DIVISION OF SYMBIOTIC SYSTEMS

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More than 80% of land plant families have symbiotic relationships with arbuscular mycorrhizal (AM) fungi. AM fungi absorb minerals, mainly phosphates, from the soil and provide them to the plants. The origin of AM symbiosis is thought to have been in the early Devonian period. On the other hand, the root nodule symbiosis that occurs between legumes and rhizobial bacteria, unlike AM symbiosis, involves host-specific recognition and postembryonic development of a nitrogen-fixing organ. Root nodule symbiosis is thought to have evolved about 60 million years ago. Despite marked differences between the fungal and bacterial symbioses, common genes required for both interactions have been identified using model legumes. Our laboratory focuses on the early stages of the interaction between these microorganisms and *Lotus japonicus* in order to reveal the molecular mechanism and the origin of these symbiotic systems.

I. Long-distance control of nodulation

Legume plants develop root nodules to recruit nitrogen-fixing bacteria called rhizobia. This symbiotic relationship allows the host plants to grow even in nitrogen poor environments. Since nodule development is an energetically expensive process, the number of nodules must be tightly controlled by the host plants. For this purpose, legume plants utilize a long-distance signaling known as autoregulation of nodulation (AON). AON signaling in legumes has been extensively studied over decades but the underlying molecular mechanism has remained largely unclear. We are trying to unveil the mechanism for AON at the molecular level.

1-1 Identification of *KLAVIER* that mediates long-distance negative regulation of nodulation and non-symbiotic shoot development

The previously isolated *klavier* (*klv*) mutant defective in long-distance negative regulation of nodulation exhibits a hypernodulating phenotype. We identified *KLAVIER* (*KLV*) as a causative gene of the *klv* hypernodulating mutant. *KLV* encoded a novel leucine-rich repeat receptor-like kinase (LRR-RLK) and mediated the systemic negative regulation of nodulation in *Lotus japonicus*. In leaves, *KLV* was predominantly expressed in the vascular tissues similar to another LRR-RLK gene, *HAR1*, which also regulates nodule number. A double mutant exhibited no additive effect on hypernodulation. This result indicated that *KLV* and *HAR1* function in the same genetic pathway that governs the negative regulation of nodulation. LjCLE-RS1 and LjCLE-RS2 represent potential root-derived mobile signals for the HAR1-mediated systemic regulation of nodulation. Overexpression of *LjCLE-RS1* or *LjCLE-RS2* did not suppress the hypernodulation phenotype of *klv* mutants, indicating that *KLV* is required and acts downstream of *LjCLE-RS1* and *LjCLE-RS2*.

In addition to the role of *KLV* in symbiosis, complementation tests and expression analysis indicated that *KLV* plays multiple roles in shoot development (Figure 1), such as maintenance of shoot apical meristem, vascular continuity, shoot growth, and promotion of flowering.

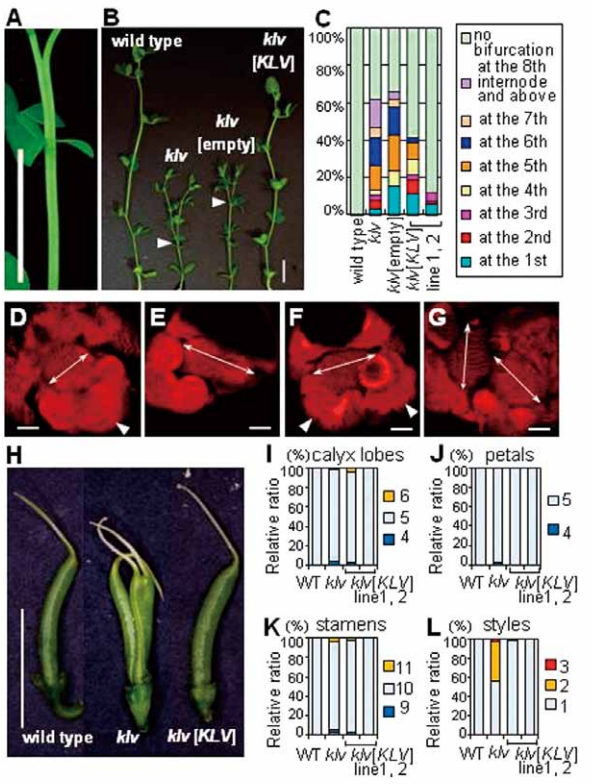

Figure 1. Morphological defects in the shoot apical meristem of *klv* and complementation of the *klv* bifurcation. (A) The bifurcated stem of a *klv* plant. (B) Shoot structures of uninoculated plants. *klv* [*KLV*] indicates a transgenic *klv* mutant introduced with the *KLV* gene, and *klv* [empty] indicates transgenic *klv* mutant with an empty vector. Each arrowhead indicates bifurcation of the stem. (C) Percentages of plants that show bifurcation. (D-G) Structures of SAMs in wild-type (D) and *klv* (E-G) plants at 4 DAG. Arrows indicate the SAM regions, and arrowheads indicate leaf primordia. (H) Close-up pictures of pistils. (I-L) Number of floral oragans.

Physical interaction analyses using transient expression in *Nicotiana benthamiana* revealed that KLV has the ability to interact with HAR1 and KLV itself. Together, these results suggest that the potential KLV-HAR1 receptor complex regulates symbiotic nodule development and that KLV is also a key component in other signal transduction pathways that mediate non-symbiotic shoot development.

1-2 *plenty*, a novel hypernodulation mutant

plenty is a novel hypernodulating mutant isolated by C^{6+} beam mutagenesis of the seeds of *L. japonicus* Miyakojima MG-20. A comparison of nodule numbers in *plenty* and wild type plants 3 weeks after inoculation with *Mesorhizobium loti* showed that the former had between 3 to 5 times more nodules. The relative range of the nodulation zone, defined as the length of the nodule-forming region to the length of the primary root, was approximately 4 times larger in the mutant than in the wild type. However, we never observed in *plenty* that nodules densely covered almost the entire root, as often seen in other hypernodulation mutants such as *har1*, *klv* or *tml*. Instead, *plenty* plants formed nodules on their lateral roots as well, while wild type plants suppressed the formation of nodules by autoregulation of nodulation (AON). Thus, it was suggested that the *plenty* mutant was defective in AON, though the magnitude of defects was not as great as in *har1*, *klv* or *tml*.

The *har1*, *klv* and *tml* mutant were reported that they formed a lot of small nodules. However, the size of the nodules was indistinguishable between wild type and *plenty* plants, even though the number of nodules increased in the *plenty* mutant. In contrast to the significant difference between wild type and *har1* plants, the *plenty* mutant did not differ from the wild type in the diameter of nodules and the nodule size distribution. Close-up pictures of nodules clearly indicate the size difference between the *plenty* mutant and other hypernodulation mutants (Fig. 2). This hypernodulation pattern of the *plenty* mutant was truly novel.

To locate the potential site of action of *PLENTY*, we conducted reciprocal grafting experiments with the wild type and *plenty* mutants. Grafting a *plenty* shoot onto a wild-type root led to nodulation in the wild-type; in contrast, grafting a wild-type shoot onto a *plenty* root resulted in an increased number of nodules, which was indistinguishable from that of *plenty* self-grafts. This root-determined hypernodulation of *plenty* indicates that unlike *HAR1* and *KLV*, *PLENTY* functions in the roots rather than in the shoots.

We further analyzed whether a root factor *PLENTY* and a shoot factor *HAR1* genetically interact with each other despite the different sites of action. We carried out reciprocal grafting using *plenty* and *har1-7* mutants. Shoot-regulated *har1-7* hypernodulation was obviously enhanced by grafting a *har1-7* shoot onto a *plenty* root, suggesting that *PLENTY* and *HAR1* might constitute the different AON signaling. This result was supported by *plenty*;*har1* double mutants that formed an additive number of nodules.

Based on these findings, PLENTY is presumed to be a root factor associated in an unknown AON pathway, whether it is local or shoot-mediated.

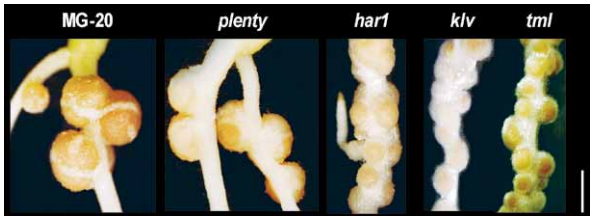

Figure 2. Close-up images of nodules. *L. japonicus* MG-20 (the wild type) and the *plenty* mutant are indistinguishable in the size of nodules. Other hypernodulation mutants form smaller nodules than MG-20 and the *plenty* mutant. Scale: 1mm.

II. Arbuscular mycorrhiza symbiosis

Mutualistic plant-fungal interaction; arbuscular mycorrhiza has several similar systems to root nodule symbiosis in host-symbiont recognition, infection process and nutrient material exchanges. Root nodule symbiosis is thought to evolve by sharing AM factors, suggesting that the AM system contains a fundamental mechanism that also regulates root nodule symbiosis. In recent studies, AM signaling molecules that conduct host-symbiont recognition were isolated from both host plant and AM fungi. These results are expected to accelerate molecular analysis of the AM signaling mechanism.

In the infection process of AM fungi, the host and the symbiont do not show obvious morphological changes, which makes it difficult to evaluate the development process of AM. In order to solve the problem, we established a molecular tool to facilitate the observation of AM fungal infection. We focused a protease gene *SbtM1* and a phosphate transporter gene *PT4*, that were highly induced during AM development and took advantage of them to established a molecular marker and visualize the infection process. These promoters and/or the open reading frames were fused with green fluorescence protein (GFP) gene or beta-glucuronidase (GUS) gene and these fusion constructs were introduced into *L. japonicus*. The transgenic plants would be good tools to observed infection process of AM fungi. We are screening AM mutants by genetic method to find novel AM signaling factors. We can analyze detailed phenotype of the symbiosis mutants and expect to find novel phenomena during AM development using these tools.

We are also conducting a screening of AM signaling factor with a different approach. Promoter analysis of *SbtM1* gene revealed AM response *cis* region in the promoter sequence. Using the *cis* sequence, we screened an AM response *trans* factor that controls transcription of AM induced genes by the yeast one hybrid system. Several candidate genes were isolated in this analysis. Reverse genetic approaches like TILLING and RNAi, and the *SbtM1* fusion constructs were used for selection of an AM response *trans* factor among the candidate genes.

III. Mathematical model of shoot apical meristem

The shoot apical meristem (SAM) of plants contains stem cells that have the ability to renew themselves and differentiate all aerial tissues such as stems and leaves. SAM

consists of a central zone (CZ) and its surrounding area named the peripheral zone (PZ) that is induced by an unknown signal from the CZ. Maintenance of the SAM essentially involves the interaction between WUS and CLV, in which WUS activates itself and CLV, and CLV inhibits WUS expression. Plants defective in *CLV* show enlarged SAM and stems with fasciation or dichotomous branching. In contrast, *wus* mutants generate a flattened SAM instead of the dome-shaped structure of the wild type, but produce many ectopic shoots across the SAM resulting in bushy plants.

Since it is not clarified how the SAM controls its proliferation and patterning, we constructed and analyzed a mathematical model that includes WUS-CLV feedback dynamics, spatial restriction of these dynamics, and area expansion by cell division. In numerical simulations, we can generate a phenotype similar to that of the wild-type plant, in which the SAM maintains a constant cell population because of the balance between cell proliferation by cell division and departure from the SAM to the outer region (Figure 3). In addition, numerical conditions corresponding to *CLV* defects result in a fasciated or dichotomously branched SAM. Furthermore, the defect in *WUS* causes proliferation of weak CZ spots. These numerical results are consistent with experimental observations in plants. Therefore, it is probable that this model captures the essence of SAM proliferation and patterning.

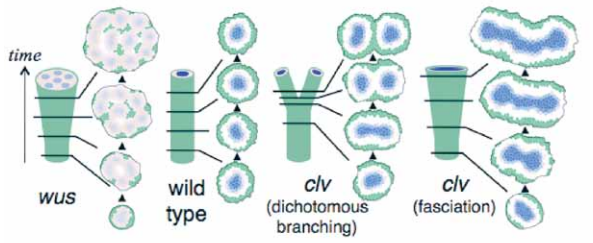

Figure 3. Numerical simulations under conditions of *wus* mutant, wild type, and *clv* mutants.

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- Ruzicka, K., Strader, L. C., Bailly, A., Yang, H., Blakeslee, J., Langowski, L., Nejedlá, E., Fujita, H., Itoh, H., Syono, K., Hejátko, J., Gray, W.M., Martinoia, E., Geisler, M., Bartel, B., Murphy, A.S., and Friml, J. (2010). Arabidopsis PIS1 encodes the ABCG37 transporter of auxinic compounds including the auxin precursor indole-3-butyric acid. Proc. Natl. Acad. Sci. USA *107*, 10749-10753.

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〔Review articles〕

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- Kawaguchi, M., and Minamisawa, K. (2010). Plant-microbe communications for symbiosis. Plant Cell Physiol. *51*, 1377-1380.
- Magori, S., and Kawaguchi, M. (2010). Analysis of two potential long-distance signaling molecules, *LjCLE-RS1/2* and jasmonic acid, in a hypernodulating mutant *too much love*. Plant Signal Behav. *5*, 403-405.
- Magori, S., Tanaka, A., and Kawaguchi, M. (2010). Physically-induced mutation: ion beam mutagenesis. In The Handbook of Plant Mutation Screening: Mining of Natural and Induced Alleles, (Meksem, K., and Kahl, G., eds.), Wiley-Blackwell-VCH. 3-16.