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Scope of Research

This laboratory aims at clarifying molecular bases of regulatory mechanisms for plant development, especially plant morphogenesis, with techniques of forward and reverse genetics, molecular biology, and biochemistry. Current major subjects are: 1) phospholipid signaling in cell morphogenesis, 2) the transcriptional network for cytokinin responses, 3) COP9 signalosome modulating signal transduction in the nuclei, and 4) the endoreduplication cell cycle in cell differentiation.

KEYWORDS

Morphogenesis
Phospholipid
Cytokinin
Signal Transduction
COP9 Signalosome

Selected Publications

Lin, Q.; Ohashi, Y.; Kato, M.; Tsuge, T.; Gu, H.; Qu, L.-J.; Aoyama, T., GLABRA2 Directly Suppresses Basic Helix-loop-helix Transcription Factor Genes with Diverse Functions in Root Hair Development, *Plant Cell*, **27,** 2894-2906 (2015).

Wada, Y.; Kusano, H.; Tsuge, T.; Aoyama, T., Phosphatidylinositol Phosphate 5-kinase Genes Respond to Phosphate Deficiency for Root Hair Elongation in *Arabidopsis thaliana, Plant J.*, **81,** 426-437 (2015).

Hayashi, K.; Nakamura, S.; Fukunaga, S.; Nishimura, T.; Jenness, M. K.; Murphy, A. S.; Motose, H.; Nozaki, H.; Furutani, M.; Aoyama, T., Auxin Transport Sites are Visualized *in planta* Using Fluorescent Auxin Analogs, *Proc. Natl. Acad. Sci. USA*, **111,** 11557-11562 (2014).

Kato, M.; Aoyama, T.; Maeshima, M., The $Ca2^{+}$-binding Protein PCaP2 Located on the Plasma Membrane is Involved in Root Hair Development as a Possible Signal Transducer, *Plant J.*, **74,** 690-700 (2013).

Aki, S.; Nakai, H.; Aoyama, T.; Oka, A.; Tsuge, T., *AtSAP130/AtSF3b-3* Function is Required for Reproduction in *Arabidopsis thaliana*, *Plant Cell Physiol.*, **52,** 1330-1339 (2011).

GLABRA2 Targets Basic Helix-Loop-Helix Transcription Factor Genes to Suppress Root Hair Development

The morphological differentiation and deposition patterns of cells are crucial determinants of plant structures. Among the plant tissues composed of multiple types of cells, the root epidermis of *Arabidopsis thaliana* has served as an excellent model system for studying morphological differentiation and pattern formation of plant cells. In *Arabidopsis*, the root epidermis is composed of non-hair (N) and hair (H) cell files, and only cells in H cell files develop root hairs. For this cell pattern formation and subsequent cell differentiation, numerous transcription factor genes, including *GLABRA2* (*GL2*), constitute the regulatory networks. *GL2* encodes a homeodomain-leucine-zipper transcription factor and is thought to be a negative regulator of root hair development because it is expressed preferentially in N cell files and mutant defects in *GL2* result in ectopic root hair formation in N cell files. Furthermore, because the expression of a modified GL2 with constitutive transactivating function (VP16-GL2ΔN) resulted in the formation of root hair-like structures from various epidermal cells, including leaf pavement cells, GL2 is thought to recognize a set of genes sufficient for root hair development. This line of evidence clarifies the role of *GL2* as a genetic switch that suppresses root hair development through the suppression of its target genes.

Despite the pivotal role of *GL2* in suppressing root hair development, the molecular basis of the suppression remains obscure because, to date, few GL2 target genes are known and none of them are connected to the gene regulatory network for root hair cell differentiation. The *PHOSPHOLIPASE Dζ1* gene is suppressed directly by GL2 in N cell files and its ectopic expression causes root hair bulges in N cell files, suggesting its involvement in promoting root hair development. *CELLULOSE SYNTHASE5* and *XYLOGLUCAN ENDOTRANSGLYCOSYLASE17*, both of which encode enzymes involved in polysaccharide synthesis, are also GL2 target genes, but their roles in root hair development are unclear. *GL2* and *MYB23* constitute a positive feedback loop in the shoot epidermis where GL2 directly recognizes *MYB23*. Upstream regions of these genes typically contain the L1 box–like sequence 5'-TAAATGT-3'. Although GL2 physically interacted with DNA regions containing this sequence, whether the sequence is necessary and/or sufficient for recognition by GL2 *in planta* remains unclear.

In this study, we identified five *Arabidopsis* bHLH transcription factor genes, *ROOT HAIR DEFECTIVE6 (RHD6), ROOT HAIR DEFECTIVE6-LIKE1 (RSL1), RSL2, Lotus japonicus ROOTHAIRLESS1-LIKE1 (LRL1)*, and *LRL2*, as direct targets of GL2 using transcriptional and post-translational induction systems with chimeric transcription factors VP16-GL2ΔN and GR-VP16-GL2ΔN, respectively. Chromatin immunoprecipitation (ChIP) analysis using a green fluorescence protein (GFP)-fused GL2 protein confirmed the physical interaction of GL2 with these genes *in planta*. Expression analyses showed that the genes are suppressed by *GL2* in N cell files and are expressed in various developmental stages of root hair development. Moreover, the phenotypes caused by the ectopic expression of their GFP-fusion proteins differed among them: *GL2* promoter-driven GFP-LRL1 and GFP-LRL2, but not the other GFP-bHLH proteins, conferred root hair development on non-hair cells. These results indicate that GL2 targets these bHLH genes, which have diverse functions, to robustly suppress root hair development via multimodal pathways.

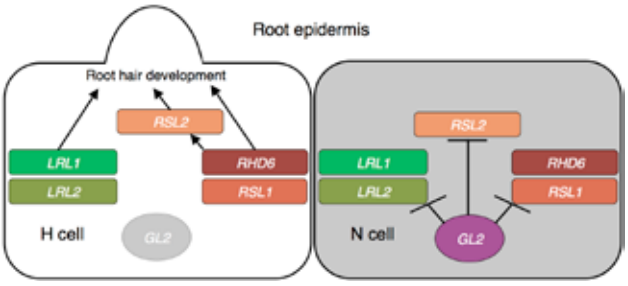

Figure 1. A model of the transcriptional network surrounding *GL2* in root epidermal cells. In N cells (gray color), GL2 directly suppresses the bHLH transcription factor genes *RHD6*, *RSL1*, *RSL2*, *LRL1*, and *LRL2*. In H cells (white), *GL2* is not activated and the bHLH genes remain active to promote root hair development.

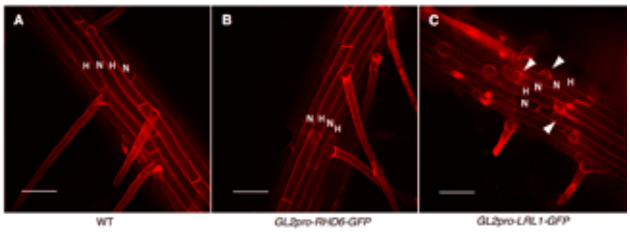

Figure 2. Root hair development patterns of transgenic plants harboring the *GL2* promoter-driven bHLH-GFP genes. PI-stained epidermal cells of wild-type **(A)**, and transgenic roots with *GL2pro-RHD6-GFP* **(B)** and *GL2pro-LRL1-GFP* **(C)** are shown. H and N cell files are marked by "H" and "N", respectively. Ectopic root hairs developing from N cell files are indicated by arrowheads. Bar = 25 μm.