PFT1-controlled ROS balance is critical for multiple stages of root hair development in *Arabidopsis*

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Reactive oxygen species (ROS) have been shown to play key roles in cellular decision making and signal integration in multicellular organisms. In roots, ROS levels are managed by the action of peroxidases and NAPDH oxidases, resulting in a distinct spatial distribution of hydrogen peroxide (H_2O_2) and superoxide (O_2^-) that is critical for the balance between cell proliferation and differentiation. In addition, ROS is required for the determination of the cell shape of root hairs. Mutations in the Mediator subunit MED25/PFT1 result in compromised root hair development, due to altered expression of a suite of H_2O_2-producing class III peroxidases. *pft1-1* mutants form shorter root hairs than wild-type plants. Analysis of *pft1-1* cross-sections revealed that also root hair initiation is compromised, probably by impeding local cell wall loosening. It is suggested that ROS homeostasis is critical throughout the development of root hairs, controlling various processes via PFT1-regulated transcription of genes encoding redox-active enzymes.

Root hairs, tubular-shaped outgrowths of specialized cells in the epidermis, have been used successfully as a model system to study cell fate acquisition and differentiation in plants. In Arabidopsis, root hairs are formed in a genetically determined pattern that is dictated by an unknown positional signal derived from the cortical cells and by communication between future hair cells and neighboring non-hair cells.[1-3] The initiation of root hair outgrowth starts with the formation of a dome-shaped structure at the basal end of the trichoblast. The subsequent elongation of root hairs involves a multitude of processes and signaling cascades orchestrating re-assembly of the cytoskeleton and the cell wall, resulting in highly polarized tip growth and rapid elongation of the hair.[4-6]

The production of reactive forms of molecular oxygen (Reactive oxygen species; ROS) is an omnipresent by-product of aerobic metabolism. During the past two decades, it became evident that ROS is not only a negative factor for cells, damaging proteins, DNA or cell structures, but constitutes also a key component in signal integration and decision making.[7,8] Multicellularity has evolved in a hypoxic environment. To cope with increasing levels of toxic oxygen derivatives, organisms evolved sophisticated scavenging mechanisms to manage ROS levels. The necessity to monitor ROS accumulation was likely the driving force that adopted controlled ROS production as a means to relay developmental and environmental clues. In roots, the two major types of ROS, hydrogen peroxide (H_2O_2) and superoxide (O_2^-), are differentially distributed and fulfill different functions.[9] We showed recently that the Mediator subunit MED25/PFT1 controls root hair differentiation by regulating the expression of several genes encoding redox-active proteins, in particular class III peroxidases and NADPH-oxidases, which critically alters the balance of H_2O_2 and O_2^-.[10] Root hair elongation was compromised in *pft1* mutants, probably caused by perturbed H_2O_2-dependent and peroxidase-mediated cross-linking of extensins.[11] The mechanism by which PFT1 controls the redox balance resembles that of the transcription factor UPBEAT1 (UPB1), which controls the transition from proliferation to differentiation through the distribution of ROS along the roots.[12] Similar to PFT1, UPB1 controls the expression of a suite of class III peroxidases genes. While UPB1 maintains the ROS balance by repressing the expression of H_2O_2-scavenging class III peroxidases, PFT1 controls the distribution of ROS by activating the gene expression of H_2O_2-generating class III peroxidases. Since peroxidases are mainly localized in the apoplast, a possible role for ROS involves both negative action on the cell cycle and supporting action on cell elongation via cell wall modification.

Table 1. Effect of the *PFT1* mutation on root morphology

Genotype	Epidermal cells	Cortical cells	Root hairs (H position)	Root hairs (N position)
Col-0	22.4 ± 0.09	8.0 ± 0.01	0.95 ± 0.04	0.02 ± 0.0
pft1-1	20.8 ± 0.08	8.0 ± 0.01	0.54 ± 0.03	0.005 ± 0.0

Fifty cross-sections per root from a total of ten roots per genotype from 7-d-old seedling were analyzed. Numbers report average per cross-section ± SE. H position, hair position; N position, non-hair position.

PFT1 has been identified as the Med25 subunit of the Mediator complex,[13] a large multiprotein protein complex that relays information from transcriptional regulators to RNA polymerase II to initiate transcription that first identified in yeast.[14] In plants, Mediator subunits have been associated with the regulation of specialized processes in growth and development; however, the exact mechanism by which PFT1 affects root hair development remains elusive.

To investigate potential effects of PFT1-controlled ROS balance on early stages of root hair development, we analyzed cross-sections from *pft1-1* roots in comparison with those from wild-type plants, counting all visible bulges as initiated root hairs. The data in **Table 1** show that the number of root hairs in the default (H) position in *pft1-1* plants was reduced to approximately half of the frequency observed in the wild-type. In addition, the relatively rare formation of ectopic root hairs in the N position was also significantly reduced in roots of the mutant. It can thus be assumed that PFT1 not only controls root hair elongation, but also earlier stages of root hair development, i.e., fate acquisition and/or root hair initiation (**Fig. 1**). Oxygen levels can influence the cell fate in mammalian cells,[15-17] and recent evidence suggest that this can be also the case in plants.

Hypoxia was shown to act as a positional signal to control the fate of maize germ cells, indicating a key role for ROS control to maintain fertility.[18] Furthermore, programmed death of cells that overlie adventitious root primordia in rice was shown to be induced by a combination of mechanical signaling and ROS.[19] A possible scenario that would explain the *pft1-1* phenotype involves impairment of lateral communication between the two cell types of the *Arabidopsis* epidermis, hair cells and non-hair cells. Such cross-talk is critical for cell fate acquisition and involves the movement of the bHLH transcription factor GL3 from hair cells to non-hair cells and the migration of the single-repeat R3 MYB transcription factors CPC, TRY and ETC1 from non-hair cells to hair cells.[5,20,21] Disruption of this cell-to-cell communication results in an altered pattern of root hair in the epidermis. For example, *cpc* mutants cannot repress the formation of GL2, a negative regulator of the root hair cell fate, resulting in the formation of sparse root hairs.[1] In an altered ROS distribution could compromise this sophisticated cell-to-cell communication, resulting in a perturbed root hair pattern. An alternate, not mutually exclusive scenario involves an effect of altered ROS management on the initiation of root hair formation. In epidermal cells of maize roots, ROS was shown to be required for peroxidase-mediated local disassembly of the cell wall to initiate bulge formation, the first stage in root hair formation after cell specification.[22] In *pft1-1* plants, reduced expression of PFT1-regulated peroxidase genes may compromise this local loosening of cell walls, resulting in trichoblasts that fail to initiate root hair formation. In addition, shorter root hairs observed on the *pft1-1* plants indicates that ROS distribution is important for peroxidase-mediated cell wall remodeling in later stages of root hair development and for RHD2 NADPH oxidase-mediated establishment of a Ca^{2+} gradient that controls the site of the outgrowth and cell shape after initiation (**Fig. 1**).[10,23,24] Taken together, our data imply a sophisticated role of ROS management in both early and late stages of root hair morphogenesis. The molecular mechanisms which translate the level and the distribution of ROS into a phenotypical readout, however, await further experimentation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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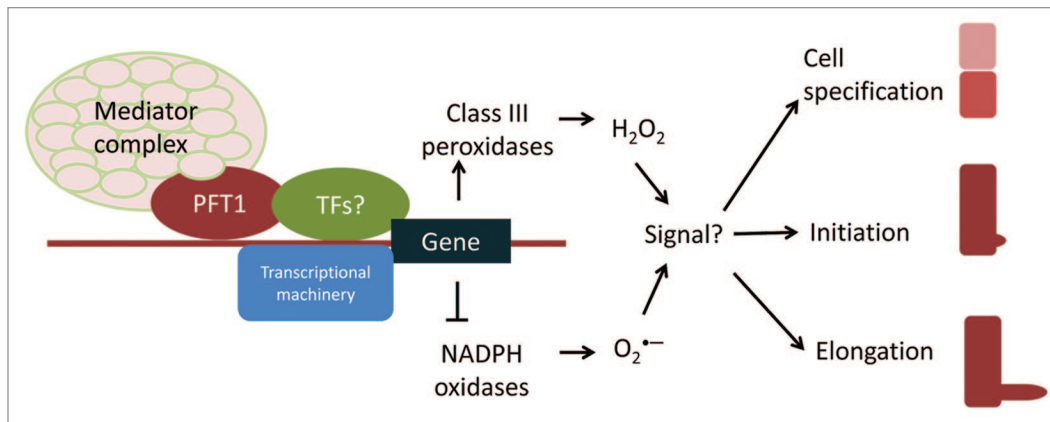
Figure 1. Possible effects of MED25/PFT1-controlled redox balance on root hair formation. The Mediator subunit PFT1 interacts with transcription factors (TFs) and the transcriptional machinery to initiate or repress transcription of H_2O_2-producing class III peroxidases (positive regulation) and O_2^--producing NADPH oxidases (negative regulation). The distribution of H_2O_2 and O_2^- may act as a signal to regulate several processes in root hair development.

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