Plant Genes Involved in Symbiotic Signal Perception/Signal Transduction

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Abstract

A host genetic programme that is initiated upon recognition of specific rhizobial Nod factors governs the symbiosis of legumes with nitrogen-fixing bacteria. This programme coordinates two major developmental processes that run in parallel in legume roots: *de novo* cortical cell division leading to nodule primordia formation, and the infection thread initiation in the root hairs guiding bacteria towards dividing cortical cells. This chapter focuses on the plant genes involved in the recognition of the symbiotic signal produced by rhizobia, and the downstream genes, which are part of a complex symbiotic signalling pathway that leads to the generation of calcium spiking in the nuclear regions and activation of transcription factors controlling symbiotic genes induction.

6.1 Perception of Symbiotic Signals at the Plasma Membrane

Genetic and molecular studies of host–microbe interaction identified a two-way signal exchange as a central mechanism for partner recognition during the symbiotic establishment. Plant-produced strigolactones are recognized and perceived by the obligate arbuscular mycorrhiza (AM) fungi, while specific flavonoids or isoflavonoids secreted from the host roots (Peters et al. 1986; Spaink et al. 1989) are recognized by the symbiotic rhizobia. It has been well established that in rhizobia, this recognition has a direct consequence, as the flavonoid-activated rhizobial NodD proteins promote transcription of bacterial *nod* genes involved in synthesis and secretion of lipochitin oligosaccharides (Nod factors). These molecules serve as major bacterial signals detected by the legume host (Mulligan and Long 1985; Spaink et al. 1991; Truchet et al. 1991). Chitin-derived signals, both in the form of GlcNac tetra/pentamers and acylated GlcNac oligomers, are also produced by the AM fungi and are perceived by the plants as symbiotic signals (Maillet et al. 2011; Genre et al. 2013), but the AM genes

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involved in synthesis of these molecules await identification.

Rhizobial Nod factors trigger physiological responses, gene expression, and cell division in susceptible legume roots during establishment of root nodule symbiosis (RNS). As a consequence, a new plant organ, the root nodule, hosting the symbiont is formed (reviewed in Oldroyd et al. 2011). The rhizobial symbiont of *Lotus japonicus*, *Mesorhizobium loti* strain R7A, produces a pentameric Nod factor with a 4-*O*-fucose at the reducing end, which either has an acetyl group or a proton in position 3 or 4. The non-reducing end is N-methylated and N-acylated (*cis*-vaccenic acid or stearic acid) and has a carbamoyl group in position 3 (Lopez-Lara et al. 1995; Bek et al. 2010). The acetylated fucosyl group is important for effective Nod factor signalling and the absence of this decoration leads to host-dependent nodulation phenotypes among different *Lotus* species: *L. japonicus*, *L. filicaulis*, *L. corniculatus* and *L. burttii* (Rodpothong et al. 2009).

Perception of Nod factors in legumes is mediated by receptor kinases containing LysM modules in their extracellular domains. In *Lotus*, the two receptor kinases perceiving the Nod factor signal, NFR1 and NFR5, are predicted to have a topology where single-pass transmembrane domains anchor the proteins to the plasma membrane exposing the LysM domains to the extracytoplasmic space, and the serine/threonine kinase to the cytoplasm (Madsen et al. 2003; Radutoiu et al. 2003; Madsen et al. 2011). Based on their domain similarity, and the fact that mutations in both *Nfr1* and *Nfr5* genes equally abolish Nod factor or rhizobia recognition, a heteromeric receptor complex (NFR1–NFR5) was proposed to initiate signal transduction following perception of a correctly decorated Nod factor (Radutoiu et al. 2003). Follow-up studies using bimolecular fluorescence complementation (BiFC) in *Nicotiana benthamiana* and leek cells revealed NFR1 and NFR5 localization at the plasma membrane and their interaction upon co-expression, supporting the originally suggested model (Madsen et al. 2011). The intracellular region of the NFR1 protein has all the subdomains of a typical kinase, and based on in vitro analyses, it has been shown to have the capacity for autophosphorylation and for NFR5 phosphorylation. On the other hand, NFR5, which lacks important kinase subdomains, and has therefore been considered a pseudokinase, failed to display kinase activity in a similar in vitro assay with myelin basic protein as a catalytic substrate (Madsen et al. 2011). The observation that a deletion of nine amino acids in the NFR5 kinase domain of the *nfr5-1* mutant abolishes the symbiotic interaction (Madsen et al. 2003) indicates that the intracellular region of NFR5 is also crucial, possibly serving as an interacting domain for downstream signalling components. A plasma membrane-associated remorin, SYMREM1, which is specifically induced during RNS, has been identified both in *Lotus japonicus* and in *Medicago truncatula*, and it has been shown to interact with Nod factor receptors in both model legumes (Levebvre et al. 2010; Tóth et al. 2012). Recently, using an Y2H approach, a ROP GTPase has been identified as an NFR5 kinase interactor, and transcriptional deregulation of this gene by RNAi led to a symbiotic defective phenotype (Ke et al. 2012). With the exception of *Lj*SYMREM1, no other interaction partners of NFR1 have been identified so far. However, the fact that particular amino acids and defined regions of the NFR1 kinase domain play a major role in downstream symbiotic signalling has been clearly shown by using point mutation constructs (Madsen et al. 2011) and domain swaps between *Lotus* NFR1 and *Arabidopsis* CERK1 (Nakagawa et al. 2011). CERK1 recognizes chitin (Miya et al. 2007; Wan et al. 2008), a microbial signal that is biochemically similar to rhizobial Nod factor. The chitin octamer is a PAMP produced by fungal pathogens, and CERK1 provides resistance in plants by activating a specific signalling cascade, leading to activation of defence genes (reviewed by Gust et al. 2012). NFR1 and CERK1 proteins show high sequence and structural similarity, especially in the intracellular region (Radutoiu et al. 2003; Miya et al. 2007; Wan et al. 2008). It is therefore unclear how specificity is achieved in the downstream signalling upon binding of symbiotic Nod factor or pathogen-derived chitin

to the ectodomain. It has been shown that a chimeric NFR1-CERK1 construct containing the YAQ amino acid sequence from NFR1 in the αEF helix of CERK1 kinase allows reconstitution of symbiotic signalling in the *nfr1* mutant (Nakagawa et al. 2011). This poses an important question: Does the YAQ sequence allow for the recruitment of specific downstream symbiotic partners or prevent the interaction with CERK1 partners acting in the defence pathway?

The presence of GlcNac-binding LysM modules in the extracellular regions of NFR1 and NFR5 represents a strong indication for the receptors' ability to bind rhizobial Nod factors. However, biochemical studies showing direct binding proved to be challenging due to the recalcitrant nature of NFR proteins and the amphiphilic properties of Nod factor ligands. Recently, a breakthrough in the biochemical analysis of NFRs-Nod factor binding ability has been achieved, and detailed studies using plant-produced proteins showed the ability of both *Lotus* receptors to directly bind, with high affinity, the Nod factors produced by *M. loti* (Broghammer et al. 2012). Using two different techniques, it has been shown that full length NFR1 and NFR5 proteins have K_d values for Nod factor binding in the nanomolar range. These are comparable with the ligand concentrations inducing membrane depolarization and calcium spiking in legume roots (Radutoiu et al. 2003; Miwa et al. 2006). These recent biochemical evidence complemented previous molecular studies that demonstrated the ability of NFR1 and NFR5 to mediate Nod factor perception and to ensure the specificity in the legume–rhizobia interaction. By transforming *M. truncatula* with the *L. japonicus Nfr1* and *Nfr5* genes, it was shown that the two receptors act in concert as host determinants, allowing *M. truncatula*, the non-host, to recognize and be infected by *M. loti,* the symbiont of *Lotus* (Radutoiu et al. 2007). Recognition of *M. loti* triggers initiation of nodule organogenesis in the root cortex as well as infection thread formation in the root hairs. This extended NFR1- and NFR5-mediated signal cascade is dependent on both Nod factor synthesis and structure. By using domain swap experiments and amino acid substitutions between NFRs of related *Lotus* species, it has been shown that a single amino acid variation, L118 to F, in the LysM2 domain of NFR5 plays a major role in discriminating *M. loti* and *R. leguminosarum* DZL Nod factors in *L. filicaulis* (Radutoiu et al. 2007). Interestingly, the same domain has been found in CERK1 to bind chitin (Liu et al. 2012), and homology modelling of the NFR5 LysM2 domain onto the CERK1 structure identified a possible binding groove, indicating a direct interaction with the ligand (M. Blaise, pers. communication). However, the presence of three LysM domains in the NFR5 and NFR1 receptors (Madsen et al. 2003; Radutoiu et al. 2003) suggests the involvement of more than one LysM domain in Nod factor perception. Three lines of evidence support this notion: (i) the non-nodulation phenotype caused by an amino acid substitution in the LysM1 domain of the *M. truncatula* homologue of NFR5 called NFP (Arrighi et al. 2006), (ii) the involvement of LysM1 of the pea SYM37 NFR1-like receptor in distinguishing 'European' and 'Middle East' *Rhizobium leguminosarum* bv. *viciae* strains (Zhukov et al. 2008) and (iii) the reported binding affinity of the CERK1 LysM2 domain to chitin (Liu et al. 2012) is significantly lower ($K_d = 44, 8$ μM) compared to the one found for Nod factor (Broghammer et al. 2012) in the case of NFR1 (K_d = 4.9 nM) and NFR5 (K_d = 10 nM). Surprisingly, a very high-affinity chitin-binding site ($K_d = 280$ pM), formed intramolecularly by the LysM1 and LysM3 domains, has been identified in the crystal structure of the *C. fulvum* fungal effector *Ecp6* (Sanchez-Vallet et al. 2013). By comparison with the CERK1 structure, the possibility of a similar LysM1–LysM3 arrangement in the ectodomain of the receptor has been excluded (Sanchez-Vallet et al. 2013). However, functionally CERK1 acts as a dimer, and NFR1–NFR5 forms a heterodimer, therefore the possibility of a corresponding high-affinity binding groove, formed in these cases intermolecularly, represents a very attractive hypothesis.

Protein–carbohydrate recognition events are central to cell–cell communication, cellular defence mechanisms, protein trafficking, and host–microbe recognition (Sacchettini et al. 2001).

In the past couple of years, an important role for chitin oligosaccharides (COs) or their derivatives, as signal molecules in plant and animal developmental processes and defence mechanisms, has emerged. In addition, carbohydrate-based microbe-associated molecular patterns (MAMPs), which are partly unidentified, are thought to be produced by microbes or plants during microbial infection (Boller and Felix 2009; Gimenez-Ibanez et al. 2009). Compared to other plant species, such as *Arabidopsis* or rice, the family of LysM receptor kinase in legumes, has expanded, but so far only *Nfr1, Nfr5* and *Lys3* (Kawaharada et al. manuscript in preparation) have an assigned function in rhizobial infection. In total, 17 loci encoding LysM proteins were identified in *Lotus* as having a typical receptor kinase structure (Lohmann et al. 2010). Analysis of the *Lys* genes location in the *L. japonicus* genome revealed the contribution of both tandem and segmental duplications for the expansion of this gene family. Seven *Lys* genes are arranged in tandem repeats (*Nfr1-Lys1-Lys2*, *Nfr5-Lys12*, *Lys13-Lys14*), while the occurrence of segmental duplication is supported by the syntenic regions on: (i) chromosome 4 containing *Lys11*, the closest paralog of *Nfr5*, and chromosome 2 where *Nfr5-Lys12* resides and (ii) chromosome 6 where *Lys6* is located and the *Nfr1-Lys1-Lys2* region. A tempting hypothesis for the evolutionary diversification of this receptor family in legumes is their unique capacity to decipher various structures of microbe-derived molecules produced by an extended spectrum of interacting organisms: associative, symbiotic, and parasitic bacteria or fungi. Gene expression studies performed on *Lotus* identified 13 *Lys* genes expressed in roots and nodules, which represent attractive candidates for the suggested cortical receptor of rhizobial Nod factors (Madsen et al. 2010) and for Myc signal(s) perception. However, the identification of receptors proves to be challenging due to the complex nature or the unknown structure of these microbial signals, and the possible redundancy among receptor genes. Rhizobial genes involved in Nod factor synthesis are transcriptionally active in the cortical infection threads (Timmers et al. 1998; Schlaman et al. 1991), but neither the structure, nor the composition of these signals is currently known. In the case of AM, both chitin oligomers and acylated forms of chitin with different decorations are able to induce calcium spiking or to activate nodulin genes (Maillet et al. 2011; Genre et al. 2013), and as a consequence of such complex signalling from the microbe, one could expect a complex deciphering mechanism in the host as well.

In both AM and RNS, the symbiotic signal perception at the plasma membrane leads to the activation of calcium spiking and ultimately to mycorrhiza- and nodulation-associated gene expression. Both symbioses share a common genetic programme, which was co-opted from a pre-existing AM pathway during the evolution of RNS (Kistner and Parniske 2002). In *Lotus japonicus,* at least eight genes of this common pathway have been identified so far: *SymRK*, *Nup85, Nup133, Nena, Castor, Pollux, CCaMK and Cyclops* (Stracke et al. 2002; Mitra et al. 2004; Kistner et al. 2005; Imaizumi-Anraku et al. 2005; Kanamori et al. 2006; Saito et al. 2007; Groth et al. 2010). This poses an interesting question: How are distinct rhizobial and AM fungal stimuli integrated by one common pathway to activate specific downstream signalling events for either symbiosis?

6.2 Signalling from Plasma Membrane to Nucleus

The receptor-like kinase (RLK) SYMRK (NORK/DMI2) mediates symbiotic signal transduction following Nod factor perception, and *symrk* mutants are deficient in both RNS and AM (Endre et al. 2002; Stracke et al. 2002). SYMRK is composed of an intracellular kinase, a transmembrane domain and an extracytoplasmic region consisting of leucine-rich repeats (LRRs) and a malectin-like domain (MLD) (Antolìn-Llovera et al. 2014). At least three distinct SYMRK variants with different sizes of the extracellular region exist in angiosperms. The longest version, containing three LRRs, is needed for RNS, while shorter versions are sufficient for

AM (Markmann et al. 2008). SYMRK interacts with both SYMREM1 and NFR5, potentially acting as a coreceptor of NFR5 in symbiotic signalling (Tóth et al. 2012; Antolìn-Llovera et al. 2014). The alteration of a conserved extracellular 'GDPC' sequence in the *symrk-14* mutant affects symbiotic development in the epidermis, but not in the cortex (Kosuta et al. 2011). Dependent on the 'GDPC' sequence, full length SYMRK is cleaved *in planta*, resulting in the release of the extracellular MLD. The cleavage product lacking the MLD (SYMRK-ΔMLD) outcompetes full length SYMRK for NFR5 interaction, suggesting that the MLD interferes with NFR5 binding (Antolìn-Llovera et al. 2014). Moreover, SYMRK-ΔMLD is rapidly degraded if the LRR region is present. Removal of the LRRs stabilizes the truncated SYMRK and results in an increased formation of infection threads (Antolìn-Llovera et al. 2014). The degradation of SYMRK-ΔMLD is potentially mediated by the E3 ligase SINA, an interactor of SYMRK, whose ectopic expression was correlated with reduced SYMRK protein levels and impaired infection thread development (Den Herder et al. 2012). Taken together, these results emphasize the importance of intricate SYMRK regulation in symbiotic signalling.

The components involved in signal transduction from the PM receptors to the nuclear calcium spiking machinery have not been determined; however, screens for interaction partners of known signalling components identified candidates for missing pieces. Among the interactors of SYMRK, a 3-hydroxy-3-methylglutaryl coenzyme A reductase1 (HMGR1) coenzyme (Kevei et al. 2007) as well as the MAP kinase kinase (MAPKK) SIP2 (Chen et al. 2012) has been identified, and silencing of either of these causes nodulation defects. MAP kinase or HMGR signalling might be involved in downstream signal transduction. HMGRs are the rate controlling enzymes of the mevalonate pathway that produces sterols, isoprenoids and in particular cytokinins, which are necessary for the induction of nodule morphogenesis (reviewed in Oldroyd et al. 2011). Metabolites of the HMGR pathway could act as symbiotic secondary messengers (Kevei et al. 2007). Although MtHMGR1 is upregulated during initial stages of mycorrhizal symbiosis (Liu et al. 2003), neither HMGR1 nor SIP2 was implicated with a function in mycorrhiza colonization, indicating that additional pathways downstream of SYMRK might be involved in AM signal transduction.

6.3 Generation of Nuclear Calcium Spiking

Nuclear and perinuclear calcium oscillations are initiated after contact with both rhizobia and AM fungi (Kosuta et al. 2008; Chabaud et al. 2011; Sieberer et al. 2012) and can be triggered directly by the addition of rhizobial lipo-chitooligosaccharide nodulation factors (NF) (Ehrhardt et al. 1996; Miwa et al. 2006; Sieberer et al. 2009) and short-chain chito-oligomers (COs), which are present in AM fungal exudates (Genre et al. 2013). Forward and reverse genetic screens in legumes have identified proteins involved in the generation of the calcium response. The closely related ion channels CASTOR and POLLUX (*M. truncatula* DMI1) are required for calcium spiking, and mutant alleles are deficient for RNS and AM colonization (Ané et al. 2004; Imaizumi-Anraku et al. 2005; Charpentier et al. 2008). The channels are located in the nuclear envelope (Riely et al. 2007; Charpentier et al. 2008), and in the case of DMI1, preferentially targeted to the inner side of the nuclear membrane (Capoen et al. 2011). Electrophysiological and functional analyses revealed that the proteins are cation channels. CASTOR showed a preference for K^+, and POLLUX could complement a yeast K^+ import and export mutant (Charpentier et al. 2008). Symbiotic signalling in *L. japonicus* requires both CASTOR and POLLUX. In *M. truncatula,* DMI1 alone is sufficient and expression of *Dmi1* in *Lotus* was able to complement a *castor/pollux* double mutant (Venkateshwaran et al. 2012). This functional difference between POLLUX and DMI1 was pinpointed to a single amino acid exchange in the putative selectivity filter region of the channels (alanine in POLLUX, serine in DMI1), which resulted in an increased mean channel opening time of DMI1 compared

with CASTOR. Exchanging the filter region of POLLUX to that of DMI1 allowed for the complementation of *dmi1* and *castor/pollux* double mutants; however, the same was not true when the change was introduced into CASTOR, which failed to rescue either *dmi1* or *castor/pollux* but was still able to rescue a *castor* single mutant (Venkateshwaran et al. 2012). This finding indicates a functional or regulatory difference between CASTOR and POLLUX/DMI1 that goes beyond their K^+ conductivity.

A mathematical model predicted that calcium-dependent activation of DMI1 and voltage-dependent opening of calcium channels in addition to the presence of a calcium pump are sufficient for sustained calcium oscillations (Granqvist et al. 2012). A SERCA type calcium pump, *M. truncatula* Calcium ATPase8 (MCA8), was localized to both the inner and the outer nuclear membrane. Silencing of *Mca8* perturbed spiking and resulted in reduced mycorrhization (Capoen et al. 2011).

Several hypotheses regarding the function of CASTOR and POLLUX/DMI1 in the calcium spiking machinery have been put forward. One model assumes that CASTOR and POLLUX/DMI1 are activated by secondary messengers, causing K^+ to flow into the perinuclear space. This would cause hyperpolarization of the nuclear membranes and in turn could lead to the opening of voltage-gated calcium channels (Venkateshwaran et al. 2012). In a slightly different model, it was suggested that for continued calcium spiking both DMI1 and calcium channels would need to be simultaneously activated by the binding of second messenger molecules. In this case, DMI1 (as well as CASTOR and POLLUX) would predominantly act as a counterion channel, but also initially contribute to the activation of the calcium channels by hyperpolarization of the nuclear membrane (Charpentier et al. 2013).

The role of the nuclear pore complex in symbiotic signalling remains poorly understood. Mutations in three nucleoporin genes, *Nup85, Nup133* and *Nena* (*Seh1*), abolish calcium spiking and cause defects in both RNS and AM symbiosis, similar to the phenotypes observed in *castor* and *pollux* mutants (Kistner et al. 2005; Kanamori et al. 2006; Saito et al. 2007; Groth et al. 2010). Yeast and vertebrate homologues of NUP85, NUP133 and NENA are part of the nuclear pore NUP107-160 subcomplex, which is an essential component of the NPC scaffold and required for NPC assembly (Walther et al. 2003; Doucet et al. 2010). Given the apparent lack of broad pleiotropic defects in the mutants, the subcomplex likely remains at least partially intact but can no longer fulfil certain functions required in symbiotic signal transduction. Aberrations in structure or distribution of the NPCs could prevent ongoing calcium oscillations by affecting the electrophysiological properties of the nucleus. Alternatively, changes in the NPC scaffold could also interfere with nucleo-cytoplasmatic transport of symbiotic proteins or messengers (reviewed in Binder and Parniske 2013). While import and export of macromolecules through the central NPC channel does not depend on the NUP107-160 subcomplex, larger membrane proteins ($>\sim$25 kDa), which are imported from the outer to the inner nuclear membrane, have to pass through both the central channel as well as the NPC scaffold (Meinema et al. 2011). As this implies a remodelling of nucleoporin connections in order to create on opening, it is conceivable that structural defects in the *nup* mutants can impair proper localization of nuclear envelope membrane proteins such as CASTOR, POLLUX, MCA8, or the calcium channels and thus affect calcium spiking.

6.4 Decoding the Calcium Signal

As the likely primary decoder of symbiotic calcium signatures, a nuclear calcium- and calmodulin-dependent kinase (CCaMK) plays a central role in symbiotic signal transduction (reviewed in Singh and Parniske 2012). *ccamk* mutants do not form infection threads, nodules and arbuscules when inoculated with rhizobia or AM fungi (Levy et al. 2004; Mitra et al. 2004). A calmodulin (CaM)-binding domain and three calcium-binding EF-hands mediate CCaMKs regulation during calcium spiking (Swainsbury

et al. 2012). Calcium binding induces a conformational change in the protein (Swainsbury et al. 2012) and promotes its autophosphorylation (Takezawa et al. 1996; Sathyanarayanan et al. 2000). Constitutive activation of CCaMK, caused by mutations in an autophosphorylation site (T265D, T265I), leads to spontaneous nodule development in the absence of rhizobia (Gleason et al. 2006; Tirichine et al. 2006). Negative regulation of CCaMK as a result of autophosphorylation within the calcium/CaM-binding domain (Liao et al. 2012; Routray et al. 2013) is also required for normal cortical infection and AM development (Liao et al. 2012), demonstrating an intricate modulation of CCaMK activity during symbiotic signalling. Autoactive CCaMK mutants are able to restore nodulation, and AM symbiosis in the mutants *symrk, castor, pollux, nup85* and *nup133* (Hayashi et al. 2010; Madsen et al. 2010). This indicates that the primary function of these genes is the activation of calcium spiking and highlights the importance of calcium signalling in symbiotic signal transduction.

It emerges that the calcium oscillations themselves may carry cell type and stage-specific information. Live cell imaging demonstrated a transition from low- to high-frequency spiking during apoplastic cell entry that was very similar for both mycorrhizal and rhizobial symbionts (Sieberer et al. 2012). Previously, calcium oscillations induced by AM fungal hyphae were described to be less regular than Nod factor-induced spiking (Kosuta et al. 2008; Chabaud et al. 2011); however, so far there has been no proof of differential decoding of AM and Nod factor-induced calcium signals by CCaMK.

CCaMK forms a complex with the nuclear protein CYCLOPS (*M. truncatula IPD3*) (Singh et al. 2014), which is essential for microbial infection (Messinese et al. 2007; Yano et al. 2008). CYCLOPS was revealed to be a novel type of transcriptional activator, which upon phosphorylation by CCaMK binds to a CYCLOPS responsive *cis* element in the *Nin* promoter and actives *Nin* gene expression (Singh et al. 2014). Phosphorylation of CYCLOPS S50 and S154 is critical for promoter binding and symbiotic development. An autoactive phosphomimetic mutant version of CYCLOPS (S50D/S154D) triggers spontaneous nodule formation independent of CCaMK, indicating that CYCLOPS acts as a master regulator of root nodule organogenesis (Singh et al. 2014).

6.5 Common Symbiosis Genes Involved in Microbial Accommodation

Several AM genes with putative functions in membrane trafficking are also involved in RNS. *M. truncatula Vapyrin* (*Petunia Pam1*) is required for arbuscule formation and efficient fungal entry of the root (Reddy et al. 2007; Feddermann et al. 2010; Pumplin et al. 2010; Murray et al. 2011) and deletion of the gene prevents rhizobial infection threads from reaching the cortical cell layer, resulting in an increased number of uninfected nodule primordia (Murray et al. 2011). *Vapyrin* encodes a protein with an N-terminal VAMP-associated protein (VAP)/major sperm protein (MSP) domain and a C-terminal ankyrin-repeat domain. Based on the domain structure and observed localization in the nucleus, cytosol and in distinct puncta in colonized cells, VAPYRIN was proposed to be involved in membrane trafficking and cellular rearrangement during symbiotic accommodation; however, this has not been experimentally verified (Pumplin et al. 2010; Murray et al. 2011). Two closely related *M. truncatula* vesicle-associated membrane proteins (VAMP) are involved in rhizobium–legume symbiosis and AM (Ivanov et al. 2012). Silencing of both *Vamp721d* and *Vamp721e* inhibits arbuscule and symbiosome formation and blocks bacterial release from the infection thread. Both gene products localize to small vesicles, which accumulate at bacterial release sites near symbiosome membranes and VAMP721e also accumulates at the tips of arbuscule branches, potentially at the periarbuscular membrane (Ivanov et al. 2012). While other VAMP72 proteins in *A. thaliana* are recruited during the interaction with biotrophic fungi (Kwon et al. 2008), VAMP721d/e are not present in the *Arabidopsis* genome. Considering

that *Arabidopsis* has lost several symbiosis genes (Zhu et al. 2006), it is possible that the exocytotic pathway involving VAMP721d/e is specific to perimicrobial membrane synthesis.

6.6 Transcription Factors Involved in Early Symbiotic Responses

Transcriptional regulation is important to integrate signalling pathways and to coordinately regulate molecular networks. The calcium signal that is decoded by CCaMK leads to the expression of subsets of genes. Autoactive CCaMK substitutes for Nod factors to activate expression of *ENOD11*, which is induced by both rhizobial and mycorrhizal infection (Gleason et al. 2006; Journet et al. 2001). CCaMK activation seems to be an intracellular switch that activates transcriptional networks.

Several transcription factors that regulate symbiotic processes have been identified so far. Among them, the GRAS family transcription factors, NSP1 and NSP2 (Nodulation Signalling Pathway 1 and 2), act furthest upstream of the CCaMK-mediated pathway. They were initially identified as factors specific to RNS (Catoira et al. 2000; Oldroyd and Long 2003; Kaló et al. 2005; Smit et al. 2005; Heckmann et al. 2006; Murakami et al. 2006), and then involvement in mycorrhizal colonization was recently identified (Maillet et al. 2011; Delaux et al. 2013). *nsp1* and *nsp2* mutations downregulate expression of *Vapyrin* and *Enod11* (Oldroyd and Long 2003; Hirsch et al. 2009; Murray et al. 2011). This is consistent with the idea that *Nsp1* and *Nsp2* are common to both AM and RNS. In nodulation processes, *nsp1* and *nsp2* mutants exhibit phenotypes similar to loss-of-function *ccamk* mutants in regard to symbiotic root hair responses as well as nodule formation. Root hairs of these mutants are deformed in response to Nod factors, but do not display root hair curling caused by rhizobial infection (Catoira et al. 2000; Oldroyd and Long 2003; Heckmann et al. 2006; Murakami et al. 2006). Autoactive CCaMK mutants do not rescue phenotypes of *nsp1* and *nsp2,* unlike those of common *SYM* mutants defective in the nuclear calcium spiking (Hayashi et al. 2010; Madsen et al. 2010). These results suggest that a site in the nodulation processes where NSP1 and NSP2 act is close to that of CCaMK and that the GRAS family proteins are required for the CCaMK-mediated pathway. How activities of the GRAS proteins are regulated is an important issue for understanding symbiotic signal transduction.

NSP1 and NSP2 form a heterodimer and bind to the promoters of the transcription factors *M. truncatula Ern1* (*ERF Required for Nodulation1*) and *Nin* (*NODULE INCEPTION*) in vitro as well as that of *Enod11* in vitro and in vivo (Hirsch et al. 2009). Expression of *Ern1* and *Nin* is induced by rhizobial infection depending on NSP1 and NSP2 (Murakami et al. 2006; Marsh et al. 2007; Hirsch et al. 2009; Cerri et al. 2012). NSP2 also interacts with an AM-specific GRAS family protein, RAM1 (Required for Arbuscular Mycorrhization1) (Gobbato et al. 2012), which directly targets *Ram2* expression. Both *Ram* genes were identified during a forward genetic screen that aimed to identify loci specifically involved in mycorrhizal signalling (Gobbato et al. 2012; Wang et al. 2012). Multiple dimerization of the GRAS family transcription factors is involved in the regulation of symbiotic processes. The heterodimerization between NSP1 and NSP2 seems to be important for NSP2 function, because an NSP2 derivative with an amino acid substitution in the domain responsible for binding with NSP1 resulted in the reduction of nodulation efficiency (Hirsch et al. 2009).

ERN1 coordinately regulates *Enod11* expression with the NSP1–NSP2 complex by targeting a *cis*-acting element different from those for NSP1–NSP2 complex. ERN1 is required to activate Nod factor-elicited *Enod11* expression during early pre-infection, while NSP1–NSP2 mediates *Enod11* expression during subsequent rhizobial infection (Cerri et al. 2012). NIN is a RWP-RK domain-containing transcription factor specific to and essential for RNS (Schauser et al. 1999). Root hairs of *nin* mutants are deformed and excessively curled in response to rhizobial infection, and failed to initiate infection thread

development. This indicates that NIN acts downstream of CCaMK and the GRAS transcription factors to regulate symbiotic root hair responses (Marsh et al. 2007). *Nin* is activated by cytokinin through a *L. japonicus* cytokinin receptor, LHK1 (Tirichine et al. 2007). Gain-of-function LHK1 spontaneously induces nodules without rhizobial infection (Tirichine et al. 2007). Ectopic expression of *Nin* also induces cortical cell divisions in the absence of rhizobia (Soyano et al. 2013). NIN regulates cortical cell divisions downstream of the cytokinin signalling.

LjNF-YA1 and *LjNF-YB1* have been identified as direct targets of NIN (Soyano et al. 2013). They are involved in stimulation of cell division. They encode different subunits of a CCAAT-box binding heterotrimeric complex. Knock-down of *LjNF-YA1* prevents the nodule formation. Co-overexpression of the two *Lotus NF-Y* genes stimulates cells division in lateral root primordia as well as cortical cell division. NF-Y regulates expression of its target genes by influencing histone modification and requires an additional transcriptional activator to efficiently activate transcription. NIN may also regulate other transcription factors that act together with the NF-Y to induce cortical cell division. *Arabidopsis* NIN-like proteins (NLPs) play a central role in the transcriptional regulation of nitrate-responsive genes and target nitrate-responsive elements (Konishi and Yanagisawa 2013), which are almost identical to NIN-binding nucleotide sequences. Nitrate is known as an inhibitor of nodulation. There may be a linkage between nodulation control and nitrate-response pathways.

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References

Ané JM, Kiss GB, Riely BK, Penmetsa RV, Oldroyd GED, Ayax C, Levy J, Debelle F, Baek JM, Kalo P, Rosenberg C, Roe BA, Long SR, Denarie J, Cook DR (2004) *Medicago truncatula* DMI1 required for bacterial and fungal symbioses in legumes. Science 303:1364–1367

Antolín-Llovera M, Ried MK, Parniske M (2014) Cleavage of the SYMBIOSIS RECEPTOR-LIKE KINASE ectodomain promotes complex formation with nod factor receptor 5. Curr Biol 24(4):422–427. doi:10.1016/j.cub.2013.12.053

Arrighi JF, Barre A, Ben Amor B, Bersoult A, Soriano LC, Mirabella R, de Carvalho-Niebel F, Journet EP, Gherardi M, Huguet T, Geurts R, Denarie J, Rouge P, Gough C (2006) The *Medicago truncatula* lysin [corrected] motif-receptor-like kinase gene family includes NFP and new nodule-expressed genes. Plant Physiol 142:265–279

Bek AS, Sauer J, Thygesen MB, Duus JO, Petersen BO, Thirup S, James E, Jensen KJ, Stougaard J, Radutoiu S (2010) Improved characterization of nod factors and genetically based variation in LysM receptor domains identify amino acids expendable for nod factor recognition in *Lotus* spp. Mol Plant Microbe Interact 23:58–66

Binder A, Parniske M (2013) The Nuclear pore complex in symbiosis and pathogen defense. In: Evans ER, Graumann K, Bryant JA (eds) Annual plant reviews: plant nuclear structure, genome architecture and gene regulation, vol 46. Wiley, Oxford, pp 229–254

Boller T, Felix G (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol 60:379–406

Broghammer A, Krusell L, Blaise M, Sauer J, Sullivan JT, Maolanon N, Vinther M, Lorentzen A, Madsen EB, Jensen KJ, Roepstorff P, Thirup S, Ronson CW, Thygesen MB, Stougaard J (2012) Legume receptors perceive the rhizobial lipochitin oligosaccharide signal molecules by direct binding. Proc Natl Acad Sci USA 109:13859–13864

Capoen W, Sun J, Wysham D, Otegui MS, Venkateshwaran M, Hirsch S, Miwa H, Downie JA, Morris RJ, Ane JM, Oldroyd GE (2011) Nuclear membranes control symbiotic calcium signaling of legumes. Proc Natl Acad Sci USA 108:14348–14353

Catoira R, Galera C, de Billy F, Penmetsa RV, Journet EP, Maillet F, Rosenberg C, Cook D, Gough C, Denarie J (2000) Four genes of Medicago truncatula controlling components of a nod factor transduction pathway. Plant Cell 12:1647–1666

Cerri MR, Frances L, Laloum T, Auriac MC, Niebel A, Oldroyd GE, Barker DG, Fournier J, de Carvalho-Niebel F (2012) *Medicago truncatula* ERN transcription factors: regulatory interplay with NSP1/NSP2 GRAS factors and expression dynamics throughout rhizobial infection. Plant Physiol 160:2155–2172

Chabaud M, Genre A, Sieberer BJ, Faccio A, Fournier J, Novero M, Barker DG, Bonfante P (2011) Arbuscular mycorrhizal hyphopodia and germinated spore exudates trigger Ca^{2+} spiking in the legume and nonlegume root epidermis. New Phytol 189:347–355

Charpentier M, Bredemeier R, Wanner G, Takeda N, Schleiff E, Parniske M (2008) *Lotus japonicus* CASTOR and POLLUX are ion channels essential for perinuclear calcium spiking in legume root endosymbiosis. Plant cell 20:3467–3479

Charpentier M, Vaz Martins T, Granqvist E, Oldroyd GED, Morris RJ (2013) The role of DMI1 in establishing Ca^{2+} oscillations in legume symbioses. Plant Signal Behav 8:e22894

Chen T, Zhu H, Ke D, Cai K, Wang C, Gou H, Hong Z, Zhang Z (2012) A MAP kinase kinase interacts with SymRK and regulates nodule organogenesis in *Lotus japonicus*. Plant Cell 24:823–838

Delaux PM, Becard G, Combier JP (2013) NSP1 is a component of the Myc signaling pathway. New Phytol 199:59–65

Den Herder G, Yoshida S, Antolin-Llovera M, Ried MK, Parniske M (2012) *Lotus japonicus* E3 ligase SEVEN IN ABSENTIA4 destabilizes the symbiosis receptor-like kinase SYMRK and negatively regulates rhizobial infection. Plant Cell 24:1691–1707

Doucet CM, Talamas JA, Hetzer MW (2010) Cell cycle-dependent differences in nuclear pore complex assembly in metazoa. Cell 141:1030–1041

Ehrhardt DW, Wais R, Long SR (1996) Calcium spiking in plant root hairs responding to Rhizobium nodulation signals. Cell 85:673–681

Endre G, Kereszt A, Kevei Z, Mihacea S, Kalo P, Kiss GB (2002) A receptor kinase gene regulating symbiotic nodule development. Nature 417:962–966

Feddermann N, Muni RR, Zeier T, Stuurman J, Ercolin F, Schorderet M, Reinhardt D (2010) The PAM1 gene of petunia required for intracellular accommodation and morphogenesis of arbuscular mycorrhizal fungi encodes a homologue of VAPYRIN. Plant J 64:470–481

Genre A, Chabaud M, Balzergue C, Puech-Pages V, Novero M, Rey T, Fournier J, Rochange S, Becard G, Bonfante P, Barker DG (2013) Short-chain chitin oligomers from arbuscular mycorrhizal fungi trigger nuclear Ca^{2+} spiking in *Medicago truncatula* roots and their production is enhanced by strigolactone. New Phytol 198:190–202

Gimenez-Ibanez S, Ntoukakis V, Rathjen JP (2009) The LysM receptor kinase CERK1 mediates bacterial perception in *Arabidopsis*. Plant Signal Behav 4:539–541

Gleason C, Chaudhuri S, Yang T, Munoz A, Poovaiah BW, Oldroyd GE (2006) Nodulation independent of rhizobia induced by a calcium-activated kinase lacking autoinhibition. Nature 441:1149–1152

Gobbato E, Marsh JF, Vernie T, Wang E, Maillet F, Kim J, Miller JB, Sun J, Bano SA, Ratet P, Mysore KS, Denarie J, Schultze M, Oldroyd GE (2012) A GRAS-type transcription factor with a specific function in mycorrhizal signaling. Curr Biol 22:2236–2241

Granqvist E, Wysham D, Hazledine S, Kozlowski W, Sun J, Charpentier M, Martins TV, Haleux P, Tsaneva-Atanasova K, Downie JA, Oldroyd GE, Morris RJ (2012) Buffering capacity explains signal variation in symbiotic calcium oscillations. Plant Physiol 160:2300–2310

Groth M, Takeda N, Perry J, Uchida H, Draxl S, Brachmann A, Sato S, Tabata S, Kawaguchi M, Wang TL, Parniske M (2010) NENA, a *Lotus japonicus* homolog of Sec13, is required for rhizodermal infection by arbuscular mycorrhiza fungi and rhizobia but dispensable for cortical endosymbiotic development. Plant Cell 22:2509–2526

Gust AA, Willmann R, Desaki Y, Grabherr HM, Nurnberger T (2012) Plant LysM proteins: modules mediating symbiosis and immunity. Trends Plant Sci 17:495–502

Hayashi T, Banba M, Shimoda Y, Kouchi H, Hayashi M, Imaizumi-Anraku H (2010) A dominant function of CCaMK in intracellular accommodation of bacterial and fungal endosymbionts. Plant J 63:141–154

Heckmann AB, Lombardo F, Miwa H, Perry JA, Bunnewell S, Parniske M, Wang TL, Downie JA (2006) *Lotus japonicus* nodulation requires two GRAS domain regulators one of which is functionally conserved in a non-legume. Plant Physiol 142:1739–1750

Hirsch S, Kim J, Munoz A, Heckmann AB, Downie JA, Oldroyd GED (2009) GRAS proteins form a DNA binding complex to induce gene expression during nodulation signaling in Medicago truncatula. Plant Cell 21:545–557

Imaizumi-Anraku H, Takeda N, Charpentier M, Perry J, Miwa H, Umehara Y, Kouchi H, Murakami Y, Mulder L, Vickers K, Pike J, Downie JA, Wang T, Sato S, Asamizu E, Tabata S, Yoshikawa M, Murooka Y, Wu GJ, Kawaguchi M, Kawasaki S, Parniske M, Hayashi M (2005) Plastid proteins crucial for symbiotic fungal and bacterial entry into plant roots. Nature 433:527–531

Ivanov S, Fedorova EE, Limpens E, De Mita S, Genre A, Bonfante P, Bisseling T (2012) Rhizobium-legume symbiosis shares an exocytotic pathway required for arbuscule formation. Proc Natl Acad Sci USA 109:8316–8321

Journet EP, El-Gachtouli N, Vernoud V, de Billy F, Pichon M, Dedieu A, Arnould C, Morandi D, Barker DG, Gianinazzi-Pearson V (2001) *Medicago truncatula* ENOD11: a novel RPRP-encoding early nodulin gene expressed during mycorrhization in arbuscule-containing cells. Mol Plant Microbe Interact 14:737–748

Kalo P, Gleason C, Edwards A, Marsh J, Mitra RM, Hirsch S, Jakab J, Sims S, Long SR, Rogers J, Kiss GB, Downie JA, Oldroyd GED (2005) Nodulation signaling in legumes requires NSP2, a member of the GRAS family of transcriptional regulators. Science 308:1786–1789

Kanamori N, Madsen LH, Radutoiu S, Frantescu M, Quistgaard EM, Miwa H, Downie JA, James EK, Felle HH, Haaning LL, Jensen TH, Sato S, Nakamura Y, Tabata S, Sandal N, Stougaard J (2006) A nucleoporin is required for induction of Ca^{2+} spiking in legume nodule development and essential for rhizobial and fungal symbiosis. Proc Natl Acad Sci USA 103:359–364

Ke D, Fang Q, Chen C, Zhu H, Chen T, Chang X, Yuan S, Kang H, Ma L, Hong Z, Zhang Z (2012) The small GTPase ROP6 interacts with NFR5 and is involved in nodule formation in *Lotus japonicus*. Plant Physiol 159:131–143

Kevei Z, Lougnon G, Mergaert P, Horvath GV, Kereszt A, Jayaraman D, Zaman N, Marcel F, Regulski K, Kiss

GB, Kondorosi A, Endre G, Kondorosi E, Ane JM (2007) 3-hydroxy-3-methylglutaryl coenzyme a reductase 1 interacts with NORK and is crucial for nodulation in *Medicago truncatula*. Plant Cell 19:3974–3989

Kistner C, Parniske M (2002) Evolution of signal transduction in intracellular symbiosis. Trends Plant Sci 7:511–518

Kistner C, Winzer T, Pitzschke A, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Webb KJ, Szczyglowski K, Parniske M (2005) Seven *Lotus japonicus* genes required for transcriptional reprogramming of the root during fungal and bacterial symbiosis. Plant Cell 17:2217–2229

Konishi M, Yanagisawa S (2013) *Arabidopsis* NIN-like transcription factors have a central role in nitrate signalling. Nature Commun 4:1617

Kosuta S, Hazledine S, Sun J, Miwa H, Morris RJ, Downie JA, Oldroyd GE (2008) Differential and chaotic calcium signatures in the symbiosis signaling pathway of legumes. Proc Natl Acad Sci USA 105:9823–9828

Kosuta S, Held M, Hossain MS, Morieri G, Macgillivary A, Johansen C, Antolin-Llovera M, Parniske M, Oldroyd GE, Downie AJ, Karas B, Szczyglowski K (2011) *Lotus japonicus* symRK-14 uncouples the cortical and epidermal symbiotic program. Plant J 67:929–940

Kwon C, Neu C, Pajonk S, Yun HS, Lipka U, Humphry M, Bau S, Straus M, Kwaaitaal M, Rampelt H, El Kasmi F, Jurgens G, Parker J, Panstruga R, Lipka V, Schulze-Lefert P (2008) Co-option of a default secretory pathway for plant immune responses. Nature 451:835–840

Lefebvre B, Timmers T, Mbengue M, Moreau S, Herve C, Toth K, Bittencourt-Silvestre J, Klaus D, Deslandes L, Godiard L, Murray JD, Udvardi MK, Raffaele S, Mongrand S, Cullimore J, Gamas P, Niebel A, Ott T (2010) A remorin protein interacts with symbiotic receptors and regulates bacterial infection. Proc Natl Acad Sci USA 107:2343–2348

Levy J, Bres C, Geurts R, Chalhoub B, Kulikova O, Duc G, Journet EP, Ane JM, Lauber E, Bisseling T, Denarie J, Rosenberg C, Debelle F (2004) A putative Ca^{2+} and calmodulin-dependent protein kinase required for bacterial and fungal symbioses. Science 303:1361–1364

Liao J, Singh S, Hossain MS, Andersen SU, Ross L, Bonetta D, Zhou Y, Sato S, Tabata S, Stougaard J, Szczyglowski K, Parniske M (2012) Negative regulation of CCaMK is essential for symbiotic infection. Plant J 72:572–584

Liu J, Blaylock LA, Endre G, Cho J, Town CD, VandenBosch KA, Harrison MJ (2003) Transcript profiling coupled with spatial expression analyses reveals genes involved in distinct developmental stages of an arbuscular mycorrhizal symbiosis. Plant Cell 15:2106–2123

Liu TT, Liu ZX, Song CJ, Hu YF, Han ZF, She J, Fan FF, Wang JW, Jin CW, Chang JB, Zhou JM, Chai JJ (2012) Chitin-induced dimerization activates a plant immune receptor. Science 336:1160–1164

Lohmann GV, Shimoda Y, Nielsen MW, Jorgensen FG, Grossmann C, Sandal N, Sorensen K, Thirup S, Madsen LH, Tabata S, Sato S, Stougaard J, Radutoiu S (2010) Evolution and regulation of the *Lotus japonicus* LysM receptor gene family. Mol Plant Microbe Interact 23:510–521

Lopez-Lara IM, van den Berg JD, Thomas-Oates JE, Glushka J, Lugtenberg BJ, Spaink HP (1995) Structural identification of the lipo-chitin oligosaccharide nodulation signals of Rhizobium loti. Mol Microbiol 15:627–638

Madsen EB, Antolin-Llovera M, Grossmann C, Ye JY, Vieweg S, Broghammer A, Krusell L, Radutoiu S, Jensen ON, Stougaard J, Parniske M (2011) Autophosphorylation is essential for the in vivo function of the *Lotus japonicus* nod factor receptor 1 and receptor-mediated signalling in cooperation with nod factor receptor 5. Plant J 65:404–417

Madsen EB, Madsen LH, Radutoiu S, Olbryt M, Rakwalska M, Szczyglowski K, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J (2003) A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals. Nature 425:637–640

Madsen LH, Tirichine L, Jurkiewicz A, Sullivan JT, Heckmann AB, Bek AS, Ronson CW, James EK, Stougaard J (2010) The molecular network governing nodule organogenesis and infection in the model legume *Lotus japonicus*. Nat Commun 1:1–12

Maillet F, Poinsot V, Andre O, Puech-Pages V, Haouy A, Gueunier M, Cromer L, Giraudet D, Formey D, Niebel A, Martinez EA, Driguez H, Becard G, Denarie J (2011) Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature 469:58–63

Markmann K, Giczey G, Parniske M (2008) Functional adaptation of a plant receptor-kinase paved the way for the evolution of intracellular root symbioses with bacteria. PLoS Biol 6(3):e68. doi:10.1371/journal.pbio.0060068

Marsh JF, Rakocevic A, Mitra RM, Brocard L, Sun J, Eschstruth A, Long SR, Schultze M, Ratet P, Oldroyd GE (2007) *Medicago truncatula* NIN is essential for rhizobial-independent nodule organogenesis induced by autoactive calcium/calmodulin-dependent protein kinase. Plant Physiol 144:324–335

Meinema AC, Laba JK, Hapsari RA, Otten R, Mulder FA, Kralt A, van den Bogaart G, Lusk CP, Poolman B, Veenhoff LM (2011) Long unfolded linkers facilitate membrane protein import through the nuclear pore complex. Science 333:90–93

Messinese E, Mun JH, Yeun LH, Jayaraman D, Rouge P, Barre A, Lougnon G, Schornack S, Bono JJ, Cook DR, Ane JM (2007) A novel nuclear protein interacts with the symbiotic DMI3 calcium- and calmodulin-dependent protein kinase of Medicago truncatula. Mol Plant Microbe Interact 20:912–921

Mitra RM, Gleason CA, Edwards A, Hadfield J, Downie JA, Oldroyd GE, Long SR (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, Sun J, Oldroyd GED, Downie JA (2006) Analysis of nod-factor-induced calcium signaling in root hairs of

symbiotically defective mutants of *Lotus japonicus*. Mol Plant Microbe Interact 19:914–923

Miya A, Albert P, Shinya T, Desaki Y, Ichimura K, Shirasu K, Narusaka Y, Kawakami N, Kaku H, Shibuya N (2007) CERK1, a LysM receptor kinase is essential for chitin elicitor signaling in *Arabidopsis*. Proc Natl Acad Sci USA 104:19613–19618

Mulligan JT, Long SR (1985) Induction of rhizobium meliloti nodC expression by plant exudate requires nodD. Proc Natl Acad Sci USA 82:6609–6613

Murakami Y, Miwa H, Imaizumi-Anraku H, Kouchi H, Downie JA, Kawaguchi M, 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

Murray JD, Muni RR, Torres-Jerez I, Tang Y, Allen S, Andriankaja M, Li G, Laxmi A, Cheng X, Wen J, Vaughan D, Schultze M, Sun J, Charpentier M, Oldroyd G, Tadege M, Ratet P, Mysore KS, Chen R, Udvardi MK (2011) Vapyrin a gene essential for intracellular progression of arbuscular mycorrhizal symbiosis is also essential for infection by rhizobia in the nodule symbiosis of *Medicago truncatula*. Plant J 65:244–252

Nakagawa T, Kaku H, Shimoda Y, Sugiyama A, Shimamura M, Takanashi K, Yazaki K, Aoki T, Shibuya N, Kouchi H (2011) From defense to symbiosis: limited alterations in the kinase domain of LysM receptor-like kinases are crucial for evolution of legume-rhizobium symbiosis. Plant J 65:169–180

Oldroyd GE, Long SR (2003) Identification and characterization of nodulation-signaling pathway 2, a gene of *Medicago truncatula* involved in nod actor signaling. Plant Physiol 131:1027–1032

Oldroyd GE, Murray JD, Poole PS, Downie JA (2011) The rules of engagement in the legume-rhizobial symbiosis. Annu Rev Genet 45:119–144

Peters NK, Frost JW, Long SR (1986) A plant flavone luteolin induces expression of rhizobium-meliloti nodulation genes. Science 233:977–980

Pumplin N, Mondo SJ, Topp S, Starker CG, Gantt JS, Harrison MJ (2010) *Medicago truncatula* vapyrin is a novel protein required for arbuscular mycorrhizal symbiosis. Plant J 61:482–494

Radutoiu S, Madsen LH, Madsen EB, Felle HH, Umehara Y, Gronlund M, Sato S, Nakamura Y, Tabata S, Sandal N, Stougaard J (2003) Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. Nature 425:585–592

Radutoiu S, Madsen LH, Madsen EB, Jurkiewicz A, Fukai E, Quistgaard EM, Albrektsen AS, James EK, Thirup S, Stougaard J (2007) LysM domains mediate lipochitin-oligosaccharide recognition and Nfr genes extend the symbiotic host range. EMBO J 26:3923–3935

Reddy DMR, Schorderet S, Feller U, Reinhardt D (2007) A petunia mutant affected in intracellular accommodation and morphogenesis of arbuscular mycorrhizal fungi. Plant J 51:739–750

Riely BK, Lougnon G, Ane JM, Cook DR (2007) The symbiotic ion channel homolog DMI1 is localized in the nuclear membrane of *Medicago truncatula* roots. Plant J 49:208–216

Rodpothong P, Sullivan JT, Songsrirote K, Sumpton D, Cheung KW, Thomas-Oates J, Radutoiu S, Stougaard J, Ronson CW (2009) Nodulation gene mutants of *Mesorhizobium loti* R7A-nodZ and nolL mutants have host-specific phenotypes on *Lotus* spp. Mol Plant Microbe Interact 22:1546–1554

Routray P, Miller JB, Du L, Oldroyd G, Poovaiah BW (2013) Phosphorylation of S344 in the calmodulin-binding domain negatively affects CCaMK function during bacterial and fungal symbioses. Plant J 76 (2):287–296

Sacchettini JC, Baum LG, Brewer CF (2001) Multivalent protein-carbohydrate interactions. A new paradigm for supermolecular assembly and signal transduction. Biochemistry 40:3009–3015

Saito K, Yoshikawa M, Yano K, Miwa H, Uchida H, Asamizu E, Sato S, Tabata S, Imaizumi-Anraku H, Umehara Y, Kouchi H, Murooka Y, Szczyglowski K, Downie JA, Parniske M, Hayashi M, Kawaguchi M (2007) NUCLEOPORIN85 is required for calcium spiking fungal and bacterial symbioses and seed production in *Lotus japonicus*. Plant Cell 19:610–624

Sanchez-Vallet A, Saleem-Batcha R, Kombrink A, Hansen G, Valkenburg DJ, Thomma BP, Mesters JR (2013) Fungal effector Ecp6 outcompetes host immune receptor for chitin binding through intrachain LysM dimerization. Elife 2:e00790

Sathyanarayanan PV, Cremo CR, Poovaiah BW (2000) Plant Chimeric Ca^{2+}/Calmodulin-dependent Protein Kinase: role of the neural visinin-like domain in regulating autophosphorylation and calmodulin affinity. J Biol Chem 275:30417–30422

Schauser L, Roussis A, Stiller J, Stougaard J (1999) A plant regulator controlling development of symbiotic root nodules. Nature 402:191–195

Schlaman HR, Horvath B, Vijgenboom E, Okker RJ, Lugtenberg BJ (1991) Suppression of nodulation gene expression in bacteroids of rhizobium leguminosarum biovar viciae. J Bacteriol 173:4277–4287

Sieberer BJ, Chabaud M, Timmers AC, Monin A, Fournier J, Barker DG (2009) A nuclear-targeted cameleon demonstrates intranuclear Ca^{2+} spiking in *Medicago truncatula* root hairs in response to rhizobial nodulation factors. Plant Physiol 151:1197–1206

Sieberer BJ, Chabaud M, Fournier J, Timmers AC, Barker DG (2012) A switch in Ca^{2+} spiking signature is concomitant with endosymbiotic microbe entry into cortical root cells of *Medicago truncatula*. Plant J 69:822–830

Singh S, Parniske M (2012) Activation of calcium- and calmodulin-dependent protein kinase (CCaMK), the central regulator of plant root endosymbiosis. Curr Opin Plant Biol 15:444–453

Singh S, Katzer K, Lambert J, Cerri M, Parniske M (2014) CYCLOPS, a DNA binding transcriptional

activator orchestrates symbiotic root nodule development. Cell Host Microbe 15(2):139–152

Smit P, Raedts J, Portyanko V, Debelle F, Gough C, Bisseling T, Geurts R (2005) NSP1 of the GRAS protein family is essential for rhizobial nod factor-induced transcription. Science 308:1789–1791

Soyano T, Kouchi H, Hirota A, Hayashi M (2013) Nodule inception directly targets NF-Y subunit genes to regulate essential processes of root nodule development in *Lotus japonicus*. PLoS Genet 9:e1003352. doi:10.1371/journal.pgen.1003352

Spaink HP, Okker RJ, Wijffelman CA, Tak T, Goosen-de Roo L, Pees E, van Brussel AA, Lugtenberg BJ (1989) Symbiotic properties of rhizobia containing a flavonoid-independent hybrid nodD product. J Bacteriol 171:4045–4053

Spaink HP, Sheeley DM, van Brussel AA, Glushka J, York WS, Tak T, Geiger O, Kennedy EP, Reinhold VN, Lugtenberg BJ (1991) A novel highly unsaturated fatty acid moiety of lipo-oligosaccharide signals determines host specificity of Rhizobium. Nature 354:125–130

Stracke S, Kistner C, Yoshida S, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Szczyglowski K, Parniske M (2002) A plant receptor-like kinase required for both bacterial and fungal symbiosis. Nature 417:959–962

Swainsbury DJ, Zhou L, Oldroyd GE, Bornemann S (2012) Calcium ion binding properties of *Medicago truncatula* calcium/calmodulin-dependent protein kinase. Biochemistry 51:6895–6907

Takezawa D, Ramachandiran S, Paranjape V, Poovaiah BW (1996) Dual regulation of a chimeric plant serine/threonine kinase by calcium and calcium/calmodulin. J Biol Chem 271:8126–8132

Timmers ACJ, Auriac MC, de Billy F, Truchet G (1998) Nod factor internalization and microtubular cytoskeleton changes occur concomitantly during nodule differentiation in alfalfa. Development 125:339–349

Tirichine L, Imaizumi-Anraku H, Yoshida S, Murakami Y, Madsen LH, Miwa H, Nakagawa T, Sandal N, Albrektsen AS, Kawaguchi M, Downie A, Sato S, Tabata S, Kouchi H, Parniske M, Kawasaki S, Stougaard J (2006) Deregulation of a Ca^{2+}/calmodulin-dependent kinase leads to spontaneous nodule development. Nature 441:1153–1156

Tirichine L, Sandal N, Madsen LH, Radutoiu S, Albrektsen AS, Sato S, Asamizu E, Tabata S, Stougaard J (2007) A gain-of-function mutation in a cytokinin receptor triggers spontaneous root nodule organogenesis. Science 315:104–107

Tóth K, Stratil TF, Madsen EB, Ye J, Popp C, Antolin-Llovera M, Grossmann C, Jensen ON, Schussler A, Parniske M, Ott T (2012) Functional domain analysis of the remorin protein LjSYMREM1 in *Lotus japonicus*. PLoS ONE 7:e30817. doi:10.1371/journal.pone.0030817

Truchet G, Roche P, Lerouge P, Vasse J, Camut S, de Billy F, Prome J-C, Denarie J (1991) Sulphated lipo-oligosaccharide signals of Rhizobium meliloti elicit root nodule organogenesis in alfalfa. Nature 351:670–673

Venkateshwaran M, Cosme A, Han L, Banba M, Satyshur KA, Schleiff E, Parniske M, Imaizumi-Anraku H, Ane JM (2012) The recent evolution of a symbiotic ion channel in the legume family altered ion conductance and improved functionality in calcium signalling. Plant Cell 24:2528–2545

Walther TC, Alves A, Pickersgill H, Loiodice I, Hetzer M, Galy V, Hulsmann BB, Kocher T, Wilm M, Allen T, Mattaj IW, Doye V (2003) The conserved Nup107-160 complex is critical for nuclear pore complex assembly. Cell 113:195–206

Wan J, Zhang XC, Neece D, Ramonell KM, Clough S, Kim SY, Stacey MG, Stacey G (2008) A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in *Arabidopsis*. Plant Cell 20:471–481

Wang E, Schornack S, Marsh JF, Gobbato E, Schwessinger B, Eastmond P, Schultze M, Kamoun S, Oldroyd GE (2012) A common signaling process that promotes mycorrhizal and oomycete colonization of plants. Curr Biol 22:2242–2246

Yano K, Yoshida S, Muller J, Singh S, Banba M, Vickers K, Markmann K, White C, Schuller B, Sato S, Asamizu E, Tabata S, Murooka Y, Perry J, Wang TL, Kawaguchi M, Imaizumi-Anraku H, Hayashi M, Parniske M (2008) CYCLOPS, a mediator of symbiotic intracellular accommodation. Proc Natl Acad Sci USA 105:20540–20545

Zhu H, Riely BK, Burns NJ, Ane JM (2006) Tracing nonlegume orthologs of legume genes required for nodulation and arbuscular mycorrhizal symbioses. Genetics 172:2491–2499

Zhukov V, Radutoiu S, Madsen LH, Rychagova T, Ovchinnikova E, Borisov A, Tikhonovich I, Stougaard J (2008) The pea Sym37 receptor kinase gene controls infection-thread initiation and nodule development. Mol Plant Microbe Interact 21:1600–1608