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
SOKENDAI Graduate Student:	TAKAHARA, Masahiro
	SASAKI, Takema
	YORO, Emiko
	TSUZUKI, Shusaku
	NISHIDA, Hanna
	FUKUHARA, Mai
Technical Assistant:	SUZAKI, Momoyo
	ICHIKAWA, Michiko
	OGAWA, Yuko
Secretary:	SANJO, Kazuko

Rhizobium–legume symbiosis is one of the most successful mutually beneficial interactions on earth. In this symbiosis, soil bacteria collectively called rhizobia supply the host legumes with ammonia produced through bacterial nitrogen fixation, in contrast to host plants providing the rhizobia with their photosynthetic products. To accomplish this biotic interaction, leguminous plants develop root nodules in which to confine rhizobia. This novel lateral organ differentiation (i.e., nodulation) is triggered by Nod factors secreted by rhizobia. 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 Endoreduplication is essential for initiation of root nodule organogenesis and rhizobial infection

During early nodule development, rhizobia-derived Nod factors induce the dedifferentiation of root cortical cells. The activated cortical cells then proliferate to form the primordium of the symbiotic nitrogen-fixing root nodule. Recent identification and functional analyses of the putative cytokinin receptors *Lotus japonicus* LOTUS HISTIDINE KINASE 1 (LHK1) and *Medicago truncatula* CYTOKININ RESPONSE 1, have led to a greater understanding of how the activation of cytokinin signaling is crucial to the initiation of nodule organogenesis. In particular, it has been shown that in the *L. japonicus spontaneous nodule formation 2* (*snf2*) mutant, a gain-of-function form of LHK1 confers the constitutive activation of cytokinin signaling, resulting in the formation of spontaneous nodule-like structures in the absence of rhizobia.

We identified a novel nodulation-deficient mutant named *vagrant infection thread 1* (*vag1*) after suppressor mutant screening of *snf2* mutants in *L. japonicus*. The *VAG1* gene encodes a protein that is putatively orthologous to *Arabidopsis* ROOT HAIRLESS 1/HYPOCOTYL 7, a component of the plant DNA topoisomerase VI that is involved in the control of endoreduplication. Nodule phenotype of the *vag1* mutant shows that VAG1 is required for the ploidy-dependent cell growth of rhizobial infected cells. Furthermore, the *VAG1* gene mediates a local emergence of endoreduplicated cortical cells during early nodule development, which is essential for the initiation of cortical cell proliferation leading to nodule primordium formation (Figure 1). In addition, a misguided infection thread formation in the *vag1* mutant indicates that the endoreduplication of cortical cells is required for the guidance of symbiotic bacteria to host meristematic cells. Overall, our results provide new insights into the role of endoreduplication in plant development and plant–microbe symbiosis.
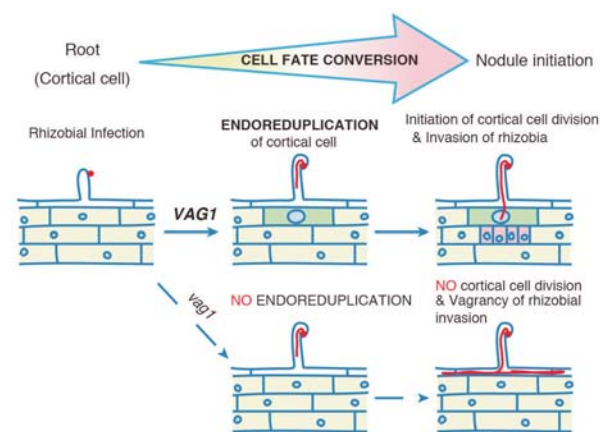

Figure 1. Model for the conversion of cortical cell fate toward root nodule initiation.

1-2 Analysis of a non-nodulating *L. japonicus* mutant *daphne* reveals a negative regulation of rhizobial infection

The main events for creation of nodules are divided into rhizobial infection and nodule organogenesis. For proper establishment of symbiosis, it is essential that the two phenomena proceed synchronously in different root tissues, the epidermis and cortex. Owing to their complexity most of those have defects in both infection and organogenesis pathways, the molecular interaction between these symbiotic signalings has still remained elusive.

Our study began with the isolation of a unique mutant from C[6+] beam mutagenized seeds of *L. japonicus*, named *daphne*. *daphne* showed a novel symbiotic phenotype, non-nodulation and increased number of rhizobial infections. This mutant allowed us to focus on the interrelationship of the two different symbiotic pathways. Characterization of the locus responsible for these phenotypes revealed a chromosomal translocation upstream of *NIN* gene in the *daphne* genome. Genetic analysis using a known *nin* mutant revealed that *daphne* is a novel *nin* mutant allele. Although the *daphne*

mutant showed reduced expression of *NIN* after rhizobial infection, the spatial expression pattern of *NIN* in epidermal cells was broader than that in the wild type. Over-expression of *NIN* strongly suppressed hyper-infection in *daphne*, and *daphne* phenotypes were partially rescued by cortex specific expression of *NIN*. These observations suggested that *daphne* mutation enhanced the role of NIN in the infection pathway due to a specific loss of the role of NIN in nodule organogenesis. Based on the results, we provide evidence that a bifunctional transcription factor NIN negatively regulates infection but positively regulates nodule organogenesis during the course of the symbiosis.
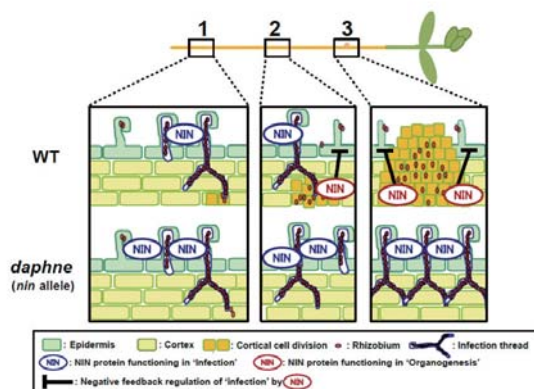

Figure 2. A model of inhibition of rhizobial infection processes mediated by NIN. In the wild type, NIN functions in both rhizobial infection (blue) and organogenesis (red). In the earlier stage, NIN (blue) is predominant but in the later stage, the proportion of NIN (red) has increased with nodule development. It is assumed that a potential negative correlation between the organogenesis and infection pathway (black bars) regulates the amount of infection and restricts the region of rhizobial susceptibility.

1-3 Nodulation by global control

In the legume-rhizobia symbiosis, the nodule number is tightly restricted by the host through a systemic suppression called Autoregulation Of Nodulation (AON). AON provides a long-distance control of nodulation via the root-to-shoot-to-root negative feedback. In *L. japonicus*, autoregulation is mediated by CLE-RS genes that are specifically expressed in the root, and the receptor kinase HAR1 that functions in the shoot. However, the mature functional structures of CLE-RS gene products and the molecular nature of CLE-RS/HAR1 signaling governed by these spatially distant components remain elusive. We showed that CLE-RS2 is a post-translationally arabinosylated glycopeptide derived from the CLE domain. Chemically synthesized CLE-RS glycopeptides cause significant suppression of nodulation and directly bind to HAR1 in an arabinose-chain and sequence-dependent manner. In addition, CLE-RS2 glycopeptide specifically produced in the root is found in xylem sap collected from the shoot. We propose that CLE-RS glycopeptides are the long sought mobile signals responsible for the initial step of autoregulation of nodulation.

II. Arbuscular mycorrhiza symbiosis

In order to obtain insights about molecular mechanisms in AM symbiosis, we performed transcriptome analysis in *L. japonicus* by RNAseq using next generation sequencing technology. This analysis revealed that phytohormone gibberellin (GA) biosynthesis genes were up-regulated during AM symbiosis (Figure 3A,B). GA has been reported to inhibit AM fungal infection by genetic and pharmacological analyses. However, our transcriptome analysis and phytohormone quantification analysis revealed GA accumulation in the roots infected with AM fungi, suggesting that *de novo* GA synthesis plays a role in AM symbiosis development.

We found pleiotropic effects of GAs on the AM fungal infection. In particular, the morphology of AM fungal colonization was altered by the status of GA signaling in the host root. Exogenous GA treatment inhibited hyphal entry into the host root and arbuscule formation, however, it enhanced hyphal branching in the cortex (Figure 3C,D). On the other hand, inhibition of GA biosynthesis inhibited hyphal branching, resulting in low hyphal density in the host root (Figure 3E). Alterations in GA conditions also affected expression of the AM symbiosis-induced genes that are required for AM fungal infection and hyphal elongation in the host root. These studies demonstrated that GA signaling interacts with symbiotic responses and regulates AM colonization of the host root.
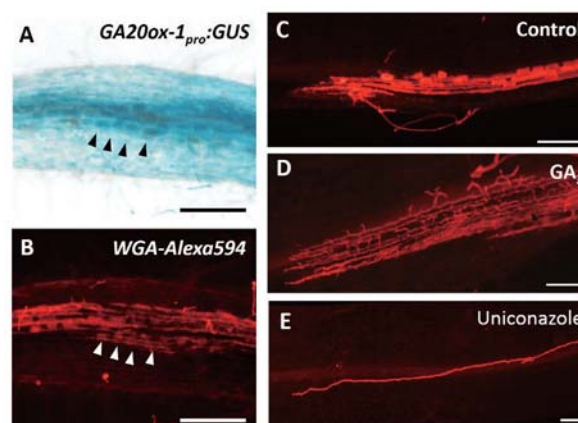

Figure 3. A GA biosynthesis gene *GA20 oxidase-1* was induced around AM inner hyphae. (A) Transgenic roots carrying the *GA20ox-1_{pro}:GUS* were stained with X-gluc. (B) The inner fungal structures were stained with WGA-Alexa594 at 3 wai with *R. irregularis*. Arrowheads indicate a symbiotic structure 'arbuscule'. (C-E) GA treatment increased hyphal branching (D) compared with mock treated roots (C). On the other hand, GA biosynthesis inhibitor 'uniconazole' treatment inhibited hyphal branching in the host roots. Scale bars = 200 μm.

III. Evolutionary dynamics of nitrogen fixation in the legume-rhizobia symbiosis

The stabilization of host-symbiont mutualism against the emergence of parasitic individuals is pivotal to the evolution of cooperation. One of the most famous symbioses occurs between legumes and their colonizing rhizobia, in which rhizobia extract nutrients (or benefits) from legume plants while supplying them with nitrogen resources (or costs) produced by nitrogen fixation. Natural environments, however, are widely populated by ineffective rhizobia that extract benefits without paying costs and thus proliferate

more efficiently than nitrogen-fixing cooperators. How and why this mutualism becomes stabilized and evolutionarily persists has been extensively discussed. To better understand the evolutionary dynamics of this symbiosis system, we constructed a simple model based on the continuous snowdrift game with multiple interacting players (Figure 4A). We investigated the model using adaptive dynamics and numerical simulations. We found that symbiotic evolution depends on the relative strength between the benefit and cost, and that cheaters can emerge when these quantities are balanced (Figure 4B). This result suggests that the symbiotic relationship is robust to the emergence of cheaters, and may explain the prevalence of cheating rhizobia in nature. Our model also predicts that, although cheaters possess a short-term advantage over cooperators, their evolutionary persistence is not guaranteed. These findings provide a theoretical basis of the evolutionary dynamics of legume-rhizobia symbioses, which is extendable to other single-host, multiple-colonizer systems.
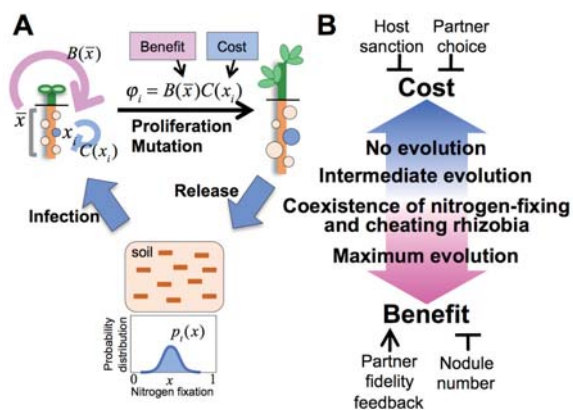

Figure 4. Schematic representation of the model. (A) The probability distribution of rhizobia strategy (i.e. nitrogen fixation) is iteratively changed in the cycle of infection, proliferation/mutation, and release steps. (B) The evolution of the legume-rhizobia symbiosis depends on the cost-benefit balance.

Publication List

〔Original papers〕

- Fujita, H., and Kawaguchi, M. 2013 . Pattern formation by two-layer Turing system with complementary synthesis. J. Theor. Biol. 322, 33-45.
- Miyata, K., Kawaguchi, M., and Nakagawa, T. (2013). Two distinct EIN2 genes cooperatively regulate ethylene signaling in *Lotus japonicus*. Plant Cell Physiol. *54*, 1469-1477.
- Murakami Y., Yokoyama H., Fukui R., and Kawaguchi, M. (2013). Downregulation of NSP2 expression in developmentally young regions of *Lotus japonicus* roots in response to rhizobial Inoculation. Plant Cell Physiol. *54*, 518-527.
- Okamoto, S., Shinohara, H., Mori, T., Matsubayashi, Y., and Kawaguchi, M. (2013). Root-derived CLE glycopeptides control nodulation by direct binding to HAR1 receptor kinase. Nature Commun. *4*, 2191.
- Soyano, T., Kouchi, H., Hirota, A., and Hayashi, M. (2013). NODULE INCEPTION directly targets NF-Y subunit genes to regulate essential processes of root nodule development in *Lotus japonicus*. PLoS Genetics *9*, e1003352.

- Suzaki, T., Ito, M., and Kawaguchi, M. (2013). Induction of localized auxin response during spontaneous nodule development in *Lotus japonicus*. Plant Signal. Behav. *8*, e23359.
- Suzaki, T., and Kawaguchi, M. (2013). Grafting analysis indicates that malfunction of *TRICOT* in the root causes a nodulation-deficient phenotype in Lotus japonicus. Plant Signal. Behav. 8, e23497.
- Suzaki, T., Kim, C.S., Takeda, N., Szczyglowski, K., and Kawaguchi, M. (2013). TRICOT encodes an AMP1-related carboxypeptidase that regulates root nodule development and shoot apical meristem maintenance in Lotus japonicus. Development 140, 353-361.
- Takahara, M., Magori, S., Soyano, T., Okamoto, S., Yoshida C., Yano, K., Sato, S., Tabata, S., Yamaguchi, K., Shigenobu, S., Takeda, N., Suzaki, T., and Kawaguchi, M. (2013). TOO MUCH LOVE, a novel kelch repeat-containing F-box protein, functions in the long-distance regulation of the legume-*Rhizobium* symmbiosis. Plant Cell Physiol. *54*, 433-447.
- Takeda, N., Tsuzuki, S., Suzaki, T., Parniske, M., and Kawaguchi, M. (2013). CERBERUS and NSP1 of *Lotus japonicus* are common symbiosis genes that modulate arbuscular mycorrhiza development. Plant Cell Physiol. *54*, 1711-1723.
- Tameshige, T., Fujita, H., Watanabe, K., Toyokura, K., Kondo, M., Tatematsu, K., Matsumoto, N., Tsugeki, R., Kawaguchi, M., Nishimura, M., and Okada, K. (2013). Pattern dynamics in adaxial-abaxial specific gene expression are modulated by a plastid retrograde signal during Arabidopsis leaf development. PLoS Genetics *9*, e1003655.
- Tisserant, E., Malbreil, M., Kuo, A., Kohler, A., Symeonidi, A., Balestrini, R., Charron, P., Duensing, N., Freidit Frey, N., Gianinazzi-Pearson, V., Gilbert, B., Handa, Y., Herr, J., Hijri, M., Koul, R., Kawaguchi, M., Krajinski, F., Lammers, P., Masclaux, F.G., Murat, C., Morin, E., Ndikumana, S., Pagni, M., Petitpierre, D., Requena, N., Rosikiewicz, P., Riley, R., Saito, K., San Clemente, H., Shapiro, H., van Tuinen, D., Bécard, G., Bonfante, P., Paszkowski, U., Shachar-Hill, Y., Tuskan, G.A., Young, J.P.W., Sanders, I.R., Henrissat, B., Rensing, S.A., Grigoriev, I.V., Corradi, N., Roux, C., and Martin, F. (2013). The genome of an arbuscular mycorrhizal fungus provides insights into the oldest plant symbiosis. Proc. Natl. Acad. Sci. USA *110*, 20117-20122.

〔Review article〕

- Suzaki, T., Ito, M., and Kawaguchi, M. (2013). Genetic basis of cytokinin and auxin functions during root nodule development. Front. Plant Sci. *4*, 42.