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Epigenetic modifications of nuclei differ between root meristematic tissues of *Hordeum vulgare*

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Abbreviations: RAM, root apical meristem; H4K5ac, histone H4 acetylation; H3K9me2, histone H3 dimethylation; 5mC, DNA methylation; EdU, 5-ethynyl-2'-deoxyuridine; TSA, trichostatin A; HDAC, histone deacetylase; DAPI, 4',6 diamidino-2-phenylindole

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Recent studies on the role of epigenetic modifications during plant development emphasize the fact that both positional information and tissue specificity are essential factors that establish epigenetic marks and thus determine cell fate and differentiation processes. The root apical meristem (RAM), which contains stem cells and generates radial patterns of tissues, is an ideal model for studying the correlation between cell position and cell-type differentiation, with particular emphasis on the patterns, global levels, and landscapes of epigenetic modifications. To date, there has been no clear evidence for differential levels of histone and DNA modification across root meristematic tissues. Our study clearly indicates that levels of modifications with potential epigenetic effects vary between RAM tissues. Of particular interest is that histone H4 acetylation in the epidermis is not simply replication-dependent and probably plays a role in epidermal cell differentiation.

Epigenetic gene regulation is a key mechanism conferring stable but reversible gene expression patterns. Cell differentiation in plants depends on transcriptional and translational regulation of gene expression, and thus involves the switch in patterns of gene activity, mainly governed by alterations in chromatin state. Epigenetic mechanisms, such as DNA and histone post-translational modifications, are key regulators of chromatin condensation. These epigenetic mechanisms play crucial roles in many aspects of plant growth and development, including flowering time control, seed development, and cell fate maintenance.[1-5] During plant development, organs are formed from a group of undifferentiated cells maintained in meristems. Continuously dividing, meristematic cells are strictly controlled by numerous and interconnected pathways involving transcriptional regulation, phytohormones, and epigenetic gene regulation. Root apical meristems (RAM) establish root conformation through the process of controlled cell proliferation and morphogenesis, which generate radial patterns of tissues within each concentric ring of cells. The fate of a given cell in a root, however, is not permanently fixed, but depends on various signals from its neighborhood.[6,7] The correlation between cell position and cell-type differentiation during the formation of the root epidermis is very well documented.[8]

We have investigated 4 epigenetic modifications in a tissue-specific manner and demonstrated for the first time that global levels of these modifications vary between meristematic tissues.[9] The combination of immunostaining techniques applied to anatomical section preparations allowed demonstration of the spatial distribution of these modifications within the cells of particular RAM tissues. We have detected the highest amount of histone H4 acetylation (H4K5ac) in the vascular tissues, while the lowest was observed in the root cap and epidermis. In contrast to acetylation, histone H3 dimethylation (H3K9me2) was the most abundant in the root cap and epidermis and considerably less prevalent in vascular tissues. DNA methylation was at its highest level in the root cap and vascular tissue, and at its lowest in the epidermis (for details, see Figures 2, 4, 5, and Table 2 in ref. 9). The most dynamic variation in epigenetic modification was in the epidermis. The proximal meristem epidermis was virtually devoid of the analyzed modifications, whereas at the boundary between the proximal meristem and elongation zone, the amount of histone H4 acetylation, histone H3 dimethylation, and DNA methylation was much higher. We postulate 2 explanations for this phenomenon; one could involve epidermal cell specification into trichoblasts and atrichoblasts through changes in gene expression patterns during differentiation. The other may be linked with DNA replication through the high level of H4K5ac. Nevertheless, DNA replication does not explain high levels of other modifications, such as H3K9me2 and DNA methylation (5mC), which are typical hallmarks of a "closed" chromatin state. We used the click-it EdU kit with

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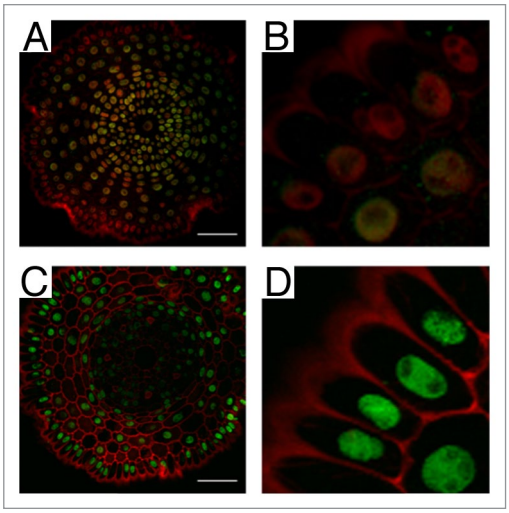

Figure 1. Acetylation of H4K5 in comparison with DNA replication. (**A**) Transverse section through proximal meristem, green fluorescence, H4K5ac; red fluorescence, DAPI. (**B**) Epidermis from proximal meristem, green fluorescence, H4K5ac; red fluorescence, DAPI. (**C**) Transverse section through proximal meristem, green fluorescence—cells with positive EdU signals. (**D**) Epidermis from proximal meristem, green fluorescence – cells with positive EdU signals. Bars: 50 μm.

5-ethynyl-2'-deoxyuridine—an analog of thymidine, which is incorporated into DNA during its synthesis, to compare the level of H4K5ac within the cells/tissues that had just undergone DNA replication. Surprisingly, the cells in proximal meristem epidermis were vigorously replicating although H4K5ac was almost undetectable within those cells **(Fig. 1 A–D)**.

Our results may indicate that epidermal cells are driven by different developmental programs than the ones in other meristematic tissues and that the epigenetic modifications may play an important role in these processes. It is known that chromatin modifications can affect gene expression in the epidermis. Treatment of germinating Arabidopsis seedlings with trichostatin A (TSA), which is an inhibitor of histone deacetylases (HDACs), promoted hair cell formation at non-hair positions.[10] Therefore, histone acetylation might function as the mechanism that regulates the position-dependent expression of the patterning genes. Interestingly, we detected a high level of H4K5ac along with a high level of 5mC in the epidermis at the boundary between proximal meristem and the elongation zone. The observed turnover of these 2 modifications may reflect the switch in the developmental programs within this tissue.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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