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Emerging concepts in chromatin-level regulation of plant cell differentiation: timing, counting, sensing and maintaining

Ana Karina Morao[1], Daniel Bouyer[1] and François Roudier[1,2]

Plants are characterized by a remarkable phenotypic plasticity that meets the constraints of a sessile lifestyle and the need to adjust constantly to the environment. Recent studies have begun to reveal how chromatin dynamics participate in coordinating cell proliferation and differentiation in response to developmental cues as well as environmental fluctuations. In this review, we discuss the pivotal function of chromatin-based mechanisms in cell fate acquisition and maintenance, within as well as outside meristems. In particular, we highlight the emerging role of specific epigenomic factors and chromatin pathways in timing the activity of stem cells, counting cell divisions and positioning cell fate transitions by sensing phytohormone gradients.

Addresses
[1] Institut de Biologie de l'Ecole Normale Supérieure, Centre National de la Recherche Scientifique (CNRS) UMR8197, Institut National de la Santé et de la Recherche Médicale (INSERM) U1024, Ecole Normale Supérieure, 46 rue d'Ulm, 75230 Paris Cedex 05, France
[2] Laboratoire de Reproduction et Développement des Plantes, Centre National de la Recherche Scientifique (CNRS) UMR5667, Institut National de la Recherche Agronomique (INRA) UMR879, Ecole Normale Supérieure de Lyon, Université Lyon 1 (UCBL), 46 Allée d'Italie, 69364 Lyon Cedex 07, France

Corresponding authors: Bouyer, Daniel (bouyer@biologie.ens.fr) and Roudier, François (roudier@biologie.ens.fr)

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Introduction

Plant development mainly occurs post-embryonically and is characterized by continuous growth and extensive phenotypic plasticity. It relies primarily on the activity of stem cell populations that are maintained within the root and shoot meristems, and fuel the differentiation pathways leading to organ formation. Numerous studies have established that plant cell identity results from a tight interplay between transcriptional regulatory networks and phytohormonal signaling [1–3]. In contrast, the mechanisms regulating the progression of a cell along a differentiation path are still poorly understood.

In recent years, chromatin organization has emerged as an important player in the regulation of cell differentiation [4]. Chromatin-level orchestration of gene expression relies on covalent modifications of histones, incorporation of histone variants, DNA methylation and other factors, such as modifying and ATP-dependent remodeling enzymes (see Box 1). Combinations of these factors contribute to defining dynamic chromatin states that modulate accessibility to DNA regulatory regions and correlate with distinct transcriptional outcomes [5,6]. Whereas some chromatin states are quite transient, others can be perpetuated through replication (see Box 1). These differential dynamics are the basis of the two main properties achieved by chromatin during differentiation: plasticity, which is required to allow cell fate change by altering gene expression profiles through the selective action of cell type specific transcription factors (TFs), and heritability, which is necessary to maintain cell identity by stabilizing transcriptional states. In this review, we discuss recent studies, mostly in Arabidopsis, revealing how chromatin dynamics contributes to the coordination of cell division with differentiation as well as to the regulation of cell fate acquisition and maintenance in plants.

Chromatin-level control of stem cell fate and activity

Within meristems, stem cells are found in specific microenvironments known as stem cell niches (SCN), in which their pluripotent state is maintained by local signals coming from an organizing center (OC) [3,7]. The homeodomain transcription factors (TFs) WUSCHEL (WUS) and its homologue WUSCHEL RELATED HOMEOBOX 5 (WOX5) are key organizers of stem cell pools that maintain stem cell identity in the shoot (SAM) and root apical meristems (RAM), respectively [8,9]. Recent findings indicate that both the spatiotemporal regulation of *WOX5* and *WUS* expression and their repressive action on differentiation factors depend on specific chromatin-based mechanisms [10•,11••,12••].

Restriction of *WOX5* expression within the RAM OC, called the quiescent center (QC), is regulated by the PHD-domain protein ROW1 (REPRESSOR OF WUS1). ROW1 is expressed in the proximal meristem, just above the QC (Figure 1). The binding of ROW1 to

Box 1 Chromatin factors and pathways

Studies in the model plant *Arabidopsis thaliana* have provided important insights into chromatin components and regulatory pathways as well as epigenome organization. The basic unit of chromatin is the nucleosome, which is composed of a protein core of two molecules of the histones H2A, H2B, H3 and H4, around which 147 bp of DNA is wrapped.

Several histone variants exist and provide specific indexing of the genome. For instance, H3.1 is incorporated during DNA replication and H3.3 is preferentially linked with transcription [56]. Additional variants of H3 or H2A are either exclusively found over pericentromeric heterochromatic regions such as H2A.W [57], at responsive genes (H2A.Z) [58] or only expressed during particular developmental phases, such as gametophyte-specific H3s [56]. Besides nucleosomal histones, linker H1 histones are important regulators of DNA accessibility [59,60].

In addition, histones can be subject to a plethora of post-translational modifications such as phosphorylation, ubiquitination, acetylation and methylation, which are found over several residues located mainly in their N-terminal extremity. Whereas acetylation is correlated with gene expression and show rather fast kinetics [61], methylation of histones can be associated with active or repressive transcriptional states, depending on the modified residue. For instance, H3K4me3 is associated with transcriptional activity, whereas H3K9me2 is found primarily at silent transposable elements and H3K27me3 marks genes repressed by the Polycomb Repressive Complex 2 (PRC2). PRC2 is evolutionary conserved and regulates most developmental phase transitions in plants through the maintenance of a repressive state [62]. In comparison to active marks, H3K27me3 and H3K9me2 tend to have lower turnover rates [61]. Moreover, H3K27me3 propagation across DNA replication through continuous modification of both parental and newly incorporated histones represents a specific mode of maintenance, which reflects the central role of PRC2-mediated gene repression in cellular memory [51]. H3K27me3 removal can be achieved either by passive, replication-coupled dilution or via the activity of specific demethylases [63,64].

DNA methylation (5mC) is another key epigenetic mark involved in the control of transposable element activity and the regulation of gene expression, which, in plants, is found in all sequence contexts through the activity of distinct enzymatic pathways [65,66]. RNA directed DNA methylation (RdDM) is a plant-specific pathway that relies on the action of small interfering RNAs (siRNAs) generated via distinct pathways [48,67]. Sequence-specific targeting of DNA methyltransferases via these siRNAs is particularly important for de novo methylation of repeat sequences [68].

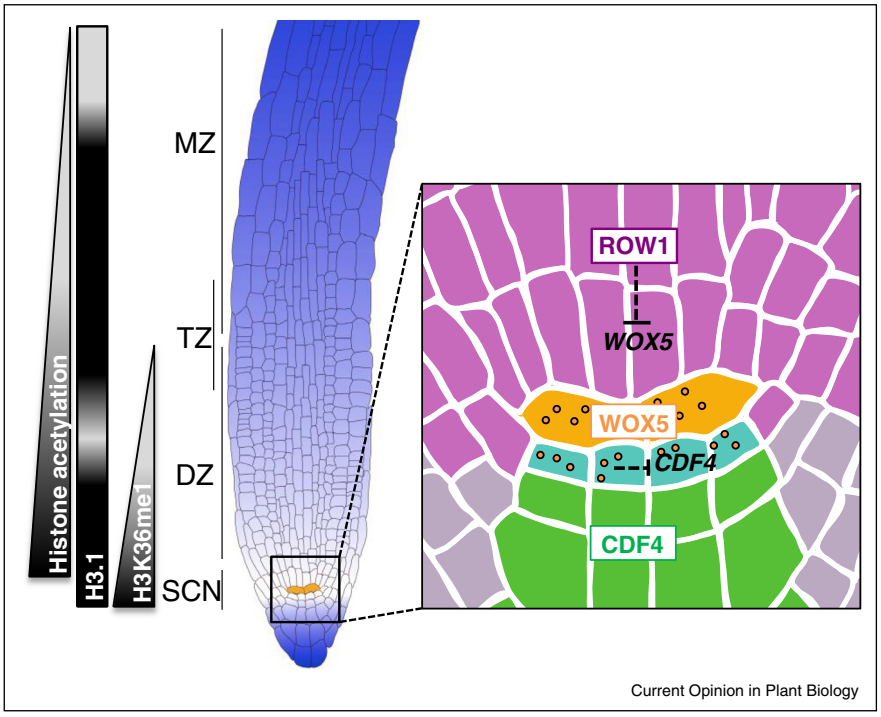
H3K4me3 marks present at the *WOX5* promoter region has been proposed to block *WOX5* transcription in adjacent cell layers located shootward to the QC [10•]. Indeed, loss of ROW1 leads to an enlargement of the *WOX5* expression domain accompanied by defects in cell differentiation. Similarly, *row1* mutants show ectopic expression of *WUS* resulting in severe SAM defects [13]. In the SAM however, ROW1 directly binds to *WUS* regulatory DNA where it is proposed to compete with the SWI/SNF chromatin remodeler SPLAYED in order to prevent WUS transcription outside of the SAM OC [13,14]. Since WOX5 and WUS are functionally interchangeable [9], it would be interesting to determine if the binding of ROW1 at the *WUS* locus also relies on the recognition of H3K4me3 or whether the ability of ROW1 to repress stem cell organizers is based on different mechanisms in the root and shoot meristems.

In addition to sustaining continuous organogenesis by stem cell maintenance, chromatin-level regulation of *WUS* also controls the transition towards determinate growth in the floral meristem. Termination of the floral stem cell pool through the stable repression of *WUS* occurs in two consecutive steps. At an early stage, the homeotic TF AGAMOUS (AG) recruits Polycomb repressive complex 2 (PRC2) to the *WUS* locus, thus initiating its downregulation via the deposition of the repressive H3K27me3 mark [15]. Full repression of *WUS* is only achieved two days later through the repressive action of *KNUCKLES* (*KNU*), which encodes a zinc finger protein. Activation of *KNU* depends on AG, which in this case triggers the eviction of PRC2 from *KNU*, thus leading to the progressive dilution of H3K27me3 across replication cycles [11••]. Thus, the chromatin-assisted delayed activation of *KNU* allows precise timing of SC termination and simultaneously provides a counting mechanism to establish the proper number of mitosis necessary for floral organ formation. It remains to be understood how the same TF is able in a very similar cell population to recruit and evict PRC2 from distinct genes, promoting or preventing H3K27me3 deposition, respectively.

Accumulating evidence indicates that WUS and WOX5 act in turn as repressors of differentiation factors in stem cells in a chromatin-dependent manner. WOX5 has been shown to move from the QC to adjacent columella stem cells (CSC) where it represses the differentiation factor CYCLING DOF FACTOR 4 (CDF4) by recruiting the co-repressor TOPLESS (TPL) and the histone deacetylase HDA19 (Figure 1) [12••]. Formation of this repressive deacetylation complex is limited to the CSC as *CDF4* is expressed in the differentiating daughter cell, revealing the remarkable spatial precision of this process. Since WUS also interacts with TPL in the SAM [16], recruitment of a TPL-HDAC19 complex could represent a general mechanism to repress differentiation-promoting genes in stem cells.

In addition to its role in stem cell maintenance, chromatin-level regulation is also required to repress pluripotency genes outside SCNs in order to allow cell differentiation. For instance, the ASYMMETRIC LEAVES complex recruits PRC2 to the promoters of cell proliferation factors at the SAM boundaries, leading to their sustained repression during leaf primordia formation and subsequent growth [17]. More generally, PRC2 is critical to maintain meristem activity and orchestrate differentiation processes over time but is largely dispensable for meristem establishment during embryogenesis [18].

Figure 1



Chromatin-level regulation of cell differentiation within the root apical meristem. Root growth homeostasis largely depends on the tight coordination between the activity of the stem cell niche (SCN), cell production in the division zone (DZ) and cell elongation as well as end-differentiation within the transition (TZ) and maturation zone (MZ). Both this shootward and the columella differentiation gradients are highlighted in purple. Stem cell maintenance requires the activity of WOX5 whose expression is confined to the quiescent, organizing center (QC) by the binding of ROW1 to H3K4me3 marks at the *WOX5* promoter in adjacent cell files. In turn, WOX5 movement from the QC to columella stem cells (turquoise) leads to direct repression of the CDF4 differentiation factor through the recruitment of a histone deacetylase complex. Transition from the division to the maturation zone is accompanied by a reduction of histone mobility and acetylation levels, as well as the rapid eviction of histone H3.1 after the last mitotic and endoreplication cycles in the DZ and MZ, respectively. The progressive reduction of H3K36me1 levels upon each replication cycle is proposed to participate in counting the remarkably stable number of mitosis within the DZ. Expression domains of *WOX5*, *ROW1* and *CDF4* are indicated in orange, pink and green, respectively.

Spatiotemporal chromatin-level regulation of cell fate transitions

Histone acetylation dynamics play an important role in translating auxin gradients into position-dependent cell fate switches during flower organogenesis. Under low auxin conditions, TPL bound to the histone deacetylase HDA19 forms a complex with Aux/IAA proteins that interacts with the MONOPTEROS/AUXIN RESPONSE FACTOR 5 (MP/ARF5) [19], thereby preventing the expression of MP targets. Higher auxin levels lead to Aux/IAA proteolysis, thus enabling MP to recruit the SWI/SNF family members SPLAYED (SYD) and BRAHMA (BRM). Remodeling of the deacetylated, repressive chromatin state into a more accessible, transcription-prone environment at MP targets then promotes flower primordium initiation [20••]. This 'opening' of chromatin likely favors in turn the binding of additional TFs and the recruitment of histone acetyltransferase activity to sustain the transcriptional activation of auxin responsive genes, as previously reported for bZIP TFs [21]. As auxin graded distribution is a major orchestrator of cell fate transitions [1–3], one can expect that this chromatin switch represents a common theme during plant organogenesis, coupling phytohormone-driven transcriptional output and positional cues.

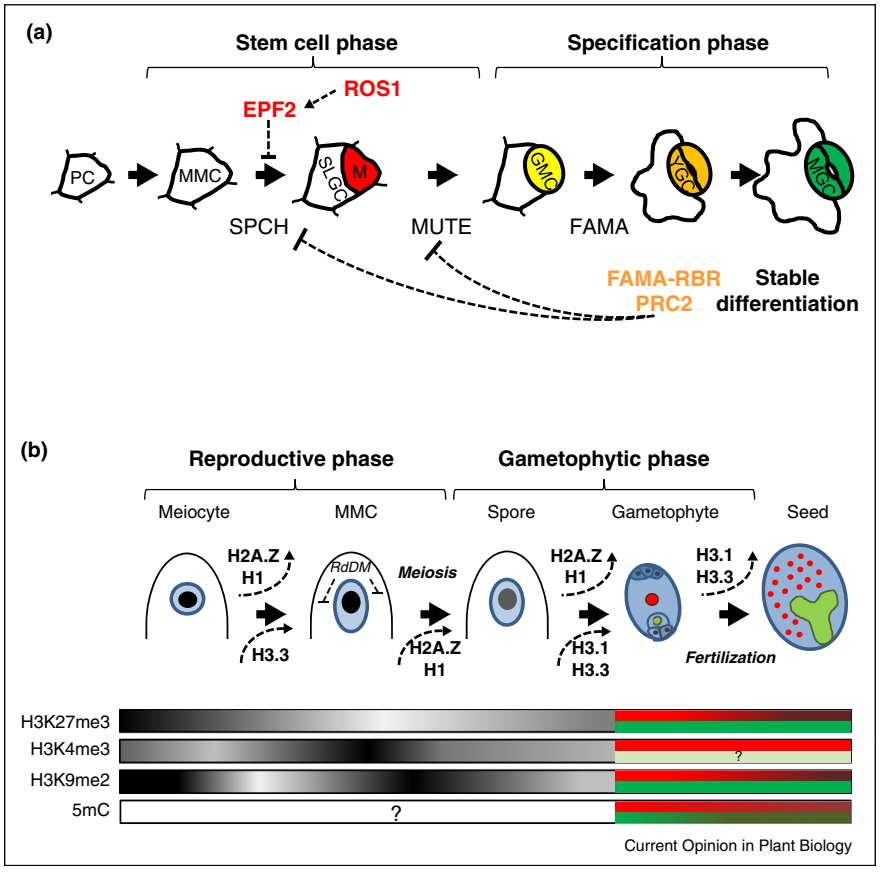
Several studies have exploited the relatively simple functional organization of the Arabidopsis root in order to monitor the dynamics of chromatin factors during the differentiation process (Figure 1). Earlier work suggested that local chromatin accessibility is rapidly altered in cells sensing new positional cues within the meristem [22]; the signaling and mechanistic pathways involved remain to be characterized.

Comparisons between the DNA methylome of different root cell types revealed some variations that had little impact on gene expression, suggesting that the role of DNA methylation dynamics in regulating transcriptional programs within somatic cells is rather limited [23•]. Of note, columella cells located at the tip showed the highest methylation levels among investigated cell types, mainly

at CHH sites over TE sequences, suggesting strong RNA-directed DNA methylation (RdDM) activity (see Box 1). Indeed, overaccumulation of 24-nt small RNA was accompanied with increased expression of RdDM components, the activity of which might be facilitated by a global decrease in heterochromatin compaction, as suggested by the absence of the nucleosome remodeller DECREASED DNA METHYLATION 1 (DDM1) and the downregulation of H1 and H2A.W histone variants [23•].

Imaging-based quantification of nucleosome dynamics along the root differentiation gradient (Figure 1) indicated that histone H2B mobility diminishes concomitantly with acetylation levels as cells transit from the division to the maturation zone [24•]. Furthermore, cells reaching the limit of the division zone are characterized by an abrupt depletion of the replication-dependent histone H3.1 variant [25•]. Eviction of this variant is also observed in cells of the maturation zone that are terminating their endoreplication cycles, which suggests a general link between H3.1 dynamics and the replication potential of cells during development [25•]. How this ultimate DNA synthesis is programmed remains to be determined, but the fact that the number of cells is remarkably stable within the division zone suggests the existence of mechanisms that count

Figure 2



Chromatin dynamics during the differentiation of lineage-committed cells. **(a)** Stomatal lineage initiates with the asymmetric division of a meristemoid mother cell (MMC) that derives from a protodermal leaf cell (PC), resulting in the generation of a stomata stem cell-like meristemoid (M) and a larger stomatal lineage ground cell (SLGC). M further differentiates into a guard mother cell (GMC), which divides symmetrically to form the two young (YGC) and then mature (MGC) guard cells. ROS1-dependent expression of EPF2 in M cells prevents the differentiation of adjacent SLGC into additional M cells thus participating in leaf epidermis patterning between stomata and pavement cells. While DNA methylation dynamics likely affects the plasticity of the stomatal lineage, repression of stem cell phase regulators by PRC2 in guard cells ensures robust fate commitment. **(b)** Female germline establishment starts with the differentiation of a subepidermal cell within the ovule primordium into a megaspore mother cell (MMC). Fate transitions are associated with successive waves of eviction of H2A.Z and H1 histones as well as incorporation of stage-specific H3.1 and H3.3 variants, specifically expressed during the reproductive or gametophytic phase. Differentiation of the MMC is restricted by the non-cell autonomous action of RdDM pathway components. Following meiosis, the resulting spore divides and differentiates into four different cell types including the egg cell (EC, green) and the central cell (CC, red), which represent the female gametes. Concomitantly to histone variant replacement, the spore-to-gametophyte transition is also potentially controlled by RdDM. In addition, variation in the abundance of specific chromatin marks occurs during the reproductive and gametophytic phases (grey heatmap). Upon fertilization, the gametophyte-specific histone H3 variants are finally exchanged with somatic H3 variants in the embryo and endosperm, which are characterized by distinct epigenomes.

mitotic or replication cycles. By analogy with the delayed activation of *KNU* mentioned above [11••], a chromatin-dilution model has been proposed whereby H3K36me1 levels, specifically deposited in the SCN by the SET-domain protein ASH1-RELATED3 (ASHR3), would passively decrease upon each cell division, reaching depletion after about four rounds of replication, when cells enter the transition zone [26].

Chromatin-based regulation in lineage-committed cells

Besides the activity of stem cell pools within meristems, de novo generation of specialized cell lineages such as stomatal differentiation at the surface of above-ground organs is also critical for tissue functioning. Stomatal development occurs in two phases: an early stem cell phase leading to the generation of a meristemoid (M) cell through asymmetric division, followed by a specification phase ending with the formation of a pair of stomatal guard cells, which are involved in gaz exchange regulation (Figure 2a). Fate transitions during stomatal development are controlled by the sequential activity of the bHLH transcription factors SPEECHLESS (SPCH), MUTE and FAMA among other factors such as EPIDERMAL PATTERNING FACTOR 2 (EPF2) [27,28]. During M cell differentiation, active demethylation of the *EPF2* promoter by the DNA glycosylase/lyase ROS1 leads to EPF2 expression, which acts as a negative regulator of meristemoid mother cell (MMC) fate through the down-regulation of *SPCH* [29•]. In addition, *SPCH* and *FAMA* are directly targeted by RdDM and repressed under low humidity conditions [30]. These findings indicate that transcriptional regulation by DNA methylation affects stomatal initiation at different levels, thus providing developmental flexibility in response to environmental cues (Figure 2a). CHH methylation has also been shown to influence differentiation and growth of epidermal fiber cells in cotton [31,32].

At the other end of the stomatal lineage, PRC2-mediated gene repression plays an important role in maintaining stable fate commitment of guard cells. Indeed, altering the expression pattern of FAMA, a master regulator of guard cell identity, leads to the failure of lineage termination and the generation of stoma within stoma [33]. Reiteration of this transcriptional program was linked with incomplete PRC2-mediated repression of the stem cell-like regulator *SPCH* and *MUTE* genes within guard cells. Interestingly, interfering with the ability of FAMA to recruit the RETINOBLASTOMA-RELATED (RBR) repressor protein at early stomatal lineage genes leads to a similar reiteration phenotype [34]. This suggests that binding of the FAMA-RBR complex recruits PRC2 at stomata-promoting TFs during late differentiation stages, thus enforcing the stable acquisition of guard cell identity [35]. To which extent these events are correlated with the observed depletion of histone H3.1 towards the end of guard cell differentiation [25•] needs to be determined.

In keeping with this model, the critical role of PRC2 activity in maintaining the identity of fully differentiated cells was recently highlighted [36••]. Thus, whereas epidermal root hairs reach full specification in the absence of PRC2, they undergo several rounds of mitosis despite their polyploidy and revert back to an unorganized, dedifferentiated cell mass, from which embryoid structures occasionally arise. Other mature cell types are affected in a similar way, suggesting that PRC2 acts as a general repressor of a dedifferentiation potential that is retained in mature cells [36••]. Further work will help determining whether these phenotypes are caused primarily by the deregulation of *LEC2*-dependent embryonic programs, reentry into mitosis or other processes controlled by PRC2.

Extensive chromatin dynamics occur during the differentiation of male and female germline cells [37,38•,39]. On the female side, meiocyte differentiation into the megaspore mother cell (MMC) is accompanied by chromatin decondensation, reduction of marks associated with pericentromeric heterochromatin (see Box 1) as well as of H3K27me3. Fate transitions in the reproductive and gametophytic phases are characterized by successive rounds of eviction of the variant histone H2A.Z as well as of the linker histone H1 and the incorporation of stage-specific H3.3 variants (Figure 2b). In addition, RdDM prevents the differentiation of surrounding cells into the MMC fate in both Arabidopsis and maize [40]. RdDM is also likely involved at the spore-to-gametophyte transition that leads to the formation of the two female gametes, the egg cell (EC) and the homo-diploid central cell (CC) [41]. These cells, and their respective fertilization products, the embryo and endosperm, have contrasted epigenomes at the cytological and molecular level. Thus, in comparison to the EC, the CC has low levels of H3K9me2 and DNA methylation, which was shown to depend on the DNA demethylase DME and to be functionally important for endosperm differentiation [42–44]. Conversely, H3K9me2 deposition in the EC seems important for early embryogenesis [45]. CC differentiation and development into endosperm is also regulated by PRC2 [46], a mechanism that appears evolutionary conserved even in plants where the gametophyte represents the dominant phase [47].

Despite these extensive dynamics, alterations of the chromatin modifiers involved have rather subtle developmental defects, which questions the relevance of chromatin-based mechanisms in germline differentiation. Although this role is potentially blurred by redundant layers of regulatory processes [48], such sequential chromatin changes might also serve as an epigenetic reprogramming step to start a new life cycle with a fresh

potential, unperturbed by the progenitors' history [49,50].

Conclusions and prospects

Recent findings have started to unveil key roles for chromatin-based mechanisms in establishing, fine-tuning and maintaining transcriptional patterns during cell differentiation. Accumulating evidence indicate that such regulations are important to achieve both developmental flexibility and robust fate commitment in time and space.

Within meristems, high chromatin state dynamics relay TF-driven decisions and phytohormonal cues by sensing signaling gradients, thus participating in the temporal stabilization of cell identities as well as the promotion of cell fate progression or switches in a position-dependent manner. This progression needs to be coupled with DNA replication, both during mitotic and endoreplication cycles, that likely represents a window of opportunity to modify the chromatin landscape and rewire cell fate [51]. Indeed, recent evidence in *Drosophila* reveals extensive remodeling of chromatin organization upon replication, especially at transcriptionally active genes [52]. Although this remains to be explored in plants, replication-associated dilution of chromatin marks has been shown to couple the timing of stem cell termination with the counting of mitosis during floral organ formation; a mechanism that was also proposed to account for the regulation of cell number within the RAM, which requires further investigation.

Outside the meristem and its predominant signaling environment, chromatin-level regulation is essential to maintain stable fate commitment, possibly independently of preceding patterning mechanisms. This has been exemplified by the role of PRC2 activity in preventing 'slipping back' into previous differentiation stages or earlier developmental programs, during late stomata or root hair differentiation, for instance.

Overall, our understanding of how chromatin-based mechanisms are integrated within developmental regulatory networks and how transcriptional decisions are maintained through replication is still very limited. Combination of quantitative live imaging techniques with cell-type specific and single cell (epi)genomic approaches [53–55] will be instrumental in outlining the regulatory principles that underpin the fascinating complexity of plant cell differentiation.

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