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Author: Elżbieta Wolny, Agnieszka Brąszewska-Zalewska, Daria Kroczek, Robert Hasterok

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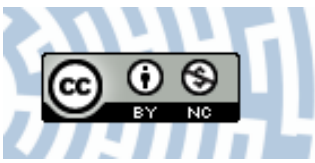

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In situ analysis of epigenetic modifications in the chromatin of *Brachypodium distachyon* embryos

Elzbieta Wolny, Agnieszka Braszewska-Zalewska, Daria Kroczek, and Robert Hasterok*

Department of Plant Anatomy and Cytology; Faculty of Biology and Environmental Protection; University of Silesia in Katowice, Katowice, Poland

Epigenetic modifications of the chromatin structure are crucial for many biological processes and act on genes during the development and germination of seeds. The spatial distribution of 3 epigenetic markers, i.e. H4K5ac, H3K4me2 and H3K4me1 was investigated in 'matured,' 'dry,' 'imbibed" and 'germinating' embryos of a model grass, Brachypodium. Our results indicate that the patterns of epigenetic modification differ in the various types of tissues of embryos that were analyzed. Such a tissue-specific manner of these modifications may be linked to the switch of the gene expression profiles in various organs of the developing embryo.

Keywords: *B. distachyon*, chromatin, embryo, epigenetic modifications, H3K4me1, H3K4me2, H4K5ac, immunostaining techniques, organ, Poaceae, tissue

Abbreviations: Brachypodium, *Brachypodium distachyon*; DAPI, 4′, 6 diamidino-2-phenylindole; H4K5ac, histone H4 acetylation at lysine 5; H3K4me2, histone H4 dimethylation at lysine 4; H3K4me1, histone H3 methylation at lysine 4; RAM, root apical meristem; SAM, shoot apical meristem.

*Correspondence to: Robert Hasterok; Email: robert.hasterok@us.edu.pl

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The seed, a structure, which originates from the fertilised ovule and includes the embryo and other maternally derived tissues, is an important stage in the life cycle of higher plants.[1] Two distinct stages, i.e., morphogenesis and maturation, can be distinguished during seed formation, which is initiated by embryogenesis in which a mature embryo develops from a single fertilised cell through multiple cell divisions and a series of morphogenetic processes. Following morphogenesis, the developing seed enters the maturation phase, which is characterized by the accumulation of storage compounds, the reorganization of the metabolism, the arrest of growth, the loss of water and the entry into a dormancy period that is broken upon germination.[2,3] There are 3 stages that can be distinguished during germination: (i) seed imbibition and the reinitiating of metabolic processes; (ii) limited water uptake and (iii) increased water uptake and the emergence of the radicle.[4,5]

Dry seeds represent the transitional state between an embryo and seedling. During the plant life cycle when the cells or tissues transform toward a new fate or function, the chromatin undergoes structural changes in its organization. The switch from one developmental phase to the next requires significant changes in both the spatial and temporal patterns of gene expression. The transcriptional reprogramming of these genes involves the active modification of their chromatin structure. Gene expression can be influenced by epigenetic modifications such as DNA methylation and histone modifications.[6] The global levels of the distribution of the histone modifications of 3 epigenetic markers, i.e., H4K5ac, H3K4me2 and H3K4me1 in 'matured,' 'dry,' 'imbibed' and 'germinating' embryos of a model grass, *Brachypodium distachyon* (Brachypodium), were studied in a tissue and organ-specific manner.[7]

Our results indicate that the abundance of these modifications differs in various organs and tissues of the 4 types of Brachypodium embryos. Embryos from matured seeds were characterized by the highest level of H4K5ac in RAM and epithelial cells of the scutellum. H3K4me2 was most evident in the epithelial cells of the scutellum. In 'dry' embryos the level of H4K5ac was the highest in the coleorhiza. H3K4me1 was the most elevated in the coleoptile, whereas H3K4me2 was the most prominent in the leaf primordia and RAM. The chromatin of 'dry' embryos, which are in a quiescent state, exhibits intensive immunofluorescence signals of H3K4me2 that correspond with transcriptionally active euchromatin (**Fig. 1**). Such an observation is intriguing since the assumption that these signals would be

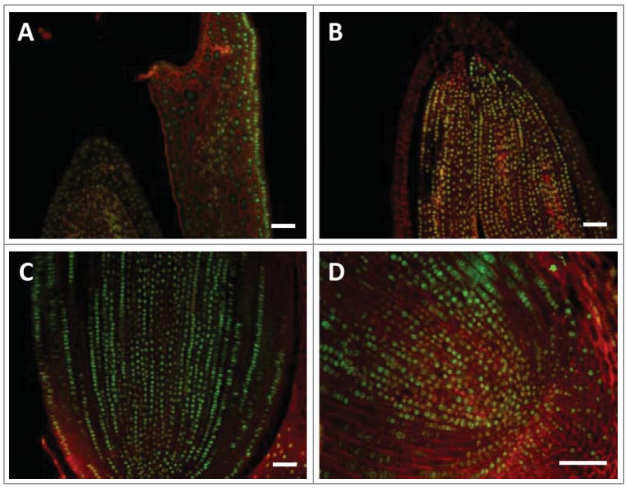

Figure 1. The immunodetection (green fluorescence) of H3K4me2 in 'matured' (**A**) 'dry' (**B, C**) and 'imbibed' (**D**) Brachypodium embryos. Cross sections through the scutellum (**A**), coleoptile and leaf primordia (**B**), the RAM (**C**), the RAM and the root cap (**D**). Red (artificial color) fluorescence, DAPI. Bars: 50 μm.

less intense or that they would be absent in these embryos. In embryos from germinating seeds, H4K5ac and H3K4me1 was the most evident in the scutellum, while the highest level of H3K4me2 was observed in the coleoptile. We did not observe significant differences in the intensity of immunofluorescence signals of histone H4 acetylation and H3 methylation between particular tissues of the embryo in 'imbibed' embryos. For more detail see **Figures 5-7**, **Figure S1** and **Table 1** in ref. 7.

The patterns of epigenetic modifications may vary not only between particular tissues of the same embryo type but also between the different types of embryos that were analyzed. The scutellum, coleorhiza and coleoptiles are the most variable organs in terms of histone H4 acetylation and histone H3 methylation in all of the types of the embryos that were analyzed. The tissue-specific manner of the modifications that were analyzed may suggest that they play an important role in the embryo during seed maturation, desiccation, and germination and may be involved in the switch of the gene expression profiles in specific organs of the developing embryo. Although the involvement of any epigenetic modifications of the chromatin in seed development is not yet well understood, it is apparent that plants modulate their physiology and development using epigenetic mechanisms.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

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