Published in final edited form as:
Nat Rev Microbiol. 2007 August ; 5(8): 619–633. doi:10.1038/nrmicro1705.

How rhizobial symbionts invade plants: the *Sinorhizobium–Medicago* model

Kathryn M. Jones, **Hajime Kobayashi**, **Bryan W. Davies**, **Michiko E. Taga**, and **Graham C. Walker**
Department of Biology, Massachusetts Institute of Technology, Building 68, Room 633, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, USA

Abstract

Nitrogen-fixing rhizobial bacteria and leguminous plants have evolved complex signal exchange mechanisms that allow a specific bacterial species to induce its host plant to form invasion structures through which the bacteria can enter the plant root. Once the bacteria have been endocytosed within a host-membrane-bound compartment by root cells, the bacteria differentiate into a new form that can convert atmospheric nitrogen into ammonia. Bacterial differentiation and nitrogen fixation are dependent on the microaerobic environment and other support factors provided by the plant. In return, the plant receives nitrogen from the bacteria, which allows it to grow in the absence of an external nitrogen source. Here, we review recent discoveries about the mutual recognition process that allows the model rhizobial symbiont Sinorhizobium meliloti to invade and differentiate inside its host plant alfalfa (Medicago sativa) and the model host plant barrel medic (Medicago truncatula).

The recent completion of the *Sinorhizobium meliloti* genome sequence, and the progress towards the completion of the *Medicago truncatula* genome sequence, have led to a surge in the molecular characterization of the determinants that are involved in the development of the symbiosis between rhizobial bacteria and leguminous plants. Aromatic compounds from legumes called flavonoids first signal the rhizobial bacteria to produce lipochitooligosaccharide compounds called Nod factors[1]. Nod factors that are secreted by the bacteria activate multiple responses in the host plant that prepare the plant to receive the invading bacteria. Nod factors and symbiotic exopolysaccharides induce the plant to form infection threads, which are thin tubules filled with bacteria that penetrate into the plant cortical tissue and deliver the bacteria to their target cells. Plant cells in the inner cortex internalize the invading bacteria in host-membrane-bound compartments that mature into structures known

Correspondence to G.C.W. gwalker@mit.edu.

Competing interests statement
The authors declare no competing financial interests.

DATABASES
The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
bacA | *bdhA* | *hemA* | *katA* | *oxyR* | *phbC* | *sitA*
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj
Lotus japonicus | *Medicago sativa* | *Medicago truncatula* | *Sinorhizobium meliloti*
Entrez Protein: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Nod D1 | Nod D2 | Nod D3
UniProtKB: http://ca.expasy.org/sprot
leghaemoglobin | MtNFP

FURTHER INFORMATION
Graham C. Walker's homepage: http://web.mit.edu/biology/www/facultyareas/facresearch/walker.html
Access to this links box is available online.

as symbiosomes. The internalized bacteria then develop into bacteroids, a differentiated form that is capable of nitrogen fixation. During invasion and symbiosis, rhizobial bacteria can evade the host plant innate immune response. In this Review, we summarize and integrate the advances that have been made towards understanding the invasion of plant tissues by rhizobia, and the differentiation of the specialized bacterial and plant structures that facilitate nutrient exchange.

Invasion of plant roots

Although plant roots are exposed to various micro-organisms in the soil, their cell walls form a strong protective barrier against most harmful species. The early steps in the invasion of barrel medic (*M. truncatula*) and alfalfa (*Medicago sativa*) roots by *S. meliloti* are characterized by the reciprocal exchange of signals that allow the bacteria to use the plant root hair cells as a means of entry.

Initial signal exchange

Flavonoid compounds (2-phenyl-1,4-benzopyrone derivatives) produced by leguminous plants are the first signals to be exchanged by host–rhizobial symbiont pairs[1] (FIG. 1). Flavonoids bind bacterial NodD proteins, which are members of the LysR family of transcriptional regulators, and activate these proteins to induce the transcription of rhizobial genes[1,2]. For example, the *M. sativa*-derived flavonoid luteolin stimulates binding of an active form of NodD1 to an *S. meliloti* '*nod*-box' promoter, which activates transcription of the downstream *nod* genes[3]. *S. meliloti* has two other NodD proteins — NodD2, which is activated by as-yet-unpurified plant compounds, and NodD3, which does not require plant-derived compounds to activate gene expression from *nod* box promoters[1]. The expression of NodD3 itself is controlled by a complex regulatory circuit[2]. Any of these NodD proteins can provide *S. meliloti* with the ability to nodulate *M. sativa*[1]. Flavonoids from non-host plants can inhibit the transcription of *S. meliloti nod* genes[3].

Among the rhizobial genes induced by flavonoid-activated NodD proteins are several *nod* genes encoding enzymes that are required for the production of lipochitooligosaccharide Nod factors[4]. Bacterially produced Nod factors induce multiple responses required for nodulation of appropriate host plants, and are the best characterized of the signals that are exchanged between plant hosts and rhizobial symbionts[1]. Nod factors consist of a backbone of β-1,4-linked *N*-acetyl-D-glucosamine residues, which can differ in number not only between bacterial species but also within the repertoire of a single species[1]. Nod factors are *N*-acylated at the non-reducing terminal residue with acyl chains that can also vary between rhizobial species[1]. The genes of the *nodABC* operon encode the proteins that are required to make the core Nod factor structure[1]. The products of other *nod* genes (and *noe* and *nol* genes) make modifications to Nod factors that impart host specificity, including the addition of fucosyl, sulphuryl, acetyl, methyl, carbamoyl and ara-binosyl residues, as well as introducing differences to the acyl chain[1,4]. Many rhizobial species produce more than one type of Nod factor, but it is not yet possible to predict the range of possible host plants from the Nod factor structure[1]. See REFS [1,4–7] for in-depth reviews of Nod factor production by bacteria, and perception and signalling by plants.

Nod factors initiate multiple responses that are essential for bacterial invasion of the plant host[4]. One of the earliest plant responses to the correct Nod factor structure is an increase in the intracellular levels of calcium in root hairs, followed by strong calcium oscillations (spiking)[4] and alterations to the root hair cytoskeleton[8–11]. These responses are followed by curling of the root hairs, which traps rhizobial bacteria within what is known as a tight colonized curled root hair (CCRH)[12,13] (FIG. 1). Simultaneously, Nod factor stimulates root cortex cells to reinitiate mitosis, a process that is dependent on the inhibition of auxin transport by rhizobia

and plant flavonoids[8,14–16]. These cells will form the nodule primordium, and give rise to the cells that will receive the invading bacteria[14].

Multiple extracellular-domain-containing receptors are required for a complete plant response to Nod factor[6] (FIG 2) and in the absence of a functional *MtNFP* (*M. truncatula* Nod factor perception) gene, *M. truncatula* cannot respond[17]. MtNFP is a member of the LysM family of receptors, and is required for root hair curling and the induction of a subset of transcriptional changes in response to Nod factor[17,18]. Recently, RNA interference (RNAi) partial depletion experiments demonstrated an additional role for MtNFP during a later step in bacterial penetration of the root hair[18] (infection thread formation; see below). Another LysM family receptor, encoded by *MtLYK3*/*HCL*, is known to be required for CCRH formation and fine-tuning of the plant responses to Nod factor during infection thread formation (P. Smit and T. Bisseling, unpublished data)[19].

Many downstream components of the Nod factor signal transduction system in leguminous plants have recently been identified[4–7] (FIG. 2). *DMI1* (does not make infections 1) encodes a ligand-gated ion channel that localizes to the nuclear membrane, and null mutations in *DMI1* eliminate the calcium spiking response to Nod factor[20,21]. *DMI3* encodes a Ca^{2+}–calmodulin-dependent protein kinase (CCaMk) that is necessary for the induction of cell division in the root cortex and for the transcriptional changes that are required for the establishment of the symbiosis[22,23]. Expression of a constitutively active allele of *DMI3* induces these cell divisions and transcriptional changes, but does not induce root hair curling nor promote entry of bacteria, suggesting that these responses require other intermediates[24]. *DMI2* encodes a leucine-rich-repeat receptor kinase that is required for tight root hair curling around the bacteria[25]. Two GRAS family transcriptional regulators, nodulation signalling pathway 1 (NSP1) and NSP2, are also required for Nod-factor-induced transcriptional changes[26,27]. Additionally, calcium spiking and transcript induction are dependent on phospholipid signalling pathways[28]. Transcriptional changes induced early in the nodulation programme, which are mainly due to the actions of Nod factor, have been characterized in several recent studies, and purified Nod factor can induce many of these signalling events in the absence of bacteria[2,8]. However, although Nod factor is necessary for nodule formation and *S. meliloti* invasion, it is not the only bacterially produced effector that is required for these symbionts to enter plant tissues and colonize plant cells.

Infection thread development

In all but the most primitive rhizobial–host symbioses, the bacteria must be internalized by plant cells in the root cortex before they can begin to fix nitrogen[1]. The bacteria penetrate these deeper plant tissues through the production of infection threads (FIG. 3). To form these structures, the bacteria must first become trapped at the root hair tip in a CCRH. Several *S. meliloti* mutants have been isolated that are inefficient in colonizing root hairs. Mutants that are unable to produce cyclic β-glucans cannot attach to root hairs effectively, a defect that might be due to a disrupted interaction with the host cell surface[29,30]. A constitutively active mutant of the transcriptional regulator *exoS* and a null mutant of the transcriptional regulator *exoR* are both less efficient than wild-type bacteria at colonizing root hairs[31]. Both of these mutants overproduce succinoglycan, but this does not appear to be the cause of their symbiotic defect, which could be caused by the lack of flagella or other currently unidentified factors[31–33].

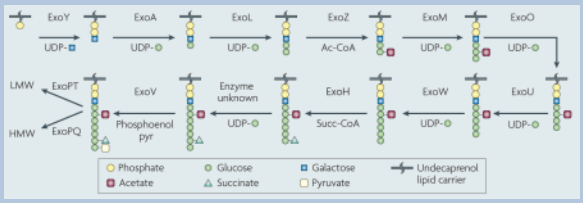
Bacteria that have become trapped in a CCRH at the root hair tip, and can produce both Nod factor and a symbiotically active exopolysaccharide, induce the progressive ingrowth of the root hair cell membrane, resulting in bacterial invasion of interior plant tissue[13]. Efficient invasion occurs even if Nod factor and exopolysaccharide are supplied by separate *S. meliloti* strains that are co-inoculated and trapped together in the same CCRH[34]. The tip of the

developing infection thread is a site of new membrane synthesis, and is proposed to involve inversion of the tip growth that is normally exhibited by the root hair and to be the result of reorganization of cellular polarity[13]. Microscopic analysis of fluorescently tagged bacteria within infection threads indicates that only the bacteria at the tip of the ingrowing infection thread are actively dividing[35]. Invasion appears to progress by continued bacterial proliferation at the tip and sustained induction of infection thread membrane synthesis.

S. meliloti produces the exopolysaccharides succinoglycan (also known as exopolysaccharide I, EPSI) and galactoglucan (EPSII), which facilitate infection thread formation[36,37]. Succinoglycan is a polymer of an octasaccharide repeating unit modified with acetyl, succinyl and pyruvyl substituents[38] (BOX 1), is more efficient than galactoglucan in mediating infection thread formation on *M. sativa* and is the only exopolysaccharide produced by *S. meliloti* that can mediate the formation of infection threads on *M. truncatula*[36,37]. An *S. meliloti exoY* mutant, which cannot produce any succinoglycan, can form CCRHs but initiates almost no infection threads — these mutants remain trapped in a microcolony at the tip of the root hair[39] (FIGS 3,4; TABLE 1).

Box 1

Sinorhizobium meliloti succinoglycan biosynthesis



Sinorhizobium meliloti strain 1021 requires the exopolysaccharide succinoglycan to establish a functional symbiosis with *Medicago truncatula* and *Medicago sativa*[36,143]. Bacterial mutants that are defective in succinoglycan production cannot initiate infection thread formation and yield nodules that are devoid of bacteria or bacteroids[36,143]. Succinoglycan is a polymer of an octasaccharide repeating unit composed of one galactose and seven glucose residues with acetyl, succinyl and pyruvyl modifications[38,144] (see figure). Succinoglycan is produced in a high molecular weight (HMW) form, as a polymer containing hundreds of the octasaccharide repeating unit, and a low molecular weight (LMW) form that is composed of monomers, dimers and trimers of the repeating unit[145]. Application of the purified trimer fraction can partially rescue infection thread formation of a succinoglycan-deficient mutant. However, the variability of invasion under these conditions suggests that succinoglycan must be applied within the colonized curled root hair to function effectively[145].

Many gene products are required for succinoglycan biosynthesis. ExoC, ExoB and ExoN are involved in the biosynthesis of the precursors UDP-galactose and UDP-glucose[146,147]. ExoY initiates the synthesis of the repeating unit, by transferring the galactosyl residue to the lipid carrier on the cytoplasmic side of the inner membrane[148], and sugar transferases encoded by *exoA, exoL, exoM, exoO, exoU* and *exoW* subsequently elongate the octasaccharide backbone[148]. ExoZ, ExoH and ExoV transfer succinyl (Succ), acetyl (Ac) and pyruvyl (pyr) groups, respectively, to the growing backbone[149,150]. The mature repeating unit (linked to the lipid carrier) is flipped to the periplasmic side of the inner membrane, and then transferred to the growing polymer of succinoglycan by ExoP, ExoQ and ExoT[148,149,151]. The high molecular weight fraction of this polymer can be further

processed by two extracellular endo-glycanases, ExoK and ExsH, to the low molecular weight form of succinoglycan[40]. UDP, uridine diphosphate.

An *S. meliloti exoH* mutant produces succinoglycan that lacks the succinyl substituent, and this mutant forms abortive, aberrant infection threads[39] (TABLE 1). Unlike the normal condition, aberrant infection threads are bulbous and winding, as if the bacteria have continued to proliferate even though the tip of the infection thread has halted its inward progression[36, 39]. In contrast to *exoY*-mutant-containing CCRHs, 70% of *exoH*-mutant-containing CCRHs initiate infection threads, but they all abort before reaching the base of the root hair[39]. Interestingly, the succinoglycan polymer produced by an *exoH* mutant is mostly in the high molecular weight form because the lack of the succinyl substituent renders it refractory to cleavage by extracellular glycanases[40]. It is not clear whether infection thread extension is dependent on the presence of the succinyl substituent of succinoglycan or on the production of low molecular weight (trimer, dimer and monomer) forms of succinoglycan. If succinoglycan has a role in signalling to the plant, perhaps the low molecular weight forms can interact more readily with the plant cell membrane, whereas the high molecular weight forms, which can be composed of polymers of hundreds of monomers, are sterically hindered by the plant cell wall. In a different host–symbiont pair, involving vetch (*Vicia sativa*) and *Rhizobium leguminosarum*, the structure of the bacterial exopolysaccharide appears to be less critical for infection thread extension[41].

As mentioned earlier, in addition to succinoglycan, Nod factor is required for active and ongoing infection thread formation. Aberrant, abortive infection threads are formed when Nod factor is supplied in a structurally incomplete form, or if Nod factor perception is disrupted[18,19,42]. An *S. meliloti nodF nodL* double mutant that produces Nod factor that lacks the acetyl substituent and has an altered acyl chain cannot effectively invade plant cells, and induces approximately 20% of colonized root hairs to form infection threads[19,42]. Those infection threads that are formed are aberrant and ‘sac-like’, and abort before reaching the base of the root hair cell[19] (FIG. 4 and TABLE 1). This is similar to the invasion phenotype of the *exoH* mutant described above[39].

In relation to the host plant, mutant isolation and RNA interference have been used to identify genes encoding proteins that are involved in infection thread formation. The *M. truncatula lin* mutant, when inoculated with wild-type *S. meliloti*, has an abortive infection thread phenotype that is similar to that of a wild-type plant inoculated with an *S. meliloti exoY* mutant[39,43] (TABLE 1). In both of these cases, infection arrests at the CCRH stage (FIG. 4). A deletion mutant of *MtBIT1*/*ERN*, which encodes a putative transcription factor involved in relay of the Nod factor signal, forms normal CCRHs but aberrant infection threads that cannot extend into deeper plant tissues[44]. RNAi knockdown has been used to assess the role of Nod factor receptors in infection thread formation. *M. truncatula* plants that are partially depleted by RNAi for the primary Nod factor receptor *MtNFP*, although capable of root hair curling and initiation of cortical cell divisions, form aberrant infection threads[18] (FIG. 4 and TABLE 1). RNAi depletion of the secondary Nod factor receptor *MtLYK3*/*HCL* (also known as the entry receptor) does not cause wild-type *S. meliloti* to form aberrant infection threads; however, it does cause an *S. meliloti nodFE* mutant (which produces Nod factor with a modified acyl chain) to form defective infection threads[19] (FIG. 4 and TABLE 1). These results indicate that the Nod factor signal requirement for infection thread formation is more stringent and perhaps of greater amplitude than that required for CCRH formation and the initiation of cortical cell divisions.

Other than the genetic and biochemical demonstrations of the requirement for succinoglycan and Nod factor, little is know about the way in which these factors mediate the extensive

cytoskeletal remodelling that occurs during infection thread formation, or how the cytoskeleton might be involved in directing vesicular traffic to this site of new membrane synthesis. The cytoskeletal changes that occur during tip growth of polarized root hairs[45] and the early effects of Nod factor on the root hair cytoskeleton that lead to root hair deformation have been subject to detailed analysis[13,46,47]. However, the cytoskeletal reorganizations involved in infection thread formation have not yet been thoroughly dissected. Inward movement of the root hair cell nucleus, in advance of the ingrowing infection thread, has been observed[8]. In these cells, a network of microtubules connects the nucleus to the tip of the infection thread and surrounds the infection thread itself [8]. As the infection thread develops, the inside of this tubule is topologically outside the root hair cell and possesses a plant cell wall[48]. Interactions between plant cell-wall material and other components of the infection thread matrix might play an important part in infection thread growth[48]. Plant cell wall remodelling during root hair colonization and infection thread formation is another process that is not well understood, but is proposed to involve plant proteins that are exported to the cell wall and the infection thread matrix[48]. Plant glycoproteins that are associated with the infection thread matrix become crosslinked, probably by reactive oxygen species (ROS) generated in the infection thread[49–51]. One hypothesis is that solidification of the infection thread matrix, as a result of this crosslinking, generates a mechanical force that helps the ingrowth of the infection thread to proceed[48]. The production of ROS by plants is often part of a defence response against invading pathogens and it can also serve as an activating signal for other plant defences (see below)[52]. One intriguing possibility is that the facilitation of infection thread progression by ROS is one way that rhizobial bacteria have adapted a plant defence response for their own benefit.

The correct balance of ROS in the infection thread might be necessary for infection thread progression, and an excess of ROS could be detrimental to the rhizobia within the infection thread. However, the level of stress experienced by bacteria in the infection thread due to ROS is unknown. Single mutants in any of the three *S. meliloti* catalases, one of the two superoxide dismutases (*sodB*) or in the global regulator of H_2O_2 protection, *oxyR*, are sensitive to ROS, but are not defective in symbiosis[53–56]. However, double mutants in the genes encoding catalase B and catalase C (*katB*/*katC*) or catalase A and catalase C (*katA*/*katC*) are compromised in their ability to invade the host plant[53,54]. Mutants in a second superoxide dismutase, *sodC*, a predicted *sodM* (Smc00911) or a secreted chloroperoxidase (Smc01944) have not yet been analysed[57]. Further work is therefore required to elucidate how ROS, plant proteins and cell-wall materials interact with exopolysaccharides or other products that are secreted by the bacteria to influence development of the infection thread.

Targeting infection threads

Once an infection thread has penetrated to the base of a root hair cell the bacteria must induce new rounds of infection thread formation in each successive cell layer. Two important questions are: how is infection thread growth targeted to the cortical tissue layer; and, following the penetration of the cortex by an infection thread, what molecular processes mediate endocytosis of bacteria by plant cells into host-membrane-bound compartments?

The plant hormone cytokinin and the Nod-factor-dependent reinitiation of the cell cycle are involved in directing infection threads to the plant cortex. RNAi-mediated depletion of the *M. truncatula* putative cytokinin receptor/histidine kinase *MtCRE1* results in a block in the reinitiation of cell division in the root cortical cells and the abortion of infection threads[58] (TABLE 1). A mutation in the *Lotus japonicus* orthologue of *MtCRE1*, *LHK1* (BOX 2), causes infection threads to arrest or loop and wander in the plant epidermal tissue instead of penetrating straight into the deeper cell layers[59]. It appears that this cytokinin receptor has an important role in initiating cortical cell division, as a constitutively active mutant of *L. japonicus LHK1* induces spontaneous initiation of cell division in the absence of infecting bacteria[60].

The induction of the putative transmembrane-domain-containing transcription factor *MtNIN* is dependent on *MtCRE1*[58]. *Mtnin* mutants are particularly interesting because they have early signalling responses to Nod factor but are impaired in both induction of cortical cell division and infection thread development, suggesting that *MtNIN* might be involved in integrating these processes[61] (TABLE 1). This is consistent with a model in which cortical cell divisions provide a signal for the initiation and maintenance of infection threads. *Mtrit1* and *Mtsli* mutants are also defective in penetration of the infection thread into deeper cortical tissue, but the genes carrying these mutations have not yet been cloned[62–64] (TABLE 1).

Box 2

Mesorhizobium loti and Lotus japonicus: model system for determinate nodules

Rhizobium–legume symbiosis research has progressed quickly using the *Mesorhizobium loti–Lotus japonicus* model system, in addition to the *Sinorhizobium meliloti–Medicago truncatula* model system as discussed in the text. The Nod factor signalling pathway in these two models is similar and has many conserved components (see table). However, what makes comparative study of both these systems most interesting is that they form different types of nodules, in which the nodule structure and the differentiation programme imposed on the symbiotic bacteria are different. *M. truncatula* and *Medicago sativa* are examples of indeterminate legumes, which form nodules that possess a persistent meristem, whereas *L. japonicus* forms determinate nodules, which lack a persistent meristem and in which all cells in the interior of the nodule proliferate, differentiate and senesce synchronously[65]. Engineering of bacterial symbionts that nodulate the opposite type of host has determined that the plant controls the degree of differentiation and the ultimate fate of the bacteria[65]. Differentiated bacterial symbionts isolated from *L. japonicus* nodules have not undergone genomic endoreduplication and can resume growth[65]. However, fully differentiated bacteroids isolated from *M. truncatula* are unable to resume growth, perhaps because of effects of extensive genomic endoreduplication on the bacterial cell cycle[65].

Medicago trunculata protein	*Lotus japonicus* orthologue	Function
MtNFP	LjNFR5 (REF. 4)	Nod factor receptor
Unknown	LjNFR1 (REF. 4)	Nod factor receptor
MtDMI2 (REF. 25)	LjSymRK[4]	Leucine-rich repeat receptor-like kinase
MtDMI1 (REFS 20,21)	LjCASTOR/POLLUX[152]	Ion channel
Unknown	LjNUP133, LjNUP85 (REFS 153, 154)	Nucleoporin
MtDMI3 (REFS 22,23)	LjSYM15/snf1 (REF. 155)	Ca^{2+}–calmodulin-dependent protein kinase
MtNSP1 (REF. 27)	LjNSP1 (REF. 156)	GRAS family transcriptional regulator
MtNSP2 (REF. 26)	LjNSP2 (REF. 156)	GRAS family transcriptional regulator
MtCRE1 (REF. 58)	LjLHK1 (REF. 59)(not required for infection thread formation)	Cytokinin receptor or histidine kinase
MtNIN[61]	LjNIN[157]	Predicted transmembrane transcription factor

The earliest infection threads that penetrate the growing *M. truncatula* or *M. sativa* nodule must grow past the actively dividing cells in the developing nodule primordium[14]. Ultimately, cells adjacent to the initial primordium will give rise to a persistent nodule meristem that maintains a population of actively dividing cells and will continue to grow outward from the root for the life of the nodule[8,14]. *M. truncatula* and *M. sativa* are examples of indeterminate legumes, which form nodules that possess a persistent meristem. Determinate legumes, such as *L. japonicus* (BOX 2), produce nodules without a persistent meristem and in which all cells in the interior of the nodule proliferate, differentiate and senesce synchronously[65]. In developing indeterminate nodules, which we focus on here, infection threads grow through the developing meristematic zone to the underlying cell layers through which the wave of mitotic activity has already passed[65]. These layers of invasion-competent cells have exited mitosis and

become polyploid owing to cycles of genomic endoreduplication without cytokinesis[14,66]. Plant cell endoreduplication is required for the formation of functional nodules, and is dependent on the degradation of mitotic cyclins by anaphase-promoting complex (APC), an E3 ubiquitin ligase[66,67]. The advantages of cellular polyploidy are a higher transcription rate and a higher metabolic rate than cells with the normal 2n DNA content[68] — characteristics that might be important for the invaded plant cells to support bacterial nitrogen fixation (discussed later).

Endocytosis of rhizobia

When the bacteria reach the target tissue layer, the inner plant cortex, they must be internalized by a cortical cell and establish a niche within that cell. Each bacterial cell is endocytosed by a target cell in an individual, unwalled membrane compartment that originates from the infection thread[48]. The entire unit, consisting of an individual bacterium and the surrounding endocytic membrane, is known as the symbiosome[48]. In indeterminate nodules, a bacterial cell and its surrounding membrane divide synchronously before the bacteria differentiate into nitrogen-fixing bacteroids[69]. New lipid and protein material targeted to the symbiosome membrane imparts a distinct biochemical identity to this compartment[69].

Both plant and bacterial defects can cause a failure of symbiosome formation. When the plant leucine-rich-repeat receptor kinase DMI2 is partially depleted by RNAi, the result is overgrown infection threads that fail to release bacteria into symbiosomes[70]. In wild-type infections, the DMI2 protein is localized to the infection thread and symbiosome membranes, which is consistent with a role for this protein in release of bacteria from infection threads and symbiosome development[70]. RNAi-mediated knockdown of the *MtHAP2-1* gene, which encodes a predicted transcription factor, also prevents bacterial release from infection threads[71]. The *M. truncatula nip* mutant also produces overgrown infection threads and the bacteria fail to release from these infection threads[72]. The *NIP* gene has not yet been cloned, but it is likely that its gene product is involved in symbiosome development. Bacteria can also fail to release from infection threads owing to bacterial defects. The *hemA* mutant of *S. meliloti*, which has a primary defect in haem biosynthesis, is not released from infection threads and is not enclosed in symbiosomes[73]. Several bacterial components that are important for symbiosis are known to require haem, such as the oxygen sensor FixL and cytochrome haem proteins, which could explain this phenotype[74,75].

Recent proteomic and immunolocalization studies have begun to define biochemical markers of the symbiosome membrane. These include previously identified nodule-specific proteins (ENOD8, ENOD16, nodulin 25 and nodulin 26), energy and transport proteins, bacterial proteins, and proteins predicted to be involved in folding, processing, targeting and storage[76]. Based on the types of protein that have been extracted from the symbiosome membrane, Catalano *et al.* proposed that proteins are added to this membrane by multiple mechanisms, one of which is being targeted by syntaxin proteins to the symbiosome via the Golgi and membrane-bound vesicles[76]. One particularly interesting protein that is predicted to be involved in the targeting of vesicles to the symbiosome membrane is the SNARE protein MtSYP132 (*M. truncatula* syntaxin of plants 132)[77]. Syntaxins are involved in the docking of vesicles at the target membrane, and might be crucial for the development of the rhizobium-containing vesicle into a mature symbiosome[78].

As rhizobial bacteria are enclosed in the symbiosome membrane by endocytosis, it has been assumed that this compartment is a derivative of the lytic vacuole (the plant counterpart to the mammalian lysosome)[79,80]. Consistent with this hypothesis, several enzymes that localize to the lumen of the soybean symbiosome, or the peribacteroid space, have an acidic pH optimum[79]. However, parallels have also been drawn between the symbiosome and plant protein-storage vacuoles that are initially a non-lytic niche[79,81].

Bacteroid differentiation and survival

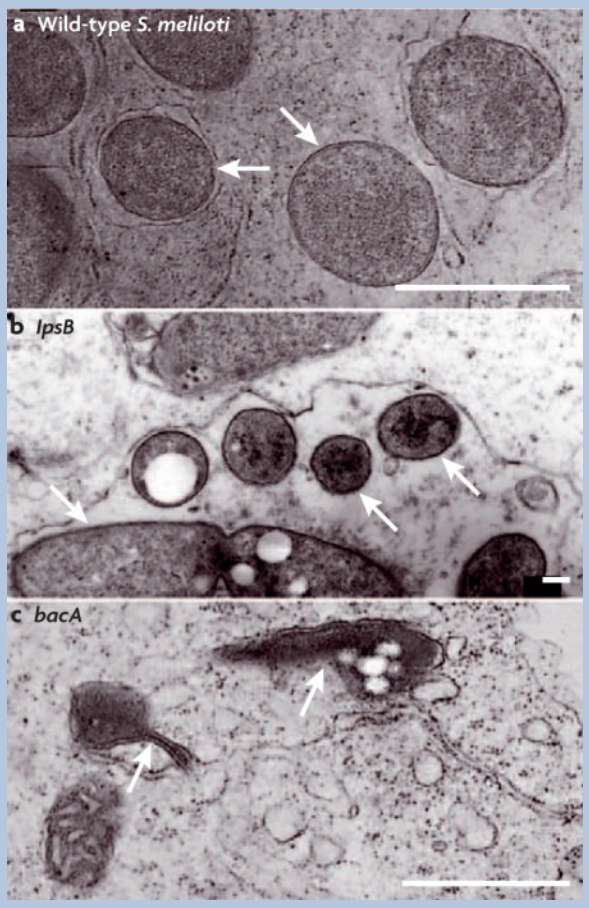
Once the bacteria have been engulfed within host cell membranes, they must survive within the symbiosome compartment and differentiate into the nitrogen-fixing bacteroid form. Both bacterial and plant factors are involved in these processes (FIG. 5).

LPS and rhizobial survival

One of the most important defence mechanisms that Gram-negative bacteria use against the extracellular environment is the lipopolysaccharide (LPS), which is a major component of the outer membrane. LPS consists of a lipid A membrane anchor attached to a polysaccharide core that in turn is attached to an O-antigen repeating unit[82]. The *S. meliloti bacA* gene is required by *S. meliloti* for the production of lipid A of the appropriate structure and for survival within host cells[83,84] (BOX 3). The *bacA* mutant cells lyse soon after endocytosis and do not exhibit any morphological changes that are characteristic of bacteroids, such as cell elongation[83]. BacA is required for modification of lipid A with a very-long-chain fatty acid moiety, and the fatty acid defect may be the cause of the *bacA* mutant symbiotic defect[84] (BOX 3). An *S. meliloti lpsB* mutant produces an LPS core with an altered sugar composition[82] (BOX 3). Although it can invade the plant root, become enclosed within a symbiosome membrane and begin to elongate into the bacteroid form, it cannot complete the bacteroid differentiation programme and fix nitrogen[82]. The *lpsB* mutant symbiotic defect is probably a result of its compromised outer membrane; however, as these bacteria also appear to lack contact with the plant symbiosome membrane, it is possible that they fail to survive because they cannot interact properly with the host[82].

Box 3

Host invasion parallels between rhizobia and animal pathogens



Symbiotic rhizobial bacteria and pathogenic *Brucella* species both form chronic infections of eukaryotic cells within a host-membrane-derived compartment[158]. *Brucella* spp. are α-proteobacteria that are closely related to *Sinorhizobium meliloti* and invade mammalian cells, causing brucellosis[159]. Both *Brucella* spp. and *S. meliloti* must gain entry to target host cells and prevent their own destruction by the lytic compartments of these cells. Bacterial production of lipopolysaccharide (LPS) with the appropriate structure (see main text) is important for survival of both *Brucella* spp. and *S. meliloti* within host cells. The *S. meliloti lpsB* mutant produces an LPS core with altered sugar composition (see figure) and the *Brucella abortus pmm* mutant produces a truncated LPS core[82,160,161]. Neither mutant can survive within host cells and this might be because their outer membranes are compromised[82,160]. These mutants are also sensitive to detergents and to antimicrobial peptides, such as polymyxin B, that resemble defence compounds produced by phagocytic cells and plant cells[82,162,163]. LPS lipid A, with the appropriate structure, might also be important for the survival of *S. meliloti* and *B. abortus* within their respective hosts[83,158]. The *bacA* gene encodes a predicted inner membrane protein with similarity to peroxisomal-membrane transporters of fatty acids and is required for the modification of lipid A with very-long-chain fatty acids in both *S. meliloti* and *B. abortus* (see figure)[84]. However, *S. meliloti* mutants that are unable to synthesize this modified lipid A in the free-living form can form an effective symbiosis, raising the possibility that another function of BacA is required[164]. The production of periplasmic cyclic β-glucans is required for *Brucella* spp.

to survive within intracellular compartments and for *S. meliloti* to adhere to root hair cells[29,165]. During host cell invasion by *B. abortus*, cyclic β-glucans prevent fusion of the bacteria-containing endocytic vacuole with the lysosome, by modulating lipid raft organization in host cell membranes[165]. This suggests that cyclic β-glucans are critical for correct cell surface interactions of *B. abortus* with endocytic vacuole membranes and of *S. meliloti* with root hair cells.

Regulatory genes conserved between *Brucella* spp. and *S. meliloti* are also important for either invasion or persistence within their respective hosts. For example, the *B. abortus bvrS/bvrR* sensor kinase/response regulator system is required for intracellular survival[31,166]. The *B. abortus bvrS/bvrR* system may be required for virulence, because it controls the expression of several outer membrane proteins and is involved in modulating the properties of bacterial lipopolysaccharide [162,167]. It has not been possible to construct null mutants of the *S. meliloti* homologues of these genes, *exoS* and *chvI*, indicating that they are essential[32,168]. However, a mutant that produces a constitutively active ExoS sensor kinase is partially impaired in its ability to infect root hairs (see main text)[31–33]. A Tn5 insertion mutant of another *S. meliloti* sensor kinase, *cbrA*, is also symbiotically defective[169]. This mutant is sensitive to agents that stress the bacterial membrane, and this may be the cause of its symbiotic defect[169]. The *B. abortus* homologue of *cbrA*, *pdhS*, is essential for viability and therefore it has not been possible to assess its requirement for pathogenesis[170]. Panels **a** and **c** reprinted with permission from REF. [83] © (1993) Cold Spring Harbor Laboratory Press. Panel **b** reprinted with permission from REF. [82] © (2002) National Academy of Sciences. The scale bars represent 1 μm for **a** and **c**, and 0.21 μm for **b**.

Non-LPS factors and rhizobial survival—*S. meliloti* mutants that are defective in other aspects of physiology are also defective in symbiosis with the plant host. The *sitA* mutant invades plant cells at a reduced efficiency, but the bacteroids formed are senescent and cannot effectively fix nitrogen[55]. The *sitA* gene encodes a component of a manganese transporter, and the mutant cannot grow in minimal medium without additional manganese[55]. Although this mutant is sensitive to ROS, this is apparently not the cause of the symbiotic defect, suggesting that an Mn^{2+}-requiring enzyme or intracellular Mn^{2+} homeostasis is important for symbiosis[55]. Mutants in the *pha* gene cluster have a symbiotic phenotype that is similar to that of *sitA* mutants, with a reduced efficiency of infection and formation of senescent bacteroids[85]. Interestingly, the product of the *pha* gene cluster is a K^+-efflux system that is active under alkaline conditions[85]. This suggests that mechanisms that cope with specific ion stress are required for the bacteria that invade, and to persist in, the intracellular environment. Mutants that are defective in global bacterial transcriptional responses under stress conditions are also defective in symbiosis. A mutant in *rpoH1*, encoding a putative σ^{32}-factor that is predicted to interact with bacterial RNA polymerase, appears to invade normally, but forms bacteroids that senesce prematurely[86]. By contrast, a mutant in *relA* is defective at multiple stages in nodule formation[87]; *relA* mutants lack the stringent response — a bacterial transcriptional response to starvation[87].

Plant control and support of bacteroid differentiation—The host plant exerts control over the survival of bacteria within the symbiosome, and must not only provide nutritional support and the correct micro-aerobic environment required for nitrogen fixation, but also provide specifications for part of the bacterial differentiation programme. In indeterminate nodules, the internalized bacteria and the symbiosome membrane divide concomitantly before bacteroid differentiation, whereas in determinate nodules, bacteria divide within the membrane compartment and form a small mass of cells[88]. Surprisingly, plants that form indeterminate nodules impose a programme of genomic endoreduplication on the invading bacterial cells[65]. Bacteroids within indeterminate nodules increase their DNA content and cell size,

which might allow them to reach a higher metabolic rate to support nitrogen fixation[65,68]. The intensive DNA synthesis that is required for endoreduplication within bacteroids requires a large quantity of dNTPs that must be supplied by ribonucleotide reductase (RNR)[89]. For many bacterial species, performing DNA synthesis in both an oxygen-rich environment, such as the infection thread, and an oxygen-depleted environment, such as the symbiosome, would present a lethal problem. This is because most aerobes possess an oxygen-requiring class I RNR, whereas obligate anaerobes typically have an exquisitely oxygen-sensitive class III RNR[90]. Some bacterial species, including rhizobia, have an adaptation that circumvents this problem — they possess a vitamin B_{12}-dependent class II RNR that functions independently of oxygen concentration. Significantly, when the class I RNR of *Escherichia coli* is substituted for the class II RNR from *S. meliloti*, survival within the nodule, but not free-living growth, is impaired (M. E. T. and G. C. W., unpublished data). Phosphate must also be obtained by bacteria during the invasion process. *S. meliloti* that is mutated in both the *phoCDET* and *pstSCAB* phosphate-transport systems forms small nodules that cannot fix nitrogen[91,92]. However, these transporters are not expressed by mature bacteroids, suggesting that the requirement for phosphate declines after differentiation[92].

Plants control bacteroid differentiation and survival in other ways that are in the process of being characterized. Bacteria fail to differentiate or senesce prematurely in several mutants of the host plants *M. truncatula* and pea (*Pisum sativum*), but none of the genes carrying these mutations has yet been cloned[62–64,93,94]. In the pea *sym13* mutant, symbiosome membranes are not closely associated with the bacteroid and the bacteroids senesce early[95]. Immunological detection of plant glycoproteins that are still attached to bacteroids isolated from pea nodules suggests that there is a close physical interaction between the symbiosome and bacteroid membranes, at least in indeterminate nodules where a single bacteroid is enclosed within each symbiosome membrane[96,97]. A close association between the bacteroid outer membrane and the symbiosome membrane might be important for nutrient exchange between the bacteria and the plant.

Nodule development and nutrient exchange—Bacteria that have been enclosed within a functional symbiosome membrane, have been provided with a low oxygen environment and have completed the bacteroid differentiation programme can express the enzymes of the nitrogenase complex and begin to fix nitrogen[98]. An oxygen-sensing bacterial regulatory cascade controls the expression of the nitrogenase complex and the microaerobic respiratory enzymes that are required to provide energy and reductant to nitrogenase[98]. This cascade is induced by the presence of low oxygen tension within the differentiating bacteroid[89,99,100]. The bacterial regulators include the oxygen-sensing two-component regulatory system FixL and FixJ, NifA, σ^{54} and FixK[98]. These regulators are responsible for many of the changes in gene and protein expression that have been detected during bacteroid differentiation[2]. In general, the transcriptional changes in bacteroids are consistent with the downregulation of most metabolic processes and a concomitant increase in the expression of the gene products involved in nitrogen fixation and respiration[2]. Respiratory activity provides nitrogenase with the 16 molecules of ATP and 8 electrons that are estimated to be required to reduce 1 molecule of N_2 to 2 molecules of NH_4^+ (REF. [101]).

The NH_4^+ produced by nitrogenase can be secreted by bacteroids. Once secreted, it is thought to be taken up by the plant through NH_4^+ channels that have been detected in the peribacteroid membrane and then assimilated[88,102]. A complex amino-acid cycling system between plant cells and bacteroids might prevent the bacteroids from assimilating NH_4^+, and allow NH_4^+ to be secreted for uptake by the plant[88,101,103,104]. Metabolite analysis and RNAi depletion experiments suggest that NH_4^+ assimilation by plant cells occurs primarily through glutamine and asparagine synthetases[105–107]. In *L. japonicus*, RNAi depletion of the next enzyme in the

nitrogen assimilation pathway, glutamate synthase, results in a reduction of nitrogenase activity and the growth of stunted plants[108].

A constant carbon supply is required to provide metabolites and energy for bacteroid differentiation and nitrogen fixation. Polyhydroxybutyrate (PHB) granules, produced by *S. meliloti* during the invasion process, are degraded during bacteroid differentiation and appear to be used preferentially as a carbon source at this stage[109]. Surprisingly, mutants in PHB synthesis (*phbC*) or degradation (*bdhA*) are symbiotically competent, indicating that alternate carbon sources are available to the bacteria during differentiation[109–111]. In a mixed infection, *phbC* and *bdhA* mutants are less competitive for nodule occupancy than the wild type, suggesting that the ability to synthesize and use PHB might allow the bacteria to compete in situations of reduced carbon availability[109,111]. Fixed carbon from the plant is provided to the bacteria in the form of dicarboxylic acids, such as malate, through the bacterial Dct uptake system[101,112]. NAD^+-malic enzyme activity, which produces pyruvate directly from malate, is required for nitrogen fixation by *S. meliloti*[113]. This suggests that the production of acetyl-CoA from malate using malic enzyme and pyruvate dehydrogenase, is important for the funnelling of carbon into the TCA cycle in bacteroids[101].

Some plant proteins, in addition to glutamate synthase, have been shown to be required to support nitrogen fixation. Leghaemoglobins are plant-produced, oxygen-binding proteins that impart a red colour to functional root nodules, and which, it has long been proposed, can adjust the microaerobic environment within nodules[114]. It has recently been confirmed by RNAi that *L. japonicus* leghaemoglobin is required for nitrogen fixation and to maintain a microaerobic environment in the nodule[114]. Another required plant protein is sucrose synthase (SS), which has been characterized in pea using the SS1-defective *rug4* mutant[115,116]. Contrary to its name, sucrose synthase in pea nodules catabolizes sucrose to UDP-glucose and fructose, which is ultimately metabolized to malate and transported to bacteroids[101,105,115]. The *L. japonicus* sulphate transporter Sst1 is also required for nitrogen fixation and has been detected in symbiosome membranes[117,118]. Sulphate is required to form iron–sulphur clusters in nitrogenase subunits[119]. Transcript profiling and proteomic analysis of plant nodules have identified new candidates for RNAi that will almost certainly lead to the discovery of more plant factors required for nodulation[2].

Bacteria in indeterminate nodules might further benefit from the symbiosis by scavenging for nutrients in the ghost plant cells that are located in the root proximal section of the nodule, in which the original bacteroids have already senesced[120]. These bacteria are released from infection threads into the decaying plant tissue and appear to proliferate. They do not differentiate into bacteroids or fix nitrogen and can probably resume a free-living lifestyle once the nodule has decayed[120].

Rhizobial evasion of plant innate immunity

It remains to be determined how the host plants tolerate such intimate contact with invading rhizobial bacteria without attacking the invaded tissues. Plants, as well as animals, can mount innate immune responses to microorganisms in response to the perception of conserved microbial factors (microorganism-associated molecular patterns or MAMPs)[121]. This usually results in the activation of signalling cascades, and the production of antimicrobial effectors that help the organism ward off microbial attack[121]. However, the plant perception of microorganisms differs from that of animals in the domain structure of receptor proteins and, in many cases, the epitopes perceived[121]. In the interaction between plants and rhizobial bacteria, some bacterial factors activate innate immune responses whereas other bacterial factors suppress these responses[122]. Defining how plants perceive the conserved microbial molecular patterns of rhizobia and respond to them is currently an active area of research.

MAMPs and host-specific elicitors—Microbial factors that induce innate immune responses in plants are classified as either general or host-specific elicitors[52]. General elicitors include flagellin, elongation factor Tu (EFTu), cold shock protein (CSP), chitin and LPS[123–125]. These compounds are conserved across multiple groups of bacteria, allowing plants to perceive and respond to an epitope that is common to many bacteria[123–125]. Plants respond to general elicitors with basal defence responses that can impart a measure of resistance to infection[52]. Basal defences include increases in extracellular pH, ethylene, ROS and reactive nitrogen species, phenolic compounds and changes in intracellular Ca^{2+}, as well as cell signalling cascades and transcriptional changes[52]. Many plant species mount basal defence responses to the broadly conserved flg22 peptide found in bacterial flagellin[123]. However, rhizobial bacteria, along with many other α-proteobacteria, such as *Agrobacterium tumefaciens* and *Brucella* spp., do not have the flg22 epitope as part of their flagellin sequence[126,127]. Two other bacterial epitopes that induce plant defence responses are the elf18 peptide of EFTu and the csp15 peptide from bacterial CSP[123]. These peptides are conserved in rhizobial bacteria; however legumes lack the ability to mount an innate response to them[123]. Comparisons have been made between plant basal defence responses to pathogens and plant responses to rhizobial Nod factor[51]. One model, suggested by the production of ROS during infection thread formation, is that rhizobial effectors, such as Nod factor, provoke a defence reaction in host plants that is attenuated or modified by other rhizobial effectors, such as LPS (see below)[51,128,129].

Unlike general elicitors, most host-specific elicitors are effector proteins that are injected by pathogens into plant cells by bacterial type 3 secretion systems (T3SSs) and which cause a hypersensitive response and programmed cell death[52]. These responses are more extreme and cause more tissue damage than basal defence responses[52]. One example is the root pathogen *Ralstonia solanacearum*, which requires the GALA7 type 3 secreted protein to cause wilting disease on *M. truncatula*[130].

Nod factor and chitin—Chitin is another general elicitor that induces plant innate immune responses, such as the oxidative burst[124]. This response helps plants defend themselves against fungal attack, as chitin is a component of fungal cell walls[124]. Intriguingly, the chitin receptor of rice (*Oryza sativa*) is a LysM-domain-containing protein, as are the Nod factor receptors MtNFP and MtLYK3[131]. One possibility is that plant proteins containing extracellular, chitin-interacting LysM domains were co-opted in the legume lineage to recognize bacterially produced lipochitooligosaccharide Nod factors.

Negative feedback on nodulation—Plant defence responses have been implicated in restricting the number of nodules that form on a colonized root. After an initial round of nodule formation has begun, subsequent nodulation events are subject to autoinhibition[132]. Signalling that is antagonistic to the early responses to Nod factor is mediated by the plant hormones ethylene and jasmonic acid, which are involved in defence signalling in other plant–microorganism interactions[52,133]. It has been proposed that autoinhibition is controlled by the plant by the abortion of infection threads[134]. Late-initiating infection threads that abort are associated with necrotic cells, and with the accumulation of defence-related proteins and phenolic compounds in plant cells and cell walls[134]. This type of cellular damage is also found near aborted infection threads and failed symbiosomes formed by *M. truncatula lin*, *nip* and *sym6* mutants, and aborted infection threads formed on wild-type *M. sativa* plants by *S. meliloti exoY* mutants[29,43,72,122,134–136]. *M. truncatula* plants inoculated with the *exoY* mutant also express several plant defence genes more strongly than plants inoculated with wild-type *S. meliloti* (K. M. J. and G. C. W., unpublished data). Another study established that plants depleted for a calcium-dependent protein kinase by RNAi over-express defence genes and have a reduced efficiency of nodulation[137]. It remains to be determined whether plant defence responses are a cause of infection thread abortion or a consequence of the failure of infection

thread progression, and how the absence of succinoglycan might influence these defence responses.

Exopolysaccharides and β-heptaglucoside—It has been proposed that the exopolysaccharide(s) produced by a rhizobial species are actively involved in suppress ing defence responses in the host plant[122]. In fact, soybean defence responses provoked by the β-heptaglucoside elicitor from the plant pathogen *Phytophthora sojae* can be suppressed by cyclic-β-glucans produced by the soybean symbiont *Bradyrhizobium japonicum*[138]. As *B. japonicum* cyclic-β-glucans are effective antagonists of the soybean β-heptaglucoside receptor, the most likely mechanism for this suppression is competition for the same binding sites[139]. Legumes are the only plant group that possess the β-heptaglucoside receptor and the ability to mount a defence response to this elicitor[140].

LPS—In most plant species, the response to bacterial LPS is different from the toxic shock that can be induced in animals[141]. Inoculation of a leaf with purified LPS from a plant pathogenic strain can often induce localized resistance in the treated tissue to subsequent infection by that strain[141]. In many cases, LPS pretreatment can prevent a plant hypersensitive response that would result in catastrophic tissue damage[141]. One possible explanation is that antimicrobials produced in the LPS-treated leaf tissue prevent the bacteria from proliferating enough to activate a hypersensitive response[141]. By contrast, plant cells in culture often respond to LPS fractions from bacteria with an oxidative burst and transcription of defence genes[125]. For example, *S. meliloti* LPS core oligosaccharide can induce an oxidative burst in cultured cells of the non-host plant tobacco (*Nicotiana tabacum*)[128]. However, the lipid A component of *S. meliloti* LPS can suppress both an oxidative burst and the expression of defence genes in cultured cells of its host plants *M. truncatula* and *M. sativa*[128,129]. An exciting area of research is in determining which LPS epitopes from rhizobial bacteria and plant pathogens elicit or suppress defence responses on plants of different lineages. Interestingly, the LPS lipid A component of *Brucella abortus*, which has a very similar structure to that of *S. meliloti*, is much less toxic to animal cells than the lipid A of other bacterial species[84,142] (BOX 3).

Future perspectives

It is safe to say that the field of rhizobial–plant symbioses is booming. The genomes of multiple rhizobial species have been sequenced and the genomes of the model host plants *M. truncatula* and *L. japonicus* are nearing completion. The cloning of plant Nod factor receptors and signal transduction intermediates, and the dissection of early plant responses to Nod factor have opened a door between symbiosis research and the field of plant signal transduction. Plant hormones and signalling components are sure to have an even more prominent role in future symbiosis studies. Dissecting the plant signalling events that permit the subsequent steps of infection thread formation and bacterial endocytosis will be equally exciting. Another conceptual advance has been the recognition that rhizobia have many features in common with their mammalian pathogen cousins that also reside within intracellular, host-membrane-derived compartments. Despite the differences in the impact on the host of symbiotic and pathogenic bacteria, strategies that mammalian pathogens use to interact with their hosts and to survive within host cells should continue to provide clues as to how rhizobial bacteria accomplish the same mission.

Acknowledgments

We are very grateful to B. K. Minesinger, S. M. Simon, R. V. Woodruff and the reviewers for their helpful comments. We would like to thank members of the Walker laboratory for helpful discussions, and the National Institutes of Health (USA), the Japan Society for the Promotion of Science, the National Science and Research Council of Canada, and the Jane Coffin Childs Fund for Medical Research for funding. G. C. W. is an American Cancer Society Research Professor.

Glossary

Flavonoid
A 2-phenyl-1,4-benzopyrone derivative, produced by plants, that serves as a defence and signalling compound

Nod factor
A lipochitooligosaccharide compound that induces multiple responses that are required for nodulation of appropriate host plants

Symbiotic exopolysaccharide
A rhizobial secreted β-glucan that is structurally distinct for different species and that mediates infection thread formation

Infection thread
An ingrowth of the root hair cell membrane, populated with rhizobial bacteria, that progresses inward by new membrane synthesis at the tip

Symbiosome
A host-derived membrane compartment that originates from the infection thread housing a bacteroid

Bacteroid
A rhizobial bacterium that has been endocytosed by a plant cell and has elongated and/or branched, and has differentiated or is differentiating into a form that can perform nitrogen fixation

Nitrogen fixation
The reduction of atmospheric dinitrogen to ammonia

Colonized curled root hair
A root hair that has been induced by Nod factor to curl around a microcolony of rhizobial bacteria and entrap it

Auxin
A plant hormone (chiefly indole acetic acid) that regulates plant growth in a concentration-dependent manner

Nodule primordium
Dedifferentiated, proliferating tissue that develops in the plant cortex during nodule initiation

Cyclic β-glucan
A cyclized β-1,2 chain of 17–25 glucose residues produced by rhizobial bacteria, *Brucella* spp. and *Agrobacterium* spp. that localizes to the periplasm and that functions in osmotolerance and in interaction with host membranes

Succinoglycan
A symbiotic exopolysaccharide produced by *S. meliloti*, also known as EPS I, that mediates infection thread formation. An octasaccharide repeating unit modified with acetyl, succinyl and pyruvyl substituents that can be polymerized into a high molecular weight or a low molecular weight form composed of monomers, dimers and trimers

Galactoglucan
A second exopolysaccharide of *S. meliloti* (EPS II) that can mediate infection thread formation on *M. sativa* at a low efficiency and that is produced when an

intact copy of the ExpR regulator is present. A disaccharide repeating unit modified with acetyl and pyruvyl substituents

Endoreduplication

Genomic replication without cytokinesis that results in greater than 2n DNA content within a cell

Indeterminate nodule

A nodule formed by plants of some clades of legumes that develops a continuously growing nodule meristem at the distal end and has zones of tissue at different stages of development

α-proteobacteria

A group of bacteria that contains several species able to persist within host-derived membrane-bound compartments in eukaryotic cells. Includes rhizobial bacteria and mammalian pathogens such as *Brucella* spp., *Bartonella* spp. and *Rickettsia* spp

References

1. Perret X, Staehelin C, Broughton WJ. Molecular basis of symbiotic promiscuity. Microbiol Mol Biol Rev 2000;64:180–201. [PubMed: 10704479]
2. Barnett MJ, Fisher RF. Global gene expression in the rhizobial–legume symbiosis. Symbiosis 2006;42:1–24.
3. Peck MC, Fisher RF, Long SR. Diverse flavonoids stimulate NodD1 binding to *nod* gene promoters in *Sinorhizobium meliloti*. J Bacteriol 2006;188:5417–5427. [PubMed: 16855231]
4. Oldroyd GE, Downie JA. Calcium, kinases and nodulation signalling in legumes. Nature Rev Mol Cell Biol 2004;5:566–576. [PubMed: 15232574]
5. Cook DR. Unraveling the mystery of Nod factor signaling by a genomic approach in *Medicago truncatula*. Proc Natl Acad Sci USA 2004;101:4339–4340. [PubMed: 15070718]
6. Geurts R, Fedorova E, Bisseling T. Nod factor signaling genes and their function in the early stages of *Rhizobium* infection. Curr Opin Plant Biol 2005;8:346–352. [PubMed: 15955723]
7. Oldroyd GE, Downie JA. Nuclear calcium changes at the core of symbiosis signalling. Curr Opin Plant Biol 2006;9:351–357. [PubMed: 16713329]
8. Timmers AC, Auriac MC, Truchet G. Refined analysis of early symbiotic steps of the *Rhizobium–Medicago* interaction in relationship with microtubular cytoskeleton rearrangements. Development 1999;126:3617–3628. [PubMed: 10409507]The definitive microscopy study of plant cytological changes during infection thread formation.
9. Sieberer BJ, Timmers AC, Emons AM. Nod factors alter the microtubule cytoskeleton in *Medicago truncatula* root hairs to allow root hair reorientation. Mol Plant Microbe Interact 2005;18:1195–1204. [PubMed: 16353554]
10. Cardenas L, et al. The role of nod factor substituents in actin cytoskeleton rearrangements in *Phaseolus vulgaris*. Mol Plant Microbe Interact 2003;16:326–334. [PubMed: 12744461]
11. de Ruijter NCA, Bisseling T, Emons AMC. *Rhizobium* Nod factors induce an increase in subapical fine bundles of actin filaments in *Vicia sativa* root hairs within minutes. Mol Plant Microbe Interact 1999;12:829–832.
12. Esseling JJ, Lhuissier FG, Emons AM. Nod factor-induced root hair curling: continuous polar growth towards the point of nod factor application. Plant Physiol 2003;132:1982–1988. [PubMed: 12913154] Demonstrated that Nod factor has a directional effect on root hair polar tip growth.
13. Gage DJ. Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes. Microbiol Mol Biol Rev 2004;68:280–300. [PubMed: 15187185]
14. Foucher F, Kondorosi E. Cell cycle regulation in the course of nodule organogenesis in *Medicago*. Plant Mol Biol 2000;43:773–786. [PubMed: 11089876]

15. Heidstra R, et al. Ethylene provides positional information on cortical cell division but is not involved in Nod factor-induced root hair tip growth in *Rhizobium*–legume interaction. Development 1997;124:1781–1787. [PubMed: 9165125]
16. Wasson AP, Pellerone FI, Mathesius U. Silencing the flavonoid pathway in *Medicago truncatula* inhibits root nodule formation and prevents auxin transport regulation by rhizobia. Plant Cell 2006;18:1617–1629. [PubMed: 16751348]
17. Amor BB, et al. 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 2003;34:495–506. [PubMed: 12753588]Identified an *M. truncatula* mutant that was completely devoid of responses to Nod factor.
18. Arrighi JF, et al. The *Medicago truncatula* lysine motif-receptor-like kinase gene family includes NFP and new nodule-expressed genes. Plant Physiol 2006;142:265–279. [PubMed: 16844829] Cloned the *NFP*-encoded Nod factor receptor and demonstrated that it has a role in infection thread formation.
19. Limpens E, et al. LysM domain receptor kinases regulating rhizobial Nod factor-induced infection. Science 2003;302:630–633. [PubMed: 12947035]Identified and cloned the MtLYK3 and MtLYK4 Nod factor receptors.
20. Ane JM, et al. *Medicago truncatula* DMI1 required for bacterial and fungal symbioses in legumes. Science 2004;303:1364–1367. [PubMed: 14963334]
21. Riely BK, Lougnon G, Ane JM, Cook DR. The symbiotic ion channel homolog DMI1 is localized in the nuclear membrane of *Medicago truncatula* roots. Plant J 2007;49:208–216. [PubMed: 17173544]
22. Mitra RM, et al. A $Ca2^{+}$/calmodulin-dependent protein kinase required for symbiotic nodule development: gene identification by transcript-based cloning. Proc Natl Acad Sci USA 2004;101:4701–4705. [PubMed: 15070781]
23. Levy J, et al. A putative $Ca2^{+}$ and calmodulin-dependent protein kinase required for bacterial and fungal symbioses. Science 2004;303:1361–1364. [PubMed: 14963335]
24. Gleason C, et al. Nodulation independent of rhizobia induced by a calcium-activated kinase lacking autoinhibition. Nature 2006;441:1149–1152. [PubMed: 16810256]
25. Esseling JJ, Lhuissier FG, Emons AM. A nonsymbiotic root hair tip growth phenotype in NORK-mutated legumes: implications for nodulation factor-induced signaling and formation of a multifaceted root hair pocket for bacteria. Plant Cell 2004;16:933–944. [PubMed: 15031407]
26. Kalo P, et al. Nodulation signaling in legumes requires NSP2, a member of the GRAS family of transcriptional regulators. Science 2005;308:1786–1789. [PubMed: 15961668]
27. Smit P, et al. NSP1 of the GRAS protein family is essential for rhizobial Nod factor-induced transcription. Science 2005;308:1789–1791. [PubMed: 15961669]
28. Charron D, Pingret JL, Chabaud M, Journet EP, Barker DG. Pharmacological evidence that multiple phospholipid signaling pathways link *Rhizobium* nodulation factor perception in *Medicago truncatula* root hairs to intracellular responses, including Ca^{2+} spiking and specific ENOD gene expression. Plant Physiol 2004;136:3582–3593. [PubMed: 15489277]
29. Dickstein R, Bisseling T, Reinhold VN, Ausubel FM. Expression of nodule-specific genes in alfalfa root nodules blocked at an early stage of development. Genes Dev 1988;2:677–687. [PubMed: 3417147]First demonstrated that cyclic-β-glucan mutants and exopolysaccharide mutants of *S. meliloti* lack infection threads.
30. Dylan T, Nagpal P, Helinski DR, Ditta GS. Symbiotic pseudorevertants of *Rhizobium meliloti ndv* mutants. J Bacteriol 1990;172:1409–1417. [PubMed: 2307652]
31. Yao SY, et al. *Sinorhizobium meliloti* ExoR and ExoS proteins regulate both succinoglycan and flagellum production. J Bacteriol 2004;186:6042–6049. [PubMed: 15342573]
32. Cheng HP, Walker GC. Succinoglycan production by *Rhizobium meliloti* is regulated through the ExoS-ChvI two-component regulatory system. J Bacteriol 1998;180:20–26. [PubMed: 9422587]
33. Zhang XS, Cheng HP. Identification of *Sinorhizobium meliloti* early symbiotic genes by use of a positive functional screen. Appl Environ Microbiol 2006;72:2738–2748. [PubMed: 16597978]
34. Klein S, Hirsch AM, Smith CA, Signer ER. Interaction of *nod* and *exo Rhizobium meliloti* in alfalfa nodulation. Mol Plant Microbe Interact 1988;1:94–100. [PubMed: 2979704]

35. Gage DJ. Analysis of infection thread development using Gfp- and DsRed-expressing *Sinorhizobium meliloti*. J Bacteriol 2002;184:7042–7046. [PubMed: 12446653]Demonstrated that only bacteria near the tip of an infection thread proliferate and progress into plant tissue.
36. Pellock BJ, Cheng HP, Walker GC. Alfalfa root nodule invasion efficiency is dependent on *Sinorhizobium meliloti* polysaccharides. J Bacteriol 2000;182:4310–4318. [PubMed: 10894742]
37. Glazebrook J, Walker GC. A novel exopolysaccharide can function in place of the calcofluor-binding exopolysaccharide in nodulation of alfalfa by *Rhizobium meliloti*. Cell 1989;56:661–672. [PubMed: 2537152]
38. Reinhold BB, et al. Detailed structural characterization of succinoglycan, the major exopolysaccharide of *Rhizobium meliloti* Rm1021. J Bacteriol 1994;176:1997–2002. [PubMed: 8144468]
39. Cheng HP, Walker GC. Succinoglycan is required for initiation and elongation of infection threads during nodulation of alfalfa by *Rhizobium meliloti*. J Bacteriol 1998;180:5183–5191. [PubMed: 9748453]Showed that succinoglycan must be succinylated and acetylated to efficiently facilitate infection thread formation
40. York GM, Walker GC. The succinyl and acetyl modifications of succinoglycan influence susceptibility of succinoglycan to cleavage by the *Rhizobium meliloti* glycanases ExoK and ExsH. J Bacteriol 1998;180:4184–4191. [PubMed: 9696768]
41. Laus MC, van Brussel AA, Kijne JW. Exopolysaccharide structure is not a determinant of host-plant specificity in nodulation of *Vicia sativa* roots. Mol Plant Microbe Interact 2005;18:1123–1129. [PubMed: 16353547]
42. Ardourel M, et al. *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 1994;6:1357–1374. [PubMed: 7994171]
43. Kuppusamy KT, et al. *LIN*, a *Medicago truncatula* gene required for nodule differentiation and persistence of rhizobial infections. Plant Physiol 2004;136:3682–3691. [PubMed: 15516512]
44. Middleton PH, et al. An ERF transcription factor in *Medicago truncatula* that is essential for Nod factor signal transduction. Plant Cell 2007;19:1221–1234. [PubMed: 17449807]
45. Sieberer BJ, Ketelaar T, Esseling JJ, Emons AM. Microtubules guide root hair tip growth. New Phytol 2005;167:711–719. [PubMed: 16101908]
46. Esseling JJ, Emons AM. Dissection of Nod factor signalling in legumes: cell biology, mutants and pharmacological approaches. J Microsc 2004;214:104–113. [PubMed: 15102059]
47. Vassileva VN, Kouchi H, Ridge RW. Microtubule dynamics in living root hairs: transient slowing by lipochitin oligosaccharide nodulation signals. Plant Cell 2005;17:1777–1787. [PubMed: 15863517]
48. Brewin NJ. Plant cell wall remodelling in the Rhizobium–Legume symbiosis. Crit Rev Plant Sci 2004;23:293–316.
49. Vandenbosch KA, et al. Common components of the infection thread matrix and the intercellular space identified by immunocytochemical analysis of pea nodules and uninfected roots. EMBO J 1989;8:335–341. [PubMed: 16453870]The first immunological detection of antigens localizing to the infection thread.
50. Rathbun EA, Naldrett MJ, Brewin NJ. Identification of a family of extensin-like glycoproteins in the lumen of rhizobium-induced infection threads in pea root nodules. Mol Plant Microbe Interact 2002;15:350–359. [PubMed: 12026173]
51. Santos R, Herouart D, Sigaud S, Touati D, Puppo A. Oxidative burst in alfalfa–*Sinorhizobium meliloti* symbiotic interaction. Mol Plant–Microbe Interact 2001;14:86–89. [PubMed: 11194876]
52. Abramovitch RB, Anderson JC, Martin GB. Bacterial elicitation and evasion of plant innate immunity. Nature Rev Mol Cell Biol 2006;7:601–611. [PubMed: 16936700]
53. Sigaud S, Becquet V, Frendo P, Puppo A, Herouart D. Differential regulation of two divergent *Sinorhizobium meliloti* genes for HPII-like catalases during free-living growth and protective role of both catalases during symbiosis. J Bacteriol 1999;181:2634–2639. [PubMed: 10198032]
54. Jamet A, Sigaud S, Van de Sype G, Puppo A, Herouart D. Expression of the bacterial catalase genes during *Sinorhizobium meliloti–Medicago sativa* symbiosis and their crucial role during the infection process. Mol Plant Microbe Interact 2003;16:217–225. [PubMed: 12650453]

55. Davies BW, Walker GC. Disruption of *sitA* compromises *Sinorhizobium meliloti* for manganese uptake required for protection against oxidative stress. J Bacteriol 2007;189:2101–2109. [PubMed: 17172335]

56. Jamet A, Kiss E, Batut J, Puppo A, Herouart D. The *katA* catalase gene is regulated by OxyR in both free-living and symbiotic *Sinorhizobium meliloti*. J Bacteriol 2005;187:376–381. [PubMed: 15601722]

57. Barloy-Hubler F, Cheron A, Hellegouarch A, Galibert F. Smc01944, a secreted peroxidase induced by oxidative stresses in *Sinorhizobium meliloti* 1021. Microbiology 2004;150:657–664. [PubMed: 14993315]

58. Gonzalez-Rizzo S, Crespi M, Frugier F. The *Medicago truncatula* CRE1 cytokinin receptor regulates lateral root development and early symbiotic interaction with *Sinorhizobium meliloti*. Plant Cell 2006;18:2680–2693. [PubMed: 17028204]

59. Murray JD, et al. A cytokinin perception mutant colonized by *Rhizobium* in the absence of nodule organogenesis. Science 2007;315:101–104. [PubMed: 17110535]

60. Tirichine L, et al. A gain-of-function mutation in a cytokinin receptor triggers spontaneous root nodule organogenesis. Science 2007;315:104–107. [PubMed: 17110537]

61. Marsh JF, et al. *Medicago truncatula* NIN is essential for rhizobial-independent nodule organogenesis induced by autoactive calcium/calmodulin-dependent protein kinase. Plant Physiol 2007;144:324–335. [PubMed: 17369436]

62. Mitra RM, Long SR. Plant and bacterial symbiotic mutants define three transcriptionally distinct stages in the development of the *Medicago truncatula*/*Sinorhizobium meliloti* symbiosis. Plant Physiol 2004;134:595–604. [PubMed: 14739349]Plant and rhizobial mutants arrested at different morphologically defined stages of symbiotic development are also arrested in the ability to undergo symbiosis-specific transcriptional changes.

63. Starker CG, Parra-Colmenares AL, Smith L, Mitra RM, Long SR. Nitrogen fixation mutants of *Medicago truncatula* fail to support plant and bacterial symbiotic gene expression. Plant Physiol 2006;140:671–680. [PubMed: 16407449]

64. Pislariu CI, Dickstein R. An IRE-like AGC kinase gene, MtIRE, has unique expression in the invasion zone of developing root nodules in *Medicago truncatula*. Plant Physiol 2007;144:682–694. [PubMed: 17237187]

65. Mergaert P, et al. Eukaryotic control on bacterial cell cycle and differentiation in the *Rhizobium*–legume symbiosis. Proc Natl Acad Sci USA 2006;103:5230–5235. [PubMed: 16547129]Showed that plants that form indeterminate nodules impose a programme of genomic endoreduplication on the bacteria, whereas plants that form determinate nodules do not.

66. Cebolla A, et al. The mitotic inhibitor ccs52 is required for endoreduplication and ploidy-dependent cell enlargement in plants. EMBO J 1999;18:4476–4484. [PubMed: 10449413]

67. Vinardell JM, et al. Endoreduplication mediated by the anaphase-promoting complex activator CCS52A is required for symbiotic cell differentiation in *Medicago truncatula* nodules. Plant Cell 2003;15:2093–2105. [PubMed: 12953113]

68. Galitski T, Saldanha AJ, Styles CA, Lander ES, Fink GR. Ploidy regulation of gene expression. Science 1999;285:251–254. [PubMed: 10398601]

69. Robertson JG, Lyttleton P. Division of peribacteroid membranes in root nodules of white clover. J Cell Sci 1984;69:147–157. [PubMed: 6490745]

70. Limpens E, et al. Formation of organelle-like N_2-fixing symbiosomes in legume root nodules is controlled by DMI2. Proc Natl Acad Sci USA 2005;102:10375–10380. [PubMed: 16006515] Demonstrated that a Nod factor signalling intermediate DMI2 is required for symbiosome formation as well as early events in Nod factor signalling.

71. Combier JP, et al. MtHAP2-1 is a key transcriptional regulator of symbiotic nodule development regulated by microRNA169 in *Medicago truncatula*. Genes Dev 2006;20:3084–3088. [PubMed: 17114582]

72. Veereshlingam H, et al. nip, a symbiotic *Medicago truncatula* mutant that forms root nodules with aberrant infection threads and plant defense-like response. Plant Physiol 2004;136:3692–3702. [PubMed: 15516506]

73. Dickstein R, Scheirer DC, Fowle WH, Ausubel FM. Nodules elicited by *Rhizobium meliloti* haem mutants are arrested at an early stage of development. Mol Gen Genet 1991;230:423–432. [PubMed: 1766439]
74. Gilles-Gonzalez MA, Ditta GS, Helinski DR. A haemoprotein with kinase activity encoded by the oxygen sensor of *Rhizobium meliloti*. Nature 1991;350:170–172. [PubMed: 1848683]
75. Kereszt A, Slaska-Kiss K, Putnoky P, Banfalvi Z, Kondorosi A. The *cycHJKL* genes of *Rhizobium meliloti* involved in cytochrome c biogenesis are required for 'respiratory' nitrate reduction *ex planta* and for nitrogen fixation during symbiosis. Mol Gen Genet 1995;247:39–47. [PubMed: 7715602]
76. Catalano CM, Lane WS, Sherrier DJ. Biochemical characterization of symbiosome membrane proteins from *Medicago truncatula* root nodules. Electrophoresis 2004;25:519–531. [PubMed: 14760646]
77. Catalano CM, Czymmek KJ, Gann JG, Sherrier DJ. *Medicago truncatula* syntaxin SYP132 defines the symbiosome membrane and infection droplet membrane in root nodules. Planta 2006;225:541–550. [PubMed: 16944200]Defined a molecular marker of symbiosome membranes.
78. Sanderfoot AA, Raikhel NV. The specificity of vesicle trafficking: coat proteins and SNAREs. Plant Cell 1999;11:629–642. [PubMed: 10213783]
79. Mellor RB. Bacteroids in the *Rhizobium*–legume symbiosis inhabit a plant internal lytic compartment: implications for other microbial endosymbioses. J Exp Bot 1989;40:831–839.
80. Roth LE, Stacey G. Bacterium release into host cells of nitrogen-fixing soybean nodules: the symbiosome membrane comes from three sources. Eur J Cell Biol 1989;49:13–23. [PubMed: 2759097]
81. Jones KM, Lloret J, Daniele JR, Walker GC. The type IV secretion system of *Sinorhizobium meliloti* strain 1021 is required for conjugation, but not for intracellular symbiosis. J Bacteriol 2006;189:2133–2138. [PubMed: 17158676]
82. Campbell GR, Reuhs BL, Walker GC. Chronic intracellular infection of alfalfa nodules by *Sinorhizobium meliloti* requires correct lipopolysaccharide core. Proc Natl Acad Sci USA 2002;99:3938–3943. [PubMed: 11904442]
83. Glazebrook J, Ichige A, Walker GC. A *Rhizobium meliloti* homolog of the *Escherichia coli* peptide-antibiotic transport protein SbmA is essential for bacteroid development. Genes Dev 1993;7:1485–1497. [PubMed: 8393417]
84. Ferguson GP, et al. Similarity to peroxisomal-membrane protein family reveals that *Sinorhizobium* and *Brucella* BacA affect lipid-A fatty acids. Proc Natl Acad Sci USA 2004;101:5012–5017. [PubMed: 15044696]
85. Putnoky P, et al. The *pha* gene cluster of *Rhizobium meliloti* involved in pH adaptation and symbiosis encodes a novel type of K^+ efflux system. Mol Microbiol 1998;28:1091–1101. [PubMed: 9680201]
86. Mitsui H, Sato T, Sato Y, Ito N, Minamisawa K. *Sinorhizobium meliloti* RpoH1 is required for effective nitrogen-fixing symbiosis with alfalfa. Mol Genet Genom 2004;271:416–425.
87. Wells DH, Long SR. The *Sinorhizobium meliloti* stringent response affects multiple aspects of symbiosis. Mol Microbiol 2002;43:1115–1127. [PubMed: 11918800]
88. Prell J, Poole P. Metabolic changes of rhizobia in legume nodules. Trends Microbiol 2006;14:161–168. [PubMed: 16520035]
89. Hunt S, Layzell DB. Gas exchange of legume nodules and the regulation of nitrogenase activity. Annu Rev Plant Physiol 1993;44:483–511.
90. Jordan A, Reichard P. Ribonucleotide reductases. Annu Rev Biochem 1998;67:71–98. [PubMed: 9759483]
91. Bardin S, Dan S, Osteras M, Finan TM. A phosphate transport system is required for symbiotic nitrogen fixation by *Rhizobium meliloti*. J Bacteriol 1996;178:4540–4547. [PubMed: 8755882]
92. Yuan ZC, Zaheer R, Finan TM. Regulation and properties of PstSCAB, a high-affinity, high-velocity phosphate transport system of *Sinorhizobium meliloti*. J Bacteriol 2006;188:1089–1102. [PubMed: 16428413]
93. Morandi D, Prado E, Sagan M, Duc G. Characterisation of new symbiotic *Medicago truncatula* (Gaertn.) mutants, and phenotypic or genotypic complementary information on previously described mutants. Mycorrhiza 2005;15:283–289. [PubMed: 15558330]

94. Sherrier DJ, Borisov AY, Tikhonovich IA, Brewin NJ. Immunocytological evidence for abnormal symbiosome development in nodules of the pea mutant line Sprint2Fix^{-} (*sym31*). Protoplasma 1997;199:57–68.

95. Voroshilova VA, et al. Effect of mutations in *Pisum sativum* L. genes blocking different stages of nodule development on the expression of late symbiotic genes in *Rhizobium leguminosarum* bv *viciae*. Mol Plant Microbe Interact 2001;14:471–476. [PubMed: 11310734]

96. Bradley DJ, Butcher GW, Galfre G, Wood EA, Brewin NJ. Physical association between the peribacteroid membrane and lipopolysaccharide from the bacteroid outer membrane in *Rhizobium*-infected pea root nodule cells. J Cell Sci 1986;85:47–61. [PubMed: 3793795]

97. Bolanos L, Redondo-Nieto M, Rivilla R, Brewin NJ, Bonilla I. Cell surface interactions of Rhizobium bacteroids and other bacterial strains with symbiosomal and peribacteroid membrane components from pea nodules. Mol Plant Microbe Interact 2004;17:216–223. [PubMed: 14964535]

98. Fischer HM. Genetic regulation of nitrogen fixation in rhizobia. Microbiol Rev 1994;58:352–386. [PubMed: 7968919]

99. Vasse J, de Billy F, Camut S, Truchet G. Correlation between ultrastructural differentiation of bacteroids and nitrogen fixation in alfalfa nodules. J Bacteriol 1990;172:4295–4306. [PubMed: 2376562]The first observation of a plant defence response associated with failed infection threads.

100. Yang WC, Horvath B, Hontelez J, van Kammen A, Bisseling T. *In situ* localization of *Rhizobium* mRNAs in pea nodules: *nifA* and *nifH* localization. Mol Plant Microbe Interact 1991;4:464–468.

101. Poole P, Allaway D. Carbon and nitrogen metabolism in Rhizobium. Adv Microb Physiol 2000;43:117–163. [PubMed: 10907556]

102. Day DA, Poole PS, Tyerman SD, Rosendahl L. Ammonia and amino acid transport across symbiotic membranes in nitrogen-fixing legume nodules. Cell Mol Life Sci 2001;58:61–71. [PubMed: 11229817]

103. Lodwig EM, et al. Amino-acid cycling drives nitrogen fixation in the legume–*Rhizobium* symbiosis. Nature 2003;422:722–726. [PubMed: 12700763]

104. de Bruijn FJ, et al. *Rhizobium meliloti* 1021 has three differentially regulated loci involved in glutamine biosynthesis, none of which is essential for symbiotic nitrogen fixation. J Bacteriol 1989;171:1673–1682. [PubMed: 2563998]

105. Kahn, ML.; McDermott, TR.; Udvardi, MK. The Rhizobiaceae. Spaink, HP.; Kondorosi, A.; Hooykaas, PJJ., editors. Kluwer Academic Publishers; Dordrecht: 1998. p. 461-485.

106. Barsch A, Carvalho HG, Cullimore JV, Niehaus K. GC-MS based metabolite profiling implies three interdependent ways of ammonium assimilation in *Medicago truncatula* root nodules. J Biotechnol 2006;127:79–83. [PubMed: 16870293]

107. Harrison J, Pou de Crescenzo MA, Sene O, Hirel B. Does lowering glutamine synthetase activity in nodules modify nitrogen metabolism and growth of *Lotus japonicus*? Plant Physiol 2003;133:253–262. [PubMed: 12970491]

108. Cordoba E, Shishkova S, Vance CP, Hernandez G. Antisense inhibition of NADH glutamate synthase impairs carbon/nitrogen assimilation in nodules of alfalfa (*Medicago sativa* L.). Plant J 2003;33:1037–1049. [PubMed: 12631328]

109. Willis LB, Walker GC. The *phbC* (poly-β-hydroxybutyrate synthase) gene of *Rhizobium* (*Sinorhizobium*) *meliloti* and characterization of *phbC* mutants. Can J Microbiol 1998;44:554–564. [PubMed: 9734305]

110. Povolo S, et al. Isolation and characterization of mutants of *Rhizobium meliloti* unable to synthesize poly-β-hydroxybutyrate. Can J Microbiol 1994;40:823–829.

111. Aneja P, Zachertowska A, Charles TC. Comparison of the symbiotic and competition phenotypes of *Sinorhizobium meliloti* PHB synthesis and degradation pathway mutants. Can J Microbiol 2005;51:599–604. [PubMed: 16175209]

112. Ronson CW, Lyttleton P, Robertson JG. C(4)-dicarboxylate transport mutants of *Rhizobium trifolii* form ineffective nodules on *Trifolium repens*. Proc Natl Acad Sci USA 1981;78:4284–4288. [PubMed: 16593058]

113. Driscoll BT, Finan TM. NAD^{+}-dependent malic enzyme of *Rhizobium meliloti* is required for symbiotic nitrogen fixation. Mol Microbiol 1993;7:865–873. [PubMed: 8387144]Showed that

rhizobial malic enzyme is required for nitrogen fixation and proposed a model for carbon assimilation by bacteroids

114. Ott T, et al. Symbiotic leghemoglobins are crucial for nitrogen fixation in legume root nodules but not for general plant growth and development. Curr Biol 2005;15:531–535. [PubMed: 15797021] Directly demonstrated that plant leghaemoglobins are required for nitrogen fixation by symbiotic bacteria.
115. Gordon AJ, Minchin FR, James CL, Komina O. Sucrose synthase in legume nodules is essential for nitrogen fixation. Plant Physiol 1999;120:867–878. [PubMed: 10398723]
116. Barratt DH, et al. Multiple, distinct isoforms of sucrose synthase in pea. Plant Physiol 2001;127:655–664. [PubMed: 11598239]
117. Krusell L, et al. The sulfate transporter SST1 is crucial for symbiotic nitrogen fixation in *Lotus japonicus* root nodules. Plant Cell 2005;17:1625–1636. [PubMed: 15805486]
118. Wienkoop S, Saalbach G. Proteome analysis. Novel proteins identified at the peribacteroid membrane from *Lotus japonicus* root nodules. Plant Physiol 2003;131:1080–1090. [PubMed: 12644660]
119. Dos Santos PC, et al. Iron–sulfur cluster assembly: NifU-directed activation of the nitrogenase Fe protein. J Biol Chem 2004;279:19705–19711. [PubMed: 14993221]
120. Timmers AC, et al. Saprophytic intracellular rhizobia in alfalfa nodules. Mol Plant Microbe Interact 2000;13:1204–1213. [PubMed: 11059487]
121. Ausubel FM. Are innate immune signaling pathways in plants and animals conserved? Nature Immunol 2005;6:973–979. [PubMed: 16177805]
122. Mithofer A. Suppression of plant defence in rhizobia–legume symbiosis. Trends Plant Sci 2002;7:440–444. [PubMed: 12399178]
123. Boller T. Peptide signalling in plant development and self/non-self perception. Curr Opin Cell Biol 2005;17:116–122. [PubMed: 15780586]
124. Shibuya N, Minami E. Oligosaccharide signalling for defence responses in plant. Physiol Mol Plant Pathol 2001;59:223–233.
125. Zeidler D, et al. Innate immunity in *Arabidopsis thaliana*: lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes. Proc Natl Acad Sci USA 2004;101:15811–15816. [PubMed: 15498873]
126. Altschul SF, Gish W, Miller W, Meyers EW, Lipmann DJ. A basic local alignment search tool. J Mol Biol 1990;215:403–410. [PubMed: 2231712]
127. Felix G, Duran JD, Volko S, Boller T. Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J 1999;18:265–276. [PubMed: 10377992]
128. Scheidle H, Gross A, Niehaus K. The Lipid A substructure of the *Sinorhizobium meliloti* lipopolysaccharides is sufficient to suppress the oxidative burst in host plants. New Phytol 2005;165:559–565. [PubMed: 15720666]
129. Tellstrom V, et al. The lipopolysaccharide of *Sinorhizobium meliloti* suppresses defense-associated gene expression in cell cultures of the host plant. Medicago truncatula Plant Physiol 2007;143:825–837.Showed that the *S. meliloti* LPS suppresses short-term *M. truncatula* defence responses such as oxidative burst and longer-term defence responses requiring transcriptional changes.
130. Angot A, et al. *Ralstonia solanacearum* requires F-box-like domain-containing type III effectors to promote disease on several host plants. Proc Natl Acad Sci USA 2006;103:14620–14625. [PubMed: 16983093]
131. Kaku H, et al. Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. Proc Natl Acad Sci USA 2006;103:11086–11091. [PubMed: 16829581]
132. van Brussel AA, Tak T, Boot KJ, Kijne JW. Autoregulation of root nodule formation: signals of both symbiotic partners studied in a split-root system of *Vicia sativa* subsp *nigra*. Mol Plant Microbe Interact 2002;15:341–349. [PubMed: 12026172]
133. Sun J, et al. Crosstalk between jasmonic acid, ethylene and Nod factor signaling allows integration of diverse inputs for regulation of nodulation. Plant J 2006;46:961–970. [PubMed: 16805730]
134. Vasse J, de Billy F, Truchet G. Abortion of infection during the *Rhizobium meliloti*–alfalfa symbiotic interaction is accompanied by a hypersensitive reaction. Plant J 1993;4:555–566.

135. Niehaus K, Kapp D, Puhler A. Plant defence and delayed infection of alfalfa pseudonodules induced by an exopolysaccharide (EPS I)-deficient *Rhizobium meliloti* mutant. Planta 1993;190:415–425.

136. Tirichine L, de Billy F, Huguet T. *Mtsym6*, a gene conditioning Sinorhizobium strain-specific nitrogen fixation in *Medicago truncatula*. Plant Physiol 2000;123:845–851. [PubMed: 10889234]

137. Ivashuta S, et al. RNA interference identifies a calcium-dependent protein kinase involved in *Medicago truncatula* root development. Plant Cell 2005;17:2911–2921. [PubMed: 16199614]

138. Mithöfer A, Bhagwat AA, Feger M, Ebel J. Suppression of fungal β-glucan-induced plant defence in soybean (*Glycine max* L.) by cyclic 1, 3-1, 6-β-glucans from the symbiont *Bradyrhizobium japonicum*. Planta 1996;199:270–275.

139. Bhagwat AA, et al. Further studies of the role of cyclic β-glucans in symbiosis. An *NdvC* mutant of *Bradyrhizobium japonicum* synthesizes cyclodecakis-(1–3)-β-glucosyl. Plant Physiol 1999;119:1057–1064. [PubMed: 10069844]

140. Mithöfer A, Fliegmann J, Neuhaus-Url G, Schwarz H, Ebel J. The hepta-β-glucoside elicitor-binding proteins from legumes represent a putative receptor family. Biol Chem 2000;381:705–713. [PubMed: 11030428]

141. Dow M, Newman MA, von Roepenack E. The induction and modulation of plant defense responses by bacterial lipopolysaccharides. Annu Rev Phytopathol 2000;38:241–261. [PubMed: 11701843]

142. Goldstein J, et al. Lipopolysaccharide (LPS) from *Brucella abortus* is less toxic than that from *Escherichia coli*, suggesting the possible use of *B. abortus* or LPS from *B. abortus* as a carrier in vaccines. Infect Immun 1992;60:1385–1389. [PubMed: 1548064]

143. Leigh JA, Signer ER, Walker GC. Exopolysaccharide-deficient mutants of *Rhizobium meliloti* that form ineffective nodules. Proc Natl Acad Sci USA 1985;82:6231–6235. [PubMed: 3862129] Demonstrated that succinoglycan is critical for successful infection thread formation.

144. Aman P, McNeil M, Franzen LE, Darvill AG, Albersheim P. Structural elucidation, using HPLC-MS and GLC-MS, of the acidic exopolysaccharide secreted by *Rhizobium meliloti* strain Rm1021. Carbohydr Res 1981;95:263–282.

145. Wang LX, Wang Y, Pellock B, Walker GC. Structural characterization of the symbiotically important low-molecular-weight succinoglycan of *Sinorhizobium meliloti*. J Bacteriol 1999;181:6788–6796. [PubMed: 10542182]

146. Buendia AM, et al. The *Rhizobium meliloti exoZl exoB* fragment of megaplasmid 2: ExoB functions as a UDP-glucose 4-epimerase and ExoZ shows homology to NodX of *Rhizobium leguminosarum* biovar *viciae* strain TOM. Mol Microbiol 1991;5:1519–1530. [PubMed: 1787800]

147. Uttaro AD, Cangelosi GA, Geremia RA, Nester EW, Ugalde RA. Biochemical characterization of avirulent *exoC* mutants of *Agrobacterium tumefaciens*. J Bacteriol 1990;172:1640–1646. [PubMed: 2307661]

148. Reuber TL, Walker GC. Biosynthesis of succinoglycan, a symbiotically important exopolysaccharide of *Rhizobium meliloti*. Cell 1993;74:269–280. [PubMed: 8343955]The *S. meliloti* biosynthetic pathway of succinoglycan was defined by biochemical analysis of *exo* mutants.

149. Glucksmann MA, Reuber TL, Walker GC. Genes needed for the modification, polymerization, export, and processing of succinoglycan by *Rhizobium meliloti*: a model for succinoglycan biosynthesis. J Bacteriol 1993;175:7045–7055. [PubMed: 8226646]

150. Leigh JA, Reed JW, Hanks JF, Hirsch AM, Walker GC. *Rhizobium meliloti* mutants that fail to succinylate their Calcofluor-binding exopolysaccharide are defective in nodule invasion. Cell 1987;51:579–587. [PubMed: 2824062]

151. Becker A, Niehaus K, Puhler A. Low-molecular-weight succinoglycan is predominantly produced by *Rhizobium meliloti* strains carrying a mutated ExoP protein characterized by a periplasmic N-terminal domain and a missing C-terminal domain. Mol Microbiol 1995;16:191–203. [PubMed: 7565082]

152. Imaizumi-Anraku H, et al. Plastid proteins crucial for symbiotic fungal and bacterial entry into plant roots. Nature 2005;433:527–531. [PubMed: 15616514]

153. Kanamori N, et al. 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 2006;103:359–364. [PubMed: 16407163]

154. Saito K, et al. NUCLEOPORIN85 is required for calcium spiking, fungal and bacterial symbioses, and seed production in *Lotus japonicus*. Plant Cell 2007;19:610–624. [PubMed: 17307929]

155. Tirichine L, et al. Deregulation of a $Ca2^{+}$/calmodulin-dependent kinase leads to spontaneous nodule development. Nature 2006;441:1153–1156. [PubMed: 16810257]

156. Heckmann AB, et al. *Lotus japonicus* nodulation requires two GRAS-domain regulators, one of which is functionally conserved in a non-legume. Plant Physiol 2006;142:1739–1750. [PubMed: 17071642]

157. Schauser L, Roussis A, Stiller J, Stougaard J. A plant regulator controlling development of symbiotic root nodules. Nature 1999;402:191–195. [PubMed: 10647012]

158. LeVier K, Phillips RW, Grippe VK, Roop RM, Walker GC. Similar requirements of a plant symbiont and a mammalian pathogen for prolonged intracellular survival. Science 2000;287:2492–2493. [PubMed: 10741969]Established that the *bacA* gene is required for survival of *B. abortus* within mammalian cells, as well as for survival of *S. meliloti* within plant cells.

159. Tsolis RM. Comparative genome analysis of the α-proteobacteria: relationships between plant and animal pathogens and host specificity. Proc Natl Acad Sci USA 2002;99:12503–12505. [PubMed: 12271145]

160. Allen CA, Adams LG, Ficht TA. Transposon-derived *Brucella abortus* rough mutants are attenuated and exhibit reduced intracellular survival. Infect Immun 1998;66:1008–1016. [PubMed: 9488389]

161. Lestrate P, et al. Attenuated signature-tagged mutagenesis mutants of *Brucella melitensis* identified during the acute phase of infection in mice. Infect Immun 2003;71:7053–7060. [PubMed: 14638795]

162. Manterola L, et al. The lipopolysaccharide of *Brucella abortus* BvrS/BvrR mutants contains lipid A modifications and has higher affinity for bactericidal cationic peptides. J Bacteriol 2005;187:5631–5639. [PubMed: 16077108]

163. Pelegrini PB, Franco OL. Plant γ-thionins: novel insights on the mechanism of action of a multi-functional class of defense proteins. Int J Biochem Cell Biol 2005;37:2239–2253. [PubMed: 16084753]

164. Ferguson GP, Datta A, Carlson RW, Walker GC. Importance of unusually modified lipid A in *Sinorhizobium* stress resistance and legume symbiosis. Mol Microbiol 2005;56:68–80. [PubMed: 15773979]

165. Arellano-Reynoso B, et al. Cyclic β-1, 2-glucan is a *Brucella* virulence factor required for intracellular survival. Nature Immunol 2005;6:618–625. [PubMed: 15880113]Demonstrated that *B. abortus* cyclic-β-glucan is required to prevent endocytic vacuole fusion with the lysosome.

166. Sola-Landa A, et al. A two-component regulatory system playing a critical role in plant pathogens and endosymbionts is present in *Brucella abortus* and controls cell invasion and virulence. Mol Microbiol 1998;29:125–138. [PubMed: 9701808]

167. Guzman-Verri C, et al. The two-component system BvrR/BvrS essential for *Brucella abortus* virulence regulates the expression of outer membrane proteins with counterparts in members of the Rhizobiaceae. Proc Natl Acad Sci USA 2002;99:12375–12380. [PubMed: 12218183]

168. Keating DH. *Sinorhizobium meliloti* SyrA mediates the transcriptional regulation of genes involved in lipopolysaccharide sulfation and exopolysaccharide biosynthesis. J Bacteriol 2007;189:2510–2520. [PubMed: 17209018]

169. Gibson KE, Campbell GR, Lloret J, Walker GC. CbrA is a stationary-phase regulator of cell surface physiology and legume symbiosis in *Sinorhizobium meliloti*. J Bacteriol 2006;188:4508–4521. [PubMed: 16740957]

170. Hallez R, et al. The asymmetric distribution of the essential histidine kinase PdhS indicates a differentiation event in *Brucella abortus*. EMBO J 2007;26:1444–1455. [PubMed: 17304218]

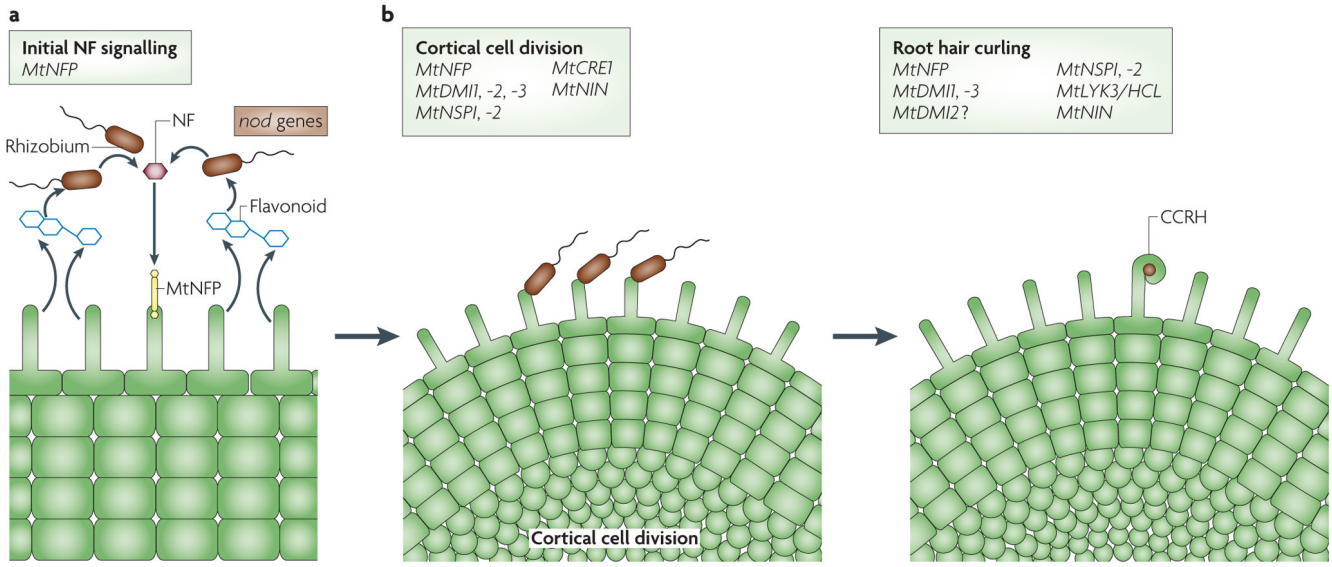


Figure 1. The initial signalling dialogue between *Sinorhizobium meliloti* and *Medicago truncatula*

a | The induction of rhizobial *nod* genes requires plant flavonoids[1,4]. The *nod* gene products produce Nod factor (NF), which is initially perceived by the *M. truncatula* MtNFP receptor[1, 4,17]. **b** | Root hair curling and cortical cell divisions require many *M. truncatula* gene products[4,13]: *MtNFP*[17]; *MtDMI1* (REF. [20]); *MtDMI2* (REF. [25]); *MtDMI3* (REFS [22,23]); *MtNSP1* (REF. [27]); *MtNSP2* (REF. [26]); *MtCRE1* (REFS [58–60]); and *MtNIN*[58,61]. *MtLYK3/HCL* is required for colonized curled root hair (CCRH) formation, but not for the induction of cortical cell divisions[19] (P. Smit and T. Bisseling, unpublished data). The required rhizobial genes are boxed in brown and the required plant genes are boxed in light green.

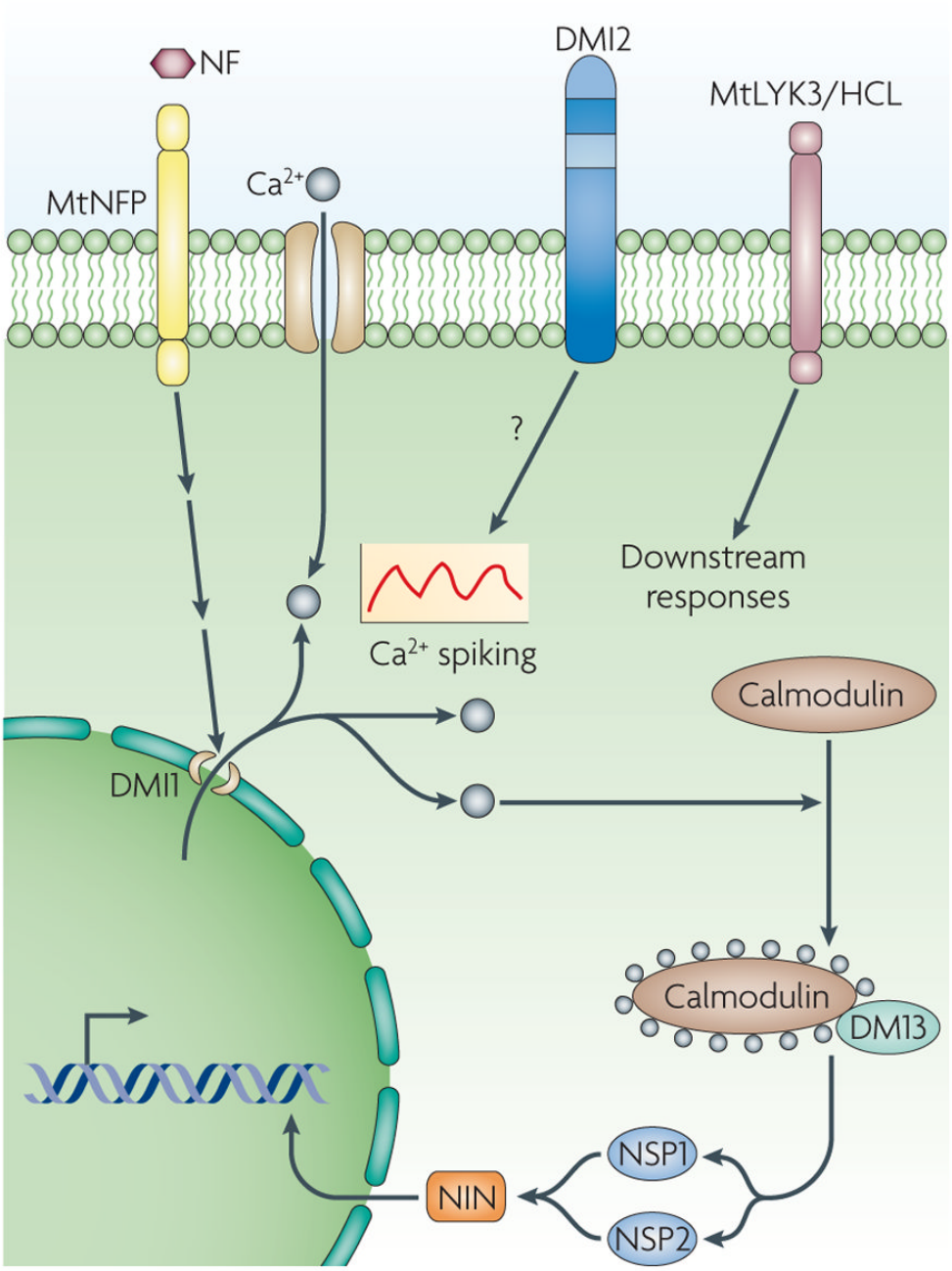


Figure 2. Downstream components of the Nod factor signal transduction system

A complete response of *Medicago truncatula* to Nod factor (NF) from *Sinorhizobium meliloti* requires multiple extracellular-domain-containing cell-surface receptors, including the LysM family receptors MtNFP and MtLYK3/HCL. *DMI2* encodes a leucine-rich-repeat receptor kinase that is localized to the membrane and is required for tight root hair curling around the bacteria. Downstream of NF, *DMI1* encodes a ligand-gated ion channel that localizes to the nuclear membrane. *DMI3* encodes a Ca^{2+}–calmodulin-dependent protein kinase that is required for the induction of cell division in the root cortex and for the transcriptional changes required for the establishment of the symbiosis. Two GRAS family transcriptional regulators, nodulation signalling pathway 1 (NSP1) and NSP2, are also required

for Nod-factor-induced transcriptional changes. The response to NF also involves Ca^{2+} spiking.

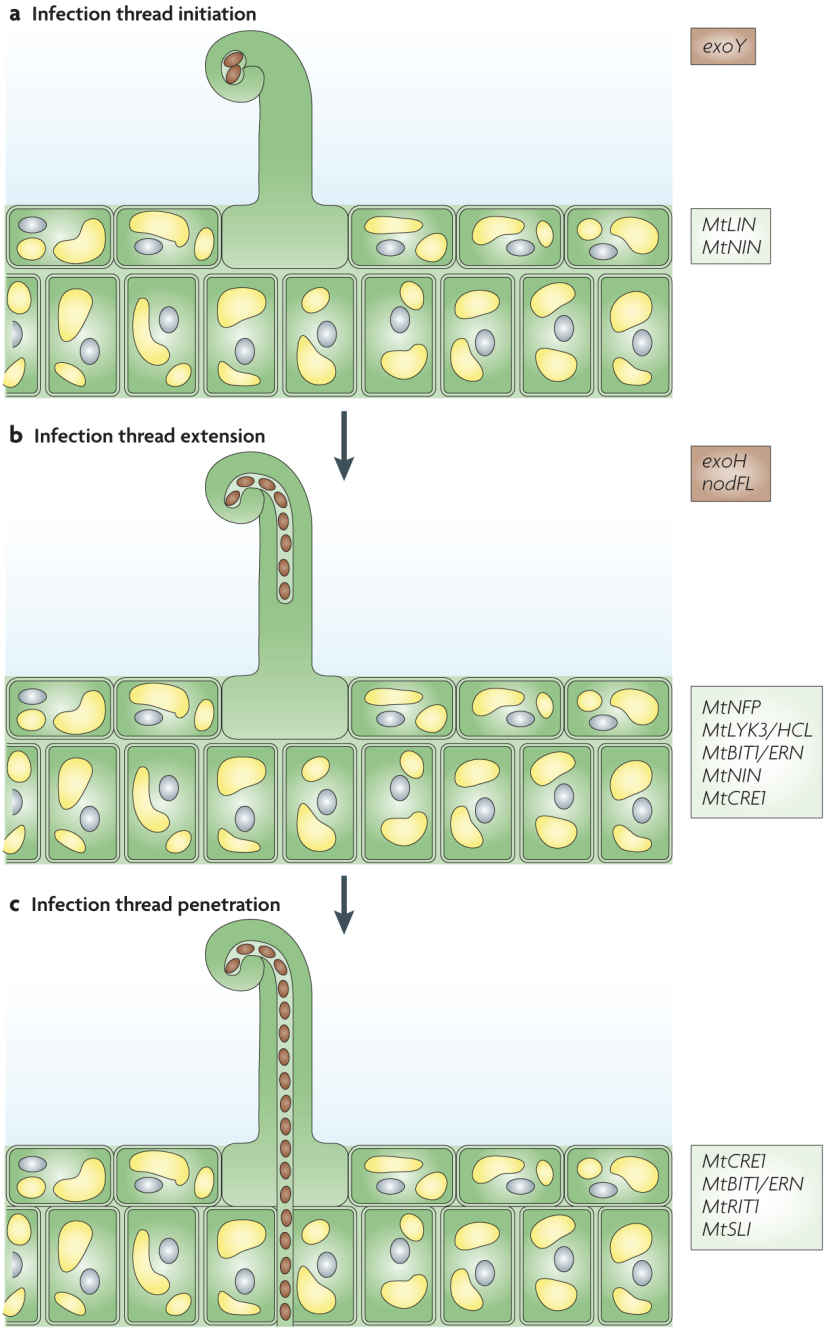


Figure 3. Root hair invasion by *Sinorhizobium meliloti*

a | *S. meliloti exoY*[36,39] and *Medicago trunculata MtLIN*[43] and *MtNIN*[61] are required for infection thread initiation. **b** | *S. meliloti exoH*[39] and *M. trunculata MtNFP*[18], *MtLYK3/HCL*[19] (P. Smit and T. Bisseling, unpublished data), *MtBIT1/ERN*[44], *MtNIN*[61] and *MtCRE1* (REFS [58–60]) are required for infection threads to extend to the base of the root hair cell. **c** | *MtCRE1* (REFS [58–60]), *MtBIT1/ERN*[44], *MtRIT1* (REF. [62]) and *MtSLI*[64] are required for infection thread penetration into the underlying cell layers. The required rhizobial genes are boxed in brown and the required plant genes are boxed in light green.

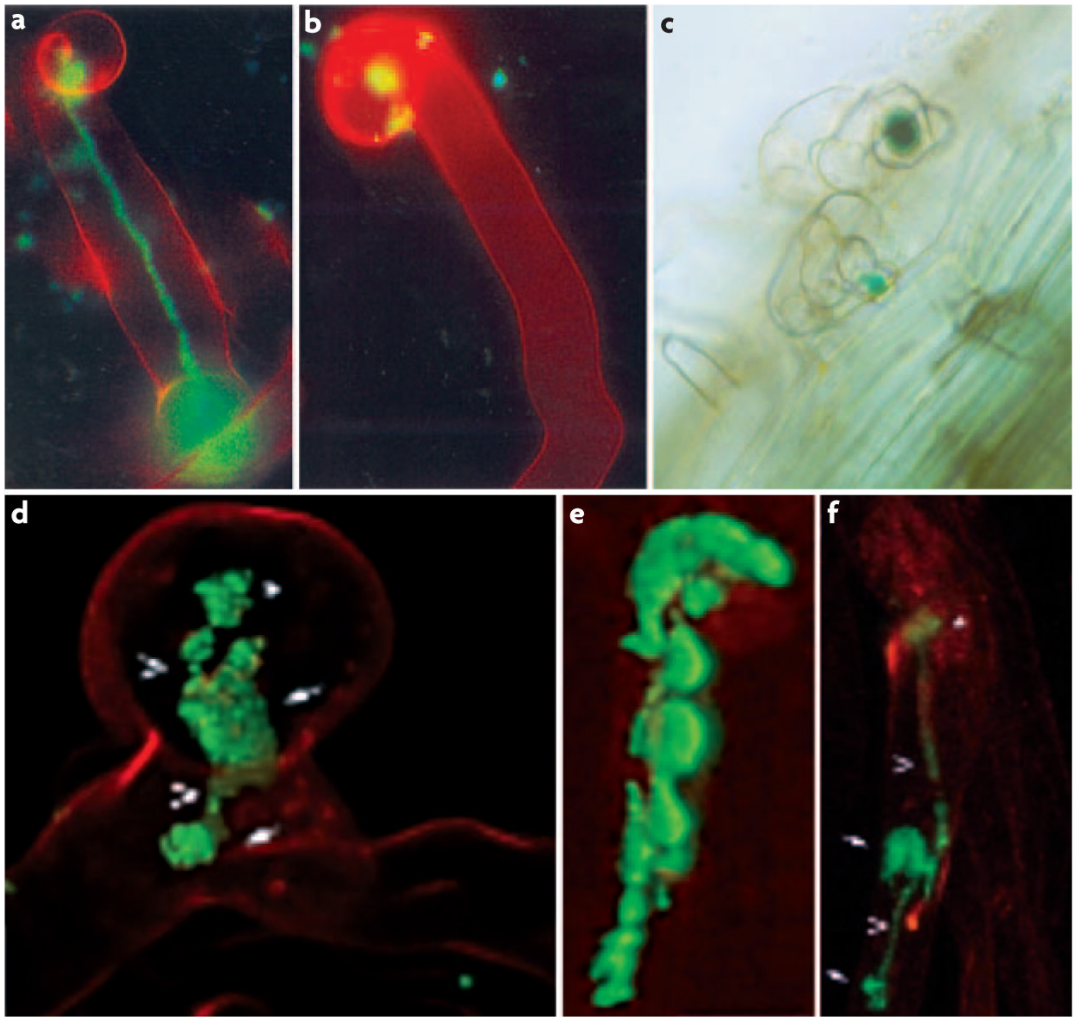


Figure 4. Infection thread failure can be caused by plant or bacterial defects

Infection thread formation during invasion of *Medicago truncatula* or *Medicago sativa* by *Sinorhizobium meliloti*. **a** | An *M. sativa* root hair cell infected by *S. meliloti* wild-type bacteria, with a fully extended infection thread[36]. **b** | An *M. sativa* root hair arrested at the colonized curled root hair (CCRH) stage during infection by an *S. meliloti exoY* mutant[36]. **c** | A *M. truncatula lin* mutant arrested at the CCRH stage during infection by wild-type *S. meliloti*[43]. **d** | An arrested CCRH, formed during infection of *M. truncatula* with an *S. meliloti nodF nodL* mutant[19]. **e** | An aberrant, aborted infection thread formed by wild-type *S. meliloti* on *M. truncatula* partially depleted of *MtNFP* mRNA by RNA interference (RNAi)[18]. **f** | An aberrant infection thread formed by an *S. meliloti nodF nodE* mutant on *M. truncatula* partially depleted for MtLYK3 by RNAi[19]. Parts **a** and **b** reprinted with permission from REF. [36] © (2000) The American Society for Microbiology. Part **c** and **e** reprinted with permission from REF. [43] © (2004) American Society of Plant Biologists. Parts **d** and **f** reprinted with permission from REF. [19] © (2003) American Association for the Advancement of Science.

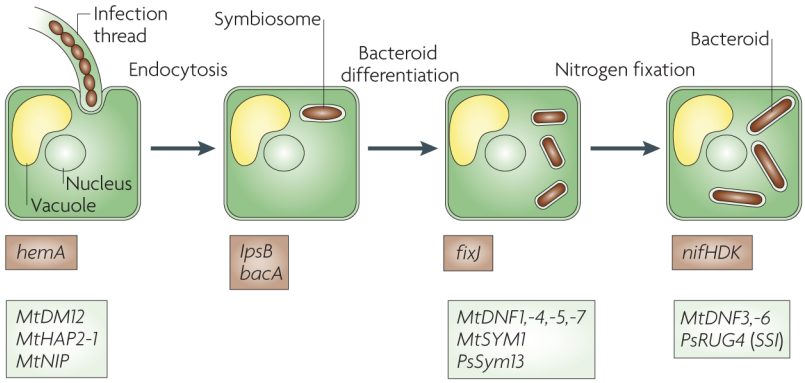


Figure 5. Endocytosis of bacteria and bacteroid differentiation

Bacterial endocytosis requires the *Sinorhizobium meliloti hemA* gene[73], the *Medicago truncatula NIP* gene[72] and wild-type expression levels of the *MtDMI2* (REF. [70]) and *MtHAP2-1* (REF. [71]) genes. *S. meliloti lpsB*[82] and *bacA*[83] are required for bacterial survival within the symbiosome membrane. *S. meliloti fixJ*[98], *M. truncatula MtSYM1* (REF. [64]), *MtDNF1*, *-4*, *-5* and *-7* (REFS [62,63]), and pea (*Pisum sativum*) *PsSYM13* (REF. [95]) are required for bacteroid differentiation. The *S. meliloti nifHDK* genes encode nitrogenase and are required for nitrogen fixation[98]. The pea *PsRUG4* gene encodes sucrose synthase and is required to support bacteroid nitrogen fixation[115,116]. The *M. truncatula* Mt*DNF3* and *-6* genes are required for the maintenance of nitrogen fixation[62,63]. The required rhizobial genes are boxed in brown and the required plant genes are boxed in light green.

Table 1

Bacterial and plant genes that are involved in infection thread development

***Sinorhizobium meliloti* 1021 genotype**	***Medicago truncatula* genotype**	**Infection thread phenotype**	**Comment**
exoY mutant: succinoglycan-deficient[39]	Wild type	None initiated	Succinoglycan is necessary for infection thread initiation
Wild type	*Mtlin* mutant[43]	Very few initiated; remainder aborted	Implies LIN is a possible plant product required for infection thread formation
exoH mutant: succinoglycan lacks succinyl modification, and consequently is mostly in HMW form[39,40]	Wild type	Few initiated; remainder aberrant or aborted	Implies succinoglycan structural features are important for infection thread extension
NodFL double mutant: Nod factor lacks the acetyl substituent and carries an altered acyl chain[19,42]	Wild type	Few initiated; remainder aberrant or aborted	Correct Nod factor structure is needed for infection thread formation as well as other responses
Wild type	RNAi knockdown of Nod factor receptor *MtNFP*[18]	Aberrant	Implies a higher threshold of Nod factor perception or transduction is needed for infection thread formation than for other Nod factor responses
NodFE double mutant: Nod factor carries an altered acyl chain[19]	RNAi knockdown of secondary Nod factor receptor *MtLYK3*/*HCL*[19]	Aberrant	Suggests that secondary Nod factor receptor and Nod factor structural features interact to facilitate infection thread formation
Wild type	Mutant of putative transcription factor *MtBIT1*/*ERN*[44]	Aberrant or aborted	Implies the function of putative transcription factor ERN is required for infection thread extension
Wild type	Mutant of *MtNIN*[61]	Very few initiated; remainder aborted	Suggests reinitiation of cell division in the plant cortex is necessary for initiation and maintenance of infection threads.
Wild type	RNAi knockdown of putative cytokinin receptor MtCRE1 (REF. [58])	Aborted	Suggests re-initiation of cell division in the plant cortex is necessary for initiation and maintenance of infection threads
Wild type	Mutants of MtRIT1 (REF. [62]) or MtSLI[64]	Extended to base of root hair cell; aborted in underlying cell layers	Implies RIT1 and SLI are possible plant products required for infection thread penetration

HMW, high molecular weight; RNAi, RNA interference.