Division of Biochemistry
- Molecular Biology -

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Dr LAPARRA, Hélène	Meristem Therapeutics, France, 1 - 8 April 2005
Prof YOSHIOKA, Keiko	University of Toronto, Canada, 19 May 2005
Assoc Prof SERINO, Giovanna	University of Rome La Sapienza, Italy, 18 August-3 September 2005

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 cellular proteins in higher plants and pathogens. The current major subjects are the two-component response regulators involved in cytokinin signaling, HD-Zip proteins required for phospholipid signaling, COP9 signalosome modulating protein degradation, and cyclines and CDKs controlling cell cycle.

Research Activities (Year 2005)

Presentations

Transcription Factor-type Response Regulator ARR1 of *Arabidopsis thaliana*: Functions and Target genes, Taniguchi M, Aoyama T, Oka A, International Symposium on Plant Axis Formation and Signal Transduction, 2 - 3 March (Tokyo).

Screening for Genes Directly Regulated by ARR1, Taniguchi M, Aoyama T, Oka A; Novel Regulation of COP9 Signalosome (CSN): a Master Regulator of Signal Transduction in Plant Morphogeneis, Tsuge T, Dohmae N, Wei N, Oka A, 2005 Ann Meeting of Jpn Soc Plant Physiol, 24 - 26 March (Niigata).

Two-component Regulatory System and Cytokinin Signaling, Oka A; COP9 Signalosome: the Key Complex Linking Environmental Signals to Morphogenesis in Plants and Mammals, Tsuge T, Memorial Seminar for the 80th Anniversary of the College of Life Science, Peking University, 29 April (Beijing, PRC).

Screening for the Direct Target Genes of ARR1, Taniguchi M, Aoyama T, Oka A; Revealing the Novel Regulation of COP9 Signalosome (CSN), Tsuge T, Dohmae N, Wei N, Oka A; Expression and Function Analyses of the *AtPLD*ζ2 Gene, Yamamoto Y, Ohashi Y, Oka A, Aoyama, T; AtCYCA2;3 is a Key Regulator in the Termination of Endoreduplication Rounds in *Arabidopsis*, Imai K, Ohashi Y, Tsuge T, Yoshizumi T, Matsui M, Oka A, Aoyama T, XVI International Conference on Arabidopsis Research, 15 - 19 June (Madison, USA).

AtCYCA2;3 is a Key Regulator in the Termination of Endoreduplication Rounds in *Arabidopsis*, Imai K, Ohashi Y, Tsuge T, Yoshizumi T, Matsui M, Oka A, Aoyama T, Rice-Arabidopsis Joint Workshop, 6 - 7 July (Nara).

To Reveal New Mechanisms of CSN: the Key Complex Linking Environmental Signals to Morphogenesis, Tsuge T,

Suppression of CRE1 Defects by Functional Alterations of the Downstream Components

Cytokinins are a class of phytohormones that induce a variety of physiological and developmental events, including cell division. These responses to cytokinins in *Arabidopsis thaliana* (Fig. 1) are triggered through the perception of cytokinin by the sensor histidine kinase CRE1. A *cre1* mutation leads to defect of vascular development, the degree of which depends on each mutant allele. Among those, the *wol* allele show severe inhibition of root growth accompanied by missing phloem, and by frequent generation of short adventitious roots in which the reduced numbers of vascular cells are included [1].

Figure 1. *Arabidopsis* cultivars.

The transcription-factor type response regulators such as ARR1 are activated by cytokinin. On the analogy of bacterial histidine kinases, these response regulators together with the AHP bridge components have been thought to be functionally located downstream of CRE1. CRE1 and ARR1 do not seem to interact directly in cells because of their respective localizations (CRE1 at the cell membrane and ARR1 in nuclei), whereas AHPs seem to be small enough for passive transport through the nuclear membrane (Fig. 2). Indeed, AHP has potential for associating with both CRE1 and ARR1 *in vitro*.

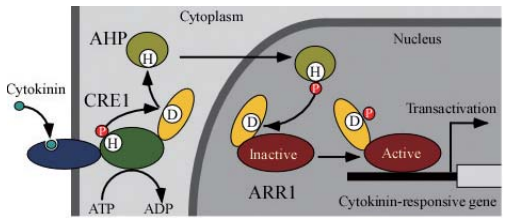

Figure 2. Framework of the intracellular cytokinin signal transduction pathway in *Arabidopsis*. Quoted and altered from ref 1.

From these observations, cytokinin signaling by phosphorelay appears to occur from CRE1 to AHP and then to ARR1 *in vivo* [1]. In order to show that this signal flow actually occurs in plant cells, an attempt was made to suppress *cre1* defects by alterations of the putative downstream components, ARR1 and AHP. Upon introduction into *wol* plants of either constitutively active ARR1ΔR or conditionally active ARR1ΔR::GR, the *wol* phenotype (inhibition of root growth and defect of vascular development) was restored partially to up to a level comparable to that of wild-type plants. These results indicate *cre1* defects being restored by activation of ARR1 without cytokinin, implying that ARR1 is functionally located downstream of CRE1 *in planta*. Another line of suppression mutants were obtained by mutagenesis with *wol* seeds. In addition to several intragenic suppressor mutations, a recessive, extragenic suppressor mutation (*ahp6*) was identified. AHP6 is a pseudo-AHP, and has been presumed to inhibit active AHPs competitively. This suppression also suggests that AHPs work downstream from CRE1. These results support the view that cytokinin signaling in plant cells actually occurs *via* CRE1-AHP-ARR1 phosphorelay, as supposed from a previous biochemical study and from the analogy of bacterial signaling.

[1] Aoyama T & Oka A, *J. Plant Res.*, **116**, 221-231 (2003).
[2] Mähönen A *et al*., *Science*, **311**, 94-98 (2006).

RIKEN Seminar, 10 March (Wako)/ COE Seminar of Nara Institute of Science and Technology, 30 August (Ikoma)/ Institute Seminar of Rome University La Sapienza, 9 September (Rome, Italy).

Characterization of an *Arabidopsis* PI4P5K3 Gene, a Candidate Regulator for Root-hair Development, Kusano H, Yasuda K, Aki S, Ohashi Y, Shimada H, Oka A, and Aoyama T, 1st International Conference on Plant Lipid-Mediated Signaling: Building Connections, 26 - 29 October (Raleigh, USA).

Phospholipid Signaling in Root-hair Cell Morphogenesis, Aoyama T, The 21st Symposium in Conjunction with Award of International Prize for Biology: Morphology, Molecules and Morphogenesis (Awardee: Prof Nam-Hai Chua, Rockefeller Univ), 30 November-1 December (Nagoya).

Novel Regulation of COP9 Signalosome: a Master Regulator of Signal Transduction in Morphogenesis, Tsuge T, Aki S, Dohmae N, Menon S, Wei N, Oka; Functional Analysis of the *AtPIPK3* Gene in Root Hair Development, Kusano H, Yasuda K, Aki S, Yoshizumi T, Matsui M, Oka A, Aoyama T, 2005 Ann Meeting of Mol Biol Soc Jpn, 7 - 10 December (Fukuoka).

Grants

Oka A, Two-component Regulatory System of Phosphorelay Involved in Cytokinin Signaling, Grant-in-Aid for Scientific Research (B), 1 April 2004 - 31 March 2007.

Aoyama T, Roles of Phospholipid Signaling in Root-hair Formation, Grant-in-Aid for Scientific Research (B), 1 April 200 - 31 March 2007.