Lipochitooligosaccharides Modulate Plant Host Immunity to Enable Endosymbioses

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Abstract

Symbiotic nitrogen-fixing rhizobium bacteria and arbuscular mycorrhizal fungi use lipochitooligosaccharide (LCO) signals to communicate with potential host plants. Upon a compatible match, an intimate relation is established during which the microsymbiont is allowed to enter root (-derived) cells. Plants perceive microbial LCO molecules by specific LysM-domain-containing receptor-like kinases. These do not only activate a common symbiosis signaling pathway that is shared in both symbioses but also modulate innate immune responses. Recent studies revealed that symbiotic LCO receptors are closely related to chitin innate immune receptors, and some of these receptors even function in symbiosis as well as immunity. This raises questions about how plants manage to translate structurally very similar microbial signals into different outputs. Here, we describe the current view on chitin and LCO perception in innate immunity and endosymbiosis and question how LCOs might modulate the immune system. Furthermore, we discuss what it takes to become an endosymbiont.

INTRODUCTION

Plants are continuously exposed to microbes that range from beneficial to pathogenic. Plant roots, in particular, encounter massive numbers of microbes, known as the soil-root microbiome (17, 118). Two of the most extensively studied examples of beneficial microbes are arbuscular mycorrhizal (AM) fungi and nitrogen-fixing rhizobium bacteria. These microbes can live in intimate contact with their plant hosts and are intracellularly accommodated to establish an endosymbiotic relation. AM fungi are hosted inside the root inner cortex cells of the vast majority of land plants. Their highly branched hyphae are contained within specialized host membrane compartments to form host-microbe interface structures called arbuscules. The resulting symbiotic interface facilitates the exchange of nutrients, such as phosphorus and nitrogen, for photosynthates (66). Rhizobium bacteria establish an endosymbiosis with leguminous plants, which results in formation of a novel organ, the root nodule. Inside nodule cells, rhizobium bacteria are hosted as transient nitrogen-fixing organelles called symbiosomes. Symbiosomes contain a plant-derived outer membrane that forms a symbiotic interface between plant cytoplasm and bacterium, which facilitates the exchange of nutrients between both partners. At the other end of the spectrum, pathogenic microbes can invade plant roots and extract nutrients at the expense of the plant. Biotrophic pathogens colonize living plant cells, where they form feeding structures, such as haustoria, that function as interfaces between plants and microbes, which are analogous to symbiotic interfaces.

Given the plethora of microbes that roots are exposed to, plants must be able to distinguish between friend and foe. Like pathogens, symbiotic microbes are initially recognized as foreign organisms, which triggers the plant innate immune system (188). Microbes typically express a range of microbe/pathogen-associated molecular patterns (MAMPs/PAMPs) that are perceived by plant pattern recognition receptors to activate immune responses. This forms a first, basal level of defense to block microbial penetration of plant cells (19, 81, 136). Therefore, endosymbionts like rhizobium and AM fungi have to modulate the innate immune system.

To establish symbiosis and evade innate immune responses, symbiotic microbes engage in a molecular dialog with potential plant hosts. Microbial lipochitooligosaccharides (LCOs) have been identified as the key signals to allow entry of the microbes into plant root cells. The use of LCOs appears to have been invented at least two times independently in evolution to establish an endosymbiosis with plants, first by AM fungi and later by rhizobium bacteria and possibly also by actinorhizal *Frankia* bacteria (168, 169). LCOs are structurally related to MAMPs such as chitin-oligosaccharides and peptidoglycans. In recent years, it has become clear that perception of all these chitin-like molecules involves related LysM (lysin motif)-domain-containing receptor-like proteins. Furthermore, recent data indicate that perception of symbiotic LCOs is much more intertwined with innate immune signaling than previously imagined. This raises questions about how plants manage to integrate very similar microbial signals to distinguish friend from foe. Here, we review our current understanding of the perception of chitin-like molecules in endosymbioses and innate immune signaling and discuss how LCOs might modulate the immune system. Furthermore, we discuss what it takes to become an endosymbiont.

The Ancient Arbuscular Mycorrhizal Symbiosis

AM symbiosis between plants and fungi of the Glomeromycota phylum is thought to have originated ~450 million years ago in the Devonian era (134, 145). Fossil records from that period suggest that since then AM symbiosis has remained largely unchanged (146). Fungal colonization of the root generally starts with formation of a hyphal hyphopodium at the epidermis. Subsequently, the plant actively facilitates the entry of fungal hyphae by formation of a prepenetration

apparatus through remodeling of its cytoskeleton (56). After crossing the epidermis, fungal hyphae mostly spread intercellularly until they reach root inner cortex cells, where the hyphae branch and form intracellular arbuscules. During arbuscule formation, the hyphae become surrounded by a specialized host membrane to form an optimized symbiotic interface that facilitates the efficient exchange of nutrients (66). This interface lacks a structured cell wall, which implies that extensive modulation of the host cell wall occurs (11). Recently, unraveling of the genome sequence of the model AM fungus *Rhizophagus irregularis* revealed that this species lacks cell wall–degrading enzymes (100, 171). This indicates that AM fungi mostly rely on their plant hosts to deliver cell wall–remodeling enzymes to facilitate infection. Such enzymes might be delivered through a symbiosis-dedicated exocytosis pathway. Two homologous v-SNARE proteins, part of a symbiotic clade, have been identified in the legume *Medicago truncatula*, which are essential for the formation of the cell wall–free interface in both AM and rhizobial symbioses (79). Because fungal cell wall–degrading enzymes can release so-called damage-associated molecular patterns that trigger defense responses (14, 47), the absence of cell wall–degrading enzymes in AM fungi may therefore be a key adaptation to avoid a strong defense response.

Signals in Arbuscular Mycorrhizal Symbiosis

AM fungi sense the presence of potential host plants by plant secreted signals such as strigolactones, 2-hydroxy fatty acids, and flavonoids (124). Perception of these molecules stimulates growth and branching of fungal hyphae, which increase the chance to contact the roots of a host plant. In turn, AM fungi release diffusible signals that are perceived by the host and induce symbiosis-specific responses. Typical responses include the induction of calcium oscillations in and around the nucleus (known as calcium spiking), stimulation of lateral root formation, activation of symbiosis-specific genes, and branching of root hairs (29, 88–91). The nature of such AM signals was recently revealed by purifying exudate fractions of in vitro root cultures infected with *R. irregularis* and from germinated fungal spores (55, 109). By testing these fractions in various bioassays for symbiotic responses, it was found that *R. irregularis* makes acylated chitin-like molecules called myc-LCOs. myc-LCOs consist of β-1-4-linked *N*-acetyl glucosamine (GlcNac) residues with an acyl chain at the nonreducing residue (109). *R. irregularis* produces a mix of sulfated and nonsulfated tetrameric and pentameric LCOs mostly acylated with either an oleic acid (C18:1) or palmitic acid (C16:0) (109). Application of such myc-LCO mixtures at subnanomolar concentrations stimulates mycorrhization in a variety of plant species. This indicates that myc-LCOs are efficiently perceived by a broad range of plants.

A key response that is triggered by myc-LCOs is the activation of calcium spiking, which forms a central response in a genetically identified signaling network. Initially, this network has been characterized in two legume species: *M. truncatula* and *Lotus japonicus*. Because the identified genes are essential for both AM and rhizobial symbioses, the genetic network is referred to as the common symbiosis signaling pathway (129, 134). This pathway consists of an LRR (leucine-rich repeat)-receptor kinase (named LjSYMRK in *L. japonicus* and MtDMI2 in *M. truncatula*) (42, 167), a putative cation channel located at the nuclear envelope (LjCASTOR, LjPOLLUX, and MtDMI1) (3, 78, 135, 151), components of the nuclear pore (64, 84, 154), and a calcium channel (MtMCA8) (27). These components are all required to induce calcium spiking (30, 160). These regular oscillations in calcium concentration induced upon LCO perception are decoded by a nuclear-localized calcium-calmodulin-dependent kinase (CCaMK; in *M. truncatula* also called MtDMI3) that interacts directly with the transcription factor LjCYCLOPS/MtIPD3 (75, 119, 185). Phylogenetic studies revealed that the core components of the common symbiosis signaling

pathway are conserved in the earliest land plants (37, 177). This is in line with the ancient origin of the AM symbiosis and the fact that AM fungi have an extremely wide host range.

In addition to LCOs, short-chain chitooligosaccharides consisting of four or five GlcNAc residues that lack an acyl chain have been proposed to play a role in AM symbiosis (55). Tetrameric and pentameric chitooligosaccharides were identified in germinated spore exudates at markedly higher concentration than LCOs and their secretion was induced by the application of strigolactones. Such short-chain chitooligosaccharides are also able to trigger calcium oscillations in epidermal cells of legume as well as nonlegume (cultured) roots (29, 55). Like myc-LCO signaling, chitooligosaccharide-induced signaling is dependent on the common symbiosis signaling pathway. However, in contrast to myc-LCOs, short-chain chitooligosaccharides fail to stimulate formation of lateral roots (109). Further, the calcium signatures that are induced by chitin oligomers differ from the calcium spikes induced upon mycorrhizal (or rhizobial) infection or LCO application. Both mycorrhizal infection (cell entry) and LCO perception are correlated with high frequency and regular calcium spiking, whereas short-chain chitin oligomers induce irregular calcium spikes that are less frequent (55, 159). Low frequency calcium spiking has been correlated with preinfection stages (159). Therefore, the output of these calcium spikes may be different. These studies also indicate that perception of LCOs and chitin oligomers in root organ cultures differs markedly from that observed in intact plants. In contrast to intact plants, root organ cultures appear to be more than 1,000 times less sensitive to LCOs. This might be explained by an altered hormone balance in the root cultures. Therefore, it remains to be determined what role short-chain chitin oligomers play in mycorrhization in intact plants, and how they cross talk with myc-LCO signaling.

Rhizobium Co-Opted Lipochitooligosaccharide Biosynthesis Through Convergent Evolution

myc-LCOs are structurally similar to LCOs produced by symbiotic nitrogen-fixing rhizobium bacteria (also known as Nod factors) (95, 109). Rhizobium bacteria secrete LCOs in response to a specific blend of among others flavonoids released by plant roots. Flavonoids activate the transcriptional activator NodD, which in turn induces rhizobial nodulation (*nod*) genes to produce LCOs (31). Rhizobium LCOs control several key processes in symbiosis. They are essential, and even sufficient, to induce formation of root nodules. Nodule organogenesis starts with the reprogramming of root cortical cells that reenter the cell cycle to form a nodule primordium (183). These primordium cells acquire the ability to take up rhizobium bacteria intracellularly. However, to ensure their uptake the bacteria need to reach the primordium cells at the right time. Therefore, the process of nodule organogenesis needs to be tightly coordinated with the entry of the bacteria into the roots, i.e., the infection process. This infection process is also controlled by rhizobium LCO signaling and can be genetically uncoupled from the organogenesis program (141). Depending on the legume species, different modes of root infection have been identified (164). The best studied and most common mechanism involves the formation of so-called infection threads that initiate in the root hairs (52). Rhizobium bacteria attach to the root hairs and upon LCO signaling induce continuous reorientation of the growth direction of the root hair to entrap the bacteria in a closed cavity. From there, a cell wall–bound tube-like structure, the infection thread, is formed that guides the bacteria to the nodule primordium cells. At this point, the bacteria are first able to release from the infection threads to be taken up into the nodule cells and form symbiosomes. Also, this process depends on rhizobium LCO signaling (123). The formation of infection threads in the root hairs is most sensitive to structural variations in the LCO molecules, which thereby play a major role in determining host specificity (8, 58, 137, 143, 161).

Table 1 Overview of symbiotic rhizobia in the orders Rhizobiales and Burkholderiales

α-Proteobacteria			
Rhizobiales	**Genera**	**Number of symbiotic genera**	**Symbiotic genera**
Aurantimonadaceae	4	–	
Bartonellaceae	1	–	
Beijerinckiaceae	8	–	
Bradyrhizobiaceae	12	2	*Bosea*, *Bradyrhizobium*
Brucellaceae	6	1	*Ochrobactrum*
Cohaesibacteraceae	2	–	
Hyphomicrobiaceae	21	1	*Devosia*
Methylobacteriaceae	3	2	*Methylobacterium*, *Microvirga*
Methylocystaceae	8	–	
Phyllobacteriaceae	12	3	*Aminobacter*, *Mesorhizobium*, *Phyllobacterium*
Rhizobiaceae	6	3	*Rhizobium/Agrobacterium*, *Sinella*, *Sinorhizobium*
Rhodobiaceae	9	–	
Xanthobacteraceae	7	1	*Azorhizobium*
β-Proteobacteria			
Burkholderiales			
Alcaligenaceae	20	–	
Burkholderiaceae	12	2	*Burkholderia*, *Cupriavidus*
Oxalobacteraceae	15	–	
Sutterellaceae	2	–	

Nitrogen-fixing rhizobium symbionts form a polyphyletic group representing 13 genera within the α-proteobacteria and 2 genera within the β-proteobacteria [**Table 1**] (115, 155). Symbiotic rhizobium bacteria have in common the fact that they combine two genetic traits, namely a set of nitrogen fixation (*nif*) genes that encode the nitrogenase enzyme complex and a set of (*nod*) genes that allow LCO biosynthesis. The nitrogen fixation trait has an ancient origin that dates back 1.5–2.2 billion years (22). This trait was repeatedly horizontally transmitted to a diverse range of microbial species, including those that have given birth to current rhizobium symbionts (39, 144). An ancestral rhizobium symbiont managed, most probably as a unique evolutionary event, to combine the nitrogen fixation trait with its ability to produce LCOs. This event must have occurred at least 60 million years ago, prior to, or coinciding with, the birth of nitrogen-fixing root nodules in legumes (40). Once established, the unique combination of nitrogen fixation and LCO biosynthesis spread via lateral gene transfer (186). Such spreading is especially prominent within the α-proteobacteria [**Table 1**]. These rhizobium symbionts have organized the nitrogen fixation and LCO biosynthesis genes on symbiotic plasmids or as symbiotic islands in their genomes.

Symbiotic rhizobium species have in common a set of only five core *nod* genes, but generally contain many more lineage-specific genes that allow variation in LCO structure. The core *nod* genes encode an N-acetylglucosaminyltransferase (NodC) that synthesizes the chitin tetramer or pentamer backbone, a chitooligosaccharide deacetylase (NodB) that removes the acetyl group of the nonreducing glucosamine, and an N-acyltransferase (NodA) that subsequently attaches a C16–C20 lipid tail to this position. Two additional core genes, *nodI* and *nodJ*, encode an ATP

binding cassette (ABC) transporter that facilitates LCO secretion (28, 46, 163). As biosynthesis of chitin oligomers is not a common feature of prokaryotes, it raises questions about the evolutionary origin of these core *nod* genes. A survey of the *R. irregularis* genome sequence failed to identify homologs of these genes. Therefore, it is unlikely that an ancestral rhizobium acquired the ability to synthesize LCOs via lateral gene transfer from AM fungi. In search for paralogs of core *nod* genes, it was found that *Burkholderia* species (β-proteobacteriales) harbor paralogs of *nodI* and *nodJ*. The genus *Burkholderia* is made up of many symbiotic rhizobia, and their symbiotic capacity is considered to be more than 50 million years old (4, 20). Interestingly, *nodI-nodJ* paralogous genes are absent in nonsymbiotic α-proteobacteria, and phylogenetic analyses suggest that at least the rhizobial LCO transport system originates from ancestral *Burkholderia* (7). However, the origin of *nodI* and *nodJ* is not necessarily indicative for the origin of LCO biosynthesis in symbiotic rhizobia. Similar studies with *nodA*, *nodB*, and *nodC* did not identify putative paralogous genes, despite massive (meta) genome sequencing programs. Therefore, an alternative scenario in which LCO biosynthesis genes evolved first in a different, yet unknown, species cannot be ruled out. Only upon emergence of the *nodABC* operon in *Burkholderia* via lateral gene transfer were the *nodI* and *nodJ* genes recruited to facilitate LCO secretion. As symbiotic α-proteobacteria display greater sequence diversity in *nodABC* compared to *Burkholderia*, it is a likely scenario that this operon evolved first in an ancestral α-proteobacterial species (7, 20).

Lipochitooligosaccharide Perception in Symbiosis Intertwines with Innate Immune Signaling

Rhizobium- and myc-LCOs are structurally very similar and both are perceived by related LysM-domain-containing receptor-like kinases (LysM-RKs). The LysM domain is a widely distributed protein domain that is generally thought to bind GlcNAc molecules (25). Unique to plants is the presence of LysM domains in the extracellular part of transmembrane receptor kinases (190). LysM-RKs are characterized by an extracellular part with one to three LysM domains for which a single evolutionary origin has been suggested (190).

Genetic and biochemical studies in *L. japonicus* and *M. truncatula* have led to the identification of two different LysM-RKs that are essential for rhizobium LCO perception and signaling: LjNFR1/MtLYK3 and LjNFR5/MtNFP (9, 24, 99, 107, 142). Strikingly, LjNFR5 and MtNFP lack critical amino acids in the kinase domain and do not show autophosphorylation, indicating that they have a dead kinase (9, 106, 107). However, deletion of the intracellular kinase domain abolishes the activity of MtNFP (93, 140), which suggests that specific binding partners confer the signaling role of these receptors. LjNFR5 and MtNFP have been shown to form a complex with LjNFR1 and MtLYK3, respectively, in addition to both proteins forming homomers (106, 123). LjNFR1 and MtLYK3 have a functional kinase domain, and heteromerization with LjNFR5/MtNFP is therefore proposed to constitute a functional rhizobium LCO receptor complex (106, 140). In addition, LjNFR1 and LjNFR5 were also found to interact with LjSYMRK, and ectopic expression of the Nod-factor receptors or SYRMK initiates spontaneous nodule organogenesis in *L. japonicus* in the absence of rhizobia (6, 150). LjNFR5 was further shown to interact with a small Rho-like GTPase LjROP6 (86). Both proteins, LjSYMRK/MtDMI2 and LjROP6, likely in conjunction with LjNFR5/MtNFP, play a role in rhizobial infection.

Interestingly, myc-LCOs are also perceived by MtNFP, as the ability of myc-LCOs to induce lateral root formation in *M. truncatula* is dependent on *MtNFP* (109). Furthermore, early transcriptional changes induced by myc-LCOs are largely dependent on MtNFP (32). Rhizobium- and myc-LCOs each trigger their own set of transcriptional changes, and differences were even observed between sulfated and nonsulfated myc-LCOs. This suggests that additional (co)receptors

are involved. The involvement of additional myc-LCO (co)receptors is also supported by the fact that *Ljnfr5/Mtnfp* knockout mutants are not impaired in mycorrhizal colonization (15, 142). In *M. truncatula*, a paralog of *MtNFP*, *MtLYR1*, is upregulated upon mycorrhization (53, 62, 74). Therefore, it has been proposed that duplication of an ancient LjNFR5/MtNFP-like myc-LCO receptor allowed one copy to obtain a new function in Nod factor signaling while the other maintained its role in mycorrhization (130). This hypothesis is supported by the observation that in *Parasponia*, the only nonlegume species able to establish a rhizobium symbiosis, only a single *LjNFR5/MtNFP* ortholog is present and is required for the intracellular accommodation of both rhizobia and AM fungi (130). As *Parasponia* obtained the ability to nodulate relatively recently and independently from legumes (13), this strongly suggests that perception of rhizobium LCOs evolved from the ancient myc-LCO perception mechanism (168). A recent origin of nodulation in *Parasponia* also fits with the primitive nature of rhizobium infection of this species. Whereas most legumes guide their rhizobial symbionts via intracellular infection threads toward the newly formed root nodule, *Parasponia* allow rhizobium to enter first apoplastically via a mechanism known as crack entry (131). Only when it reaches the nodule is rhizobium hosted intracellularly. However, it is not hosted as a symbiosome but rather in so-called fixation threads that have some resemblance to mycorrhizal arbuscules. Knockdown of the *Parasponia andersonii NFP* ortholog (*PaNFP*) specifically blocks the formation of fixation threads as well as arbuscules, suggesting that this process may in fact be the generic function of symbiotic LCO signaling. In legumes, this mechanism evolved to allow more strict control over rhizobium infection and in some legumes coevolved into a highly specific plant-rhizobium partner selection mechanism (137). Whether MtLYR1 is indeed involved in myc-LCO perception remains to be demonstrated, but additional LCO (co)receptors may also be involved. Recently, it was shown that MtLYR3 has a high affinity for LCOs and is expressed in roots and nodules (48). MtLYR3 and MtNFP are the result of ancient tandem duplication, and their genomic location also shows synteny with the MtLYR1 genomic region (9). Therefore, MtLYR3 may represent an additional coreceptor for LCO perception.

Analogous to LjNFR5/NFP-type LysM-RKs, it was found that LjNFR1 and MtLYK3 also play a role in mycorrhization. *Mtlyk3* and *Ljnfr1* mutants are impaired in mycorrhizal infection (189). However, infection is not completely blocked, again suggesting that additional LysM-RKs are involved. These data further suggest evolution of the rhizobium LCO perception mechanism from the ancient mycorrhizal signaling machinery. Intriguingly, it was found in rice (*Oryza sativa*) that a single receptor, OsCERK1, facilitates infection of AM fungi but is also essential for chitin-triggered defense responses (122, 189). OsCERK1 is a close homolog, if not an ortholog, of the chitin innate immune receptor AtCERK1 of *Arabidopsis thaliana* (*Arabidopsis*) and the legume rhizobium LCO receptors LjNFR1 and MtLYK3. The ability of LjNFR1/MtLYK3 and OsCERK1 to function in symbiotic signaling correlates with the presence of a YAQ/YAR motif in the kinase domain (38, 122, 125). Defense-related chitin signaling does not require this motif, as *Arabidopsis* AtCERK1 appears to have lost it (38, 125). Furthermore, the kinase domain of OsCERK1 when fused to the extracellular part of LjNFR1 was able to fully complement the nodulation defect of the *Ljnfr1* mutant (122). This indicates that no additional evolution of the kinase domain is required to function in nodulation and underlines that symbiotic LCO perception and innate immune signaling intertwine.

Chitin-Induced Innate Immune Signaling Shows Analogies to Symbiotic Lipochitooligosaccharide Signaling

In contrast to tetrameric and pentameric chitin oligomers, longer chain chitin oligomers (hexa- to octamers) or peptidoglycans are typically perceived as MAMPs that trigger innate immunity

(156, 166). Perception of these chitin-like molecules also involves LysM-RKs. The functioning of these receptors has been best studied in *Arabidopsis*, a species that lost the AM symbiosis, and in rice, which does form an AM symbiosis.

In *Arabidopsis*, at least three LysM-RKs are involved in chitin-induced innate immune signaling: AtCERK1, AtLYK4, and AtLYK5 (26, 77, 121, 138, 158, 175, 176). As mentioned above, AtCERK1 is a close homolog, or even an ortholog, of LjNFR1/MtLYK3 and was originally identified as the key chitin receptor on the basis of its mutant phenotype and its ability to bind chitin oligomers. Octameric chitin oligomers cause dimerization of AtCERK1, leading to activation of its kinase domain (101). Short-chain chitin oligomers also bind to the extracellular part of AtCERK1 but fail to induce dimerization. Interestingly, such short-chain chitin oligomers could attenuate AtCERK1 signaling in a dose-dependent manner (101). Therefore, it is possible that the short-chain chitin oligomers found in the exudates of AM fungi (55) actually interfere with defense activation by longer chain oligomers. The relative abundance of the different chitin oligomers may in such cases play an important role in determining the outcome of the responses. AtCERK1 can form a heteromer complex with AtLYK5 and AtLYK4 (26, 175). AtLYK5 and AtLYK4 belong to the same LysM-RK subfamily as LjNFR5/MtNFP and likewise do not have an active kinase domain. AtLYK5 binds chitin oligomers with much higher affinity than AtCERK1, indicating that AtLYK5 is in fact the major chitin receptor in *Arabidopsis*. AtLYK5 functions partly redundantly with AtLYK4, as both bind chitin and only a double mutant completely blocks chitin signaling, similar to *Atcerk1* mutants. Binding of chitin oligomers by AtLYK5 induces heteromerization with AtCERK1, resulting in activation of its kinase domain (26). This indicates the involvement of a receptor complex analogous to the LjNFR5-LjNFR1 and MtNFP-MtLYK3 receptor complexes in symbiosis.

In contrast to AtCERK1, OsCERK1 was not found to bind chitin oligomers, indicating differences in affinity for chitin-like molecules (82, 158). To perceive myc-LCOs, it is likely that OsCERK1 pairs with LysM coreceptors, for which homologs of LjNFR5/MtNFP in rice, such as OsLYR1, are key candidates. To perceive chitooligosaccharides, rice relies on the LysM-domain-containing CHITIN ELICITOR BINDING PROTEIN (OsCEBiP) which lacks an intracellular kinase domain (82). OsCEBiP binds chitin and forms a heteromer complex with OsCERK1 to activate chitin-triggered defense responses (71, 157). Notably, homologs of OsCEBiP in *Arabidopsis*, AtLYM1, AtLYM2, and AtLYM3, are not involved in AtCERK1-mediated innate immunity (158, 175). Nevertheless, AtLYM2 was shown to bind chitin and contributed to resistance against the fungal pathogen *Alternaria brassicicola* in an AtCERK1-independent manner (126, 138). Additionally, AtLYM2 was shown to control the transport ability of plasmodesmata in a chitin-dependent manner, which plays a role in plant immunity to pathogenic fungi such as *Botrytis cinerea* (43). This suggests that at least two different pathways (AtCERK1 dependent and AtCERK1 independent) are operational in chitin-based defense. It is currently not known with which coreceptor(s) AtLYM2 interacts to relay signaling. In contrast, AtLYM1 and AtLYM3 were shown to form a complex with AtCERK1 to trigger immunity in response to peptidoglycans (182). In rice, the LYM homologs OsLYP4 and OsLYP6 play a role in peptidoglycan perception as well as chitin perception (101). Overall, these data indicate that CERK1 homologs function as a coreceptor in diverse signaling complexes related to perception of chitin-like molecules, including LCOs.

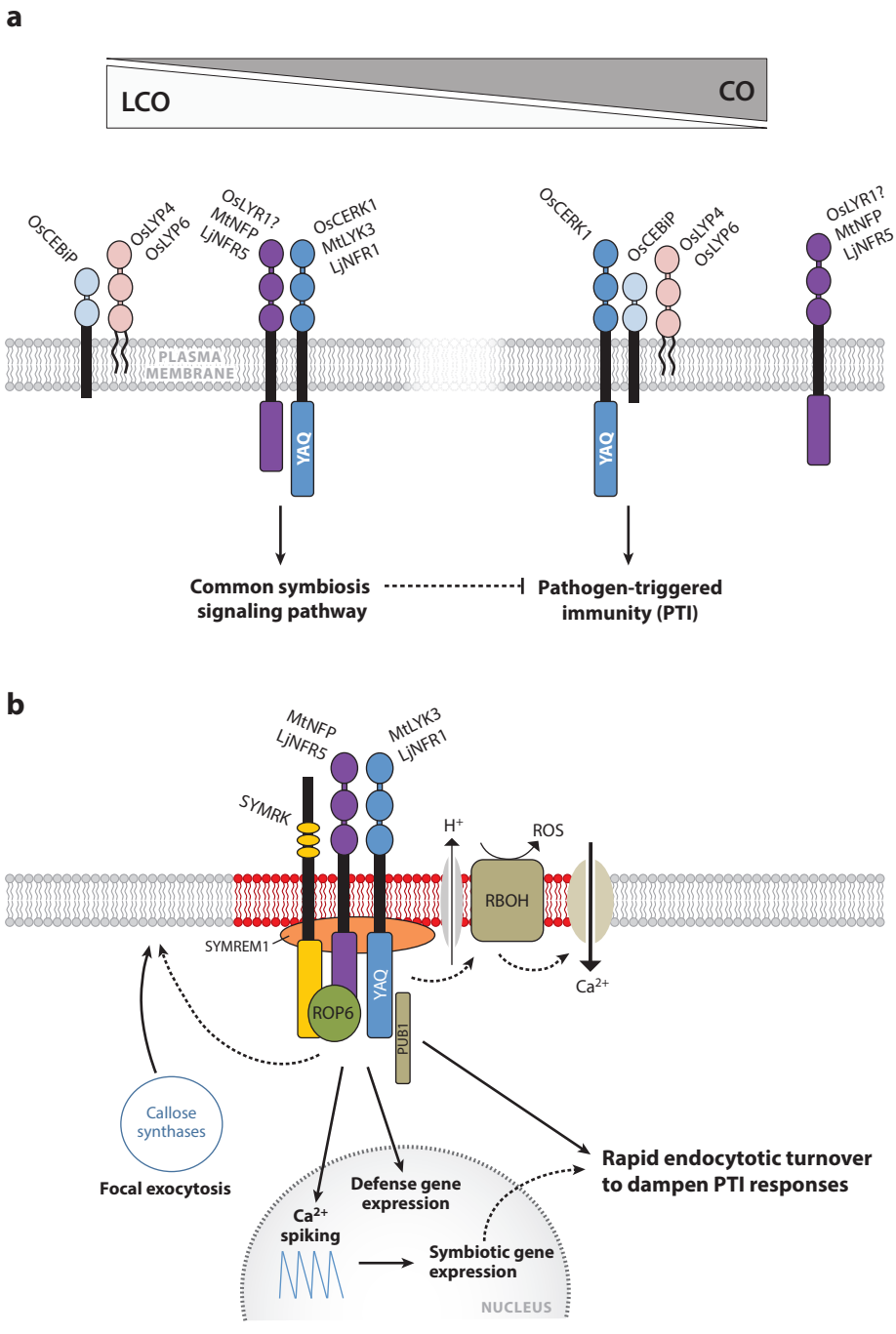
Two Models for Dual Functioning of Lipochitooligosaccharide LysM-RKs

Specific gene duplication events early in legume evolution provided the freedom to evolve symbiosis specific functions of LjNFR1/MtLYK3 and LjNFR5/MtNFP (38, 130, 190). However, three lines of evidence indicate that LjNFR1/MtLYK3 and LjNFR5/MtNFP still have a dual function

in symbiosis and defense signaling, similar to the dual function of OsCERK1. (*a*) Rhizobium LCOs transiently trigger defense-related gene expression in an LjNFR1-dependent manner (125). (*b*) MtNFP acts, independent of MtLYK3, in defense against fungal pathogens, including *Verticillium albo-atrum* and *Colletotrichum trifolii*, or the oomycete *Aphanomyces euteiches* (16, 148). A lack of MtNFP makes plants more susceptible to such pathogens, whereas ectopic expression of MtNFP increases resistance (148). (*c*) A strong coexpression of both receptors—LjNFR5-LjNFR1 or MtNFP-MtLYK3—in *Nicotiana benthamiana* leaves triggered a spontaneous cell death response (24, 140). This response requires an active LjNFR1/MtLYK3 kinase domain. An early cell death response was also observed in *M. truncatula* nodules when *MtNFP* was overexpressed (123). This work further showed that both receptors are under tight posttranslational control in legume roots and nodules, likely to prevent premature induction of defense responses. Taken together, these data suggest that dual functioning of LCO receptors in defense and symbiosis is not unique for rice (and other nonlegumes) but has also been maintained in legumes to function in rhizobium symbiosis. This implies that genetic constraints preserve this dual functioning (122). The biological meaning of this dual functioning can be explained in two ways. A first model is based on competition for CERK1/NFR1/LYK3-type proteins by different receptor complexes. High affinity for LCOs (and/or short-chain chitin oligomers) leads to the preferential formation of symbiotic receptor complexes at the expense of the formation of complexes that act in defense signaling (122) (**Figure 1*a***). Variations in affinities for different chitin-like molecules encoded by the extracellular receptor domains determine which complexes are formed. This would automatically block chitin-induced defense responses, without the need to independently suppress chitin-triggered immunity. This model is in line with the finding that LCOs can repress innate immunity (96). As an alternative model, the MAMP-triggered responses may actually be recruited to facilitate/regulate the infection process by symbiotic microbes (**Figure 1*b***). For example, several typical early MAMP-triggered responses, such as calcium influx, production of reactive oxygen species (ROS), and focal exocytosis, have been implicated in the formation and growth of infection threads during the rhizobium interaction (23). Infection threads are tip-growing structures, and ROS production is thought to facilitate the oxidative cross-linking of the infection thread matrix to allow the formation of a tube-like infection thread. The spatiotemporal regulation of receptor complexes may then be important to fine-tune the responses to prevent too-strong defense responses (123). This hypothesis is also in line with the essential role for symbiotic LCO receptors in infection thread formation (8, 9, 58, 99, 161), which is independent from their role in activating the common symbiosis signaling pathway (108). Several studies suggest that symbiotic LCO receptors are located in lipid-raft-like membrane domains in the plasma membrane, which is thought to play an important role in controlling their turnover and their ability to form specific complexes (68, 80, 94). Localization of MtNFP and MtLYK3 in the nodule showed that these receptors accumulate in a very narrow zone at the nodule apex and are rapidly removed from the infection thread membrane to allow the release of the bacteria from the infection threads (123).

Lipochitooligosaccharide Perception Is Not Sufficient to Evade Innate Immunity

LCO perception may play an even broader role in suppression of PAMP-triggered immune responses, as LCOs were shown to suppress flg22 (a 22 amino acid peptide fragment of flagellin)-induced immune responses in various plant species. In *Arabidopsis* this response depends specifically on AtLYK3 but not on AtCERK1 (96). *Atlyk3* mutants appear to be more resistant to fungal pathogens such as *B. cinerea* and *Pectobacterium carotovorum*, suggesting that these pathogens exploit this receptor kinase to infect plants (132). These data led to the speculation that suppression


a
LCO
CO
OsCEBiP
OsLYP4
OsLYP6
OsLYR1?
MtNFP
LjNFR5
OsCERK1
MtLYK3
LjNFR1
PLASMA MEMBRANE
YAQ
OsCERK1
OsCEBiP
OsLYP4
OsLYP6
OsLYR1?
MtNFP
LjNFR5
YAQ
Common symbiosis signaling pathway
Pathogen-triggered immunity (PTI)
b
MtNFP
LjNFR5
MtLYK3
LjNFR1
SYMRK
H+
ROS
RBOH
SYMREM1
YAQ
ROP6
PUB1
Ca2+
Callose synthases
Focal exocytosis
Rapid endocytotic turnover to dampen PTI responses
Defense gene expression
Ca2+ spiking
Symbiotic gene expression
NUCLEUS

of innate immunity may have been the original/first function of LCOs (97). However, it is difficult to imagine why nonsymbiotic plants such as *Arabidopsis* would have maintained a LCO perception system to repress innate immunity. It is also difficult to reconcile with the observation that LCOs do trigger defense responses (125). Clearly, a better understanding of the cross talk between the various receptor complexes, potentially via shared downstream partners, involved in LCO and MAMP perception is required to resolve this apparent paradox.

Hijacking the LysM-RK-activated common symbiosis signaling pathway, which is operational in the vast majority of land plants, would seem to be an attractive strategy for biotrophic pathogens to colonize root cells (133, 149). However, studies that indicate that pathogens make use of this strategy are exceptionally scarce. Root knot nematodes have been suggested to secrete signal molecules, which trigger root hair deformations similar to those triggered by LCOs (179). This ability is (partially) dependent on LjNFR1-LjNFR5 and LjSYMRK, and correspondently absent in root hairs of *Arabidopsis*. This led to the suggestion that root knot nematodes may produce LCO-like molecules to make use of the common symbiosis signaling pathway (179). However, a clear involvement of the common symbiosis signaling pathway in root knot nematode infection has not been shown, and the nature of the nematode signals remains to be determined.

In *M. truncatula*, the *Mtccamk* mutant fails to show cytoplasmic aggregation at attempted penetration sites of *C. trifolii* and *Phytophthora medicaginis* (57), a response that is typically triggered upon fungal contact (including AM fungi) or touch (69). However, this defect does not affect overall fungal colonization, suggesting that MtCCaMK is not required for infection by these pathogens. In fact, most studies on (hemi)biotrophic pathogenic fungi or oomycetes suggest that such pathogens do not hijack the common symbiosis signaling pathway. In *L. japonicus* leaf cells, haustorium formation by rust fungi is not dependent on the common symbiosis signaling pathway (117).

Figure 1

Two hypothetical models to explain the evolutionarily conserved dual function of lipochitooligosaccharide (LCO) receptors in symbiosis and immune signaling. Dashed arrows represent hypothesized interactions, whereas solid arrows represent proven interactions. Different receptor types are shown in different colors. (*a*) Chitooligosaccharides (COs) and LCOs are perceived by LysM-domain-containing receptors OsCERK1/LjNFR1/MtLYK3. These LysM-RKs (receptor-like kinases) function as coreceptors in various complexes. Different affinities for the various chitin-like molecules determine which complexes are formed. In rice, chitin perception involves a complex between OsCERK1 and the membrane-anchored OsCEBiP. Additionally, the GPI-anchored OsLYP4 and OsLYP6 are involved in chitin and peptidoglycan perception. LCOs preferentially recruit the coreceptor CERK1/NFR1/LYK3 into the symbiotic complex at the expense of the complexes involved in MAMP-triggered immune responses. LCO perception involves a complex between CERK1/NFR1/LYK3 and NFR5/NFP homologs, which have a dead kinase. This leads to the activation of the common symbiosis signaling pathway, which in turn may affect MAMP-triggered immunity. (*b*) Symbiotic LCO receptor complexes activate early immune responses to facilitate symbiont infection. Several early MAMP-triggered immune responses, such as ion changes (including calcium influx), ROS (reactive oxygen species) production via respiratory burst oxidases (RBOH), and focal exocytosis, are triggered upon LCO perception, have been implicated in rhizobial infection. LCO receptor heteromer complexes may be formed especially at lipid-raft-like membrane domains (*marked in red*). Such domains can be marked by the symbiotic remorin SYMREM1 or flotillins, which controls their signaling potential and endocytotic turnover to prevent too-strong defense responses. In addition to forming a heteromer complex with the CERK1-orthologous LysM-RK LjNFR1, the kinase-dead LjNFR5 has been found to interact with the common symbiosis signaling pathway LRR receptor kinase SYMRK as well as with the small GTPase ROP6, which control rhizobial infection. Additionally, a U-box E3-ubiquitin ligase (PUB1) has been found to interact with MtLYK3, which may control its ubiquitination-dependent turnover, similar to the role of PUB homologs in immune receptor complexes. Activation of the common symbiosis signaling pathway, marked by calcium spiking, may further influence the posttranslational turnover of the receptor complexes.

Similarly, intracellular colonization of rice roots by the biotrophic rice blast fungus *Magnaporthe oryzae* does not require OsCCaMK (110). Also, the oomycete *Phytophthora palmivora* does not require any components of the common symbiosis signaling pathway to infect and form haustoria in *M. truncatula* roots (147). Taken together, it appears that pathogenic fungi and oomycetes do not exploit the common symbiosis signaling pathway by producing LCOs, or short-chain chitin oligosaccharides, to facilitate plant colonization. Likewise, no bacterial pathogens have been identified that gained the core *nod* genes via horizontal gene transfer, allowing them to exploit these to colonize/parasitize potential host plants. This suggests that there may be as yet unknown penalties associated with LCO-induced signaling that have been overcome by Glomeromycota and symbiotic rhizobia. It suggests that additional adaptations are required to benefit from LCO signaling. Such adaptations may include the evasion of recognition by the innate immune system. For example, in the case of Glomeromycota, the lack of cell wall–degrading enzymes is thought to minimize their perception as potential pathogens (171), and secreted effector proteins have evolved to modulate defense (87). In the case of rhizobium (*Sinorhizobium meliloti* and *Mesorhizobium loti*), it was suggested that flagellin has been adapted so that it does not trigger defense responses (44, 103). Furthermore, rhizobial surface polysaccharides play important roles in the suppression of defense responses in legume roots (51). Additionally, adaptations in the rhizobial type III secretion system (T3SS) and its associated effector proteins appear to play a key role, which is further discussed below.

From Pathogen to Symbiont

As lateral gene transfer is a common mechanism in prokaryotes (33), it can be envisioned that new potential symbionts may arise. However, the restricted number of actual rhizobium symbionts suggests that evolution of de novo symbionts is hampered by genetic barriers that affect the relation with potential host plants. To get insights into such barriers, comparative studies of a relatively young symbiont with its nonsymbiotic relatives have been conducted. For this, *Cupriavidus taiwanensis* is used, as it likely represents a recently evolved nitrogen-fixing symbiont. *C. taiwanensis* is the only symbiotic species in the genus *Cupriavidus* and sister genus *Ralstonia*. *Cupriavidus* and *Ralstonia* are diverged from *Burkholderia* and form a distinct lineage in the β-proteobacteriales (20, 173). *C. taiwanensis* contains a 35-kb symbiotic region on a self-transferrable plasmid. This region contains 31 symbiosis genes organized in operons interspersed with retroelements, which may be indicative for recent structural rearrangements (2). *C. taiwanensis* contains only a single operon, *nodBCIJHASUQ*, of 10 *nod* genes that are essential for LCO biosynthesis. This enables *C. taiwanensis* to produce pentameric LCOs with either a vaccenic acid (C18:1) or palmitic acid (C16:0) as the lipid tail. Additionally, an O-carbamoyl (determined by *nodU*), an N-methyl (determined by *nodS*), and/or 6-O-sulfate group (determined by *nodH* and *nodQ*) can be present (2). This pallet of LCOs is similar, as it is produced by a broad range of different symbiotic rhizobia in the α-proteobacteriales (10, 105). But in contrast to several of these species, *C. taiwanensis* has a restricted host range and nodulates only the legume *Mimosa pudica*. This suggests that the host range of *C. taiwanensis* is constrained by other factors. In the search for these factors, it was found that the host-range of *C. taiwanensis* can be extended by mutating its T3SS (153). Wild-type *C. taiwanensis* forms only infective nodules with degenerative symbiosomes on *Leucaena leucocephala*, whereas the T3SS knockout mutant forms fully functional nodules. This suggests that wild-type *C. taiwanensis* secretes components during its symbiotic lifestyle that hamper symbiosome development in *L. leucocephala*. Interestingly, the organization of the T3SS operon in *C. taiwanensis* is atypical for symbiotic rhizobia, but more similar to what is found in the opportunistic pathogen *Burkholderia cenocepacia* (153). The biological process in which the T3SS of

C. taiwanensis functions remains unknown. However, these studies make apparent that the symbiotic lifestyle of *C. taiwanensis* is hampered by a not yet adapted T3SS, thereby restricting its symbiotic potential.

A similar finding was obtained in an experimental evolutionary approach in which the symbiotic plasmid of *C. taiwanensis* was introduced into its relative, *Ralstonia solanacearum* (111). *R. solanacearum* is a broad host range plant pathogen that invades roots intercellularly and causes wilting upon colonization of the vascular system (54). The chimeric *R. solanacearum* produced LCOs but was not able to trigger root nodule formation on *M. pudica*. However, this dramatically changed upon inactivation of its T3SS and the master virulence regulator hrpG. Now the mutated chimeric *R. solanacearum* strain could trigger infected root nodules, although the bacteria did not yet fix nitrogen and triggered defense responses in the nodules (111). Again, this finding illustrates that adaptive changes in the secretome are essential for a successful transfer of the nitrogen-fixing symbiosis trait. Subsequent selection (co-culturing) cycles in *M. pudica* further increased the symbiotic capacity of the chimeric *R. solanacearum*, indicating that plants can select mutations in the bacteria that further reduce the activation of host defenses (111, 112).

Besides potential negative effects, T3SS-dependent effector proteins can also be used by rhizobium to facilitate symbiosis, and as such play a role, in addition to LCOs, as host range determinants (113). The broad host range strain *Sinorhizobium fredii* NGR234 has been shown to use its T3SS to inject so-called nodulation outer Proteins (Nops) into plant cells to suppress defense responses (12, 35). Transcriptional activation of the T3SS-encoding genes was shown to be induced by flavonoids in a NodD-dependent manner, indicating a common regulatory mechanism with LCO biosynthesis genes (90, 178). Strikingly, some rhizobia have even evolved T3SS effector proteins that allow them to bypass LCO signaling to enable nodulation. The LCO-deficient *Bradyrhizobium elkanii nodC* mutant BEnodC was shown to induce nodulation in soybean by making use of its T3SS (128). This suggests that this strain evolved effectors that when injected into the plant cells can bypass the LCO receptors. It is currently not known how such effectors trigger nodulation. It would be interesting to see whether they signal via the common symbiosis signaling pathway or activate nodulation downstream of this pathway. The LCO-deficient *B. elkanii* strain failed to form infection threads in roots hairs but instead invaded roots via crack entry (128). This is in line with a specific role for rhizobium LCO receptors in infection thread formation, as discussed above. Most *Bradyrhizobium* species rely on LCOs to nodulate, which suggests that this LCO-independent nodulation arose later in evolution. Furthermore, although the LCO-deficient strain could nodulate, its effectiveness was much improved upon addition of LCOs, indicating that LCO-induced signaling is a more efficient strategy.

PERSPECTIVE: HORMONAL RESPONSES MAY BE KEY TO SYMBIOTIC LIPOCHITOOLIGOSACCHARIDE SIGNALING

The studies described above make clear that symbiotic rhizobia have not only gained the capacity to produce LCOs but also need to reset their T3SS secretome to overcome pathogenicity. Furthermore, we have presented two models that show how perception of LCOs can modulate the balance between different LysM-RK receptor complexes and/or use the readout of innate immune signaling to benefit rhizobial infection (**Figure 1**). It remains unclear, however, to what extent symbiotic LCO signals can also actively repress innate immunity. In this last section, we focus on the potential role of plant hormones in such active repression of innate immunity. On the basis of recent studies, we speculate on a central role for DELLA proteins in hormonal cross talk to repress defense responses in symbiosis. However, it should be noted that regulation and cross talk between various hormones, especially in roots, is far from understood.

Symbiotic LCO signaling causes changes in the hormonal landscape of the host plant. This is most obvious in the case of rhizobium-induced nodule organogenesis, but myc-LCOs also induce changes in root architecture. In fact, all classical plant hormones have been implicated in nodulation as well as mycorrhization (45, 50, 120), including the major defense hormones salicylic acid (SA), jasmonic acid (JA), and ethylene (139). Therefore, cross talk between the LCO-induced common symbiosis signaling pathway and various hormonal networks is likely to influence innate immune signaling.

SA is an important regulator of resistance to biotrophic pathogens (60), and SA has also been shown to have a negative effect on both rhizobial and mycorrhizal symbioses (18, 73, 114, 165, 174). AM fungi and rhizobia seem to employ LCO signaling to suppress SA-dependent defense responses. Inoculation of alfalfa (*Medicago sativa*) plants with LCO-deficient rhizobia or an incompatible rhizobium strain induces accumulation of SA, whereas a slight reduction in SA levels is observed upon inoculation with compatible rhizobia (114). Likewise, inoculation of tomato with the rather incompatible AM fungal species *Glomus mosseae* elevates SA concentrations in the root and expression of the SA-responsive gene *PR1a*, whereas this is not observed upon inoculation with the very compatible fungal species *R. irregularis* (104). Studies in pea (*Pisum sativum*) revealed that mycorrhization induces a transient rise in SA levels, which is repressed during prolonged colonization. In contrast, a constant rise in SA levels is induced in pea plants mutated in CCaMK, suggesting that repression of SA biosynthesis is based on activation of the common symbiosis signaling network (18).

Whereas SA is a major signal in resistance to biotrophic pathogens, defense against necrotrophic pathogens requires JA as a signal (139). Both hormones antagonize each other, such that activation of JA signaling can compromise SA-dependent defense. Several biotrophic pathogens exploit this antagonism to suppress SA-mediated defense (85). For example, several *Pseudomonas syringae* strains produce the effector coronatine (COR), which acts as an analog of jasmonate-isoleucine (JA-Ile), the bioactive form of JA (81). By binding the JA receptor COI1, COR induces proteosomal degradation of the JAZ proteins that function as negative regulators (transcriptional repressors) of JA signaling (139). COR-deficient strains of *P. syringae* have been shown to produce other effectors to activate JA responses; e.g., HopX1. *HopX1* encodes a cysteine protease that degrades JAZ, thereby activating JA signaling in order to antagonize SA-mediated defense (59).

Recently, DELLA proteins have been implicated as important regulators of the JA-SA balance (127). DELLAs belong to the GRAS family of transcriptional regulators and were originally identified as gibberellin (GA)-sensitive repressors of plant growth (34, 70). However, DELLA proteins appear to form signaling hubs involved in various hormone signaling pathways, including in auxin, ethylene, and JA signaling (180). DELLAs promote JA signaling by competing for JAZ binding with MYC2, a key transcriptional activator of JA signaling (21, 76). Activation of JA signaling by MYC2 promotes DELLA accumulation, further attenuating MYC2 repression by JAZ proteins (181, 184). Recent data suggest that AM fungi may exploit this DELLA function to reduce SA-mediated defense responses. Experiments in rice and *M. truncatula* revealed that DELLA mutants in rice (*Osslr1*) and *M. truncatula* (*Mtdella1/Mtdella2* double mutant) are severely impaired in root infection and/or arbuscule formation (49, 187), indicating that DELLA proteins are essential transcriptional regulators of this endosymbiosis. In line with this, GA application at high concentrations was shown to block colonization, whereas lower concentrations were already sufficient to block arbuscule formation (41). It is therefore tempting to speculate that DELLAs might promote colonization and arbuscule formation through immune modulation by sequestering JAZ proteins, affecting the JA-SA signaling balance.

Ectopic expression in *M. truncatula* of a nondegradable *MtDELLA1* allele (*Mtdella1-Δ18*) in the vasculature and endodermis is sufficient to sustain arbuscule formation in the root cortex of the

Mtdella1/Mtdella2 double mutant or upon GA application (50). Additionally, ectopic expression of *Mtdella1-Δ18* enables arbuscule formation in an *L. japonicus Ljcyclops* mutant. The CCaMK-interacting protein LjCYCLOPS/MtIDP3 is a key protein of the common symbiotic signaling pathway (185), suggesting that DELLA proteins intersect at some point with LCO signaling but do so in a non-cell-autonomous fashion (49). Intriguingly, expression of the dominant active *Mtdella1-Δ18* allele in an *M. truncatula Mtccamk* mutant did not rescue the mycorrhizal colonization defect, which indicates that additional input of CCAMK is required. In rice, the DELLA OsSLR1 was shown to interact with the GRAS family OsDIP1 (DELLA interacting protein 1), which is induced upon mycorrhization (187). DIP1 was also shown to interact with the GRAS protein RAM1 (required for arbuscular mycorrhization 1), which is essential for root colonization by AM fungi (61). This led to the suggestion that DELLA, DIP1, and RAM1 are part of a larger GRAS protein regulatory complex (65). Other GRAS family proteins that play a role in both mycorrhization and nodulation include the transcriptional regulators NSP1 and NSP2 (36, 83, 102, 162). Both proteins are required for the production of strigolactones (102), which act as plant signals that promote hyphal branching of AM fungi as well as root and shoot architecture (1, 63, 152, 172). Both NSP1 and NSP2 have been shown to control mycorrhizal colonization (36, 92, 102, 109, 170). However, the relatively mild effects on mycorrhizal colonization contrast with the essential role for NSP1 and NSP2 during nodulation (72, 83, 162). This suggests that different GRAS complexes are involved in the control of the two symbioses. However, our understanding of the functioning of these GRAS complexes in relation to symbiotic LCO signaling remains scant (98) and will remain a major objective in future research.

As has become apparent, the mechanisms behind LCO-mediated modulation of innate immunity are far from clear. However, recent studies not only provided novel insight but also extended the studies to other, nonlegume plant species, such as rice and *Arabidopsis*. This will open new frontiers to unravel the fundamentals of symbiotic signaling.

SUMMARY POINTS

1. Combining the nitrogen fixation trait with the capacity to synthesize LCOs marked the origin of nitrogen-fixing rhizobia. The ability to make LCOs enabled rhizobia to activate the signaling pathway that is used by host plants to establish an endosymbiosis with AM fungi. This common symbiosis signaling pathway is essential for the formation of a symbiotic host-microbe interface. In legumes, this signaling pathway evolved to allow formation of root nodules and a more strict control over rhizobium infection.
2. CERK1-type LysM-RKs have dual functions in immune signaling and symbiosis, indicating that LCO signaling and chitin-based immune signaling are intertwined.
3. Two putative models have been put forward to explain this evolutionarily conserved dual function. (*a*) Perception of LCOs modulates the balance between different LysM-RK receptor complexes, favoring a symbiotic complex at the expense of complexes required for immune responses. (*b*) Early immune responses are co-opted to facilitate symbiont infection. Tight regulation of the receptor complexes at the posttranslational level, involving rapid endocytotic turnover, subsequently prevents activation of defense responses.
4. Microbial activation of the common symbiosis signaling pathway is not sufficient to evade innate immunity. Therefore, microbes need to evolve additional genetic adaptations to benefit from LCO signaling. In rhizobia, the T3SS especially needs to be adapted to evade immune responses.

5. Microbial activation of the common symbiosis signaling pathway can modulate innate immune responses through its effect on the hormonal landscape. On the basis of recent studies, we speculate on a central role for DELLA proteins in hormonal cross talk to repress defense responses in symbiosis, in part by influencing the SA-JA balance.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Akiyama K, Matsuzaki K, Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435:824–27
2. Amadou C, Pascal G, Mangenot S, Glew M, Bontemps C, et al. 2008. Genome sequence of the β-rhizobium *Cupriavidus taiwanensis* and comparative genomics of rhizobia. *Genome Res.* 18:1472–83
3. Ane J, Baek J, Kalo P, Rosenberg C, Roe BA, et al. 2004. *Medicago truncatula DMI1* required for bacterial and fungal symbioses in legumes. *Science* 303:1364–68
4. Angus AA, Hirsch AM. 2010. Insights into the history of the legume-betaproteobacterial symbiosis. *Mol. Ecol.* 19:28–30
5. Antolín-Llovera M, Ried MK, Binder A, Parniske M. 2012. Receptor kinase signaling pathways in plant-microbe interactions. *Annu. Rev. Phytopathol.* 50:451–73
6. 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.* 24:422–27
7. Aoki S, Ito M, Iwasaki W. 2013. From β- to α-proteobacteria: the origin and evolution of rhizobial nodulation genes *nodIJ*. *Mol. Biol. Evol.* 30:2494–508
8. Ardourel M, Demont N, Debellé F, Maillet F, De Billy F, et al. 1994. *Rhizobium meliloti* lipooligosaccharide nodulation factors: different structural requirements for bacterial entry into target root hair cells and induction of plant symbiotic developmental responses. *Plant Cell* 6:1357–74
9. Arrighi J-F, Barre A, Ben Amor B, Bersoult A, Soriano LC, et al. 2006. The *Medicago truncatula* lysin motif-receptor-like kinase gene family includes *NFP* and new nodule-expressed genes. *Plant Physiol.* 142:265–79
10. Ba S, Willems A, De Lajudie P, Jeder H, Quatrini P, et al. 2002. Symbiotic and taxonomic diversity of rhizobia isolated from *Acacia tortilis* subsp. *raddiana* in Africa. *Syst. Appl. Microbiol.* 145:130–45
11. Balestrini R, Bonfante P. 2014. Cell wall remodeling in mycorrhizal symbiosis: a way towards biotrophism. *Front. Plant Sci.* 5:237
12. Bartsev A, Deakin W, Boukli N. 2004. NopL, an effector protein of *Rhizobium* sp. NGR234, thwarts activation of plant defense reactions. *Mol. Plant-Microbe Interact.* 134:871–79
13. Behm JE, Geurts R, Kiers ET. 2014. *Parasponia*: a novel system for studying mutualism stability. *Trends Plant Sci.* 19:757–63
14. Bellincampi D, Cervone F, Lionetti V. 2014. Plant cell wall dynamics and wall-related susceptibility in plant-pathogen interactions. *Front. Plant Sci.* 5:228

15. Ben Amor B, Shaw S, Oldroyd G. 2003. The *NFP* locus of *Medicago truncatula* controls an early step of Nod factor signal transduction upstream of a rapid calcium flux and root hair deformation. *Plant J.* 34:495–506
16. Ben C, Toueni M, Montanari S, Tardin MC, Fervel M, Negahi A, et al. 2012. Natural diversity in the model legume *Medicago truncatula* allows identifying distinct genetic mechanisms conferring partial resistance to Verticillium wilt. *J. Exp. Bot.* 63:695–709
17. Berendsen RL, Pieterse CMJ, Bakker PAHM. 2012. The rhizosphere microbiome and plant health. *Trends Plant Sci.* 17:478–86
18. Blilou I, Ocampo JA, García-Garrido M. 1999. Resistance of pea roots to endomycorrhizal fungus or rhizobium correlates with enhanced levels of endogenous salicylic acid. *J. Exp. Bot.* 50:1663–68
19. 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
20. Bontemps C, Elliott GN, Simon MF, Dos Reis Júnior FB, Gross E, et al. 2010. *Burkholderia* species are ancient symbionts of legumes. *Mol. Ecol.* 19:44–52
21. Boter M, Ruí O, Abdeen A. 2004. Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and *Arabidopsis*. *Genes Dev.* 2:1577–91
22. Boyd ES, Peters JW. 2013. New insights into the evolutionary history of biological nitrogen fixation. *Front. Microbiol.* 4:201
23. Brewin NJ. 2004. Plant cell wall remodelling in the rhizobium-legume symbiosis. *CRC Crit. Rev. Plant Sci.* 23:293–316
24. Broghammer A, Krusell L, Blaise M, Sauer J, Sullivan JT, et al. 2012. Legume receptors perceive the rhizobial lipochitin oligosaccharide signal molecules by direct binding. *Proc. Natl. Acad. Sci. USA* 109:13859–64
25. Buist G, Steen A, Kok J, Kuipers OP. 2008. LysM, a widely distributed protein motif for binding to (peptido)glycans. *Mol. Microbiol.* 68:838–47
26. Cao Y, Liang Y, Tanaka K, Nguyen CT, Jedrzejczak RP, et al. 2014. The kinase LYK5 is a major chitin receptor in *Arabidopsis* and forms a chitin-induced complex with related kinase CERK1. *eLife* 3:1–19
27. Capoen W, Sun J, Wysham D, Otegui MS, Venkateshwaran M, et al. 2011. Nuclear membranes control symbiotic calcium signaling of legumes. *Proc. Natl. Acad. Sci. USA* 108:14348–53
28. Cardenas L, Dominguez J, Santana O, Quint C. 1996. The role of the *nodI* and *nodJ* genes in the transport of Nod metabolites in *Rhizobium etli*. *Gene* 173:183–87
29. Chabaud M, Genre A, Sieberer J, Faccio A, Novero M, et al. 2011. Arbuscular mycorrhizal hyphopodia and germinated spore exudates trigger Ca^{2+} spiking in the legume and nonlegume root epidermis. *New Phytol.* 189:347–55
30. 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
31. Cooper JE. 2007. Early interactions between legumes and rhizobia: disclosing complexity in a molecular dialogue. *J. Appl. Microbiol.* 103:1355–65
32. Czaja LF, Hogekamp C, Lamm P, Maillet F, Martinez EA, et al. 2012. Transcriptional responses toward diffusible signals from symbiotic microbes reveal MtNFP- and MtDMI3-dependent reprogramming of host gene expression by arbuscular mycorrhizal fungal lipochitooligosaccharides. *Plant Physiol.* 159:1671–85
33. Darmon E, Leach DRF. 2014. Bacterial genome instability. *Microbiol. Mol. Biol. Rev.* 78:1–39
34. Davière J-M, Achard P. 2013. Gibberellin signaling in plants. *Development* 140:1147–51
35. Deakin WJ, Broughton WJ. 2009. Symbiotic use of pathogenic strategies: rhizobial protein secretion systems. *Nat. Rev. Microbiol.* 7:312–20
36. Delaux P, Bécard G, Combier J. 2013. NSP1 is a component of the Myc signaling pathway. *New Phytol.* 199:59–65
37. Delaux P-M, Séjalon-Delmas N, Bécard G, Ané J-M. 2013. Evolution of the plant-microbe symbiotic "toolkit". *Trends Plant Sci.* 18:298–304
38. De Mita S, Streng A, Bisseling T, Geurts R. 2014. Evolution of a symbiotic receptor through gene duplications in the legume-rhizobium mutualism. *New Phytol.* 201:961–72

39. Dos Santos PC, Fang Z, Mason SW, Setubal JC, Dixon R. 2012. Distribution of nitrogen fixation and nitrogenase-like sequences amongst microbial genomes. *BMC Genomics* 13:162
40. Doyle JJ. 2011. Phylogenetic perspectives on the origins of nodulation. *Mol. Plant-Microbe Interact.* 24:1289–95
41. El Ghachtouli N, Martin-Tanguy J, Paynot M, Gianinazzi S. 1996. First report of the inhibition of arbuscular mycorrhizal infection of *Pisum sativum* by specific and irreversible inhibition of polyamine biosynthesis or by gibberellic acid treatment. *FEBS J.* 385:189–92
42. Endre G, Kereszt A, Kevei Z, Mihacea S, Kalo P, Kiss GB. 2002. A receptor kinase gene regulating symbiotic nodule development. *Nature* 417:962–66
43. Faulkner C, Petutschnig E, Benitez-Alfonso Y, Beck M, Robatzek S, et al. 2013. Lym2-dependent chitin perception limits molecular flux via plasmodesmata. *Proc. Natl. Acad. Sci. USA* 110:9166–70
44. Felix G, Duran JD, Volko S, Boller T. 1999. Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J.* 18:265–76
45. Ferguson BJ, Mathesius U. 2014. Phytohormone regulation of legume-rhizobia interactions. *J. Chem. Ecol.* 40:770–90
46. Fernandez-Lopez M, Haeze WD, Mergaert P, Verplancke C, Prome J, et al. 1996. Role of *NodI* and *NodJ* in lipo-oligosaccharide secretion in *Azorhizobium caulinodans* and *Escherichia coli*. *Mol. Microbiol.* 20:993–1000
47. Ferrari S, Savatin DV, Sicilia F, Gramegna G, Cervone F, De Lorenzo G. 2013. Oligogalacturonides: plant damage-associated molecular patterns and regulators of growth and development. *Front. Plant Sci.* 4:49
48. Fliegmann J, Canova S, Lachaud C, Uhlenbroich S, Gasciolli V, et al. 2013. Lipo-chitooligosaccharidic symbiotic signals are recognized by LysM receptor-like kinase LYR3 in the legume *Medicago truncatula*. *ACS Chem. Biol.* 8:1900–6
49. Floss DS, Levy JG, Lévesque-Tremblay V, Pumplin N, Harrison MJ. 2013. Della proteins regulate arbuscule formation in arbuscular mycorrhizal symbiosis. *Proc. Natl. Acad. Sci. USA* 110:E5025–34
50. Foo E, Ferguson BJ, Reid JB. 2014. Common and divergent roles of plant hormones in nodulation and arbuscular mycorrhizal symbioses. *Plant Signal. Behav.* 9:e29593
51. Fraysse N, Couderc F, Poinsot V. 2003. Surface polysaccharide involvement in establishing the rhizobium-legume symbiosis. *Eur. J. Biochem.* 270:1365–80
52. Gage DJ. 2004. Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes. *Microbiol. Mol. Biol. Rev.* 68:280–300
53. Gaude N, Bortfeld S, Duensing N, Lohse M, Krajinski F. 2012. Arbuscule-containing and non-colonized cortical cells of mycorrhizal roots undergo extensive and specific reprogramming during arbuscular mycorrhizal development. *Plant J.* 69:510–28
54. Genin S, Boucher C. 2004. Lessons learned from the genome analysis of *Ralstonia solanacearum*. *Annu. Rev. Phytopathol.* 42:107–34
55. Genre A, Chabaud M, Balzergue C, Puech-Pagés V, Novero M, et al. 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.* 198:179–89
56. Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG. 2005. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *Plant Cell* 17:3489–99
57. Genre A, Ortu G, Bertoldo C, Martino E, Bonfante P. 2009. Biotic and abiotic stimulation of root epidermal cells reveals common and specific responses to arbuscular mycorrhizal fungi. *Plant Physiol.* 149:1424–34
58. Geurts R, Heidstra R, Hadri A, Downie JA, Franssen H, et al. 1997. Sym2 of pea is involved in a nodulation factor–perception mechanism that controls the infection process in the epidermis. *Plant Physiol.* 115:351–59
59. Gimenez-Ibanez S, Boter M, Fernández-Barbero G, Chini A, Rathjen JP, Solano R. 2014. The bacterial effector HopX1 targets JAZ transcriptional repressors to activate jasmonate signaling and promote infection in *Arabidopsis*. *PLOS Biol.* 12:e1001792

60. Glazebrook J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* 43:205–27
61. Gobbato E, Marsh JF, Vernié T, Wang E, Maillet F, et al. 2012. A GRAS-type transcription factor with a specific function in mycorrhizal signaling. *Curr. Biol.* 22:2236–41
62. Gomez SK, Javot H, Deewatthanawong P, Torres-Jerez I, Tang Y, et al. 2009. *Medicago truncatula* and *Glomus intraradices* gene expression in cortical cells harboring arbuscules in the arbuscular mycorrhizal symbiosis. *BMC Plant Biol.* 9:10
63. Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pagès V, Dun EA, et al. 2008. Strigolactone inhibition of shoot branching. *Nature* 455:189–94
64. Groth M, Takeda N, Perry J, Uchida H, Dräxl S, et al. 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* 22:2509–26
65. Gutjahr C. 2014. Phytohormone signaling in arbuscular mycorhiza development. *Curr. Opin. Plant Biol.* 20:26–34
66. Gutjahr C, Parniske M. 2013. Cell and developmental biology of arbuscular mycorrhiza symbiosis. *Annu. Rev. Cell Dev. Biol.* 29:593–617
67. Haney CH, Long SR. 2010. Plant flotillins are required for infection by nitrogen-fixing bacteria. *Proc. Natl. Acad. Sci. USA* 107:478–83
68. Haney CH, Riely BK, Tricoli DM, Cook DR, Ehrhardt DW, Long SR. 2011. Symbiotic rhizobia bacteria trigger a change in localization and dynamics of the *Medicago truncatula* receptor kinase LYK3. *Plant Cell* 23:2774–87
69. Hardham AR, Takemoto D, White RG. 2008. Rapid and dynamic subcellular reorganization following mechanical stimulation of *Arabidopsis* epidermal cells mimics responses to fungal and oomycete attack. *BMC Plant Biol.* 8:63
70. Hauvermale AL, Ariizumi T, Steber CM. 2012. Gibberellin signaling: a theme and variations on DELLA repression. *Plant Physiol.* 160:83–92
71. Hayafune M, Berisio R, Marchetti R, Silipo A, Kayama M, et al. 2014. Chitin-induced activation of immune signaling by the rice receptor CEBiP relies on a unique sandwich-type dimerization. *Proc. Natl. Acad. Sci. USA* 111:E404–13
72. Heckmann AB, Lombardo F, Miwa H, Perry JA, Bunnewell S, et al. 2006. *Lotus japonicus* nodulation requires two GRAS domain regulators, one of which is functionally conserved in a non-legume. *Plant Physiol.* 142:1739–50
73. Herrera Medina M, Gagnon H, Piche Y, Ocampo JA, Garcia Garrido J, Vierheilig H. 2003. Root colonization by arbuscular mycorrhizal fungi is affected by the salicylic acid content of the plant. *Plant Sci.* 164:993–98
74. Hogekamp C, Arndt D, Pereira PA, Becker JD, Hohnjec N, Küster H. 2011. Laser microdissection unravels cell-type-specific transcription in arbuscular mycorrhizal roots, including CAAT-box transcription factor gene expression correlating with fungal contact and spread. *Plant Physiol.* 157:2023–43
75. Horváth B, Yeun LH, Domonkos A, Halasz G, Gobbato E, et al. 2011. *Medicago truncatula IPD3* is a member of the common symbiotic signaling pathway required for rhizobial and mycorrhizal symbioses. *Mol. Plant-Microbe Interact.* 11:1345–58
76. Hou X, Lee LYC, Xia K, Yan Y, Yu H. 2010. DELLAs modulate jasmonate signaling via competitive binding to JAZs. *Dev. Cell* 19:884–94
77. Iizasa E, Mitsutomi M, Nagano Y. 2010. Direct binding of a plant LysM receptor-like kinase, LysM RLK1/CERK1, to chitin in vitro. *J. Biol. Chem.* 285:2996–3004
78. Imaizumi-Anraku H, Takeda N, Charpentier M, Perry J, Miwa H, et al. 2005. Plastid proteins crucial for symbiotic fungal and bacterial entry into plant roots. *Nature* 433:527–31
79. Ivanov S, Fedorova EE, Limpens E, De Mita S, Genre A, Bonfante P. 2012. Rhizobium-legume symbiosis shares an exocytotic pathway required for arbuscule formation. *Proc. Natl. Acad. Sci. USA* 109:8316–21
80. Jarsch IK, Konrad SS, Stratil TF, Urbanus SL, Szymanski W, et al. 2014. Plasma membranes are subcompartmentalized into a plethora of coexisting and diverse microdomains in *Arabidopsis* and *Nicotiana benthamiana*. *Plant Cell* 26:1698–711

81. Jones JDG, Dangl JL. 2006. The plant immune system. *Nature* 444:323–29
82. Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiyama C, Dohmae N, et al. 2006. Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *Proc. Natl. Acad. Sci. USA* 103:11086–91
83. Kalo P, Gleason C, Edwards A, Marsh J, Mitra RM, et al. 2005. Nodulation signaling in legumes requires NSP2, a member of the GRAS family of transcriptional regulators. *Science* 308:1786–89
84. Kanamori N, Madsen LH, Radutoiu S, Frantescu M, Quistgaard EMH, et al. 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* 103:359–64
85. Kazan K, Lyons R. 2014. Intervention of phytohormone pathways by pathogen effectors. *Plant Cell* 26:2285–309
86. Ke D, Fang Q, Chen C, Zhu H, Chen T, et al. 2012. The small GTPase ROP6 interacts with NFR5 and is involved in nodule formation in *Lotus japonicus*. *Plant Physiol.* 159:131–43
87. Kloppholz S, Kuhn H, Requena N. 2011. A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. *Curr. Biol.* 21:1204–9
88. Kosuta S, Chabaud M, Gough C, Dénairé J, Barker DG. 2003. A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific *MtENOD11* expression in roots of *Medicago truncatula*. *Plant Physiol.* 131:952–62
89. Kosuta S, Hazledine S, Sun J, Miwa H, Morris RJ, et al. 2008. Differential and chaotic calcium signatures in the symbiosis signaling pathway of legumes. *Proc. Natl. Acad. Sci. USA* 105:9823–28
90. Krause A, Doerfel A, Göttfert M. 2002. Mutational and transcriptional analysis of the type III secretion system of *Bradyrhizobium japonicum*. *Mol Plant-Microbe Interact.* 15:1228–35
91. Kuhn H, Kuester H, Requena N. 2010. Membrane steroid-binding protein 1 induced by a diffusible fungal signal is critical for mycorrhization in *Medicago truncatula*. *New Phytol.* 185:716–33
92. Lauressergues D, Delaux P-M, Formey D, Lelandais-Briere C, Fort S, et al. 2012. The microRNA miR171h modulates arbuscular mycorrhizal colonization of *Medicago truncatula* by targeting NSP2. *Plant J.* 72:512–22
93. Lefebvre B, Klaus-Heisen D, Pietraszewska-Bogiel A, Hervé C, Camut S, et al. 2012. Role of N-glycosylation sites and CXC motifs in trafficking of *Medicago truncatula* Nod factor perception protein to plasma membrane. *J. Biol. Chem.* 287:10812–23
94. Lefebvre B, Timmers T, Mbengue M, Moreau S, Hervé C, et al. 2010. A remorin protein interacts with symbiotic receptors and regulates bacterial infection. *Proc. Natl. Acad. Sci. USA* 107:2343–48
95. Lerouge P, Roche P, Faucher C, Maillet F, Truchet G, et al. 1990. Symbiotic host-specificity of *Rhizobium meliloti* is determined by a sulphated and acylated glucosamine oligosaccharide signal. *Nature* 344:781–84
96. Liang Y, Cao Y, Tanaka K, Thibivilliers S, Wan J, et al. 2013. Nonlegumes respond to rhizobial Nod factors by suppressing the innate immune response. *Science* 341:1384–87
97. Liang Y, Tóth K, Cao Y, Tanaka K, Espinoza C, Stacey G. 2014. Lipochitooligosaccharide recognition: an ancient story. *New Phytol.* 204:289–96
98. Limpens E, Bisseling T. 2014. Cyclops: a new vision on rhizobium-induced nodule organogenesis. *Cell Host Microbe* 15:127–29
99. Limpens E, Franken C, Smit P, Willemse J, Bisseling T, Geurts R. 2003. LysM domain receptor kinases regulating rhizobial Nod factor–induced infection. *Science* 302:630–33
100. Lin K, Limpens E, Zhang Z, Ivanov S, Saunders DGO, et al. 2014. Single nucleus genome sequencing reveals high similarity among nuclei of an endomycorrhizal fungus. *PLOS Genet.* 10:e1004078
101. Liu T, Liu Z, Song C, Hu Y, Han Z, et al. 2012. Chitin-induced dimerization activates a plant immune receptor. *Science* 336:1160–64
102. Liu W, Kohlen W, Lillo A, Op den Camp R, Ivanov S, et al. 2011. Strigolactone biosynthesis in *Medicago truncatula* and rice requires the symbiotic GRAS-type transcription factors NSP1 and NSP2. *Plant Cell* 23:3853–65
103. Lopez-Gomez M, Sandal N, Stougaard J, Boller T. 2012. Interplay of flg22-induced defence responses and nodulation in *Lotus japonicus*. *J. Exp. Bot.* 63:393–401

104. López-Ráez JA, Verhage A, Fernández I, García JM, Azcón-Aguilar C, et al. 2010. Hormonal and transcriptional profiles highlight common and differential host responses to arbuscular mycorrhizal fungi and the regulation of the oxylipin pathway. *J. Exp. Bot.* 61:2589–601
105. Lorquin J, Lortet G, Ferro M, Mear N, Boivin C. 1997. *Sinorhizobium teranga* bv. *acacia* ORS1073 and *Rhizobium* sp. strain ORS1001, two distantly related *Acacia*-nodulating strains, produce similar Nod factors that are O carbamoylated, N methylated, and mainly sulfated. *J. Bacteriol.* 179:3079–83
106. Madsen EB, Antolín-Llovera M, Grossmann C, Ye J, Vieweg S, et al. 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.* 65:404–17
107. Madsen EB, Madsen LH, Radutoiu S, Sato S, Kaneko T, et al. 2003. A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals. *Nature* 425:637–40
108. Madsen LH, Tirichine L, Jurkiewicz A, Sullivan JT, Heckmann AB, et al. 2010. The molecular network governing nodule organogenesis and infection in the model legume *Lotus japonicus*. *Nat. Commun.* doi:10.1038/ncomms1009
109. Maillet F, Poinsot V, André O, Puech-Pagès V, Haouy A, et al. 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. *Nature* 469:58–63
110. Marcel S, Sawers R, Oakeley E, Angliker H, Paszkowski U. 2010. Tissue-adapted invasion strategies of the rice blast fungus *Magnaporthe oryzae*. *Plant Cell* 22:3177–87
111. Marchetti M, Capela D, Glew M, Cruveiller S, Chane-Woon-Ming B, et al. 2010. Experimental evolution of a plant pathogen into a legume symbiont. *PLOS Biol.* 8:e1000280
112. Marchetti M, Jauneau A, Capela D, Remigi P, Gris C, et al. 2014. Shaping bacterial symbiosis with legumes by experimental evolution. *Mol. Plant-Microbe Interact.* 27:956–64
113. Marie C, Broughton WJ, Deakin WJ. 2001. *Rhizobium* type III secretion systems: legume charmers or alarmers? *Curr. Opin. Plant Biol.* 4:336–42
114. Martinez-Abarca F, Herrera-Cervera JA, Bueno P, Sanjuan J, Bisseling T, Olivares J. 1998. Involvement of salicylic acid in the establishment of the *Rhizobium meliloti*–alfalfa symbiosis. *Mol. Plant-Microbe Interact.* 11:153–55
115. Masson-Boivin C, Giraud E, Perret X, Batut J. 2009. Establishing nitrogen-fixing symbiosis with legumes: How many rhizobium recipes? *Trends Microbiol.* 17:458–66
116. Mbengue M, Camut S, de Carvalho-Niebel F, Deslandes L, Froidure S, et al. 2010. The *Medicago truncatula* E3 ubiquitin ligase PUB1 interacts with the LYK3 symbiotic receptor and negatively regulates infection and nodulation. *Plant Cell* 22:3474–88
117. Mellersh D, Parniske M. 2006. Common symbiosis genes of *Lotus japonicus* are not required for intracellular accommodation of the rust fungus *Uromyces loti*. *New Phytol.* 170:641–44
118. Mendes R, Garbeva P, Raaijmakers JM. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol. Rev.* 37:634–63
119. Messinese E, Mun J, Yeun LH, Jayaraman D, Rougé P, et al. 2007. A novel nuclear protein interacts with the symbiotic DMI3 calcium- and calmodulin-dependent protein kinase of *Medicago truncatula*. *Mol. Plant-Microbe Interact.* 20:912–21
120. Miransari M, Abrishamchi A, Khoshbakht K, Niknam V. 2014. Plant hormones as signals in arbuscular mycorrhizal symbiosis. *Crit. Rev. Biotechnol.* 34:123–33
121. Miya A, Albert P, Shinya T, Desaki Y, Ichimura K, et al. 2007. Cerk1, a LysM receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 104:19613–18
122. Miyata K, Kozaki T, Kouzai Y, Ozawa K, Ishii K, et al. 2014. Bifunctional plant receptor, OsCERK1, regulates both chitin-triggered immunity and arbuscular mycorrhizal symbiosis in rice. *Plant Cell Physiol.* 55:1864–72
123. Moling S, Pietraszewska-Bogiel A, Postma M, Fedorova E, Hink MA, et al. 2014. Nod factor receptors form heteromeric complexes and are essential for intracellular infection in *Medicago* nodules. *Plant Cell* 26:4188–99
124. Nadal M, Paszkowski U. 2013. Polyphony in the rhizosphere: presymbiotic communication in arbuscular mycorrhizal symbiosis. *Curr. Opin. Plant Biol.* 16:473–79

125. Nakagawa T, Kaku H, Shimoda Y, Sugiyama A, Shimamura M, et al. 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.* 65:169–80
126. Narusaka Y, Shinya T, Narusaka M, Motoyama N, Shimada H, et al. 2013. Presence of LYM2 dependent but CERK1 independent disease resistance in *Arabidopsis*. *Plant Signal. Behav.* 8:10–12
127. Navarro L, Bari R, Achard P, Lisón P, Nemri A, et al. 2008. DELLAs control plant immune responses by modulating the balance of jasmonic acid and salicylic acid signaling. *Curr. Biol.* 18:650–55
128. Okazaki S, Kaneko T, Sato S, Saeki K. 2013. Hijacking of leguminous nodulation signaling by the rhizobial type III secretion system. *Proc. Natl. Acad. Sci. USA* 110:17131–36
129. Oldroyd GED. 2013. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nat. Rev. Microbiol.* 11:252–63
130. Op den Camp R, Streng A, De Mita S, Cao Q, Polone E, et al. 2011. LysM-type mycorrhizal receptor recruited for rhizobium symbiosis in nonlegume *Parasponia*. *Science* 331:909–12
131. Op den Camp RHM, Polone E, Fedorova E, Roelofsen W, Squartini A, et al. 2012. Nonlegume *Parasponia andersonii* deploys a broad rhizobium host range strategy resulting in largely variable symbiotic effectiveness. *Mol. Plant-Microbe Interact.* 25:954–63
132. Paparella C, Savatin DV, Marti L, De Lorenzo G, Ferrari S. 2014. The *Arabidopsis* LYSIN MOTIF-CONTAINING RECEPTOR-LIKE KINASE3 regulates the cross talk between immunity and abscisic acid responses. *Plant Physiol.* 165:262–76
133. Parniske M. 2000. Intracellular accommodation of microbes by plants: a common developmental program for symbiosis and disease? *Curr. Opin. Plant Biol.* 3:320–28
134. Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat. Rev. Microbiol.* 6:763–75
135. Peiter E, Sun J, Heckmann AB, Venkateshwaran M, Riely BK, et al. 2007. The *Medicago truncatula* DMI1 protein modulates cytosolic calcium signaling. *Plant Physiol.* 145:192–203
136. Pel MJC, Pieterse CMJ. 2013. Microbial recognition and evasion of host immunity. *J. Exp. Bot.* 64:1237–48
137. Perret X, Staehelin C, Broughton WJ. 2000. Molecular basis of symbiotic promiscuity. *Microbiol. Mol. Biol. Rev.* 64:180–201
138. Petutschnig EK, Jones AME, Serazetdinova L, Lipka U, Lipka V. 2010. The lysin motif receptor-like kinase (LysM-RLK) CERK1 is a major chitin-binding protein in *Arabidopsis thaliana* and subject to chitin-induced phosphorylation. *J. Biol. Chem.* 285:28902–11
139. Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SCM. 2012. Hormonal modulation of plant immunity. *Annu. Rev. Cell Dev. Biol.* 28:489–521
140. Pietraszewska-Bogiel A, Lefebvre B, Koini M a, Klaus-Heisen D, Takken FLW, et al. 2013. Interaction of *Medicago truncatula* lysin motif receptor-like kinases, NFP and LYK3, produced in *Nicotiana benthamiana* induces defence-like responses. *PLOS ONE* 8:e65055
141. Popp C, Ott T. 2011. Regulation of signal transduction and bacterial infection during root nodule symbiosis. *Curr. Opin. Plant Biol.* 14:458–67
142. Radutoiu S, Madsen LH, Madsen EB, Felle HH, Umehara Y, et al. 2003. Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. *Nature* 425:585–92
143. Radutoiu S, Madsen LH, Madsen EB, Jurkiewicz A, Fukai E, et al. 2007. LysM domains mediate lipochitin-oligosaccharide recognition and Nfr genes extend the symbiotic host range. *EMBO J.* 26:3923–35
144. Raymond J, Siefert JL, Staples CR, Blankenship RE. 2004. The natural history of nitrogen fixation. *Mol. Biol. Evol.* 21:541–54
145. Redecker D, Kodner R, Graham LE. 2000. Glomalean fungi from the Ordovician. *Science* 289:1920–21
146. Remy W, Taylor TN, Hass H, Kerp H. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proc. Natl. Acad. Sci. USA* 91:11841–43
147. Rey T, Chatterjee A, Buttay M, Toulotte J, Schornack S. 2015. *Medicago truncatula* symbiosis mutants affected in the interaction with a biotrophic root pathogen. *New Phytol.* 206:497–500
148. Rey T, Nars A, Bonhomme M, Bottin A, Balzergue S, et al. 2013. Nfp, a LysM protein controlling Nod factor perception, also intervenes in *Medicago truncatula* resistance to pathogens. *New Phytol.* 198:875–86

149. Rey T, Schornack S. 2013. Interactions of beneficial and detrimental root-colonizing filamentous microbes with plant hosts. *Genome Biol.* 14:121
150. Ried MK, Antolín-Llovera M, Parniske M. 2014. Spontaneous symbiotic reprogramming of plant roots triggered by receptor-like kinases. *Elife* 25:3
151. Riely BK, Lougnon G, Ané J-M, Cook DR. 2007. The symbiotic ion channel homolog DMI1 is localized in the nuclear membrane of *Medicago truncatula* roots. *Plant J.* 49:208–16
152. Ruyter-Spira C, Kohlen W, Charnikhova T, van Zeijl A, van Bezouwen L, et al. 2011. Physiological effects of the synthetic strigolactone analog GR24 on root system architecture in *Arabidopsis*: another belowground role for strigolactones? *Plant Physiol.* 155:721–34
153. Saad MM, Crèvecoeur M, Masson-Boivin C, Perret X. 2012. The type 3 protein secretion system of *Cupriavidus taiwanensis* strain LMG19424 compromises symbiosis with *Leucaena leucocephala*. *Appl. Environ. Microbiol.* 78:7476–79
154. Saito K, Yoshikawa M, Yano K, Miwa H, Uchida H, et al. 2007. NUCLEOPORIN85 is required for calcium spiking, fungal and bacterial symbioses, and seed production in *Lotus japonicus*. *Plant Cell* 19:610–24
155. Sawada H, Kuykendall LD, Young JM. 2003. Changing concepts in the systematics of bacterial nitrogen-fixing legume symbionts. *J. Gen. Microbiol.* 49:155–79
156. Shibuya N, Minami E. 2001. Oligosaccharide signalling for defence responses in plant. *Physiol. Mol. Plant Pathol.* 59:223–33
157. Shimizu T, Nakano T, Takamizawa D, Desaki Y, Ishii-Minami N, et al. 2010. Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. *Plant J.* 64:204–14
158. Shinya T, Motoyama N, Ikeda A, Wada M, Kamiya K, et al. 2012. Functional characterization of CEBiP and CERK1 homologs in *Arabidopsis* and rice reveals the presence of different chitin receptor systems in plants. *Plant Cell Physiol.* 53:1696–706
159. Sieberer BJ, Chabaud M, Fournier J, Timmers ACJ, 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.* 69:822–30
160. 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.* 15:444–53
161. Smit P, Limpens E, Geurts R, Fedorova E, Dolgikh E, et al. 2007. *Medicago* LYK3, an entry receptor in rhizobial nodulation factor signaling. *Plant Physiol.* 145:183–91
162. Smit P, Raedts J, Portyanko V, Debellé F, Gough C, et al. 2005. Nsp1 of the GRAS protein family is essential for rhizobial Nod factor–induced transcription. *Science* 308:1789–91
163. Spaink HP, Wijfjes AHM, Lugtenberg BJJ. 1995. Rhizobium NodI and NodJ proteins play a role in the efficiency of secretion of lipochitin oligosaccharides. *J. Appl. Microbiol.* 177:6276–81
164. Sprent JI. 2007. Evolving ideas of legume evolution and diversity: a taxonomic perspective on the occurrence of nodulation. *New Phytol.* 174:11–25
165. Stacey G, Bickley C, Alvin M, Kim S. 2006. Effects of endogenous salicylic acid on nodulation in the model legumes *Lotus japonicus* and *Medicago truncatula*. *Plant Physiol.* 141:1473–81
166. Stacey G, Shibuya N. 1997. Chitin recognition in rice and legumes. *Plant Soil* 194:161–69
167. Stracke S, Kistner C, Yoshida S, Mulder L, Sato S, et al. 2002. A plant receptor-like kinase required for both bacterial and fungal symbiosis. *Nature* 417:959–62
168. Streng A, Op den Camp R, Bisseling T, Geurts R. 2011. Evolutionary origin of rhizobium Nod factor signaling. *Plant Signal. Behav.* 6:1510–14
169. Svistoonoff S, Hocher V, Gherbi H. 2014. Actinorhizal root nodule symbioses: What is signalling telling on the origins of nodulation? *Curr. Opin. Plant Biol.* 20:11–18
170. Takeda N, Tsuzuki S, Suzaki T, Parniske M, Kawaguchi M. 2013. *CERBERUS* and *NSP1* of *Lotus japonicus* are common symbiosis genes that modulate arbuscular mycorrhiza development. *Plant Cell Physiol.* 54:1711–23
171. Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, et al. 2013. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. *Proc. Natl. Acad. Sci. USA* 110:20117–22

172. Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, et al. 2008. Inhibition of shoot branching by new terpenoid plant hormones. *Nature* 455:195–200
173. Vandamme P, Coenye T. 2004. Taxonomy of the genus *Cupriavidus*: a tale of lost and found. *Int. J. Syst. Evol. Microbiol.* 54:2285–89
174. Van Spronsen PC, Tak T, Rood AMM, van Brussel AAN, Kijne JW, Boot KJM. 2003. Salicylic acid inhibits indeterminate-type nodulation but not determinate-type nodulation. *Mol. Plant-Microbe Interact.* 16:83–91
175. Wan J, Tanaka K, Zhang X-C, Son GH, Brechenmacher L, et al. 2012. Lyk4, a lysin motif receptor-like kinase, is important for chitin signaling and plant innate immunity in *Arabidopsis*. *Plant Physiol.* 160:396–406
176. Wan J, Zhang X-C, Neece D, Ramonell KM, Clough S, et al. 2008. A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in *Arabidopsis*. *Plant Cell* 20:471–81
177. Wang B, Yeun L, Xue J, Liu Y. 2010. Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. *New Phytol.* 186:514–25
178. Wassem R, Kobayashi H, Kambara K, Le Quéré A, Walker GC, et al. 2008. TtsI regulates symbiotic genes in *Rhizobium* species NGR234 by binding to tts boxes. *Mol. Microbiol.* 68:736–48
179. Weerasinghe RR, Bird DM, Allen NS. 2005. Root-knot nematodes and bacterial Nod factors elicit common signal transduction events in *Lotus japonicus*. *Proc. Natl. Acad. Sci. USA* 102:3147–52
180. Weiss D, Ori N. 2007. Mechanisms of cross talk between gibberellin and other hormones. *Plant Physiol.* 144:1240–46
181. Wild M, Davière J-M, Cheminant S, Regnault T, Baumberger N, et al. 2012. The *Arabidopsis* DELLA RGA-LIKE3 is a direct target of MYC2 and modulates jasmonate signaling responses. *Plant Cell* 24:3307–19
182. Willmann R, Lajunen HM, Erbs G, Newman M, Kolb D, Tsuda K. 2011. *Arabidopsis* lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. *Proc. Natl. Acad. Sci. USA* 108:19824–29
183. Xiao T, Schilderink S, Moling S, Deinum EE, Kondorosi E, et al. 2014. Fate map of *Medicago truncatula* root nodules. *Development* 141:3517–28
184. Yang D, Yao J, Mei C, Tong X, Zeng L, et al. 2012. Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proc. Natl. Acad. Sci. USA* 109:E1192–200
185. Yano K, Yoshida S, Müller J, Singh S, Banba M, et al. 2008. CYCLOPS, a mediator of symbiotic intracellular accommodation. *Proc. Natl. Acad. Sci. USA* 105:20540–45
186. Young JPW, Haukka KE. 1996. Diversity and phylogeny of rhizobia. *New Phytol.* 133:87–94
187. Yu N, Luo D, Zhang X, Liu J, Wang W, et al. 2014. A DELLA protein complex controls the arbuscular mycorrhizal symbiosis in plants. *Cell Res.* 24:130–33
188. Zamioudis C, Pieterse CMJ. 2012. Modulation of host immunity by beneficial microbes. *Mol. Plant-Microbe Interact.* 25:139–50
189. Zhang X, Dong W, Sun J, Feng F, Deng Y, et al. 2015. The receptor kinase CERK1 has dual functions in symbiosis and immunity signalling. *Plant J.* 81:258–67
190. Zhang X-C, Wu X, Findley S, Wan J, Libault M, et al. 2007. Molecular evolution of lysin motif-type receptor-like kinases in plants. *Plant Physiol.* 144:623–36