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Poster Abstract – D.17

SUBCELLULAR LOCALIZATION OF MAIZE RPD3-LIKE HISTONE DEACETYLASES

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Histone acetylation and deacetylation play an essential role in modifying chromatin structure and regulating gene expression in eukaryotes during cell differentiation and development. Acetylation/deacetylation of histone tails constitutes a signal that may function in combination with other covalent modifications to generate an epigenetic code. Histone deacetylases (HDACs) are grouped into three classes based on their similarity to known yeast histone deacetylases, and it is well-known that HDACs work in large multiprotein complexes with transcriptional co-repressors.

Basic features of histone deacetylation in plants resemble those of other eukariotes, but there are also some differences, reflecting that growth and morphogenesis in plants continue throughout their life.

While human class I deacetylases localize in the nucleus, with the exception of HDAC3, a peculiar feature of class II HDACs is their ability to shuttle between the nucleus and the cytoplasm in a cell-type and signal-dependent manner. Recent studies have demonstrated that HDACs subcellular localization is a key element in regulating their activity. This nucleocytoplasmic shuttling of HDACs is mediated by a phosphorylation-dependent binding of 14-3-3 protein to the N-termini of class II HDACs.

Maize represents one of the best-characterized system for studies of plant HDACs. In a previous work we investigated the role of the maize Rpd3-like HDAC genes *ZmHDA101, ZmHDA102* and *ZmHDA108*, during plant development and differentiation. This study showed that the three *ZmHDA* transcrips are ubiquitously expressed in all the analyzed maize organs and that the proteins localize in all cellular domains during different stages of shoot, anther and kernel development.

Recently, it has been demostrated that *Arabidopsis thaliana* histone deacetylases 1 (AtHD1 or AtHDA19), an ortholog of yeast RPD3, is localized in the nucleus presumably in the euchromatic region but not in the nucleolus, while AtHDA6 and AtHD2 are present in the nucleolus. However, a few studies have been performed so far concerning the subcellular localization of plant HDACs and the regulation of their activity.

In this study we investigate the subcellular localization of ZmHDA101 and ZmHDA108 by transient transformation of tobacco and maize protoplasts with ZmHDA101/108-GFP chimeric constructs. Immunolabeling experiments are also performed on different maize tissues of B73 line and of antisense and overexpression mutants of ZmHDA101, using a specific anti-ZmHDA antibody. These experiments reveal that the two ZmHDA have a peculiar behavior, different from the ortholog AtHD1 and human class I HDACs, as they localize in both the nucleus and cytoplasm, frequently at the same time. This suggests a kind of nucleocytoplasmic shuttling, although no

nuclear localization and/or nuclear exporting signals has been identified in these maize deacetylases so far. The nuclear localization seems to exclude the nucleolus as AtHD1. The cytoplasmic localization is not diffuse, but shows a *"foci"*-like and/or a cytoskeleton-like pattern, thus suggesting a precise modulation of ZmHDA activity, maybe in a cell-cycle and cell-type dependent manner. The immunolocalization experiments confirm the presence of HDACs in both the nucleus and/or cytoplasm in cells of different tissues, indicating a cell-type preferential protein localization.