Division of Symbiotic Systems

Professor
KAWAGUCHI, Masayoshi

Assistant Professor:	*TAKEDA, Naoya*
	SUZAKI, Takuya
Technical Staff:	*FUKADA-TANAKA, Sachiko*
NIBB Research Fellow:	*SOYANO, Takashi*
Postdoctoral Fellow:	*FUJITA, Hironori*
	TATEMATSU, Kiyoshi
	HANDA, Yoshihiro
	KOBAYASHI, Yuki
	NAGAE, Miwa
	MATSUOKA, Hikaru
SOKENDAI Graduate Student:	*SASAKI, Takema*
	YORO, Emiko
	TSUZUKI, Shusaku
	NISHIDA, Hanna
	FUKUHARA, Mai
	LIU, Meng
Visiting Undergraduate:	*MIZUTANI, Yoshiyuki*
Technical Assistant:	*SUZAKI, Momoyo*
	ICHIKAWA, Michiko
	OGAWA, Yuko
	YOSHINORI, Yumi
Secretary:	*SANJO, Kazuko*
	OKUBO, Masayo

Rhizobium–legume symbiosis is one of the most successful mutually beneficial interactions on earth. In this symbiosis, soil bacteria called rhizobia supply the host legumes with ammonia produced through bacterial nitrogen fixation. In return, host plants provide the rhizobia with their photosynthetic products. To accomplish this biotic interaction, leguminous plants develop nodules on their roots. On the other hand, more than 80% of land plant families have symbiotic relationships with arbuscular mycorrhizal (AM) fungi. Despite marked differences between the fungal and bacterial symbioses, common genes are required for both interactions. Using a model legume *Lotus japonicus*, we are trying to unveil the mechanisms of both symbiotic systems.

I. Nodulation

1-1 Genetic mechanism involved in early and late nodule development

In response to appropriate inductive conditions, plants have the capacity of forming new organs from differentiated cells. Root nodulation is one such unique developmental process that predominantly occurs in leguminous plants. In this process, signaling initiated by symbiotic bacterial infection alters the fate of differentiated cortical cells and causes formation of new organs.

The *L. japonicus vagrant infection thread 1* (*vag1*) is a novel mutant involved in nodule development. *VAG1* encodes a protein orthologous to *Arabidopsis* ROOT HAIRLESS 1, which functions as a subunit of DNA topoisomerase VI. Analyses focusing on nuclear size found the emergence of a few cortical cells with enlarged nuclei during initiation of cortical cell division in wild type. In the *vag1* mutants, these potentially endoreduplicated cells are not observed and subsequent cortical cell division is severely compromised. Thus, it is possible that DNA topoisomerase VI is involved in the endoreduplication of cortical cells, which can trigger the onset of cortical cell division. During late nodule development, endoreduplication results in the formation of enlarged rhizobia-colonized cells in mature nodules. In wild type *L. japonicus*, there are enlarged rhizobia-colonized cells in the inner region of the nodule, and smaller rhizobia-infected (as yet uncolonized) cells in the surrounding region (Figure 1). In *vag1* nodules, the number of these small rhizobia-infected cells is higher whereas the number of rhizobia-colonized cells is lower (Figure 1). This suggests that the *vag1* mutant has a defect in differentiation from small rhizobia-infected cells to enlarged rhizobia-colonized cells. Overall, our data indicate that endoreduplication mediated by DNA topoisomerase VI may be a prerequisite regulator for the control of two key nodule developmental processes: the first is related to the onset of nodule organogenesis during early nodule development, and the second is associated with the differentiation of rhizobia-colonized infected cells in late nodule development.

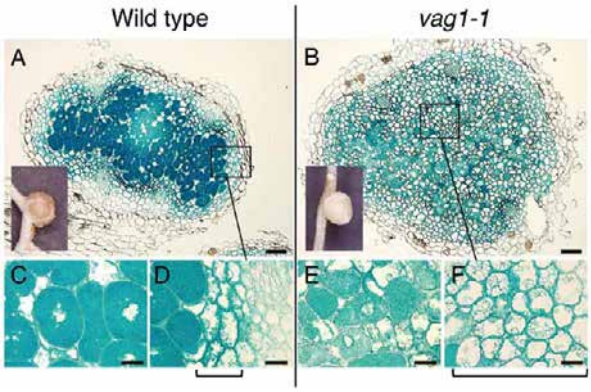

Figure 1. The effect of the *vag1* mutation on nodule structure. Sections through nodules of wild type (A, C, D) and *vag1-1* (B, E, F) at 21 days after inoculation with rhizobia that constitutively express the *LacZ* reporter gene. (C, E) Rhizobia-colonized infected cells located at the inner region of nodules. (D, F) In wild type, small rhizobia-infected (as yet uncolonized) cells (bracket) are located surrounding the region of rhizobia-colonized cells. In contrast, the inner region of *vag1-1* nodule comprises a large number of these small rhizobia-infected cells. Scale bars: 100 μm in A, B; 20 μm in C-F.

1-2 A presumptive post-translational modification enzyme, PLENTY controls nodulation and root growth in *L. japonicus*

Legumes can survive even in nitrogen-deficient environments depending on root nodules symbiosis with rhizobia; however, forming nodules consumes energy, requiring nodule number to be strictly controlled. The previous studies of hypernodulation mutants, *har1*, *klv*, and *tml*, have proposed long-distance control of nodulation (Figure 2), via systemic mobile signals, CLE-RS1/2 peptides. Recently, we found that, at least, the CLE-RS2 peptide is arabinosylated and this modification is essential for nodule inhibition (Okamoto et al., 2013). In *Arabidopsis*, the post-translational modifications of hormone-like small peptides and their critical roles in biological activities have been

gradually found. Further, one of the modification enzymes, hydroxyproline O-arabinosyltransferase (HPAT), is identified and its homolog in *L. japonicus* is thought to be a strong candidate for the modification enzyme of CLE-RS2.

We identified the HPAT homolog in *L. japonicus*, PLENTY, as a responsible factor for the already isolated *plenty* mutant. PLENTY is localized to the Golgi, suggesting a similarity of protein functions between *Lj*PLENTY and *At*HPAT. However, overexpression of *CLE-RS1/2* is still effective on nodule inhibition in *plenty*, suggesting that PLENTY itself does not mainly mediate the arabinosylation of CLE-RS1/2. In addition, *plenty har1* double mutant showed an additive nodulation. These suggest a novel HAR1-independent nodulation controlling pathway mediated by an unknown possible substrate of PLENTY which is a putative post-translational modification enzyme.

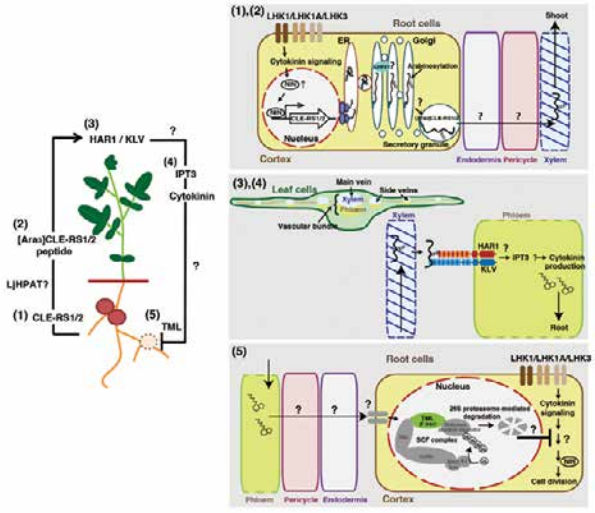

Figure 2. A model of the long-distance control of nodulation. (1) Nodulation signaling pathway downstream of cytokinin receptors activates *NIN* expression. The NIN transcription factor activates *CLE-RS1/2* expression through direct binding to their promoter. (2) It is likely that the CLE-RS1/2 peptides are posttranslationally modified with triarabinoside, a reaction mediated by an enzyme similar to HPAT in the Golgi apparatus. These modified CLE-RS peptides are transported to the xylem. (3) These peptides are transmitted from roots to shoots and directly bind to HAR1 in the phloem of leaf cells. (4) Downstream of the CLE-RS/HAR1 signaling pathway, activated IPT3 produces cytokinin, which is transported to roots through phloem tissue. (5) Shoot-derived cytokinin is directly or indirectly involved in proteasome-mediated degradation of an unidentified positive regulator of nodule organogenesis.

II. Arbuscular mycorrhiza symbiosis

Arbuscular mycorrhiza is a plant-fungus interaction that confers great advantage to growth and survival on the land. AM fungi enter into the host root and elongate the hyphae between the root cells. The intraradical hyphae form symbiotic structures called 'arbuscule' and 'vesicle' (Figure 3A). AM fungi supply phosphate to the host plant through the symbiotic structures and in return, they obtain photosynthetic products form the host. To obtain insights about molecular mechanisms of AM development, we are studying symbiotic signaling factors that regulate symbiotic gene expression and AM fungal infection.

We performed transcriptome analysis in wild-type and symbiotic mutants of *L. japonicus*. This analysis showed that plant hormone gibberellin (GA) biosynthesis and metabolism genes were induced during AM development. The GA biosynthesis gene expression was disturbed in the symbiotic mutants that showed abnormal AM fungal colonization in the host roots, indicating that *de novo* biosynthesis of GAs has some function in AM development. Functional analysis of GA in AM development revealed that GA has a negative effect on some AM-induced gene expressions, but also has a positive effect on other expressions (Figure 3B). *RAM1* and *RAM2* that function in AM entry processes are suppressed by GA signaling, on the other hand, GA signaling promotes or maintains *SbtM1* expression that is required for AM fungal colonization in the host root. This indicated that GA signaling interferes with the symbiotic signaling pathway, which decreases or enhances expression levels of AM-induced genes. In addition, treatment with GA or GA biosynthesis inhibitor disturbed the GA signaling and caused an inhibitory effect on AM hyphal entry into the host root or the hyphal branching in the root cortical cell layer. These studies revealed the host plant controls GA signaling level by induction of GA biosynthesis and metabolism genes during AM development and the GA signaling regulates AM fungal colonization in the host root.

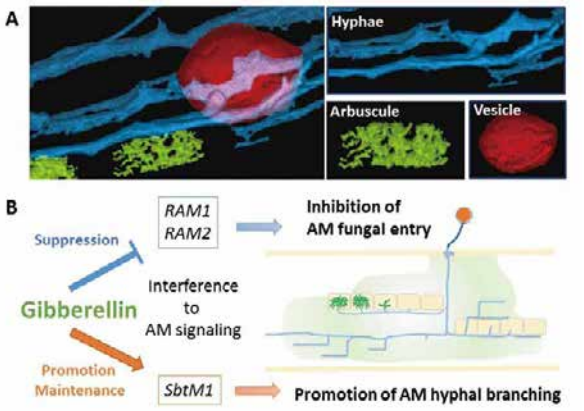

Figure 3. AM fungal colonization and interference of GA signaling with the symbiotic gene expression. (A) AM fungal structures in the host root. (B) GA signaling is enhanced by GA biosynthesis during AM development and differentially interferes with symbiotic signaling pathways. The interference affects and regulates the AM colonization in the host root.

III. Computational modeling for pattern formation of stomatal differentiation in *Arabidopsis* leaves

Stomata are epidermal structures that mediate gas exchange in plants, and are usually formed separately from each other (Figure 4A). In the initial step of stomatal development, stomatal lineage cells are self-organized from a homogeneous field, and thus this is a good example for two-dimensional pattern formation in living systems. Stomatal differentiation is promoted by transcription factors SPCH and SCRM, but in contrast is suppressed by diffusible peptide EPF2, which interacts with its membrane receptors to stimulate the degradation of SPCH/SCRM. In addition, this regulatory network involves feedback loops; SPCH•SCRM

heterodimer activates SCRM expression (positive feedback), and also stimulates EPF2 expression leading to suppression of SPCH/SCRM (negative feedback). This regulatory framework is similar to that of the activator–inhibitor system, a well-known model for pattern formation, and is predicted to be essential for stomatal patterning.

Thus, we constructed and examined a mathematical model, which is based on experimental results. Our model can explain many experimental observations, such as that stomatal lineage cells are formed separately in the wild type but are clustered in *tmm* mutant and *er erl1 erl2* mutants (Figure 4B). Furthermore, our model also explains the opposite response that exogenous application of EPF2 peptide completely eliminates stomatal differentiation in the wild type but does not affect the *tmm* mutant (Figure 5C). These results suggest that stomatal pattern formation is basically understood by the activator-inhibitor mechanism, that is, local activation and lateral inhibition.

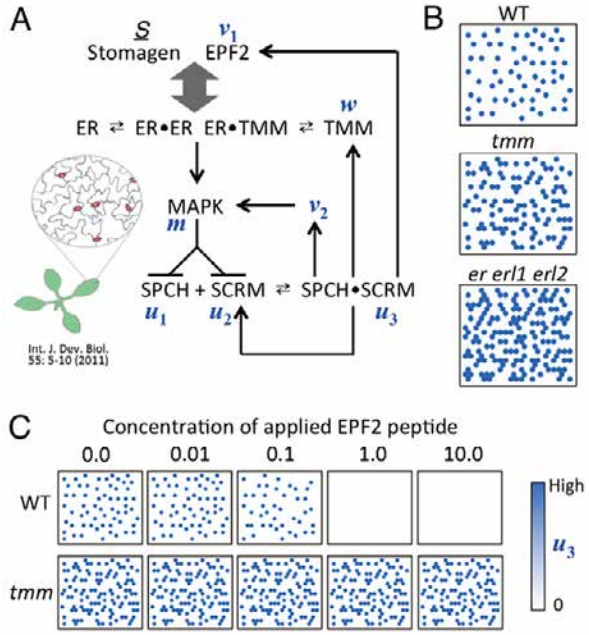

Figure 4. (A) Regulatory network for the differentiation of stomatal lineage cells. Our model can generate various stomatal patterns including the wild type, *tmm*, and *er erl1 erl2* (B), and also explains the opposite response to EPF2 peptide between the wild type and *tmm* (C).

Publication List

〔Original papers〕

- Chungopast, S., Hirakawa, H., Sato, S., Handa, Y., Saito, K., Kawaguchi, M., Tajima, S., and Nomura, M. (2014). Transcriptomic profiles of nodule senescence in *Lotus japonicus* and *Mesorhizobium loti* symbiosis. Plant Biotech. *31*, 345-349.
- Daum, G., Medzihradszky, A., Suzaki, T., and Lohmann J.U. (2014). A mechanistic framework for noncell autonomous stem cell induction in Arabidopsis. Proc. Natl. Acad. Sci. USA *111*, 14619-14624.
- Fujita, H., Aoki, S., and Kawaguchi, M. (2014). Evolutionary dynamics of nitrogen fixation in the legume-rhizobia symbiosis. PLoS One *9*, e93670.
- Kikuchi, Y., Hijikata, N., Yokoyama, K., Ohtomo, R., Handa, Y., Kawaguchi, M., Saito, K., and Ezawa, T. (2014). Polyphosphate accumulation is driven by transcriptome alterations that lead to near-synchronous and near-equivalent uptake of inorganic cations in an arbuscular mycorrhizal fungus. New Phytol. *204*, 638-649.
- Kojima,T., Saito, K., Oba, H., Yoshida, Y., Terasawa, J., Umehara, Y., Suganuma, N., Kawaguchi, M., and Ohtomo, R. (2014). Isolation and phenotypic characterization of *Lotus japonicus* mutants specifically defective in arbuscular mycorrhizal formation. Plant Cell Physiol. *55*, 928-941
- Nagae, M., Takeda, N., and Kawaguchi, M. (2014). Common symbiosis genes CERBERUS and NSP1 provide additional insight into the establishment of arbuscular mycorrhizal and root nodule symbioses. Plant Signal. Behav. *9*, e28544.
- Sasaki, T., Suzaki, T., Soyano, T., Kojima, M., Sakakibara, H., and Kawaguchi, M. (2014). Shoot-derived cytokinins systemically regulate root nodulation. Nature Commun. *5*, 4983.
- Soyano, T., Hirakawa, H., Sato, S., Hayashi, M., and Kawaguchi, M. (2014). NODULE INCEPTION creates a long-distance negative feedback loop involved in homeostatic regulation of nodule organ production. Proc. Natl. Acad. Sci. USA *111*, 14607-14612.
- Suzaki, T., Ito, M., Yoro, E., Sato, S., Hirakawa, H. Takeda, N., and Kawaguchi, M. (2014). Endoreduplication- mediated initiation of symbiotic organ development in *Lotus japonicus*. Development *141*, 2441-2445.
- Wakabayashi, T., Oh, H., Kawaguchi, M., Harada, K., Sato, S., Ikeda, H., and Setoguchi, H. (2014). Polymorphisms of E1 and GIGANTEA in wild populations of *Lotus japonicus*. J. Plant Res. *127*, 651-660.
- Yoro, E., Suzaki, T., Toyokura, K., Miyazawa, H., Fukaki, H., and Kawaguchi, M. (2014). A positive regulator of nodule organogenesis, NODULE INCEPTION, acts as a negative regulator of rhizobial infection in *Lotus japonicus*. Plant Physiol. *165*, 747-758.

〔Original paper (E-publication ahead of print)〕

- Takeda, N., Handa, Y., Tsuzuki, S., Kojima, M., Sakakibara, H., and Kawaguchi, M. Gibberellins interfere with symbiosis signaling and gene expression, and alter colonization by arbuscular mycorrhizal fungi in *Lotus japonicus*. Plant Physiol. 2014 Dec 19.

〔Review articles〕

- Kawaguchi, M. (2014). Genes for autoregulaton of nodulation. In The *Lotus japonicus* Genome, S. Tabata and J. Stougaard, eds. (Springer), pp. 73-78.
- Kawaguchi, M., and Sandal, N. (2014). Wild accessions and mutant resources. In The *Lotus japonicus* Genome, S. Tabata and J. Stougaard, eds. (Springer), pp. 211-220.
- Soyano, T., and Kawaguchi, M. (2014). Systemic regulation of root nodule formation. Review. In Advances in Biology and Ecology of Nitrogen Fixation, T. Ohyama, ed. (InTech), pp. 89-109.
- Suzaki, T., and Kawaguchi, M. (2014). Root nodulation: a developmental program involving cell fate conversion triggered by symbiotic bacterial infection. Curr. Opin. Plant Biol. *21*, 16-22.