the HMT activity. Besides the four core subunits, regulatory subunits (PcG cofactors) have been described in animals that can associate with the PRC2 complex and influence its function, such as Polycomb like (PCL) or JARID2, a histone demethylase essential for developmental control despite impaired enzymatic activity (Landeira and Fisher, 2011).

In *A. thaliana*, extensive genetic analysis of the PcG proteins has been conducted, revealing that PRC2 is structurally conserved and comprises homologs of the four core subunits (reviewed in Köhler and Hennig, 2010). One of the PRC2 core subunits, the Extra sex combs (Esc) homolog, is encoded by a unique gene, FERTILIZATION INDEPENDENT ENDOSPERM (FIE), whereas the three other PRC2 core subunits are encoded by small gene families up to five members. The MULTICOPY SUPPRESSOR OF IRA 1 to 5 genes (MSI1–5) encode the PRC2 p55 homolog, whereas EMBRYONIC FLOWER2 (EMF2), FERTILIZATION INDEPENDENT SEED2 (FIS2) and VERNALIZATION2 (VRN2) are the homologs of the drosophila Su(Z)12 protein. CURLY LEAF (CLF), SWINGER (SWN, also known as EZA1), and MEDEA (MEA) are the homologs of the E(Z) PRC2 histone methyltransferase. The three E(Z) HMTs differ by their expression patterns throughout the plant life cycle. MEA is expressed predominantly in the female gametophyte and developing seeds but not in mature seeds and seedlings, whereas CLF and SWN are present in vegetative tissues. Redundancy issues are certainly responsible for the difficulty of an unambiguous identification of complex composition, and as yet scant biochemical data exist on the characterization of the specific complexes. Three main PRC2 complexes have been characterized (Hennig and Derkacheva, 2009; Köhler and Hennig, 2010) and associated with different plant developmental stages (the EMBRYONIC FLOWER (EMF), VERNALIZATION (VRN) and FERTILIZATION INDEPENDENT SEED (FIS) complexes). PcG cofactors similar to PCL have been described in plants. The H3K27 trimethylation activity of the VRN-PRC2 complex is increased by the interaction with three related plant homeodomain (PHD) finger proteins, VEL1, VRN5 and VIN3, that form the PHD-PCR2 complex involved in the vernalization process (De Lucia et al., 2008) (Figure 3.2).

While PRC2 complexes are evolutionarily conserved, PRC1 complexes are more plastic and heterogeneous in eukaryotes, suggesting that they form a small family (Schuettengruber and Cavalli, 2009). The four core components of the drosophila PRC1 complex are: Polycomb (Pc), a protein whose chromodomain recognizes the silencing mark H3K27me3, Polyhomeotic (Ph) (a protein containing SAM and C2-C2 zinc finger

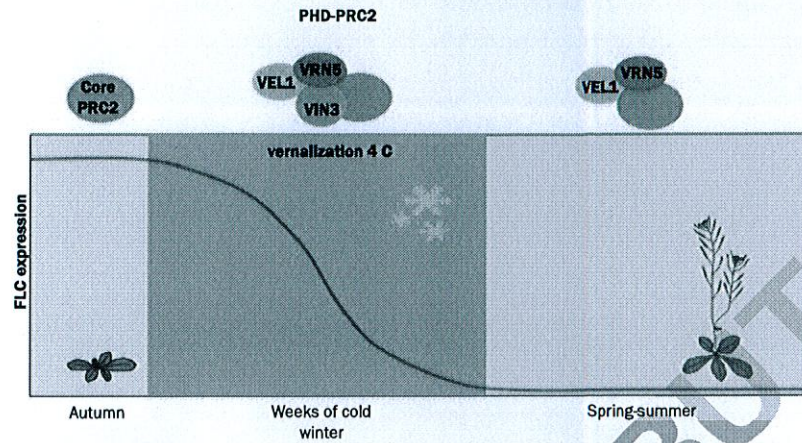


Figure 3.2 The vernalization process and the PRC2 complexes

domains), and two PRC1 RING proteins, drosophila Ring finger protein 1/Sex combs extra (dRing/Sce) and Posterior sex combs (Psc).

The PRC1 RING proteins contain a Ring-finger domain at the N-terminal region and the RAWUL (Ring-finger and WD40 associated Ubiquitin-Like) domain at the C-terminal region, and they are further subdivided into RING subfamily (dRing/RING1A/RING1B) and BMI subfamily (Psc/Polycomb group RING fingers (PCGFs)/BMI proteins) (Sanchez-Pulido et al., 2008).

Some of the PRC1 subunits are encoded by small families in mammals and plants, giving rise to a diversity of PRC1 complexes (Figure 3.1). In an extensive proteomic study, six mammalian PRC1 complexes have been reported, corresponding to combinatorial associations of the different PcG homologs, all of which contain RING1A/B and are distinguishable by their Psc homologs (PCGF1 to 6) (Gao et al., 2012). By analysing the genomic localization of different subunits of the six PRC1 complexes, regions bound exclusively or predominantly by one of the six PCGF were identified, highlighting that the different complexes have distinct genomic localizations (Gao et al., 2012). Additional components are also found associated with PRC1 in substoichiometric amounts, such as the PcG protein Sex Comb on Midleg (Scm), and an atypical PRC1 complex, dRING associated Factors (dRAF), comprising dRING, Psc, and the H3K36me2 histone demethylase KDM2 has been reported (Lagarou et al., 2008). Interestingly, the Psc subunit is crucial for inheritance of chromatin features throughout DNA replication (Lo et al., 2012).

In plants, recent data indicate that the chromodomain protein LIKE HETEROCHROMATIN PROTEIN1 (LHP1) (Gaudin et al., 2001) and homologs of the two drosophila RING-finger domain proteins form a complex similar in domain composition and function to animal PRC1 (Bratzel et al., 2010; Chen et al., 2010; Xu and Shen, 2008; Zhang et al., 2007b). The chromodomain of LHP1 binds to trimethylated lysines H3K27 and H3K9me3 *in vitro* (Zhang et al., 2007b). However, LHP1 is mainly localized in euchromatin *in vivo* (Libault et al., 2005), and its genome-wide distribution indicated that LHP1 mainly co-localizes with H3K27me3 *in vivo*, suggesting that LHP1 is a functional homolog of Pc (Turck et al., 2007; Zhang et al., 2007b). Recent works have identified 5 RING-finger domain proteins interacting with LHP1, AtRING1A/B and AtBMI1A/B/C, AtBMI1A/B having a H2A monoubiquitination activity (Bratzel et al., 2010; Chen et al., 2010; Xu and Shen, 2008). The plant-specific EMF1 protein interacts physically with some of the plant PRC1 subunits, thus participating in a PRC1-like complex (Calonje et al., 2008). Recently, EMF1 was shown to bind tightly to free DNA and to inhibit chromatin remodeling, having a Psc-like activity (Beh et al., 2012). Beside the chromodomain, LHP1 possesses a chromoshadow domain with protein-protein interaction properties (Gaudin et al., 2001). Consistently, several LHP1 partners have been found, such as transcription factors (SCARECROW (Cui and Benfey, 2009) and SHORT VEGETATIVE PHASE (SVP) (Liu et al., 2009)), as well as various chromatin-associated proteins (del Olmo et al., 2010; Li and Luan, 2011; Gaudin, unpublished data). These associated proteins, such as transcription factors, could participate in specifying sets of PcG target genes, as already reported in animals (Yu et al., 2012), giving rise to PRC1 variant complexes, or modulating the plant PRC1 activity.

In the coming years, we expect a rapid evolution of the biochemical complexity of the plant PRC complexes by the identification of homologous proteins or additional regulatory partners, to fulfil the diverse functions of these complexes in planta. The diversity of the complexes may also be accompanied by variations in the recruitment pathways (histone marks, Polycomb-response elements (PREs), transcription factors, RNA cofactors ...) as well as transcription repression mechanisms (direct interference with the transcription machinery (Lehmann et al., 2012), modulation of chromatin compaction, inhibition of chromatin remodelling, effects on higher-order nuclear structures).

3.3 Polycomb functions in plant development

Plants, which evolved multicellularity independently of animals, utilize the PcG system in strikingly similar ways (Meyerowitz, 2002). The developmental functions of the plant PcG are evident from the multitude of developmental defects exhibited by mutants of various PcG subunit genes (Bratzel et al., 2010; Gaudin et al., 2001; Goodrich et al., 1997; Grossniklaus et al., 1998; Hennig et al., 2003; Luo et al., 1999; Ohad et al., 1999; Xu and Shen, 2008), indicating that PcG complexes play a crucial role in all major stages of plant development and progressions through the plant life cycle. A complete loss of PcG function in plant cells inhibits cell differentiation and activates an embryo-like state (Bratzel et al., 2010; Chanvivattana et al., 2004; Chen et al., 2010), suggesting that PcG is required to control cell identity and the ability to form different tissues with diverse functions. PcG function is also required to maintain gametophytic identity of cells (for review see Köhler and Hennig, 2010). Moreover, the system controls one of the most important developmental transitions plants make, the floral transition, from vegetative growth to reproductive flowers. The PcG system regulates flowering through a process called vernalization that allows plants to flower after long periods of cold in more favourable conditions in spring (Kim et al., 2009). This is achieved via down-regulation of *FLOWERING LOCUS C (FLC)*, whose repression is maintained by the Polycomb system (Bastow et al., 2004; De Lucia et al., 2008; Gendall et al., 2001; Greb et al., 2007; Mylne et al., 2006); for review see Kim and Sung (2012) (Figure 3.2).

With the identification of PcG targets at the genomic scale via the analysis of the trimethylated H3K27, signature of Polycomb repression, and the in-depth studies on a few PcG targets in plants, the crucial role of the PcG in plant development is highly appreciated. In *A. thaliana*, H3K27me₃ is found at approximately 20% of annotated genes (Zhang et al., 2007a), essentially transcriptional regulators but also genes encoding microRNAs, and fluctuates according to tissues and developmental stages, thus allowing fine-tuning of gene expression during plant differentiation (Lafos et al., 2011). The genome-wide distribution of the PRC1 subunit LHP1 by DamID approach (Germann and Gaudin, 2011) identified about 2300 target regions and 4000 genes distributed over the five chromosomes, of which 90% are associated with H3K27me₃ (Zhang et al., 2007). These

data confirm the hierarchical mechanism of PRC complexes, but also suggest that PRC1 might act independently of PRC2 for a tiny set of target regions devoid of H3K27me3 (Zhang et al., 2007b).

3.4 Non-coding RNAs as regulatory cofactors of Polycomb complexes

Recent high throughput sequencing approaches have listed thousands of non-coding RNAs (ncRNAs) in mammals with an extraordinary diversity, these molecules being transcribed either from intergenic regions or from intragenic regions in sense or in antisense orientation. As their functionalities were questioned, the last few years saw mounting evidence of regulatory roles of ncRNAs, ranging from 50 to several kbp in length, as signals, decoys, guides and/or scaffolds in assembling and regulating various protein complexes and thus impacting gene expression (Wang and Chang, 2011). In particular, their association with chromatin-modifying enzymes and PRC2 complexes has been recently reported (for reviews see Bracken and Helin, 2009; De Lucia and Dean, 2011; Guttman and Rinn, 2012; Nagano and Fraser, 2011). This emerging theme already has many examples in the animal fields. The pioneer HOTAIR lncRNA regulating the HOX-D locus (Rinn et al., 2007; Tsai et al., 2010), the RepA/Xist RNA involved in X chromosome inactivation (Zhao et al., 2008), and Kcnq1ot1 (Pandey et al., 2008) participating in repression of imprinted genes have all been implicated in Polycomb control. Actually, a significant part of the long interspersed non-coding RNAs (lncRNAs) in the human genome seems to be physically associated with PRC2 (Guttman and Rinn, 2012; Khalil et al., 2009). Control of PRC1 may also involve ncRNA (Yap et al., 2010). Recent work has also identified several shorter RNAs (50–200 nt) as candidate PRC2 regulators, which are transcribed from the 5' end of PcG target genes (Kanhere et al., 2010). While HOTAIR works in *trans* (Gupta et al., 2010; Rinn et al., 2007), a large number of non-coding transcripts seem to act in *cis* (Figure 3.3). This type of silencing is likely to have widespread consequences, and ncRNAs can now be considered as PcG cofactors that could guide site-specific recruitment of PRC complexes to target genes.

RNA-mediated recruitment is especially attractive for PcG proteins due to the absence of specific DNA-binding components of most PRC complexes. Thus, PcG proteins must rely on other factors that can recognize specific DNA sequences. *Drosophila* biologists defined small stretches of DNA associated with target genes that are necessary and

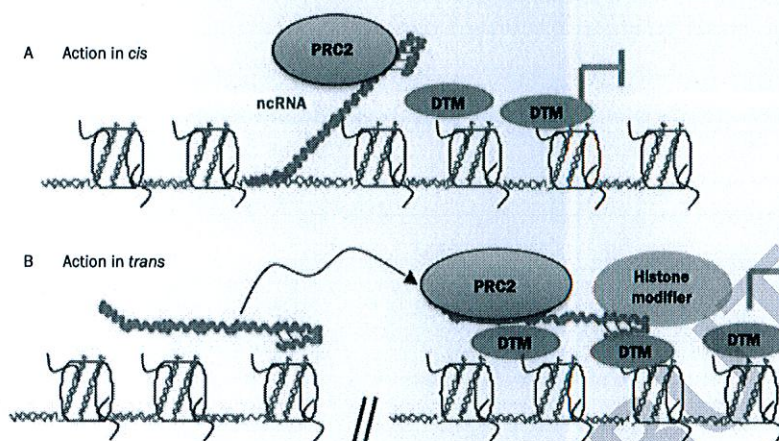


Figure 3.3 Models of ncRNA acting *in cis* (A) and *trans* (B) to recruit PRC2 complexes

sufficient to perform the silencing and maintenance tasks of the PcG system. These sequences have been called Polycomb-response elements (PREs). In flies, Polycomb complexes may contain sequence specific DNA-binding factors, such as Zeste, Pipsqueak (PSQ) or Polyhomeotic (Pho), to help bind PREs (Ringrose and Paro, 2004; Schwartz and Pirrotta, 2008). By contrast, mammalian and plant Polycomb complexes are not thought to contain such subunits (DNA binding proteins) or PREs, though a few PRE-like elements have recently been reported in mammals (Sing et al., 2009; Woo et al., 2010) and the JARID2 histone demethylase may facilitate binding (Li et al., 2010; Pasini et al., 2010; Peng et al., 2009). Therefore, the mechanism of PcG recruitment to thousands of genomic locations remains poorly understood. In this respect, ncRNAs may be playing a crucial role (Bracken and Helin, 2009; Guenther and Young, 2010). RNA-mediated recruitment represents an ideal mechanism for targeting chromatin modifiers *in cis* to specific alleles or unique locations in the genome, as they remain tethered to the site of transcription and can co-transcriptionally direct enzymatic activities to a unique region (Lee, 2009, 2010). They can also be complementary to genomic sequences and act *in trans*. Besides guiding functions allowing recruitment of chromatin complexes to specific genomic locations, ncRNAs can also act as scaffold elements to assemble large complexes in various processes (Guettg et al., 2012; Wang and Chang, 2011). A recent study has demonstrated a role of HOTAIR in scaffolding PRC2 complex with H3K4 demethylase complex (Tsai et al., 2010). Furthermore, HOTAIR has been shown to interact

with thousands of genomic loci, binding very short regions that serve as nucleation sites for PRC silencing (Chu et al., 2011).

To date there is a very limited number of examples of ncRNAs interacting with Polycomb complexes in plants (De Lucia and Dean, 2011; Kim and Sung, 2012; Muller and Goodrich, 2011). Non-coding RNAs have a central role in the regulation of *FLOWERING LOCUS C (FLC)*, a transcriptional regulator that delays the transition to flowering. *FLC* expression is repressed by prolonged cold experienced during winter (Figure 3.2). This silencing is epigenetically stable and involves a Polycomb-like mechanism and ncRNAs. The ncRNA *COOLAIR* is expressed from a promoter located in the 3'-flanking non-coding region of the *FLC* gene, it is partially spliced and it is involved in the early repression of *FLC* expression during vernalization (Swiezewski et al., 2009). By native RNA immunoprecipitation (RIP), the antisense *COOLAIR* transcript was found interacting with PRC2 (De Lucia et al., unpublished). As *COOLAIR* reaches its peak of expression, the amount of *FLC* mRNA starts to fall and transcription of *COLD AIR* is initiated (Heo and Sung, 2011). *COLD AIR* is a non-coding transcript that links decreased *FLC* expression to the maintenance of its repression by PcG protein complexes in *A. thaliana*. *COLD AIR* is encoded at the *FLC* locus as an intragenic sense RNA located in the first intron of the *FLC* gene. Its expression is increased by cold and it is reported to bind to CLF, the HMT subunit of plant PRC2 complex (Heo and Sung, 2011). However, Heo and Sung show that other proteins are required to ensure specificity of this RNA-protein interaction. Thus it appears that PRC2-lncRNA association is an evolutionarily conserved mechanism in PcG-mediated gene repression in eukaryotes. As for PRC1, a RNA binding protein interacts with LHP1, which may suggest some indirect implication of RNA in gene regulation by plant PRC1-like complex (Latrasse et al., 2011).

From all these data, it seems that non-coding RNAs are at the heart of developmental regulation, determining the epigenetic status and transcriptional network in any given cell type, and they provide a means to integrate external differentiation cues with dynamic nuclear responses.

3.5 Emerging roles of PcG and ncRNAs in responses to environmental stress

The Polycomb system is not only involved in the regulation of plant development. Recent studies show that cold-induced genes (Kwon et al.,

2009) and drought-stress response genes are regulated by PcG (Alexandre et al., 2009; Finnegan et al., 2011). Expression of the genes coding for Polycomb components in barley was found to be responsive to the abiotic stress-related hormone abscisic acid (ABA), implying an association with ABA-mediated processes during seed development and stress responses. Transcriptome analyses highlighted enrichment in stress response genes in *lhp1* mutant (Latrasse et al., 2011).

Besides emerging evidence of a role of PcG proteins in response to environmental stress, ncRNAs may also play a critical role in the adaptation of developmental processes rather than in differentiation per se, as very few ncRNAs involved in development were previously revealed by classical genetic approaches. Like some miRNAs, certain ncRNAs are induced in various developmental processes as well as during abiotic stress responses in plants and animals (Jones-Rhoades et al., 2006; Prasanth and Spector, 2007; Sunkar et al., 2007; Yaish et al., 2011). In *A. thaliana*, Crespi's group identified ncRNAs displaying diverse regulation by environmental stimuli (Hirsch et al., 2006). A subsequent study based on genome-wide bioinformatic analysis identified 76 *Arabidopsis* ncRNAs, among which a significant fraction have an altered accumulation in response to abiotic stress (Ben Amor et al., 2009). For some of them the change in expression observed after salt treatment was maintained, while for others the induction was transient. Overexpression of two of these ncRNAs suggested that at least a subset of these newly identified ncRNAs have roles in developmental or stress adaptation programs. The number of examples of ncRNAs responding to stress conditions in plants is definitely increasing (Wu et al., 2012), suggesting that lncRNAs remain an underexplored class of ncRNAs that could be far more prevalent than previously appreciated to adapt growth and development to abiotic interactions. In animals, transcription of lncRNAs within gene regulation elements can modulate activity in response DNA damage (Hung et al., 2011). Hence, lncRNAs represent a class of factors that can be modulated by external stimuli.

3.6 PcG protein functions in three-dimensional nuclear organization

The eukaryotic genome is organized into functionally distinct segments, interspersed and intertwined, that undergo dynamic changes at different phases of the cell cycle and in response to various intracellular and extracellular signals. Furthermore, besides a compartmentalization of the

genome into chromosome territories, recent studies using chromosome conformation capture (3C) and derivative techniques have revealed a three-dimensional folding of chromatin into functional globules (Lieberman-Aiden et al., 2009) and an important role of PcG proteins in higher levels of chromatin organization. The three-dimensional organization of Polycomb target genes in the cell nucleus has recently been revealed as an important parameter in their regulation in animals (for review see Bantignies and Cavalli, 2011; Delest et al., 2012). Long-distance interactions in *cis* or in *trans* (on different chromosomes) among the major elements bound by PcG proteins, including PREs and core promoters, have been visualized by microscopy-based techniques, 3C and derivative techniques (Comet et al., 2011; Sexton et al., 2012; Tolhuis et al., 2011). Importantly, these interactions change upon activation of a certain target gene, and they can be implicated in co-regulation of different target genes by PcG proteins via association in three-dimensional nuclear space (Bantignies et al., 2011). Thus, specific nuclear organization imposed by PcG proteins influences the maintenance of epigenetic states and regulates the co-repression of target genes. Among these kinds of contact, 'gene kissing' (between copies of a certain allele) also occurs in *FLC* silencing (De Lucia et al., unpublished), suggesting that they are a general mechanism of PcG regulation in eukaryotes. Thus, besides the classical linear epigenetic organization of the genome, the 3D nuclear architecture has to be taken into account to better understand the complex mechanisms that fine-tune the genetic element activities in various processes and in response to external stimuli.

3.7 Perspectives: the role of Polycomb in abiotic and biotic stress response

Stress puts cells at risk, and adaptation is crucial for maximizing cell survival. Cellular adaptation mechanisms include modification of certain aspects of cell physiology, such as the induction of efficient changes in gene expression programs by intracellular signalling networks. Recent studies using genome-wide approaches as well as single-cell transcription measurements, in combination with classical genetics, have shown that specific modulation of gene expression can be accomplished by several different strategies. How organisms can achieve generic and specific responses to different stresses by regulating gene expression at multiple stages of mRNA biogenesis, from chromatin structure to transcription, mRNA stability and translation, is nowadays a central question.

In the light of the recent evidence concerning the involvement of the Polycomb system and ncRNAs in the abiotic stress response, the identification of Polycomb target genes and the Polycomb RNA partners under different stress conditions is an interesting area to explore. In addition, it has to be taken into account that changes in nuclear organization under stress conditions can allow coordinated co-regulation of a number of genes that need to be modulated in response to stress. Therefore, the recent progress in the identification of a 3D interactome for Polycomb target genes will be crucial for further investigation.

At the same time, the role of chromatin in the memory of the immune response is currently under intense investigation. Priming is a phenomenon that enables cells to respond more rapidly and/or sensitively during a second exposure to a stimulus than during the initial exposure. It is likely that a role of the Polycomb system will be revealed in the next future, in the light of the presence of H3K27me3 at many defence-related genes in *Arabidopsis*, exploring a Polycomb-mediated chromatin memory for biotic stress responses.

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