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Immunolocalization of Histone Modifications as a Tool to Visualize Chromatin Dynamics in Plants

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The nuclear DNA of eukaryotic organisms is associated with several proteins. These proteins together with the DNA form chromatin. In all eukaryotes, DNA is folded around a core of histones to form nucleosomes. Chromatin was previously believed to serve mainly to organize and compact the genetic material [1], [2]. However, in recent years, chromatin has been regarded has a highly dynamic structure responsible for controlling gene expression. Therefore, chromatin is highly flexible, to make genetic information accessible when needed, and the degree of compaction has to be tightly regulated. DNA staining, using 4',6-diamidino-2-phenylindole (DAPI), provided the basis for a cytological distinction between weakly stained euchromatin, now known to be gene rich, and brightly stained heterochromatin, which usually contains various repetitive sequences [3]. In many organisms, heterochromatic DNA is hypermethylated and this is used as an additional mechanism to regulate transcription. Histones can be modified by acetylation, phosphorylation, methylation or ubiquitination. These mechanisms provide signals to which other factors can bind and additionally alter the biochemical properties of chromatin (i.e., "histone code") [4]. In general, acetylation of histones is correlated with active genes, whereas methylation of histones at different positions results in changes in gene expression. The methylation of lysine 9 in histone 3 (H3K9) is associated with heterochromatin formation while methylation of lysine 4 of histone 3 (H3K4) methylation is related to gene activation and positioned in euchromatin [5]. DNA and histone modifications recruit various non-histone proteins to specific chromosomal regions and eventually create a defined nuclear structure that is able to affect gene expression [6].

Our aim is to understand how chromatin structure and organization differs between plant species. We are investigating how the pattern of histone modifications (methylation and acetylation) varies with different genome size and complexity and are investigating the mechanisms involved in the different chromatin organization in plant species with very similar genome sizes. Immunofluorescence studies were carried out in the model plant species *Oryza sativa* (Rice) and *Medicago truncatula* (Barrel Medic) using root sections. We used commercial antibodies raised against histone marks (H3k9me2, H3k27me2) and DNA methylation (5-mC) associated with heterochromatin, and the histone mark (H3k4me2) associated with euchromatin. Confocal and fluorescence microscopy were used in conjunction with three dimensional reconstruction to visualize intact well-preserved nuclei.

Our results indicate that plant species with similar genome sizes may have very different chromatin organization and different patterns of histone modifications in the nucleus. Despite the similar genome sizes of Medicago (~525Mbp) and Rice (~430Mbp), the genome organization and distribution of the chromatin in the nucleus is quite different. Medicago has clearly defined compacted regions of heterochromatin, while Rice has a more homogenous distribution of heterochomatin, as visualized by DAPI staining (Figure 1). The immunolabelling patterns also

differ in the two species and correlate with the euchromatin and heterochromatin distribution in the nucleus (Figure 2). However, H3k27me2 does not have a diffuse pattern in Rice, which probably indicates that this mark is not essential for the distribution of the chromatin in Rice (Figure 2 b). This difference in the distribution of the histone and DNA methylation is better visualized in the Z axis projection of a series of confocal sections, obtained with the imaging of immunolabelled root sections from the two species (Figure 3).

Plant genomes, in line with other higher eukaryotes genomes, consist of repetitive and low or single copy DNA sequences. It has been shown that the variation in plant genome size is largely due to differences in the amount of repetitive sequences. In the highest plants studied so far, LTR-retrotransposons were found to be the major components of repetitive DNA. Extensive fluorescence in situ hybridization (FISH) and sequencing data indicates that both Medicago and Rice genomes are organized into gene rich euchromatic chromosome arms and distinct gene-poor centromeric/pericentromeric regions. However, in Rice some chromosomes have few satellites repeats. The lack of extensive amounts of satellite repeats allowed sequencing of the entire centromere of rice chromosome 8 and chromosome 3 [7]. Therefore, it is possible that during evolution, Medicago and Rice have adopted different mechanisms to compact the chromatin in the nucleus and silence their genome repeat sequences. *Oryza sativa* (*Poaceae* family) and *Medicago truncatula* (*Fabaceae* family) diverged around 155 million years ago [8], when the divergence between monocots and dicots occurred. The divergence between monocots and dicots represents a major event in higher plant evolution. Despite having very similar genome sizes, Medicago (dicot) and Rice (monocot) utilise different mechanisms of condensing and silencing the heterochromatin in the nucleus. These results will be important to understand the evolution of genomes and the strategies that different species adopted to silence and regulate gene expression.

Acknowledgements

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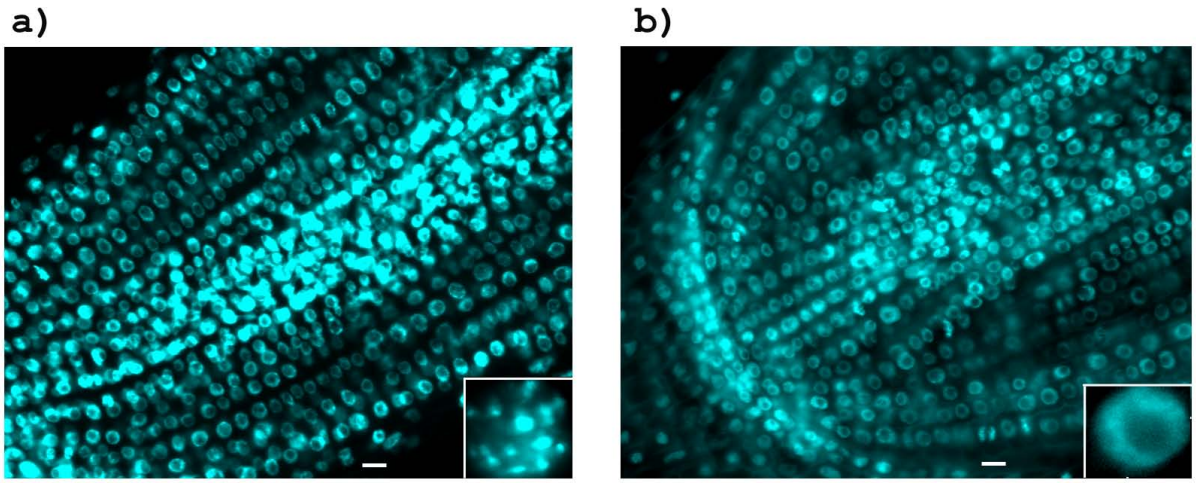

Figure 1. *Medicago truncatula* (a) and *Oryza sativa* (b) root sections labelled with 4',6-diamidino-2-phenylindole (DAPI). 20 µm thick sections were obtained using a vibratome and stained afterwards using DAPI (blue). It is possible to visualize brighter regions, which correspond to more compacted chromatin (Heterochromatin). In the nucleus of *Medicago truncatula* clear heterochromatin compacted areas are visible, while in *Oryza sativa* the pattern is more dispersed. Scale bar = 20 µm.

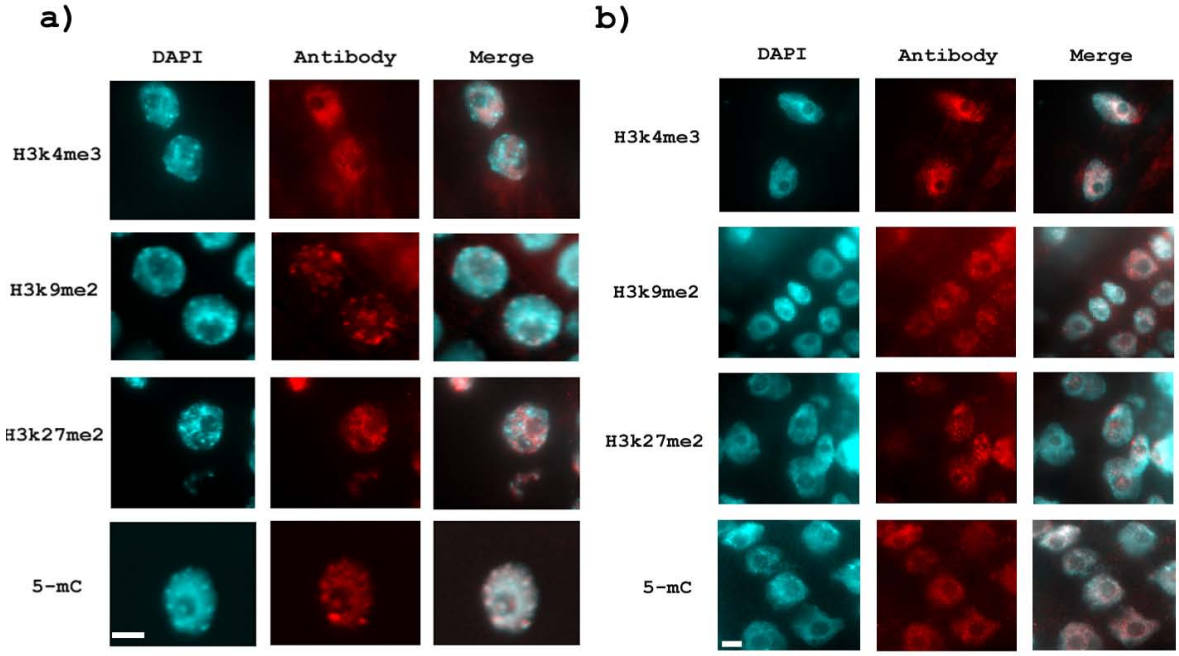

Figure 2. Whole nucleus distribution of DNA 5-mC, H3k9 dimethylation, H3k4 trimethylation and H3k27 dimethylation in interphase on *Medicago truncatula* (a) and *Oryza sativa* (b). Antibody labelling against Methylated histone H3 or Cytosines is shown in red. DAPI (DNA) is shown in blue. Images were obtained using a Leica DRM2 fluorescence**.** Right columns correspond to merged images. Scale bar = 5 µm

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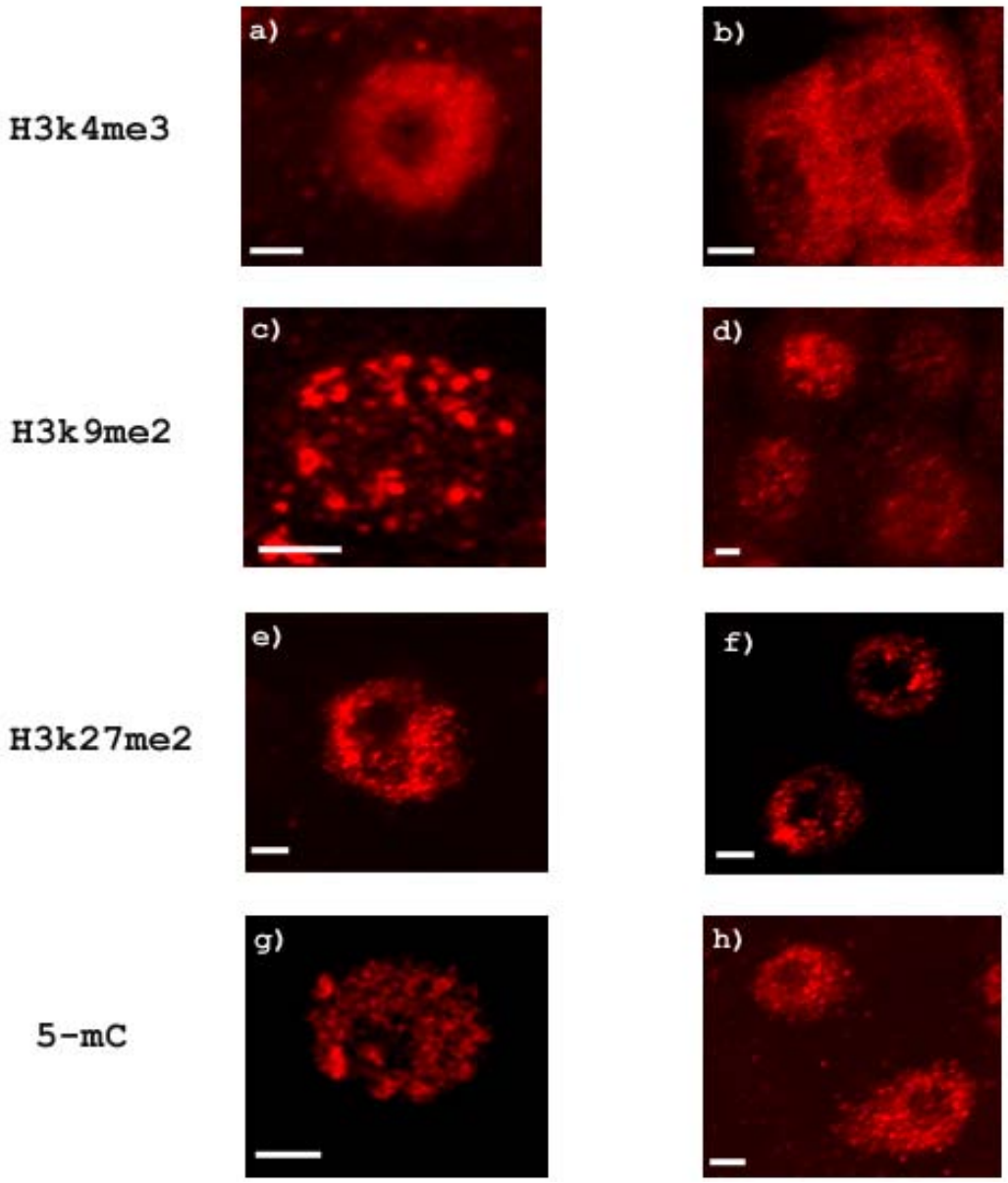

Figure 3. Immunofluorescence of root sections from *Medicago truncatula* (a, c, e, g) and *Oryza sativa* (b, d, f, h) obtained with confocal microscopy. Projections of consecutive confocal sections are shown, with a section spacing of 0.5 µm. The antibodies for histone and DNA methylation are shown in red. Labeling for H3k4me3 and H3k27me2 (Alexa Fluor 594, Invitrogen) H3k9me2 and 5-mC (Cy3, Sigma). Scale bar = 5 µm

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