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学 位 論 文 題 目	Molecular genetic studies on regulatory mechanisms for infection thread and root nodule formation in *Lotus japonicus*
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論文内容の要旨

Summary of thesis contents

In the process of establishing legume–rhizobium symbiosis, two specialized symbiotic structures, infection thread (IT) and root nodule, are formed. In this study, I analyzed two mutants of *Lotus japonicus*, *daphne* and *plenty*, in order to study regulatory mechanisms of the number of ITs and nodules, respectively.

For successful symbiosis, it is essential that rhizobial infection in epidermis and organogenesis of nodules in root cortex proceed mostly at the same time. Although several symbiotic genes have been identified by genetic screening of nonsymbiotic mutants, most of the mutants harbor defects in both the infection and organogenesis pathways, leading to experimental difficulty in investigating the molecular genetic relationships between the two pathways in different tissues separately. To focus only on the infection pathway, I analyzed *daphne* mutant, which shows a non-nodulation phenotype but a dramatically increased number of ITs. Characterization of the *daphne* locus revealed a chromosomal translocation upstream of *NODULE INCEPTION (NIN)* gene, already identified as a gene for a key transcription factor required for both the infection and organogenesis. Genetic analysis using a known *nin* mutant revealed that *daphne* was a novel *nin* mutant allele. In *daphne*, the gene expression level of *NIN* in whole roots was decreased. In contrast, an epidermal expression pattern of *NIN* was broader in *daphne* than that in the wild type. These indicate that gene expression patterns of *NIN* in epidermis and cortex are disorganized due to the chromosomal translocation in *daphne*. In addition, overexpression of *NIN* strongly suppressed hyper-formation of ITs, and *NIN* driven by cortex-specific enhancer rescued the non-nodulation phenotype of *daphne*, suggesting that epidermal infection was enhanced by a malfunction of NIN functioning in the cortex. Taken together, I proposed a NIN-mediated IT inhibition, which is a new negative feedback regulation from cortex to epidermis during nodule development. The hyper-formation of ITs in *daphne* enabled identification of this new inhibitory pathway.

I further compared newly identified NIN-mediated IT inhibition pathways and a previously well-characterized mechanism for regulating nodule numbers called a long-distance control of nodulation. The nodulation controlling mechanism comprises CLE-root signal 1/2 (CLE-RS1/2) and its receptors, *HYPERNODULATION AND ABERRANT ROOT FORMATION1* (*HAR1*) and *KLAVIER* (*KLV*). CLE-RS1/2 peptides expressed in roots are transported to shoots as a long-distance signal, and received by these receptors in shoots. Overexpression of *CLE-RS1/2* peptide genes suppressed hyper-formation of ITs in *daphne* in a HAR1/KLV-dependent manner, indicating that the components of the long-distance control of nodulation also function in inhibiting ITs. However, even in the presence of *har1*, overexpression of *NIN* locally suppressed

hyper-formation of ITs in *daphne*. This result indicates that the NIN-mediated IT inhibition is established not only by the long-distance control but also by an unknown local pathway. Altogether, the NIN-mediated IT inhibition includes both systemic and local pathways for inhibiting ITs, and the systemic pathway is the same as the long-distance control of nodulation.

Secondly, I identified *PLENTY* gene responsible for a hypernodulation *plenty* mutant, as a new piece of regulation of nodule numbers. Phylogenetic analysis revealed that *PLENTY* shares approximately 70% amino acid sequence similarity with genes encoding Arabidopsis post-translational modification enzyme, hydroxyproline O-arabinosyltransferase (HPAT). PLENTY is localized to the Golgi as Arabidopsis HPATs are. Further, it is known that at least CLE-RS2 peptide, which is a systemic root-derived signal in the long-distance control of nodulation, is arabinosylated and the modification is essential for its inhibitory effect of nodulation. Therefore, I investigated whether PLENTY mediates the modification of CLE-RS1/2. Unlike *har1* and *klv* mutants, overexpression of *CLE-RS1/2* strongly suppressed the hypernodulation phenotype of *plenty*, suggesting that PLENTY does not mainly mediate the modification. In addition, the *plenty har1* double mutant showed additive nodulation, suggesting that PLENTY controls nodule numbers independently from the HAR1-mediated long-distance control. Thus, it is possible that a putative post-translational modification enzyme PLENTY mediates the modification of unknown signals, but not CLE-RS1/2, for inhibiting nodulation.

In this study, I identified a new negative feedback loop that controls the amount of rhizobial infection depending on nodule development, and a new component of nodulation control which functions independently from the known HAR1-mediated pathway. These accelerate further study on the regulatory mechanisms for maintaining the symbiotic balance between legumes and rhizobia.