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Current Opinion in Plant Biology

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

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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.

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Current Opinion in Plant Biology 2016, **34**:27–34

This review comes from a themed issue on **Cell biology**

Edited by **Keiko Sugimoto** and **Arp Schnittger**

http://dx.doi.org/10.1016/j.pbi.2016.07.010

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].