HD-ZIP III TRANSCRIPTION FACTORS AND ACL5 ARE INVOLVED IN A REGULATORY LOOP CONTROLLING VASCULAR DEVELOPMENT

BAIMA S.*, FORTE V.*, POSSENTI M.*, FELICI B.*,**, RUBERTI I.***, MORELLI G.*

*) National Research Institute for Food and Nutrition, Via Ardeatina 546, 00178 Roma, Italy
**) CRA-RPS, Via della Navicella 2, 00184 Roma, Italy
***) Molecular Biology and Pathology Institute, CNR, P.le A. Moro 5, 00185 Roma, Italy

HD-ZIP III transcription factors, thermospermine, ACAULIS5, vascular development

The Arabidopsis HD-ZIP III transcription factor (TF) family consists of five highly related proteins (ATHB-8, CNA, PHB, PHV, REV) involved in several developmental processes including vascular development. In particular the ATHB-8 gene promotes the proliferation and differentiation of vascular precursor cells (Baima et al., 2001). The ACAULIS5 (ACL5) gene, encoding a thermospermine synthase, is also involved in the regulation of the vascular system as a acl5 loss-of-function mutant is characterized by the formation of an increased number of veins and vascular elements in leaves and stems (Hanzawa et al., 1997, 2000; Clay et al., 2005). In addition, HD-ZIP III genes expression was found to be affected in the acl5 mutant (Imai et al. 2006; Kakhei et al., 2008). Very recently it has been proposed that ACL5 controls xylem specification by preventing premature cell death of tracheary elements (Knott et al., 2007; Muniz et al., 2008).

Interestingly, we found that increased levels of ACL5 expression likely producing higher amount of thermospermine can delay or even totally inhibit the differentiation of procambial cells into differentiated tracheary elements. We also found ACL5 among the 390 Arabidopsis putative target genes containing the 11 bp pseudo-palindromic sequence (BS-III) recognized by the HD-ZIP III proteins in vitro. To further investigate the functional relationship between these genes we first demonstrated by EMSA that the HD-ZIPIII domain specifically bind the wt ACL5 promoter but not a derivative carrying mutations in the BS-III element. To confirm the functional role of this observation in vivo, transgenic Arabidopsis plants expressing the GUS reporter gene under the control of either the wt or the mutated ACL5 promoter have been generated and characterized. Histochemical analysis of the expression pattern has revealed that ATHB-8, an early marker of pre-procambial cells differentiation, is expressed earlier than ACL5 and that mutations in the BS-III element strongly reduces GUS expression in the very early phases of vascular development in leaf and root primordia, as well as in the primary root meristem. In addition, gene expression analysis by qPCR revealed that ACL5 is up-regulated upon induction of ATHB-8 in Arabidopsis transgenic inducible lines. Taken together, these data indicate that ACL5 is a downstream target of the HD-ZIPIII family. Nonetheless, a careful analysis of the phenotype of double, triple and quadruples mutant combinations of acl5-1 with HD-ZIP III mutants have shown that some of the developmental defects caused by the loss of ACL5 function are compensated for by the loss of HD-ZIP III function. These observations suggest that HD-ZIP III proteins take part to the developmental regulatory events downstream of ACL5 action, although functional HD-ZIPIII genes are required to sustain the ACL5 autoregulatory negative feedback loop. To explain these data a model will be presented.