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Cellular insights into legume root infection by rhizobia

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Abstract

Legume plants establish an endosymbiosis with nitrogen-fixing rhizobia bacteria, which are taken up from the environment anew by each host generation. This requires a dedicated genetic program on the host side to control microbe invasion, involving coordinated reprogramming of host cells to create infection structures that facilitate inward movement of the symbiont. Infection initiates in the epidermis, with different legumes utilizing distinct strategies for crossing this cell layer, either between cells (intercellular infection) or transcellularly (infection thread infection). Recent discoveries on the plant side using fluorescent-based imaging approaches have illuminated the spatiotemporal dynamics of infection, underscoring the importance of investigating this process at the dynamic single-cell level. Extending fluorescence-based live-dynamic approaches to the bacterial partner opens the exciting prospect of learning how individual rhizobia reprogram from rhizospheric to a host-confined state during early root infection.

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Root nodule symbiosis, legumes, rhizobia, infection thread, intercellular infection.

Introduction

Certain plant lineages establish symbiotic relationships with nitrogen-fixing bacteria, that are hosted in specialised root organs known as nodules. Here, the products of bacterial nitrogen fixation are exchanged for plant photosynthates. Building on an ancient fungal symbiosis and lateral root programmes through transcriptional rewiring or gene refinement, root nodule symbioses have evolved in species of closely-related angiosperms [1,2], including legumes interacting with rhizobia proteobacteria [3,4], the focus of this review.

Rhizobial root entry is a critical stage in which the plant selectively identifies and guides compatible bacteria from the root surface to the developing nodule [5]. This plant-directed process occurs via transcellular or intercellular routes [6,7]. While molecular understanding of intercellular infection is now emerging [8,9], knowledge of rhizobia infection mainly comes from extensive studies of transcellular infection in the models *Medicago* and *Lotus* [5]. This involves *de novo* assembly of apoplastic tubular compartments, infection threads (ITs), which separate incoming rhizobia from the host cell cytoplasm by a specific cell wall/membrane interface.

Genetic and transcriptomic studies, including recent cell-type-specific research, have uncovered key plant and bacterial genes, as well as regulatory networks that support IT development [10–15]. However, these methods cannot easily capture the dynamic spatiotemporal cellular changes critical for IT development. Resolutive live and clearing-based microscopy methods have made these dynamic processes accessible and provided new insights into plant cellular responses, including the transient changes that shape IT assembly and polar growth. This review summarises the latest advances in this field and discusses the great potential of combining *in vivo* microscopy in different models to integrate rhizobial infection mechanisms from plant and bacterial perspectives.

Plant cellular needs for transcellular infection thread development

IT formation involves sequential, well-defined steps [5]. Rhizobia secrete Nodulation factors that trigger

Abbreviations

TGN/EE	trans-Golgi network/early endosome
IT	infection thread
IC	infection chamber
PIT	pre-infection thread
TEM	transmission electron microscopy
MT	microtubules
ER	endoplasmic reticulum

early signalling and morphological changes in host root hairs, leading to entrapment of rhizobia in a curled root hair. They are then enclosed in an infection chamber (IC) that expands radially before polar IT growth is initiated [16] (Fig. 1a). Perception of rhizobial Nodulation factors and exopolysaccharides by lysin motif (LysM) receptor complexes is required for effective IT development [17,18]. Downstream of signal perception, infection-related gene expression is orchestrated by key plant transcription factors [19]. This leads to a plethora of cellular changes. Fluorescence microscopy in live or fixed/cleared tissues [16,20–24] has been instrumental in elucidating stage-specific plant cellular responses involved in IT development. These mark the commitment of cells to transcellular infection and the dynamic changes at the cell wall interface and cytoplasm to guide IT polar growth, as detailed below and in Fig. 1.

Coordinated cell wall assembly and infection thread polar growth

Targeted secretion of cell wall components creates a suitable apoplastic environment for bacterial growth. *In vivo* localization of exocytic (Vesicle associated membrane protein 21e or VAMP21e) and cell wall (Early nodulin 11 or ENOD11, Nodule pectate lyase or NPL and Symbiosis-specific pectin methylesterase or SyPME1) fluorescent protein fusions in early infection sites supports active exocytosis and cell wall remodelling in radially-expanding chambers prior to IT growth [13,16,25]. Next, the localization of SyPME1 along the IT and NPL close to the tip of growing ITs further establishes dynamic spatial regulation of pectin metabolism for IT growth [22,25]. Pectin de-esterification by SyPME may favour wall stiffness by complexing calcium ions, while degradation by NPL likely facilitates wall plasticity for IT growth.

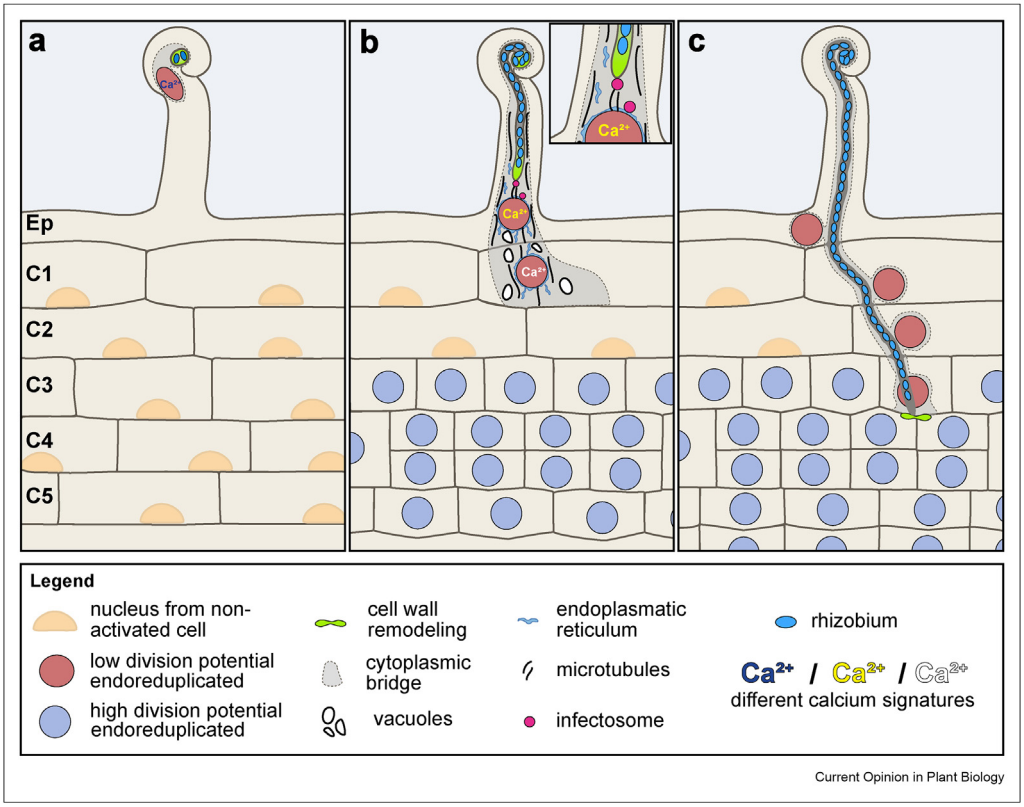
Actin rearrangements appear essential for IT formation [33–36], as mutations in key regulators like Nck-associated protein 1 (*NAP1*), *PIR1* (for 121F-specific p53 inducible RNA), Symbiotic formin 1 (*SYFO1*) or SCAR/WAVE complex members cause impaired infection phenotypes. Some of them also regulate endomembrane trafficking or cell wall function [34,36], such as the plasma membrane-localized SYFO1 [36], which is proposed to act as a bridge component via interactions with actin and the cell wall. The microtubule (MT)-interacting Developmentally-regulated plasma membrane polypeptide (DREPP) and the polarity-associated Rho GTPase 6 (LjROP6) and its activator Spike 1 (LjSPK1) may also control cytoskeletal rearrangements necessary for IT development [37,38]. However, their localisation at the root hair membrane instead of the IT interface suggests a role in regulating root hair morphogenesis rather than IT growth.

The key regulatory switch from radial to polar IT growth is under the regulation of Vapyrin (VPY) [26]. VPY localizes *in vivo* to specific cytoplasmic puncta at the tip of growing ITs or near the nucleus with its partners Lumpy infection (LIN), Exocyst subunit (EXO70H4), and Rhizobium-directed polar growth (RPG). This so-called infectosome protein complex is key for regulating vesicle trafficking for IT polar growth (Fig. 1) [22,26]. RPG was recently identified as a critical component regulating VPY puncta localization [22]. RPG co-localization with a TGN/EE marker in *Nicotiana* supports its role in regulating endomembrane trafficking [39]. Refined studies with NPL and a MT-associated GFP fusion in *rpg* revealed the importance of RPG for NPL-polarized cell wall secretion and tip-to-nucleus microtubule connection [22]. In *rpg-1* infected root hairs, microtubules appear less dense and do not properly connect the IT tip and the nucleus. Since MTs are tightly associated with the ER-enriched cytoplasmic bridge, the question remains whether the cytoplasmic bridge structure is itself also affected in the MT-dispersed *rpg* mutant configuration.

Acquiring competence and paving the road for transcellular IT passage

In root hairs, IT progression is preceded by a broad cytoplasmic column connecting the nucleus to the elongating IT tip (Fig. 1b). Cytoplasmic rearrangements also occur in adjacent cortical cells, forming a transvacuolar bridge called pre-infection thread (PIT) [40], that anticipates the future passage of the IT. These cytoplasmic strands are lined by arrays of MTs and decorated with endoplasmic reticulum (ER), as shown by immunostaining (with anti-tubulin antibodies) and live microscopy of fluorescent fusions (GFP-HDEL-ER and TUA6 tubulin GFP) [20,28,29]. Transmission electron microscopy (TEM) studies allowed visualizing the ER network at ultrastructural resolution and revealed the highly enriched fragmented vacuole composition of PITs [27]. This process appears to be genetically dependent on Nodule inception (NIN) and linked to specific calcium responses [27]. While epidermal calcium spiking is an important hallmark of early recognition of endosymbiotic microbes, other calcium spiking signatures accompany rhizobial root hair infection and cortical reprogramming (Fig. 1). These infection-related calcium spiking profiles were tightly coupled to fluorescent Annexin 1 calcium binding protein (MtAnn1) fusion cellular dynamics [27]. *MtAnn1*

Fig. 1



Plant cellular responses underlying polar infection thread growth. Schematic representation of early stages of transcellular rhizobia infection in *Medicago*. Successive formation of IC **(a)**, ITs in root hairs **(b)**, and IT progression through root cortex **(c)** are described. **(a)** Rhizobia are enclosed in a curled root hair after initial molecular dialogue and signalling. Once curling is complete, rhizobia become enclosed in an apoplastic infection chamber that expands radially by active cell wall remodelling, as evidenced by localized accumulation of exocytic (VAMP21e) and cell wall (ENOD11, NPL and SyPME1) proteins [16,25] (green) prior to bacterial proliferation. **(b)** IT transcellular tubular structures are formed by polar growth from expanded ICs and progress inward through a broad cytoplasmic column (in grey) connecting the nucleus to the elongating IT tip. The transition from a radial to a tip-growing IT structure is under the regulatory control of the infectosome complex, which consists of the Vapyrin protein VPY, the putative E3 ligase LIN, the exocyst complex subunit EXO70H4, and RPG [22,26]. Infectosome players localize to cytoplasmic puncta (in pink) positioned at the IT growing tip and in the nuclear periphery to direct IT elongation. As evidenced by localized accumulation of pectin-modifying enzymes (such as NPL) at the tip of growing ITs [22,25], polarized IT growth likely involves targeted secretion of apoplastic components at the tip. **(b)** The wide cytoplasmic column ahead of the extending IT tip is enriched in ER and fragmented vacuoles [20,27]. A dense array of MTs (black lines) provides support for the cytoplasmic bridge [28,29] and mediates IT tip to nucleus connectivity for efficient IT progression in an RPG-dependent manner [22,26]. Mitotic AUR1 kinase emerges as a novel regulator of MT function to support IT and bridge assembly [30]. Cytoplasmic bridges precede IT growth in root hairs and pave the way for IT progression in one or two adjacent cortical cells, forming the so-called PIT bridges (in the C1 cell layer in **b**). High-frequency nuclear calcium spiking is a central component of early microbial recognition signalling in the host epidermis, while cells engaged in infection show stage-specific calcium spiking signatures [27]. The transition from radial (in **a**) to polar IT growth (in **b**) is marked by a regulatory switch from high to low calcium spike amplitude profiles [27,31]. Cortical cells preparing for IT passage exhibit unique low frequency calcium spiking profiles. **(b)** Plant cells engaged to rhizobial infection enter the cell cycle and arrest in a G2 post-replicative phase without mitosis (red nuclei) for transcellular infection. This involves the recruitment of cell cycle specific players and auxin plays a consistent role in this process [10,23,32]. **(c)** The acquisition of a pre-mitotic cell cycle arrest state is consistently observed in the trajectory of infected cortical cells [21].

mutation results in deregulated calcium spiking and altered cytoplasmic composition, possibly required for a suitable cytoplasmic environment for efficient infection.

PITs are structurally related to premitotic phragmosome transvacuolar bridges formed in cells preparing for mitosis (Fig. 1b). A parallel has been drawn between these processes, supported by *in situ* hybridization of histone and cell-cycle related markers in *Pisum* showing that PIT cells, though non-dividing, enter the cell cycle [41]. Cytoplasmic bridges resembling PITs also form in infected root hairs, and evidence from root hair transcriptomics also supports a cell cycle-regulated status in these cells. Furthermore, cell cycle gene activation coincides with the expression of several auxin-responsive genes [10], indicating a key role for auxin in rhizobia-infected root hair cells that do not divide. Consistently, the auxin-responsive DII biosensor and a DR5 signalling reporter confirmed local auxin accumulation and signalling in rhizobia-infected cells [23,32]. The role of auxin in IT development is further supported by the reduced infection phenotypes observed when auxin biosynthesis is

disrupted, either through chemical inhibition of YUCCA activity in *Lotus* or by mutation of auxin response factor 16 (ARF16) in *Medicago* [10,32]. As a major hormone regulator of cell division, auxin promotes cortical nodule organogenesis and is associated with mitotic cell reactivation in this context [42,43]. Thus, auxin may help root hairs to acquire an analogous pre-mitotic state. Other possible roles for auxin in the epidermis include suppression of cytokinin biosynthesis and signalling to allow early infection [32], or acting as a mobile signal (in its IAA or methylated form) that can travel through transporter-dependent or independent pathways to coordinate infection with nodule organogenesis [44–46].

Further evidence that infected root hair cells enter the cell cycle and adopt a mitotic-like state was provided by the discovery of the novel rhizobial-induced mitotic α-Aurora kinase regulator AUR1 [30]. AUR1 is localised to the ER and together with its interactors TPX (Targeting protein for Xenopus kinesin-like protein) and MAP65 (Microtubule-associated protein 65) is presumably involved in the regulation of MT function to support IT and cytoplasmic bridge assembly. *AUR1* is key to the formation of infection structures, and when mutated leads to branched IT phenotypes, possibly due to misdirected cell wall deposition, but this has yet to be shown.

Recently, elegant studies combining fluorescent reporters (e.g. histone and cyclin-based) with powerful clearing methods provided crucial information on the cell cycle status of *Medicago* IT-containing deep cortical cells [21]. These cells exhibited reduced proliferation and post-replicative cell cycle exit. The Nuclear transcription factor Y A1 (NF-YA1) was involved in this switch, as supported by mutant analysis and its ability to interfere with *Nicotiana* mitotic cell entry [21]. In *Lotus* and *Phaseolus* legumes, G2/M transition markers revealed cell cycle reactivation and plate formation in cortical cells near infected root hairs [23,47]. Whether strict mitotic control and arrest occurs in these models during IT passage remains unknown. Moreover, polyploidization of nodule primordia cells, under the likely control of VAG1 (Vagrant infection thread 1), a putative component of the DNA topoisomerase VI complex, seems required for infection in *Lotus* [48]. Together, these findings establish a close link between infection and cell cycle regulation (whether in cells preparing for infection or IT-traversed) in a wide diversity of legumes, both indeterminate (*Medicago*, *pea*) or determinate (*Lotus*) types (i.e. nodules with or without a persistent apical meristem, respectively).

Interaction of rhizobia with the infection chamber and infection thread environment

The rhizobial path to an endosymbiotic nitrogen-fixing form (bacteroids) commences after the early signal exchange in the rhizosphere, usually with a single rhizobial cell attached to the root hair tip, although also mixed infections originating from a few founder cells can occur [16,49]. Attachment usually involves rhicadhesin and cell envelope polysaccharides, and can be supported by plant lectins [50]. The attached rhizobial cell becomes enclosed in the IC created by root hair curling including cell wall remodelling [16]. This is initiated before and later accompanied by rhizobial multiplication. Initiation of the inward growing IT involves local remodelling of the plant cell wall. A possible contribution of a bacterial plant cell wall degrading enzyme was suggested for the cell-bound bacterial cellulase CelC2 from *Rhizobium leguminosarum* bv. trifolii [51]. Rhizobial IT colonization involves bacterial cell growth and division as well as bacterial movement in the IT [20,49]. It has been speculated that this involves bacterial gliding, and it is unknown if flagellar motility [52,53] and biosurfactants [54–56] have a role in the IT. A model has been suggested that bacteria near the IT tip grow actively, while bacteria in the distal area of the IT appear to have stopped growth [49]. A small space at the tip of the IT is usually free of bacteria [20,57]. IT tip progression therefore does not require direct contact to rhizobial cells.

Rhizobia are exposed to a variety of fluctuating stress factors in the soil as well as in the rhizosphere and *in planta*, including low pH, oxidative and osmotic stress, and antimicrobial compounds. Stress adaptation programs ensure rhizobial survival and growth in these different habitats. Moreover, these programs are also part of the dialogue with the host. This connection and relevant rhizobial stress adaptation programs have recently been reviewed in detail [58]. It is known that IT progression requires rhizobial extracellular polysaccharides and continued production of Nodulation factors. The perception of these molecules and associated plant responses have been summarized above. In addition, rhizobial oxidative stress response reflecting reactive oxygen species (ROS) accumulation [59–62] and processes dependent on the intracellular stress signal (p)ppGpp [63] were found to be important for different steps of host colonization, including the IT stage [15]. It has been suggested that rhizobial catalase activity influences the IT wall and matrix texture [64].

Little is known about which nutrients are available to and utilized by the rhizobia to support their growth and survival in the IT. A metabolome analysis of soybean root hairs upon inoculation with rhizobia revealed that amino acids, disaccharides, and lactic acid among other compounds are significantly enriched in inoculated root hairs compared to non-inoculated root hairs and stripped roots [65]. However, whether these metabolites are exuded to the rhizosphere or transported into the IT is not known. Moreover, on the bacterial side, current knowledge is derived either from studies on individual mutants, often struggling to determine the affected symbiotic interaction stage(s), or from global monitoring

or screening approaches which have limited spatial and temporal resolution. Laser-capture microdissection coupled to RNA-sequencing [66], transposon mutant competition experiments coupled to insertion-sequencing (INSeq) [15] and genome-scale metabolic modelling [67] have improved throughput and resolution in defining bacterial genes expressed during or required for different stages of symbiosis. The INSeq approach identified about 140 genes putatively important specifically for bacteria within or recently released from ITs, or for regrowth after nodule senescence. These include genes related to chemotaxis and motility supporting a possible role for bacterial movement through ITs, as well as several defense- and stress-related genes and ABC uptake transporter genes. The metabolic modelling approach predicted malate, fructose, xylose, *myo*-inositol, γ-aminobutyrate (GABA), 2-isopropylmalate, serine, nucleobases, and nucleosides to be taken up by undifferentiated nodule bacteria [67].

Intercellular infection in the legume-rhizobia symbiosis

IT formation is common in most studied legumes. However, in less investigated tribes such as Dalbergiae (*Arachis*, *Aeschynomene*) and Genisteae (*Lupinus*) nodules can be infected without epidermal ITs, by a process called intercellular infection [68,69]. For instance, in *Arachis hypogea* and *Aeschynomene afraspera*, bacteria accumulate at the base of lateral roots, entering through natural opening or "cracks" in the root. In *A. afraspera* entry occurs between an axillary hair and an epidermal cell of the primary root [70], while in *A. hypogea* it is often described to occur through the middle lamella of two adjacent root hair cells [71—73]. In both cases, bacteria spread through the cortex's apoplastic space, and are internalized by cortical cells [70—73]. In contrast, in *Lupinus*, after intercellular penetration of the epidermis bacteria enter host cells of the first cortical layer and spread occurs through cell division of these cells [74]. These observations suggest variance even within intercellular infection.

Intercellular infection has been proposed as a primitive form of infection [75,76]. While *Lotus* is usually infected through ITs, some mutants lacking epidermal ITs still form nodules and internalize rhizobia, albeit at a low frequency, indicating the presence of alternative pathways [75,77,78]. Infection without ITs has also been observed in *Lotus* inoculated with subcompatible rhizobia, such as *Sinorhizobium fredii* HH103 [79], *Rhizobium leguminosarum* Norway [80] and *Rhizobium* sp. IRGB74 [8]. In contrast to Dalbergiae, *Lotus* nodules form irrespective of lateral roots, with bacteria crossing the epidermis, accumulating in infection pockets below the epidermis, and spreading from there into the cortex [80]. This, along with the observation that intercellular infection occurs in some actinorhizal plants within the Rosales order, further supports the widespread phylogenetic distribution of this infection mode [81—83]. For instance, Frankia infects *Elaeagnus* via the middle lamella [81], while rhizobia induce in *Parasponia* epidermal and outer cortical cell divisions giving rise to openings in the epidermis through which bacteria infect [84].

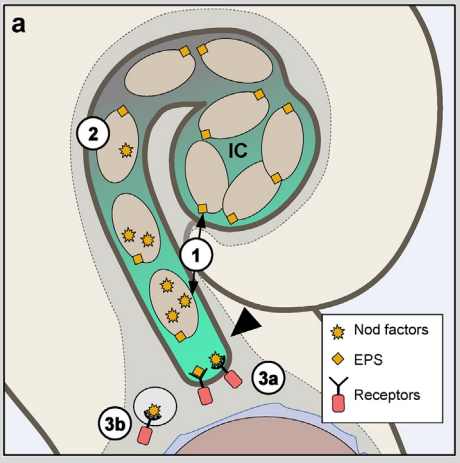
Recent reviews have highlighted the similarities and differences between intercellular and IT-mediated infection [6,85]. Genes like *NPL* and *RPG*, crucial for IT formation, are often either absent or not induced during intercellular infection [8,85—87]. Moreover, specific genes such as *Nodule enhanced Glycosyl Hydrolase* (*NGH*) are induced near intercellular entry sites but not during IT infection [88]. Lastly, key genes like *NIN* show tissue-specific expression variations during IT and intercellular infection. For instance, NIN is expressed in both the cortex and epidermis during IT infection but only expressed in developing nodule primordia in *A. hypogea* [88]. Other key determinants of IT polar growth such as *VPY* are also up-regulated in *Aeschynomene evenia*, raising the question of their tissue/cell-specific expression profiles in this IT-independent interaction [9]. Overall, this suggests that intercellular infection requires a dedicated genetic program with specific or adapted genetic functions.

Conclusions and perspectives

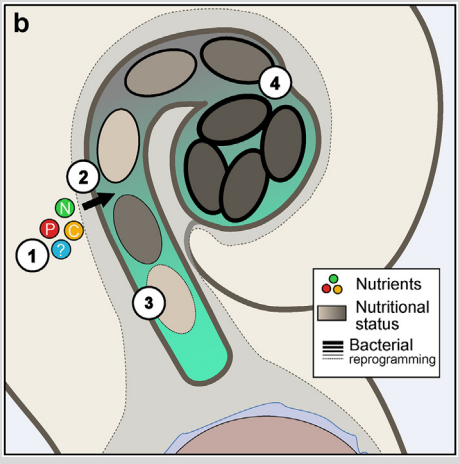
The plant host orchestrates sequential cellular processes to pave the way and control the invasion of its root by the microbial partner. Specific recognition of the symbiont triggers entrapment of the bacteria, after which the formation of ITs is initiated. The inward polar growth of these structures involves spatiotemporal changes at the cell wall interface with likely delivery of cell wall materials to the tip of the growing IT. This spatially regulated process appears to be facilitated by the infectosome complex, which acts in concert with the tip-to-nucleus MT network for regulated polar secretion. The growth of the IT is preceded by a cytoplasmatic bridge that connects the IT tip to the nucleus, during which acquisition of a mitotic state without division and specific calcium spiking signatures matter. Dynamic changes in the plant cell wall interface likely create a unique environmental gradient in the apoplast, to which rhizobia need to adapt in terms of motility and stress coping mechanisms. We have not only just begun to understand the spatiotemporal dynamics of this symbiosis at cellular resolution, but we are just scratching the surface of the vast untapped natural diversity of symbiotic associations with different modes of infection.

Technological advancements, such as super resolution microscopy [89], single particle tracking [90], multi-imaging analysis [91] and a growing number of genetically encoded sensors (e.g., calcium, ROS, pH, etc.) offer unprecedented opportunities to study the cellular dynamics of key proteins (e.g., transcription factors and receptor complexes) and processes (e.g., cell wall remodelling) during IT progression in model organisms

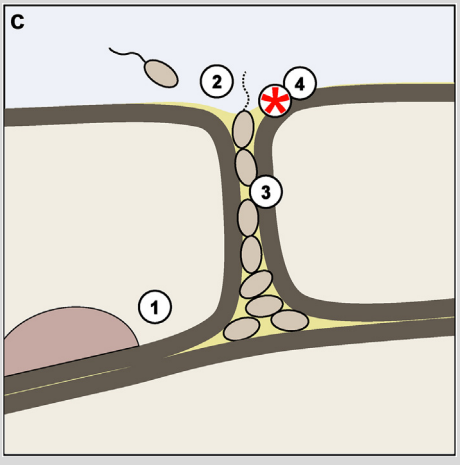
Box 1. Outstanding open questions. An unexplored question is how individual rhizobia cells reprogram during the switch from a free-living to a restricted host environment, whether within the IT (a,b) or in the apoplastic spaces (c) between cells. Understanding this process will require a higher spatiotemporal resolution and real-time parallel observation of plant and bacterial functions at the single cell level. By combining powerful live-cell imaging strategies developed for plants and free-living rhizobia, we can gain crucial insights into the status of individual bacterial cells and its link to specific plant responses. Outstanding questions that can be addressed in this context are described below. IT, infection thread; IC, infection chamber; EPS, exopolysaccharide; arrowhead indicates IT tip.



1. How is the production and perception of rhizobia signals, such as Nod factors and EPS, regulated throughout the progression of infection? Do bacteria in the IC and in the IT tip produce the same type of signal?
2. How is the expression of core biosynthetic and/or modifying genes that control signal production regulated during IT-progression?
3. How does the spatiotemporal regulation of rhizobial signalling molecules relate to the localisation (a) and turnover (b) of receptor complexes during infection?



1. Which nutrients are available to and utilised by rhizobia during early infection within ITs?
2. Is there evidence of bacterial feeding by the host?
3. Do rhizobia face nutritional limitations inside ITs?
4. How does the rhizobia nutritional status correlate with rhizobial cell proliferation and movement within the confined plant compartments?



1. What is the host cell-specific signalling and transcriptional response at intercellular infection sites?
2. Is there reprogramming of bacterial cells at intercellular infection sites?
3. What are the changes in the cell wall integrity and composition?
4. Are the common cellular remodelling principles across different intercellular infection systems?

*Entry site depends on the system: e.g. between root hairs, middle lamella, at the junction of a lateral root site.

and their diversification in non-model systems. While a wealth of information is available on genes required for the establishment of symbiotic infection, the dynamics of proteins and protein complexes in a cellular context are poorly investigated. Single particle tracking and super-resolution microscopy of receptor proteins could shed light how symbiotic complexes are formed at the IT interface. Live cell microscopy of transcription factors at different stages of infection, coupled with single-cell RNA sequencing would be extremely informative for inferring the timing of complex formation and transcriptional output. Moreover, cell-based studies in different intercellular models could help answer key questions regarding the basic toolkit to allow symbiotic root infection. Finally, the use of fluorescence-based reporters and sensors in both the host and the bacterial symbiont promises to uncover the reprograming of bacteria in the plant environment during early root infection and the crosstalk with their plant partners (Box 1).

Statement

During the preparation of this work the authors used ChatGPT3.5 in order to reduce the number of words. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Huisman R, Geurts R: **A roadmap toward engineered nitrogen-fixing nodule symbiosis**. *Plant Commun* 2020, **1**, 100019.

2. Lebreton AKJ: **At the root of plant symbioses: untangling the genetic mechanisms behind mutualistic associations**. *Curr Opin Plant Biol* 2024, **77**:102448.

3. Masson-Boivin C, Sachs JL: **Symbiotic nitrogen fixation by rhizobia-the roots of a success story**. *Curr Opin Plant Biol* 2018, **44**:7–15.

4. Mathesius U: **Are legumes different? Origins and consequences of evolving nitrogen fixing symbioses**. *J Plant Physiol* 2022, **276**, 153765.

5. ** Gao JP, Liang W, Liu CW, Xie F, Murray JD: **Unraveling the rhizobial infection thread**. *J Exp Bot* 2024, **75**:2235–2245.
This review highlights early research milestones and two recent outstanding discoveries in rhizobial symbiosis related to the recruitment of a conserved mitotic module and infectosome protein complex to coordinate polar infection thread growth.

6. Ibáñez F, Wall L, Fabra A: **Starting points in plant-bacteria nitrogen-fixing symbioses: intercellular invasion of the roots**. *J Exp Bot* 2016, **68**:1905–1918.

7. Parniske M: **Uptake of bacteria into living plant cells, the unifying and distinct feature of the nitrogen-fixing root nodule symbiosis**. *Curr Op Plant Biol* 2018, **44**:164–174.

8. Montiel J, Reid D, Grønbæk TH, Benfeldt CM, James EK, Ott T, Ditengou FA, Nadzieja M, Kelly S, Stougaard J: **Distinct signaling routes mediate intercellular and intracellular rhizobial infection in *Lotus japonicus***. *Plant Physiol* 2021, **185**:1131–1147.

9. Quilbé J, Lamy L, Brottier L, Leleux P, Fardoux J, Rivallan R, Benichou T, Guyonnet R, Becana M, Villar I, *et al.*: **Genetics of nodulation in *Aeschynomene evenia* uncovers mechanisms of the rhizobium–legume symbiosis**. *Nat Commun* 2021, **12**:829.

10. Breakspear A, Liu C, Roy S, Stacey N, Rogers C, Trick M, Morieri G, Mysore KS, Wen J, Oldroyd GE, *et al.*: **The root hair "infectome" of *Medicago truncatula* uncovers changes in cell cycle genes and reveals a requirement for auxin signaling in rhizobial infection**. *Plant Cell* 2014, **26**:4680–4701.

11. Cervantes-Pérez SA, Thibivilliers S, Laffont C, Farmer AD, Frugier F, Libault M: **Cell-specific pathways recruited for symbiotic nodulation in the *Medicago truncatula* legume**. *Mol Plant* 2022, **15**:1868–1888.

12. Frank M, Fechete LI, Tedeschi F, Nadzieja M, Nørgaard MMM, Montiel J, Andersen KR, Schierup MH, Reid D, Andersen SU: **Single-cell analysis identifies genes facilitating rhizobium infection in *Lotus japonicus***. *Nat Commun* 2023, **14**:7171.

13. Liu CW, Breakspear A, Guan D, Cerri MR, Jackson K, Jiang S, Robson F, Radhakrishnan GV, Roy S, Bone C, *et al.*: **NIN acts as a network hub controlling a growth module required for rhizobial infection**. *Plant Physiol* 2019, **179**:1704–1722.

14. Pereira WJ, Boyd J, Conde D, Triozzi PM, Balmant KM, Dervinis C, Schmidt HW, Boaventura-Novaes C, Chakraborty S, Knaack SA, *et al.*: **The single-cell transcriptome program of nodule development cellular lineages in *Medicago truncatula***. *Cell Rep* 2024, **43**, 113747.

15. Wheatley RM, Ford BL, Li L, Aroney STN, Knights HE, Ledermann R, East AK, Ramachandran VK, Poole PS: **Lifestyle adaptations of *Rhizobium* from rhizosphere to symbiosis**. *Proc Natl Acad Sci U S A* 2020, **117**:23823–23834.

16. Fournier J, Teillet A, Chabaud M, Ivanov S, Genre A, Limpens E, de Carvalho-Niebel F, Barker DG: **Remodeling of the infection chamber before infection thread formation reveals a two-step mechanism for rhizobial entry into the host legume root hair**. *Plant Physiol* 2015, **167**:1233–1242.

17. Kelly S, Radutoiu S, Stougaard J: **Legume LysM receptors mediate symbiotic and pathogenic signalling**. *Curr Opin Plant Biol* 2017, **39**:152–158.

18. Krönauer C, Radutoiu S: **Understanding Nod factor signalling paves the way for targeted engineering in legumes and non-legumes**. *Curr Opin Plant Biol* 2021, **62**, 102026.

19. Chakraborty S, Valdés-López O, Stonoha-Arther C, Ané JM: **Transcription factors controlling the Rhizobium-legume symbiosis: integrating infection, organogenesis, and the abiotic environment**. *Plant Cell Physiol* 2022, **63**:1326–1343.

20. Fournier J, Timmers ACJ, Sieberer BJ, Jauneau A, Chabaud M, Barker DG: **Mechanism of infection thread elongation in root hairs of *Medicago truncatula* and dynamic interplay with associated rhizobial colonization**. *Plant Physiol* 2008, **148**:1985–1995.

21. ** Batzenschlager M, Lace B, Zhang N, Su C, Egli S, Krohn P, Salfeld J, Ditengou FA, Laux T, Ott T: **Competence for transcellular infection in the root cortex involves a post-replicative, cell-cycle exit decision in *Medicago truncatula***. *eLife* 2023, **12**, RP88588.
Combining fluorescent reporters with powerful clearing methods, this work provided outstanding results on how root cortical cells enter the cell cycle and pre-mitotic arrest during rhizobial infection.

22. * Lace B, Su C, Invernot Perez D, Rodriguez-Franco M, Vernié T, Batzenschlager M, Egli S, Liu CW, Ott T: **RPG acts as a central determinant for infectosome formation and cellular polarization during intracellular rhizobial infections**. *eLife* 2023, **12**, e80741.
In this paper, the RPG protein is revealed as a core component of the infectosome complex, essential for polar elongation of infection threads.

23. Nadzieja M, Stougaard J, Reid D: **A toolkit for high resolution imaging of cell division and phytohormone signaling in legume roots and root nodules**. *Front Plant Sci* 2019, **10**:1000.

24. Rae AE, Rolland V, White RG, Mathesius U: **New methods for confocal imaging of infection threads in crop and model legumes**. *Plant Methods* 2021, **17**:24.

25. * Su C, Zhang G, Rodriguez-Franco M, Hinnenberg R, Wietschorke J, Liang P, Yang W, Uhler L, Li X, Ott T: **Transcellular progression of infection threads in *Medicago truncatula* roots is associated with locally confined cell wall modifications**. *Curr Biol* 2023, **33**:533–542.e535.
This paper showed the spatial localization of specific cell wall enzymes, Pectin Methyl Esterase (SyPME1) and Nodulation Pectate Lyase (NPL) during rhizobia root transcellular infection. This together with ultrastructural and functional studies provided crucial information on local pectin cell wall modifications required for IT growth and cell-to-cell progression.

26. Liu CW, Breakspear A, Stacey N, Findlay K, Nakashima J, Ramakrishnan K, Liu M, Xie F, Endre G, de Carvalho-Niebel F, *et al.*: **A protein complex required for polar growth of rhizobial infection threads**. *Nat Commun* 2019, **10**:2848.

27. Guillory A, Fournier J, Kelner A, Hobecker K, Auriac M-C, Frances L, Delers A, Ru AL, Keller J, Delaux P-M, *et al.*: **Annexin and calcium-regulated priming of legume root cells for endosymbiotic infection**. *bioRxiv* 2024. 2024.2001.2010.574854.

28. Perrine-Walker FM, Lartaud M, Kouchi H, Ridge RW: **Microtubule array formation during root hair infection thread initiation and elongation in the *Mesorhizobium-Lotus* symbiosis**. *Protoplasma* 2014, **251**:1099–1111.

29. 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.

30. ** Gao JP, Jiang S, Su Y, Xu P, Wang J, Liang W, Liu CW, Murray JD: **Intracellular infection by symbiotic bacteria requires the mitotic kinase AURORA1**. *Proc Natl Acad Sci U S A* 2022, **119**, e2202606119.
This paper reports a novel regulatory module, the mitotic α-Aurora kinase (AUR1) and its transcriptional regulator MYB3R1, to control rhizobial root hair infection. It also provided evidence for the involvement of AUR1 in the regulation of microtubule function to support IT and cytoplasmic bridge assembly.

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

32. Nadzieja M, Kelly S, Stougaard J, Reid D: **Epidermal auxin biosynthesis facilitates rhizobial infection in *Lotus japonicus***. *Plant J* 2018, **95**:101–111.

33. Yokota K, Fukai E, Madsen LH, Jurkiewicz A, Rueda P, Radutoiu S, Held M, Hossain MS, Szczyglowski K, Morieri G, *et al.*: **Rearrangement of actin cytoskeleton mediates invasion of *Lotus japonicus* roots by *Mesorhizobium loti***. *Plant Cell* 2009, **21**:267–284.

34. Gavrin A, Rey T, Torode TA, Toulotte J, Chatterjee A, Kaplan JL, Evangelisti E, Takagi H, Charoensawan V, Rengel D: **Developmental modulation of root cell wall architecture confers resistance to an oomycete pathogen**. *Curr Biol* 2020, **30**:4165–4176.

35. Qiu L, Lin JS, Xu J, Sato S, Parniske M, Wang TL, Downie JA, Xie F: **SCARN a novel class of SCAR protein that is required for root-hair infection during legume nodulation**. *PLoS Genet* 2015, **11**, e1005623.

36. Liang P, Schmitz C, Lace B, Ditengou FA, Su C, Schulze E, Knerr J, Grosse R, Keller J, Libourel C, *et al.*: **Formin-mediated bridging of cell wall, plasma membrane, and cytoskeleton in symbiotic infections of *Medicago truncatula***. *Curr Biol* 2021, **31**:2712–2719.e2715.

37. Liu J, Liu MX, Qiu LP, Xie F: **SPIKE1 activates the GTPase ROP6 to guide the polarized growth of infection threads in *Lotus japonicus***. *Plant Cell* 2020, **32**:3774–3791.

38. Su C, Klein M-L, Hernández-Reyes C, Batzenschlager M, Ditengou FA, Lace B, Keller J, Delaux P-M, Ott T: **The *Medicago truncatula* DREPP protein triggers microtubule fragmentation in membrane nanodomains during symbiotic infections**. *Plant Cell* 2020, **32**:1689–1702.

39. Li X, Liu M, Cai M, Chiasson D, Groth M, Heckmann AB, Wang TL, Parniske M, Downie JA, Xie F: **RPG interacts with E3-ligase CERBERUS to mediate rhizobial infection in *Lotus japonicus***. *PLoS Genet* 2023, **19**, e1010621.

40. Van Brussel AAN, Bakhuizen R, Van Spronsen PC, Spaink HP, Tak T, Lugtenberg BJJ, Kijne JW: **Induction of preinfection thread structures in the leguminous host plant by mitogenic lipooligosaccharides of *Rhizobium***. *Science* 1992, **257**:70–72.

41. Yang WC, de Blank C, Meskiene I, Hirt H, Bakker J, van Kammen A, Franssen H, Bisseling T: ***Rhizobium* Nod factors reactivate the cell cycle during infection and nodule primordium formation, but the cycle is only completed in primordium formation**. *Plant Cell* 1994, **6**:1415–1426.

42. Velandia K, Reid JB, Foo E: **Right time, right place: the dynamic role of hormones in rhizobial infection and nodulation of legumes**. *Plant Commun* 2022, **3**, 100327.

43. Xiao T, Shen D, Müller S, Liu J, van Seters A, Franssen H, Bisseling T, Kulikova O, Kohlen W: **Nodule organogenesis in *Medicago truncatula* requires local stage-specific auxin biosynthesis and transport**. *bioRxiv* 2023. 2023.2012.2007.570677.

44. Gaudioso-Pedraza R, Beck M, Frances L, Kirk P, Ripodas C, Niebel A, Oldroyd GED, Benitez-Alfonso Y, de Carvalho-Niebel F: **Callose-regulated symplastic communication coordinates symbiotic root nodule development**. *Curr Biol* 2018, **28**:3562–3577.e3566.

45. Goto T, Soyano T, Liu M, Mori T, Kawaguchi M: **Auxin methylation by IAMT1, duplicated in the legume lineage, promotes root nodule development in *Lotus japonicus***. *Proc Natl Acad Sci U S A* 2022, **119**, e2116549119.

46. Tee EE, Faulkner C: **Plasmodesmata and intercellular molecular traffic control**. *New Phytol* 2024, **243**:32–47.

47. Monroy-Morales E, Dávila-Delgado R, Ayala-Guzmán E, Gamboa-deBuen A, Sánchez-López R: **Visualization of the crossroads between a nascent infection thread and the first cell division event in *Phaseolus vulgaris* nodulation**. *Int J Mol Sci* 2022, **23**:5267.

48. Suzaki T, Ito M, Yoro E, Sato S, Hirakawa H, Takeda N, Kawaguchi M: **Endoreduplication-mediated initiation of symbiotic organ development in *Lotus japonicus***. *Development* 2014, **141**:2441–2445.

49. Gage DJ: **Analysis of infection thread development using Gfp- and *Ds*Red-Expressing *Sinorhizobium meliloti***. *J Bacteriol* 2002, **184**:7042–7046.

50. Downie JA: **The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots**. *FEMS Microbiol Rev* 2010, **34**:150–170.

51. Robledo M, Jiménez-Zurdo JI, Velázquez E, Trujillo ME, Zurdo-Piñeiro JL, Ramírez-Bahena MH, Ramos B, Díaz-Mínguez JM, Dazzo F, Martínez-Molina E, Mateos PF: ***Rhizobium* cellulase**

$CelC_2$ is essential for primary symbiotic infection of legume host roots. *Proc Natl Acad Sci U S A* 2008, **105**:7064–7069.

52. Aroney STN, Pini F, Kessler C, Poole PS, Sánchez-Cañizares C: **The motility and chemosensory systems of *Rhizobium leguminosarum*, their role in symbiosis, and link to PTSNtr regulation**. *Environ Microbiol* 2024, **26**, e16570.

53. Aroney STN, Poole PS, Sánchez-Cañizares C: **Rhizobial chemotaxis and motility systems at work in the soil**. *Front Plant Sci* 2021, **12**.

54. Covelli JM, Althabegoiti MJ, López MF, Lodeiro AR: **Swarming motility in *Bradyrhizobium japonicum***. *Res Microbiol* 2013, **164**:136–144.

55. Daniels R, Reynaert S, Hoekstra H, Verreth C, Janssens J, Braeken K, Fauvart M, Beullens S, Heusdens C, Lambrichts I, *et al.*: **Quorum signal molecules as biosurfactants affecting swarming in *Rhizobium etli***. *Proc Natl Acad Sci* 2006, **103**: 14965–14970.

56. Tambalo DD, Yost CK, Hynes MF: **Characterization of swarming motility in *Rhizobium leguminosarum* bv. *viciae***. *FEMS Microbiol Lett* 2010, **307**:165–174.

57. Brewin NJ: **Plant cell wall remodelling in the *Rhizobium*–legume symbiosis**. *Crit Rev Plant Sci* 2004, **23**:293–316.

58. Hawkins JP, Oresnik IJ: **The *Rhizobium*-legume symbiosis: co-opting successful stress management**. *Front Plant Sci* 2022, **12**.

59. Santos R, Hérouart D, Sigaud S, Touati D, Puppo A: **Oxidative burst in alfalfa-*Sinorhizobium meliloti* symbiotic interaction**. *Mol Plant Microbe Interact* 2001, **14**:86–89.

60. Rubio MC, James EK, Clemente MR, Bucciarelli B, Fedorova M, Vance CP, Becana M: **Localization of superoxide dismutases and hydrogen peroxide in legume root nodules**. *Mol Plant Microbe Interact* 2004, **17**:1294–1305.

61. Jamet A, Sigaud S, Van de Sype G, Puppo A, Hérouart 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.

62. Jamet A, Mandon K, Puppo A, Hérouart D: **H_2O_2 is required for optimal establishment of the *Medicago sativa*/*Sinorhizobium meliloti* symbiosis**. *J Bacteriol* 2007, **189**:8741–8745.

63. Krol E, Becker A: **ppGpp in *Sinorhizobium meliloti*: biosynthesis in response to sudden nutritional downshifts and modulation of the transcriptome**. *Mol Microbiol* 2011, **81**: 1233–1254.

64. Wisniewski J-P, Rathbun EA, Knox JP, Brewin NJ: **Involvement of diamine oxidase and peroxidase in insolubilization of the extracellular matrix: implications for pea nodule initiation by *Rhizobium leguminosarum***. *Mol Plant Microbe Interact* 2000, **13**:413–420.

65. Brechenmacher L, Lei Z, Libault M, Findley S, Sugawara M, Sadowsky MJ, Sumner LW, Stacey G: **Soybean metabolites regulated in root hairs in response to the symbiotic bacterium *Bradyrhizobium japonicum***. *Plant Physiol* 2010, **153**: 1808–1822.

66. Roux B, Rodde N, Jardinaud M-F, Timmers T, Sauviac L, Cottret L, Carrère S, Sallet E, Courcelle E, Moreau S, *et al.*: **An integrated analysis of plant and bacterial gene expression in symbiotic root nodules using laser-capture microdissection coupled to RNA sequencing**. *Plant J* 2014, **77**:817–837.

67. ** Schulte CCM, Ramachandran VK, Papachristodoulou A, Poole PS: **Genome-scale metabolic modelling of lifestyle changes in *Rhizobium leguminosarum***. *mSystems* 2022, **7**, e00975-00921.
This study provides genome-scale metabolic models of rhizosphere rhizobia, undifferentiated nodule bacteria, and bacteroids, and suggests compounds taken up by bacteria in these states.

68. Venado RE, Liang J, Marín M: **Rhizobia infection, a journey to the inside of plant cells**. In *Advances in botanical research: regulation of nitrogen-fixing symbioses in legumes*. Edited by Frendo P, Frugier F, Masson-Boivin C; 2020:97–118.

69. Sprent JI: **Development and functioning of nodules**. In *Legume nodulation: a global perspective*. Edited by Sprent JI, John Wiley & Sons Ltd.; 2009:79–95.

70. Bonaldi K, Gargani D, Prin Y, Fardoux J, Gully D, Nouwen N, Goormachtig S, Giraud E: **Nodulation of *Aeschynomene afraspera* and *A. indica* by photosynthetic *Bradyrhizobium* sp. strain ORS285: the nod-dependent versus the nod-independent symbiotic interaction**. *Mol Plant Microbe Interact* 2011, **24**:1359–1371.

71. Boogerd FC, van Rossum D: **Nodulation of groundnut by *Bradyrhizobium*: a simple infection process by crack-entry**. *FEMS Microbiol Rev* 1997, **21**:5–27.

72. Chandler MR: **Some observations on infection of *Arachis hypogaea* L. by *Rhizobium***. *J Exp Bot* 1978, **29**:749–755.

73. Uheda E, Daimon H, Yoshizako F: **Colonization and invasion of peanut (*Arachis hypogaea* L.) roots by *gusA*-marked *Bradyrhizobium* sp**. *Can J Bot* 2001, **79**:733–738.

74. González-Sama A, Lucas MM, De Felipe MR, Pueyo JJ: **An unusual infection mechanism and nodule morphogenesis in white lupin (*Lupinus albus*)**. *New Phytol* 2004, **163**:371–380.

75. Madsen LH, Tirichine L, Jurkiewicz A, Sullivan JT, Heckmann AB, Bek AS, Ronson CW, James EK, Stougaard J: **The molecular network governing nodule organogenesis and infection in the model legume *Lotus japonicus***. *Nat Commun* 2010, **1**:10.

76. Held M, Hossain MS, Yokota K, Bonfante P, Stougaard J, Szczyglowski K: **Common and not so common symbiotic entry**. *Trends Plant Sci* 2010, **15**:540–545.

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

78. Suzaki T, Takeda N, Nishida H, Hoshino M, Ito M, Misawa F, Handa Y, Miura K, Kawaguchi M: **Lack of symbiont accommodation controls intracellular symbiont accommodation in root nodule and arbuscular mycorrhizal symbiosis in *Lotus japonicus***. *PLoS Genet* 2019, **15**, e1007865.

79. Acosta-Jurado S, Rodríguez-Navarro DN, Kawaharada Y, Perea JF, Gil-Serrano A, Jin H, An Q, Rodríguez-Carvajal MA, Andersen SU, Sandal N, *et al.*: ***Sinorhizobium fredii* HH103 invades *Lotus burttii* by crack entry in a Nod factor-and surface polysaccharide-dependent manner**. *Mol Plant Microbe Interact* 2016, **29**:925–937.

80. Liang J, Klingl A, Lin YY, Boul E, Thomas-Oates J, Marín M: **A sub-compatible rhizobium strain reveals infection duality in *Lotus***. *J Exp Bot* 2019, **70**:1903–1913.

81. Miller IM, Baker DD: **The initiation, development and structure of root nodules in *Elaeagnus angustifolia* L. (Elaeagnaceae)**. *Protoplasma* 1985, **128**:107–119.

82. Fournier J, Imanishi L, Chabaud M, Abdou-Pavy I, Genre A, Brichet L, Lascano HR, Muñoz N, Vayssières A, Pirolles E, *et al.*: **Cell remodeling and subtilase gene expression in the actinorhizal plant *Discaria trinervis* highlight host orchestration of intercellular *Frankia* colonization**. *New Phytol* 2018, **219**: 1018–1030.

83. Pawlowski K, Demchenko KN: **The diversity of actinorhizal symbiosis**. *Protoplasma* 2012, **249**:967–979.

84. Bender GL, Nayudu M, Goydych W, Rolfe BG: **Early infection events in the nodulation of the non-legume *Parasponia andersonii* by *Bradyrhizobium***. *Plant Sci* 1987, **51**:285–293.

85. Quilbé J, Montiel J, Arrighi JF, Stougaard J: **Molecular mechanisms of intercellular rhizobial infection: novel findings of an ancient process**. *Front Plant Sci* 2022, **13**, 922982.

86. Raul B, Bhattacharjee O, Ghosh A, Upadhyay P, Tembhare K, Singh A, Shaheen T, Ghosh AK, Torres-Jerez I, Krom N, *et al.*: **Microscopic and transcriptomic analyses of Dalbergoid legume peanut reveal a divergent evolution leading to Nod**

factor-dependent epidermal crack-entry and terminal bacteroid differentiation. *Mol Plant Microbe Interact* 2022, **35**: 131–145.

87. Gully D, Czernic P, Cruveiller S, Mahé F, Longin C, Vallenet D, François P, Nidelet S, Rialle S, Giraud E, *et al.*: **Transcriptome profiles of Nod factor-independent symbiosis in the tropical legume *Aeschynomene evenia***. *Sci Rep* 2018, **8**, 10934.

88. * Bhattacharjee O, Raul B, Ghosh A, Bhardwaj A, Bandyopadhyay K, Sinharoy S: **Nodule INception-independent epidermal events lead to bacterial entry during nodule development in peanut (*Arachis hypogaea*)**. *New Phytol* 2022, **236**:2265–2281.

This study provides evidence for the diversification in the regulatory networks controlling transcellular and intercellular epidermal infection.

89. Prakash K, Diederich B, Heintzmann R, Schermelleh L: **Super-resolution microscopy: a brief history and new avenues**. *Philos Trans R Soc A Math Phys Eng Sci* 2022, **380**, 20210110.

90. Nguyen TD, Chen Y-I, Chen LH, Yeh H-C: **Recent advances in single-molecule tracking and imaging techniques**. *Annu Rev Anal Chem* 2023, **16**:253–284.

91. Aoyama T, Sugimoto N, Sato Y: **Application of fluorescence lifetimes to multi-imaging analysis in plant cells**. *bioRxiv* 2023. 2023.2008.2028.555227.