The involvement of 5-methyl cytosine DNA Demethylases in the dormant-growth transition in poplar

Daniel Conde, Alicia Moreno-Cortés, Tamara Hernández-Verdeja, Jose M. RamosSánchez, Mariano Perales, Pablo González-Melendi, Isabel Allona.

Centro de Biotecnología y Genómica de Plantas (CBGP, UPM-INIA), Departamento de Biotecnología-Biología Vegetal. Universidad Politécnica de Madrid. Campus de Montegancedo, Pozuelo de Alarcón, E-28223 Madrid, Spain. daniel.conde@upm.es
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Background

Woody species are highly adapted to their habitats. In response to environmental cues woody perennials trigger self-protective developmental programmes, in which signal transduction, transcriptional reprogramming and epigenetic regulation could participate in defining the winter dormancy state. Winter dormancy is the mechanism used by perennial plants to survive the harsh conditions of winter in temperate and cold regions and determines the geographical distribution of tree species (Chuine and Beaubien 2001; Horvath et al. 2003; Allona et al. 2008). Epigenetic control of winter dormancy in woody plants is barely known. Among the important epigenetic marks, 5-methyl cytosine (5mC) regulates gene expression in animals and plants. Global changes in 5mC DNA methylation have been shown in the transition of developmental stages in plants such as chestnut bud set and burst, flowering in azalea, aging in pine trees among other. However, the mechanism and the enzymes involved in the modification of the methylome and its control over those development processes remain to be identified. Our previous results showed higher DNA methylation and less acetylated Lys 8 of histone H4 global levels in poplar stem during winter dormancy compared to active growing season (Conde et al. 2013). In this study we focus in the understanding of the molecular mechanism behind these changes in DNA methylation profile and their role in the control of winter dormancy.

Methods

Analysis of the 5-methyl cytosine levels by the application of the immunofluorescence-based method set up in our lab, in stem vibratome sections cut from hybrid poplar (Populus tremula x alba) growing in the field at different stages of winter dormancy process.
To develop a protocol for buds paraffin wax embedding to analyze the level of 5-methyl cytosine by applying our immunofluorescence-based method in poplar apex microtome sections in diferents stages of winter dormancy.
RT-PCR analysis to determine the profile of gene expresion at diferent stages of winter dormancy involved in modification of DNA methylation profile.
Hybrid poplar transformation to obtain transgenic lines with modified expression of a demethylase and phenological experiments with selected lines.

Results and Conclusions

The immunolocalization assays performed in poplar stem sections showed that DNA methylation leves fall suddenly when trees coming from the dormant state are near to restore the growing season. We have determined the spatial distribution of DNA methylation changes in this organ.
We have identified two poplar homologs to Arabidopsis DME gene: PtaDML8/PtaDML10. The DME protein promotes global DNA demethylation along the genome during endosperm development. Our RT-PCR analyses indicate that the expression of PtaDML8/PtaDML10 genes increases significantly when trees are near to restart growing after winter dormancy. The phenologycal assays showed that PtaDML8/PtaDML10 knockdown plants have a delayed in resuming of growth after dormancy.
Taken together, we hypothesize that an active control of the 5mC DNA methylation might play a key role in winter dormancy and that 5mC demethylases would be crucial in this process.

Competing interests

The author declares that they have no competing interests.

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