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Minireview

Molecules and Cells

Long-Distance Control of Nodulation: Molecules and Models

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Legume plants develop root nodules to recruit nitrogen-fixing bacteria called rhizobia. This symbiotic relationship allows the host plants to grow even under nitrogen limiting environment. Since nodule development is an energetically expensive process, the number of nodules should 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 had been largely unclear until recently. With the advent of the model legumes, *L. japonicus* and *M. truncatula*, we have been seeing a great progress including isolation of the AON-associated receptor kinase. Here, we summarize recent studies on AON and discuss an updated view of the long-distance control of nodulation.

INTRODUCTION

Legumes develop root nodules to establish a symbiotic interaction with soil bacteria called rhizobia. In nodules, rhizobia provide the host plants with ammonia, which is produced through bacterial nitrogen fixation. In return, legumes supply the bacteria with photosynthetic products. It appears that leguminous plants have evolved a complex signaling pathway to regulate nodule formation (i.e. nodulation). The signal transduction for nodulation in plants is triggered by lipochito-oligosaccharide molecules called Nod factor (NF). NF is synthesized and secreted by rhizobia in response to plant-derived flavonoids (D'Haeze and Holsters, 2002). Recent studies using two model legume species, *Lotus japonicus* and *Medicago truncatula*, have elucidated multiple molecules essential for the NF signaling cascade. Such signaling components include the putative NF receptors (NFR1/5; Madsen et al., 2003; Radutoiu et al., 2003), the Ca^{2+}/calmodulin-dependent protein kinase (CCaMK; Lévy et al., 2004; Mitra et al., 2004; Tirichine et al., 2006a), the primary transcriptional regulators (NSP1/2; Heckmann et al., 2006; Kaló et al., 2005; Murakami et al., 2006; Smit et al., 2005), etc. Activation of the NF pathway leads to enhanced cell division in root cortex as well as bacterial entry from root hairs into nodule primordia. These two morphological changes are coordinately regulated, resulting in successful nodule development (for recent review, see Geurts et al., 2005; Oldroyd and Downie, 2008; Stacey et al., 2006).

Although this nodule formation is beneficial for host plants to secure a nitrogen resource, overproduction of nodules could have deleterious effects on plant growth. To avoid this problem, legume plants utilize a negative feedback regulation, where early nodulation events rapidly trigger a systemic signaling and repress further nodulation on younger root regions (Kosslak and Bohlool, 1984; Malik and Bauer, 1988; Nutman, 1952; Pierce and Bauer, 1983). This regulation is well known as autoregulation of nodulation (AON). In *L. japonicus*, AON is mediated primarily by *HAR1* gene, which encodes a receptor kinase (RK) with a high homology to *Arabidopsis thaliana* CLAVATA1 (Krusell et al., 2002; Nishimura et al., 2002). This gene and its roles in AON are conserved among other legume species such as *M. truncatula* (*SUNN*; Schnabel et al., 2005), *Pisum sativum* (*SYM29*; Krusell et al., 2002), and *Glycine max* (*NARK*; Searle et al., 2003). Interestingly, grafting experiments have shown that the CLV1-like RK functions in shoots but not in roots, indicating that long-distance communication between shoots and roots is critical for proper nodule number control (Delves et al., 1986; Krusell et al., 2002; Nishimura et al., 2002; Penmetsa et al., 2003; Sagan and Duc, 1996; Sheng and Harper, 1997). The existence of the systemic signaling in nodulation suggests that legumes optimize nodule number probably in concert with endogenous and environmental inputs. Therefore, AON is not just a legume-specific regulation but rather it should be viewed as a good example of the as-yet-unclear whole-body homeostasis in higher plants. In this review, we focus on the molecular basis underlying AON and highlight recent progress in characterization of the potential signaling components required for this long-distance signaling.

AON overview

The AON model has its origin in studies done by Nutman in 1952. He found that excision of nodules from red clover roots causes a transient increase in the number of subsequently-developed nodules (Nutman, 1952). This observation suggests that old nodules (i.e. early nodulation) posses an inhibitory effect on further nodulation and that a homeostatic control over

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nodule number may exist in legumes. Consistent with this notion, double inoculation analyses using soybeans elucidated that pre-inoculation of one part of roots with rhizobia reduces the number of nodules induced by the subsequent rhizobial inoculation (Pierce and Bauer, 1983). Moreover, split-root experiments revealed that this inhibitory effect on nodulation can be transmitted systemically between two physically-separated root compartments (Kosslak and Bohlool, 1984). Identification of the CLV1-like RK and its role exclusively in shoots for AON, further supported the systemic control of nodule number (Krusell et al., 2002; Nishimura et al., 2002; Schnabel et al., 2005; Searle et al., 2003).

Based on these observations, AON is likely to be mediated by a long-distance signaling circuit as proposed in Fig. 1. First, early nodulation events in one part of roots rapidly induce "root-derived signals", which are transported to shoots. Then, the root-derived signals directly or indirectly activate the CLV1-like RK and other unknown shoot factors, leading to production of "shoot-derived signals". The shoot-derived signals are delivered to a whole root system via phloem. Finally, the shoot-derived signals are perceived and decoded in roots, resulting in inhibition of further nodulation (Fig. 1). This AON signaling enables host plants to restrict the infection/nodulation-susceptible zone in a narrow area of roots. However, mutants defective in this control, such as *L. japonicus har1* (*hypernodulation aberrant root formation*; Wopereis et al., 2000), cannot arrest further nodule formation even in young developing root portions. As a result, AON mutants cause overproduction of nodules, which cover a wide portion of roots (i.e. hypernodulation or supernodulation; Fig. 2). Studies using such hypernodulation mutants have been making great strides in better understanding of AON at the molecular level. Here, we will describe each step of the AON signaling scheme by citing recent molecular genetic studies on nodule number control.

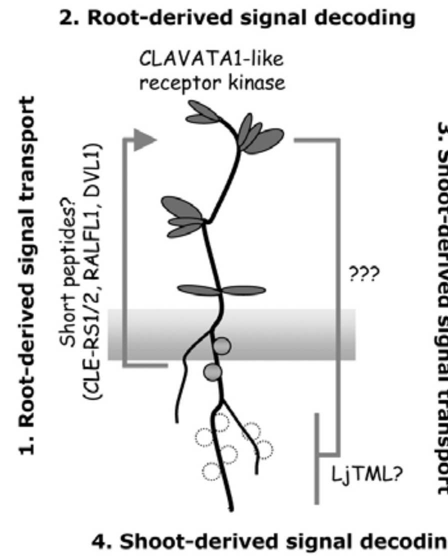

Fig. 1. Model of AON signaling. Early nodulation events in one part of roots rapidly induce "root-derived signals", which are transported to shoots (1). Short peptide-coding genes, such as *CLE-RS1/2*, *RALFL1*, and *DVL1*, may be involved in this process. Then, the root-derived signals directly or indirectly activate the CLV1-like RK and other unknown shoot factors, leading to production of "shoot-derived signals" (2). The shoot-derived signals are delivered to a whole root system via phloem (3). The molecular characteristics of the shoot-derived signal are largely unknown. Finally, the shoot-derived signals are perceived and decoded in roots, resulting in inhibition of further nodulation (4). The shoot-derived signal decoding is mediated by the root regulator TML.

Root-derived signal induction

LjHAR1 and *HAR1* orthologs from other legumes (*MtSUNN*/*PsSYM29*/*GmNARK*) encode a leucine-rich repeat (LRR) receptor-like kinase, which has a high homology to *A. thaliana* CLV1 (Krusell et al., 2002; Nishimura et al., 2002; Schnabel et al., 2005; Searle et al., 2003). AtCLV1 has been widely recognized as a critical factor that regulates shoot apical meristem size (Williams and Fletcher, 2005). It has been shown that AtCLV1 directly binds a 12 amino acid peptide processed from CLAVATA3 (CLV3), a member of the CLV3/ERS-related (CLE) family (Ogawa et al., 2008). Therefore, it would be reasonable to postulate that the AON signaling in legumes also utilizes CLE peptides to activate LjHAR1 and HAR1 orthologs. In *L. japonicus*, at least 39 potential *CLE* genes have been identified and among them, two *CLE* genes (*LjCLE-RS1* and *LjCLE-RS2*) have been implicated for AON signaling (Okamoto et al., 2009). Several lines of evidence suggest that both CLE-RS1 and CLE-RS2 may act as the AON-associated root-derived signals; (1) *CLE-RS1/2* are rapidly upregulated in roots within 24 h of rhizobial inoculation; (2) overexpression of *CLE-RS1/2* in wild-type roots strongly suppresses nodulation; (3) this inhibitory effect can be systemically transmitted to even non-transformed roots; and (4) the nodulation suppression by *CLE-RS1/2* requires LjHAR1, the AON-associated CLV1-like RK (Okamoto et al., 2009). It remains elusive how LjCLE-RS1/2 are processed into active peptides and whether the active form of the CLEs can be delivered from roots to shoots. Nevertheless, these findings strongly indicate that *CLE* genes possess important roles not only in apical meristem maintenance but also in lateral organ homeostasis.

In addition to *CLE* genes, two small peptide-coding genes, *Rapid alkalinization factor-like 1* (*RALF-like 1*) and *Devil 1* (*DVL1*), may be involved in nodule number control. Both *RALFL1* and *DVL1* were initially identified from *M. truncatula* as genes upregulated upon NF application (Combier et al., 2008). Overexpression of *MtRALFL1* and *MtDVL1* in wild-type roots caused a drastic decrease in nodule number, suggesting their roles in AON signaling (Combier et al., 2008). However, it has been unclear whether *MtRALF1* and *MtDVL1* exert the inhibitory effect on nodulation locally or systemically. It is well known that a plant hormone ethylene functions as a local inhibitor of nodulation in an AON-independent manner. Indeed, the ethylene-insensitive mutant, *sickle*, leads to hyperinfection of rhizobia but still maintains the AON systemic regulation (Penmetsa and Cook, 1997). Therefore, we cannot exclude the possibility that *MtRALF1* and *MtDVL1* might function in the ethylene-related pathway rather than AON signaling. To understand the precise mode of action of these two genes, their possible genetic interaction with the CLV1-like RK needs to be examined.

Root-derived signal decoding

As discussed above, LjHAR1 and HAR1 orthologs are indispensible shoot factors in AON signaling. It has been shown that *LjHAR1* promoter is active mainly in phloem tissues of leaves, stems, and nodules (Nontachaiyapoom et al., 2007). Phloem is generally considered as a conduit for long-distance signaling (Lough and Lucas, 2006). Thus, the primary function of the CLV1-like RK may be to mediate production and loading of the

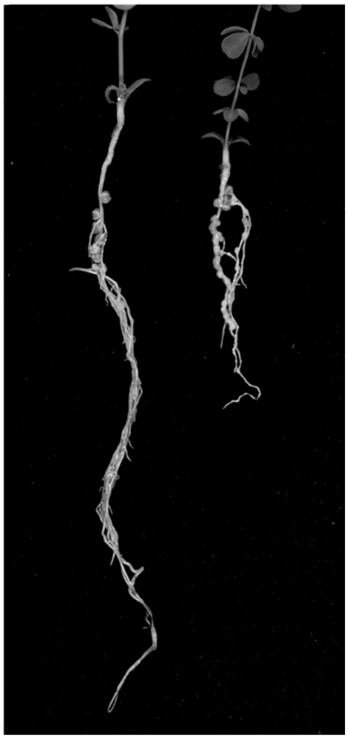

Fig. 2. Defects in AON lead to hypernodulation. Example of AON mutants. *L. japonicus* wild-type plants (left) restrict nodulation in a narrow portion of the root by the effect of AON signaling. On the other hand, *har1* mutants (right) cause a drastic increase in nodule number as well as an expanded nodulation zone.

shoot-derived signal molecules into phloem in response to the root-derived signal. During this process, the activity of the CLV1-like RK may be modulated by kinase-associated protein phosphatases (KAPP; Miyahara et al., 2008).

The question is, however, whether this CLV1-like RK alone can accomplish the root-derived signal decoding in shoots. It is possible that other unknown receptor-like kinases (RLKs) might have functional overlap with LjHAR1 and HAR1 orthologs. This notion comes from the variable severity of hypernodulation phenotype among *L. japonicus har1* alleles. At present, 5 *har1* alleles in the same accession background are available. All of the *har1* mutations except *har1-4* are found in the intracellular domain of HAR1 (Fig. 3A; Krusell et al., 2002; Nishimura et al., 2002). Interestingly, the *har1-4* mutation, a missense mutation located in the LRR domain, causes more severe hypernodulation compared to at least *har1-5* (Kawaguchi et al., 2002). This observation is reminiscent of the strong *clv1* alleles of *A. thaliana*, which coincidentally posses a missense mutation in the LRR domain of CLV1 (Diévart et al., 2003). Since the null alleles of *clv1* exhibit only a mild phenotype in stem cell maintenance, the *clv1* mutations in the LRR are likely to act as gain-of-function mutations (Diévart et al., 2003). Therefore, it was proposed that the *clv1* gain-of-function mutations may interfere with action of other RLKs that have overlapped roles with CLV1 (Diévart et al., 2003). Such RLKs might include CORYNE, a putative RLK that functions independently of CLV1 (Miwa et al., 2008; Müller et al., 2008).

By analogy with these findings from the *clv1* alleles, we hypothesize that the *har1-4* mutation might have a similar gain-of-function effect and negatively modulate as-yet-unidentified RLKs that act redundantly of HAR1 (Fig. 3B). On the other hand, the *har1-5* mutation, a missense mutation at the conserved residue in the kinase domain, might have no effect on such redundant RLKs, resulting in relatively weak nodulation phenotype (Fig. 3B). To test this hypothesis, it is inevitable to isolate additional *har1* alleles including null mutants and compare them with regard to phenotypic severity. If this prediction is indeed correct, it would be fruitful to reverse-genetically examine other RLKs for involvement in nodule number control.

In addition to the CLV1-like RK, KLAVIER (KLV) of *L. japonicus* is also important for AON signaling. The *klv* mutation causes hypernodulation phenotype similar to that of *har1* (Oka-Kira et al., 2005). Grafting experiments clearly showed that *klv* hypernodulation is determined by the shoot genotype (Oka-Kira et al., 2005). This suggests that KLV is another shoot factor participated in the root-derived signal decoding. However, functional relationship between KLV and HAR1 remains elusive. Considering that *klv* mutants exhibit pleiotropic phenotypes such as stem fasciation and aberrant leaf veins, KLV could be a more general factor that regulates multiple aspects of plant development (Oka-Kira et al., 2005).

Shoot-derived signal induction

The most controversial issue on AON signaling is the molecular property of the shoot-derived signal. Up to date, multiple plant hormones have been implicated for AON. For example, it was revealed that exogenous application of jasmonic acid (JA) to wild-type plants leads to a decrease in nodule number in *L. japonicus* as well as *M. truncatula* (Nakagawa and Kawaguchi, 2006; Sun et al., 2006). In the case of *L. japonicus*, shoot application of JA has been shown to rescue hypernodulation of *har1*, the AON mutant (Nakagawa and Kawaguchi, 2006). This suggests that shoot-derived JA might function as an inhibitor of nodulation, potentially downstream of HAR1. On the other hand, it has been elucidated that application of a JA biosynthesis inhibitor to soybean leaves dramatically suppresses nodulation in *nark* mutants (corresponds to *Ljhar1*; Kinkema and Gresshoff, 2008). In this case, JA seems to act as a positive regulator of nodulation. Thus, downregulation of JA level or signaling could be the primary function of the CLV1-like RK. Given this discrepancy between the two studies, it remains inconclusive whether JA is actually one of the AON signaling components.

Auxin is another controversial plant hormone which might be involved in AON signaling. van Noorden et al. (2006) found that auxin loading from a shoot to a root is transiently reduced within 24 hr of rhizobial inoculation in *M. truncatula* wild-type plants. In contrast, the AON mutant, *sunn*, fails to repress long-distance auxin transport, leading to constitutively high level of auxin accumulation in the root (van Noorden et al., 2006). Based of these observations, it was hypothesized that auxin is a positive regulator of nodulation and that AON signaling repress nodulation by downregulating polar auxin transport (van Noorden et al., 2006). However, such auxin transport reduction after rhizobial inoculation was not observed in another model legume, *L. japonicus* (Pacios-Bras et al., 2003). Moreover, the ethylene-insensitive mutant *sickle*, which shows AON-independent hypernodulation, also results in failure of the auxin transport inhibition similar to that of *sunn* (Prayitno et al., 2006). Therefore, the polar auxin transport change during nodulation might not be a direct effect of AON.

Most of the efforts to identify the AON-associated shoot-derived signal largely rely on physiological analyses. This could be a reason why inconsistent results have been often obtained among different legume species or methodologies. To isolate

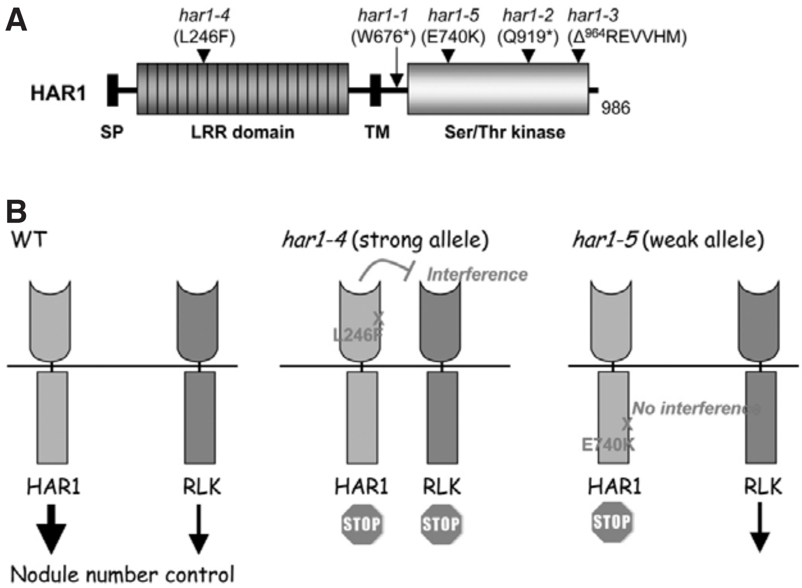

Fig. 3. Comparison among different *har1* alleles. (A) Summary of *har1* alleles of *L. japonicus* based on previous studies (Krusell et al., 2002; Nishimura et al., 2002). *HAR1* encodes a putative receptor protein with an extracellular leucine-rich repeat (LRR) domain and an intracellular serine/threonine kinase domain. SP, signal peptide; TM, transmembrane domain. The *har1-1* and *har1-2* mutations are nonsense mutations in the intracellular domain. The *har1-3* mutation is a deletion of 6 amino acids starting from the 964^{th} arginine. The *har1-5* mutation is a missense mutation at the conserved glutamate in the kinase domain. The *har1-4* is a missense mutation located in the LRR domain. (B) Potential mode of action of *har1-4* (strong allele) and *har1-5* (weak allele) mutations. AON is primarily mediated by the HAR1 RK, but another receptor-like kinase (RLK) may be also involved to a lesser extent. The strong hypernodulation phenotype of *har1-4* may be achieved by interference of such a redundant RLK. On the other hand, the *har1-5* does not affect other RLKs, leading to the weak hypernodulation.

the bona fide shoot-derived signal molecule, molecular and genetic approach will be more important in the near future.

Shoot-derived signal decoding

The as-yet-unknown shoot-derived signal is presumably transported to roots and decoded by root factors. A strong candidate for such a root regulator is TOO MUCH LOVE (TML) of *L. japonicus*. It has been shown that *tml* mutants cause hypernodulation, which is not enhanced by the *har1* mutation (Magori et al., 2009). This observation indicates that TML is another component of the HAR1-mediated AON signaling cascade. More importantly, in contrast to *har1*, the hypernodulation of *tml* is determined by the root genotype (Magori et al., 2009). Further, grafting experiments with two roots revealed that the suppressive effect of TML on nodulation cannot be systemically propagated from one root to another (Magori et al., 2009). Taken together, TML is likely to function downstream of HAR1, probably as a receptor/mediator of unknown shoot-derived signal molecules. The *NOD3* gene of *P. sativum* could be compatible with *TML* as *nod3* mutants show similar root-regulated hypernodulation (Postma et al., 1988). To analyze the possible relationship, molecular cloning of the two genes and further comparative study will be needed.

In *tml* mutant roots, the NF-inducible genes, such as *NIN* and *ENOD40*, are excessively upregulated (Magori, unpublished data). Thus, TML may act on any of the NF signaling cascade components and negatively regulate its expression or activity. Among the signaling components, the factors upstream of CCaMK are unlikely to be the TML targets. It was found that gain-of-function mutations in CCaMK lead to nodule development even in the absence of rhizobia or the NF receptor (i.e. spontaneous nodulation; Gleason et al., 2006; Tirichine et al., 2006a; 2006b). Importantly, the number of spontaneous nodules can be further increased by the *Ljhar1* mutation (2006a). This result suggests that AON signaling is still functional even when the mutated CCaMK is constitutively active. Therefore, the root regulator, TML, is likely to target CCaMK or other factors downstream of CCaMK. At the same time, we cannot rule out another possibility that TML negatively regulates unknown factors that function independently of the NF signaling cascade. To gain insights into the potential targets, further analyses such as suppressor mutant screening of *tml* may be helpful.

Concluding remarks

Higher plants utilize whole-body signaling to coordinate their development in response to endogenous and environmental information. AON signaling in legumes is a good example of such long-distance regulation. Studies on AON have a long history but we are just beginning to understand the molecular basis underlying AON. Owing to the model legumes, *L. japonicus* and *M. truncatula*, several players associated with AON have been identified in the past 5 years. However, the precise machinery of AON is relatively unclear compared with the NF signaling cascade, partly due to a paucity of hypernodulating mutant lines. This suggests that functional redundancy may exist among AON-related genes. Thus, reverse genetic approach will be increasingly important in the next decade. To this end, research platform in the model legumes needs to be further improved. For example, establishment of T-DNA knock-out mutant resources may facilitate isolation of novel AON-associated molecules.

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REFERENCES

Combier, J.P., Küster, H., Journet, E.P., Hohnjec, N., Gamas, P., and Niebel, A. (2008). Evidence for the involvement in nodulation of the two small putative regulatory peptide-encoding genes *MtRALFL1* and *MtDVL1*. Mol. Plant Microbe. Interact. *21*, 1118-1127.

Delves, A.C., Mathews, A., Day, D.A., Carter, A.S., Carroll, B.J., and Gresshoff, P.M. (1986). Regulation of the soybean-Rhizobium nodule symbiosis by shoot and root factors. Plant Physiol. *82*, 588-590.

D'Haeze, W., and Holsters, M. (2002). Nod factor structures, re-

sponses, and perception during initiation of nodule development. Glycobiology *12*, 79-105.
Diévart, A., Dalal, M., Tax, F.E., Lacey, A.D., Huttly, A., Li, J., and Clark, S.E. (2003). *CLAVATA1* dominant-negative alleles reveal functional overlap between multiple receptor kinases that regulate meristem and organ development. Plant Cell *15*, 1198-1211.
Geurts, R., Fedorova, E., and Bisseling, T. (2005). Nod factor signaling genes and their function in the early stages of *Rhizobium* infection. Curr. Opin. Plant Biol. *8*, 346-352.
Gleason, C., Chaudhuri, S., Yang, T., Muñoz, A., Poovaiah, B.W., and Oldroyd, G.E. (2006). Nodulation independent of rhizobia induced by a calcium-activated kinase lacking autoinhibition. Nature *441*, 1149-1152.
Heckmann, A.B., Lombardo, F., Miwa, H., Perry, J.A., Bunnewell, S., Parniske, M., Wang, T.L., and Downie, J.A. (2006). *Lotus japonicus* nodulation requires two GRAS domain regulators, one of which is functionally conserved in a non-legume. Plant Physiol. *142*, 1739-1750.
Kaló, P., Gleason, C., Edwards, A., Marsh, J., Mitra, R.M., Hirsch, S., Jakab, J., Sims, S., Long, S.R., Rogers, J., et al. (2005). Nodulation signaling in legumes requires NSP2, a member of the GRAS family of transcriptional regulators. Science *308*, 1786-1789.
Kawaguchi, M., Imaizumi-Anraku, H., Koiwa, H., Niwa, S., Ikuta, A., Syono, K., and Akao, S. (2002). Root, root hair, and symbiotic mutants of the model legume *Lotus japonicus*. Mol. Plant-Microbe Interact. *15*, 17-26.
Kinkema, M., and Gresshoff, P.M. (2008). Investigation of downstream signals of the soybean autoregulation of nodulation receptor kinase GmNARK. Mol. Plant-Microbe Interact. *21*, 1337-1348.
Kosslak, R.M., and Bohlool, B.B. (1984). Suppression of nodule development of one side of a split-root system of soybeans caused by prior inoculation of the other side. Plant Physiol. *75*, 125-130.
Krusell, L., Madsen, L.H., Sato, S., Aubert, G., Genua, A., Szczyglowski, K., Duc, G., Kaneko, T., Tabata, S., de Bruijn, F., et al. (2002). Shoot control of root development and nodulation is mediated by a receptor-like kinase. Nature *420*, 422-426.
Lévy, J., Bres, C., Geurts, R., Chalhoub, B., Kulikova, O., Duc, G., Journet, E.P., Ané, J.M., Lauber, E., Bisseling, T., et al. (2004). A putative Ca^{2+} and calmodulin-dependent protein kinase required for bacterial and fungal symbioses. Science *303*, 1361-1364.
Lough, T.J., and Lucas, W.J. (2006). Integrative plant biology: role of phloem long-distance macromolecular trafficking. Annu. Rev. Plant Biol. *57*, 203-232.
Madsen, E.B., Madsen, L.H., Radutoiu, S., Olbryt, M., Rakwalska, M., Szczyglowski, K., Sato, S., Kaneko, T., Tabata, S., Sandal, N., et al. (2003). A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals. Nature *425*, 637-640.
Magori, S., Oka-Kira, E., Shibata, S., Umehara, Y., Kouchi, H., Hase, Y., Tanaka, A., Sato, S., Tabata, S., and Kawaguchi, M. (2009). *TOO MUCH LOVE*, a root regulator associated with the long-distance control of nodulation in *Lotus japonicus*. Mol. Plant-Microbe Interact. (in press).
Malik, N.S., and Bauer, W.D. (1988). When does the self-regulatory response elicited in soybean root after Inoculation occur? Plant Physiol. *88*, 537-539.
Mitra, R.M., Gleason, C.A., Edwards, A., Hadfield, J., Downie, J.A., Oldroyd, G.E., and Long, S.R. (2004). A Ca^{2+}/calmodulin-dependent protein kinase required for symbiotic nodule development: Gene identification by transcript-based cloning. Proc. Natl. Acad. Sci. USA *101*, 4701-4705.
Miwa, H., Betsuyaku, S., Iwamoto, K., Kinoshita, A., Fukuda, H., and Sawa, S. (2008). The receptor-like kinase SOL2 mediates CLE signaling in Arabidopsis. Plant Cell Physiol. *49*, 1752-1757.
Miyahara, A., Hirani, T.A., Oakes, M., Kereszt, A., Kobe, B., Djordjevic, M.A., and Gresshoff, P.M. (2008). Soybean nodule autoregulation receptor kinase phosphorylates two kinase-associated protein phosphatases *in vitro*. J. Biol. Chem. *283*, 25381-25391.
Müller, R., Bleckmann, A., and Simon, R. (2008). The receptor kinase CORYNE of Arabidopsis transmits the stem cell-limiting signal CLAVATA3 independently of CLAVATA1. Plant Cell *20*, 934-946.
Murakami, Y., Miwa, H., Imaizumi-Anraku, H., Kouchi, H., Downie, J.A., Kawaguchi, M., and Kawasaki, S. (2006). Positional cloning identifies *Lotus japonicus* NSP2, a putative transcription factor of the GRAS family, required for *NIN* and *ENOD40* gene expression in nodule initiation. DNA Res. *13*, 255-265.
Nakagawa, T., and Kawaguchi, M. (2006). Shoot-applied MeJA suppresses root nodulation in *Lotus japonicus*. Plant Cell Physiol. *47*, 176-180.
Nishimura, R., Hayashi, M., Wu, G.J., Kouchi, H., Imaizumi-Anraku, H., Murakami, Y., Kawasaki, S., Akao, S., Ohmori, M., Nagasawa, M., et al. (2002). HAR1 mediates systemic regulation of symbiotic organ development. Nature *420*, 426-429.
Nontachaiyapoom, S., Scott, P.T., Men, A.E., Kinkema, M., Schenk, P.M., and Gresshoff, P.M. (2007). Promoters of orthologous *Glycine max* and *Lotus japonicus* nodulation autoregulation genes interchangeably drive phloem-specific expression in transgenic plants. Mol. Plant-Microbe Interact. *20*, 769-780.
Nutman, P.S. (1952). Studies on the physiology of nodule formation. III. Experiments on the excision of root-tips and nodules. Ann. Bot. *16*, 79-101.
Ogawa, M., Shinohara, H., Sakagami, Y., and Matsubayashi, Y. (2008). *Arabidopsis* CLV3 peptide directly binds CLV1 ectodomain. Science *319*, 294.
Oka-Kira, E., Tateno, K., Miura, K., Haga, T., Hayashi, M., Harada, K., Sato, S., Tabata, S., Shikazono, N., Tanaka, A., et al. (2005). *klavier* (*klv*), a novel hypernodulation mutant of *Lotus japonicus* affected in vascular tissue organization and floral induction. Plant J. *44*, 505-515.
Okamoto, S., Ohnishi, E., Sato, S., Takahashi, H., Nakazono, M., Tabata, S., and Kawaguchi, M. (2009). Nod factor/nitrate-induced *CLE* genes that drive HAR1-mediated systemic regulation of nodulation. Plant Cell Physiol. *50*, 67-77.
Oldroyd, G.E., and Downie, J.A. (2008). Coordinating nodule morphogenesis with rhizobial infection in legumes. Annu. Rev. Plant Biol. *59*, 519-546.
Pacios-Bras, C., Schlaman, H.R., Boot, K., Admiraal, P., Langerak, J.M., Stougaard, J., and Spaink, H.P. (2003) Auxin distribution in *Lotus japonicus* during root nodule development. Plant Mol. Biol. *52*, 1169-1180.
Penmetsa, R.V., and Cook, D.R. (1997). A legume ethylene-insensitive mutant hyperinfected by its rhizobial symbiont. Science *275*, 527-530.
Penmetsa, R.V., Frugoli, J.A., Smith, L.S., Long, S.R., and Cook, D.R. (2003). Dual genetic pathways controlling nodule number in *Medicago truncatula*. Plant Physiol. *131*, 998-1008.
Pierce, M., and Bauer, W.D. (1983). A rapid regulatory response governing nodulation in soybean. Plant Physiol. *73*, 286-290.
Postma, J.G., Jacobsen, E., and Feenstra, W. (1988). Three pea mutants with an altered nodulation studied by genetic analysis and grafting. J. Plant Physiol. *132*, 424-430.
Prayitno, J., Rolfe, B.G., and Mathesius, U. (2006). The Ethylene-insensitive *sickle* mutant of *Medicago truncatula* shows altered auxin transport regulation during nodulation. Plant Physiol. *142*, 168-180.
Radutoiu, S., Madsen, L.H., Madsen, E.B., Felle, H.H., Umehara, Y., Grønlund, M., Sato, S., Nakamura, Y., Tabata, S., Sandal, N., et al. (2003). Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. Nature *425*, 585-592.
Sagan, M., and Duc, G. (1996). *Sym28* and *Sym29*, two new genes involved in regulation of nodulation in pea (*Pisum sativum* L.). Symbiosis *20*, 229-245.
Schnabel, E., Journet, E.P., de Carvalho-Niebel, F., Duc, G., and Frugoli, J. (2005). The *Medicago truncatula SUNN* gene encodes a *CLV1*-like leucine-rich repeat receptor kinase that regulates nodule number and root length. Plant Mol. Biol. *58*, 809-822.
Searle, I.R., Men, A.E., Laniya, T.S., Buzas, D.M., Iturbe-Ormaetxe, I., Carroll, B.J., and Gresshoff, P.M. (2003). Long-distance signaling in nodulation directed by a CLAVATA1-like receptor kinase. Science *299*, 109-112.
Sheng, C., and Harper, J.E. (1997). Shoot versus root signal involvement in nodulation and vegetative growth in wild-type and hypernodulating soybean genotypes. Plant Physiol. *113*, 825-831.
Smit, P., Raedts, J., Portyanko, V., Debellé, F., Gough, C., Bisseling, T., and Geurts, R. (2005). NSP1 of the GRAS protein family is essential for rhizobial Nod factor-induced transcription. Sci-

ence *308*, 1789-1791.
Stacey, G., Libault, M., Brechenmacher, L., Wan, J., and May, G.D. (2006). Genetics and functional genomics of legume nodulation. Curr. Opin. Plant Biol. *9*, 110-121.
Sun, J., Cardoza, V., Mitchell, D.M., Bright, L., Oldroyd, G., and Harris, J.M. (2006). Crosstalk between jasmonic acid, ethylene and Nod factor signaling allows integration of diverse inputs for regulation of nodulation. Plant J. *46*, 961-970.
Tirichine, L., Imaizumi-Anraku, H., Yoshida, S., Murakami, Y., Madsen, L.H., Miwa, H., Nakagawa, T., Sandal, N., Albrektsen, A.S., Kawaguchi, M., et al. (2006a). Deregulation of a Ca^{2+}/calmodulin-dependent kinase leads to spontaneous nodule development. Nature *441*, 1153-1156.
Tirichine, L., James, E.K., Sandal, N., and Stougaard, J. (2006b). Spontaneous root-nodule formation in the model legume *Lotus japonicus*: a novel class of mutants nodulates in the absence of rhizobia. Mol. Plant-Microbe. *19*, 373-382.
van Noorden, G.E., Ross, J.J., Reid, J.B., Rolfe, B.G., and Mathesius, U. (2006). Defective long-distance auxin transport regulation in the *Medicago truncatula super numeric nodules* mutant. Plant Physiol. *140*, 1494-1506.
Williams, L., and Fletcher, J.C. (2005). Stem cell regulation in the *Arabidopsis* shoot apical meristem. Curr. Opin. Plant Biol. *8*, 582-586.
Wopereis, J., Pajuelo, E., Dazzo, F.B., Jiang, Q., Gresshoff, P.M., De Bruijn, F.J., Stougaard, J., and Szczyglowski, K. (2000). Short root mutant of *Lotus japonicus* with a dramatically altered symbiotic phenotype. Plant J. *23*, 97-114.