Complex posttranslational modifications in EXT proteins that control root hair tip-growth in *Arabidopsis*

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Root hairs are single cells, responsible for the absorption of water and nutrients in the plant. Their cell walls are composed of polysaccharides and hydroxyproline (Hyp)-rich glycoproteins that include collagen-like extensins (EXTs) proteins that unlike collagen are highly O-glycosylated. Through previous work we had demonstrated that O-glycosylation on EXTs is essential for cell-wall self-assembly and, hence, root hair elongation. We successfully determined several players involved in this process that consists of sequential steps of post-translational modifications (PMTs): proline hydroxilation and Hyp-O-arabinosylation. We identified 3 prolyl-4-hydroxilases (P4H2, 5 and 13) out of the 13 present in *Arabidopsis thaliana*, 2 arabinosyltransferases (XEG113-2 and RRA3) and several EXTs in the process of root hair growth. In our current study we address the physiological significance of these PMTs present in EXTs together with the characterization of the enzymes involved. We showed that of the three P4Hs, P4H5 has a pivotal role in the determination of Hyp-O-arabinosylation, being able to restore the wild type phenotype of *p4h2* and *p4h13* and also *in vivo* interacting with P4H2 and P4H13. We further show that arabinosylation is required for proper EXT function in root hair growth since our mutant lines presented lower levels of arabinose residues. We also identified, by co-expression analysis and mutant phenotypes, two apoplastic type-III peroxidases (PERs) related to EXTs that could involved in the assembly of the EXT-extracellular network through the crosslinking of Tyr residues present in EXTs. Finally, through a live imaging approach we were able to determine the growth dynamics of our root mutants and therefore establish that the root hairs are shorter through a combined effect of reduced growth rate and reduced growth time. Together these data represent a major breakthrough in our understanding of how PMTs affects EXTs function in tip-growing cells and how complex they can be.
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