Decoding the Epigenetic Language of Plant Development

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ABSTRACT Epigenetics refers to the study of heritable changes in gene expression or cellular phenotype without changes in DNA sequence. Epigenetic regulation of gene expression is accomplished by DNA methylation, histone modifications, histone variants, chromatin remodeling, and may involve small RNAs. DNA methylation at cytosine is carried out by enzymes called DNA Methyltransferases and is involved in many cellular processes, such as silencing of transposable elements and pericentromeric repeats, X-chromosome inactivation and genomic imprinting, etc. Histone modifications refer to posttranslational covalent attachment of chemical groups onto histones such as phosphorylation, acetylation, and methylation, etc. Histone variants, the non-canonical histones with amino acid sequences divergent from canonical histones, can have different epigenetic impacts on the genome from canonical histones. Higher-order chromatin structures maintained or modified by chromatin remodeling proteins also play important roles in regulating gene expression. Small non-coding RNAs play various roles in the regulation of gene expression at pre- as well as posttranscriptional levels. A special issue of \textit{Molecular Plant} on 'Epigenetics and Plant Development' (Volume 4, Number 2, 2009) published a variety of articles covering many aspects of epigenetic regulation of plant development. We have tried here to present a bird's-eye view of these credible efforts towards understanding the mysterious world of epigenetics. The majority of the articles are about the chromatin modifying proteins, including histone modifiers, histone variants, and chromatin remodeling proteins that regulate various developmental processes, such as flowering time, vernalization, stem cell maintenance, and response to hormonal and environmental stresses, etc. Regulation of expression of seed transcriptome, involvement of direct tandem repeat elements in the \textit{PHE1} imprinting in addition to PcG proteins activity, paramutation, and epigenetic barriers in species hybridization are described well. The last two papers are about the Pol V-mediated heterochromatin formation independent of the 24nt-siRNA and the effect of genome position and tissue type on epigenetic regulation of gene expression. These findings not only further our current understanding of epigenetic mechanisms involved in many biological phenomena, but also pave the path for the future work, by raising many new questions that are discussed in the following lines.

Key words: Chromatin structure and remodeling; epigenetics; gene silencing; flowering.

HISTONE MODIFICATIONS TAKE CHARGE IN FLOWERING-TIME REGULATION

Flowering is the transition from vegetative to reproductive phase in the plant lifecycle. In \textit{Arabidopsis}, distinct pathways, including vernalization, photoperiod, gibberellin, and autonomous pathways, form a regulatory network that controls the timing of flowering to ensure maximal reproductive success (Baurle and Dean, 2006; Williams et al., 2005). In such a network, \textit{FLOWERING LOCUS C} (\textit{FLC}) plays a central role in repressing the floral transition largely by reducing the expression of three key floral integrators: \textit{FLOWERING LOCUS T} (\textit{FT}), \textit{SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1} (\textit{SOC1}), and \textit{FLOWERING LOCUS D} (\textit{FD}) (Michaels and Amasino, 1999; Searle et al., 2006). Reports from various labs have revealed that \textit{FLC} and \textit{FT} are regulated by various chromatin modifications. Recently, lines of evidences found that an RNA-binding protein directs the 3' processing of \textit{FLC} antisense transcripts, which triggers localized histone demethylation to negatively regulate \textit{FLC} sense transcription (Hornyik et al., 2010; Liu et al., 2010;
Swiezewski et al., 2009). In this special issue, Yihui He reviewed recent discoveries about the regulation of FLC by chromatin modifications (He, 2009). Histone H3 lysine-4 (H3K4) tri-methylation mediated by ATX1 (Pien et al., 2008), H2B mono-ubiquitination (Cao et al., 2008; Gu et al., 2009), histone H3K36 di- and tri-methylation (Xu et al., 2008), and deposition of the histone variant H2A.Z (Deal et al., 2007; Zilberman et al., 2008) are regarded as active marks for FLC transcription, whereas histone deacetylation (Ausin et al., 2004; He et al., 2003), H3K4 demethylation (Jiang et al., 2007; Liu et al., 2007b), histone H3K9 tri-methylation (Liu et al., 2004; Swiezewski et al., 2007), H3K27 tri-methylation (Jiang et al., 2008), and H4R3 symmetric di-methylation (H4R3sme2) (Wang et al., 2007) repress FLC transcription. Vernalization is a process that suppresses FLC expression through distinct histone modifications in the FLC chromatin, including H3K9 and H3K27 di- and tri-methylation, H4R3sme2, histone deacetylation, and H3K4 demethylation (Bastow et al., 2004; Finnegan and Dennis, 2007; Greb et al., 2007; Schmitz et al., 2008; Sung and Amasino, 2004; Sung et al., 2006). It is intriguing that the FLC chromatin undergoes distinct modifications in response to developmental and environmental signals. Further studies, aimed to characterize more chromatin modifiers and the interaction networks among these factors, will explain how these modifications are regulated and coordinated in the regulation of flowering time and will help us to better understand the underlying chromatin mechanisms and, at the same time, will uncover other important gene regulation networks.

As mentioned above, vernalization promotes flowering largely by repressing FLC expression. As the upstream component of the vernalization pathway, *VERNALIZATION INSENSITIVE 3* (*VIN3*), a chromatin remodeling Plant Homeo Domain (PHD) finger protein, is induced in response to cold, binds to the FLC chromatin, and interacts with components of the Polycomb-group Repressive Complex 2 (PRC2), which catalyzes histone H3K27 tri-methylation (De Lucia et al., 2008; Liu et al., 2010; Schubert et al., 2006; Sung and Amasino, 2004). Induction of *VIN3* is associated with histone H3 and H4 acetylation (Jean Finnegan et al., 2005). In this special issue, Bond et al. investigated the regulation of *VIN3* by histone acetylation in response to short- and long-term cold treatment. They have shown that there are two spatially and temporally distinct phases of acetylation of *VIN3* chromatin during cold exposure. They have also shown that the cold acclimation pathway and the cold-induction of *VIN3* are regulated by different mechanisms, since *VIN3* is not induced by the SAGA-like transcriptional activator complex. Treatment of *Arabidopsis* seedlings with the histone deacetylase inhibitor, nicotinamide, causes induction of *VIN3* and repression of FLC; however, the repression of FLC is independent of *VIN3* activity, suggesting that a novel pathway is involved in suppressing FLC (Bond et al., 2009). It will be interesting to unravel this pathway.

Once the transition from vegetative to reproductive growth is induced by external signals, such as inductive photoperiod or vernalization, *Arabidopsis* continues flowering in the absence of primary signal(s); such ability is termed as floral commitment. In the review by Adrian et al., different molecular scenarios are discussed to illustrate the molecular memory of flowering. In some cases, this memory is mediated by epigenetic mechanisms (e.g. the stable repression of FLC by vernalization (De Lucia et al., 2008; Greb et al., 2007; Sung and Amasino, 2004; Sung et al., 2006)), whereas in other cases, floral commitment can be mediated by a transcriptional network (back-locked feed-forward loop by *AGAMOUS-LIKE 24 (AGL24)/SOC1, LEAFY (LFY) and APETALA1 (AP1)*, etc. (Lee et al., 2008; Liu et al., 2008, 2007a)). And, interestingly, some of the genes (*FT, SOC1, AGL24, etc.*) that build up these transcriptional loops are regulated by epigenetic mechanisms (H3K27me3, H3K4me3, etc. (Jiang et al., 2008; Oh et al., 2008; Zhang et al., 2007a)). Apparently, as the authors have said, how to combine these two models into one function will be the future goal (Adrian et al., 2009).

**FUNCTIONS, MECHANISMS, AND EVOLUTION OF POLYCOMB GROUP PROTEIN-LIKE COMPLEXES IN PLANTS**

Polycomb group proteins (PcGs) are a family of proteins first identified in *Drosophila* that play important roles in regulating gene expression through chromatin remodeling. In *Drosophila* and mammalian cells, there are at least two PcG complexes, namely Polycomb Repressive Complex 1 (PRC1) and Polycomb Repressive Complex 2 (PRC2). Enhancer of Zeste (E(z)), Suppressor of Zeste (12) (Su(z)12), MULTICOPY SUPPRESSOR OF IRA (MSI), and Extra Sex Combs (ESC) of the PRC2 complex are conserved among plants and animals (Jullien and Berger, 2009; Pien and Grossniklaus, 2007). In *Arabidopsis*, there are more than one PRC2 complexes, each of which contains the ESC homolog, FERTILIZATION-INDEPENDENT ENDOSPERM (FIE) protein in combination with different homologs of E(z) and Su(z)12. These different complexes control different developmental processes in *Arabidopsis*. By searching the rice genomic databases, Luo et al., in this special issue, have shown that the rice genome contains two E(z)-like genes (*ORYZA SATIVA E(z)1 (OsiEZ1)* and *ORYZA SATIVA CURLY LEAF (OsCLF)*), two Su(z)12-like genes (*ORYZA SATIVA EMBRYONIC FLOWER 2a (OsEMF2a)* and *ORYZA SATIVA EMBRYONIC FLOWER 2b (OsEMF2b)*), and two homologs of ESC (*OsFIE1* and *OsFIE2*). Expression analysis shows that the endosperm-specific gene, *OsFIE1*, is maternally expressed but paternally silenced, whereas *OsiEZ1*, *OsCLF*, *OsEMF2a*, *OsEMF2b*, and *OsFIE2* are expressed in all tissues and both maternal and paternal copies are expressed. Evolutionary analysis suggests that *OsiEZ1* and *OsCLF* seem to have duplicated before the separation of monocots and dicots; *OsEMF2a* and *OsEMF2b* appear to have arisen from a recent duplication in the Gramineae; and two ESC genes in rice possibly have duplicated in the ancestor of grasses. Phenotypic analysis of T-DNA insertion mutants of *OsFIE1*, *OsEMF2b*, and *OsCLF* genes shows no autonomous endosperm development in these mutants, and no morphological changes except...
for the *Osemf2b* mutants, which exhibit pleiotropic phenotypes including early-flowering and abnormal floral organs (Luo et al., 2009b). Future work with emphasis on characterization of more mutants of the rice PcG genes and their downstream targets will help to uncover the conserved as well as the specific roles of the rice PcG proteins.

In contrast to rice, there are four Su(z)12-like proteins in *Arabidopsis*: VERNALIZATION2 (VRN2), EMBRYONIC FLOWER2 (EMF2), FERTILIZATION-INDEPENDENT SEED2 (FIS2), and VEF-L36. They all share a conserved VEF domain that is found in chromatin proteins required for gene silencing across eukaryotic organisms. VRN2, EMF2, and FIS2 have been implicated in vernalization-mediated flowering, vegetative development, and seed development, respectively (Chanvivattana et al., 2004; Kohler et al., 2003b; Sung and Amasino, 2004). To get a better understanding of how these VEF-domain-containing genes (VEF genes) evolved and diversified, Chen et al., in this special issue, have analyzed sequences related to *VEF* genes in *Arabidopsis* and other land plants. Based on their investigations, *EMF2* appears to be the prototype in the generation of the *VEF* gene family. *VRN2* may have arisen from a duplication of the ancestral *EMF2*-like gene followed by deletion, insertion, or exon-skipping events. *FIS2* and *VEF-L36* may have derived from a VRN2-like ancestral sequence in *Arabidopsis* or in other angiosperms. Although the hypothesis may not represent complete genomic data due to limitations in taxon sampling and in character sampling, the work by Chen et al. has demonstrated an evolutionary history of the *VEF* genes largely consistent with the taxonomic history of the plants (Chen et al., 2009).

**EMF1, WHEN AND WHERE TO PLAY**

*EMF1* and *EMF2* encode a transcriptional regulator and a PcG protein, respectively. As mentioned above, *EMF2* is the *Arabidopsis* homolog of Su(z)12. Although there seems to be no PRC1 counterparts in *Arabidopsis*, EMF1 plays a PRC1-like role and participates in the PcG-mediated gene silencing by acting downstream of EMF2 to affect transcriptional repression of the floral homeotic genes *AGAMOUS, PISTILLATA, and APETALA3* (Calonje et al., 2008). The *emf1* and *emf2* mutant plants display pleiotropic phenotypes (Kim et al., 2010). These mutants produce flowers without much of vegetative development, suggesting that these genes repress the genes required for the transition to flowering. Histone H3K27me3 was found to be reduced in the regulatory regions of the AG gene in *emf1* and *emf2* mutants (Calonje et al., 2008), implying that repression of this gene is achieved by PcG-directed histone modification. In this special issue of *Molecular Plant*, Sanchez et al. ingeniously found the *EMF1* gene activity in epigenetic regulation of plant development by carefully controlling the gene expression in different organs and at different stages of development using tissue and stage-specific promoters to drive the sense or antisense cDNA of the gene. Plants with decreased level of *EMF1* in the SAM and leaf primordia fail to develop rosette leaves and normal leaves, respectively. However, depletion of *EMF1* in flower did not affect flower development, probably due to the pre-existing *EMF1* protein or because it is not required for flower development. Similarly, reduction of gene activity in the early developmental stages leads to the production of normal rosettes but causes early flowering, which might be due to a perturbation in cellular memory. Broader spectrum temporal and spatial expression of *EMF1* is required for the normal development because localized expression is not sufficient to rescue the loss-of-function mutant (Sanchez et al., 2009). This shows how *EMF1* regulates the expression of the coordinately repressed genes, through H3K27 methylation, in different stages of plant development. However, finding the genome-wide tissue-specific *EMF1* targets (by Chromatin immunoprecipitation (ChiP)-chip or ChiP-sequencing) in wild-type as well as H3K27me3 patterns in *emf1* mutants will further deepen our knowledge of the relationship between *EMF1* and H3K27me3 in the epigenetic regulation of target genes.

**EPIGENETIC REGULATION BY HISTONE VARIANTS IN PLANTS**

‘Histone replacement’ is a process in which canonical histones are replaced by histone variants, H2A by H2A.Z, for instance. Incorporation of histone variants in place of the canonical histones at certain loci confers specific structural and functional features to the local chromatin (Bernstein and Hake, 2006; Kamakaka and Biggins, 2005; Sarma and Reinberg, 2005) and hence is implicated in a myriad of biological processes, including the regulation of gene expression (Adam et al., 2001; Brickner et al., 2007; Larochelle and Gaudreau, 2003; Li et al., 2005; Raisner et al., 2005; Santitissaran et al., 2000; Zhang et al., 2005), prevention of heterochromatin spreading to euchromatin regions (Dhillon and Kamakaka, 2000; Krogan et al., 2003; Meneghini et al., 2003), cell cycle progression (Dhillon et al., 2006), genome stability (Carr et al., 1994; Downs et al., 2004; Keogh et al., 2006; Krogan et al., 2004), suppression of antisense RNAs (Zofall, 2009), stabilizing the association of condensin with mitotic chromosomes (Kim et al., 2009), and plant thermosensory perception (Franklin, 2010; Kumar and Wigge, 2009). Recent work in different labs found that SWR1, an ATp-dependant chromatin remodeling complex, is involved in the deposition of H2A.Z in yeast and animals. Although the presence of this complex is not yet shown in plants, functional subunits of SWR1 have been characterized recently (He, 2009). In this special issue, Rosana March-Diaz and Jose C. Reyes reviewed the recent advances in investigating the functions of H2A.Z histone and the proteins responsible for its deposition in plants. The *Arabidopsis* genome contains 13 genes for histone H2A, which are differentially expressed throughout the cell cycle (March-Diaz and Reyes, 2009). *Arabidopsis* contains orthologs of the 11 known components of yeast and human SWR1/SRCAP complexes, including PHOTOPERIOD
INDEPENDENT EARLY FLOWERING1 (PIE1) (Noh and Amasino, 2003), SERRATED AND EARLY FLOWERING (SEF) (March-Diaz et al., 2007), ACTIN-RELATED PROTEIN 6 (ARP6) (Choi et al., 2005), etc. The pie1, sef, and arp6 mutants display pleiotropic phenotypes. Pieces of evidence for the presence of a SWR1-like complex in Arabidopsis include (1) the presence of orthologs of many of the known subunits with similar characteristics of this complex; (2) identical interactions of these proteins with other proteins/complexes to that of the known SWR1 members in yeast and animals; and (3) significant overlap between the genes regulated by H2A.Z and putative SWR1 complex members and similarity between phenotypes provoked by their loss-of-function mutations. However, it has been challenging for plant biologists to purify a functional SWR1 complex in plants that would, probably, lead to the identification of more plant-specific components (March-Diaz and Reyes, 2009). The authors also pointed to some as yet unanswered questions: (1) Is H2A.Z essential in Arabidopsis? (2) By what mechanism do histone variants affect transcription? (3) How are H2A.Z and SWR1 recruited to the chromatin? In addition, finding the protein partners in the deposition and removal of H2A.Z or in recruiting other factors to H2A.Z-containing nucleosomes (histone-modifying enzymes, chromatin remodelers, and possibly others) will provide further information about how histone variants influence biological processes.

CHROMATIN REMODELING IN STEM CELL MAINTENANCE

Stem cells are able to renew themselves and to generate cells destined to form new tissues and organs. In higher plants, the shoot apical meristem (SAM) and the root apical meristem (RAM) are the two most intensively studied regions containing stem cells, which form the aboveground part and the underground root system of the plant, respectively. Recent studies have indicated crucial roles of chromatin remodeling in the regulation of stem cell activity. In this special issue on ‘Epigenetics and Plant Development’, Shen and Xu highlighted some of the chromatin remodeling factors in Arabidopsis, such as the nucleosome assembly/disassembly factors, the ATP-dependent chromatin remodeling complexes, and the histone covalent modifiers (e.g., histone acetyltransferases and lysine methyltransferases) and summarized their roles in regulating the maintenance of stem cell niche in the SAM, leaf initiation and boundary establishment, floral organ identity, RAM organization and root development, and the SAM–RAM polarity establishment (Shen and Xu, 2009). Although powerful genetic approaches have enabled us to characterize so many chromatin-remodeling factors, their molecular mechanisms of action still need to be elucidated, with respect to how these factors coordinate with each other, whether these factors are directly or indirectly involved in the activation or repression of their target genes, and whether these factors may provide means to regulate different sets of targets or to regulate the same targets at different cell types or times during plant development.

REGULATION OF PLANT HORMONAL AND STRESS RESPONSES BY CHROMATIN REMODELING

Being sessile organisms, unlike animals that can move freely and respond to the environmental stresses, plants have adopted to respond to stresses by the chromatin remodeling. Nucleosome Assembly Protein 1 (NAP1) family proteins are considered as histone chaperones, which are conserved from yeast to human and are proposed to facilitate the assembly of newly synthesized histone H2A and H2B into a dimer, transfer the dimer to replicating DNA in the nucleus, and act synergistically with ATP-dependent chromatin remodeling factors to facilitate the assembly and remodeling of chromatin (Dong et al., 2005). In Arabidopsis, four NAP1 (NAP1;1–4) (Dong et al., 2003) and two NAP1-related proteins (NRP1/2) have been discovered so far (Zhu et al., 2006). Recently, one paper implicated NAP1 proteins in transcription and nucleotide excision repair (Liu et al., 2009) and in another study from the same laboratory, reported in this issue of Molecular Plant, a truncated NAP1 protein, AtNAP1;3T (lacking C-terminal 34 amino acids), was found to alter plant’s response to ABA and salt stress. Furthermore, AtNAP1;3T functions as a dominant negative factor controlling the ABA response (Liu et al., 2009). This work provided a novel link between chromatin remodeling and hormonal and stress responses. Further investigations into the chromatin composition and structural changes in the NAP1 mutants will enhance our understanding of the role of nucleosome assembly in regulating the expression of various genes.

MSI-like proteins, which form a subgroup of WD40 proteins, are present in all eukaryotes. The Arabidopsis genome contains five MSI1-like genes (MSI1-5) (Ach et al., 1997; Hennig et al., 2003; Kenzior and Folk, 1998). Complete loss of AtMSI1 protein is lethal, which is consistent with its being an essential component of chromatin assembly factor 1 (CAF1) (Kaya et al., 2001) and participating in polycomb group repressive complexes, such as FIS2 (Kohler et al., 2003a) and EMF2 complexes (Schonrock et al., 2006). AtMSI1 function is required for the maintenance of homeotic genes expression through inheritance of epigenetic states during mitosis (Hennig et al., 2003). MSI1 regulates flowering time by induction of SOC1 through H3K4 dimethylation and H3K9 acetylation (Bouveret et al., 2006). Lars Hennig’s group has been exploring the diverse roles played by MSI1 in epigenetic regulation of differentiation and development. In this special issue, this group (Alexandre et al., 2009) reports that a large number of ABA-responsive genes are specifically activated in msi1-cs plants, which points to a hidden role of this gene in the regulation of plants’ responses to drought stress. MSI1 was found to directly target RESPONSIVE TO DISSICATION20 (RD20) and negatively regulate plants’ tolerance to drought stress (Alexandre et al., 2009). Transcriptome analysis of the msi-cs plants indicated that the known functions of this protein only represent a small fraction of the diverse roles played by this protein and...
further studies are needed to decipher the important biological roles of this gene in plant development.

EPIGENETIC REGULATION OF SEED DEVELOPMENT

Plant development takes place in distinct phases, each of which is characterized by the expression of a particular set of genes, while keeping the others repressed. Seed development is an important trait of flowering plants that gives it dominance over the other land plants. The expression of seed-specific genes at a specific time in the Arabidopsis life cycle raises two related questions: What drives the expression of the seed transcriptome? And what prevents the expression of the seed transcriptome during other stages of plant development? The studies by Joe Ogas et al., in this issue of Molecular Plant, attempt to address these two questions. The first question has been partly answered by the identification of several genes that act as positive regulators of the seeds development program, such as LEAFY COTYLEDON (LEC), ABSCISIC ACID INSENSITIVE3 (ABI3), BABY BOOM (BBM), AGAMOUS-LIKE15 (AGL15), WUSCHEL (WUS), and SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE (SERK), etc. (Boutilier et al., 2002; Heck et al., 1995; Koornneef et al., 1984; Laux et al., 1996; Meinke, 1992; Schmidt et al., 1997). Efforts towards addressing the second question are still in progress. However, so far, three categories of proteins have been characterized to have roles in repressing the seed transcriptome in other stages of plant development. These include (1) histone modifiers (PcG and HISTONE DEACETYLASE6/19 (HDAC6/HDA19), (2) chromatin remodelers (PICKLE (PKL) and BRAHMA (AtBRM1)), and (3) transcription factors (VP1/ABSCISIC ACID INSENSITIVE 3-LIKE (VAL)) (Zhang and Ogas, 2009). Future investigations into how PKL promotes deposition of H3K27me3 and how the restricted expression of seed genes is released in the next generation as well as functional analysis of the PcG-associated machinery that represses seed-specific transcriptional programs will undoubtedly accelerate our understanding of developmental regulation of seed-specific genes.

REQUIREMENT OF DIRECT TANDEM REPEATS FOR PHERES1 IMPRINTING IN ARABIDOPSIS

Imprinting is an epigenetic phenomenon in plants and animals, which refers to monoallelic expression of specific genes in a parent-of-origin manner. Imprinted genes are maternally expressed but paternally silenced or vice versa. DNA methylation and histone modifications were found to have role in monoallelic gene expression. Once established in the germ line, these epigenetic marks are maintained throughout the lifecycle. In plants, imprinting is confined to the endosperm (Jullien and Berger, 2009; Makarevich et al., 2008). The imprinted genes share a common feature that they are usually less methylated in the endosperm than embryo, exhibit endosperm-specific expression and low expression in other tissues of the plant (Day et al., 2008; Gehring et al., 2009). By genome-wide DNA methylation profiling in Arabidopsis, five new imprinted genes were verified out of ~50 candidate imprinted genes fitting these criteria (Gehring et al., 2009). Now, there are at least 10 known imprinted genes in Arabidopsis, amongst which MEDEA (MEA), FWA, FIS2, MATERNALLY EXPRESSED PAB C-TERMINAL (MPC), HOMEODOMAIN GLABROUS8 (HDG8), HOMEODOMAIN GLABROUS9 (HDG9), and AtMYB3R2 are maternally expressed, whereas PHERES1 (PHE1), HDG3, and At5G62110 are paternally expressed. Imprinting of PHE1 requires histone H3K27me3 directed by the FERTILIZATION INDEPENDENT SEED (FIS) Polycomb group (PcG) complex as well as DNA methylation of a distantly located region downstream of the PHE1 locus (Villar et al., 2009). However, which element within this region contributes to the establishment of imprinting is unknown. By comparing such distal region between PHE1 and its close homolog PHERES2 (PHE2), and by investigating this region in different Arabidopsis accessions, Villar et al., in this special issue on ‘Epigenetics and Plant Development’, demonstrated that the presence of tandem repeats and methylation of these sequences are conserved in many Arabidopsis accessions. In concert with this finding, they have shown that PHE2, which lacks the tandem repeat, is not regulated by genomic imprinting, as PHE2 is equally expressed from maternal and paternal alleles, although it is a direct target of the FIS PcG complex. Deletion of the tandem repeats and the region downstream of the repeats results in biallelic expression of PHE1 (Villar et al., 2009). The work by Villar et al. not only broadens our knowledge about imprinting, by uncovering the importance of a tandem repeat sequence in the establishment of genomic imprinting, but also strengthens the notion that genomic imprinting and gene silencing are not the necessary consequences of FIS PcG targeting; additional mechanisms may be required for the PcG-directed silencing. As the authors have mentioned, further investigations on how differentially methylated region interacts with the FIS PcG complex would specifically unravel the molecular basis of PHE1 imprinting in Arabidopsis.

PARAMUTATION

Paramutation is an epigenetic phenomenon mediated by interactions between two alleles of a single locus, which results in a heritable change in gene expression of paramutable allele induced by the paramutagenic allele. It was initially discovered and studied in plants and later reported in other species such as fungi and mammals (Chandler and Stam, 2004). Although great progress, almost exclusively in the model plant maize, has been achieved, the specific mechanisms of paramutation remain to be clarified. In this special issue, Maike Stam has elegantly reviewed some of the most important findings on paramutation and summarized three models explaining various paramutation phenomena, namely
the RNA model, the physical interaction model, and the RNA-physical interaction model (Stam, 2009). In the RNA model, siRNAs derived from the paramutagenic repeats turn the paramutable allele into paramutagenic allele by RNA-directed DNA methylation (RdDM) and chromatin silencing, which results in the production of more siRNAs. In the physical interaction model, a protein complex directs the pairing between the paramutagenic repeats and the repeatable repeats. Once paired, the epigenetic state can be transferred from the paramutagenic to the paramutable allele and the newly silenced allele can further silence new targets. In the RNA-physical interaction model, both siRNAs and physical pairing are required. The pairing is directed by the siRNAs and facilitates the transition of paramutable repeats into paramutagenic repeats. Nevertheless, continued efforts should be paid to enhance our understanding of paramutation. Although the cloning of the RNA-dependent RNA polymerase, MOP1 gene indicates that RNAi machinery plays a critical role in paramutation (Alleman et al., 2006), exactly how the RNAs cause paramutation has not been fully demonstrated. In addition to RNAs, it is likely that certain proteins are involved in physical interactions between homologous alleles. It is crucial to identify these proteins. Like other epigenetic changes, the roles of covalent modifications of DNA and/or the histones and the chromatin structure involved in paramutation are still unknown. Further investigations will hopefully shed light on this mystery.

**EPIGENETIC BARRIERS IN SPECIES HYBRIDIZATION**

Hybridization is a process of mating organisms of different varieties or species to create a hybrid. Species hybridization results in the formation of new species and phenotypic traits; however, there are many genetics and epigenetic barriers that hinder a successful hybridization. Ryo Ishikawa and Tetsu Kinoshita, in this special issue of *Molecular Plant*, outlined the current knowledge on the epigenetic events impeding species hybridization. There are two well-studied genomic events—transposon silencing and genomic imprinting—resisting species hybridization, which are controlled epigenetically via DNA methylation (Gehring et al., 2009), histone modifications (Slotkin and Martienssen, 2007), and small RNAs (Girard and Hannon, 2008; Ishikawa and Kinoshita, 2009). Studies, both in plants and animals, show that genome expansion and loss of transposon silencing occur after hybridization, such as in sunflower (Rieseberg et al., 1995), mammals (O'Neill et al., 1998), wheat (Kashkush et al., 2003), and *Arabidopsis* (Josefsson et al., 2006). Successful hybridization, in some instances, results in heterosis such that the progeny display superior characteristics as compared to either parent. Very little is known, until now, about the molecular mechanisms controlling species hybridization or heterosis, processes of pivotal importance in agriculture.

**24nt-siRNA-INDEPENDENT HETEROCHROMATIN ORGANIZATION BY Pol V**

The 24nt-siRNAs, generated by Nuclear RNA polymerase IV (Pol IV), RNA-DEPENDENT RNA POLYMERASE 2 (RDR2), and DICER-LIKE 3 (DCL3), play an essential role in heterochromatin formation at pericentromeric repeats, retroelements, silenced rRNA genes, and other genomic loci. The 24nt-siRNAs in association with AGONAUTE 4 (AGO4) bind to RNA Polymerase V (Pol V) transcripts and subsequently recruit DOMAINS REARRANGED METHYLASE 2 (DRM2) and histone modifying machinery to the heterochromatin loci (Wierzbicki et al., 2008). In this issue of *Molecular Plant*, Olga Pontes et al. have carefully analyzed the role of Pol V and the plant-specific SWI/SNF2 chromatin remodeling protein DEFICIENT IN RNA-DEPENDENT DNA METHYLATION 1 (DRD1) in the condensation of pericentromeric repeats (Pontes et al., 2009). Decondensation and coincident reactivation of pericentromeric repeats were observed specifically in pol V and drd1 mutants. Furthermore, this role of the Pol V in heterochromatin organization was found to be independent of the Pol IV, RDR2, and DCL3-mediated siRNA production, pointing to either a novel mechanism of silencing pericentromeric repeats or the involvement of other small RNA pathways (Pontes et al., 2009). Future studies of (1) how pericentromeric repeats are transcribed, (2) the nature of these non-coding RNAs, and (3) the nature of any interacting protein(s) would likely provide new insights into the epigenetic regulation of pericentromeric regions.

**GENOME POSITION AND TISSUE TYPE ALSO COUNT IN EPIGENETIC REGULATION**

In eukaryotes, transcriptional regulation is achieved either by transcription factors or epigenetic regulators. Thousands of genes are epigenetically regulated in *Arabidopsis* that control many essential biological processes (Schubert et al., 2006; Turck et al., 2007; Zhang et al., 2007b). In contrast to transcription factors, the recruitment of which to DNA requires only interaction with a specific DNA sequence, targeting of an epigenetic regulator to a particular site involves complex interactions between DNA, RNA, histones, and other regulatory proteins (Lindroth et al., 2004; Simon et al., 2005; Turck et al., 2007; Zhang et al., 2007b). Since loci with identical sequences could acquire different combinations of epigenetic regulators (Lewis et al., 2007; Rangwala and Richards, 2007), genome position-related and tissue-specific factors may affect
the targeting of the epigenetic regulators. One paper in this issue of *Molecular Plant* from Eric Lam’s lab shows how factors such as genome position, sequence context, and organ type affect the targeting of epigenetic regulators. By comparing the *in vivo* bioluminescence in four CCLs (Chromatin Charting Lines), containing an identical T-DNA containing the *LUCIFERASE* (*LUC*) and *NEOMYCIN PHOSPHOTRANSFERASE II* (*NPTII*) genes inserted into different loci on *Arabidopsis* chromosome 2, it was found that the expression level of *LUC* gene was different not only in these lines, but even in different tissues of the same plant. In order to further characterize the genome-locus and tissue dependence on epigenetic regulators, the eight well known epigenetic regulators *LIKE HETEROCROMATIN PROTEIN1* (*LHP1*), *MORPHEUS’ MOLECULE1* (*MOM1*), *CYTOSINE METHYLTRANSFERASE3* (*CMT3*), *DRD1*, *DRM2*, *SU(VAR)3–9 HOMOLOG 2* (*SUHV2*), *CLF*, and *HEADING DATE 1* (*HD1*) were down-regulated in these CCLs using RNAi against these regulators. Different patterns of *LUC* expression in a genome-locus and tissue-specific manner were observed, which implies that these regulators target the same DNA sequences in a genome-locus and tissue-specific manner. Finally, using a novel approach of comparing the expression level of seven known epigenetically silenced loci AG, FLC, 180bps, *CYCLOPHILIN 40* (*CyP40*), *ARABIDOPSIS THALIANA MUTATOR1* (*AtMU1*), *ARABIDOPSIS THALIANA SINE1* (*AtS1N1*), and *Athila* LHP1 in four transgenic CCLs harboring the RNAi constructs for the eight epigenetic regulators in roots and shoots revealed a possible interaction network among these epi-regulators that explains the similarity of their molecular phenotype and targets (Luo et al., 2009a). Further analysis of siRNA accumulation, DNA methylation, and histone modifications at the *LUC* gene cassette of CCLs will help to unveil the molecular basis of the observed genome-locus and tissue-specific deposition of the epigenetic regulators. Additionally, applying the analysis to larger datasets obtained from microarray and high-throughput sequencing technologies may help to unravel the genome-wide functional network of epigenetic regulators.

**CONCLUDING REMARKS**

The term ‘epigenetic’ was first coined by Waddington more than half a century ago. Now, it has emerged as a broad field of science that investigates a myriad of biological phenomena governed by versatile molecular mechanisms. Through a combination of genetic, biochemical, genomic, and computational approaches, tremendous achievements have been made in uncovering the molecular basis of many epigenetic processes in both animals and plants. ‘Histone code hypothesis’ was first proposed by Strahl and Allis about a decade ago; to date, many of the histone-modifying proteins have been characterized, although still many more remain to be discovered and the sequence/priority order of the histone modifications remains to be elucidated. The mechanism and outcomes of many of the histone modifiers have been established but how these modifications are maintained during continuous cell division also needs to be addressed. In addition, the role of small RNAs in transcriptional and posttranscriptional gene silencing has been established recently, but how the small RNAs’ activity is maintained in cell divisions is still to be unveiled. In animals, the developmental program is planned in the embryo but in plants, it is designed post-embryonically through a yet not fully understood mechanism. Recently, the RNA splicing was found to be regulated by the epigenetic regulators (histone modifications and chromatin remodeling) but the underlying molecular events are largely unknown. To sum up, what has been done so far is just the onset of a seemingly endless effort to explore the unknown epigenetic mechanisms regulating the behavior of life.

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