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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 Signaling
RNA
Signal Transduction
COP9 Signalosome

Selected Publications

Wu, Z.; Zhu, D.; Lin, X.; Miao, J.; Gu, L.; Deng, X.; Yang, Q.; Zhu, D.; Cao, X.; Tsuge, T.; Dean, C.; Aoyama, T.; Gu, H.; Qu, L.-J., RNA Binding Proteins RZ-1B and RZ-1C Play Critical Roles in Regulating Pre-mRNA Splicing and Gene Expression during Development in *Arabidopsis*, *Plant Cell*, **28,** 55-73 (2016).

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 Ca^{2+}-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).

Genetic Analysis of RNA-Binding Protein Genes in *Arabidopsis thaliana*

Some classes of RNA binding proteins (RBPs) play pivotal roles in regulating gene expression, both co-transcriptionally and post-transcriptionally. The *Arabidopsis thaliana* genome encodes more than 600 putative RBPs, but the detailed functions of the vast majority of these RBPs remain unclear. Serine/arginine-rich (SR) proteins belong to a group of RBPs. Among the 19 SR proteins in *Arabidopsis*, only a few have been studied functionally. Gain-of-function analyses of SR protein genes RS2Z33 and SR30, as well as loss-of-function analyses of SR45, SCL33, SCL33a, RS40, and RS41, have revealed that the proteins encoded by these genes regulate alternative splicing of their own pre-mRNA and that of other SR genes. Glycine-rich RNA binding proteins (GRPs), a small group of plant RBPs, contain an N-terminal RRM domain and a C-terminal glycine-rich stretch. GRPs in *Arabidopsis* include GRP1-8 and RZ-1A-C (Fig 1). GRP7 and GRP8 are important regulators of circadian oscillations, flowering time, responses to plant pathogens, and cold stress. RZ-1A-C has a zinc finger motif between the RRM domain and C terminus. RZ-1, which was first reported in the tobacco, belongs to a subgroup of GRPs and binds to a large ribonucleoprotein particle localized in the nucleus. Homologs of RZ-1 in *Arabidopsis* were shown to have RNA chaperone activity in *Escherichia coli*. Overexpression of RZ-1A, but not homologous genes RZ-1B and RZ-1C, confers freezing tolerance in transgenic *Arabidopsis*. However, there is no direct genetic evidence to support the apparently important roles of these GRPs in plant growth and development and in responses to environmental stimuli.

In this study, we provide functional evidence that *Arabidopsis* RZ-1B and RZ-1C represent a unique group of GRPs. rz-1b rz-1c double mutants displayed a wide variety of defects, including delayed germination, retarded development of root and shoot meristems, late flowering, reduced stature, and serrated leaves (Fig 2). RZ-1B and RZ-1C interact with SR proteins through their C-terminal domains, which are also essential for nuclear speckle localization. High-throughput RNA-seq analysis of rz-1b rz-1c double mutants revealed perturbation of the splicing of many genes. Defective splicing was also observed in plants overexpressing the C-terminal domain of RZ-1C, confirming that RZ-1B/1C regulates splicing via interaction with SR proteins. RZ-1B and RZ-1C were found to be required for maintaining the optimal expression levels of more than 3000 genes, including many developmental regulators. Taken together, these findings reveal the essential roles of RZ-1B and RZ-1C as regulators of plant development, splicing, and general gene expression via interaction with proteins including SR proteins.

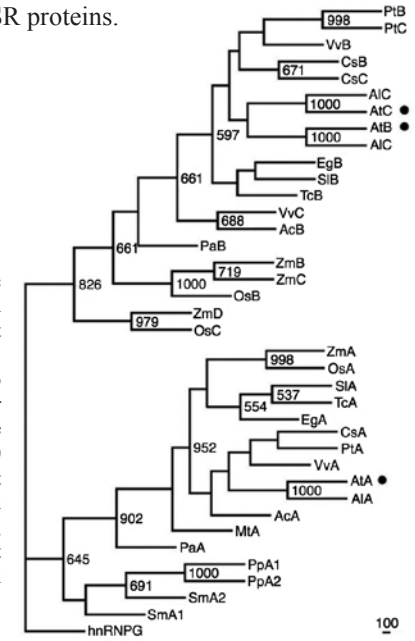

Figure 1. Phylogenetic tree of At RZ-1-related proteins from different plant species.
The tree consists of two large clades. The number at each node indicates the bootstrap value (1000 replicates). Three At RZ-1 proteins are marked with black circles. At A, At B, and At C refer to At RZ-1A, At RZ-1B, and At RZ-1C, respectively.

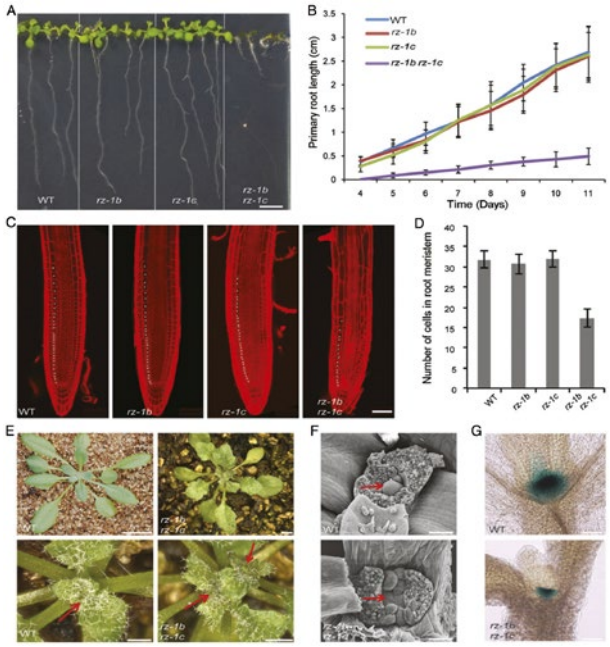

Figure 2. Pleotropic Defects of the rz-1b rz-1c Double Mutant.
(A) Image of seedlings grown vertically for 10 d after germination. Bar = 0.5 cm. (B) Statistics of the primary root length from day 4 to day 11 after germination. Error bars represent SD (n = 20). (C) Confocal image of root meristems from different genotypes. The image shows the representative root meristems from seedlings at 4 d after germination. The roots were stained with propidium iodide. Asterisks indicate the dividing cells in the endodermis. Bar = 50 mm. (D) Statistics of endodermal cells from different genotypes within the meristematic regions. Error bars represent SD (n = 15). (E) rz-1b rz-1c generates multiple SAMs during vegetative growth. The images show wild-type and rz-1b rz-1c seedlings 3 weeks after germination. The lower panels show magnified images of the upper panels. Arrows indicate the positions of SAMs. Bars = 5 mm (upper panel) and 1 mm (lower panel). (F) Scanning electron microscopy images of the SAMs of the wild type and rz-1b rz-1c. Arrows indicate the SAM in the wild type and the corresponding region in rz-1b rz-1c. Bar = 50 mm. (G) Histochemical staining of CLAVATA3:GUS reporter in wild-type and rz-1b rz-1c backgrounds. Bar = 80 mm.