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Prof
OKA, Atsuhiro
(D Sc)

Assoc Prof
SUGISAKI, Hiroyuki
(D Sc)

Assoc Prof
AOYAMA, Takashi
(D Sc)

Instr
SAKAI, Hiroe
(D Sc)

Assoc Instr
OHASHI, Yohei
(D Sc)

Techn
YASUDA, Keiko

Lecturer (pt)

TABATA, Satoshi (D Sc)
Kazusa DNA Research Institute

Technician (pt)

NAKAGAWA, Shuko

Students

TANIGUCHI, Masatoshi (D2)
IMAI, Kumiko (D1)
YAMAMOTO, Yukimi (M2)

Scope of Research

This laboratory aims at clarifying the framework of regulatory network between genetic programs and environmental stress responses through the study on structure-function relationships of genetic materials and cellualr proteins in higher plants and pathogens. The major subjects are mechanisms involved in intracellular signal transduction and regulation of gene expression responsive to environmental stimuli, differentiation and development of plant organs, and plant-microbe interaction. As of December 2003, study is being concentrated on the roles of two-component response regulators involved in cytokinin signaling for cell growth and division and of homeodomain proteins modulating phospholipid signaling for root-hair development.

Research Activities (Year 2003)

Presentations

Analysis of function of CDKA expressed in Arabidopsis trichome, Imai K, Ohashi Y, Aoyama T, Oka A, 2003 Ann Meeting (Nara) of Jpn Soc Plant Physiol, 27 - 29 March (Osaka).

GLABRA2 regulates root-hair development by modulating phospholipid signaling, Ohashi Y, Oka A, Pousada R, Possenti M, Ruberti I, Morelli G, Aoyama T, VII International Congress of Plant Molecular Biology, 23 - 28 June (Barcelona, Spain),

Cytokinin signaling and two-component regulatory system, Oka A, Gamma Field Symposia No.42 (Institute of Radiation Breeding, NIAR MAFF) 'Molecular mechanisms of and mutations in plant hormone responses and signal transduction', 16 - 17 July (Mito).

Function of phospholipase D in root-hair development, Aoyama T, 16th Plant Lipid Symposium, 28 - 29 November (Yamaguchi).

Interactions between plant transcription factors and their target genes, Aoyama T, Taniguchi M, Ohashi Y, Oka A; Functinal analysis of A2 cyclins expressed in Arabidopsis trichome cells, Imai K, Ohashi Y, Aoyama T, Oka A, 2003 Ann Meeting of Mol Biol Soc Jpn, 10 - 13 December 2003 (Kobe).

Grants

Oka A and Aoyama T, Molecular basis of cytokinin signaling in plant cells, Grant-in-Aid for Scientific Research on Priority Areas (A), 1 April 2003 - 31 March 2005.

Aoyama T and Oka A, Molecular mechanism of adaptive responses controlled by *Arabidopsis* His-Asp phosphorelay signal transduction, Grant-in-Aid for Scientific Research on Priority Areas (B), 1 April 2000 - 31 March 2005.

Award

Ohashi Y, The ICR Award for Young Scientists, Functinal analysis of the *Arabidopsis GLABRA2* gene that contributes root-hair development through the modulation of phospholipid signaling, ICR, 5 December 2003.

Phospholipid signaling modulated by GLABRA2 contributes root-hair development

Cell morphogenesis and its disposition pattern are crucial for plants to ensure functional tissue and organ structures because the relative positions of plant cells do not change after proliferation. The *Arabidopsis* root epidermis is composed of two types of cell files, only one of which produces root hairs. Each hair cell file makes contact with two adjacent underlying cortical cell files, whereas each hairless cell file makes contact with a single cortical cell file. Mutations in the *GLABRA2 (GL2), WEREWOLF (WER),* and *TRANSPARENT TESTA GLABRA 1 (TTG1)* genes lead to the generation of root hairs from all the cell files. Conversely, mutations in *CAPRICE (CPC)* reduce the frequency of root hairs in hair cell files. The homeodomain protein GL2 is synthesized predominantly in hairless cell files. TTG1 positively controls *GL2* expression. The Myb-type transcription factors WER and CPC, which cross-control each other in a feedback loop, positively and negatively regulate the position-specific expression of *GL2*, respectively. Thus a transcriptional regulatory circuit appears to be involved in determining root-hair pattern formation. We have now analyzed their downstream events by identifying genes targeted by GL2 [1], the transcription factor farthest downstream in the known feedback loop.

We conducted a subtraction experiment using cDNAs prepared from plants synthesizing a modified version of GL2 (constitutively active for transactivation) and the native GL2 (repressor) to detect GL2-target genes that promote root-hair development. One of such genes encoded a phospholipase D (PLD) named AtPLDζ1. GL2 bound to a promoter region of *AtPLDζ1*. Furthermore, *AtPLD ζ1* expression in developing wild-type root epidermis was higher in hair cell files than in hairless cell files. In contrast, mutations in *GL2* as well as in the GL2-binding region upstream of *AtPLDζ1* abolished this expression specificity by elevating transcription levels in hairless cell files. It was thus concluded that GL2 works as a repressor for the *AtPLDζ1* gene.

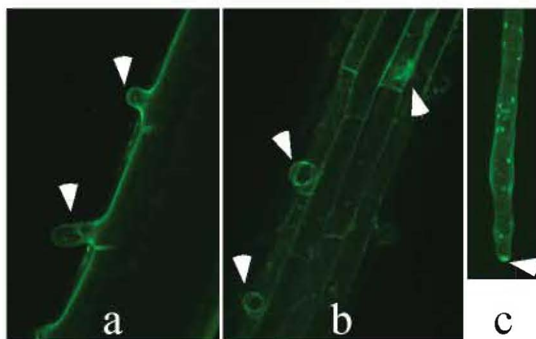

Figure 1. The intracellular localization of AtPLDζ1-GFP fusion protein, visualized by its autofluorescence under a confocal laser-scanning microscope. The arrowheads indicate relatively strong fluorescence signals around bulges (a, b) and the root-hair apex (c). Quoted from ref 1.

AtPLDζ1 (a fusion derivative with green fluorescent protein GFP) was localized in vesicles mainly at the cell cortical region (Figure 1). At early stages of root-hair development, the fusion protein was localized preferentially around bulges (Figure 1a, b). In growing root hairs, relatively strong fluorescence was often observed around the root-hair apex (Figure 1c). Both elevation and reduction of *AtPLDζ1* expression led to various abnormalities in root-hair development. PLD hydrolyzes phosphatidylcholine to generate phosphatidic acid and choline. *Arabidopsis* encodes two types of PLDs, plant-specific type and universal type for eukaryotes. AtPLDζ1 is of the latter type. The universal type PLDs in animal and yeast cells modulate membrane-trafficking events to and from the plasma membrane. By analogy, AtPLDζ1 might regulate vesicle trafficking and exocytosis in root-hair tip growth. The localization pattern of the GFP-fusion protein supports this postulate.

1. Ohashi, Y. *et al. Science*, **300**, 1427-1430 (2003).

Genes encoding enzymes involved in protocatechuate *meta*-degradation pathway of *Pseudomonas ochraceae*

Protocatechuate (PCA) is a common intermediate in the biodegradation of various aromatic compounds including phthalate, fluorene, and various methoxy derivatives of benzoate. Some microorganisms *e.g.* lignin-assimilating *Sphingomonas paucimobilis* and phthalate-assimilating *Arthrobacter keyseri*, metabolize PCA through *meta*-degradation pathway (α-keto acid pathway), in which PCA undergoes the 4,5-cleavage by PCA 4,5-dioxygenase to give 4-carboxy-2-hydroxymuconate-6-semialdehyde (CHMS). CHMS is then converted to 2-pyrone-4,6-dicarboxylate (PDC) by $NAD(P)^+$-dependent CHMS dehydrogenase. PDC is hydrolyzed by PDC lactonase to give 4-ozalomesaconate, which is hydrated by 4-oxalomesaconate hydratase, and finally cleaved to pyruvate and oxaloacetate by 4-hydroxy-4-methyl-2-oxoglutarate aldolase [2]. The molecular mechanism of each enzyme-catalyzed reaction is hampered owning to the deficient information of the enzyme structure and the lack of an over-expression system of the enzyme.

A 7.5-kb *EcoRI* DNA fragment from the *P. ochraceae* NGJ1 chromosome was previously shown to carry a promoter-like sequence and genes encoding transporter, 4-oxalomesaconate hydratase, 4-hydroxy-4-methyl-2-oxoglutarate aldolase, and 3'-truncated PDC lactonase. To elucidate the whole genetic structure corresponding to enzymes involved in the pathway, a DNA fragment downstream of the *EcoRI* fragment was cloned. As a result, this region corresponded to the α and β subunits of PCA 4,5-dioxygenase, CHMS dehydrogenase, and the 3'-half of PDC lactonase. These enzymes were excessively synthesized in *E. coli* cells, and purified to homogeneity through several steps of chromatography. Their molecular and catalytic properties were found to be indistinguishable from those isolated from *P. ochraceae* cells.

2. Maruyama, K. *et al. Biosci. Biotech. Biochem.* **65**, 2701-2709 (2001).