PROGRESS REPORT for Mizutani Foundation Research Grant

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Progress Report:

Root hairs are single cells specialized in the absorption of water and nutrients that develop by tip-growth, a process shared with pollen tubes, axons, and fungal hyphae. However, structural cell walls imposed constraints prompt utilization of new molecules and mechanisms to accomplish tip-growth in plants. Root hairs have been used as a single-cell model to study cell wall biosynthesis during tip-growth. They can reach high growth rates and to expand, their cell walls have to accommodate extensive structural changes. From a biological standpoint, root hairs contribute an increased root surface area that may more efficiently absorb water and nutrients from the soil, and that also anchor the plant in the soil during germination and growth. Root hair 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.

Goals: understand at a single plant cell level the molecular mechanisms that control cell expansion. We have studied in detail the enzymes (P4Hs, prolyl4-hydroxylases; GTs, glycosyltransferases; PERs, peroxidases) that introduce several post-translational modifications (PMTs) in the cell wall *O*-glycoprotein Extensins.

Methods: by using mutants isolated for each P4H, GTs and PERs responsible for each post-translational modification present in EXTs as well as overexpressors of some of them, we could identify which are the most important ones that are relevant for EXTs function in the root hair cell expansion.

Results and Discussion: First, I have addressed the physiological significance of several post-translational modifications (PTMs) of EXTs during tip-growth such as such as proline hydroxylation, Hyp-O-arabinosylation, serine-O-galactosylation and Tyr-crosslinking. Then, I characterized the P4H5 enzyme as a main regulator of peptidyl-proline hydroxylation of EXTs specifically in the root hairs (1). I also found that both, *O*-glycosylation types, Hyp-*O*-arabinosylation and serine-*O*-galactosylation, are required and have additive effects for correct EXT function in root hair cell expansion (1). Finally, I have identified six root hair specific peroxidases (PERs) as candidates for the Tyr-crosslinking of EXTs.

Conclusion: Overall our results emphasize that these complex PMTs on EXTs would ultimately modulate their supramolecular collagen-like self-assembly and in turn control root hair tip-growth and possibly many other rapidly expanding plant cells (1).

(1) Velasquez S.M., *et al.* & **J. M. Estevez**. 2014. Decoding the functions of posttranslational modifications on extensin proteins single cell growth. Sent to *Cell Reports*.