Chromatin Remodeling in Stem Cell Maintenance in *Arabidopsis thaliana*

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**ABSTRACT** Pluripotent stem cells are able to both self-renew and generate undifferentiated cells for the formation of new tissues and organs. In higher plants, stem cells found in the shoot apical meristem (SAM) and the root apical meristem (RAM) are origins of organogenesis occurring post-embryonically. It is important to understand how the regulation of stem cell fate is coordinated to enable the meristem to constantly generate different types of lateral organs. Much knowledge has accumulated on specific transcription factors controlling SAM and RAM activity. Here, we review recent evidences for a role of chromatin remodeling in the maintenance of stable expression states of transcription factor genes and the control of stem cell activity in *Arabidopsis*.

Key words: chromatin structure and remodeling; epigenetics; meristem development; histone chaperone; histone modification.

**INTRODUCTION**

Stem cells found in multi-cellular organisms are characterized by the ability to renew themselves and to differentiate into a diverse range of specialized cell types. In higher plants that lack cell migration, stem cells are confined in spatially fixed zones in a microenvironment called the stem cell niche (Scheres, 2007), which displays remarkable longevity, allowing plants in some species to grow for hundreds of years. The most intensely investigated plant stem cells are located at the tips of shoots and roots, within the zones respectively called shoot apical meristem (SAM) and root apical meristem (RAM). While the RAM forms the underground root system, the SAM continuously produces leaves and stems, forming the aboveground phyllotaxy of the plant. The SAM can also be reprogrammed to produce flowers, ensuring plant reproduction. The balances between cell division for stem cell renewal and cell differentiation for organ formation are controlled by specific transcription factors. In this review, we focus on recent studies in *Arabidopsis* highlighting crucial roles of chromatin remodeling in the maintenance of expression pattern of these transcription factor genes and meristem activity during plant growth and development.

**CHROMATIN REMODELING FACTORS**

Chromatin represents the physiological template of genetic information in all eukaryotes. The basic unit of chromatin is the nucleosome, which is composed of ~146 base pairs of DNA wrapped around an octamer of two molecules of each of the histones H2A, H2B, H3, and H4. Chromatin structure carries information of epigenetic memory. Epigenetics refer to heritable changes (during mitosis and sometimes meiosis) of genome function that occur without a change in DNA sequence. The advantage of epigenetic changes in a stem-cell context is that they are stable and also reversible. Stable changes might be necessary for a cellular memory that allows the determined cells to retain their developmental identity through successive mitotic cycles; and reversibility might provide plasticity that is important, particularly in plants, allowing differentiated cells to recover totipotency under certain physiological and environmental conditions. The chromatin remodeling factors discussed in this review are implicated in several processes, including nucleosome assembly/disassembly, ATP-dependent chromatin remodeling, and covalent modifications of histones (e.g. acetylation and methylation).

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Chromatin Assembly

Nucleosome assembly/disassembly is mediated by histone chaperones and occurs during DNA replication as well as during gene transcription, DNA repair, and recombination. CHROMATIN ASSEMBLY FACTOR-1 (CAF-1) and NUCLEOSOME ASSEMBLY PROTEIN1 (NAP1) are two types of evolutionarily conserved histone chaperones, which are primarily responsible for deposition of H3/H4 and H2A/H2B, respectively (De Koning et al., 2007). The Arabidopsis CAF-1 is composed of three subunits: FASCIATA1 (FAS1), FASCIATA2 (FAS2), and MULTICOPY SUPPRESSOR OF IRA1 (MS11) (Kaya et al., 2001). MS11 is also found in other complexes, such as with histone deacetylases and histone methyltransferases (Rossi et al., 2003; Hennig et al., 2005). In addition, four homologs of MS11 exist in the Arabidopsis genome. Arabidopsis contains four genes encoding NAP1 and two genes encoding NAP1 RELATED PROTEIN1 and 2 (NRP1 and NRP2) (Zhu et al., 2006; Liu et al., 2009). A gene encoding a homolog of HISTONE REPRESSION A FACTOR (HIRA) was also characterized in Arabidopsis (Phelps-Durr et al., 2005). In yeast and animals, HIRA functions as a chaperone of the variant histone H3.3 in chromatin remodeling (De Koning et al., 2007).

ATP-Dependent Chromatin Remodeling

ATP-depndant chromatin remodeling complexes utilize ATP hydrolysis to remodel or reposition nucleosomes. The Arabidopsis genome encodes more than 40 different proteins belonging to ATP-depndant chromatin remodeling complexes, which can be grouped into three major types, namely SWI/SNF, ISWI, and CHD. The SWI/SNF-type SPLAYED (SYD), BRAHMA (BRM), and AtSWI3C, and the CHD-type PICKLE (PKL) are among the ones with best characterized biological functions (Hsieh and Fischer, 2005; Jerzmanowski, 2007).

Histone Acetylation

The histone tails protrude outward from the nucleosomes and are subject to different types of covalent modifications including acetylation, methylation, phosphorylation, and ubiquitylation. Acetylation is catalyzed by histone acetyltransferases (HATs) and is generally associated with transcriptional activation, whereas deacetylation catalyzed by histone deacetylases (HDACs) is associated with transcriptional suppression. The Arabidopsis genome encodes 12 HATs and 18 HDACs. In vitro enzyme assay revealed substrate preferences of some examined HATs, such as HAG1/AtGCN5 on histone H3 lysine 14 (H3K14), HAG2 on H4K12, and HAG4 on H4K5 (Earley et al., 2007). Substrate specificities of HDACs are less well known. In contrast, several HDACs including HDA18 and HDA19 had been characterized for their function in plant growth and development (Hollender and Liu, 2008).

Histone Methylation and Polycomb Silencing

Histone methylation (with addition of mono-, di-, or trimethyl group) can occur at different lysine residues, which has distinct implication for chromatin activity. While methylations on H3K4 and H3K36 are generally associated with transcriptional activation, those on H3K9, H3K27, and H4K20 are associated with transcriptional suppression. The Arabidopsis genome encodes 47 SET-domain proteins, which are potential lysine methyltransferases and can be classed into seven groups (Ng et al., 2007). The SET-domain proteins discussed in this review belong to TRITHORAX (TRX) and Polycomb group (PCG). Similar to their homologous TRX proteins in animals, ATX1 and ATX2 in Arabidopsis were shown to be involved in H3K4 methylation in activation of gene transcription (Alvarez-Venegas et al., 2003; Pien et al., 2008; Saleh et al., 2008). In animals, PcG proteins act antagonistically to TRX in transcriptional suppression through formation of at least two types of complexes: the Polycomb repressive complex 1 and 2 (PRC1 and PRC2) (Martin and Zhang, 2005; Weake and Workman, 2008). PRC2 catalyzes H3K27 methylation, resulting in the recruitment of PRC1 through binding of the chromodomain protein Polycomb (Pc) with trimethyl-H3K27. PRC1 acts to establish stable suppression by catalyzing H2A monoubiquitylation via its RING-domain subunits and/or by formation of higher order chromatin repressive structures.

In Arabidopsis, PRC2 is structurally conserved and most of its components are encoded by small gene families. Among different possible combinations of components forming PRC2-like complexes, those containing the core proteins CURLY LEAF (CLF) or SWINGER (SWN), EMBRYONIC FLOWER2 (EMF2) or VERNALIZATION2 (VRN2), FERTILISATION INDEPENDENT ENDOSPERM (FIE) and MS1 play important roles in different processes of the plant lifecycle (Pien and Grossniklaus, 2007). The SET-domain proteins CLF and SWN are the catalytic active subunits of the complexes in H3K27 methylation. CLF and SWN, and similarly EMF2 and VRN2, have partially redundant functions. In contrast to the previous belief that plants do not have homologs of PRC1 components, recent studies indicate that a PRC1-like complex functions in conjunction with PRC2 in transcription suppression in Arabidopsis. Genome-wide analysis revealed that the binding sites of the chromodomain protein LIKE-HETEROCHROMATIN PROTEIN1 (LHP1), also called TERMINAL FLOWER2 (TFL2), is co-localized with trimethyl-H3K27-enriched chromatin regions in Arabidopsis (Turck et al., 2007; Zhang et al., 2007), suggesting that LHP1/TFL2 may play a similar function to animal Pc in gene silencing. We reported that LHP1/TFL2 binds AtRING1a and AtRING1b, two homologs containing each a RING-domain showing highest homologies with the animal RING1, one of the core components of PRC1 (Xu and Shen, 2008). Finally, it had been shown that EMBRYONIC FLOWER1 (EMF1) interacts with MS11, binds DNA, and is necessary for H3K27 trimethylation (Calonje et al., 2008). EMF1 is a plant-specific PcG protein, which does not display significant homology with animal proteins (Moon et al., 2003).

Remarks

Several genes encoding cofactors of chromatin remodeling complexes will be described later when their roles in
regulation of stem cell activity are discussed. It is useful to note that most, if not all, chromatin remodeling factors regulate a number of genes and are known or supposed to function in several biological processes. We here below focus our discussion on their function in the regulation of stem cell activity.

ORGANIZING CENTER IN SAM: CHROMATIN REMODELING IN REGULATION OF WUSCHEL EXPRESSION

The structure of SAM can be subdivided into three zones: the central zone (CZ), the peripheral zone (PZ), and the rib zone (RZ) (Figure 1A). CZ is the core domain of SAM, in which cells maintain a relatively undifferentiated state and divide infrequently to replenish the pool of stem cells and to provide cells replacing their neighbors in PZ and RZ. PZ is active in cell division and is the site from which lateral organs (such as leaves) initiate. RZ is active in production of the inner part of the stem. According to the tunica-corpus structure, SAM can also be subdivided into three layers: the epidermal layer (L1), the subepidermal layer (L2), and the corpus (L3). While L1 and L2 manifest anticlinal cell divisions, L3 exhibits variable directions of cell divisions. The organizing center (OC) located in the CZ of L3 (Figure 1A) is composed of a few cells exhibiting a relatively low cell division rate. WUSCHEL (WUS) encodes a homeodomain transcription factor, is specifically expressed in the OC, and plays a key role in positioning the stem cells and in the maintenance of SAM organization (Laux et al., 1996; Mayer et al., 1998). Several chromatin regulators are involved in confining WUS expression in the OC region (Figure 1B).

Mutations in FAS1 or FAS2 resulted in a fasciated stem and a disorganized SAM with unstable expression pattern of WUS (Kaya et al., 2001). Ectopic expression of WUS was observed in L2 and L1 tissue layers, indicating a role of CAF-1 in suppression of WUS transcription. Release of WUS suppression was also observed by mutation in CLF, EMF2, LHP1/TFL1, or ICU2 (Barrero et al., 2007). ICU2 encodes a putative catalytic subunit of DNA polymerase α, which physically binds LHP1, and icu2 mutant

Figure 1. Chromatin Remodeling Factors in Control of Vegetative Shoot Apical Meristem Activity.
(A) Structure of Arabidopsis shoot apical meristem (SAM). SAM can be divided into different zones (CZ, PZ, RZ) and tissue layers (L1, L2, L3). The organizing center (OC), composed of cells with a relatively low rate of cell divisions, contains important positional information for SAM organization.
(B) Summary of chromatin regulators involved in regulation of WUS expression in OC maintenance.
(C) A model of suppression of Class I KNOX genes by Polycomb-like complexes, which confines SAM activity.
(D) A flowchart highlighting chromatin remodeling factors involved in regulation of the transcription factor genes AS1 and CUC in the initiation and boundary establishment of leaf primordia.
Positive actions are represented by arrows and negative actions by bars. Question marks indicate factors unidentified to date.
showed a synergistic mutant phenotype with fas1, clf, lhp1 tfl2, and is epistatic to emf2 (Barrero et al., 2007). Mutations in BRUSHY1 (BRUI), also named MGOUN3 (MGO3) and TON-SOKU (TSK), encoding a protein involved in the post-replicative stabilization of chromatin structure, showed very similar effects to fas1 and fas2 mutants on SAM and WUS expression (Gyomarc’h et al., 2004; Suzuki et al., 2004; Takeda et al., 2004). Together, these observations suggest a possible link between DNA replication and chromatin remodeling by CAF-1 and PcG complexes in the maintenance of WUS suppression. Histone acetylation seems also to play a role in the regulation of WUS expression; mutations in HAG1/AtGCN5 resulted in expansion of the WUS expression domain throughout whole floral meristems (Bertrand et al., 2003). It is currently unknown, however, whether the suppression of WUS expression by these above listed factors is direct or indirect.

WUS expression in the OC region is activated by the SWI/SNF-type ATPase SYD (Kwon et al., 2005). Mutations in SYD resulted in reduction of WUS expression and SAM size. The SYD protein directly binds to the WUS promoter, supporting the notion that ATP-dependent chromatin remodeling directly regulates WUS activation (Kwon et al., 2005). Recently, BRCA1-associated RING domain 1 (BARD1) was identified as a SYD-binding protein (Han et al., 2008). Mutations in BARD1 resulted in expansion of WUS expression into the L1 and L2 layers of the whole SAM, whereas overexpression of BARD1 resulted in reduced WUS expression. It was proposed that BARD1 represses WUS transcription through inhibition of the chromatin remodeling factor SYD (Han et al., 2008).

**SAM MAINTENANCE: CONFINING KNOX Expression Pattern by Polycomb Group Proteins**

Class I KNOTTED1-like homeobox (KNOX) genes act independently and complementarily to WUS in the maintenance of stem cell niche in the SAM. Class I KNOX genes include SHOOTMERISTEMLESS (STM), BRUVIPEDICELLUS (BP) also named KNOTTED-like gene from Arabidopsis thaliana 1 (KNAT1), KNAT2, and KNAT6. The STM gene is activated early during embryogenesis, concomitant with the initiation of cotyledons and SAM formation; it is expressed in the whole region of SAM but silenced in lateral organ primordia (Barton and Poethig, 1993; Long et al., 1996). Loss-of-function of STM eliminates stem cell activity in the SAM, resulting in a shoot-meristemless phenotype. STM is necessary in the maintenance of the indeterminate cell fate and the prevention of cell differentiation in the meristem (Lenhard et al., 2002). BP/KNAT1, KNAT2, and KNAT6 are also specifically expressed in the SAM and have partially redundant roles with STM in SAM maintenance (reviewed in Scofield and Murray, 2006). Ectopic expression of these Class I KNOX genes in other tissues causes abnormal organogenesis, indicating that their expression pattern determines their function. Recent studies revealed that histone methylation and PcG proteins play crucial functions to avoid expression of Class I KNOX genes in differentiated cells (Figure 1C).

Ectopic expression of STM and KNAT2 was observed in leaves of the clf mutant (Katz et al., 2004). Transgenic plants with co-suppressed FIE showed ectopic expression of STM and KNAT2 as well as BP/KNAT1 (Katz et al., 2004). A higher level of ectopic expression of STM was detected in the double mutant clf swn, indicating redundant function of CLF and SWN in suppression of STM (Schubert et al., 2006). The CLF protein was shown to bind at STM chromatin, and levels of H3K27 methylation were decreased at STM chromatin in the clf and emf2 mutants and more drastically in the clf swn and emf2 vrn2 mutants (Schubert et al., 2006). Taken together, these studies indicate that PRC2 is directly involved in suppression of Class I KNOX genes. Release of suppression of Class I KNOX genes was also observed in the lhp1 and AtRING1a−/− AtRING1b−/− mutants (Xu and Shen, 2008). Inspection of the LHP1-binding site profiling data (Zhang et al., 2007; http://epigenomics.mcdb.ucla.edu/LHP1) revealed that LHP1 binds at or at proximal regions of chromatin of Class I KNOX genes. It thus suggests that the PRC1-like complex containing LHP1, AtRING1a, and AtRING1b acts in conjunction with PRC2-catalyzed H3K27-methylation in suppression of Class I KNOX genes. Consistent with their action downstream of PRC2, AtRING1a and AtRING1b are not required for H3K27 trimethylation at chromatin of Class I KNOX genes (Xu and Shen, 2008). The WD40-domain cyclophilin CYP71 was identified as a repressor of STM, BP/KNAT1, and KNAT2 expression, and was shown to positively regulate H3K27 di- and tri-methylation at target loci (Li et al., 2007). The relationship between CYP71 and the PRC2/PRC1-mediated silencing is, however, currently unclear.

**CHROMATIN REMODELING IN REGULATION OF LEAF INITIATION AND BOUNDARY ESTABLISHMENT**

The leaf primordium initiates from PZ of the SAM. This process requires activation of leaf identity genes and suppression of Class I KNOX genes (Figure 1D). ASYMMETRIC LEAVES1 (AS1), encoding a MYB domain transcription factor, is a leaf identity gene that is expressed starting from the P0 stage of leaf formation but silenced in the SAM (Byrne et al., 2000; Sun et al., 2002). GENERAL TRANSCRIPTION FACTOR GROUP E6 (GTE6), a bromodomain protein, directly facilitates AS1 expression by targeting the AS1 locus and mediating histone H3/H4 acetylation (Chua et al., 2005). The identity of histone acetyltransferase or deacetylase involved is currently unknown.

AS1 is able to physically interact with the LATERAL ORGAN BOUNDARIES (LOB) domain transcription factor AS2 (Xu et al., 2003), and binds and suppresses transcription of BP/KNAT1, KNAT2, and KNAT6 in lateral organs (Byrne et al., 2000; Ori et al., 2000; Semiarti et al., 2001; Lin et al., 2003; Guo et al., 2008). Mutations in either AS1 or AS2 resulted in ectopic expression of BP/KNAT1, KNAT2, and KNAT6 in leaves with
undifferentiated meristem characters. A yeast two-hybrid screen using AS1 as a prey identified the histone chaperone HIRA as a binding protein of AS1 (Phelps-Durr et al., 2005). The null allele mutant hira is embryo lethal, and co-suppression of HIRA in transgenic plants resulted in a phenotype similar to that of as1, which is accompanied by ectopic expression of BP/KNAT1 and KNAT2 in leaves. It thus suggests that AS1, AS2, and HIRA form a chromatin-remodeling complex involved in suppression of BP/KNAT1 and KNAT2 expression (Phelps-Durr et al., 2005). The null allele mutant as1 is embryo lethal, and co-suppression of HIRA in transgenic plants resulted in a phenotype similar to that of hira, which is accompanied by ectopic expression of BP/KNAT1 and KNAT2 in leaves. Recent studies indicated that PKL is involved in H3K27 methylation (Zhang et al., 2008), suggesting a possible link between PKL and the PcG-silencing pathway. Whether such a link exists between the AS1–AS2 pathway and the PRC2/PRC1-mediated silencing pathway in KNOX suppression remains to be investigated.

CUP-SHAPED COTYLEDON genes (CUC1, CUC2, and CUC3) encode NAC domain transcription factors, and are expressed in the boundaries between lateral organs and the SAM (Aida et al., 1997; Takada et al., 2001; Vroemen et al., 2003; Hibara et al., 2006). Mutations in any two of the three CUC genes caused a strong defect in boundary formation and resulted in a fused cotyledon phenotype. Overexpression of CUC1 was able to induce ectopic expression of class I KNOX genes in the cotyledon, resulting in ectopic meristem formation on the cotyledon. The CUC genes thus establish organ boundaries by locally inhibiting cell proliferation. Both the SWI/SNF-type ATPases SYD and BRM are involved in the CUC pathway in defining meristem boundaries (Kwon et al., 2006). brm was identified from a mutant screen for enhancers of the cuc2 mutant phenotype. The brm mutant was able to enhance the phenotype of cuc1 and cuc3. In contrast, mutations in SYD strongly enhanced the cuc1 phenotype, had only a modest enhancer effect on cuc3, and had no effect on cuc2. Consistently, molecular data revealed that BRM activates transcription of all three CUC genes, whereas SYD only activates CUC2 transcription (Kwon et al., 2006). It is currently unclear whether BRM and SYD are directly or indirectly involved in activation of CUC genes.

**CHROMATIN REMODELING IN REGULATION OF FLORAL ORGAN IDENTITY**

Floral meristem (FM) identity is conferred by LEAFY (LFY) and APETALA1 (AP1), which are expressed in FM but not in SAM (for a review, see Zik and Irish, 2003). The syd mutants were first isolated in a screen to identify phenotypic enhancers of the weak leafy-5 allele (Wagner and Meyerowitz, 2002). SYD acts with LEAFY to regulate shoot meristem identity. FM sequentially generates three types of lateral organs (sepal, petals, and stamens) and then terminates in the formation of carpels in the center of the flower (Figure 2A). Flower organ identity is determined by three classes of homeotic genes: the class-A genes APETALA1 (AP1) and APETALA2 (AP2), the class-B genes PISTILLATA (PI) and APETALAS (AP3), and the class-C gene AGAMOUS (AG), together with the SEPALLATA (SEP1, SEP2, and SEP3) genes (Zik and Irish, 2003). Several chromatin

![Figure 2](image-url)
regulators are involved in the regulation of expression of these floral homeotic genes (Figure 2B).

Chromatin-remodeling complexes containing BRM and AtSWI3C were shown to positively regulate expression of AP2, AP3, and PI (Sarnowski et al., 2005; Hurtado et al., 2006). The H3K4 methyltransferase ATX1 is also involved in activation of expression of floral homeotic genes. Mutations in ATX1 caused down-regulation of AP1, AP2, PI, and AG, but not of AP3 and SEP3 (Alvarez-Venegas et al., 2003). ATX1 protein was shown to bind chromatin at AG but not at AP1, suggesting that ATX1 regulates directly AG but indirectly AP1 expression (Saleh et al., 2007).

Mutations in CLF or down-regulation of FIE resulted in ectopic expression of AG and AP3 (Goodrich et al., 1997; Katz et al., 2004). Ectopic overexpression of AG and AP3 as well as AP1, PI, SEP2, and SEP3 was also observed in the emf2 mutant (Moon et al., 2003). The CLF protein has been shown to bind the AG locus, and its binding co-localized with H3K27me3 marks (Schubert et al., 2006). Simultaneous loss of ATX1 and CLF restored AG repression and normalized leaf phenotypes (Saleh et al., 2007), indicating antagonistic functions of ATX1 and CLF in AG regulation. Mutations in EMF2 also drastically decreased H3K27me3 levels at the AG locus (Schubert et al., 2006). MS11 also functions in flower homeotic gene repression (Hennig et al., 2003). Down-regulation of MS11 released AG suppression, which is independent of CAF-1 function, since even the fas1 fas2 double mutant maintains the repressed state of AG in leaves (Hennig et al., 2003). Current models propose that CLF or SWN, FIE, EMF2, and MS11 form PRC2-like complexes acting on repression of floral homeotic genes.

LHP1/TFL2 is involved in repression of AG, AP3, and PI (Kotake et al., 2003), and binds the promoter and transcribed regions of these genes (Germann et al., 2006). Interestingly, repression of AG and AP3 is maintained in the Atring1a−/−Atring1b−/− mutant leaves (Xu and Shen, 2008). This contrasts with the repression of Class I KNOX genes where AtRING1a and AtRING1b act with LHP1 in a PRC1-like complex. Nonetheless, homeotic conversion has been observed on flowers of the Atring1a−/−Atring1b−/− mutant (Xu and Shen, 2008), and release of AG repression was detectable in flowers (our unpublished data). Whether AtRING1a and AtRING1b directly repress AG expression specifically in flowers remains to be verified. The icu2 mutant exhibited ectopic expression of AG, AP1, AP3, PI, and SEP3 (Barrero et al., 2007), suggesting that LHP1/TFL2 and ICU2 may form a PRC1-like complex in suppression of floral homeotic genes. EMF1 is necessary for repression of AP1, PI, AP3, AG, SEP2, and SEP3 (Moon et al., 2003) and for H3K27 trimethylation at the AG locus (Calonje et al., 2008). Taken together, it appears that different PcG proteins form sub-complexes, which play redundant or non-redundant roles in the suppression of different homeotic genes.

**CHROMATIN REMODELING IN REGULATION OF RAM ORGANIZATION AND ROOT DEVELOPMENT**

In RAM, the quiescent centre (QC) is surrounded by a population of initial cells that act as stem cells for the formation of each of the different tissues in the root (Figure 3A). PLETHORA1 (PLT1) and PLT2 genes, which encode two redundant AP2-domain transcription factors, are expressed specifically in the RAM and are essential for meristem cell niche organization in response to auxin signals (Aida et al., 2004). SCARECROW (SCR) and SHORTROOT (SHR), which encode members...
of the GRAS family of transcription factors, are required for radial patterning and QC identity (Di Laurenzio et al., 1996; Helariutta et al., 2000). While SHR is expressed in the innermost tissue of the root, the stele, SCR is specifically expressed in the endodermis. Loss of PLT1 and PLT2 or loss of either SCR or SHR results in RAM termination.

Both SCR and PLT2 have been shown to be regulated by chromatin remodeling factors (Figure 3B). In addition to SAM defects, fas1 and fas2 mutants have defects in RAM; the normal restricted spatial expression pattern of SCR is partially lost; ectopic expression of SCR in adjacent cells was observed in the mutant RAM (Kaya et al., 2001). RAM organization and SCR expression were also affected in the bru1/mgo3/ tsk mutants (Guyomarç’h et al., 2004; Suzuki et al., 2004). It is currently unknown, however, whether chromatin structure at SCR is changed in the fas1, fas2, or bru1/mgo3/ tsk mutants. RETINOBLASTOMA-RELATED (RBR) was shown to act downstream of SCR and play a critical role in coordinating cell division with cell differentiation in the RAM (Wildwater et al., 2005). RBR protein binds MS1 and CLF; by analogy to animal Rb functions, it was speculated that RBR regulates transcription through interactions with several chromatin-remodeling complexes (Shen, 2002). Furthermore, loss of RBR resulted in an increase in expression of several PRC2-genes (Johnston et al., 2008). The Brassica napus SCR can binds HDA19 (Gao et al., 2004) and, more recently, the Arabidopsis SCR was reported to bind LHP1 (Cui and Benfey, 2009). It was proposed that epigenetic regulation by LHP1 and histone deacetylases forms the common basis for SCR and the phytohormone gibberellin activity in root cortex cell proliferation (Cui and Benfey, 2009).

While the single mutants nrp1-1 and nrp2-1 did not exhibit any obvious phenotype, the double mutant nrp1-1 nrp2-1 had a short-root phenotype and showed disorganized RAM and arrest of cell cycle progression at G2/M in the root tip. NPR1 and NRP2 are required for suppression of PLT2, and NPR1 and NRP2 proteins were shown to bind chromatin at PLT2 (Zhu et al., 2006). It is thus likely that NPR1 and NRP2 regulate directly the expression of PLT2.

Root epidermal hair/non-hair cell fate is triggered by a positional cue delivered by the cortical cell layer. GLABRA2 (GL2), which encodes a homeodomain transcription factor, is required for repression of root hair formation (Ohashi et al., 2003). Expression of GL2 was down-regulated in nrp1-1 nrp2-1, and NPR1 and NRP2 binds chromatin at GL2 (Zhu et al., 2006). The chromatin at GL2 had been shown to be dynamic and reorganized upon cell division in response to local positional information in roots (Costa and Shaw, 2006). Chromatin organization at GL2 was also affected in fas2 mutant roots (Costa and Shaw, 2006). Histone acetylation and histone methylation were also shown to be involved in the regulation of root patterning genes including GL2 (Xu et al., 2005; Caro et al., 2007). At least three histone deacetylase genes including HDA18 were shown to have effects on root-hair numbers (Xu et al., 2005). Increased levels of H3 acetylation and trimethyl-H3K9 and decreased levels of dimethyl-H3K9 were observed to correlate with increased levels of expression for GL2 in the gem mutants (Caro et al., 2007). The enzymes involved in these changes of histone acetylation and methylation in the gem mutants are, however, currently unknown.

Lateral roots are initiated from the anti-clinal cell divisions in the pericycle. The CHD-type ATPase PKL and histone deacetylation seem to be involved in lateral root formation and root elongation. PKL negatively regulates the auxin-induced pericycle cell divisions, possibly through regulation of transcription of AUXIN RESPONSE FACTOR genes (ARF7 and ARF19) (Fukaki et al., 2006). Mutation in LSD1-LIKE1 (LDL1, also named SWP1) induced histone hyperacetylation at promoter chromatin and activation of expression of LATERAL ROOT PRIMORDIUM1 (LRP1), a gene encoding a putative zinc finger transcription factor (Krichevsky et al., 2009). More investigations will be necessary to better understand the chromatin remodeling factors involved as well as the role of the target transcription factors in the regulation of RAM formation.

**SAM–RAM POLARITY ESTABLISHMENT: A TOPLESS STORY**

SAM–RAM polarity is established during the early stage of embryo development. TOPLESS (TPL), which encodes a WD40-domain protein, plays a critical role in the establishment of SAM–RAM polarity (Long et al., 2002, 2006). The tpl-1 mutant, which is a gain-of-function (dominant negative) mutant, showed a strong phenotype of conversion of the shoot pole into a second root pole, resulting in two RAMs instead of one SAM and one RAM. A genetic screen identified an important function of the histone acetyltransferase HAG1/AtGCN5 and the histone deacetylase HDA19 in the establishment of the tpl-1 mutant phenotype. Mutations in HAG1/AtGCN5 could suppress the tpl-1 phenotype, while the penetrance of the tpl-1 phenotype was increased by mutations in HDA19 (Long et al., 2006). It was proposed that a silencing mechanism related with histone deacetylation is required for the maintenance of the SAM identity at the shoot pole (Long et al., 2006). Nevertheless, the molecular mechanisms underlying the function of HAG1/AtGCN5 and HDA19 with TPL in the establishment of SAM–RAM polarity remain currently scarce.

**PERSPECTIVES**

In the past few years, the powerful genetic approach in Arabidopsis allows an exponentially increased number of chromatin remodeling genes to be examined for their biological roles. Chromatin remodeling factors come in different flavors, with diverse functions in stem cell maintenance and organogenesis. A general feature appears that chromatin-remodeling factors are required not for pattern initiation, but rather to ensure that the transcriptional output of early patterning events is stably inherited through cell divisions. Although, in many cases, modifications of chromatin structure at target
genes remain to be investigated and the molecular mechanisms need to be elucidated into more details, the diversity of chromatin remodeling factors may provide a means to regulate different sets of target genes or to regulate a same target gene at different times or in different types of cells during plant development. This diversity as well as the reversible nature of epigenetic modifications might allow rapid and diverse responses to environmental or developmental stimuli. This may be particularly important for a sessile life strategy, where adverse conditions need to be dealt with by modulation of developmental programs to determine a final architecture.

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