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REVIEW

Gene Expression in Nitrogen-Fixing Symbiotic Nodule Cells in *Medicago truncatula* and Other Nodulating Plants

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Short title: The nodule transcriptome

One-sentence summary: Gene expression analyses of nodules of the nitrogen-fixing clade of plants have advanced our understanding of nodule organogenesis and functioning and the evolution of symbiosis.

ABSTRACT

Root nodules formed by plants of the nitrogen-fixing clade (NFC) are symbiotic organs that function in the maintenance and metabolic integration of large populations of nitrogen-fixing bacteria. These organs feature unique characteristics and processes, including their tissue organization, the presence of specific infection structures called infection threads, endocytotic uptake of bacteria, symbiotic cells carrying thousands of intracellular bacteria without signs of immune responses, and the integration of symbiont and host metabolism. The early stages of nodulation are governed by a few well-defined functions, which together constitute the common symbiosis-signaling pathway (CSSP). The CSSP activates a set of transcription factors (TFs) that orchestrate nodule organogenesis and infection. The later stages of nodule development require the activation of hundreds to thousands of genes, mostly expressed in symbiotic cells. Many of these genes are only active in symbiotic cells, reflecting the unique nature of nodules as plant structures. Although how the nodule-specific transcriptome is activated and connected to early CSSP-signaling is poorly understood, candidate TFs have been identified using transcriptomic approaches, and the importance of epigenetic and chromatin-based regulation has been demonstrated. We discuss how gene regulation analyses have advanced our understanding of nodule organogenesis, the functioning of symbiotic cells, and the evolution of symbiosis in the NFC.

1 INTRODUCTION

- 2 In response to nitrogen starvation and the presence of specific compatible nitrogen-
- 3 fixing bacteria in the rhizosphere, plants of the nitrogen-fixing clade (NFC) in the

4 Rosid clade form symbiotic organs on their roots known as nodules. Nodules are
5 infected by a large population of these bacteria, which convert atmospheric nitrogen
6 gas to ammonia. The ammonia is taken up by the plant to satisfy its nitrogen needs
7 for growth. The NFC, which originated over 100 million years ago (MYA), is
8 composed of four orders, the Fabales, Cucurbitales, Fagales, and Rosales (Soltis et
9 al., 1995). Nodulation is widespread in the Leguminosae family of the Fabales
10 (although not present in all legume species), while it is scattered among species of
11 the three other orders. Leguminosae and species of the *Parasponia* genus of the
12 Rosales are infected with phylogenetically diverse bacteria, collectively named
13 rhizobia, belonging to the Rhizobiales of the alphaproteobacteria (alpha-rhizobia) or
14 the Burkholderiales of the betaproteobacteria (beta-rhizobia). The other nodulating
15 genera, collectively known as actinorhizal plants, interact with nitrogen-fixing
16 actinobacteria of the genus *Frankia*.

17 Although they are diverse, all rhizobia, as typical Gram-negative bacteria, have
18 (broadly speaking) a similar type of cell envelope structure, which is a crucial attribute
19 for effective nodule colonization (Haag et al., 2013). At least one other shared feature
20 of rhizobia — with one reported exception (Giraud et al., 2007) — is the capacity to
21 produce so-called Nod factors, the bacterial signals that trigger the nodulation
22 program of the host plant via the activation of the common symbiosis signaling
23 pathway (CSSP). Nod factors are lipochitooligosaccharides with a characteristic
24 molecular structure and are similar only to the Myc factors produced by mycorrhizal
25 fungi (Oldroyd, 2013; Cope et al., 2019). The capacity to produce Nod factors is
26 encoded by the nodulation or *nod* genes located on symbiotic islands or on mobile
27 plasmids in the rhizobial genomes (Poole et al., 2018).

28 By contrast, *Frankia* are Gram-positive bacteria with a fundamentally different cell
29 envelope from rhizobia. Although very little is known about the requirements of cell
30 envelopes for symbiosis in *Frankia*, these envelope differences likely have profound
31 effects on the colonization of nodules. The nodule-inducing factors produced by
32 *Frankia* strains are currently unknown (Cissoko et al., 2018), although some
33 particular *Frankia* strains possess genes homologous to the *nod* genes of rhizobia
34 (Persson et al., 2015). It is thus conceivable that *Frankia* strains produce nodule-
35 inducing signals that are structurally homologous to Nod factors.

The nodule can be viewed as a plant organ that relies on the integration or recruitment of existing processes (meristem formation, endoreduplication, polar growth, endocytosis and exocytosis, nitrogen and sugar metabolism) in a new context, in conjunction with the deployment of seemingly completely new features (infection threads and uptake of bacteria, meristem organization, formation of new organellar structures, maintenance of bacteria inside plant cells and immune suppression and metabolic integration of a bacterial endosymbiont). Thus, depending on whether one considers the glass half-empty or half-full, nodules can be viewed as “novel” or “not-so-novel” plant organs. The latter point of view — particularly motivated by the finding that nodule formation is controlled by the CSSP, which is widely conserved in land plants that form symbiotic relationships with arbuscular mycorrhizal fungi — has led to the suggestion that engineering nodulation and symbiotic nitrogen-fixation to currently non-nodulating plants would require a minimal set of genes and is therefore an attainable objective (Markmann and Parniske, 2009; Oldroyd and Dixon, 2014; Stokstad, 2016). However, nodule formation is accompanied by massive transcriptional reprogramming involving the activation and repression of hundreds of genes. Remarkably, this nodule transcriptional program is entirely different from the mycorrhizal transcriptional program controlled by the same CSSP. This highlights the uniqueness of nodules as plant organs and emphasizes the challenges faced when trying to transfer nodulation to non-legume crops. Here we review what we have learned from transcriptome approaches aimed at characterizing the specific features of nodules, focusing mostly but not exclusively on the transcriptional reprogramming taking place during the formation of symbiotic nodule cells in the model legume, *Medicago truncatula*.

NODULE DEVELOPMENT AND MORPHOLOGY: NODULES ARE UNIQUE STRUCTURES

Nodules in different lineages of the NFC can differ from one another in terms of development, infection mechanisms, final structure, and metabolism. However, nodule organogenesis and functioning commonly calls upon several processes, which do not have equivalents in other plant developmental programs. Nodule formation in *M. truncatula* is among the best described (Figure 1). This process is governed by a number of unique signaling cascades that utilize inter-specific

(bacterium to plant and *vice versa*) and intra-specific (plant to plant) signaling molecules including flavonoids (plant to bacterium) and Nod factors (bacterium to plant) (Oldroyd, 2013) (Figure 1A), Type III, IV, and VI secretion system effectors (bacterium to plant) (Nelson and Sadowski, 2015; Teulet et al., 2019), bacterial surface polysaccharides (exopolysaccharides, lipopolysaccharides) (bacterium to plant) (Haag et al., 2013), NCR (nodule-specific cysteine-rich) peptides (plant to bacterium) (Kondorosi et al., 2013), phytohormones (intra-plant) (Gamas et al., 2017; Schiessl et al., 2019), CLE and CEP peptides (intra-plant) (Taleski et al., 2018), and small RNAs (intra-plant and bacterium to plant) (Tsikou et al., 2018; Ren et al., 2019). Each of these signaling systems seems to be variations on a theme of widely used regulatory mechanisms in plants. For example, similar to Myc factors, Nod factors activate the CSSP (Figure 1A), which thus controls not only nodulation but also mycorrhization (Oldroyd, 2013). However, the output of the signaling in response to Nod factors and Myc factors is completely different (see below). It is a long-lasting conundrum how this differential output is achieved. Other key signals of nodulation are used in plant development (e.g. phytohormones) or in biotic interactions (e.g. immunity) but with a unique output in nodulation. Thus, nodulation relies upon a unique rewiring of signaling pathways.

The nodule primordium and the persistent meristem of indeterminate *M. truncatula* nodules have features reminiscent of the formation of lateral roots and the root apical meristem (Franssen et al., 2015; Couzigou et al., 2012; Xiao et al., 2014), including overlapping genetic and hormonal control mechanisms (Schiessl et al., 2019). However, the root apical meristem is organized in a different manner and leads to the formation of one central vascular bundle, while in legumes, the nodule meristem generates several peripheral vascular bundles (Figure 1B). Nevertheless, actinorhizal and *Parasponia* nodules have a central vasculature (Downie, 2014; Behm et al., 2014). The other cell types derived from the nodule meristem are also fundamentally different from root cells. Endoreduplication drives the differentiation of post-meristematic cells into symbiotic nodule cells, which become infected by rhizobium bacteria (Kondorosi et al., 2013). Endoreduplication is common in plants and can be observed in differentiating root cells and other plant tissues in a species-specific manner, as well as in many other biotrophic interactions in plants (Wildermuth et al., 2017; Edgar et al., 2014). In nodules, endoreduplication is tightly coupled to infection

and the passage of so-called infection threads (van Brussel et al., 1992; Yang et al., 1994; Suzaki et al., 2014). Determinate nodules, which form in some legume clades, differ fundamentally from the indeterminate nodules of *M. truncatula*, because they do not develop a meristem. Nevertheless, cells of determinate nodules also undergo endoreduplication after infection with rhizobia (González-Sama et al., 2006).

Consequently, and contrary to meristem-containing indeterminate nodules, these nodules have a determinate growth, which is limited by early cell divisions during the primordium stage and the subsequent cell enlargement of rhizobium-infected cells driven by their endoreduplication. Well-studied examples of determinate nodules are formed by *Lotus japonicus* and soybean (*Glycine max*).

Infection threads are tubular structures containing infecting rhizobia that conduct these bacteria inside the nodule tissues (Figure 1C). Infection thread formation initiates in growing root hairs due to the activation of the CSSP by rhizobial Nod factors. These structures share some similarities with pollen tubes and root hairs, which both display polar growth, as do infection threads. The polar growth of infection threads, pollen tubes, and root hairs indeed relies on common molecular mechanisms (Yang, 2008; Ke et al., 2012; Kiirika et al., 2012; Montiel et al., 2012; Morieri et al., 2013). However, contrary to pollen tubes and root hairs, which are autonomous cells, infection threads are trans-cellular structures that grow from cell to cell, hijacking the cellular machinery of their temporal host cells. Infection thread growth is a process also derived from the eukaryotic cell cycle (van Brussel et al., 1992; Yang et al., 1994; Timmers et al., 1999). Before penetration by an infection thread, the targeted vacuolated cortical root cell undergoes cytological reorganization, forming a so-called pre-infection thread or PIT (Figure 1C) (van Brussel et al., 1992; Timmers et al., 1999). The PIT is structurally related to the phragmosome, a cytoplasmic bridge through the vacuole formed by cells in preparation for mitosis. An analysis of the expression of cell cycle marker genes revealed that PIT-forming cells indeed enter into the cell cycle and duplicate their genomes but are arrested in the G2 phase, before the initiation of mitosis (Yang et al., 1994). More recently, it was shown that root hairs also prepare for infection thread formation by activating cell cycle genes (Breakspear et al., 2014). PITs are reminiscent of the pre-penetration apparatus in root cells prior to infection with the hyphae of arbuscular mycorrhizal fungi (Genre et al., 2008).

Infection threads grow towards young, differentiating symbiotic cells where they release rhizobia through an endocytotic process (Figure 1D). Endocytosis, a well-known process in plants (Paez Valencia et al., 2016), is essential for controlling the turnover of plasma membrane proteins such as receptors and transporters. However, the uptake of whole bacteria by plant cells as during rhizobium (or *Frankia*) infection of nodule cells is unknown, except for the case of the nitrogen-fixing symbiosis of the plant genus *Gunnera* with cyanobacteria. Nevertheless, the infection of nodule cells shares some similarities with the infection of plant cells by fungal pathogens and mycorrhizal symbionts (Harrison and Ivanov, 2017; Parniske, 2018). Endocytotic uptake does not release rhizobia freely in the cytosol of nodule cells, instead releasing them inside organelle-like vesicles known as symbiosomes, which have a plasmalemma-like membrane (Figure 1D). Within symbiosomes, the rhizobia grow and differentiate into nitrogen-fixing structures called bacteroids. The latter process is enabled by the exocytosis pathway, which traffics specific proteins to the symbiosomes (Figure 1D). The exocytosis pathway is strongly conserved in eukaryotes, but some components are nodule-specific and are encoded by duplicated genes or by nodule-specific splice variants (Stonoha-Arther and Wang, 2018).

Among the unique features of nodules, the nitrogen-fixation process by symbiotic nodule cells is certainly the most prominent. This process requires the maintenance of thousands of bacterial endosymbiont-containing symbiosomes within individual plant cells (Figure 1E). During this process, symbiosomes acquire a specific vesicular identity and avoid a vacuolar identity, which would lead to the elimination of the symbiont (Limpens et al., 2009; Sinharoy et al., 2013). In addition, symbiotic cells contain specific machinery that suppresses innate immunity responses against their many endosymbionts (Figure 1D) (Gourion et al., 2015; Wang et al., 2016; Domonkos et al., 2017; Yu et al., 2018). In certain legumes, such as *Medicago* spp. in the IRLC clade and *Aeschynomene* spp. in the Dalbergoid branch, symbiosome formation also involves the production of symbiosis-specific peptides called NCRs, which target the bacteroids and control their extreme, terminal differentiation (Mergaert, 2018). Finally, the functioning of symbiotic cells requires bacterial metabolism to be integrated with the metabolism of the host cells (Figure 1F). Bacteroids generate ammonia in large excess to their own needs and release this

ammonia to the plant. Major features of the metabolism of symbiotic cells include i) the supply of carbon sources derived from photosynthesis in the shoots to the energy-devouring bacteroids, ii) the maintenance of a low oxygen concentration to avoid the irreversible oxidation and inactivation of the nitrogenase enzyme, and iii) the assimilation of ammonium provided by nitrogen-fixing bacteroids. This specific metabolism requires the activation of a suite of enzymes and transporters and the massive production of leghemoglobin to bind oxygen (Figure 1D,F) (Udvardi and Poole 2013; Poole et al., 2018; Roy et al., 2019).

NODULE TRANSCRIPTOME DATABASES

Transcriptional reprogramming associated with nodule formation has been studied in many legume species, including *M. truncatula* (see below), *Medicago sativa* (O'Rourke et al., 2015), pea (*Pisum sativum*) (Alves-Carvalho et al., 2015), chickpea (*Cicer arietinum*) (Kant et al., 2016; Kudapa et al., 2018), *Trifolium pratense* (Trujillo et al., 2019), *L. japonicus* (Verdier et al., 2013; Høglund et al., 2009; Takanashi et al., 2012; Colebatch et al., 2004; Kelly et al., 2018), *G. max* (Brechenmacher et al., 2008; Severin et al., 2010), common bean (*Phaseolus vulgaris*) (O'Rourke et al., 2014), *Sesbania rostrata* (Capoen et al., 2007), *Lupinus* spp. (Keller et al., 2017), *Aeschynomene evenia* (Gully et al., 2018), and peanut (*Arachis hypogaea*), *Arachis duranensis*, *Arachis ipaensis* (Clevenger et al., 2016; Peng et al., 2017; Karmakar et al., 2019), in the non-legume *Parasponia andersonii* (van Velzen et al., 2018), as well as in the actinorhizal plant species *Casuarina glauca* (Hoher et al., 2011), *Alnus glutinosa* (Hoher et al., 2011), *Datisca glomerata* (Demina et al., 2013; Battenberg et al., 2018; Salgado et al., 2018) and *Ceanothus thyrsiflorus* (Battenberg et al., 2018; Salgado et al., 2018).

Transcriptomic approaches to study nodule formation have been extensively applied in *M. truncatula*, in large part due to the development of whole genome Affymetrix *Medicago* GeneChips. These GeneChips have been used in 274 different experimental conditions, which can be found in the *Medicago truncatula* Gene Expression Atlas (MtGEA) repository (<https://mtgea.noble.org/v3/index.php>; Benedito et al., 2008). MtGEA compiles a broad range of developmental and environmental conditions, including different plant organs and tissue types, biotic-, abiotic stress

conditions, and hormone treatments and is therefore currently the richest *M. truncatula* resource for the analysis of expression patterns and specificity of genes of interest and the discovery of genes expressed under certain conditions or in organs of interest. Ordering the MtGEA dataset with a clustering algorithm results in the identification of tissue-specific gene sets (Figure 2A). The heat map discloses large clusters of genes that are expressed in different plant organs, leaves, stems, flowers, seeds, hypocotyls, roots, mycorrhiza, and nodules. This analysis also revealed that nodules and mycorrhiza are primarily typified by the expression of different sets of genes (Figure 2B), despite the shared CSSP module of regulators that controls both nodule and mycorrhiza formation and a number of common target genes that are activated immediately downstream of the CSSP during both types of interactions (Hogekamp et al., 2011). For research on rhizobium-legume symbiosis, the MtGEA dataset can be complemented by expression data derived from laser-capture microdissection (LCM)-transcriptome analysis (see below) available in the SYMBIMICS database (<https://iant.toulouse.inra.fr/symbimics/>; Roux et al., 2014). Together, the MtGEA and SYMBIMICS databases are priceless resources for studying *M. truncatula* biology, particularly nodulation.

Datasets similar to MtGEA are available for two alfalfa subspecies, *M. sativa* ssp. *sativa* (B47) and *M. sativa* ssp. *falcata* (F56) (Alfalfa Gene Index and Expression Atlas Database or AGED; <http://plantgrn.noble.org/AGED/>; O'Rourke et al., 2015), *L. japonicus* (LjGEA; <https://ljgea.noble.org/v2/>; <https://lotus.au.dk/expat/>; Verdier et al., 2013), *P. vulgaris* (Common Bean Gene Expression Atlas or PvGEA; <http://plantgrn.noble.org/PvGEA/>; O'Rourke et al., 2014), soybean (SoyBase; <https://soybase.org/soyseq/>; Severin et al., 2010) and *Arachis* spp. (PeanutBase; <https://www.peanutbase.org/>; Clevenger et al., 2016). Except for LjGEA, which reports approximately 80 experimental conditions, including organs and physiological conditions, the other databases are less well furnished and focus on the principal plant organs. LjGEA was generated with Affymetrix gene chips while the AGED, PvGEA, SoyBase, and PeanutBase datasets were generated by RNA-seq.

NOVEL INSIGHTS FROM NODULE TRANSCRIPTOME ANALYSES

Because nodules form *de novo* on roots starting from fully differentiated cortical, endodermal, pericycle, and epidermal cells, the nodule transcriptome is usually defined by the genes showing differential expression in nodules versus roots. A recurring theme in each transcriptome study on legumes, *Parasponia*, and actinorhizal plants is that nodule formation is accompanied by the differential expression of hundreds to thousands of genes. These sets of nodule-enhanced genes are composed in part of genes that are more strongly expressed in nodules than in other organs but importantly, these gene sets also contain large numbers of absolutely nodule-specific genes (see below). Conversely, a transcriptome analysis in *M. truncatula* revealed that the larger part of the gene expression changes during lateral root initiation also occur during nodule organogenesis (Schiessl et al., 2019). These common differentially expressed genes are related to auxin biosynthesis and signaling and transcriptional regulation. This observation suggests that lateral root organogenesis has been recruited into the symbiotic program to which a large panel of nodule-specific features was added. The nodule-specific gene sets are composed of genes with homologs in other plants or paralogs expressed in other plant tissues as well as orphan genes. These findings further underscore the unique nature of nodules and the very complex genetic program governing the formation of this symbiotic organ. Thus, from this perspective, the glass might be half-empty.

The Specificity of the Nodule Transcriptome

The analysis presented in Figure 2 suggests that some organs, particularly nodules, are associated with the expression of genes that are extraordinarily specifically expressed. This is obvious when zooming in on the expression of NCR genes (Figure 3A) (Guefrachi et al., 2014). This extremely large gene family encodes nodule-specific cysteine-rich peptides, which target endosymbionts and induce them into a terminally differentiated state, are required for maintaining bacteroids, or are involved in the selection of well-adapted rhizobia in nodules (Mergaert, 2018). All but five of the over 300 analyzed NCR genes are exclusively expressed in nodules and in no other plant organ or in response to any other biotic interaction or abiotic stress. To express the apparent tissue specificity of genes expressed in nodules and of the NCRs in a quantitative manner, a metric called Shannon entropy was used (Guefrachi et al., 2014). Shannon entropy is a parameter derived from information theory that is applied to gene expression analysis to characterize the uniformity (high

Shannon entropy) or specificity (low Shannon entropy) of the expression pattern of a gene of interest over a number of tested conditions. Taking into account ten different tissues or organs of *M. truncatula*, the Shannon entropy was calculated for all genes in MtGEA. Ordering the MtGEA genes according to expression profile (Figure 3B) or ranking them according to their Shannon entropy value (Figure 3C) makes it clear that flowers, seeds, pods, and mycorrhiza and most particularly nodules are characterized by large gene sets with very low Shannon entropy that are thus (almost) uniquely expressed in these organs. Thus, nodulation in *M. truncatula* strongly depends on dedicated gene sets that do not function elsewhere in the plant (Guefrachi et al., 2014). As discussed below, the large majority of these genes are expressed in differentiating or mature symbiotic cells (Figure 3C).

Shannon entropy analysis of the AGED, PvGEA, LjGEA, and SoyBase datasets (Figure 3D) confirmed the notion that nodule formation in *M. sativa*, *P. vulgaris*, *L. japonicus*, and soybean is also associated with the expression of large numbers of low Shannon entropy genes that are (nearly) uniquely expressed in that organ. Soybean/*P. vulgaris*, *L. japonicus* and *M. truncatula*/*M. sativa* belong to different clades of legumes: the Milletiods, Robiniods, and IRLC (inverted repeat-lacking clade), respectively. Thus, the reliance on large gene sets that are uniquely expressed in the nodule is not restricted to *Medicago* spp. and is not a specific feature of the particular nodule types formed by these species, which are of the indeterminate type and induce terminal bacteroid differentiation. Both of these features are not present in bean, soybean, or *L. japonicus* nodules. However, the nodule-specific gene sets in *Medicago* spp. appear to be significantly larger than those in the other legumes (Figure 3). This difference is largely due to the extremely high number of *Medicago* genes encoding NCR peptides (Mergaert et al., 2003). In *M. truncatula*, the NCR gene family is composed of approximately 600 genes and in tetraploid *M. sativa*, the number of NCR genes increases to 1300 (O'Rourke et al., 2015), whereas these genes are absent in the three other legumes.

Besides the NCR gene family, there are many other nodule-specific, low entropy genes. Among these are well-studied genes, some with essential functions in symbiosis (e.g. genes encoding leghemoglobin, SNARPs [small nodulin acidic RNA-binding protein, also known as LEED..PEEDs], the phospholipase C-like DNF2 (defective in nitrogen fixation), the cysteine-rich receptor-like kinase SymCRK,

metabolite transporters, and so on) and many genes with only predicted or uncharacterized functions (e.g. putative transporter encoding genes, GRPs (glycine-rich proteins), early nodulins ENOD8 and ENOD16, calmodulin-like proteins, and so on) or genes of unknown function. Importantly, the nodule-specific transcriptome contains several families of orphan genes or taxonomically restricted genes (genes without homologs in other lineages), including the NCRs, SNARPs, and GRPs, which are restricted to the IRLC legumes (Mergaert et al., 2003; Kevei et al., 2002; Laporte et al., 2010; Trujillo et al., 2014), the NCR genes of *Aeschynomene* spp. (Czernic et al., 2015), the NAD1 (nodules with activated defense 1) genes conserved only in plants from the NFC (Wang et al., 2016), and the nodulin 20 family, first identified in soybean (Richter et al., 1991), which is specific for the Phaseoleae (Trujillo et al., 2019). Such genes could have arisen from the duplication and rearrangement of ancestral genes followed by rapid diversification. Alternatively, orphan genes could have evolved *de novo* from non-coding genomic regions believed to be used as raw materials for the evolution of new genes (Tautz and Domazet-lošo 2011).

A number of low-entropy nodule-specific genes encode proteins with unique combinations of existing domains, likely resulting from gene recombination. For example, the symbiosis-specific thioredoxin Trx s, Calmodulin-like protein, and ENOD8 families evolved from fusions of known protein domains with an N-terminal signal peptide, resulting in unique secretory proteins targeted to symbiosomes and bacteroids. There are a remarkably high number of low-entropy genes with signal peptides, suggesting that symbiotic cells target many proteins and peptides to the symbiosomes (Figure 1D) (e.g. NCRs, GRPs, SNARPs, calmodulin-like proteins, ENOD8, ENOD16, ENOD20, DNF2, SymCRK, Trx s, nodulin 25, MtN22, MtN11, lipase/lipoxygenases (also known as nodule-specific PLAT domain proteins or NPDs), basic blue-like protein, NCR-like peptides, MBP-1, and CAP antimicrobial peptides in the Dalbergioid lineage, nodulin 20 family proteins of the Phaseoleae, APN1 of *L. japonicus*, and so on) (Maunoury et al., 2010; Alunni et al., 2007; Liu et al., 2006; Dickstein et al., 2002; Laporte et al., 2010; Trujillo et al., 2014; Pecrix et al., 2018; Trujillo et al., 2019; Pislariu et al., 2019; Yamaya-Ito et al., 2017). The high level of protein secretion to symbiosomes and bacteroids in symbiotic cells of *M. truncatula*, particularly in developing and young mature cells, is further supported by the strongly developed endoplasmic reticulum in these cells (Maunoury et al., 2010)

and by the requirement of symbiotic cell-specific regulators of the secretory pathway for normal bacteroid differentiation and nodule development (reviewed in Stonoh-Arther and Wang, 2018).

Other genes resulted from duplications and modifications with neofunctionalization, such as leghemoglobin genes. Oxygen-binding leghemoglobins are essential for protecting the oxygen-sensitive enzyme nitrogenase. Their recruitment for symbiosis required mutations (replacement of a few amino acids) of non-oxygen-transporting hemoglobins present in all plants to create an oxygen-transporting hemoglobin, as well as nodule-specific expression of the corresponding genes. Other genes also resulted from duplication, whereby one paralog obtained a new, nodule-specific expression profile but without a new function. Genes encoding subunits of a nodule-specific signal peptidase complex, including *Dnf1*, fall in this category (Wang et al., 2010). This endoplasmic reticulum-localized enzyme complex of the secretory pathway is required for removing the signal peptide during trafficking of secretory proteins. The NPDs in the IRLCs and the MBP-1 and CAP peptides in the Dalbergioids are encoded by multigene families including nodule-specific subsets as well as subsets with constitutive expression (Trujillo et al., 2019). Other examples are the many small metabolite transporter genes that are essential for the formation of functional nodules (Figure 1F) (e.g. Sugiyama et al., 2017; Frare et al., 2018; Senovilla et al., 2018; Chen et al., 2019). These genes belong to small multigene families, with one copy having nodule-specific or nodule-enhanced expression. The encoded transporters assure the transport of metabolites to and from the symbiotic cells and bacteroids and are essential for the metabolic integration of bacteroids with symbiotic nodule cells. A similar situation holds true for the many genes encoding the enzymes involved in the specific carbon and nitrogen metabolism of symbiotic cells (Figure 1F). Carbon metabolism provides the carbon source to bacteroids in the form of dicarboxylic acids and synthesizes the carbon backbone for assimilation of the ammonium derived from nitrogen fixation in bacteroids. Nitrogen metabolism assures that this nitrogen is assimilated into amino acids or in some legumes into ureides. A large body of evidence indicates that the key enzymes of carbon and nitrogen metabolism in symbiotic cells are encoded by nodule-specific genes. Like small-metabolite transporters, metabolic genes are also members of small gene families,

with one copy having a nodule-specific or -enhanced expression (e.g. Miller et al., 1998; Hakoyama et al., 2009; Nouwen et al., 2017).

The large number of rhizobia in symbiotic cells implies the massive presence of PAMPs (pathogen-associated molecular patterns) and the potential to induce strong PAMP triggered immunity (PTI). Several symbiotic cell-specific proteins are required to suppress the PTI response and to enable the long-term maintenance of rhizobia (Figure 1D). In the absence of the receptor-like kinase SymCRK, the phospholipase C-like DNF2, the PLAT Domain protein NPD1, the transcription factor RSD, or the endoplasmic reticulum located small protein NAD1, a strong immune response is induced, as evidenced by the induction of immunity marker genes, nodule necrosis, and a complete halting of symbiosis (Bourcy et al., 2013; Sinharoy et al., 2013; Berrabah et al., 2014; Wang et al., 2016; Domonkos et al., 2017; Pislariu et al., 2019). The genes encoding these immune repressors are low entropy genes that are only expressed in symbiotic nodule cells. *SymCRK*, *DNF2*, *NPD1*, and *RSD* are nodule-specific paralogs, whereas *NAD1* is unique and appears to be specific to the NFC (Wang et al., 2016), suggesting that it was present in the ancestor of the NFC.

Examples of Functional Insights from Nodule Transcriptomics

As mentioned above, nodule formation has been studied by transcriptomics in many legumes and non-legume species, and several studies have focused on specific aspects of the symbiotic process in these NFC plants (e.g. Høglund et al., 2009; Libault et al., 2009a; Libault et al., 2010; Yuan et al., 2017; van Velzen et al., 2018). Undoubtly, transcriptomics have been used most extensively in *M. truncatula* to study nodule development, from very early responses induced by Nod factors and gene activation in root hairs (Combier et al., 2008; Breakspear et al., 2014; Czaja et al., 2012; Jardinaud et al., 2016; Van Zeijl et al., 2015; Larrainzar et al., 2015) to the development of mature nodules (El Yahyaoui et al., 2004; Maunoury et al., 2010; Moreau et al., 2011). Analyses focusing on the early responses to Nod factors and rhizobia have used clever tricks to overcome the limited sensitivity in detecting transcriptional responses in the small number of Nod factor- or rhizobium-responsive cells in roots. These solutions included preparing RNA from the roots of supernodulating plant mutants, which have a strongly enhanced nodulation response (Combier et al., 2008; Larrainzar et al., 2015). Alternatively, analysis has been performed using as a starting material purified root hairs, the first target cells of Nod

factors and rhizobia, obtained by breaking them off from roots frozen in liquid nitrogen (Breakspear et al., 2014; Damiani et al., 2016; Liu et al., 2019a) or root epidermal cells obtained by LCM (Jardinaud et al., 2016). Analysis of the early symbiotic signaling responses highlighted the importance of cytokinin, auxin, ethylene, strigolactone, abscisic acid, gibberellic acid, and brassinosteroid phytohormone signaling pathways. This observation suggests that an important rewiring of the interconnected phytohormone pathways is a key step in the initiation of nodule organogenesis (Breakspear et al., 2014; Czaja et al., 2012; Jardinaud et al., 2016; Van Zeijl et al., 2015; Larrainzar et al., 2015; Liu et al., 2019a; reviewed in Gamas et al., 2017; Liu et al., 2018; Buhian and Bensmihen, 2018). These studies confirmed the activation of cell cycle-related processes early in the nodulation signaling pathway (Breakspear et al., 2014; Jardinaud et al., 2016; Larrainzar et al., 2015), which is in agreement with the known role of the cell cycle in the establishment of a nodule primordium (Foucher and Kondorosi, 2000), cell differentiation (Vinardell et al., 2003; Suzaki et al., 2014), and infection (Van Brussel et al., 1992; Yang et al., 1994; Suzaki et al., 2014). Another striking finding is the identification of the gene encoding the rapid alkanization factor MtRALF1 peptide as a Nod factor-induced gene controlling infection thread development (Combier et al., 2008). Peptides of this family control cell expansion in plants, notably during root hair and pollen tube growth (Murphy and De Smet, 2014; Haruta et al., 2014; Mecchia et al., 2017; Ge et al., 2017), the two plant cell types that show the highest similarity to the infection thread structure. Notably, these RALF peptides interact with receptor-like kinases containing a so-called malectin-like domain within their ectodomain (Franck et al., 2018). Such a domain is also present and plays a key role in the ectodomain of the receptor-like kinase SYMRK/DMI2/NORK (Antolin-Llovera et al., 2014; Pan and Wang, 2018), a component of the CSSP, suggesting that MtRALF1 targets the Nod factor receptor complex, a hypothesis that might be worth pursuing.

Using time series of wild-type nodule growth combined with collections of both rhizobial and plant mutants arrested at different stages of nodule development, transcriptome dynamics during nodule organogenesis was dissected into distinct transcriptional waves composed of the induction and repression of genes that are consecutively initiated during organogenesis (Starker et al., 2006; Maunoury et al., 2010; Moreau et al., 2011; Lang and Long, 2015). Four major waves of gene

activation were uncovered using these approaches. The first wave is associated with early signaling and bacterial infections, the second with symbiotic cell differentiation, the third with the differentiation of bacteroids, and the fourth with the activation of nitrogen fixation.

In addition to studies that focused on the overall transcriptome dynamics associated with nodule development, other studies have used transcriptomics to address more specific questions. The transcriptomes in non-functional nodules formed by *M. truncatula* mutants in transcriptional regulators were used to characterize the stages of nodule development affected by the mutations at the molecular level and to delineate the sets of potential target genes of these transcriptional regulators (see below; Vernié et al., 2008; Satgé et al., 2016; Sinharoy et al., 2013; Deng et al., 2019). Another type of study used transcriptomics to investigate particular physiological conditions in nodules. During nodule senescence, nodule cells break down and their macromolecular components are degraded and remobilized to other plant organs (Van de Velde et al., 2006). The process can be developmentally induced by the aging of nodules or when the host plants need to shift their nutrient resources for seed production. Nodule senescence can also be induced by particular physiological conditions, including reduced photosynthesis, nutrient starvation, or a shift in the nitrogen status of the plant. Nodule senescence under these different conditions has been studied intensively by transcriptomics (Van de Velde et al., 2006; Seabra et al., 2012; Cabeza et al., 2013; Cabeza et al., 2014; Liese et al., 2017; Deng et al., 2019), which has led to the identification of marker genes and actors in the process. Cysteine proteinases appear to be key players in nodule senescence: manipulating their expression causes delayed or premature senescence, thus affecting nitrogen fixation (Pierre et al., 2014; Deng et al., 2019).

Other studies have used transcriptomics and dedicated Affymetrix gene chips to focus on the expression of NCR and other defensin-like peptide genes in *M. truncatula* (Nallu et al., 2013; Tesfaye et al., 2013). The results confirmed the notion that NCRs are nodule-specific peptides and revealed a distinct subset of defensin-like genes unrelated to the NCRs that are specifically expressed in seeds. Like *M. truncatula*, many defensin-like peptides are also expressed in both the inflorescences and seeds of *Arabidopsis*. The peptides in these organs may play roles in defense. Indeed, the abundant expression of defensin-like peptides may reflect a specific,

strongly protected immune status of reproductive organs. In an RNA-seq study focusing on small, secreted peptides that act via receptor-mediated signaling, 365 peptides belonging to 29 different putative functional peptide families were identified whose expression changed during nodule formation, suggesting that peptide signaling plays a prominent role in the coordination of nodulation (de Bang et al., 2017). This data set produced in this study represents a rich resource for future work aiming to decipher the functions of these peptides.

The genotypes of both the *Rhizobium* and legume influence the outcome of the interaction and the efficiency of the nitrogen-fixation process (Sugawara et al., 2013; Kazmierczak et al., 2017). Transcriptomics were used to gain insights in the molecular basis of these genotype-by-genotype variations in *M. truncatula*-*Sinorhizobium* symbiosis (Heath et al., 2012; Nallu et al., 2014; Burghardt et al., 2017). These studies revealed a high degree of genotype-by-genotype variability in the transcriptomes that was more dependent on the host genotype than the bacterial symbiont genotype. In particular, NCR expression is highly variable, suggesting that the NCRs and the differentiation of the bacteroids induced by NCRs are important determinants of the efficiency of symbiosis (Kazmierczak et al., 2017). In agreement with this finding, the orthologous NCR genes within a collection of *M. truncatula* accessions showed a high degree of nucleotide variation with signatures of diversifying selection pressure, suggesting that NCR genes are rapidly and recently evolving, possibly to help plants adapt to diversifying rhizobium symbionts (Branca et al., 2011; Nallu et al., 2014; Zhou et al., 2017).

Many transcriptome studies have been performed using whole nodules and did not provide information on the cell type-specific expression of nodule-enhanced or nodule-specific genes. Indeterminate *M. truncatula* nodules are complex organs composed of many cell types. Differentially expressed genes could be specifically expressed in one or several of these cell types. However, some studies have provided spatial resolution at the level of cell type or nodule tissue (Maunoury et al., 2010, Roux et al., 2014, Limpens et al., 2013). These studies demonstrated that the large majority of nodule-enhanced or nodule-specific genes are expressed in differentiating or mature symbiotic cells. By analyzing different stages of nodule development in conjunction with a large panel of symbiotic mutants arrested in nodule development, two major transcriptome switches (regulating a large proportion

of nodule-enhanced or -specific genes) were revealed, one that correlated with symbiotic cell maturation and another one with bacteroid differentiation (Maunoury et al., 2010). An impressive improvement in transcriptome analysis was achieved by combining LCM of nodule tissues with Affymetrix gene chips (Limpens et al., 2013) or RNA-seq analysis (Roux et al., 2014; Jardinaud et al., 2016), providing spatial resolution to global gene expression analysis in nodules (Figure 4). The dissection of nodules by LCM-RNA-seq confirmed the conclusion that a large majority (over 90%) of the 1200 nodule-enhanced or -specific genes are expressed in differentiating or mature symbiotic cells (Roux et al., 2014) (Figure 3C). This observation reinforces the notion that symbiotic nodule cells are unique cell types that have complex needs for their formation and maintenance and for the metabolic integration of endosymbionts.

New technologies could in the (near) future further improve the spatial resolution to the level of nodule tissue subdomains or even single cells, which would resolve non-uniform responses that are averaged over the population of cells in multi-cell samples or unmask unique biological properties of single cells that are too diluted in populations to be discovered in multi-cell samples. These new methods include single cell-type or single cell transcriptome analysis. Single cell types or single cells or the corresponding nuclei could be obtained through LCM, through isolating nuclei tagged in specific cell types (INTACT), or through flow cytometry and sorting of nuclei based on genome size (Figure 4; Libault, 2018). The latter technique could also be combined with fluorescent labeling of nuclei in specific cells in transgenic plant lines with cell type-specific promoters driving the expression of a fluorescent marker. Other new technologies, including spatial transcriptomics and Slide-seq, that could be applied to nodule gene expression analysis, are based on the transfer of RNA from tissue sections onto a surface coated with spatially structured molecular barcodes that are sequenced together with the transferred RNA, generating spatially resolved RNA-seq transcriptome data (Figure 4) (Ståhl et al., 2016; Rodriques et al., 2019).

Genome Organization of Nodule-Specific Genes

Nodule-specific genes often form small, intermediate, or even very large gene families, as observed for the NCR genes in many IRLC legumes (Montiel et al., 2017). After their birth, the NCR genes in these plants must have spread massively through the genome by repetitive gene duplications. NCR genes appear in small

clusters, which are spread across the genome in *M. truncatula* (Graham et al., 2004; Alunni et al., 2007; Zhou et al., 2017), indicating that long-distance duplications followed by local duplications must have played a role in the spreading of NCR genes. A well-documented mechanism for generating gene duplications is based on transposon-mediated non-homologous recombination and retrotranscription (Galindo-González et al., 2017). Many NCR genes in *M. truncatula* are in the vicinity of transposable elements, indicating transposons were (and possibly still are) involved in the explosion of the NCR gene family in the *M. truncatula* genome (Graham et al., 2004; Satgé et al., 2016).

Besides NCR genes, other nodule-specific genes in *M. truncatula*, particularly those expressed in differentiating and mature symbiotic cells, also display local duplications, including genes encoding GRPs, SNARPs, NPDs, calmodulin-like proteins, ENOD8, leghemoglobins, the ammonium channel NOD26, and others (Alunni et al., 2007; Liu et al., 2006; Dickstein et al., 2002; Trujillo et al., 2014; Frare et al., 2018; Pecrix et al., 2018; Trujillo et al., 2019). These clusters are distributed all over the *M. truncatula* genome and strikingly, in addition to locally duplicated genes, they often contain different types of nodule-specific genes (Liu et al., 2006; Pecrix et al., 2018). These genome clusters of nodule-specific genes are called symbiosis-related islands (SRIs). Almost 300 SRIs were identified in the *M. truncatula* genome, which on average contain more than seven nodule-enhanced genes and more than five distinct gene families. Besides the above-mentioned genes, several other well-known, important symbiotic genes are located within SRIs (for example, *RPG* [*RHIZOBIUM-DIRECTED POLAR GROWTH*], *ENOD40*, *ENOD11*, *ENOD12*, *IRE* [*INCOMPLETE ROOT HAIR ELONGATION*], *SymCRK*, *RSD*, and *SYMREM1* [*SYMBIOTIC REMORIN1*]). This clustered gene organization is thought to contribute to the nodule-specific regulation of SRI-localized genes (Pecrix et al., 2018).

The Evolution of Nodulation

To date, no systematic meta-analysis has been performed that compares the details of the transcriptomes of all analyzed NFC plants to identify (for example) the common nodule-enhanced genes. The upstream regulatory hierarchy that orchestrates the genetic program underlying nodulation in the host, the CSSP, is functionally conserved between legumes, *Parasponia*, and actinorhizal plants (Gherbi et al., 2008; Markmann et al., 2008; Svistoonoff et al., 2013; Clavijo et al. 2015; Granqvist

et al., 2015; van Zeijl et al., 2018; Battenberg et al 2018; van Velzen et al., 2018). However, the extent of conservation of the other actors in nodulation downstream of these master regulators is currently unknown. Nevertheless, analyses have recently started to address this question by defining orthologous groups between NFC plant species and identifying common nodule-enhanced genes. One study compared root and nodule transcriptomes in the actinorhizal plants *C. thyrsiflorus* and *D. glomerata* and the legume *M. truncatula* and identified a set of only 51 genes commonly induced in the nodules of these three NFC plants (Battenberg et al 2018). A comparison focusing on the low-entropy nodule-specific gene sets in soybean, *L. japonicus*, *P. vulgaris* and *M. truncatula* found only 12 common orthologous genes between the four species and 34 to 70 common genes in pairwise comparisons (Wu et al., 2019). These results suggest that these nodule-specific gene sets appeared late in evolution and independently in the legume clades. However, another study comparing the *P. andersonii*-nodule transcriptome obtained by RNA-seq with comparable *M. truncatula* data found a more substantial overlap (van Velzen et al., 2018). Among the 1719 and 2753 nodule-enhanced genes in *P. andersonii* and *M. truncatula*, respectively (Roux et al., 2014) (genes that are upregulated at least twofold in young mature nodules vs. uninfected roots), 290 putative orthologs were found. This common set of genes activated in *P. andersonii* and *M. truncatula* nodules includes genes encoding several known symbiosis regulators, signaling proteins, and TFs, as well as metabolic proteins such as enzymes and metabolite transporters. The common genes were further classified into two functional categories based on their expression profiles in nitrogen-fixing *P. andersonii* nodules and in uninfected non-functional nodules. Of the 290 common genes, 126 were upregulated in both functional and non-functional nodules, suggesting they play roles related to nodule organogenesis. The 164 remaining genes were only induced in infected and functional nodules and are therefore associated with infection or nitrogen fixation (van Velzen et al., 2018).

At first glance, the list of 290 common genes between these two nodule transcriptomes appears to be relatively small in light of the 1719 and 2753 nodule-enhanced genes in these species, suggesting that the functions of these nodules primarily involve different gene sets. However, the two plants diverged approximately 100 MYA, which may have brought about this functional divergence. Moreover, this

comparison has to be placed in the context of the evolution of nodulation and symbiotic nitrogen fixation. Two opposing evolutionary models for nodulation have been proposed (Figure 5A,B). Until recently, the prevailing hypothesis (based on phylogenetic ancestral state reconstruction and the strong differences in nodules of different lineages) considered the evolution of nodulation to be a two-step process. The initial step was the acquisition of an uncharacterized predisposition for nodulation by the ancestor of the NFC over 100 MYA, which was followed (at least 30 MY later) by the independent acquisition or convergent evolution of nodulation in six to ten different lineages of the NFC (Figure 5A) (Soltis et al., 1995; Werner et al., 2014; Doyle, 2016). This hypothesis implies multiple independent recruitments for nodulation of the CSSP, that pre-existed and functioned in mycorrhization in these lineages, an evolutionary scenario known as deep homology that has repeatedly been identified for animal structures (e.g. eyes and limbs) (Shubin et al., 2009). Under this scenario, nodulation evolved independently in *Medicago* and *Parasponia*, implying that the 290 common genes in *P. andersonii* and *M. truncatula* nodules arose through complex convergent evolution.

Therefore, van Velzen et al. proposed the challenging hypothesis that nodulation arose already in the common ancestor of the NFC (van Velzen et al., 2018; van Velzen et al., 2019). Besides the substantial overlap in the nodule transcriptomes of *P. andersonii* and *M. truncatula*, including 126 putative organogenesis genes, this new hypothesis is also based on the independent and recent losses or pseudogenizations of the essential symbiosis genes *NFP*, *NIN*, and *RPG* in non-nodulating plants of multiple lineages of the NFC (van Velzen et al., 2018; Griesmann et al., 2018). Together, these observations can be interpreted as a single gain of nodulation at the origin of the NFC and subsequent multiple losses in the current non-nodulating lineages (Figure 5B). Since the divergence of the four orders of the NFC occurred very rapidly (~104 MYA) after the divergence of the NFC itself from its sister clades (~110 MYA), a period of only a few MYs (Wang et al., 2009; Li et al., 2015), this hypothesis implies that nodule organogenesis evolved in a very short time, within these few MYs. Alternatively, perhaps nodulation and nitrogen-fixing symbiosis are much more ancient than previously thought and have been lost altogether in the NFC sister clades.

A possible evolutionary scenario leading to nodule formation is that the existing interaction with mycorrhiza in the ancestor for nodulation was extended to a bacterial nitrogen-fixing symbiont, resulting in the infection of root cortical cells with bacteria rather than fungi. This interaction used the already present CSSP and recruited additional genes. One of the new key functions could have been the suppression of immunity against bacteria in infected root cells. NAD1, a conserved protein specific to the NFC, might represent an early recruited immunity suppressor (Wang et al., 2016). Perhaps the ammonium channel NOD26, which is involved in the uptake of ammonium produced by bacteroids from the symbiosomes into the symbiotic cells, represents another early innovation (Frare et al., 2018). This transporter, along with ammonium assimilation enzymes, is required for the metabolic integration of the nitrogen fixing symbiont. At the regulatory level, the TF NIN, which is required for nodule organogenesis and infection in present-day plants, might have been recruited early on by the CSSP from a pre-existing TF family of NIN-like proteins (NLPs) implicated in the regulation of nitrogen homeostasis in plants (Soyano et al., 2014; Clavijo et al., 2015). Another innovation must have been the activation of the cell cycle in differentiated root cells to increase the number of infected cells by cell proliferation and to direct organogenesis. This step could have been accomplished through the recruitment of cytokinin signaling by NIN, a crucial step in the initiation of root cortical cell division in legumes (Schauser et al., 1999; Vernié et al., 2015; Gamas et al., 2017), although the role of cytokinin signaling in nodulation has not been confirmed in the other clades of the NFC (Van Zeijl et al., 2018; Gauthier-Coles et al., 2019). Subsequently, the evolution of nodulation may have occurred via a gradual process that created an ever increasing mass of infected symbiotic cells, providing more and more nitrogen to the host until the full nitrogen needs of the plant were met (Figure 5B).

The new model of van Velzen et al. (2019) further proposes that symbiosis with *Frankia* occurred at the NFC origin and subsequent independent switches to rhizobia occurred in the ancestor of legumes and *Parasponia*. This proposition is based on the finding that legumes and *Parasponia* use paralogous and not homologous hemoglobin genes in nodules to protect the oxygen-sensitive nitrogenase enzyme of the rhizobium symbionts (Sturms et al., 2010). Nitrogen fixation by *Frankia* does not require an external oxygen scavenger because these bacteria are able to create their

own oxygen barrier by forming a shell of hopanoid lipids. Legumes recruited class 2 hemoglobins and *Parasponia* independently recruited a class 1 hemoglobin to function as oxygen transporters in nitrogen-fixing symbiotic nodule cells. Thus, in this second evolutionary scenario, nodulation with *Frankia* in proto-legumes and *Parasponia* shares a relatively short common evolutionary history from the origin of nodulation to the diversification of the two lineages, followed by 100 MY of separation during which both lineages independently switched from hosting *Frankia* to rhizobia. The symbiont exchange could have been achieved through a temporary phase of co-infection of nodules by the primary *Frankia* symbiont and a secondary rhizobium symbiont, followed by the full overtaking of *Frankia* by the rhizobia. Indeed, co-infection of nodules by a nodule-inducing bacterium and a non-nodulating bacterium is often observed in legumes and actinorhizal plants (Martínez-Hidalgo and Hirsch, 2017).. Furthermore, the replacement of the symbiont likely required new host functions for recognizing the new symbionts (which have a distinct cell envelope) and for suppressing immune responses against the rhizobia. From the bacterial side, the rhizobia acquired the nodulation genes required for Nod factor synthesis (Figure 1A), although the origin of these genes is still an enigma. Nod factors were required to replace the *Frankia* factors that triggered the nodulation program, and they also helped suppress immune responses by the host (Liang et al., 2013).

One could also envision a third, in-between scenario in which the ancestor of the NFC established an interaction with *Frankia* spp. without nodule formation and organogenesis, instead forming nitrogen-fixing structures similar to arbuscular mycorrhiza (structures formed without organogenesis) with little or no cell division, facilitating bacterial uptake and forming infection foci (Figure 5C) (see Parniske 2018 for an inspiring discussion on this scenario). In other words, in this third scenario, the hypothetical “predisposition of nodulation” of scenario 1 would correspond to the replacement (or extension) of a mycorrhizal fungus with a filamentous bacterial symbiont of the *Frankia* genus. During the second stage, *Frankia* were replaced by rhizobia independently in legumes and *Parasponia*, as described in scenario 2. Finally, during the third stage, nodule organogenesis evolved independently in multiple lineages. The timing of stage two and three could also be inverted. The present day absence of nodulation in many lineages of the NFC could correspond to the loss of nodulation or the loss of the more ancient nitrogen-fixing *Frankia*

symbiosis without organogenesis. It is harder to reconcile this third scenario with the presence of putative organogenesis-related genes in the common gene set of the *Medicago* and *Parasponia* transcriptomes. However, this overlap could represent the combined result of the early shared evolution towards a simple infection focus in the root cortex and the later convergent recruitment of orthologous gene functions after the separation of the two lineages. Moreover, the three symbiosis genes (*NFP*, *NIN* and *RPG*) that were lost in non-nodulating plants of the NFC are involved in infection and are thus not necessarily (exclusive) markers of nodule organogenesis. On the other hand, the third scenario fits well with the existing differences in infection, ontogeny, and physiology of nodules formed by plants in the different lineages of the NFC. For example, the nodules of *Parasponia* and actinorhizal plants have a central vascular bundle, whereas the nodules of legumes contain multiple peripheral vascular bundles (Downie, 2014; Behm et al., 2014). In addition, legumes and non-legumes of the NFC differ in their reliance on cytokinin signaling (Van Zeijl et al., 2018; Gauthier-Coles et al., 2019). Finally, the nodule-specific transcriptomes of different plants are largely dissimilar (Battenberg et al 2018; Wu et al., 2019) (despite the overlap of a subset of nodule-enhanced genes in *Medicago* and *Parasponia*; van Velzen et al., 2018), suggesting that organogenesis in these plants followed distinct evolutionary paths.

Investigating whether the large overlap between nodule transcriptomes observed between *P. andersonii* and *M. truncatula* could be extended to all nodulating plants could be informative. The available nodule transcriptomes from legumes cover all the major clades of the Papilionoideae subfamily, the hologalegina (*Medicago*, *Pisum*, *Lotus*, *Sesbania*), the millettoids (*Phaseolus*, *Glycine*), and the genistoids (*Lupinus*), but no data are available for species of the other large clade of the Leguminosae, the Mimosoideae-Caesalpineae-Cassieae (MCC) clade. In the non-legume NFC plants, the Cucurbitales are represented by the *Datsica* transcriptome, the Fagales by the *Alnus* and *Casuarina* transcriptomes, and the Rosales by *C. thyrsiflorus* and the rhizobium-infected *Parasponia* transcriptomes. A systematic comparative analysis identifying orthologous genes in the available transcriptome data, complemented with new data from the unexplored branches of the legume family, would certainly provide insights into the evolutionary scenario of nodulation. Such analysis would further

highlight the mechanisms in nodulation that are ancestral and those that are more recent innovations specific to particular lineages of the NFC.

REGULATION OF THE NODULE TRANSCRIPTOME

Transcription Factors

The stimulation of the CSSP by Nod factors results in the activation of a TF network composed of IPD3 (CYCLOPS), IPD3L, NIN, NSP1, NSP2, DELLA proteins, ERN1, ERN2, NF-YA1, and potentially other yet-to-be identified TFs (Schauser et al., 1999; Kalo et al., 2005; Smit et al., 2005; Asamizu et al., 2008; Jin et al., 2018; Marsh et al., 2007; Combiér et al., 2006; Andrianakaja et al., 2007; Middleton et al., 2007; Cerri et al., 2012; Fonouni-Farde et al., 2016; Jin et al., 2016; Singh et al., 2014; for reviews on the topic, see Oldroyd, 2013; Soyano and Hayashi, 2014; Roy et al., 2019). This TF network activates the infection process, nodule organogenesis, and autoregulation of nodulation (AON, a long-distance negative-feedback pathway that controls nodule number based on the number of already formed or initiated nodules and the nitrogen needs of the plant) (Figure 1A). The NIN TF emerged from these studies as a master regulator of nodulation that coordinates these processes (Soyano et al., 2013; Vernié et al., 2015; Liu et al., 2019a; Liu et al., 2019b), which is in agreement with the proposed early recruitment of NIN during the evolution of nodulation in the NFC. Several direct target genes of this network have been identified (*e.g.* *ENOD11*, *CRE1*, *NPL1* [*NODULATION PECTATE LYASE 1*], *CLE*), but how this TF network further connects to the downstream nodule transcriptome and the massive reprogramming in the symbiotic cells (Figure 2B) remains to be clarified. This regulation most likely involves the generation of secondary signals (such as phytohormones and others) as well as a downstream TF network.

Nevertheless, some TFs involved in late stages of nodule formation have been identified, but they have no direct link to transcription in symbiotic cells or their roles remain to be pinpointed. A few early studies have identified potential regulators in symbiotic nodule cells, which were not further characterized (Heard and Dunn, 1995; Heard et al., 1997; Jørgensen et al., 1999). Other transcriptional regulators, such as the *Medicago* Krüppel TF MtZPT2, the *Medicago* basic helix-loop-helix TF MtbHLH1, NOOT in *Medicago* and *Lotus* (belonging to the NPR1-like BTB/POZ-Ankyrin domain

protein family), and soybean GmbHLHm1 (formerly GmSAT1) are important for nodule formation but are not directly involved in symbiotic cell-specific gene regulation. Their genes are not expressed in symbiotic cells but are instead expressed in roots and nodule vascular bundles or nodule parenchyma, where they affect nodule vasculature patterning and nutrient transport between nodules and roots (Frugier et al., 2000; Godiard et al., 2011; Couzigou et al., 2012; Chiasson et al., 2014; Magne et al., 2018).

Other characterized TFs have demonstrated roles in symbiotic cell formation. The TF EFD, belonging to the ethylene response factor family, controls symbiotic cell formation, bacteroid differentiation and the expression of a subset of nodule-specific genes, including some NCR genes (Vernié et al., 2008). However, this regulation of nodule-specific genes could be indirect, as *MtRR4* is the only gene found to be directly regulated by EFD (Vernié et al., 2008). *MtRR4* is a type A cytokinin response regulator induced by cytokinins that is part of a negative feedback loop involved in repressing the cytokinin pathway. Cytokinins are key signals that activate cell divisions in the root cortex to establish a nodule primordium (Gamas et al., 2017). The repression of cytokinin signaling mediated by EFD through *MtRR4* might be essential for promoting symbiotic cell differentiation. Furthermore, a group of three class 2 KNOX homeodomain TFs directly or indirectly regulate *EFD* and *MtRR4* expression (Di Giacomo et al., 2017; Azarakhsh et al., 2015). One of these KNOX TFs, *MtKNOX3*, is strongly induced in nodules and is active in differentiating symbiotic cells. This TF could be involved in regulating other genes, including symbiotic cell-specific genes.

The C₂H₂ TF RSD is expressed in differentiating symbiotic cells and is required for symbiosome formation. However, data suggest that RSD act as a repressor of a small set of genes to influence vesicle transport in symbiotic cells and avoid mis-targeting of specific vesicles and their cargo to symbiosomes (Sinharoy et al., 2013). Thus, the present data on RSD are not in agreement with a role of this TF in positively activating symbiotic cell-specific gene expression. Similarly, the *Medicago* TF *MtbHLH2* might be expressed in infected nodule cells but is thought to act as a negative regulator of genes that stimulate nodule senescence (Deng et al., 2019). A small set of APETALA2 TFs in soybean, *L. japonicus*, and *P. vulgaris* might have a negative effect on nodulation, including symbiotic cell functioning. These TF were

identified because they are targeted by a nodule-enhanced miRNA (see below), and their upregulation in symbiotic cells may be correlated with the activation of senescence-associated genes (Wang et al., 2014; Holt et al., 2015; Nova-Franco et al., 2015).

In a large-scale effort in soybean, the expression profiles of approximately 1000 genes encoding putative transcriptional regulators were analyzed during nodule formation via RT-qPCR (Libault et al., 2009a). This systematic screening identified 39 genes that are upregulated during nodule formation. A gene encoding a MYB TF was found to be expressed in vascular tissues and not in symbiotic nodule cells. Although the remaining genes were not further characterized, they constitute a collection of putative regulators in symbiotic nodule cells.

Overall, very little is thus known about how the massive transcriptome switch in symbiotic nodule cells is directly regulated. Libault et al. (2009b) and Young et al. (2011) cataloged all TFs in the *M. truncatula* genome. Analyzing the MtGEA and Symbimics databases using nodule-specific or nodule-enhanced expression and expression in symbiotic cells (IZ and zone III) as criteria resulted in the identification of a set of interesting TFs that might be involved in gene-specific regulation in symbiotic cells (Supplemental Figure 1). These TFs include NIN (the above-mentioned master regulator of nodulation) and IPD3. IPD3 directly interacts with the CSSP component calcium- and calmodulin-dependent protein kinase (CCaMK) and functions in Nod factor activation of the CSSP, infection thread formation and the release of rhizobia from infection threads, organogenesis, and symbiotic cell formation (Yano et al., 2008; Maunoury et al., 2010; Horváth et al., 2011; Ovchinnikova et al., 2011; Singh et al., 2014). The transcriptome data show that *IPD3* is most strongly expressed in differentiating and mature symbiotic cells of the interzone and zone III. This finding corresponds with the results of *IPD3* promoter-GUS fusion analysis (Messinese et al., 2007). *CCaMK* is also expressed in these nodule zones but has a uniform expression pattern across all nodule tissues. These expression patterns suggest that IPD3 and NIN also play important roles in controlling gene expression in differentiating and mature symbiotic cells. Therefore, NIN and IPD3 might regulate symbiotic cell-specific genes directly or indirectly (through downstream TFs). In agreement with this hypothesis, the nodule transcriptome is not activated in the nodule-like structures formed in the *M. truncatula*

ipd3 mutant TR3 (Maunoury et al., 2010). Interestingly, genetic analyses also uncovered a role for other components of the CSSP in multiple stages of nodulation (Capoen et al., 2005; Limpens et al., 2005; Moling et al., 2014). These observations suggest that the CSSP is a regulatory module that is used and re-used to control all stages of nodulation, from the first response to Nod factors to infection thread formation to the endocytotic uptake of rhizobia and to symbiotic cell differentiation and functioning. During each step, this pathway appears to activate different gene sets depending on the cellular context.

Cis-Regulatory Elements in the Promoters of Nodule-Specific Genes

Leghemoglobin genes, the prototypical symbiotic cell-specific genes, were the first legume genes cloned (Sullivan et al., 1981). Their identification was rapidly followed by the identification of other nodule-specific genes encoding nodulins (Legocki and Verma, 1980; Sandal et al., 1987). The promoters of these genes were experimentally screened for *cis*-acting elements required for high level, nodule-specific expression (Stougaard et al., 1987; Jensen et al., 1988; Metz et al., 1988; Szabados et al., 1990; Szczyglowski et al., 1994; Fröhling et al., 2000; Nallu et al., 2013). These elements were used to demonstrate the existence of nodule-specific trans-acting factors. Unfortunately, these efforts were not further pursued, and the putative TFs were not characterized (Jensen et al., 1988; Metz et al., 1988).

The precise identification of *cis*-acting elements should in principle facilitate the straightforward screening of TFs using methods such as yeast-one-hybrid analysis. Thus far, this strategy has not led to the identification symbiotic cell-specific genes. Such an approach has only been performed on *cis*-acting elements of the Nod factor-responsive promoter of the very early activated gene *ENOD11*, resulting in the identification of the TF ERN1 (Andriankaja et al., 2007). Certainly, this approach holds promise for elucidating the transcriptional network in symbiotic nodule cells.

Chromatin-Based Regulation of Nodule-Specific Gene Expression

The tight regulation of nodule-specific genes clearly requires specific TFs but in addition, regulation at the chromatin level might also be important for keeping these genes silent during all stages of plant development. Genes with high tissue-specific expression are often actively silenced throughout much of the plant lifecycle via

epigenetic mechanisms. Chromatin-based silencing of nodule-specific genes could be released during symbiotic cell differentiation, making them accessible to TFs.

The soybean protein CPP1 (cysteine-rich polycomb-like protein 1) is a DNA binding protein that interacts with the promoter of the leghemoglobin gene *Gmlbc3* (Cvitanich et al., 2000). CCP1 contains conserved CXC domains homologous to a domain in some polycomb proteins. CCP proteins are widely distributed in plants and animals where they form small families. CCP proteins Tso1 in Arabidopsis, TESMIN in mammals, Mip120 and Tombola in Drosophila, and LIN-54 in *Caenorhabditis elegans* function in chromatin complexes and are involved in cell cycle regulation, cell differentiation, and reproduction (Sijacic et al., 2011; Jiang et al., 2007; Beall et al., 2007). Despite some initial characterization in various organisms, much remains to be learned about the mechanisms of these transcriptional regulators and their biological roles. Soybean CCP1 binds the *Gmlbc3* promoter and represses its expression, which is in agreement with the notion that CCP1 is part of a chromatin-related repression complex.

Further evidence for the key role of chromatin-based regulation in the massive gene activation during symbiotic cell differentiation in *M. truncatula* was obtained by analyzing the chromatin features of nodule-specific genes during symbiotic cell differentiation. This differentiation is driven by endoreduplication whereby post-meristematic nodule cells switch their cell cycle to an endocycle and multiply their genome from a 2C/4C DNA content in meristem cells, over 8C and 16C in differentiating cells, to 32C and 64C in maturing and mature symbiotic cells (Vinardell et al., 2003). Gene expression analysis of purified nodule nuclei of increasing ploidy levels revealed a correlation between the ploidy level and the sequential activation of nodule-specific genes in these nuclei, suggesting that endoreduplication is involved in this process (Nagymihály et al., 2017). The expression levels of nodule-specific genes in nodule nuclei with different ploidy levels correlated with the opening of chromatin as well as with reduced H3K27me3 (trimethylation of lysine 27 in histone H3) levels in a subset of tested genes (Nagymihály et al., 2017). Similarly, the above-mentioned SRIs showed reduced H3K27me3 levels in nodules vs., roots (Pecrix et al., 2018). H3K27me3 is a repressive chromatin mark introduced by the Polycomb complex, a regulatory complex involved in the epigenetic silencing of chromatin. Thus, the activation of nodule-specific genes could rely on the opening of the

chromatin and the release of H3K37me3-mediated repression during symbiotic cell differentiation.

DNA methylation represents another level of epigenetic control of the expression of symbiotic nodule cell-specific genes. DNA methylation of promoters usually leads to gene silencing. A comparison of DNA methylation levels in whole roots vs. nodules or in nodule nuclei of different ploidy levels showed that many symbiotic nodule cell-specific genes, including genes encoding NCRs, calmodulin-like proteins, leghemoglobin, GRPs, and many others, as well as many transposons, become demethylated, which correlates with the activation of their expression (Satgé et al., 2016; Nagymihály et al., 2017). Remarkably, SRIs show differential methylation patterns in roots vs. nodules, and the borders of the SRIs sharply define the limits of differentially methylated regions, with the flanking regions showing very low and non-differential methylation (Pecrix et al., 2018). The DNA demethylation of nodule-specific genes is largely regulated by the activation of the DNA demethylase gene *DEMETETER* (*DME*) during symbiotic nodule cell differentiation (Satgé et al., 2016). Remarkably, DME activity also results in the loss of DNA methylation and the expression of *NCR*-flanking transposons during symbiotic cell differentiation (Guefrachi et al., 2014; Satgé et al., 2016). Since transposons are epigenetically silenced, the activation of these transposons in symbiotic cells confirms the notion that the re-structuring of chromatin occurs in these loci during symbiotic cell differentiation.

Thus, epigenetic control via DNA methylation and histone modifications appears to maintain the nodule-specific genes and SRIs silenced. During the endoreduplication-driven differentiation of symbiotic cells, epigenetic reprogramming of these loci renders them accessible and competent for transcription. However, the current data only establish correlations between gene expression, chromatin modifications, and endoreduplication. A future challenge will be to establish causal relationships between these processes.

The Regulation of Gene Expression by Alternative Splicing, Small RNAs, and Small Peptides

Additional mechanisms that control symbiotic cell-specific expression have been reported. One of these mechanisms is the alternative splicing of the *Syntaxin 132*

(*SYP132* or *SYP13II*) gene (Pan et al., 2016; Huisman et al., 2016). *SYP132* encodes a t-SNARE protein that functions in the secretory pathway controlling the fusion of secretory vesicles with the membrane of the target organelle. In *M. truncatula*, *SYP132* is transcribed into two different transcripts due to alternative splicing. The *SYP132 β* (or *SYP132C*) transcript is ubiquitously expressed, while the *SYP132 α* (or *SYP132A*) transcript is preferentially expressed in symbiotic nodule cells and in arbuscular mycorrhiza cells. The two transcripts differ in their 13th terminal exon, which in the *SYP132 β* transcript is located immediately downstream of the previous exon. By contrast, in the *SYP132 α* transcript, this canonical 13th exon is skipped and replaced by an exon located approximately 2000 base pairs downstream. The two encoded proteins are therefore almost identical, differing only in 60 C-terminal amino acids. RNAi experiments specifically targeting one of these transcripts revealed that *SYP132 β* is essential for plant growth, whereas nodule- and mycorrhiza-specific *SYP132 α* is essential for arbuscule formation and bacteroid differentiation. The *SYP132* proteins are transported to the symbiosomes in symbiotic nodule cells. Downregulating the *SYP132 α* transcript arrests bacteroid differentiation, presumably due to a blockage in the transport of nodule-specific secretory proteins, including NCRs, to the symbiosomes. It is not yet understood how this alternative splicing is regulated and if this is a common mechanism for nodule-specific gene expression or unique to *SYP132*. However, the finding that alternative splicing of *SYP132* is conserved in most dicots including plants not in the NFC and is absent in dicot species that have lost mycorrhizal symbiosis suggests that this mechanism is related to mycorrhiza formation and has been co-opted for nodule symbiosis.

MicroRNAs (miRNAs) are small regulatory RNA molecules that control the expression of specific target genes by binding to their mRNAs and affecting mRNA translation or stability.. miRNAs control early stages of nodulation before the establishment of symbiotic cells (reviewed in Couzigou and Combier, 2016). For example, the TF NF-YA1 in *M. truncatula*, which controls nodule meristem function, is negatively regulated by the miRNA miR169 (Combier et al., 2006). In addition, the class-III homeodomain-leucine zipper TFs MtHB8, MtCNA1, and MtCNA2, which are involved in root and nodule meristem formation as well as vascular bundle differentiation, are negatively regulated by miR166 (Boualem et al., 2008). In *L. japonicus* miR2111 undergoes shoot-to-root translocation and controls the AON

950 response in roots by negatively regulating the expression of *TML*, encoding an F-box
951 protein that suppresses symbiosis (Tsikou et al., 2018). In soybean, a whole array of
952 miRNAs affect the early stages of nodule induction and therefore nodule number
953 (Yan et al., 2015; Wang et al., 2014; Wang et al., 2015; Hossain et al., 2019). In
954 agreement with this role of miRNAs, Argonaute5, a small RNA-guided silencing
955 protein involved in mediating miRNA activity is required for nodule induction (del
956 Rocio Reyero-Saavedra et al., 2017).

957 miRNAs are processed from primary transcripts resulting from the transcription of
958 miRNA genes by RNA polymerase II. These primary miRNA transcripts often encode
959 small peptides called miPEPs. miPEPs enhance the transcription of their cognate
960 primary transcript and thereby the accumulation of the corresponding miRNA
961 (Lauressergues et al., 2015). In soybean, miR172c targets the APETALA 2 TF
962 NNC1, which negatively regulates nodule number (Wang et al., 2014; Wang et al.,
963 2019). The miPEP172c peptide is encoded by the primary transcript of miR172c. This
964 peptide stimulates the expression of this primary transcript, thereby positively
965 regulating nodulation (Couzigou et al., 2016). Regulatory small peptides can also be
966 encoded by protein encoding mRNAs. An alternatively spliced mRNA of the TF NF-
967 YA1 encodes such a peptide, called uORF1p (Comber et al., 2008). The alternatively
968 spliced mRNA and uORF1p are expressed in nodule tissues adjacent to the
969 meristem. uORF1p negatively regulates the expression of *NF-YA1* and thus, in
970 combination with the above-mentioned miR169, restricts the expression of this gene
971 to the nodule meristem.

972 Thus, the initial stages of nodule formation are regulated by miRNAs and small
973 peptides that control the transcript levels of their target TFs. Later stages of the
974 nodule development are also regulated by miRNAs in *L. japonicus*, soybean, and *P.*
975 *vulgaris*. In *L. japonicus*, miR397 is systemically induced only by the presence of
976 functional, nitrogen-fixing nodules and it downregulates a member of the laccase
977 protein family (De Luis et al., 2012). miR397 is conserved in many plants and is
978 involved in regulating copper homeostasis. However, whether this regulation takes
979 place in symbiotic cells or in other nodule cells remains to be confirmed. The *P.*
980 *vulgaris* miRNA homolog of the above-mentioned miR172c plays a regulatory role in
981 a late stage of nodulation. miR172c represses the expression of the APETALA2 TF
982 gene *AP2-1* (Nova-Franco et al., 2015). An analysis of the roles of AP2-1 and

miR172c suggested that AP2-1 activates senescence-related genes in symbiotic cells and that miR172c is required to downregulate *AP2-1* in active nodules. By contrast, in non-active nodules infected with a non-functional rhizobium strain or induced by nitrate application, miR172c expression is reduced, leading to the induction of *AP2-1* and senescence. Corresponding miRNAs in soybean and *L. japonicus* are expressed in symbiotic cells of mature nodules; these miRNA172s and their AP2 targets could play similar roles to those in *P. vulgaris* (Wang et al., 2014; Holt et al., 2015). Finally, miR393j-3p targets and represses the expression of *ENOD93* in soybean; this gene is specifically expressed in nodules and symbiotic cells (Yan et al., 2015).

Small RNAs derived from endonuclease-mediated tRNA cleavage (tRNA fragments or tRFs) are a recently described class of small RNAs that associate with Argonaute proteins and regulate gene expression (Kumar et al., 2016). A recent analysis of small RNA transcriptomes from soybean nodules and cultures of its symbiotic partner *Bradyrhizobium* revealed numerous *Bradyrhizobium*-derived tRFs, most with higher abundance in nodules than in culture (Ren et al., 2019). Twenty-five of these tRFs were predicted to target 52 different soybean genes, suggesting that they contribute to the post-transcriptional regulation of these genes. Three selected rhizobial tRFs were indeed shown to be loaded on soybean Argonaute protein 1 and to downregulate their predicted target genes *GmRHD3a/GmRHD3b*, *GmHAM4a/GmHAM4b*, and *GmLRX5*. The silencing of these target genes by the rhizobial tRFs occurred during the early stages of infection and enhanced nodulation. These findings point to a new level of interspecies communication between the rhizobia and their legume hosts. The finding that additional *Bradyrhizobium* tRFs are predicted to target other soybean genes and that a different set of rhizobial tRFs potentially targeting a different set of host genes was identified in a small RNA transcriptome from the legume *P. vulgaris* suggests that this type of signaling is not restricted to these three reported examples. Although it is too early to fully understand the implications of this new signaling mechanism, it is tempting to speculate that it introduces yet another level of control to host-specificity to match the right symbiont with the right plant. Another intriguing question is how these rhizobial small RNAs reach their target genes in the plant cell. An exciting possibility is that they might be delivered via outer membrane vesicles (OMVs) produced by the

1016 rhizobia, a mechanism similar to that used by the human pathogen *Pseudomonas*
1017 *aeruginosa*, which delivers a tRF via OMVs to epithelial cells in the human airway
1018 (Koeppen et al., 2016).

1019 Taken together, these pioneering studies have made it clear that miRNAs and other
1020 small RNAs play a role in coordinating and fine-tuning gene expression in symbiotic
1021 nodule cells. Accordingly, downregulating *Argonaute5* expression in *P. vulgaris* and
1022 soybean not only reduces nodule initiation but also affects the formation of symbiotic
1023 cells (del Rocio Reyero-Saavedra et al., 2017). Dedicated transcriptome analyses
1024 focusing on small RNAs expressed in nodules and infected root hairs have been
1025 performed for several legumes, including *M. truncatula* (Lelandais-Brière et al., 2009;
1026 Formey et al., 2014; Pecrix et al., 2018), soybean (Subramanian et al., 2008; Joshi et
1027 al., 2010; Yan et al., 2015; Yan et al., 2016), *P. vulgaris* (Peláez et al., 2012;
1028 Fourmey et al., 2016), and *L. japonicus* (Holt et al., 2015). The corresponding
1029 databases represent promising sources for the future discovery of novel RNAs that
1030 control gene expression in nodules.

1031 Finally, long non-coding and antisense RNAs (lncRNAs) might also be involved
1032 regulating nodule-specific gene expression. LCM RNA-seq analysis of *M. truncatula*
1033 nodules (SYMBIMICS dataset) has revealed a surprisingly large number (over 1000)
1034 of lncRNAs expressed in nodules. Most of these are active in differentiating or mature
1035 symbiotic cells. Many are located in SRIs, and the expression profiles of many of
1036 these lncRNAs were correlated with the expression of the closest mRNA-encoding
1037 genes in the genome, suggesting these lncRNAs play regulatory roles (Pecrix et al
1038 2018). For example, the TF EFD is expressed in young symbiotic cells in the distal
1039 and proximal infection zones of the nodule. Strikingly, an antisense transcript of *EFD*
1040 showed a complementary expression profile, i.e., in older symbiotic cells in the
1041 interzone and fixation zones. This finding suggests that *EFD* is downregulated by
1042 antisense transcripts in order to promote the final stages of symbiotic cell
1043 differentiation. Indeed, a large set of the above-mentioned nodule-specific genes in
1044 *M. truncatula* (e.g. genes encoding NCRs, GRPs, ENOD8, ENOD16, ENOD20,
1045 calmodulin-like proteins, leghemoglobin, nodulin 25 IPD3, NIN, SymCRK, DME,
1046 DNF1, RSD, and ERN2) as well as many others have associated lncRNAs or
1047 antisense transcripts. The biological meaning of this will certainly be worth
1048 deciphering.

1049

1050 **CONCLUDING REMARKS**

1051 Transcriptomic analysis of nodule formation has been instrumental in the discovery of
1052 new genes that were subsequently shown by reverse genetics to be important for
1053 nodule formation. Thus, transcriptomics have played a complementary role (and will
1054 probably continue to do so) to the many forward genetic screens that have been
1055 successfully conducted by several laboratories to identify key regulators of nodulation
1056 (reviewed by Roy et al., 2019). However, transcriptomics analysis also provides
1057 broader insights into the nodulation process. Here, we argue that nodule formation is
1058 a unique developmental process that is supported by drastic transcriptional
1059 reprogramming. This reprogramming involves the activation of many hundreds of
1060 genes, many of which are exclusively expressed in these organs and are novel
1061 genes that have been shaped by evolution since the origins of nodulation. A
1062 remarkable number of nodule-specific genes have signal peptides and are therefore
1063 transported to bacteroid-containing symbiosomes, suggesting that a major adaptation
1064 of nodule-forming plants is the massive production of a large variety of proteins that
1065 interact with endosymbionts. We have some knowledge about the functions of some
1066 of these proteins (e.g. the NCRs), but at the moment, we have no clue about most of
1067 their roles.

1068 Even though the CSSP is the upstream regulatory apparatus that activates both
1069 nodule formation and mycorrhiza formation, the nodule-specific and mycorrhiza-
1070 specific transcriptomes are dramatically different. How the different read-outs of this
1071 conserved module are achieved is likely one of the most important questions to be
1072 solved in the field. Perhaps the components of the CSSP are arranged in multiprotein
1073 signaling modules anchored in the cell membrane through receptor complexes
1074 located in nanoclusters organized by microdomain-associated proteins, including
1075 remorins, flotillins, and FW2.2-like proteins (Haney and Long, 2010; Haney et al.,
1076 2011; Levebvre et al., 2010; Ott, 2017; Qiao et al., 2017; Liang et al., 2018). Distinct
1077 nodulation signaling modules and mycorrhization signaling modules could activate
1078 the appropriate responses.

1079 The regulatory mechanisms that underlie symbiotic cell formation and control the
1080 transcriptome switch are also poorly understood. With the recent identification of

candidate TFs, the discovery of SRIs, the description of epigenetic modifications, and the documentation of transcriptional fine-tuning mechanisms by alternative splicing, miRNAs, miPEPs, tRFs, and antisense RNAs, we are only starting to decipher this process, and we can hope that the key regulators will soon be discovered. An intriguing observation from nodule transcriptomes is that the TF gene *IPD3* is most strongly expressed in symbiotic cells. It would be interesting to determine its specific role in these cells and how this role relates to its transcriptional activity during the very early stages of the nodulation process.

An important implication of the current analysis is that nodules are unique organs, not only from the perspective of organogenesis and histological organization but also from a genetic perspective. All attempts to transfer nodule formation to non-legume crops that are currently underway in different laboratories should take into account the finding that “naturally” evolved nodule formation relies on the concerted action of hundreds of genes, many of which appear to function specifically in nodules. It will be important in future efforts to understand in great detail not only the regulatory mechanisms of nodule-specific genes, but also their function as well as their evolution. A key issue for the future will be to understand how adaptive the nodule transcriptome response is. Gene regulation is usually considered to be an adaptive response of the organism, organ, or cell to a specific environmental or developmental condition so that genes are expressed when and where they are required. The validity of this presumption has not been tested thoroughly in plants. In bacteria, however, the picture has emerged that transcriptional responses are in large part suboptimal or even maladaptive (Price et al., 2013). Striking examples come from the rhizobium bacteroids in nodules themselves. Similar to their host cells, bacteroids undergo a major transcriptomic switch during their formation, with the induction of many hundred of genes and the downregulation of the rest of the genome. Some of these upregulated genes are essential for symbiosis, such as genes encoding nitrogenase and accessory functions. However, disappointingly from a researcher’s perspective, in various studies, the mutation of many selected genes that were strongly upregulated in mature bacteroids resulted in no symbiotic phenotype for any of the tested genes (Karunakaran et al., 2009; Lamouche et al., 2019). Similarly, in the yeast *Saccharomyces cerevisiae*, only a small portion of genes upregulated under a particular growth condition contributed to the fitness of the yeast under that

condition (Giaever et al., 2002). Thus, genes may be upregulated in a specific context only as a side-effect without having a clear functional contribution. Whether this is the case in nodules remains to be analyzed, as it is possible that part of the nodule transcriptome contributes little to symbiotic nitrogen fixation.

Clearly, it is not possible, at least with currently available technology, to engineer something as complex as nodules of the NFC. Thus, it will be important to understand to what extent the complexity present in current day nodules is required for nitrogen fixation, even at levels below the optimal level reached in legume nodules. Much attention has been paid to the early stages of nodule formation, but we would like to advocate for more intense efforts towards understanding symbiotic nodule cell formation. These unique cell types are at the heart of symbiosis and determine the high efficiency of the nitrogen-fixation process.

Supplemental Data

Supplemental Figure 1. Expression of *M. truncatula* TFs.

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AUTHOR CONTRIBUTIONS

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REFERENCES

1143 **Alunni, B., Kevei, Z., Redondo-Nieto, M., Kondorosi, A., Mergaert, P., and**
1144 **Kondorosi, E.** (2007). Genomic organization and evolutionary insights on *GRP*
1145 and *NCR* genes, two large nodule-specific gene families in *Medicago truncatula*.
1146 Mol. Plant.-Microbe. Interact. **20**: 1138–1148.

1147 **Alves-Carvalho, S. et al.** (2015). Full-length *de novo* assembly of RNA-seq data in
1148 pea (*Pisum sativum* L.) provides a gene expression atlas and gives insights into
1149 root nodulation in this species. Plant J. **84**: 1–19.

1150 **Andriankaja, A., Boisson-Dernier, A., Frances, L., Sauviac, L., Jauneau, A.,**
1151 **Barker, D.G., and de Carvalho-Niebel, F.** (2007). AP2-ERF transcription
1152 factors mediate Nod factor dependent Mt*ENOD11* activation in root hairs via a
1153 novel cis-regulatory motif. Plant Cell **19**: 2866–2885.

1154 **Antolín-Llovera, M., Ried, M. K., and Parniske, M.** (2014). Cleavage of the
1155 SYMBIOSIS RECEPTOR-LIKE KINASE ectodomain promotes complex
1156 formation with Nod Factor Receptor 5. Curr. Biol. **24**: 422–427.

1157 **Asamizu, E., Shimoda, Y., Kouchi, H., Tabata, S., and Sato, S.** (2008). A positive
1158 regulatory role for LjERF1 in the nodulation process is revealed by systematic
1159 analysis of nodule-associated transcription factors of *Lotus japonicus*. Plant
1160 Physiol. **147**: 2030-2040.

1161 **Azarakhsh, M., Kirienko, A.N., Zhukov, V.A., Lebedeva, M.A., Dolgikh, E.A., and**
1162 **Lutova, L.A.** (2017). KNOTTED1-LIKE HOMEODOMAIN 3 : a new regulator of
1163 symbiotic nodule development. J. Exp. Bot. **66**: 7181–7195.

1164 **Battenberg, K., Potter, D., Tabuloc, C.A., Chiu, J.C., and Berry, A.M.** (2018).
1165 Comparative transcriptomic analysis of two actinorhizal plants and the legume
1166 *Medicago truncatula* supports the homology of root nodule symbioses and is
1167 congruent with a two-step process of evolution in the nitrogen-fixing clade of
1168 angiosperms. Front. Plant Sci. **9**: 1256.

1169 **Beall, E.L., Lewis, P.W., Bell, M., Rocha, M., Jones, D.L., and Botchan, M.R.**
1170 (2007). Discovery of tMAC : a *Drosophila* testis-specific meiotic arrest complex
1171 paralogous to Myb Muv B. Genes Dev. **21**: 904–919.

- 1172 **Behm, J.E., Geurts, R., and Kiers, E.T.** (2014). *Parasponia*: a novel system for
1173 studying mutualism stability. *Trends Plant Sci.* **19**: 757–763.
- 1174 **Benedito, V.A. et al.** (2008). A gene expression atlas of the model legume *Medicago*
1175 *truncatula*. *Plant J.* **55**: 504–513.
- 1176 **Berrabah, F., Bourcy, M., Eschstruth, A., Cayrel, A., Guefrachi, I., Mergaert, P.,**
1177 **Wen, J., Jean, V., Mysore, K.S., Gourion, B., and Ratet, P.** (2014). A nonRD
1178 receptor-like kinase prevents nodule early senescence and defense-like
1179 reactions during symbiosis. *New Phytol.* **203**: 1305–1314.
- 1180 **Boualem, A., Laporte, P., Jovanovic, M., Laffont, C., Plet, J., Combier, J.P.,**
1181 **Niebel, A., Crespi, M., and Frugier, F.** (2008). MicroRNA166 controls root and
1182 nodule development in *Medicago truncatula*. *Plant J.* **54**: 876–887.
- 1183 **Bourcy, M., Brocard, L., Pislariu, C.I., Cosson, V., Mergaert, P., Tadege, M.,**
1184 **Mysore, K.S., Udvardi, M.K., Gourion, B., and Ratet, P.** (2013). *Medicago*
1185 *truncatula* DNF2 is a PI-PLC-XD-containing protein required for bacteroid
1186 persistence and prevention of nodule early senescence and defense-like
1187 reactions. *New Phytol.* **197**: 1250–1261.
- 1188 **Branca, A. et al.** (2011). Whole-genome nucleotide diversity, recombination, and
1189 linkage disequilibrium in the model legume *Medicago truncatula*. *Proc. Natl.*
1190 *Acad. Sci. USA* **108**: E864–E870.
- 1191 **Breakspear, A., Liu, C., Roy, S., Stacey, N., Rogers, C., Trick, M., Morieri, G.,**
1192 **Mysore, K.S., Wen, J., Oldroyd, G.E.D., Downie, J.A., and Murray, J.D.**
1193 (2014). The root hair “infectome” of *Medicago truncatula* uncovers changes in
1194 cell cycle genes and reveals a requirement for auxin signaling in rhizobial
1195 infection. *Plant Cell* **26**: 4680–4701.
- 1196 **Brechenmacher, L. et al.** (2008). Transcription profiling of soybean nodulation by
1197 *Bradyrhizobium japonicum*. *Mol. Plant. Microbe. Interact.* **21**: 631–645.

1198 **Burghardt, L.T., Guhlin, J., Chun, C.L., Liu, J., Sadowsky, M.J., Stupar, R.M.,**
 1199 **Young, N.D., and Tiffin, P. (2017).** Transcriptomic basis of genome by genome
 1200 variation in a legume-rhizobia mutualism. *Mol. Ecol.*

1201 **Buhian, W.P., and Bensmihen, S. (2018).** Nod factor regulation of phytohormone
 1202 signaling and homeostasis during rhizobia-legume symbiosis. *Front. Plant Sci.* **9**:
 1203 1247.

1204 **Cabeza, R., Koester, B., Liese, R., Lingner, A., Baumgarten, V., Dirks, J.,**
 1205 **Salinas-Riester, G., Pommerenke, C., Dittert, K., and Schulze, J. (2014).** An
 1206 RNA sequencing transcriptome analysis reveals novel insights into molecular
 1207 aspects of the nitrate impact on the nodule activity of *Medicago truncatula*. *Plant*
 1208 *Physiol.* **164**: 400–411.

1209 **Cabeza, R.A., Liese, R., Lingner, A., von Stieglitz, I., Neumann, J., Salinas-**
 1210 **Riester, G., Pommerenke, C., Dittert, K., and Schulze, J. (2014).** RNA-seq
 1211 transcriptome profiling reveals that *Medicago truncatula* nodules acclimate N₂
 1212 fixation before emerging P deficiency reaches the nodules. *J. Exp. Bot.* **65**:
 1213 6035–6048.

1214 **Capoen, W., Goormachtig, S., De Rycke, R., Schroeyers, K., and Holsters, M.**
 1215 **(2005).** SrSymRK, a plant receptor essential for symbiosome formation. *Proc.*
 1216 *Natl. Acad. Sci. USA* **102**: 10369–10374.

1217 **Capoen, W., Den Herder, J., Rombauts, S., De Gussem, J., De Keyser, A.,**
 1218 **Holsters, M., and Goormachtig, S. (2007).** Comparative transcriptome analysis
 1219 reveals common and specific tags for root hair and crack-entry invasion. *Plant*
 1220 *Physiol.* **144**: 1878–1889.

1221 **Cerri, M.R., Frances, L., Laloum, T., Auriac, M.-C., Niebel, A., Oldroyd, G.E.D.,**
 1222 **Barker, D.G., Fournier, J., and de Carvalho-Niebel, F. (2012).** *Medicago*
 1223 *truncatula* ERN transcription factors: regulatory interplay with NSP1/NSP2 GRAS
 1224 factors and expression dynamics throughout rhizobial infection. *Plant Physiol.*
 1225 **160**: 2155–2172.

1226 **Chen, L., Qin, L., Zhou, L., Li, X., Chen, Z., Sun, L., Wang, W., Lin, Z., Zhao, J.,**
1227 **Yamaji, N., Ma, JF., Gu, M., Xu, G., and Liao, H. (2019).** A nodule-localized
1228 phosphate transporter GmPT7 plays an important role in enhancing symbiotic
1229 N₂ fixation and yield in soybean. *New Phytol.* **221**: 2013-2025.

1230 **Chiasson, D.M. et al. (2014).** Soybean SAT1 (Symbiotic Ammonium Transporter 1)
1231 encodes a bHLH transcription factor involved in nodule growth and NH₄⁺
1232 transport. *Proc. Natl. Acad. USA* **111**: 4814-4819.

1233 **Cissoko, M. et al. (2018)** Actinorhizal signaling molecules: *Frankia* root hair
1234 deforming factor shares properties with NIN inducing factor. *Front. Plant Sci.* **9**:
1235 1494.

1236 **Clavijo, F. et al. (2015).** The *Casuarina NIN* gene is transcriptionally activated
1237 throughout *Frankia* root infection as well as in response to bacterial diffusible
1238 signals. *New Phytol.* **208**: 887-903.

1239 **Clevenger, J., Chu, Y., Scheffler, B., and Ozias-Akins, P. (2016).** A developmental
1240 transcriptome map for allotetraploid *Arachis hypogaea*. *Front Plant Sci.* **7**: 1446.

1241 **Colebatch, G., Desbrosses, G., Ott, T., Krusell, L., Montanari, O., Kloska, S.,**
1242 **Kopka, J., and Udvardi, M.K. (2004).** Global changes in transcription
1243 orchestrate metabolic differentiation during symbiotic nitrogen fixation in *Lotus*
1244 *japonicus*. *Plant J.* **39**: 487–512.

1245 **Combier, J.-P., Frugier, F., De Billy, F., Boualem, A., El-Yahyaoui, F., Moreau, S.,**
1246 **Vernié, T., Ott, T., Gamas, P., Crespi, M., and Niebel, A. (2006).** *MtHAP2-1* is
1247 a key transcriptional regulator of symbiotic nodule development regulated by
1248 microRNA169 in *Medicago truncatula*. *Genes Dev.* **20**: 3084–3088.

1249 **Combier, J.-P., De Billy, F., Gamas, P., Niebel, A., and Rivas, S. (2008).** *Trans-*
1250 *regulation of the expression of the transcription factor MtHAP2-1 by a uORF*
1251 *controls root nodule development. Genes Dev.* **22**: 1549–1559.

1252 **Cope, K.R. et al.** (2019). The ectomycorrhizal fungus *Laccaria bicolor* produces
1253 lipochitooligosaccharides and uses the common symbiosis pathway to colonize
1254 *Populus* roots. *Plant Cell*. doi:10.1105/tpc.18.00676.

1255 **Couzigou, J.-M. et al.** (2012). *NODULE ROOT* and *COCHLEATA* maintain nodule
1256 development and are legume orthologs of *Arabidopsis* *BLADE-ON-PETIOLE*
1257 genes. *Plant Cell* **24**: 4498-4510.

1258 **Couzigou, J.-M., and Combier, J.-P.** (2016). Plant microRNAs : key regulators of
1259 root architecture and biotic interactions. *New Phytol.* **212**: 22–35.

1260 **Cvitanich, C, Pallisgaard, N, Nielsen, KA, Chemnitz Hansen, A, Larsen, K.,**
1261 **Pihakaski-Maunsbach, K., Marcker K.A., and Jensen, E.Ø.** (2000). CPP1, a
1262 DNA-binding protein involved in the expression of a soybean *leghemoglobin c3*
1263 gene. *Proc. Natl. Acad. USA* **97**: 8163-8168.

1264 **Czaja, L.F., Hogekamp, C., Lamm, P., Maillet, F., Andres Martinez, E., Samain,**
1265 **E., Dénarié, J., Küster, H., and Hohnjec, N.** (2012). Transcriptional responses
1266 toward diffusible signals from symbiotic microbes reveal MtNFP- and MtDMI3-
1267 dependent reprogramming of host gene expression by arbuscular mycorrhizal
1268 fungal lipochitooligosaccharides. *Plant Physiol.* **159**: 1671–1685.

1269 **Czernic, P. et al.** (2015). Convergent evolution of endosymbiont differentiation in
1270 Dalbergioid and inverted repeat-lacking clade legumes mediated by nodule-
1271 specific cysteine-rich peptides. *Plant Physiol.* **169**: 1254-1265.

1272 **Damiani, I., et al.** (2016). Nod factor effects on root hair-specific transcriptome of
1273 *Medicago truncatula*: Focus on plasma membrane transport systems and
1274 reactive oxygen species networks. *Front. Plant Sci.* **7**: 794.

1275 **de Bang, T. et al.** (2017). Genome-wide identification of *Medicago* peptides involved
1276 in macronutrient responses and nodulation. *Plant Physiol.* **175**: 1669–1689.

1277 **De Luis, A., Markmann, K., Cognat, V., Holt, D.B., Charpentier, M., Parniske, M.,**
1278 **Stougaard, J., and Voinnet, O.** (2012). Two microRNAs linked to nodule

1279 infection and nitrogen-fixing ability in the legume *Lotus japonicus*. Plant Physiol.
1280 **160**: 2137–2154.

1281 **del Rocio Reyero-Saavedra, M., Qiao, Z., del Socorro Sánchez-Correa, M., Díaz-**
1282 **Pineda, M.E., Reyes, J.L., Covarrubias, A.A., Libault, M., and Valdés-López,**
1283 **O.** (2017). Gene silencing of *Argonaute5* negatively affects the establishment of
1284 the legume-rhizobia symbiosis. Genes **8**: 352.

1285 **Demina, I.V, Persson, T., Santos, P., Plaszczyca, M., and Pawlowski, K.** (2013).
1286 Comparison of the nodule vs. root transcriptome of the actinorhizal plant *Datisca*
1287 *glomerata*: Actinorhizal nodules contain a specific class of defensins. PLoS One
1288 **8**: e72442.

1289 **Deng, J., Zhu, F., Liu, J., Zhao, Y., Wen, J., Wang, T., and Dong, J.** (2019).
1290 Transcription factor bHLH2 represses *CYSTEINE PROTEASE 77* to negatively
1291 regulate nodule senescence. Plant Physiol. doi:10.1104/pp.19.00574.

1292 **Di Giacomo, E., Laffont, C., Sciarra, F., Iannelli, M.A., Frugier, F., and Frugis, G.**
1293 (2017). KNAT3 / 4 / 5-like class 2 KNOX transcription factors are involved in
1294 *Medicago truncatula* symbiotic nodule organ development. New Phytol. **213**:
1295 822–837.

1296 **Dickstein, R., Hu, X., Yang, J., Ba, L., Coque, L., Kim, D.-J., Cook, D.R., and**
1297 **Yeung, A.T.** (2002). Differential expression of tandemly duplicated Enod8 genes
1298 in *Medicago*. Plant Sci. **163**: 333-343.

1299 **Domonkos, Á. et al.** (2017). NAD1 controls defense-like responses in *Medicago*
1300 *truncatula* symbiotic nitrogen fixing nodules following rhizobial colonization in a
1301 BacA-independent manner. Genes **8**: 387.

1302 **Downie, J.A.** (2014). Legume nodulation. Curr. Biol. **24**: R184–R190.

1303 **Doyle, J.J.** (2016). Chasing unicorns: Nodulation origins and the paradox of novelty.
1304 Am. J. Bot. **103**: 1865–1868.

1305 **Edgar, B.A., Zielke, N., and Gutierrez, C.** (2014). Endocycles: a recurrent
 1306 evolutionary innovation for post-mitotic cell growth. *Nat. Rev. Mol. Cell Biol.* **15**:
 1307 197-210.

1308 **El Yahyaoui, F., Küster, H., Ben Amor, B., Hohnjec, N., Pühler, A., Becker, A.,**
 1309 **Gouzy, J., Vernié, T., Gough, C., Niebel, A., Godiard, L., and Gamas, P.**
 1310 (2004). Expression profiling in *Medicago truncatula* identifies more than 750
 1311 genes differentially expressed during nodulation, including many potential
 1312 regulators of the symbiotic program. *Plant Physiol.* **136**: 3159–3176.

1313 **Fonouni-Farde, C., Tan, S., Baudin, M., Brault, M., Wen, J., Mysore, K.S., Niebel,**
 1314 **A., Frugier, F., and Diet, A.** (2016). DELLA-mediated gibberellin signalling
 1315 regulates Nod factor signalling and rhizobial infection. *Nat. Commun.* **7**: 12636.

1316 **Formey, D. et al.** (2014). The small RNA diversity from *Medicago truncatula* roots
 1317 under biotic interactions evidences the environmental plasticity of the
 1318 miRNAome. *Genome Biol.* **15**: 457.

1319 **Formey, D., Martín-Rodríguez, J.Á., Leija, A., Santana, O., Quinto, C., Cárdenas,**
 1320 **L., and Hernández, G.** (2016). Regulation of small RNAs and corresponding
 1321 targets in Nod factor-induced *Phaseolus vulgaris* root hair cells. *Int. J. Mol. Sci.*
 1322 **17**: 887.

1323 **Foucher, F., and Kondorosi, E.** (2000). Cell cycle regulation in the course of nodule
 1324 organogenesis in *Medicago*. *Plant Mol. Biol.* **43**: 773–786.

1325 **Franck, C.M., Westermann, J., and Boisson-Dernier A.** (2018). Plant malectin-like
 1326 receptor kinases: from cell wall integrity to immunity and beyond. *Annu. Rev.*
 1327 *Plant Biol.* **69**: 301-328.

1328 **Franssen, H.J., Xiao, T.T., Kulikova, O., Wan, X., Bisseling, T., Scheres, B., and**
 1329 **Heidstra, R.** (2015). Root developmental programs shape the *Medicago*
 1330 *truncatula* nodule meristem. *Development* **142**: 2941–2950.

1331 **Frare, R., Ayub, N., Alleva, K., and Soto, G.** (2018). The ammonium channel
 1332 NOD26 is the evolutionary innovation that drives the emergence, consolidation,

1333 and dissemination of nitrogen-fixing symbiosis in angiosperms. *J. Mol. Evol.* **86**:
1334 554-565.

1335 **Frugier, F., Poirier, S., Satiat-Jeunemaître, B., Kondorosi, A. and Crespi, M.**
1336 (2000). A Krüppel-like zinc finger protein is involved in nitrogen-fixing root nodule
1337 organogenesis. *Genes Dev.* **14**: 475–482.

1338 **Frühling, M., Albus, U., Hohnjec, N., Geise, G., Pühler, A., and Perlick, A.M.**
1339 (2000). A small gene family of broad bean codes for late nodulins containing
1340 conserved cysteine clusters. *Plant Sci.* **152**: 67–77.

1341 **Galindo-González, L., Mhiri, C., Deyholos, M.K., and Grandbastien, M.A.** (2017).
1342 LTR-retrotransposons in plants: Engines of evolution. *Gene* **626**: 14–25.

1343 **Gamas, P., Brault, M., Jardinaud, M., and Frugier, F.** (2017). Cytokinins in
1344 symbiotic nodulation: When, where, what for? *Trends Plant Sci.* **22**: 792-802.

1345 **Gauthier-Coles, C., White, R.G., and Mathesius, U.** (2019) Nodulating legumes are
1346 distinguished by a sensitivity to cytokinin in the root cortex leading to
1347 pseudonodule development. *Front. Plant Sci.* **9**: 1901.

1348 **Ge, Z., et al.** (2017). *Arabidopsis* pollen tube integrity and sperm release are
1349 regulated by RALF-mediated signaling. *Science* **358**: 1596–1600.

1350 **Genre, A., Chabaud, M., Faccio, A., Barker, D.G., and Bonfante, P.** (2008).
1351 Prepenetration apparatus assembly precedes and predicts the colonization
1352 patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago*
1353 *truncatula* and *Daucus carota*. *Plant Cell.* **20**: 1407-1420.

1354 **Gherbi, H., Markmann, K., Svistoonoff, S., Estevan, J., Autran, D., Giczey, G.,**
1355 **Auguy, F., Peret, B., Laplaze, L., Franche, C., Parniske, M., and Bogusz, D.**
1356 (2008). SymRK defines a common genetic basis for plant root endosymbioses
1357 with arbuscular mycorrhiza fungi, rhizobia, and *Frankia* bacteria. *Proc. Natl.*
1358 *Acad. Sci. USA* **105**: 4928–4932.

1359 **Giaever, G. et al.** (2002). Functional profiling of the *Saccharomyces cerevisiae*
1360 genome. *Nature* **418**: 387-391.

1361 **Giraud, E. et al.** (2007). Legumes Symbioses: Absence of *nod* genes in
 1362 photosynthetic bradyrhizobia. *Science* **316**: 1307-1312.

1363 **Godiard, L., Lepage, A., Moreau, S., Laporte, D., Verdenaud, M., Timmers, T.,**
 1364 **and Gamas, P.** (2011). MtbHLH1, a bHLH transcription factor involved in
 1365 *Medicago truncatula* nodule vascular patterning and nodule to plant metabolic
 1366 exchanges. *New Phytol.* **191**: 391–404.

1367 **González-Sama, A., de la Peña, T.C., Kevei, Z., Mergaert, P., Lucas, M.M., de**
 1368 **Felipe, M.R., Kondorosi, E., and Pueyo, J.J.** (2006). Nuclear DNA
 1369 endoreduplication and expression of the mitotic inhibitor Ccs52 associated to
 1370 determinate and lupinoid nodule organogenesis. *Mol Plant-Microbe Interact.* **19**:
 1371 173-180.

1372 **Gourion, B., Berrabah, F., Ratet, P., and Stacey, G.** (2015). Rhizobium–legume
 1373 symbioses: the crucial role of plant immunity. *Trends Plant Sci.* **20**: 186–194.

1374 **Graham, M.A., Silverstein, K.A.T., Cannon, S.B., and VandenBosch, K.A.** (2004).
 1375 Computational identification and characterization of novel genes from legumes.
 1376 *Plant Physiol.* **135**: 1179–1197.

1377 **Granqvist, E., Sun, J., Op den Camp, R., Pujic, P., Hill, L., Normand, P., Morris,**
 1378 **R.J., Downie, J.A., Geurts, R., and Oldroyd, G.E.D.** (2015). Bacterial-induced
 1379 calcium oscillations are common to nitrogen-fixing associations of nodulating
 1380 legumes and nonlegumes. *New Phytol.* **207**: 551–558.

1381 **Griesmann, M. et al.** (2018). Phylogenomics reveals multiple losses of nitrogen-
 1382 fixing root nodule symbiosis. *Science* **361**: eaat1743.

1383 **Guefrachi, I., Nagymihaly, M., Pislariu, C.I., Van de Velde, W., Ratet, P., Mars,**
 1384 **M., Udvardi, M.K., Kondorosi, E., Mergaert, P., and Alunni, B.** (2014).
 1385 Extreme specificity of NCR gene expression in *Medicago truncatula*. *BMC*
 1386 *Genomics* **15**: 712.

1387 **Gully, D. et al.** (2018). Transcriptome profiles of Nod factor-independent symbiosis in
 1388 the tropical legume *Aeschynomene evenia*. *Sci. Rep.* **8**: 10934.

1389 **Haag, A.F., Arnold, M.F.F., Myka, K.K., Kerscher, B., Dall'Angelo, S., Zanda, M.,**
1390 **Mergaert, P., and Ferguson, G.P.** (2013). Molecular insights into bacteroid
1391 development during Rhizobium- legume symbiosis. *FEMS Microbiol. Rev.* **37**:
1392 364–383.

1393 **Hakoyama, T. et al.** (2009). Host plant genome overcomes the lack of a bacterial
1394 gene for symbiotic nitrogen fixation. *Nature* **462**: 514–518.

1395 **Haney, C.H., Riely, B.K., Tricoli, D.M., Cook, D.R., Ehrhardt, D.W., and Long,**
1396 **S.R.** (2011). Symbiotic rhizobia bacteria trigger a change in localization and
1397 dynamics of the *Medicago truncatula* receptor kinase LYK3. *Plant Cell* **23**: 2774-
1398 2787.

1399 **Haney, C.H., and Long, S.R.** (2010). Plant flotillins are required for infection by
1400 nitrogen-fixing bacteria. *Proc. Natl. Acad. Sci. USA* **107**: 478-483.

1401 **Hansen, A.C., Busk, H., Marcker, A., Marcker, K.A., and Jensen, E.Ø** (1999).
1402 VsENBP1 regulates the expression of the early nodulin *PsENOD12B*. *Plant Mol.*
1403 *Biol.* **40**: 495-506.

1404 **Harrison, M.J., and Ivanov, S.** (2017). Exocytosis for endosymbiosis: membrane
1405 trafficking pathways for development of symbiotic membrane compartments.
1406 *Curr. Opin. Plant Biol.* **38**: 101-108.

1407 **Haruta, M., Sabat, G., Stecker, K., Minkoff, B.B., and Sussman, M.R.** (2014). A
1408 peptide hormone and its receptor protein kinase regulate plant cell expansion.
1409 *Science* **343**: 408–412.

1410 **Heard, J. and Dunn, K.** (1995). Symbiotic induction of a MADS-box gene during
1411 development of alfalfa root nodules. *Proc. Natl. Acad. Sci. USA* **92**: 5273–5277.

1412 **Heard, J., Caspi, M., Dunn, K., College, B., and Hill, C.** (1997). Evolutionary
1413 diversity of symbiotically induced nodule MADS Box genes: Characterization of
1414 *nmhC5*, a member of a novel subfamily. *Mol. Plant-Microbe Interact.* **10**: 665–
1415 676.

1416 **Heath, K.D., Burke, P. V., and Stinchcombe, J.R.** (2012). Coevolutionary genetic
1417 variation in the legume-rhizobium transcriptome. *Mol. Ecol.* **21**: 4735–4747.

1418 **Hoher, V., Alloisio, N., Auguy, F., Fournier, P., Dumas, P., Pujic, P., Gherbi,**
1419 **H., Queiroux, C., Da Silva, C., Wincker, P., Normand, P., and Bogusz, D.**
1420 (2011). Transcriptomics of actinorhizal symbioses reveals homologs of the whole
1421 common symbiotic signaling cascade. *Plant Physiol.* **156**: 700–711.

1422 **Hogekamp, C., Arndt, D., Pereira, P.A., Becker, J.D., Hohnjec, N., and Küster, H.**
1423 (2011). Laser microdissection unravels cell-type-specific transcription in
1424 arbuscular mycorrhizal roots, including CAAT-box transcription factor gene
1425 expression correlating with fungal contact and spread. *Plant Physiol.* **157**: 2023-
1426 2043.

1427 **Høglund, N., Radutoiu, S., Krusell, L., Voroshilova, V., Hannah, M.A., Goffard,**
1428 **N., Sanchez, D.H., Lippold, F., Ott, T., Sato, S., and Tabata, S.** (2009).
1429 Dissection of symbiosis and organ development by integrated transcriptome
1430 analysis of *Lotus japonicus* mutant and wild-type plants. *PLoS One* **4**: e6556.

1431 **Holt, D.B., Gupta, V., Abel, N.B., Andersen, S.U., Stougaard, J., and Markmann,**
1432 **K.** (2015). micro RNA 172 (miR172) signals epidermal infection and is
1433 expressed in cells primed for bacterial invasion in *Lotus japonicus* roots and
1434 nodules. *New Phytol.* **172**: 241–256.

1435 **Horváth, B. et al.** (2011). *Medicago truncatula* *IPD3* is a member of the common
1436 symbiotic signaling pathway required for rhizobial and mycorrhizal symbioses.
1437 *Mol. Plant-Microbe Interact.* **24**: 1345–1358.

1438 **Hossain, M.S., Hoang, N.T., Yan, Z., Tóth, K., Meyers, B.C., and Stacey, G.**
1439 (2019). Characterization of the spatial and temporal expression of two soybean
1440 miRNAs identifies SCL6 as a novel regulator of soybean nodulation. *Front Plant*
1441 *Sci.* **10**: 475.

1442 **Huisman, R., Hontelez, J., Mysore, K.S., Wen, J., Bisseling, T., and Limpens, E.**
1443 (2016). A symbiosis-dedicated SYNTAXIN OF PLANTS 13II isoform controls the

1444 formation of a stable host–microbe interface in symbiosis. *New Phytol.* **211**:
1445 1338-1351.

1446 **Jardinaud, M.-F. et al.** (2016). A laser dissection-RNAseq analysis highlights the
1447 activation of cytokinin pathways by Nod factors in the *Medicago truncatula* root
1448 epidermis. *Plant Physiol.* **171**: 2256–2276.

1449 **Jensen, E. Ø., Marcker, K.A., Schell, J., and de Bruijn, F.J.** (1988). Interaction of a
1450 nodule specific, *trans*-acting factor with distinct DNA elements in the soybean
1451 leghaemoglobin *lbc₃* 5' upstream region. *EMBO J.* **7**: 1265-1271.

1452 **Jiang, J., Benson, E., Bausek, N., Doggett, K., and White-Cooper, H.** (2007).
1453 Tombola, a tesmin/TSO1-family protein, regulates transcriptional activation in the
1454 *Drosophila* male germline and physically interacts with Always early.
1455 *Development* **1559**: 1549–1559.

1456 **Jin, Y., Liu, H., Luo, D., Yu, N., Dong, W., Wang, C., Zhang, X., Dai, H., Yang, J.,**
1457 **and Wang, E.** (2016). DELLA proteins are common components of symbiotic
1458 rhizobial and mycorrhizal signalling pathways. *Nat. Commun.* **7**: 12433.

1459 **Jin, Y., Chen, Z., Yang, J., Mysore, K.S., Wen, J., Huang, J., Yu, N. and Wang, E.**
1460 (2018). IPD3 and IPD3L function redundantly in rhizobial and mycorrhizal
1461 symbioses. *Front. Plant Sci.* **9**: 267.

1462 **Jørgensen, J., Grønlund, M., Pallisgaard, N., Larsen, K., Marcker, K.A., and**
1463 **Jensen, E.Ø** (1999). A new class of plant homeobox genes is expressed in
1464 specific regions of determinate symbiotic root nodules. *Plant Mol. Biol.* **40**: 65–77.

1465 **Joshi, T., Yan, Z., Libault, M., Jeong, D.H., Park, S., Green, P.J., Sherrier, D.J.,**
1466 **Farmer, A., May, G., Meyers, B.C., Xu, D., and Stacey, G.** (2010). Prediction of
1467 novel miRNAs and associated target genes in *Glycine max*. *BMC Bioinformatics.*
1468 **11**: S14.

1469 **Kaló, P. et al.** (2005). Nodulation signaling in legumes requires NSP2, a member of
1470 the GRAS family of transcriptional regulators. *Science* **308**: 1786-1789.

1471 **Kant, C., Pradhan, S., and Bhatia, S.** (2016). Dissecting the root nodule
1472 transcriptome of chickpea (*Cicer arietinum* L.). PLoS One **11**: e0157908.

1473 **Karmakar, K., Kundu, A., Rizvi, A.Z., Dubois, E., Severac, D., Czernic, P.,**
1474 **Cartieaux, F., and DasGupta, M.** (2019). Transcriptomic analysis with the
1475 progress of symbiosis in ‘crack-entry’ legume *Arachis hypogaea* highlights its
1476 contrast with ‘Infection thread’ adapted legumes. Mol. Plant-Microbe Interact. **32**:
1477 271-285.

1478 **Karunakaran, R., Ramachandran, V.K., Seaman, J.C., East, A.K., Mouhsine, B.,**
1479 **Mauchline, T.H., Prell, J., Skeffington, A., and Poole, P.S.** (2009).
1480 Transcriptomic analysis of *Rhizobium leguminosarum* biovar *viciae* in symbiosis
1481 with host plants *Pisum sativum* and *Vicia cracca*. J. Bacteriol. **191**: 4002–4014.

1482 **Kazmierczak, T., Nagymihaly, M., Lamouche, F., Barrière, Q., Guefrachi, I.,**
1483 **Alunni, B., Ouadghiri, M., Ibijbjen, J., Kondorosi, É., Mergaert, P., and**
1484 **Gruber, V.** (2017). Specific host-responsive associations between *Medicago*
1485 *truncatula* accessions and *Sinorhizobium* strains. Mol. Plant-Microbe Interact.
1486 **30**: 399–409.

1487 **Ke, D., Fang, Q., Chen, C., Zhu, H., Chen, T., Chang, X., Yuan, S., Kang, H., Ma,**
1488 **L., Hong, Z., and Zhang, Z.** (2012). The small GTPase ROP6 interacts with
1489 NFR5 and is involved in nodule formation in *Lotus japonicus*. Plant Physiol. **159**:
1490 131–143.

1491 **Keller, J., Imperial, J., Ruiz-Argüeso, T., Privet, K., Lima, O., Michon-coudouel,**
1492 **S., Biget, M., Salmon, A., Aïnouche, A., and Cabello-Hurtado, F.** (2018). RNA
1493 sequencing and analysis of three *Lupinus* nodulomes provide new insights into
1494 specific host-symbiont relationships with compatible and incompatible
1495 *Bradyrhizobium* strains. Plant Sci. **266**: 102–116.

1496 **Kelly, S., Mun, T., Stougaard, J., Ben, C., and Andersen, S.U.** (2018). Distinct
1497 *Lotus japonicus* transcriptomic responses to a spectrum of bacteria ranging from
1498 symbiotic to pathogenic. Front. Plant Sci. **9**: 1218.

1499 **Kevei, Z., Vinardell, J.M., Kiss, G.B., Kondorosi, A., and Kondorosi, E. (2002).**
1500 Glycine-rich proteins encoded by a nodule-specific gene family are implicated in
1501 different stages of symbiotic nodule development in *Medicago* Spp.. *Mol. Plant-*
1502 *Microbe Interact.* **15**: 922–931.

1503 **Kiirika, L.M., Bergmann, H.F., Schikowsky, C., Wimmer, D., Korte, J., Schmitz,**
1504 **U., Niehaus, K., and Colditz, F. (2012).** Silencing of the Rac1 GTPase *MtROP9*
1505 in *Medicago truncatula* stimulates early mycorrhizal and oomycete root
1506 colonizations but negatively affects rhizobial infection. *Plant Physiol.* **159**: 501–
1507 516.

1508 **Koeppen K, Hampton TH, Jarek M, Scharfe M, Gerber SA, Mielcarz DW,**
1509 **Demers, E.G., Dolben, E.L., Hammond, J.H., Hogan, D.A., and Stanton, B.A.**
1510 (2016) A novel mechanism of host-pathogen interaction through sRNA in
1511 bacterial outer membrane vesicles. *PLoS Pathog.* **12**: e1005672.

1512 **Kondorosi, E., Mergaert, P., and Kereszt, A. (2013).** A paradigm for endosymbiotic
1513 life: Cell differentiation of *Rhizobium* bacteria provoked by host plant factors.
1514 *Annu. Rev. Microbiol.* **67**: 611–628.

1515 **Kudapa, H., Garg, V., Chitikineni, A., and Varshney, R.K. (2019).** The RNA-Seq-
1516 based high resolution gene expression atlas of chickpea (*Cicer arietinum* L.)
1517 reveals dynamic spatio-temporal changes associated with growth and
1518 development. *Plant Cell Environ.* **41**: 2209-2225.

1519 **Kumar, P., Kuscu, C., and Dutta, A. (2016).** Biogenesis and function of transfer
1520 RNA-related fragments (tRFs). *Trends Biochem. Sci.* **41**: 679-689.

1521 **Lamouche, F. et al. (2019).** Transcriptomic dissection of *Bradyrhizobium* sp. strain
1522 ORS285 in symbiosis with *Aeschynomene* spp. inducing different bacteroid
1523 morphotypes with contrasted symbiotic efficiency. *Environ. Microbiol.* **21**: 3244–
1524 3258.

1525 **Lang, C., and Long, S.R. (2015).** Transcriptome analysis of *Sinorhizobium meliloti*
1526 and *Medicago truncatula* symbiosis using nitrogen fixation-deficient nodules.
1527 *Mol. Plant-Microbe Interact.* **28**: 856-868.

1528 **Laporte, P., Satiat-Jeunemaître, B., Velasco, I., Csorba, T., Van de Velde, W.,**
1529 **Campalans, A., Burgyan, J., Arevalo-Rodriguez, M., and Crespi, M.,** (2010).
1530 A novel RNA-binding peptide regulates the establishment of the *Medicago*
1531 *truncatula*–*Sinorhizobium meliloti* nitrogen-fixing symbiosis. *Plant J.* **62**: 24–38.

1532 **Larrainzar, E. et al.** (2015). Deep sequencing of the *Medicago truncatula* root
1533 transcriptome reveals a massive and early interaction between nodulation factor
1534 and ethylene signals. *Plant Physiol.* **169**: 233-265.

1535 **Lauressergues, D., Couzigou, J.-M., San Clemente, H. Martinez, Y., Dunand, Y.,**
1536 **Bécard, G., and Combier, J.-P.** (2015). Primary transcripts of microRNAs
1537 encode regulatory peptides. *Nature* **520**: 90-93.

1538 **Legocki, R.P., and Verma, D.P.S.** (1980). Identification of “Nodule-Specific” host
1539 proteins (nodulins) involved in the development of symbiosis. *Cell* **20**: 153–163.

1540 **Lelandais-Brière, C., Naya, L., Sallet, E., Calenge, F., Frugier, F., Hartmann, C.,**
1541 **Gouzy, J., and Crespi M.** (2009). Genome-wide *Medicago truncatula* small RNA
1542 analysis revealed novel microRNAs and isoforms differentially regulated in roots
1543 and nodules. *Plant Cell* **21**: 2780–2796.

1544 **Lefebvre, B. et al** (2010). A remorin protein interacts with symbiotic receptors and
1545 regulates bacterial infection. *Proc. Natl. Acad. Sci. USA* **107**: 2343-2348.

1546 **Li, H., Wang, W., Mortimer, P.E., Li, R., Li, D., Hyde, K.D., Xu, J., Soltis, D.E., and**
1547 **Chen, Z.** (2015). Large-scale phylogenetic analyses reveal multiple gains of
1548 actinorhizal nitrogen-fixing symbioses in angiosperms associated with climate
1549 change. *Sci. Rep.* **5**: 14023.

1550 **Liang, P., Stratil, T.F., Popp, C., Marin, M., Folgmann, J., Mysore, K.S., Wen, J.,**
1551 **and Ott, T.** (2018). Symbiotic root infections in *Medicago truncatula* require
1552 remorin-mediated receptor stabilization in membrane nanodomains. *Proc. Natl.*
1553 *Acad. Sci. USA* **115**: 5289-5294.

1554 **Liang, Y., Cao, Y., Tanaka, K., Thibivilliers, S., Wan, J., Choi, J., ho Kang, C.,**
1555 **Qiu, J., and Stacey, G.** (2013). Nonlegumes respond to rhizobial Nod factors by
1556 suppressing the innate immune response. *Science* **341**: 1384–1387.

1557 **Libault, M., Joshi, T., Takahashi, K., Hurley-Sommer, A., Puricelli, K., Blake, S.,**
1558 **Finger, R.E., Taylor, C.G., Xu, D., Nguyen, H.T., and Stacey, G.** (2009a).
1559 Large-scale analysis of putative soybean regulatory gene expression identifies a
1560 *Myb* gene involved in soybean nodule development. *Plant Physiol.* **151**: 1207–
1561 1220.

1562 **Libault, M., Joshi, T., Benedito, V.A., Xu, D., Udvardi, M.K., and Stacey, G.**
1563 (2009b). Legume transcription factor genes: What makes legumes so special?
1564 *Plant Physiol.* **151**: 991–1001.

1565 **Libault, M., Farmer, A., Brechenmacher, L., Drnevich, J., Langley, R.J., Bilgin,**
1566 **D.D., Radwan, O., Neece, DJ, Clough, S.J., May, G.D., and Stacey, G.** (2010).
1567 Complete transcriptome of the soybean root hair cell, a single-cell model, and its
1568 alteration in response to *Bradyrhizobium japonicum* infection. *Plant Physiol.* **152**:
1569 541-552.

1570 **Libault, M.** (2018). Transcriptional reprogramming of legume genomes: Perspective
1571 and challenges associated with single-cell and single cell-type approaches
1572 during nodule development. *Front Plant Sci.* **9**: 1600.

1573 **Liese, R., Schulze, J., and Cabeza, R.A.** (2017). Nitrate application or P deficiency
1574 induce a decline in *Medicago truncatula* N₂-fixation by similar changes in the
1575 nodule transcriptome. *Sci. Rep.* **7**: 46264.

1576 **Limpens, E., Mirabella, R., Fedorova, E., Franken, C., Franssen, H., Bisseling, T.**
1577 **and Geurts, R.** (2005). Formation of organelle-like N₂-fixing symbiosomes in
1578 legume root nodules is controlled by *DMI2*. *Proc. Natl. Acad. USA* **102**: 10375–
1579 10380.

1580 **Limpens, E., Ivanov, S., Esse, W. Van, Voets, G., Fedorova, E., and Bisseling, T.**
1581 (2009). *Medicago* N₂-fixing symbiosomes acquire the endocytic identity marker
1582 Rab7 but delay the acquisition of vacuolar identity. *Plant Cell* **21**: 2811–2828.

1583 **Limpens, E., Moling, S., Hooiveld, G., Pereira, P.A., Bisseling, T., Becker, J.D.,**
1584 **and Küster, H.** (2013). Cell- and tissue-specific transcriptome analyses of
1585 *Medicago truncatula* root nodules. PLoS One **8**: e64377.

1586 **Liu, J., Miller, S.S., Graham, M., Bucciarelli, B., Catalano, C.M., Sherrier, D.J.,**
1587 **Samac, D.A., Ivashuta, S., Fedorova, M., Matsumoto, P., Gantt, J.S., Vance,**
1588 **C.P.** (2006). Recruitment of novel calcium-binding proteins for root nodule
1589 symbiosis in *Medicago truncatula*. Plant Physiol. **141**:167-77.

1590 **Liu, H., Zhang, C., Yang, J., Yu, N., and Wang, E.** (2018). Hormone modulation of
1591 legume-rhizobial symbiosis. J. Integr. Plant Biol. **60**: 632-648.

1592 **Liu, C.W. et al.** (2019a). NIN acts as a network hub controlling a growth module
1593 required for rhizobial infection. Plant Physiol. **179**: 1704-1722.

1594 **Liu, J., Rutten, L., Limpens, E., van der Molen, T.V., van Velzen, R.V., Chen, R.,**
1595 **Chen, Y., Geurts R., Kohlen W., Kulikova O., and Bisseling T.** (2019b). A
1596 remote cis-regulatory region is required for *NIN* expression in the pericycle to
1597 initiate nodule primordium formation in *Medicago truncatula*. Plant Cell **31**: 68-
1598 83.

1599 **Magne, K., George, J., Berbel Tornero, A., Broquet, B., Madueño, F., Andersen,**
1600 **S.U., and Ratet, P.** (2018). *Lotus japonicus* *NOOT-BOP-COCH-LIKE1* is
1601 essential for nodule, nectary, leaf and flower development. Plant J. **94**: 880-894.

1602 **Markmann, K., Giczey, G., and Parniske, M.** (2008). Functional adaptation of a
1603 plant receptor-kinase paved the way for the evolution of intracellular root
1604 symbioses with bacteria. PLoS Biol. **6**: e68.

1605 **Markmann, K., and Parniske, M.** (2009). Evolution of root endosymbiosis with
1606 bacteria: how novel are nodules? Trends Plant Sci. **14**: 77–86.

1607 **Marsh, J., Rakocevic, A., Mitra, R.M., Brocar L., Sun, J., Eschstruth, A., Long,**
1608 **S.R., Schultze, M., Ratet, P., and Oldroyd, G.E.D.** (2007). *Medicago truncatula*
1609 *NIN* is essential for rhizobial-independent nodule organogenesis induced by

1610 autoactive calcium/calmodulin-dependent protein kinase. *Plant Physiol.* **144**:
 1611 324-335.

1612 **Martínez-Hidalgo, P., and Hirsch, A.M.** (2017). The nodule microbiome: N₂-fixing
 1613 rhizobia do not live alone. *Phytobiomes J.* **1**: 70-82.

1614 **Maunoury, N. et al.** (2010). Differentiation of symbiotic cells and endosymbionts are
 1615 coupled to two transcriptome-switches in *Medicago truncatula* nodulation. *PLoS*
 1616 *One* **5**: e9519.

1617 **Mecchia, M.A., Santos-fernandez, G., Duss, N.N., Somoza, S.C., Boisson-**
 1618 **dernier, A., Gagliardini, V., Martínez-bernardini, A., Fabrice, T.N., Ringli, C.,**
 1619 **Muschietti, J.P., and Grossniklaus, U.** (2017). RALF4/19 peptides interact with
 1620 LRX proteins to control pollen tube growth in *Arabidopsis*. *Science* **358**: 1600–
 1621 1603.

1622 **Mergaert, P.** (2018). Role of antimicrobial peptides in controlling symbiotic bacterial
 1623 populations. *Nat. Prod. Rep.* **35**: 336-356.

1624 **Mergaert, P., Nikovics, K., Kelemen, Z., Maunoury, N., Vaubert, D., Kondorosi,**
 1625 **A., and Kondorosi, E.** (2003). A novel family in *Medicago truncatula* consisting
 1626 of more than 300 nodule-specific genes coding for small, secreted polypeptides
 1627 with conserved cysteine motifs. *Plant Physiol.* **132**: 161–173.

1628 **Messinese, E., Mun, J.H., Yeun, L.H., Jayaraman, D., Rougé, P., Barre, A.,**
 1629 **Lougnon, G., Schornack, S., Bono, J.J., Cook, D.R., and Ané, J.M.** (2007). A
 1630 novel nuclear protein interacts with the symbiotic DMI3 calcium- and calmodulin-
 1631 dependent protein kinase of *Medicago truncatula*. *Mol. Plant-Microbe Interact.*
 1632 **20**: 912–921.

1633 **Metz, B.A., Welters, P., Hoffmann, H.J., Jensen, E.Ø, Schell, J., and de Bruijn,**
 1634 **F.J.** (1988). Primary structure and promoter analysis of leghemoglobin genes of
 1635 the stem-nodulated tropical legume *Sesbania rostrata*: conserved coding
 1636 sequences, cis-elements and trans-acting factors. *Mol Gen Genet.* **214**: 181-91.

1637 **Middleton, P.H. et al.** (2007). An ERF transcription factor in *Medicago truncatula* that
 1638 is essential for Nod factor signal transduction. *Plant Cell* **19**: 1221-1234.

- 1639 **Miller, S.S., Driscoll, B.T., Gregerson, R.G., Gantt, J.S., and Vance, C.P.** (1998).
 1640 Alfalfa malate dehydrogenase (MDH): molecular cloning and characterization of
 1641 five different forms reveals a unique nodule-enhanced MDH. *Plant J.* **15**: 173–
 1642 184.
- 1643 **Moling, S., Pietraszewska-Bogiel, A., Postma, M., Fedorova, E., Hink, M.A.,**
 1644 **Limpens, E., Gadella, T.W., and Bisseling, T.** (2014). Nod factor receptors form
 1645 heteromeric complexes and are essential for intracellular infection in *Medicago*
 1646 nodules. *Plant Cell* **26**: 4188-4199.
- 1647 **Montiel, J., Nava, N., Cárdenas, L., Sánchez-López, R., Arthikala, M.-K.,**
 1648 **Santana, O., Sánchez, F., and Quinto, C.** (2013). A *Phaseolus vulgaris* NADPH
 1649 oxidase gene is required for root infection by rhizobia. *Plant Cell Physiol.* **53**:
 1650 1751–1767.
- 1651 **Montiel, J., Downie, J.A., Farkas, A., Bihari, P., Herczeg, R., Balint, B., Mergaert,**
 1652 **P., Kereszt, A., and Kondorosi, E.** (2017). Morphotype of bacteroids in different
 1653 legumes correlates with the number and type of symbiotic NCR peptides. *Proc.*
 1654 *Natl. Acad. Sci. USA* **114**: 5041-5046.
- 1655 **Moreau, S., Verdenaud, M., Ott, T., Letort, S., de Billy, F., Niebel, A., Gouzy, J.,**
 1656 **de Carvalho-Niebel, F., Gamas, P.** (2011). Transcription reprogramming during
 1657 root nodule development in *Medicago truncatula*. *PLoS One* **6**: e16463.
- 1658 **Morieri, G., Martinez, E.A., Jarynowski, A., Driguez, H., Morris, R., Oldroyd, G.E.,**
 1659 **and Downie J.A.** (2013). Host-specific Nod-factors associated with *Medicago*
 1660 *truncatula* nodule infection differentially induce calcium influx and calcium spiking
 1661 in root hairs. *New Phytol.* **200**: 656-662.
- 1662 **Murphy, E. and De Smet, I.** (2014). Understanding the RALF family: a tale of many
 1663 species. *Trends Plant Sci.* **19**: 664–671.
- 1664 **Nagymihály, M., Veluchamy, A., Györgypál, Z., Ariel, F., Jégu, T., Benhamed, M.,**
 1665 **Szűcs, A., Kereszt, A., Mergaert, P., and Kondorosi, É.** (2017). Ploidy-
 1666 dependent changes in the epigenome of symbiotic cells correlate with specific
 1667 patterns of gene expression. *Proc. Natl. Acad. Sci. USA* **114**: 4543–4548.

1668 **Nallu, S., Silverstein, K.A.T., Samac, D.A., Bucciarelli, B., Vance, C.P., and**
1669 **VandenBosch K.A.** (2013). Regulatory patterns of a large family of defensin-like
1670 genes expressed in nodules of *Medicago truncatula*. PLoS One **8**: e60355.

1671 **Nallu, S., Silverstein, K.A.T., Zhou, P., Young, N.D., and VandenBosch, K.A.**
1672 (2014). Patterns of divergence of a large family of nodule cysteine-rich peptides
1673 in accessions of *Medicago truncatula*. Plant J. **78**: 697–705.

1674 **Nelson, M.S., and Sadowsky, M.J.** (2015). Secretion systems and signal exchange
1675 between nitrogen-fixing rhizobia and legumes. Front. Plant Sci. **6**: 491.

1676 **Nouwen, N., Arrighi, J.F., Cartieaux, F., Chaintreuil, C., Gully, D., Klopp, C., and**
1677 **Giraud, E.** (2017). The role of rhizobial (NifV) and plant (FEN1) homocitrate
1678 synthases in *Aeschynomene*/photosynthetic *Bradyrhizobium* symbiosis. Sci.
1679 Rep. **7**: 448.

1680 **Nova-franco, B., Íñiguez, L.P., Valdés-lópez, O., Alvarado-affantranger, X., Leija,**
1681 **A., Fuentes, S.I., Ramírez, M., Paul, S., Reyes, J.L., Girard, L., and**
1682 **Hernández, G.** (2015). The Micro-RNA172c-APETALA2-1 node as a key
1683 regulator of the common bean-*Rhizobium etli* nitrogen fixation symbiosis. Plant
1684 Physiol. **168**: 273–291.

1685 **O’Rourke, J.A. et al.** (2014). An RNA-Seq based gene expression atlas of the
1686 common bean. BMC Genomics **15**: 866

1687 **O’Rourke, J.A. et al.** (2015). The *Medicago sativa* gene index 1.2: a web-accessible
1688 gene expression atlas for investigating expression differences between
1689 *Medicago sativa* subspecies. BMC Genomics **16**: 502.

1690 **Oldroyd, G.E.D.** (2013). Speak, friend, and enter: signalling systems that promote
1691 beneficial symbiotic associations in plants. Nat. Rev. Microbiol. **11**: 252-263.

1692 **Oldroyd, G.E.D., and Dixon, R.** (2014). Biotechnological solutions to the nitrogen
1693 problem. Curr. Opin. Biotechnol. **26**: 19–24.

1694 **Ott, T.** (2017). Membrane nanodomains and microdomains in plant–microbe
1695 interactions. Curr. Opin. Plant Biol. **40**: 82–88.

1696 **Ovchinnikova, E. et al.** (2011). IPD3 controls the formation of nitrogen-fixing
1697 symbiosomes in pea and *Medicago* spp.. Mol. Plant-Microbe Interact. **24**: 1333–
1698 1344.

1699 **Paez Valencia, J., Goodman, K., and Otegui, M.S.** Endocytosis and endosomal
1700 trafficking in plants. Annu. Rev. Plant Biol. **67**: 309-335.

1701 **Pan, H., Oztas, O., Zhang, X., Wu, X., Stonoha, C., Wang, E., Wang, B., and**
1702 **Wang, D.** (2016). A symbiotic SNARE protein generated by alternative
1703 termination of transcription. Nat. Plants **2**: 15197.

1704 **Pan, H., and Wang, D.** (2018). *Medicago* plants control nodulation by regulating
1705 proteolysis of the receptor-like kinase DMI2. Plant Physiol. **177**: 792-802.

1706 **Parniske, M.** (2018). Uptake of bacteria into living plant cells, the unifying and
1707 distinct feature of the nitrogen-fixing root nodule symbiosis. Curr. Opin. Plant
1708 Biol. **44**: 164-174.

1709 **Pecrix, Y. et al.** (2018). Whole-genome landscape of *Medicago truncatula* symbiotic
1710 genes. Nat. Plants **4**: 1017-1025.

1711 **Peláez, P., Trejo, M.S., Iñiguez, L.P., Estrada-Navarrete, G., Covarrubias, A.A.,**
1712 **Reyes, J.L. and Sanchez, F.** (2012). Identification and characterization of
1713 microRNAs in *Phaseolus vulgaris* by high-throughput sequencing. BMC
1714 Genomics **13**: 83.

1715 **Peng, Z., Liu, F., Wang, L., Zhou, H., Paudel, D., Tan, L., Maku, J., Gallo, M., and**
1716 **Wang, J.** (2017). Transcriptome profiles reveal gene regulation of peanut
1717 (*Arachis hypogaea* L.) nodulation. Sci. Rep. **7**: 40066.

1718 **Persson, T. et al.** (2015). *Candidatus* Frankia datiscae Dg1, the actinobacterial
1719 microsymbiont of *Datisca glomerata*, expresses the canonical *nod* genes
1720 *nodABC* in symbiosis with its host. PLoS One **10**: e0127630.

1721 **Pierre, O., Hopkins, J., Combier, M., Baldacci, F., Engler, G., Brouquisse, R.,**
1722 **Hérouart, D., and Boncompagni, E.** (2014). Involvement of papain and

1723 legumain proteinase in the senescence process of *Medicago truncatula* nodules.
 1724 New Phytol. **202**: 849-863.

1725 **Poole, P., Ramachandran, V., and Terpolilli, J.** (2018). Rhizobia: from saprophytes
 1726 to endosymbionts. Nat. Rev. Microbiol. **16**: 291-303.

1727 **Ren, B., Wang, X., Duan, J., and Ma, J.** (2019). Rhizobial tRNA-derived small RNAs
 1728 are signal molecules regulating plant nodulation. Science **365**: 919-922.

1729 **Price, M.N., Deutschbauer, A.M., Skerker, J.M., Wetmore, K.M., Ruths, T., Mar,**
 1730 **J.S., Kuehl, J. V, Shao, W., and Arkin, A.P.** (2013). Indirect and suboptimal
 1731 control of gene expression is widespread in bacteria. Mol. Syst. Biol. **9**: 660.

1732 **Pislariu, C.I., Sinharoy, S., Torres-Jerez, I., Nakashima, J., Blancaflor, E.B., and**
 1733 **Udvardi, M.K.** (2019). The nodule-specific PLAT domain protein NPD1 is
 1734 required for nitrogen-fixing symbiosis. Plant Physiol. **180**: 1480-1497.

1735 **Qiao, Z., Brechenmacher, L., Smith, B., Strout, G.W., Mangin, W., Taylor, C.,**
 1736 **Russell, S.D., Stacey, G., and Libault, M.** (2017). The GmFWL1 (FW2-2-like)
 1737 nodulation gene encodes a plasma membrane microdomain-associated protein.
 1738 Plant Cell Environ. **40**: 1442-1455.

1739 **Richter, H.E., Sandal, N.N., Marcker, K.A., and Sengupta-Gopalan, C.** (1991).
 1740 Characterization and genomic organization of a highly expressed late nodulin
 1741 gene subfamily in soybeans. Mol. Gen. Genet. **20**: 445–452.

1742 **Rodrigues, S.G., Stickels, R.R., Goeva, A., Martin, C.A., Murray, E., Vanderburg,**
 1743 **C.R., Welch, J., Chen, L.M., Chen, F., and Macosko, E.Z.** (2019). Slide-seq: A
 1744 scalable technology for measuring genome-wide expression at high spatial
 1745 resolution. Science **363**: 1463-1467.

1746 **Roux, B. et al.** (2014). An integrated analysis of plant and bacterial gene expression
 1747 in symbiotic root nodules using laser-capture microdissection coupled to RNA
 1748 sequencing. Plant J. **77**: 817–837.

1749 **Roy, S., Liu, W., Nandety, R.S., Crook, A., Mysore, K.S., Pislariu, C.I., Frugoli, J.,**
 1750 **Dickstein, R., and Udvardi, M.K.** (2019). Celebrating 20 years of genetic

discoveries in legume nodulation and symbiotic nitrogen fixation. *Plant Cell* in press.

Salgado, M.G., van Velzen, R., Nguyen, T.V., Battenberg, K., Berry, A.M., Lundin, D., and Pawlowski, K. (2018). Comparative analysis of the nodule transcriptomes of *Ceanothus thyrsiflorus* (Rhamnaceae, Rosales) and *Datisca glomerata* (Datiscaceae, Cucurbitales). *Front. Plant Sci.* **9**: 1629.

Sandal, N.N., Bojsen, K., and Marcker, K.J. (1987). A small family of nodule specific genes from soybean. *Nucleic Acids Res.* **15**: 1507–1519.

Satgé, C., Moreau, S., Sallet, E., Lefort, G., Auriac, M.-C., Remblière, C., Cottret, L., Gallardo, K., Noirot, C., Jardinaud, M.-F., and Gamas, P. (2016). Reprogramming of DNA methylation is critical for nodule development in *Medicago truncatula*. *Nat. Plants* **2**: 16166.

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

Schiessl, K. et al. (2019). *NODULE INCEPTION* recruits the lateral root developmental program for symbiotic nodule organogenesis in *Medicago truncatula*. *Curr Biol.* doi:10.1016/j.cub.2019.09.005.

Seabra, A.R., Pereira, P.A., Becker, J.D., and Carvalho, H.G. (2012). Inhibition of glutamine synthetase by phosphinothricin leads to transcriptome reprogramming in root nodules of *Medicago truncatula*. *Mol. Plant-Microbe Interact.* **25**: 976–992.

Senovilla, M., Castro-Rodríguez, R., Abreu, I., Escudero, V., Kryvoruchko, I., Udvardi, M.K., Imperial, J., and González-guerrero, M. (2018). *Medicago truncatula* copper transporter 1 (MtCOPT1) delivers copper for symbiotic nitrogen fixation. *New Phytol.* **218**: 696-709.

Severin, A.J. et al. (2010). RNA-Seq Atlas of *Glycine max*: A guide to the soybean transcriptome. *BMC Plant Biol.* **10**: 160.

Shubin, N., Tabin, C., and Carroll, S. (2009). Deep homology and the origins of evolutionary novelty. *Nature* **457**: 818-823.

1779 **Sijacic, P., Wang, W., and Liu, Z.** (2011). Recessive antimorphic alleles overcome
1780 functionally redundant loci to reveal TSO1 function in *Arabidopsis* flowers and
1781 meristems. PLoS Genet. **7**: e1002352.

1782 **Singh, S., Katzer, K., Lambert, J., Cerri, M., and Parniske, M.** (2014). CYCLOPS,
1783 A DNA-binding transcriptional activator, orchestrates symbiotic root nodule
1784 development. Cell Host Microbe **15**: 139-152.

1785 **Sinharoy, S., Torres-Jerez, I., Bandyopadhyay, K., Kereszt, A., Pislariu, C.I.,**
1786 **Nakashima, J., Benedito, V.A., Kondorosi, E., and Udvardi, M.K.** (2013). The
1787 C2H2 transcription factor REGULATOR OF SYMBIOSOME DIFFERENTIATION
1788 represses transcription of the secretory pathway gene VAMP721a and promotes
1789 symbiosome development in *Medicago truncatula*. Plant Cell **25**: 3584–3601.

1790 **Smit, P., Raedts, J., Portyanko, V., Debellé, F., Gough, C., Bisseling, T., and**
1791 **Geurts, R.** (2005). NSP1 of the GRAS protein family is essential for rhizobial
1792 Nod factor-induced transcription. Science **308**: 1789-1791.

1793 **Soltis, D.E., Soltis, P.S., Morgan, D.R. Swensen, S.M., Mullin, B.C., Dowd, J.M.,**
1794 **Martin, P.G.** (1995). Chloroplast gene sequence data suggest a single origin of
1795 the predisposition for symbiotic nitrogen fixation in angiosperms. Proc. Natl.
1796 Acad. Sci. USA **92**: 2647–2651.

1797 **Soyano, T., Kouchi, H., Hirota, A., and Hayashi, M.** (2013) Nodule inception
1798 directly targets NF-Y subunit genes to regulate essential processes of root
1799 nodule development in *Lotus japonicus*. PLoS Genet. **9**: e1003352.

1800 **Soyano, T., and Hiyashi, M.** (2014). Transcriptional networks leading to symbiotic
1801 nodule organogenesis. Curr. Opin. Plant Biol. **20**: 146-54.

1802 **Ståhl, P.L. et al.** (2016). Visualization and analysis of gene expression in tissue
1803 sections by spatial transcriptomics. Science **353**: 78-82.

1804 **Starker, C.G., Parra-Colmenares, A.L., Smith, L., Mitra, R.M., and Long, S.R.**
1805 (2006). Nitrogen fixation mutants of *Medicago truncatula* fail to support plant and
1806 bacterial symbiotic gene expression. Plant Physiol. **140**: 671-680.

1807 **Stokstad, E.** (2016). The nitrogen fix. *Science* **353**: 1225–1228.

1808 **Stonoha-Arther, C., and Wang, D.** (2018). Though love: accommodating
 1809 intracellular bacteria through directed secretion of antimicrobial peptides during
 1810 the nitrogen-fixing symbiosis. *Curr. Opin. Plant Biol.* **44**: 155–163.

1811 **Stougaard, J., Sandal, N.N., Groen, A., Kühle, A., and Marcker, K.A.** (1987).
 1812 Analysis of the soybean leghemoglobin *lbc3* gene: regulatory elements required
 1813 for promoter activity and organ specificity. *EMBO J.* **6**: 3565–3569.

1814 **Sturms, R., Kakar, S., Trent III, J., and Hargrove, M.S.** (2010). *Trema* and
 1815 *Parasponia* hemoglobins reveal convergent evolution of oxygen transport in
 1816 plants. *Biochemistry* **49**: 4085–4093.

1817 **Subramanian, S., Fu, Y., Sunkar, R., Barbazuk, W.B., Zhu, J.K., and Yu, O.**
 1818 (2008). Novel and nodulation-regulated microRNAs in soybean roots. *BMC*
 1819 *Genomics.* **9**: 160.

1820 **Sugawara, M. et al.** (2013). Comparative genomics of the core and accessory
 1821 genomes of 48 *Sinorhizobium* strains comprising five genospecies. *Genome*
 1822 *Biol.* **14**: R17.

1823 **Sugiyama, A., Saida, Y., Yoshimizu, M., Takanashi, K., Sosso, D., Frommer,**
 1824 **W.B., and Yazaki, K.** (2017). Molecular characterization of LjSWEET3, a sugar
 1825 transporter in nodules of *Lotus japonicus*. *Plant Cell Physiol.* **58**: 298–306.

1826 **Sullivan, D., Brisson, N., Goodchild, B., and Verma, D.P.S.** (1981). Molecular
 1827 cloning and organization of two leghaemoglobin genomic sequences of soybean.
 1828 *Nature* **289**: 516–518.

1829 **Suzaki, T., Ito, M., Yoro, E., Sato, S., Hirakawa, H., Takeda, N., and Kawaguchi,**
 1830 **M.** (2014). Endoreduplication-mediated initiation of symbiotic organ development
 1831 in *Lotus japonicus*. *Development* **141**: 2441–2445.

1832 **Svistoonoff, S. et al.** (2013). The independent acquisition of plant root nitrogen-
 1833 fixing symbiosis in Fabids recruited the same genetic pathway for nodule
 1834 organogenesis. *PLoS One* **8**: e64515.

1835 **Szabados, L., Ratet, P., Grunenber, B., and de Bruijn, F.J.** (1990). Functional
1836 analysis of the *Sesbania rostrata* leghemoglobin *glb3* gene 5'-upstream region in
1837 transgenic *Lotus corniculatus* and *Nicotiana tabacum* plants. *Plant Cell* **2**: 973-
1838 986.

1839 **Szczyglowski, K., Szabados, L., Fujimoto, S.Y., Silver, D., and de Bruijn, F.J.**
1840 (1994). Site-specific mutagenesis of the nodule-infected cell expression (NICE)
1841 element and the AT-rich element ATRE-BS2 of the *Sesbania rostrate*
1842 leghemoglobin *glb₃* promoter. *Plant Cell* **6**: 317–332.

1843 **Takanashi, K., Takahashi, H., Sakurai, N., Sugiyama, A., Suzuki, H., Shibata, D.,**
1844 **Nakazono, M., and Yazaki, K.** (2012). Tissue-specific transcriptome analysis in
1845 nodules of *Lotus japonicus*. *Mol. Plant-Microbe Interact.* **25**: 869–876.

1846 **Taleski, M., Imin, N., and Djordjevic, M.A.** (2018). CEP peptide hormones: key
1847 players in orchestrating nitrogen-demand signalling, root nodulation, and lateral
1848 root development. *J. Exp. Bot.* **69**: 1829-1836.

1849 **Tautz, D. and Domazet-lošo, T.** (2011). The evolutionary origin of orphan genes.
1850 *Nat. Rev. Genet.* **12**: 692–702.

1851 **Temple, S.J., Vance, C.P., and Gantt, J.S.** (1998). Glutamate synthase and
1852 nitrogen assimilation. *Trends Plant Sci.* **3**: 51-56.

1853 **Tesfaye, M. et al.** (2013). Spatio-temporal expression patterns of *Arabidopsis*
1854 *thaliana* and *Medicago truncatula* defensin-like genes. *PLoS One* **8**: e58992.

1855 **Teulet, A., et al.** (2019). The Bradyrhizobial type III effector BtnA confers the ability
1856 to form nodules in legumes. *Proc. Natl. Acad. Sci. USA*.
1857 doi:10.1073/pnas.1904456116.

1858 **Timmers, A.C.J., Auriac, M., and Truchet, G.** (1999). Refined analysis of early
1859 symbiotic steps of the Rhizobium-Medicago interaction in relationship with
1860 microtubular cytoskeleton rearrangements. *Development* **126**: 3617–3628.

1861 **Trujillo, D.I., Silverstein, K.A., and Young, N.D.** (2014). Genomic characterization
 1862 of the LEED..PEEDs, a gene family unique to the *Medicago* lineage. *G3* **4**: 2003-
 1863 2012.

1864 **Trujillo, D.I., Silverstein, K.A.T., and Young, N.D.** (2019). Nodule-specific PLAT
 1865 domain proteins are expanded in the *Medicago* lineage and required for
 1866 nodulation. *New Phytol.* **222**: 1538–1550.

1867 **Tsikou, D., Yan, Z., Holt, D.B., Abel, N.B., Reid, D.E., Madsen, L.H., Bhasin H.,**
 1868 **Sexauer, M., Stougaard, J., and Markmann, K.** (2018). Systemic control of
 1869 legume susceptibility to rhizobial infection by a mobile microRNA. *Science* **362**:
 1870 233-236.

1871 **Udvardi, M. and Poole, P.S.** (2013). Transport and metabolism in legume-rhizobia
 1872 symbioses. *Annu. Rev. Plant Biol.* **64**: 781–805.

1873 **van Brussel, A.A.N., Bakhuizen, R., van Spronsen, P.C., Spaink, H.P., Tak, T.,**
 1874 **Lugtenberg, B.J.J., and Kijne, J.W.** (1992). Induction of pre-infection thread
 1875 structures in the leguminous host plant by mitogenic lipo-oligosaccharides of
 1876 *Rhizobium*. *Science* **257**: 70–72.

1877 **Van de Velde, W., Pérez Guerra, J.C., De Keyser, A., De Rycke, R., Rombauts,**
 1878 **S., Maunoury, N., Mergaert, P., Kondorosi, E., Holsters, M., and**
 1879 **Goormachtig, S.** (2006). Aging in legume symbiosis. A molecular view on
 1880 nodule senescence in *Medicago truncatula*. *Plant Physiol.* **141**: 711–720.

1881 **van Velzen, R. et al.** (2018). Comparative genomics of the nonlegume *Parasponia*
 1882 reveals insights into evolution of nitrogen-fixing rhizobium symbioses. *Proc. Natl.*
 1883 *Acad. Sci. USA* **115**: E4700-E4709.

1884 **van Velzen, R., Doyle, J.J., and Geurts, R.** (2019). A resurrected scenario: single
 1885 gain and massive loss of nitrogen-fixing nodulation. *Trends Plant Sci.* **24**: 49-57.

1886 **Van Zeijl, A., Op den Camp, R.H.M., Deinum, E.E., Charnikhova, T., Franssen,**
 1887 **H., Op den Camp, H.J.M., Bouwmeester, H., Kohlen, W., Bisseling, T., and**
 1888 **Geurts, R.** (2015). Rhizobium lipo-chitooligosaccharide signaling triggers

1889 accumulation of cytokinins in *Medicago truncatula* roots. Mol. Plant **8**: 1213–
1890 1226.

1891 **van Zeijl, A., Wardhani, T.A.K., Seifi Kalhor, M., Rutten, L., Bu, F., Hartog, M.,**
1892 **Linders, S., Fedorova, E.E., Bisseling, T., Kohlen, W., and Geurts, R. (2018).**
1893 CRISPR/Cas9-mediated mutagenesis of four putative symbiosis genes of the
1894 tropical tree *Parasponia andersonii* reveals novel phenotypes. Front. Plant Sci.
1895 **9**: 284.

1896 **Verdier, J. et al. (2013).** A regulatory network-based approach dissects late
1897 maturation processes related to the acquisition of desiccation tolerance and
1898 longevity of *Medicago truncatula* seeds. Plant Physiol. **163**: 757–774.

1899 **Vernié, T., Moreau, S., de Billy, F., Plet, J., Combier, J.-P., Rogers, C. Oldroyd,**
1900 **G., Frugier, F., Niebel, A., and Gamas, P. (2008).** EFD Is an ERF transcription
1901 factor involved in the control of nodule number and differentiation in *Medicago*
1902 *truncatula*. Plant Cell **20**: 2696–2713.

1903 **Vernié, T., Kim, J., Frances, L., Ding, Y., Sun, J., Guan, D., Niebel, A., Gifford,**
1904 **M.L., de Carvalho-Niebel, F., and Oldroyd, G.E.D. (2015).** The NIN
1905 transcription factor coordinates diverse nodulation programs in different tissues
1906 of the *Medicago truncatula* root. Plant Cell **27**: 3410–3424.

1907 **Vinardell, J.M., Fedorova, E., Cebolla, A., Kevei, Z., Horváth, G., Kelemen, Z.,**
1908 **Tarayre, S., Roudier, F., Mergaert, P., Kondorosi, A., and Kondorosi, E.**
1909 (2003). Endoreduplication mediated by the anaphase-promoting complex
1910 activator CCS52A is required for symbiotic cell differentiation in *Medicago*
1911 *truncatula* nodules. Plant Cell **15**: 2093–2105.

1912 **Wang, H., Moore, M.J., Soltis, P.S., Bell, C.D., Brockington, S.F., Alexandre, R.,**
1913 **Davis, C.C., Latvis, M., Manchester, S.R., and Soltis, D.E. (2009).** Rosid
1914 radiation and the rapid rise of angiosperm-dominated forests. Proc. Natl. Acad.
1915 Sci. USA **106**: 3853–3858.

- 1916 **Wang, D., Griffitts, J., Starker, C., Fedorova, E., Limpens, E., Ivanov, S.,**
 1917 **Bisseling, T., and Long, S.** (2010). A nodule-specific protein secretory pathway
 1918 required for nitrogen-fixing symbiosis. *Science* **327**: 1126–1129.
- 1919 **Wang, Y., Wang, L., Zou, Y., Chen, L., Cai, Z., Zhang, S., Zhao, F., Tian, Y.,**
 1920 **Jiang, Q., Ferguson, B.J., Gresshoff, P.M., and Li, X.** (2014). Soybean
 1921 miR172c targets the repressive AP2 transcription factor NNC1 to activate
 1922 *ENOD40* expression and regulate nodule initiation. *Plant Cell* **26**: 4782–4801.
- 1923 **Wang, Y., Li, K., Chen, L., Zou, Y., Liu, H., Tian, Y., Li, D., Wang, R., Zhao, F.,**
 1924 **Ferguson, B.J., Gresshoff, P.M., and Li, X.** (2015). MicroRNA167-directed
 1925 regulation of the auxin response factors GmARF8a and GmARF8b is required
 1926 for soybean nodulation and lateral root development. *Plant Physiol.* **168**: 984-
 1927 999.
- 1928 **Wang, C. et al.** (2016). *NODULES WITH ACTIVATED DEFENSE 1* is required for
 1929 maintenance of rhizobial endosymbiosis in *Medicago truncatula*. *New Phytol.*
 1930 **212**: 176–191.
- 1931 **Wang L., Sun Z., Su C., Wang Y., Yan Q., Chen J., Ott T., and Li X.** (2019). A
 1932 GmNINa-miR172c-NNC1 regulatory network coordinates the nodulation and
 1933 autoregulation of nodulation pathways in soybean. *Mol. Plant.*
 1934 doi:10.1016/j.molp.2019.06.002.
- 1935 **Werner, G.D.A., Cornwell, W.K., Sprent, J.I., Kattge, J., and Kiers, E.T.** (2014). A
 1936 single evolutionary innovation drives the deep evolution of symbiotic N₂-fixation
 1937 in angiosperms. *Nat. Commun.* **5**: 4087.
- 1938 **Wildermuth, M.C., Steinwand, M.A., McRae, A.G., Jaenisch, J., and Chandran,**
 1939 **D.** (2017). Adapted biotroph manipulation of plant cell ploidy. *Annu. Rev.*
 1940 *Phytopathol.* **55**: 537-564.
- 1941 **Wu Z., Wang M., Yang S., Chen S., Chen X., Liu C., Wang S., Wang H., Zhang B.,**
 1942 **Liu H., Qin R., and Wang X.** (2019). A global coexpression network of soybean
 1943 genes gives insight into the evolution of nodulation in non-legumes and
 1944 legumes. *New Phytol.* doi:10.1111/nph.15845.

1945 **Xiao, T.T., Schilderink, S., Moling, S., Deinum, E.E., Kondorosi, E., Franssen, H.,**
 1946 **Kulikova, O., Niebel A., and Bisseling, T.** (2014). Fate map of *Medicago*
 1947 *truncatula* root nodules. *Development* **141**: 3517–3528.

1948 **Yan, Z., Shakhawat Hossain, M., Arikiti, S., Valdés-López, O., Zhai, J., Wang, J.,**
 1949 **Libault, M., Ji, T., Qiu, L., Meyers, B.C., and Stacey, G.** (2015). Identification
 1950 of microRNAs and their mRNA targets during soybean nodule development:
 1951 functional analysis of the role of miR393j-3p in soybean nodulation. *New Phytol.*
 1952 **207**: 748–759.

1953 **Yan, Z., et al.** (2016). Identification and functional characterization of soybean root
 1954 hair microRNAs expressed in response to *Bradyrhizobium japonicum* infection.
 1955 *Plant Biotechnol. J.* **14**: 332-341.

1956 **Yang, W.-C., de Blank, C., Meskiene, I., Hirt, H., Bakker, J., van Kammen, A.,**
 1957 **Franssen, H., and Bisseling, T.** (1994). *Rhizobium* Nod factors reactivate the
 1958 cell cycle during infection and nodule primordium formation, but the cycle is only
 1959 completed in primordium formation. *Plant Cell* **6**: 1415–1426.

1960 **Yang, Z.** (2008). Cell polarity signaling in Arabidopsis. *Annu. Rev. Cell Dev. Biol.* **24**:
 1961 551–575.

1962 **Yano, K. et al.** (2008). CYCLOPS, a mediator of symbiotic intracellular
 1963 accommodation. *Proc. Natl. Acad. Sci. USA* **105**: 20540-20545.

1964 **Young, N.D. et al.** (2011). The *Medicago* genome provides insight into the evolution
 1965 of rhizobial symbioses. *Nature* **480**: 520-524.

1966 **Yu, H., Xiao, A., Dong, R., Fan, Y., Zhang, X., Liu, C., Wang, C., Zhu, H.,**
 1967 **Duanmu, D., Cao, Y., and Zhang, Z.** (2018). Suppression of innate immunity
 1968 mediated by the CDPK-Rboh complex is required for rhizobial colonization in
 1969 *Medicago truncatula* nodules. *New Phytol.* **220**: 425-434.

1970 **Yuan, S.L., Li, R., Chen, H.F., Zhang, C.J., Chen, L.M., Hao, Q.N., Chen, S.L.,**
 1971 **Shan, Z.H., Yang, Z.L., Zhang, X.J., Qiu, Z., and Zhou, X.A.** (2017). RNA-Seq
 1972 analysis of nodule development at five different developmental stages of

1973 soybean (*Glycine max*) inoculated with *Bradyrhizobium japonicum* strain 113-2.
1974 Sci Rep. 7: 42248.

1975 **Zhou, P. et al.** (2017). Exploring structural variation and gene family architecture with
1976 *De Novo* assemblies of 15 *Medicago* genomes. BMC Genomics 18: 261.

1977

1978 **Figure legends**

1979 **Figure 1. Unique features of nodules. A.** Nod factor activation of the common
1980 symbiosis signaling pathway (CSSP). Brown, signaling in the rhizobium symbiont
1981 resulting in Nod factor production. Grey, CSSP in the host cells activates nodule
1982 organogenesis, infection and autoregulation of nodulation. **B.** Organization of nodule
1983 tissues. Tissues are color-coded. The dividing meristem cells (yellow) give rise at the
1984 edge to several vascular bundles (blue) concentrically organized around the central
1985 nodule tissue. The meristem cells (2C to 4C DNA content) give also rise to the
1986 central nodule tissue, which is mainly composed of infected symbiotic cells formed
1987 from post-meristematic cells by successive rounds of endoreduplication (shades of
1988 grey from light to dark indicate cells with increasing ploidy from 8C to 64C). Infection
1989 threads (green) deliver rhizobia to early differentiating symbiotic cells. **C.** Infection
1990 threads, filled with rhizobium bacteria, are initiated in a root hair and have polar
1991 growth (red). The underlying cortical cells are activated and initiate a cell cycle. Outer
1992 cortical cells, adjacent to an infection thread-carrying cell, do not complete the cell
1993 cycle but form a cytoplasmic bridge (related to a phragmosome) that will be
1994 penetrated by the growing infection thread, which is referred to as a preinfection
1995 thread. Inner cortical cells complete the cell cycle and the newly generated cells
1996 constitute the nodule primordium. Drawings are based on Van Brussel et al., 1992. **D.**
1997 An infection thread reaching a target cell in a nodule primordium or growing nodule
1998 releases rhizobia by endocytosis. The exocytosis pathway of the host cell targets
1999 proteins and peptides to bacteria located in vesicles called symbiosomes, resulting in
2000 the differentiation of these bacteria into nitrogen-fixing bacteroids. PAMP Triggered
2001 Immunity (PTI) is actively repressed in symbiotic cells. Specific expression in
2002 symbiotic cells is required for the production of symbiosome-targeted secretory
2003 proteins (green and orange), for immune suppression functions (yellow), and for
2004 metabolism (purple, see panel F.). **E.** A fully mature symbiotic cell is entirely filled

with bacteroid-containing symbiosomes and has a central vacuole and a polyploid nucleus (blue). **F.** Integration of the bacteroid metabolism (brown) with symbiotic cell metabolism (grey) relies on complementarity of the metabolic functions in both symbiotic partners and a complex exchange of small molecules mediated by a multitude of transporters. Some metabolic steps require a third partner, the plastid (green). The drawing is based on Temple et al., 1998, Udvardi and Poole, 2013 and Poole et al., 2018.

Figure 2. Gene expression in *M. truncatula*. **A.** Hierarchical clustering of the MtGEA dataset (Benedito et al., 2008). Genes are shown in rows and experiments in columns. On the top, the experiment types are indicated. To the left, gene clusters are labeled based on organ-specific expression. In the expression heatmap, yellow indicates high expression and black indicates low expression. In the “NCR” column, the NCR genes are indicated by a yellow line. In the “Shannon” columns, yellow indicates high specificity (*i.e.* low Shannon entropy) and black indicates low specificity, with more uniform expression (*i.e.* high Shannon entropy). **B.** An enlargement of the red squared area of the heatmap in panel A showing nodule-specific (nod) and mycorrhizal-specific (myc) genes (lines) and experiments (columns). **C.** The SYMBIMICS dataset (Roux et al., 2014) for nodule-specific genes from MtGEA (gene cluster labeled in red in panel A). The columns correspond to the nodule tissues: I, meristem; IId, distal infection zone or nodule zone II; IIp, proximal infection zone; IZ, interzone between nodule zones II and III; III, fixation zone or nodule zone III. Color-codes as in panel A. The data sets used to generate the heatmaps are available upon request.

Figure 3. Nodule-specific gene expression. Extreme specificity of nodule-specific genes in *M. truncatula* (**A-C**). Expression of NCR genes (**A**). Clusters of tissue-specific genes (**B**). Expression patterns of genes ordered according to specificity (Shannon entropy) (**C**). Panels A-C are adapted from Guefrachi et al. (2014), [licensed under the Creative Commons Attribution 4.0 International License](#). Nodule-specific gene expression in different legumes (**D**). The heatmaps show hierarchical cluster analysis of gene expression in the indicated legumes using data from the SoyBase (Severin et al., 2010), LjGEA (Verdier et al., 2013), AGED (O’Rourke et al., 2015), and PvGEA (O’Rourke et al., 2014) databases. Genes are shown in rows and tissue types in columns. The Shannon entropy of the genes (calculated based on the

expression profile) is displayed to the right of each gene expression heatmap. The nodule-specific gene clusters mostly comprising low Shannon entropy genes are highlighted by a red rectangle. Color codes: in the expression heatmaps, yellow indicates high expression and black indicates low expression; in the “NCR” columns, the NCR genes are indicated by a yellow line; in the “Shannon” columns, yellow indicates high specific expression (*i.e.* low Shannon entropy) and black indicates low specificity, more uniform expression (*i.e.* high Shannon entropy). The data sets used to generate the heatmaps are available upon request.

Figure 4. Spatial resolution of gene expression analysis in nodules. **A.** Purified nodule cell types can be obtained by laser-capture microdissection (LCM). This technology uses a laser guided by a microscope to cut cells in a precise, contamination-free manner out of their surrounding tissues from an organ section. **B.** A second methodology exploits the varying DNA content in the nuclei of differentiating symbiotic nodule cells and separates these nuclei using flow cytometry and a cell sorter. Flow-cytometry is a laser-assisted technology that analyzes the biophysical features of small particles, such as cells or nuclei, in a capillary stream. The graph on the right shows a typical flow cytometry measurement of the DNA content in nodule nuclei. A cell sorter coupled to the flow cytometer separates these nuclei. Both methods, LCM and flow cytometry, yield purified materials that are compatible with downstream molecular biology methods such as gene expression analysis (RNA-seq, microarray hybridization, RT-qPCR) and chromatin analysis (DNA methylation analysis, ChIP-seq). Both methods can be used to isolate single cells or single cell types from nodules and can also be combined with the use of fluorescent markers expressed in cells of interest. **C.** In spatial transcriptomics or Slide-seq, a nodule section is placed on a support that carries a defined array of barcodes. After the RNA is transferred from the tissue to the array, the barcode sequence is introduced during cDNA synthesis, and RNA-seq sequencing libraries are produced from the whole sample, preserving the spatial information through barcodes.

Figure 5. Models for the evolution or nodulation in the nitrogen-fixing clade. **A.** The convergent evolution hypothesis proposes the evolution of a cryptic innovation or predisposition for nodulation (red asterisk) followed by independent acquisitions of nodule formation (indicated by colored dots on tree branches). Green and blue dots

signify the acquisition of nodulation with *Frankia* and *Rhizobium*, respectively. **B.** The model of a single acquisition of nodulation with *Frankia* at the origin of the nitrogen-fixing clade (NFC) and multiple losses in the non-nodulating lineages (red crosses). This acquisition required the suppression of PAMP-triggered immunity against the infecting bacteria, a process involving the NAD1 protein. The evolution of nodulation might have involved a rapid succession of stepwise innovations including the replacement of mycorrhizal fungal infection by *Frankia* infection, followed by cortical cell divisions resulting in the production of small foci of infected cells before the development of nodules. Two independent switches of symbionts occurred (indicated by green to blue dot), once in the *Parasponia* lineage of the Rosales and once in the ancestor of the legumes in the Fabales. These switches to a *Rhizobium* symbiont were possible due to the recruitment and mutation of a non-symbiotic, non-oxygen transporting class 1 or class 2 hemoglobin (nsHb1 or nsHb2), forming the oxygen-transporting *Parasponia* hemoglobin (Parab) or leghemoglobin (Lb), respectively. **C.** An alternative model proposes that the ancestor of the NFC acquired the possibility to extend mycorrhizal infection with *Frankia* infection of root cortical cells. The subsequent stages imply multiple independent acquisitions of nodule organogenesis as in panel A and two independent symbiont switches as in panel B. The trees are schematic representations based on published chronograms (Doyle, 2016). The present status of clades is indicated by a blue or green nodule for *Rhizobium*-infected or *Frankia*-infected nodulating clades and by a black cross for non-nodulating clades.

Figure 1. Unique features of nodules. **A.** Nod factor activation of the common symbiosis signaling pathway (CSSP). Brown, signaling in the rhizobium symbiont resulting in Nod factor production. Grey, CSSP in the host cells activates nodule organogenesis, infection and autoregulation of nodulation. **B.** Organization of nodule tissues. Tissues are color-coded. The dividing meristem cells (yellow) give rise at the edge to several vascular bundles (blue) concentrically organized around the central nodule tissue. The meristem cells (2C to 4C DNA content) give also rise to the central nodule tissue, which is mainly composed of infected symbiotic cells formed from post-meristematic cells by successive rounds of endoreduplication (shades of grey from light to dark indicate cells with increasing ploidy from 8C to 64C). Infection threads (green) deliver rhizobia to early differentiating symbiotic cells. **C.** Infection threads, filled with rhizobium bacteria, are initiated in a root hair and have polar growth (red). The underlying cortical cells are activated and initiate a cell cycle. Outer cortical cells, adjacent to an infection thread-carrying cell, do not complete the cell cycle but form a cytoplasmic bridge (related to a phragmosome) that will be penetrated by the growing infection thread, which is referred to as a preinfection thread. Inner cortical cells complete the cell cycle and the newly generated cells constitute the nodule primordium. Drawings are based on Van Brussel et al., 1992. **D.** An infection thread reaching a target cell in a nodule primordium or growing nodule releases rhizobia by endocytosis. The exocytosis pathway of the host cell targets proteins and peptides to bacteria located in vesicles called symbiosomes, resulting in the differentiation of these bacteria into nitrogen-fixing bacteroids. PAMP Triggered Immunity (PTI) is actively repressed in symbiotic cells. Specific expression in symbiotic cells is required for the production of symbiosome-targeted secretory proteins (green and orange), for immune suppression functions (yellow), and for metabolism (purple, see panel F.). **E.** A fully mature symbiotic cell is entirely filled with bacteroid-containing symbiosomes and has a central vacuole and a polyploid nucleus (blue). **F.** Integration of the bacteroid metabolism (brown) with symbiotic cell metabolism (grey) relies on complementarity of the metabolic functions in both symbiotic partners and a complex exchange of small molecules mediated by a multitude of transporters. Some metabolic steps require a third partner, the plastid (green). The drawing is based on Temple et al., 1998, Udvardi and Poole, 2013 and Poole et al., 2018.

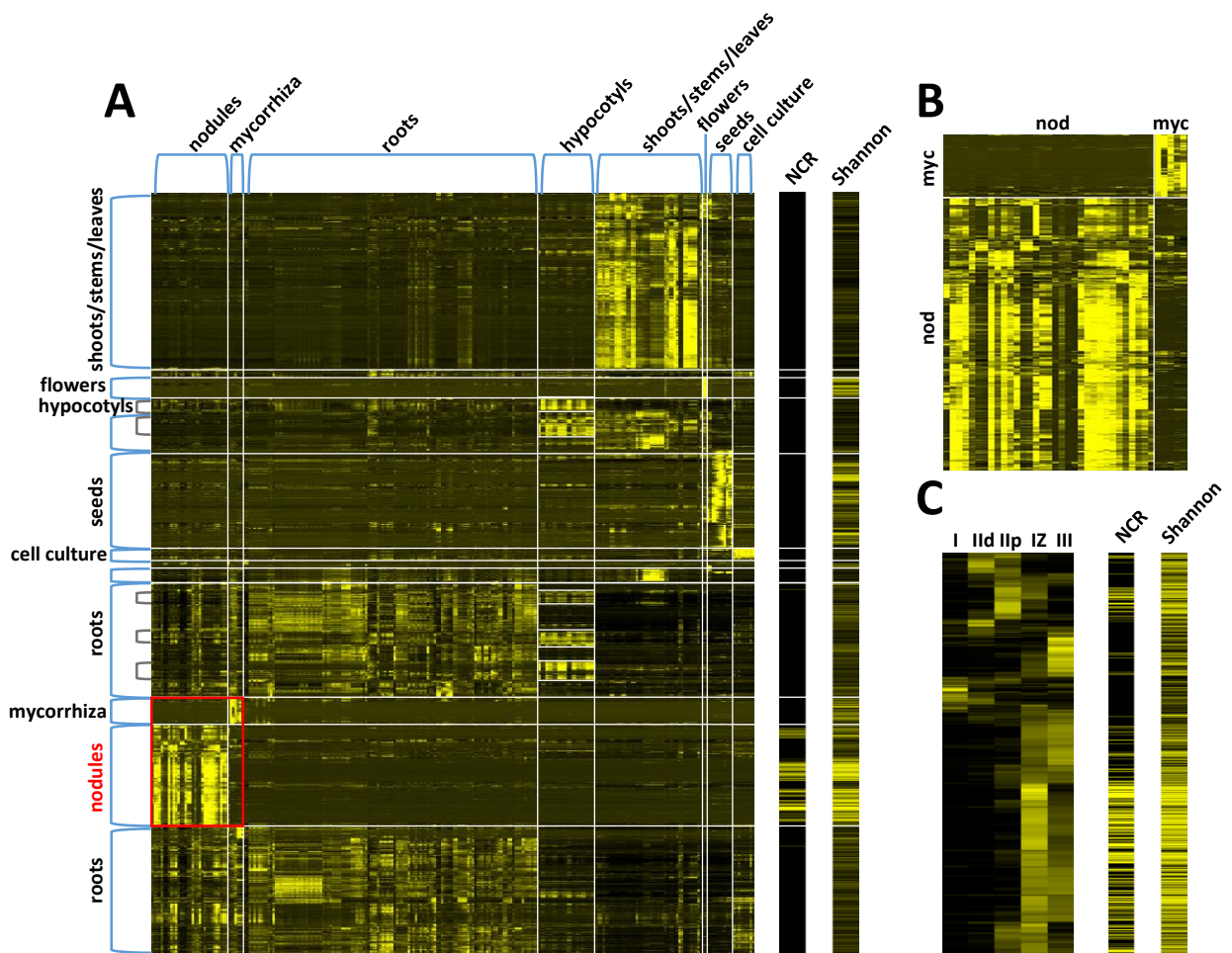


Figure 2. Gene expression in *M. truncatula*. **A.** Hierarchical clustering of the MtGEA dataset (Benedito et al., 2008). Genes are shown in rows and experiments in columns. On the top, the experiment types are indicated. To the left, gene clusters are labeled based on organ-specific expression. In the expression heatmap, yellow indicates high expression and black indicates low expression. In the “NCR” column, the NCR genes are indicated by a yellow line. In the “Shannon” columns, yellow indicates high specificity (*i.e.* low Shannon entropy) and black indicates low specificity, with more uniform expression (*i.e.* high Shannon entropy). **B.** An enlargement of the red squared area of the heatmap in panel A showing nodule-specific (nod) and mycorrhizal-specific (myc) genes (lines) and experiments (columns). **C.** The SYMBIMICS dataset (Roux et al., 2014) for nodule-specific genes from MtGEA (gene cluster labeled in red in panel A). The columns correspond to the nodule tissues: I, meristem; IId, distal infection zone or nodule zone II; IIp, proximal infection zone; IZ, interzone between nodule zones II and III; III, fixation zone or nodule zone III. Color-codes as in panel A. The data sets used to generate the heatmaps are available upon request.

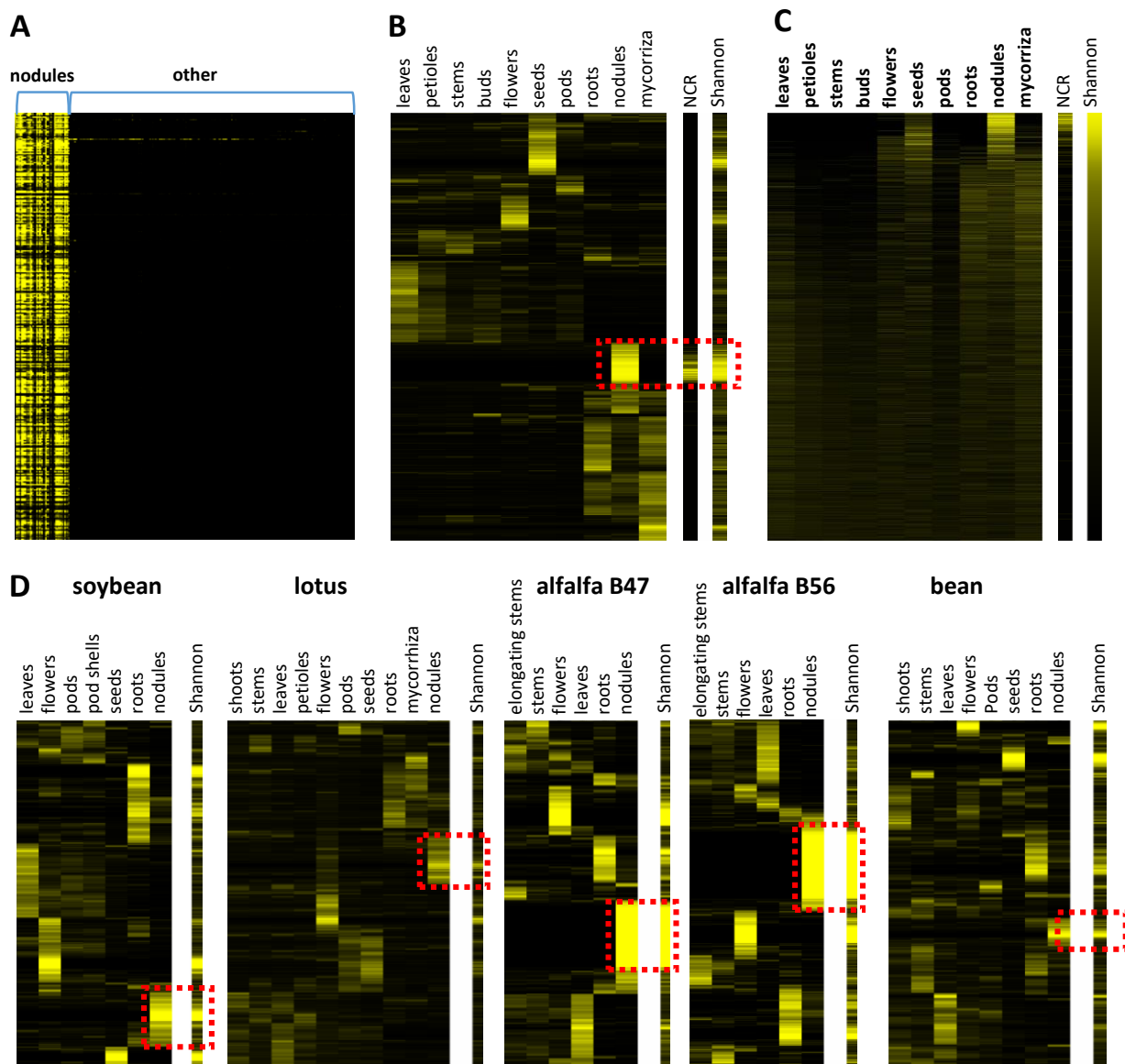


Figure 3. Nodule-specific gene expression. Extreme specificity of nodule-specific genes in *M. truncatula* (A-C). Expression of NCR genes (A). Clusters of tissue-specific genes (B). Expression patterns of genes ordered according to specificity (Shannon entropy) (C). Panels A-C are adapted from Guefrachi et al. (2014), licensed under the Creative Commons Attribution 4.0 International License. Nodule-specific gene expression in different legumes (D). The heatmaps show hierarchical cluster analysis of gene expression in the indicated legumes using data from the SoyBase (Severin et al., 2010), LJGEA (Verdier et al., 2013), AGED (O'Rourke et al., 2015), and PvGEA (O'Rourke et al., 2014) databases. Genes are shown in rows and tissue types in columns. The Shannon entropy of the genes (calculated based on the expression profile) is displayed to the right of each gene expression heatmap. The nodule-specific gene clusters mostly comprising low Shannon entropy genes are highlighted by a red rectangle. Color codes: in the expression heatmaps, yellow indicates high expression and black indicates low expression; in the "NCR" columns, the NCR genes are indicated by a yellow line; in the "Shannon" columns, yellow indicates high specific expression (*i.e.* low Shannon entropy) and black indicates low specificity, more uniform expression (*i.e.* high Shannon entropy). The data sets used to generate the heatmaps are available upon request.

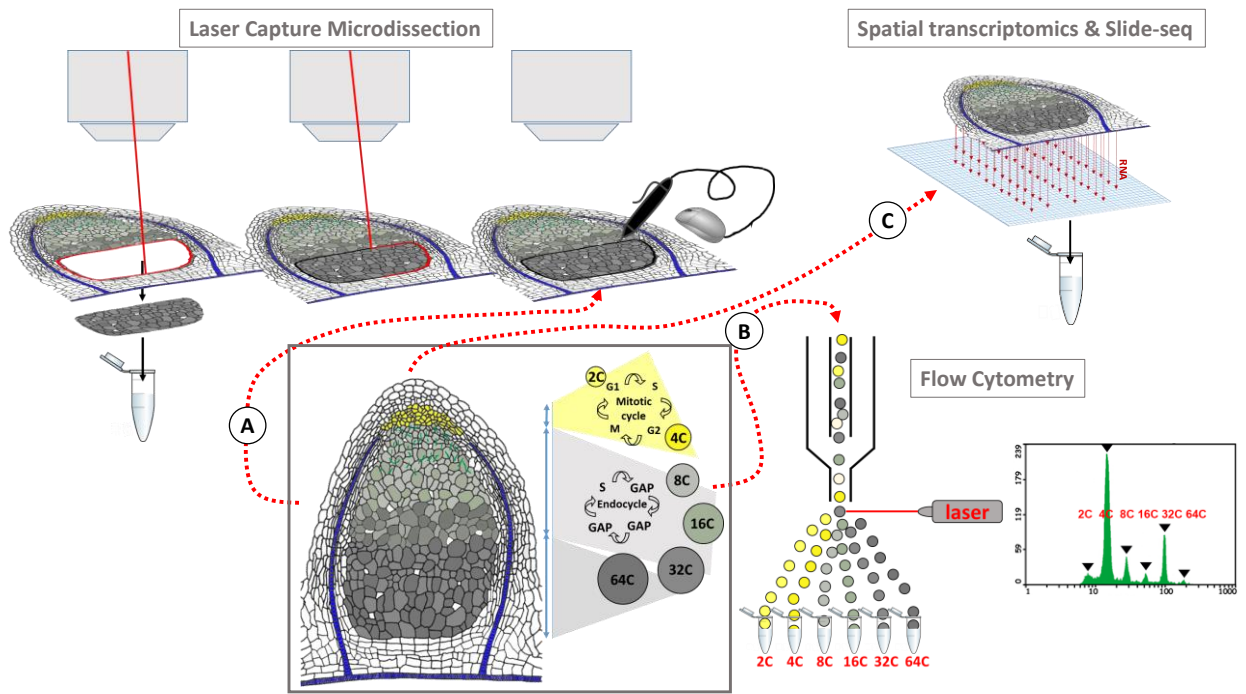


Figure 4. Spatial resolution of gene expression analysis in nodules. **A.** Purified nodule cell types can be obtained by laser-capture microdissection (LCM). This technology uses a laser guided by a microscope to cut cells in a precise, contamination-free manner out of their surrounding tissues from an organ section. **B.** A second methodology exploits the varying DNA content in the nuclei of differentiating symbiotic nodule cells and separates these nuclei using flow cytometry and a cell sorter. Flow-cytometry is a laser-assisted technology that analyzes the biophysical features of small particles, such as cells or nuclei, in a capillary stream. The graph at the right shows a typical flow cytometry measurement of the DNA content in nodule nuclei. A cell sorter coupled to the flow cytometer separates these nuclei. Both methods, LCM and flow cytometry, yield purified materials that are compatible with downstream molecular biology methods such as gene expression analysis (RNA-seq, microarray hybridization, RT-qPCR) and chromatin analysis (DNA methylation analysis, ChIP-seq). Both methods can be used to isolate single cells or single cell types from nodules and can also be combined with the use of fluorescent markers expressed in cells of interest. **C.** In spatial transcriptomics or Slide-seq, a nodule section is placed on a support that carries a defined array of barcodes. After the RNA is transferred from the tissue to the array, the barcode sequence is introduced during cDNA synthesis, and RNA-seq sequencing libraries are produced from the whole sample, preserving the spatial information through barcodes.

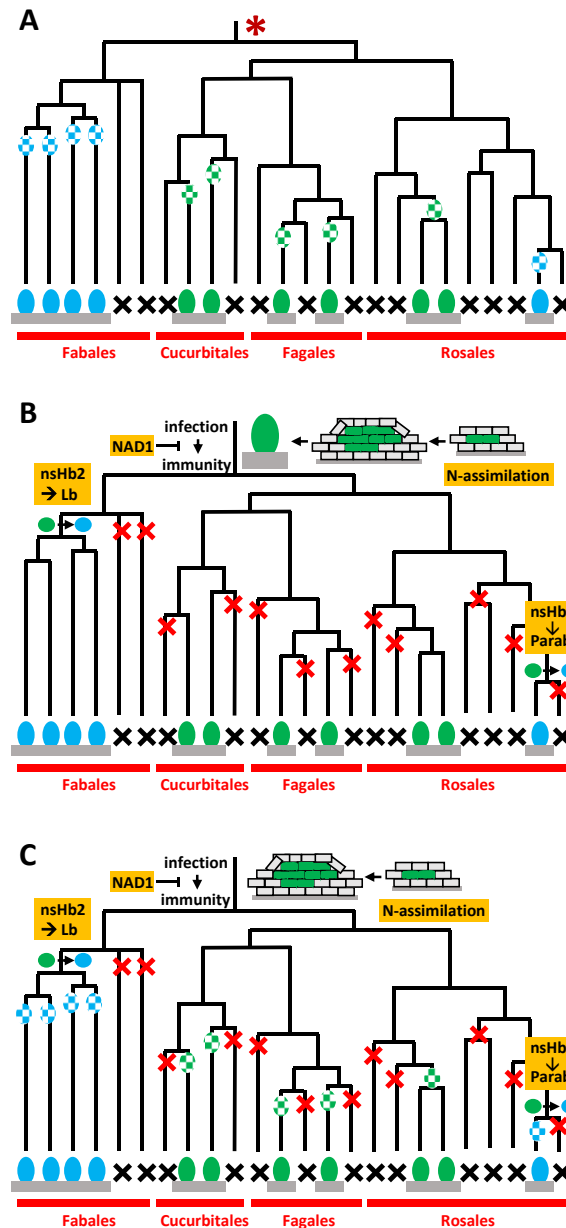


Figure 5. Models for the evolution of nodulation in the nitrogen-fixing clade. **A.** The convergent evolution hypothesis proposes the evolution of a cryptic innovation or predisposition for nodulation (red asterisk) followed by independent acquisitions of nodule formation (indicated by colored dots on tree branches). Green and blue dots signify the acquisition of nodulation with *Frankia* and *Rhizobium*, respectively. **B.** The model of a single acquisition of nodulation with *Frankia* at the origin of the nitrogen-fixing clade (NFC) and multiple losses in the non-nodulating lineages (red crosses). This acquisition required the suppression of PAMP-triggered immunity against the infecting bacteria, a process involving the NAD1 protein. The evolution of nodulation might have involved a rapid succession of stepwise innovations including the replacement of mycorrhizal fungal infection by *Frankia* infection, followed by cortical cell divisions resulting in the production of small foci of infected cells before the development of nodules. Two independent switches of symbionts occurred (indicated by green to blue dot), once in the *Parasponia* lineage of the Rosales and once in the ancestor of the legumes in the Fabales. These switches to a *Rhizobium* symbiont were possible due to the recruitment and mutation of a non-symbiotic, non-oxygen transporting class 1 or class 2 hemoglobin (nsHb1 or nsHb2), forming the oxygen-transporting *Parasponia* hemoglobin (Parab) or leghemoglobin (Lb), respectively. **C.** An alternative model proposes that the ancestor of the NFC acquired the possibility to extend mycorrhizal infection with *Frankia* infection of root cortical cells. The subsequent stages imply multiple independent acquisitions of nodule organogenesis as in panel A and two independent symbiont switches as in panel B. The trees are schematic representations based on published chronograms (Doyle, 2016). The present status of clades is indicated by a blue or green nodule for *Rhizobium*-infected or *Frankia*-infected nodulating clades and by a black cross for non-nodulating clades.

Parsed Citations

Alunni, B., Kevei, Z., Redondo-Nieto, M., Kondorosi, A., Mergaert, P., and Kondorosi, E. (2007). Genomic organization and evolutionary insights on GRP and NCR genes, two large nodule-specific gene families in *Medicago truncatula*. *Mol. Plant.-Microbe. Interact.* 20: 1138–1148.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Alves-Carvalho, S. et al. (2015). Full-length de novo assembly of RNA-seq data in pea (*Pisum sativum* L.) provides a gene expression atlas and gives insights into root nodulation in this species. *Plant J.* 84: 1–19.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Andriankaja, A., Boisson-Dernier, A., Frances, L., Sauviac, L., Jauneau, A., Barker, D.G., and de Carvalho-Niebel, F. (2007). AP2-ERF transcription factors mediate Nod factor dependent MtENOD11 activation in root hairs via a novel cis-regulatory motif. *Plant Cell* 19: 2866–2885.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Antolín-Llovera, M., Ried, M. K., and Parniske, M. (2014). Cleavage of the SYMBIOSIS RECEPTOR-LIKE KINASE ectodomain promotes complex formation with Nod Factor Receptor 5. *Curr. Biol.* 24: 422–427.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Asamizu, E., Shimoda, Y., Kouchi, H., Tabata, S., and Sato, S. (2008). A positive regulatory role for LjERF1 in the nodulation process is revealed by systematic analysis of nodule-associated transcription factors of *Lotus japonicus*. *Plant Physiol.* 147: 2030–2040.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Azaraksh, M., Kirienko, A.N., Zhukov, V.A., Lebedeva, M.A., Dolgikh, E.A., and Lutova, L.A. (2017). KNOTTED1-LIKE HOMEODOMAIN 3: a new regulator of symbiotic nodule development. *J. Exp. Bot.* 66: 7181–7195.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Battenberg, K., Potter, D., Tabuloc, C.A., Chiu, J.C., and Berry, A.M. (2018). Comparative transcriptomic analysis of two actinorhizal plants and the legume *Medicago truncatula* supports the homology of root nodule symbioses and is congruent with a two-step process of evolution in the nitrogen-fixing clade of angiosperms. *Front. Plant Sci.* 9: 1256.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Beall, E.L., Lewis, P.W., Bell, M., Rocha, M., Jones, D.L., and Botchan, M.R. (2007). Discovery of tMAC: a *Drosophila* testis-specific meiotic arrest complex paralogous to Myb Muv B. *Genes Dev.* 21: 904–919.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Behm, J.E., Geurts, R., and Kiers, E.T. (2014). Parasponia: a novel system for studying mutualism stability. *Trends Plant Sci.* 19: 757–763.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Benedito, V.A. et al. (2008). A gene expression atlas of the model legume *Medicago truncatula*. *Plant J.* 55: 504–513.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Berrabah, F., Bourcy, M., Eschstruth, A., Cayrel, A., Guefrachi, I., Mergaert, P., Wen, J., Jean, V., Mysore, K.S., Gourion, B., and Ratet, P. (2014). A non-ND receptor-like kinase prevents nodule early senescence and defense-like reactions during symbiosis. *New Phytol.* 203: 1305–1314.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Boualem, A., Laporte, P., Jovanovic, M., Laffont, C., Plet, J., Combier, J.P., Niebel, A., Crespi, M., and Frugier, F. (2008). MicroRNA166 controls root and nodule development in *Medicago truncatula*. *Plant J.* 54: 876–887.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Bourcy, M., Brocard, L., Pislariu, C.I., Cosson, V., Mergaert, P., Tadege, M., Mysore, K.S., Udvardi, M.K., Gourion, B., and Ratet, P. (2013). *Medicago truncatula* DNF2 is a PI-PLC-XD-containing protein required for bacteroid persistence and prevention of nodule early senescence and defense-like reactions. *New Phytol.* 197: 1250–1261.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Branca, A. et al. (2011). Whole-genome nucleotide diversity, recombination, and linkage disequilibrium in the model legume *Medicago truncatula*. *Proc. Natl. Acad. Sci. USA* 108: E864–E870.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Breakspear, A, Liu, C., Roy, S., Stacey, N., Rogers, C., Trick, M., Morieri, G., Mysore, K.S., Wen, J., Oldroyd, G.E.D., Downie, J.A, and Murray, J.D. (2014). 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* 26: 4680–4701.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Brechenmacher, L. et al. (2008). Transcription profiling of soybean nodulation by *Bradyrhizobium japonicum*. *Mol. Plant. Microbe. Interact.* 21: 631–645.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Burghardt, L.T., Guhlin, J., Chun, C.L., Liu, J., Sadowsky, M.J., Stupar, R.M., Young, N.D., and Tiffin, P. (2017). Transcriptomic basis of genome by genome variation in a legume-rhizobia mutualism. *Mol. Ecol.*

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Buhian, W.P., and Bensmihen, S. (2018). Nod factor regulation of phytohormone signaling and homeostasis during rhizobia-legume symbiosis. *Front. Plant Sci.* 9: 1247.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Cabeza, R., Koester, B., Liese, R., Lingner, A., Baumgarten, V., Dirks, J., Salinas-Riester, G., Pommerenke, C., Dittert, K., and Schulze, J. (2014). An RNA sequencing transcriptome analysis reveals novel insights into molecular aspects of the nitrate impact on the nodule activity of *Medicago truncatula*. *Plant Physiol.* 164: 400–411.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Cabeza, R.A, Liese, R., Lingner, A., von Stieglitz, I., Neumann, J., Salinas-Riester, G., Pommerenke, C., Dittert, K., and Schulze, J. (2014). RNA-seq transcriptome profiling reveals that *Medicago truncatula* nodules acclimate N₂ fixation before emerging P deficiency reaches the nodules. *J. Exp. Bot.* 65: 6035–6048.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Capoen, W., Goormachtig, S., De Rycke, R., Schroevers, K., and Holsters, M. (2005). SrSymRK, a plant receptor essential for symbiosome formation. *Proc. Natl. Acad. Sci. USA* 102: 10369–10374.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Capoen, W., Den Herder, J., Rombauts, S., De Gussem, J., De Keyser, A., Holsters, M., and Goormachtig, S. (2007). Comparative transcriptome analysis reveals common and specific tags for root hair and crack-entry invasion. *Plant Physiol.* 144: 1878–1889.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

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

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Chen, L., Qin, L., Zhou, L., Li, X., Chen, Z., Sun, L., Wang, W., Lin, Z., Zhao, J., Yamaji, N., Ma, J.F., Gu, M., Xu, G., and Liao, H. (2019). A nodule-localized phosphate transporter GmPT7 plays an important role in enhancing symbiotic N₂ fixation and yield in soybean. *New Phytol.* 221: 2013–2025.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Chiasson, D.M. et al. (2014). Soybean SAT1 (Symbiotic Ammonium Transporter 1) encodes a bHLH transcription factor involved in nodule growth and NH₄⁺ transport. *Proc. Natl. Acad. Sci. USA* 111: 4814–4819.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Cissoko, M. et al. (2018) Actinorhizal signaling molecules: Frankia root hair deforming factor shares properties with NIN inducing factor. *Front. Plant Sci.* 9: 1494.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Clavijo, F. et al. (2015). The *Casuarina* NIN gene is transcriptionally activated throughout Frankia root infection as well as in response to bacterial diffusible signals. *New Phytol.* 208: 887–903.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Clevenger, J., Chu, Y., Scheffler, B., and Ozias-Akins, P. (2016). A developmental transcriptome map for allotetraploid *Arachis hypogaea*. *Front Plant Sci.* 7: 1446.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Colebatch, G., Desbrosses, G., Ott, T., Krusell, L., Montanari, O., Kloska, S., Kopka, J., and Udvardi, M.K. (2004). Global changes in transcription orchestrate metabolic differentiation during symbiotic nitrogen fixation in *Lotus japonicus*. *Plant J.* 39: 487–512.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Combiér, J.-P., Frugier, F., De Billy, F., Boualem, A., El-Yahyaoui, F., Moreau, S., Vernié, T., Ott, T., Gamas, P., Crespi, M., and Niebel, A. (2006). MtHAP2-1 is a key transcriptional regulator of symbiotic nodule development regulated by microRNA169 in *Medicago truncatula*. *Genes Dev.* 20: 3084–3088.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Combiér, J.-P., De Billy, F., Gamas, P., Niebel, A., and Rivas, S. (2008). Trans-regulation of the expression of the transcription factor MtHAP2-1 by a uORF controls root nodule development. *Genes Dev.* 22: 1549–1559.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Cope, K.R. et al. (2019). The ectomycorrhizal fungus *Laccaria bicolor* produces lipochitooligosaccharides and uses the common symbiosis pathway to colonize *Populus* roots. *Plant Cell*. doi:10.1105/tpc.18.00676.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Couzigou, J.-M. et al. (2012). NODULE ROOT and COCHLEATA maintain nodule development and are legume orthologs of *Arabidopsis* BLADE-ON-PETIOLE genes. *Plant Cell* 24: 4498–4510.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Couzigou, J.-M., and Combiér, J.-P. (2016). Plant microRNAs : key regulators of root architecture and biotic interactions. *New Phytol.* 212: 22–35.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Cvitanich, C., Pallisgaard, N., Nielsen, K.A., Chemnitz Hansen, A., Larsen, K., Pihakaski-Maunsbach, K., Marcker K.A., and Jensen, E.Ø. (2000). CPP1, a DNA-binding protein involved in the expression of a soybean leghemoglobin c3 gene. *Proc. Natl. Acad. USA* 97: 8163–8168.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Czaja, L.F., Hogekamp, C., Lamm, P., Maillet, F., Andres Martinez, E., Samain, E., Dénarié, J., Küster, H., and Hohnjec, N. (2012). Transcriptional responses toward diffusible signals from symbiotic microbes reveal MtNFP- and MtDMI3-dependent reprogramming of host gene expression by arbuscular mycorrhizal fungal lipochitooligosaccharides. *Plant Physiol.* 159: 1671–1685.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Czernic, P. et al. (2015). Convergent evolution of endosymbiont differentiation in Dalbergioid and inverted repeat-lacking clade legumes mediated by nodule-specific cysteine-rich peptides. *Plant Physiol.* 169: 1254–1265.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Damiani, I., et al. (2016). Nod factor effects on root hair-specific transcriptome of *Medicago truncatula*: Focus on plasma membrane transport systems and reactive oxygen species networks. *Front. Plant Sci.* 7: 794.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

de Bang, T. et al. (2017). Genome-wide identification of *Medicago* peptides involved in macronutrient responses and nodulation. *Plant Physiol.* 175: 1669–1689.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

De Luis, A., Markmann, K., Cognat, V., Holt, D.B., Charpentier, M., Parniske, M., Stougaard, J., and Voinnet, O. (2012). Two microRNAs linked to nodule infection and nitrogen-fixing ability in the legume *Lotus japonicus*. *Plant Physiol.* 160: 2137–2154.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

del Rocio Reyero-Saavedra, M., Qiao, Z., del Socorro Sánchez-Correa, M., Díaz-Pineda, M.E., Reyes, J.L., Covarrubias, A.A., Libault, M., and Valdés-López, O. (2017). Gene silencing of *Argonaute5* negatively affects the establishment of the legume-rhizobia symbiosis. *Genes* 8: 352.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Derrina, I.V., Persson, T., Santos, P., Plaszczyca, M., and Pawlowski, K. (2013). Comparison of the nodule vs. root transcriptome of the actinorhizal plant *Datisca glomerata*: Actinorhizal nodules contain a specific class of defensins. *PLoS One* 8: e72442.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Deng, J., Zhu, F., Liu, J., Zhao, Y., Wen, J., Wang, T., and Dong, J. (2019). Transcription factor bHLH2 represses CYSTEINE PROTEASE 77 to negatively regulate nodule senescence. *Plant Physiol.* doi:10.1104/pp.19.00574.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Di Giacomo, E., Laffont, C., Sciarra, F., Iannelli, M.A., Frugier, F., and Frugis, G. (2017). KNAT3 / 4 / 5-like class 2 KNOX transcription factors are involved in *Medicago truncatula* symbiotic nodule organ development. *New Phytol.* 213: 822–837.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Dickstein, R., Hu, X., Yang, J., Ba, L., Coque, L., Kim, D.-J., Cook, D.R., and Yeung, A.T. (2002). Differential expression of tandemly duplicated *Enod8* genes in *Medicago*. *Plant Sci.* 163: 333-343.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Domonkos, Á et al. (2017). NAD1 controls defense-like responses in *Medicago truncatula* symbiotic nitrogen fixing nodules following rhizobial colonization in a *BacA*-independent manner. *Genes* 8: 387.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Downie, J.A. (2014). Legume nodulation. *Curr. Biol.* 24: R184–R190.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Doyle, J.J. (2016). Chasing unicorns: Nodulation origins and the paradox of novelty. *Am. J. Bot.* 103: 1865–1868.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Edgar, B.A., Zielke, N., and Gutierrez, C. (2014). Endocycles: a recurrent evolutionary innovation for post-mitotic cell growth. *Nat. Rev. Mol. Cell Biol.* 15: 197-210.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

El Yahyaoui, F., Küster, H., Ben Amor, B., Hohnjec, N., Pühler, A., Becker, A., Gouzy, J., Vernié, T., Gough, C., Niebel, A., Godiard, L., and Gamas, P. (2004). Expression profiling in *Medicago truncatula* identifies more than 750 genes differentially expressed during nodulation, including many potential regulators of the symbiotic program. *Plant Physiol.* 136: 3159–3176.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Fonouni-Farde, C., Tan, S., Baudin, M., Brault, M., Wen, J., Mysore, K.S., Niebel, A., Frugier, F., and Diet, A. (2016). DELLA-mediated gibberellin signalling regulates Nod factor signalling and rhizobial infection. *Nat. Commun.* 7: 12636.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Formey, D. et al. (2014). The small RNA diversity from *Medicago truncatula* roots under biotic interactions evidences the environmental plasticity of the miRNAome. *Genome Biol.* 15: 457.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Formey, D., Martín-Rodríguez, J.Á., Leija, A., Santana, O., Quinto, C., Cárdenas, L., and Hernández, G. (2016). Regulation of small RNAs and corresponding targets in Nod factor-induced *Phaseolus vulgaris* root hair cells. *Int. J. Mol. Sci.* 17: 887.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Foucher, F., and Kondorosi, E. (2000). Cell cycle regulation in the course of nodule organogenesis in *Medicago*. *Plant Mol. Biol.* 43: 773–786.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Franck, C.M., Westermann, J., and Boisson-Dernier A. (2018). Plant malectin-like receptor kinases: from cell wall integrity to immunity and beyond. *Annu. Rev. Plant Biol.* 69: 301-328.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Franssen, H.J., Xiao, T.T., Kulikova, O., Wan, X., Bisseling, T., Scheres, B., and Heidstra, R. (2015). Root developmental programs shape the *Medicago truncatula* nodule meristem. *Development* 142: 2941–2950.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Frare, R., Ayub, N., Aleeva, K., and Soto, G. (2018). The ammonium channel NOD26 is the evolutionary innovation that drives the emergence, consolidation, and dissemination of nitrogen-fixing symbiosis in angiosperms. *J. Mol. Evol.* 86: 554-565.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Frugier, F., Poirier, S., Satiat-Jeunemaitre, B., Kondorosi, A. and Crespi, M. (2000). A Krüppel-like zinc finger protein is involved in nitrogen-fixing root nodule organogenesis. *Genes Dev.* 14: 475–482.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Frühling, M., Albus, U., Hohnjec, N., Geise, G., Pühler, A., and Perlick, A.M. (2000). A small gene family of broad bean codes for late nodulins containing conserved cysteine clusters. *Plant Sci.* 152: 67–77.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Galindo-González, L., Mhiri, C., Deyholos, M.K., and Grandbastien, M.A. (2017). LTR-retrotransposons in plants: Engines of evolution. *Gene* 626: 14–25.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Gamas, P., Brault, M., Jardinaud, M., and Frugier, F. (2017). Cytokinins in symbiotic nodulation: When, where, what for? *Trends Plant Sci.* 22: 792-802.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Gauthier-Coles, C., White, R.G., and Mathesius, U. (2019) Nodulating legumes are distinguished by a sensitivity to cytokinin in the root cortex leading to pseudonodule development. *Front. Plant Sci.* 9: 1901.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ge, Z., et al. (2017). Arabidopsis pollen tube integrity and sperm release are regulated by RALF-mediated signaling. *Science* 358: 1596–1600.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Genre, A., Chabaud, M., Faccio, A., Barker, D.G., and Bonfante, P. (2008). Prepenetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago truncatula* and *Daucus carota*. *Plant Cell.* 20: 1407-1420.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Gherbi, H., Markmann, K., Svistoonoff, S., Estevan, J., Autran, D., Giczey, G., Auguy, F., Peret, B., Laplace, L., Franche, C., Parniske, M., and Bogusz, D. (2008). SymRK defines a common genetic basis for plant root endosymbioses with arbuscular mycorrhiza fungi, rhizobia, and Frankia bacteria. *Proc. Natl. Acad. Sci. USA* 105: 4928–4932.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Giaever, G. et al. (2002). Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* 418: 387-391.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Giraud, E. et al. (2007). Legumes Symbioses: Absence of nod genes in photosynthetic bradyrhizobia. *Science* 316: 1307-1312.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Godiard, L., Lepage, A., Moreau, S., Laporte, D., Verdenaud, M., Timmers, T., and Gamas, P. (2011). MtbHLH1, a bHLH transcription factor involved in *Medicago truncatula* nodule vascular patterning and nodule to plant metabolic exchanges. *New Phytol.* 191: 391–404.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

González-Sama, A., de la Peña, T.C., Kevei, Z., Mergaert, P., Lucas, M.M., de Felipe, M.R., Kondorosi, E., and Pueyo, J.J. (2006). Nuclear DNA endoreduplication and expression of the mitotic inhibitor Ccs52 associated to determinate and lupinoid nodule organogenesis. *Mol Plant-Microbe Interact.* 19: 173-180.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Gourion, B., Berrabah, F., Ratet, P., and Stacey, G. (2015). Rhizobium-legume symbioses: the crucial role of plant immunity. *Trends Plant Sci.* 20: 186–194.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Graham, M.A., Silverstein, K.A.T., Cannon, S.B., and VandenBosch, K.A. (2004). Computational identification and characterization of novel genes from legumes. *Plant Physiol.* 135: 1179–1197.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Granqvist, E., Sun, J., Op den Camp, R., Pujic, P., Hill, L., Normand, P., Morris, R.J., Downie, J.A., Geurts, R., and Oldroyd, G.E.D. (2015). Bacterial-induced calcium oscillations are common to nitrogen-fixing associations of nodulating legumes and nonlegumes. *New Phytol.*

207: 551–558.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Griesmann, M. et al. (2018). Phylogenomics reveals multiple losses of nitrogen-fixing root nodule symbiosis. *Science* 361: eaat1743.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Guefrachi, I., Nagymihaly, M., Pislariu, C.I., Van de Velde, W., Ratet, P., Mars, M., Udvardi, M.K., Kondorosi, E., Mergaert, P., and Alunni, B. (2014). Extreme specificity of NCR gene expression in *Medicago truncatula*. *BMC Genomics* 15: 712.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Gully, D. et al. (2018). Transcriptome profiles of Nod factor-independent symbiosis in the tropical legume *Aeschynomene evenia*. *Sci. Rep.* 8: 10934.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Haag, A.F., Arnold, M.F.F., Myka, K.K., Kerscher, B., Dall'Angelo, S., Zanda, M., Mergaert, P., and Ferguson, G.P. (2013). Molecular insights into bacteroid development during *Rhizobium*-legume symbiosis. *FEMS Microbiol. Rev.* 37: 364–383.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hakoyama, T. et al. (2009). Host plant genome overcomes the lack of a bacterial gene for symbiotic nitrogen fixation. *Nature* 462: 514–518.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Haney, C.H., Riely, B.K., Tricoli, D.M., Cook, D.R., Ehrhardt, D.W., and Long, S.R. (2011). Symbiotic rhizobia bacteria trigger a change in localization and dynamics of the *Medicago truncatula* receptor kinase LYK3. *Plant Cell* 23: 2774–2787.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Haney, C.H., and Long, S.R. (2010). Plant flotillins are required for infection by nitrogen-fixing bacteria. *Proc. Natl. Acad. Sci. USA* 107: 478–483.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hansen, A.C., Busk, H., Marcker, A., Marcker, K.A., and Jensen, E.Ø (1999). VsENBP1 regulates the expression of the early nodulin PsENOD12B. *Plant Mol. Biol.* 40: 495–506.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Harrison, M.J., and Ivanov, S. (2017). Exocytosis for endosymbiosis: membrane trafficking pathways for development of symbiotic membrane compartments. *Curr. Opin. Plant Biol.* 38: 101–108.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Haruta, M., Sabat, G., Stecker, K., Minkoff, B.B., and Sussman, M.R. (2014). A peptide hormone and its receptor protein kinase regulate plant cell expansion. *Science* 343: 408–412.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Heard, J. and Dunn, K. (1995). Symbiotic induction of a MADS-box gene during development of alfalfa root nodules. *Proc. Natl. Acad. Sci. USA* 92: 5273–5277.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Heard, J., Caspi, M., Dunn, K., College, B., and Hill, C. (1997). Evolutionary diversity of symbiotically induced nodule MADS Box genes: Characterization of nmhC5, a member of a novel subfamily. *Mol. Plant-Microbe Interact.* 10: 665–676.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Heath, K.D., Burke, P. V., and Stinchcombe, J.R. (2012). Coevolutionary genetic variation in the legume-rhizobium transcriptome. *Mol. Ecol.* 21: 4735–4747.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hocher, V., Aloisio, N., Auguy, F., Fournier, P., Doumas, P., Pujic, P., Gherbi, H., Queiroux, C., Da Silva, C., Wincker, P., Normand, P., and Bogusz, D. (2011). Transcriptomics of actinorhizal symbioses reveals homologs of the whole common symbiotic signaling cascade. *Plant Physiol.* 156: 700–711.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hogekamp, C., Arndt, D., Pereira, P.A., Becker, J.D., Hohnjec, N., and Küster, H. (2011). Laser microdissection unravels cell-type-

specific transcription in arbuscular mycorrhizal roots, including CAAT-box transcription factor gene expression correlating with fungal contact and spread. *Plant Physiol.* 157: 2023-2043.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Høgslund, N., Radutoiu, S., Krusell, L., Voroshilova, V., Hannah, M.A., Goffard, N., Sanchez, D.H., Lippold, F., Ott, T., Sato, S., and Tabata, S. (2009). Dissection of symbiosis and organ development by integrated transcriptome analysis of *Lotus japonicus* mutant and wild-type plants. *PLoS One* 4: e6556.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Holt, D.B., Gupta, V., Abel, N.B., Andersen, S.U., Stougaard, J., and Markmann, K. (2015). micro RNA 172 (miR172) signals epidermal infection and is expressed in cells primed for bacterial invasion in *Lotus japonicus* roots and nodules. *New Phytol.* 172: 241–256.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Horváth, B. et al. (2011). *Medicago truncatula* IPD3 is a member of the common symbiotic signaling pathway required for rhizobial and mycorrhizal symbioses. *Mol. Plant-Microbe Interact.* 24: 1345–1358.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hossain, M.S., Hoang, N.T., Yan, Z., Tóth, K., Meyers, B.C., and Stacey, G. (2019). Characterization of the spatial and temporal expression of two soybean miRNAs identifies SCL6 as a novel regulator of soybean nodulation. *Front Plant Sci.* 10: 475.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Huisman, R., Hontelez, J., Mysore, K.S., Wen, J., Bisseling, T., and Limpens, E. (2016). A symbiosis-dedicated SYNTAXIN OF PLANTS 13II isoform controls the formation of a stable host–microbe interface in symbiosis. *New Phytol.* 211: 1338-1351.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jardinaud, M.-F. et al. (2016). A laser dissection-RNAseq analysis highlights the activation of cytokinin pathways by Nod factors in the *Medicago truncatula* root epidermis. *Plant Physiol.* 171: 2256–2276.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jensen, E. Ø., Marcker, K.A., Schell, J., and de Bruijn, F.J. (1988). Interaction of a nodule specific, trans-acting factor with distinct DNA elements in the soybean leghaemoglobin *lbc3* 5' upstream region. *EMBO J.* 7: 1265-1271.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jiang, J., Benson, E., Bausek, N., Doggett, K., and White-Cooper, H. (2007). Tombola, a tesmin/TSO1-family protein, regulates transcriptional activation in the *Drosophila* male germline and physically interacts with Always early. *Development* 1559: 1549–1559.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jin, Y., Liu, H., Luo, D., Yu, N., Dong, W., Wang, C., Zhang, X., Dai, H., Yang, J., and Wang, E. (2016). DELLA proteins are common components of symbiotic rhizobial and mycorrhizal signalling pathways. *Nat. Commun.* 7: 12433.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jin, Y., Chen, Z., Yang, J., Mysore, K.S., Wen, J., Huang, J., Yu, N. and Wang, E. (2018). IPD3 and IPD3L function redundantly in rhizobial and mycorrhizal symbioses. *Front. Plant Sci.* 9: 267.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jørgensen, J., Grønlund, M., Pallisgaard, N., Larsen, K., Marcker, K.A., and Jensen, E.Ø (1999). A new class of plant homeobox genes is expressed in specific regions of determinate symbiotic root nodules. *Plant Mol. Biol.* 40: 65–77.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Joshi, T., Yan, Z., Libault, M., Jeong, D.H., Park, S., Green, P.J., Sherrier, D.J., Farmer, A., May, G., Meyers, B.C., Xu, D., and Stacey, G. (2010). Prediction of novel miRNAs and associated target genes in *Glycine max*. *BMC Bioinformatics.* 11: S14.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kaló, P. et al. (2005). Nodulation signaling in legumes requires NSP2, a member of the GRAS family of transcriptional regulators. *Science* 308: 1786-1789.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kant, C., Pradhan, S., and Bhatia, S. (2016). Dissecting the root nodule transcriptome of chickpea (*Cicer arietinum* L.). *PLoS One* 11: e0157908.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Karmakar, K., Kundu, A., Rizvi, A.Z., Dubois, E., Severac, D., Czernic, P., Cartieaux, F., and DasGupta, M. (2019). Transcriptomic analysis with the progress of symbiosis in 'crack-entry' legume *Arachis hypogaea* highlights its contrast with 'Infection thread' adapted legumes. *Mol. Plant-Microbe Interact.* 32: 271-285.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Karunakaran, R., Ramachandran, V.K., Seaman, J.C., East, A.K., Mouhsine, B., Mauchline, T.H., Prell, J., Skeffington, A., and Poole, P.S. (2009). Transcriptomic analysis of *Rhizobium leguminosarum biovar viciae* in symbiosis with host plants *Pisum sativum* and *Vicia cracca*. *J. Bacteriol.* 191: 4002-4014.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kazmierczak, T., Nagymihaly, M., Lamouche, F., Barrière, Q., Guefrachi, I., Alunni, B., Ouadghiri, M., Ibijbjen, J., Kondorosi, É., Mergaert, P., and Gruber, V. (2017). Specific host-responsive associations between *Medicago truncatula* accessions and *Sinorhizobium* strains. *Mol. Plant-Microbe Interact.* 30: 399-409.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

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

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Keller, J., Imperial, J., Ruiz-Argüeso, T., Privet, K., Lima, O., Michon-coudouel, S., Biget, M., Salmon, A., Ainouche, A., and Cabello-Hurtado, F. (2018). RNA sequencing and analysis of three *Lupinus nodulomes* provide new insights into specific host-symbiont relationships with compatible and incompatible *Bradyrhizobium* strains. *Plant Sci.* 266: 102-116.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kelly, S., Mun, T., Stougaard, J., Ben, C., and Andersen, S.U. (2018). Distinct *Lotus japonicus* transcriptomic responses to a spectrum of bacteria ranging from symbiotic to pathogenic. *Front. Plant Sci.* 9: 1218.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kevei, Z., Vinardell, J.M., Kiss, G.B., Kondorosi, A., and Kondorosi, E. (2002). Glycine-rich proteins encoded by a nodule-specific gene family are implicated in different stages of symbiotic nodule development in *Medicago* Spp.. *Mol. Plant-Microbe Interact.* 15: 922-931.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kiirika, L.M., Bergmann, H.F., Schikowsky, C., Wimmer, D., Korte, J., Schmitz, U., Niehaus, K., and Colditz, F. (2012). Silencing of the Rac1 GTPase MtROP9 in *Medicago truncatula* stimulates early mycorrhizal and oomycete root colonizations but negatively affects rhizobial infection. *Plant Physiol.* 159: 501-516.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Koeppen K, Hampton TH, Jarek M, Scharfe M, Gerber SA, Mielcarz DW, Demers, E.G., Dolben, E.L., Hammond, J.H., Hogan, D.A., and Stanton, B.A. (2016) A novel mechanism of host-pathogen interaction through sRNA in bacterial outer membrane vesicles. *PLoS Pathog.* 12: e1005672.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kondorosi, E., Mergaert, P., and Kereszt, A. (2013). A paradigm for endosymbiotic life: Cell differentiation of *Rhizobium* bacteria provoked by host plant factors. *Annu. Rev. Microbiol.* 67: 611-628.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kudapa, H., Garg, V., Chitkineni, A., and Varshney, R.K. (2019). The RNA-Seq-based high resolution gene expression atlas of chickpea (*Cicer arietinum* L.) reveals dynamic spatio-temporal changes associated with growth and development. *Plant Cell Environ.* 41: 2209-2225.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kumar, P., Kuscu, C., and Dutta, A. (2016). Biogenesis and function of transfer RNA-related fragments (tRFs). *Trends Biochem. Sci.* 41: 679-689.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lamouche, F. et al. (2019). Transcriptomic dissection of *Bradyrhizobium* sp. strain ORS285 in symbiosis with *Aeschynomene* spp. inducing different bacteroid morphotypes with contrasted symbiotic efficiency. *Environ. Microbiol.* 21: 3244-3258.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lang, C., and Long, S.R. (2015). Transcriptome analysis of *Sinorhizobium meliloti* and *Medicago truncatula* symbiosis using nitrogen

fixation-deficient nodules. Mol. Plant-Microbe Interact. 28: 856-868.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Laporte, P., Satiat-Jeunemaître, B., Velasco, I., Csorba, T., Van de Velde, W., Campalans, A., Burgyan, J., Arevalo-Rodriguez, M., and Crespi, M., (2010). A novel RNA-binding peptide regulates the establishment of the Medicago truncatula–Sinorhizobium meliloti nitrogen-fixing symbiosis. Plant J. 62: 24–38.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Larrainzar, E. et al. (2015). Deep sequencing of the Medicago truncatula root transcriptome reveals a massive and early interaction between nodulation factor and ethylene signals. Plant Physiol. 169: 233-265.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Lauressergues, D., Couzigou, J.-M., San Clemente, H. Martinez, Y., Dunand, Y., Bécard, G., and Combier, J.-P. (2015). Primary transcripts of microRNAs encode regulatory peptides. Nature 520: 90-93.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Legocki, R.P., and Verma, D.P.S. (1980). Identification of "Nodule-Specific" host proteins (nodulins) involved in the development of symbiosis. Cell 20: 153–163.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Lelandais-Brière, C., Naya, L., Sallet, E., Calenge, F., Frugier, F., Hartmann, C., Gouzy, J., and Crespi M. (2009). Genome-wide Medicago truncatula small RNA analysis revealed novel microRNAs and isoforms differentially regulated in roots and nodules. Plant Cell 21: 2780–2796.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Lefebvre, B. et al (2010). A remorin protein interacts with symbiotic receptors and regulates bacterial infection. Proc. Natl. Acad. Sci. USA 107: 2343-2348.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Li, H., Wang, W., Mortimer, P.E., Li, R., Li, D., Hyde, K.D., Xu, J., Soltis, D.E., and Chen, Z (2015). Large-scale phylogenetic analyses reveal multiple gains of actinorhizal nitrogen-fixing symbioses in angiosperms associated with climate change. Sci. Rep. 5: 14023.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Liang, P., Stratil, T.F., Popp, C., Marin, M., Folgmann, J., Mysore, K.S., Wen, J., and Ott, T. (2018). Symbiotic root infections in Medicago truncatula require remorin-mediated receptor stabilization in membrane nanodomains. Proc. Natl. Acad. Sci. USA 115: 5289-5294.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Liang, Y., Cao, Y., Tanaka, K., Thibivilliers, S., Wan, J., Choi, J., ho Kang, C., Qiu, J., and Stacey, G. (2013). Nonlegumes respond to rhizobial Nod factors by suppressing the innate immune response. Science 341: 1384–1387.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Libault, M., Joshi, T., Takahashi, K., Hurley-Sommer, A, Puricelli, K., Blake, S., Finger, R.E., Taylor, C.G., Xu, D., Nguyen, H.T., and Stacey, G. (2009a). Large-scale analysis of putative soybean regulatory gene expression identifies a Myb gene involved in soybean nodule development. Plant Physiol. 151: 1207–1220.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Libault, M., Joshi, T., Benedito, V.A, Xu, D., Udvardi, M.K., and Stacey, G. (2009b). Legume transcription factor genes: What makes legumes so special? Plant Physiol. 151: 991–1001.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Libault, M., Farmer, A, Brechenmacher, L., Drnevich, J., Langley, R.J., Bilgin, D.D., Radwan, O., Neece, DJ, Clough, S.J., May, G.D., and Stacey, G. (2010). Complete transcriptome of the soybean root hair cell, a single-cell model, and its alteration in response to Bradyrhizobium japonicum infection. Plant Physiol. 152: 541-552.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Libault, M. (2018). Transcriptional reprogramming of legume genomes: Perspective and challenges associated with single-cell and single cell-type approaches during nodule development. Front Plant Sci. 9: 1600.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Liese, R., Schulze, J., and Cabeza, R.A (2017). Nitrate application or P deficiency induce a decline in Medicago truncatula N2-fixation

by similar changes in the nodule transcriptome. *Sci. Rep.* 7: 46264.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Limpens, E., Mirabella, R., Fedorova, E., Franken, C., Franssen, H., Bisseling, T. and Geurts, R. (2005). Formation of organelle-like N₂-fixing symbiosomes in legume root nodules is controlled by DMI2. *Proc. Natl. Acad. USA* 102: 10375–10380.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Limpens, E., Ivanov, S., Esse, W. Van, Voets, G., Fedorova, E., and Bisseling, T. (2009). Medicago N₂-fixing symbiosomes acquire the endocytic identity marker Rab7 but delay the acquisition of vacuolar identity. *Plant Cell* 21: 2811–2828.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Limpens, E., Moling, S., Hooiveld, G., Pereira, P.A, Bisseling, T., Becker, J.D., and Küster, H. (2013). Cell- and tissue-specific transcriptome analyses of Medicago truncatula root nodules. *PLoS One* 8: e64377.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Liu, J., Miller, S.S., Graham, M., Bucciarelli, B., Catalano, C.M., Sherrier, D.J., Samac, D.A., Ivashuta, S., Fedorova, M., Matsumoto, P., Gantt, J.S., Vance, C.P. (2006). Recruitment of novel calcium-binding proteins for root nodule symbiosis in Medicago truncatula. *Plant Physiol.* 141:167-77.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Liu, H., Zhang, C., Yang, J., Yu, N., and Wang, E. (2018). Hormone modulation of legume-rhizobial symbiosis. *J. Integr. Plant Biol.* 60: 632-648.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Liu, C.W. et al. (2019a). NIN acts as a network hub controlling a growth module required for rhizobial infection. *Plant Physiol.* 179: 1704-1722.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Liu, J., Rutten, L., Limpens, E., van der Molen, T.V., van Velzen, R.V., Chen, R., Chen, Y., Geurts R., Kohlen W., Kulikova O., and Bisseling T. (2019b). A remote cis-regulatory region is required for NIN expression in the pericycle to initiate nodule primordium formation in Medicago truncatula. *Plant Cell* 31: 68-83.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Magne, K., George, J., Berbel Tornero, A., Broquet, B., Madueño, F., Andersen, S.U., and Ratet, P. (2018). Lotus japonicus NOOT-BOP-COCH-LIKE1 is essential for nodule, nectary, leaf and flower development. *Plant J.* 94: 880-894.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Markmann, K., Giczey, G., and Parniske, M. (2008). Functional adaptation of a plant receptor-kinase paved the way for the evolution of intracellular root symbioses with bacteria. *PLoS Biol.* 6: e68.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Markmann, K., and Parniske, M. (2009). Evolution of root endosymbiosis with bacteria: how novel are nodules? *Trends Plant Sci.* 14: 77–86.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Marsh, J., Rakocevic, A., Mitra, R.M., Brocar L., Sun, J., Eschstruth, A, Long, S.R., Schultze, M., Ratet, P., and Oldroyd, G.E.D. (2007). Medicago truncatula NIN is essential for rhizobial-independent nodule organogenesis induced by autoactive calcium/calmodulin-dependent protein kinase. *Plant Physiol.* 144: 324-335.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Martínez-Hidalgo, P., and Hirsch, A.M. (2017). The nodule microbiome: N₂-fixing rhizobia do not live alone. *Phytobiomes J.* 1: 70-82.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Maunoury, N. et al. (2010). Differentiation of symbiotic cells and endosymbionts are coupled to two transcriptome-switches in Medicago truncatula nodulation. *PLoS One* 5: e9519.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Mecchia, M.A., Santos-fernandez, G., Duss, N.N., Somoza, S.C., Boisson-dernier, A, Gagliardini, V., Martínez-bernardini, A, Fabrice, T.N., Ringli, C., Muschietti, J.P., and Grossniklaus, U. (2017). RALF4/19 peptides interact with LRX proteins to control pollen tube growth in Arabidopsis. *Science* 358: 1600–1603.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Mergaert, P. (2018). Role of antimicrobial peptides in controlling symbiotic bacterial populations. *Nat. Prod. Rep.* 35: 336-356.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Mergaert, P., Nikovics, K., Kelemen, Z., Maunoury, N., Vauvert, D., Kondorosi, A., and Kondorosi, E. (2003). A novel family in *Medicago truncatula* consisting of more than 300 nodule-specific genes coding for small, secreted polypeptides with conserved cysteine motifs. *Plant Physiol.* 132: 161–173.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Messinese, E., Mun, J.H., Yeun, L.H., Jayaraman, D., Rougé, P., Barre, A., Loughon, G., Schornack, S., Bono, J.J., Cook, D.R., and Ané, J.M. (2007). A novel nuclear protein interacts with the symbiotic DMI3 calcium- and calmodulin-dependent protein kinase of *Medicago truncatula*. *Mol. Plant-Microbe Interact.* 20: 912–921.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Metz, B.A., Welters, P., Hoffmann, H.J., Jensen, E.Ø, Schell, J., and de Bruijn, F.J. (1988). Primary structure and promoter analysis of leghemoglobin genes of the stem-nodulated tropical legume *Sesbania rostrata*: conserved coding sequences, cis-elements and trans-acting factors. *Mol Gen Genet.* 214: 181-91.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Middleton, P.H. et al. (2007). An ERF transcription factor in *Medicago truncatula* that is essential for Nod factor signal transduction. *Plant Cell* 19: 1221-1234.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Miller, S.S., Driscoll, B.T., Gregerson, R.G., Gantt, J.S., and Vance, C.P. (1998). Alfalfa malate dehydrogenase (MDH): molecular cloning and characterization of five different forms reveals a unique nodule-enhanced MDH. *Plant J.* 15: 173–184.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Moling, S., Pietraszewski-Bogiel, A., Postma, M., Fedorova, E., Hink, M.A., Limpens, E., Gadella, T.W, and Bisseling, T. (2014). Nod factor receptors form heteromeric complexes and are essential for intracellular infection in *Medicago* nodules. *Plant Cell* 26: 4188-4199.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Montiel, J., Nava, N., Cárdenas, L., Sánchez-López, R., Arthikala, M.-K., Santana, O., Sánchez, F., and Quinto, C. (2013). A *Phaseolus vulgaris* NADPH oxidase gene is required for root infection by rhizobia. *Plant Cell Physiol.* 53: 1751–1767.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Montiel, J., Downie, J.A., Farkas, A., Bihari, P., Herczeg, R., Balint, B., Mergaert, P., Kereszt, A., and Kondorosi, E. (2017). Morphotype of bacteroids in different legumes correlates with the number and type of symbiotic NCR peptides. *Proc. Natl. Acad. Sci. USA* 114: 5041-5046.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Moreau, S., Verdenaud, M., Ott, T., Letort, S., de Billy, F., Niebel, A., Gouzy, J., de Carvalho-Niebel, F., Gamas, P. (2011). Transcription reprogramming during root nodule development in *Medicago truncatula*. *PLoS One* 6: e16463.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Morieri, G., Martinez, E.A., Jarynowski, A., Driguez, H., Morris, R., Oldroyd, G.E., and Downie J.A. (2013). Host-specific Nod-factors associated with *Medicago truncatula* nodule infection differentially induce calcium influx and calcium spiking in root hairs. *New Phytol.* 200: 656-662.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Murphy, E. and De Smet, I. (2014). Understanding the RALF family: a tale of many species. *Trends Plant Sci.* 19: 664–671.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Nagyimihály, M., Veluchamy, A., Györgypál, Z., Ariel, F., Jégu, T., Benhamed, M., Szűcs, A., Kereszt, A., Mergaert, P., and Kondorosi, É. (2017). Ploidy-dependent changes in the epigenome of symbiotic cells correlate with specific patterns of gene expression. *Proc. Natl. Acad. Sci. USA* 114: 4543–4548.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Nallu, S., Silverstein, K.A.T., Samac, D.A., Bucciarelli, B., Vance, C.P., and VandenBosch K.A. (2013). Regulatory patterns of a large family of defensin-like genes expressed in nodules of *Medicago truncatula*. *PLoS One* 8: e60355.

- Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- Nallu, S., Silverstein, K.A.T., Zhou, P., Young, N.D., and VandenBosch, K.A. (2014).** Patterns of divergence of a large family of nodule cysteine-rich peptides in accessions of *Medicago truncatula*. *Plant J.* 78: 697–705.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- Nelson, M.S., and Sadowsky, M.J. (2015).** Secretion systems and signal exchange between nitrogen-fixing rhizobia and legumes. *Front. Plant Sci.* 6: 491.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- Nouwen, N., Arrighi, J.F., Cartieaux, F., Chaintreuil, C., Gully, D., Klopp, C., and Giraud, E. (2017).** The role of rhizobial (NifV) and plant (FEN1) homocitrate synthases in *Aeschynomene*/photosynthetic *Bradyrhizobium* symbiosis. *Sci. Rep.* 7: 448.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- Nova-franco, B., Iñiguez, L.P., Valdés-lópez, O., Alvarado-affantranger, X., Leija, A., Fuentes, S.I., Ramírez, M., Paul, S., Reyes, J.L., Girard, L., and Hernández, G. (2015).** The Micro-RNA172c-APETALA2-1 node as a key regulator of the common bean-Rhizobium etli nitrogen fixation symbiosis. *Plant Physiol.* 168: 273–291.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- O'Rourke, J.A. et al. (2014).** An RNA-Seq based gene expression atlas of the common bean. *BMC Genomics* 15: 866
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- O'Rourke, J.A. et al. (2015).** The *Medicago sativa* gene index 1.2: a web-accessible gene expression atlas for investigating expression differences between *Medicago sativa* subspecies. *BMC Genomics* 16: 502.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- Oldroyd, G.E.D. (2013).** Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nat. Rev. Microbiol.* 11: 252-263.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- Oldroyd, G.E.D., and Dixon, R. (2014).** Biotechnological solutions to the nitrogen problem. *Curr. Opin. Biotechnol.* 26: 19–24.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- Ott, T. (2017).** Membrane nanodomains and microdomains in plant–microbe interactions. *Curr. Opin. Plant Biol.* 40: 82–88.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- Ovchinnikova, E. et al. (2011).** IPD3 controls the formation of nitrogen-fixing symbiosomes in pea and *Medicago* spp.. *Mol. Plant-Microbe Interact.* 24: 1333–1344.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- Paez Valencia, J., Goodman, K., and Otegui, M.S. Endocytosis and endosomal trafficking in plants. *Annu. Rev. Plant Biol.* 67: 309-335.**
- Pan, H., Oztas, O., Zhang, X., Wu, X., Stonoha, C., Wang, E., Wang, B., and Wang, D. (2016).** A symbiotic SNARE protein generated by alternative termination of transcription. *Nat. Plants* 2: 15197.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- Pan, H., and Wang, D. (2018).** *Medicago* plants control nodulation by regulating proteolysis of the receptor-like kinase DMI2. *Plant Physiol.* 177: 792-802.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- Parniske, M. (2018).** Uptake of bacteria into living plant cells, the unifying and distinct feature of the nitrogen-fixing root nodule symbiosis. *Curr. Opin. Plant Biol.* 44: 164-174.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- Pecrix, Y. et al. (2018).** Whole-genome landscape of *Medicago truncatula* symbiotic genes. *Nat. Plants* 4: 1017-1025.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only Title Only Author and Title](#)
- Peláez, P., Trejo, M.S., Iñiguez, L.P., Estrada-Navarrete, G., Covarrubias, A.A., Reyes, J.L. and Sanchez, F. (2012).** Identification and characterization of microRNAs in *Phaseolus vulgaris* by high-throughput sequencing. *BMC Genomics* 13: 83.
 Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Peng, Z., Liu, F., Wang, L., Zhou, H., Paudel, D., Tan, L., Maku, J., Gallo, M., and Wang, J. (2017). Transcriptome profiles reveal gene regulation of peanut (*Arachis hypogaea* L.) nodulation. *Sci. Rep.* 7: 40066.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Persson, T. et al. (2015). Candidatus *Frankia datiscae* Dg1, the actinobacterial microsymbiont of *Datisca glomerata*, expresses the canonical nod genes *nodABC* in symbiosis with its host. *PLoS One* 10: e0127630.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Pierre, O., Hopkins, J., Combier, M., Baldacci, F., Engler, G., Brouquisse, R., Hérout, D., and Boncompagni, E. (2014). Involvement of papain and legumain proteinase in the senescence process of *Medicago truncatula* nodules. *New Phytol.* 202: 849-863.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Poole, P., Ramachandran, V., and Terpolilli, J. (2018). Rhizobia: from saprophytes to endosymbionts. *Nat. Rev. Microbiol.* 16: 291-303.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ren, B., Wang, X., Duan, J., and Ma, J. (2019). Rhizobial tRNA-derived small RNAs are signal molecules regulating plant nodulation. *Science* 365: 919-922.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Price, M.N., Deutschbauer, A.M., Skerker, J.M., Wetmore, K.M., Ruths, T., Mar, J.S., Kuehl, J. V., Shao, W., and Arkin, A.P. (2013). Indirect and suboptimal control of gene expression is widespread in bacteria. *Mol. Syst. Biol.* 9: 660.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Pislariu, C.I., Sinharoy, S., Torres-Jerez, I., Nakashima, J., Blancaflor, E.B., and Udvardi, M.K. (2019). The nodule-specific PLAT domain protein NPD1 is required for nitrogen-fixing symbiosis. *Plant Physiol.* 180: 1480-1497.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Qiao, Z., Brechenmacher, L., Smith, B., Strout, G.W., Mangin, W., Taylor, C., Russell, S.D., Stacey, G., and Libault, M. (2017). The *GmFWL1* (FW2-2-like) nodulation gene encodes a plasma membrane microdomain-associated protein. *Plant Cell Environ.* 40: 1442-1455.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Richter, H.E., Sandal, N.N., Marcker, K.A., and Sengupta-Gopalan, C. (1991). Characterization and genomic organization of a highly expressed late nodulin gene subfamily in soybeans. *Mol. Gen. Genet.* 20: 445-452.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Rodrigues, S.G., Stickels, R.R., Goeva, A., Martin, C.A., Murray, E., Vanderburg, C.R., Welch, J., Chen, L.M., Chen, F., and Macosko, E.Z. (2019). Slide-seq: A scalable technology for measuring genome-wide expression at high spatial resolution. *Science* 363: 1463-1467.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Roux, B. et al. (2014). An integrated analysis of plant and bacterial gene expression in symbiotic root nodules using laser-capture microdissection coupled to RNA sequencing. *Plant J.* 77: 817-837.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Roy, S., Liu, W., Nandety, R.S., Crook, A., Mysore, K.S., Pislariu, C.I., Frugoli, J., Dickstein, R., and Udvardi, M.K. (2019). Celebrating 20 years of genetic discoveries in legume nodulation and symbiotic nitrogen fixation. *Plant Cell* in press.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Salgado, M.G., van Velzen, R., Nguyen, T.V., Battenberg, K., Berry, A.M., Lundin, D., and Pawlowski, K. (2018). Comparative analysis of the nodule transcriptomes of *Ceanothus thyrsiflorus* (Rhamnaceae, Rosales) and *Datisca glomerata* (Datisceae, Cucurbitales). *Front. Plant Sci.* 9: 1629.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Sandal, N.N., Boisen, K., and Marcker, K.J. (1987). A small family of nodule specific genes from soybean. *Nucleic Acids Res.* 15: 1507-1519.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Satgé, C., Moreau, S., Sallet, E., Lefort, G., Auriac, M.-C., Rembrière, C., Cottret, L., Gallardo, K., Noirot, C., Jardinaud, M.-F., and Gamas, P. (2016). Reprogramming of DNA methylation is critical for nodule development in *Medicago truncatula*. *Nat. Plants* 2: 16166.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

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

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Schiessl, K. et al. (2019). NODULE INCEPTION recruits the lateral root developmental program for symbiotic nodule organogenesis in *Medicago truncatula*. *Curr Biol.* doi:10.1016/j.cub.2019.09.005.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Seabra, A.R., Pereira, P.A., Becker, J.D., and Carvalho, H.G. (2012). Inhibition of glutamine synthetase by phosphinothricin leads to transcriptome reprogramming in root nodules of *Medicago truncatula*. *Mol. Plant-Microbe Interact.* 25: 976–992.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Senovilla, M., Castro-Rodríguez, R., Abreu, I., Escudero, V., Kryvoruchko, I., Udvardi, M.K., Imperial, J., and González-guerrero, M. (2018). *Medicago truncatula* copper transporter 1 (MtCOPT1) delivers copper for symbiotic nitrogen fixation. *New Phytol.* 218: 696-709.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Severin, A.J. et al. (2010). RNA-Seq Atlas of *Glycine max*: A guide to the soybean transcriptome. *BMC Plant Biol.* 10: 160.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Shubin, N., Tabin, C., and Carroll, S. (2009). Deep homology and the origins of evolutionary novelty. *Nature* 457: 818-823.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Sijacic, P., Wang, W., and Liu, Z. (2011). Recessive antimorphic alleles overcome functionally redundant loci to reveal TSO1 function in *Arabidopsis* flowers and meristems. *PLoS Genet.* 7: e1002352.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Singh, S., Katzer, K., Lambert, J., Cerri, M., and Parniske, M. (2014). CYCLOPS, a DNA-binding transcriptional activator, orchestrates symbiotic root nodule development. *Cell Host Microbe* 15: 139-152.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Sinharoy, S., Torres-Jerez, I., Bandyopadhyay, K., Kereszt, A., Pislariu, C.I., Nakashima, J., Benedito, V.A., Kondorosi, E., and Udvardi, M.K. (2013). The C2H2 transcription factor REGULATOR OF SYMBIOSOME DIFFERENTIATION represses transcription of the secretory pathway gene VAMP721a and promotes symbiosome development in *Medicago truncatula*. *Plant Cell* 25: 3584–3601.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

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

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Soltis, D.E., Soltis, P.S., Morgan, D.R., Swensen, S.M., Mullin, B.C., Dowd, J.M., Martin, P.G. (1995). Chloroplast gene sequence data suggest a single origin of the predisposition for symbiotic nitrogen fixation in angiosperms. *Proc. Natl. Acad. Sci. USA* 92: 2647–2651.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

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

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Soyano, T., and Hayashi, M. (2014). Transcriptional networks leading to symbiotic nodule organogenesis. *Curr. Opin. Plant Biol.* 20: 146-54.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ståhl, P.L. et al. (2016). Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science* 353: 78-82.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Starker, C.G., Parra-Colmenares, A.L., Smith, L., Mitra, R.M., and Long, S.R. (2006). Nitrogen fixation mutants of *Medicago truncatula* fail to support plant and bacterial symbiotic gene expression. *Plant Physiol.* 140: 671-680.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Stokstad, E. (2016). The nitrogen fix. *Science* 353: 1225–1228.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Stonoha-Arther, C., and Wang, D. (2018). Though love: accommodating intracellular bacteria through directed secretion of antimicrobial peptides during the nitrogen-fixing symbiosis. *Curr. Opin. Plant Biol.* 44: 155-163.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Stougaard, J., Sandal, N.N., Groen, A., Kühle, A., and Marcker, K.A. (1987). Analysis of the soybean leghemoglobin lbc3 gene: regulatory elements required for promoter activity and organ specificity. *EMBO J.* 6: 3565–3569.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Sturms, R., Kakar, S., Trent III, J., and Hargrove, M.S. (2010). Trema and Parasponia hemoglobins reveal convergent evolution of oxygen transport in plants. *Biochemistry* 49: 4085–4093.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Subramanian, S., Fu, Y., Sunkar, R., Barbazuk, W.B., Zhu, J.K., and Yu, O. (2008). Novel and nodulation-regulated microRNAs in soybean roots. *BMC Genomics.* 9: 160.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Sugawara, M. et al. (2013). Comparative genomics of the core and accessory genomes of 48 Sinorhizobium strains comprising five genospecies. *Genome Biol.* 14: R17.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Sugiyama, A., Saida, Y., Yoshimizu, M., Takanashi, K., Sosso, D., Frommer, W.B., and Yazaki, K. (2017). Molecular characterization of LjSWEET3, a sugar transporter in nodules of *Lotus japonicus*. *Plant Cell Physiol.* 58: 298–306.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Sullivan, D., Brisson, N., Goodchild, B., and Verma, D.P.S. (1981). Molecular cloning and organization of two leghaemoglobin genomic sequences of soybean. *Nature* 289: 516-518.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

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

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Svistonoff, S. et al. (2013). The independent acquisition of plant root nitrogen-fixing symbiosis in Fabids recruited the same genetic pathway for nodule organogenesis. *PLoS One* 8: e64515.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Szabados, L., Ratet, P., Grunenberg, B., and de Bruijn, F.J. (1990). Functional analysis of the *Sesbania rostrata* leghemoglobin glb3 gene 5'-upstream region in transgenic *Lotus corniculatus* and *Nicotiana tabacum* plants. *Plant Cell* 2: 973-986.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Szczyglowski, K., Szabados, L., Fujimoto, S.Y., Silver, D., and de Bruijn, F.J. (1994). Site-specific mutagenesis of the nodule-infected cell expression (NICE) element and the AT-rich element ATRE-BS2 of the *Sesbania rostrata* leghemoglobin glb3 promoter. *Plant Cell* 6: 317–332.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Takanashi, K., Takahashi, H., Sakurai, N., Sugiyama, A., Suzuki, H., Shibata, D., Nakazono, M., and Yazaki, K. (2012). Tissue-specific transcriptome analysis in nodules of *Lotus japonicus*. *Mol. Plant-Microbe Interact.* 25: 869–876.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Taleski, M., Imin, N., and Djordjevic, M.A. (2018). CEP peptide hormones: key players in orchestrating nitrogen-demand signalling, root nodulation, and lateral root development. *J. Exp. Bot.* 69: 1829-1836.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Tautz, D. and Domazet-Lošo, T. (2011). The evolutionary origin of orphan genes. *Nat. Rev. Genet.* 12: 692–702.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Temple, S.J., Vance, C.P., and Gantt, J.S. (1998). Glutamate synthase and nitrogen assimilation. *Trends Plant Sci.* 3: 51-56.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Tesfaye, M. et al. (2013). Spatio-temporal expression patterns of *Arabidopsis thaliana* and *Medicago truncatula* defensin-like genes. PLoS One 8: e58992.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Teulet, A, et al. (2019). The Bradyrhizobial type III effector BtnA confers the ability to form nodules in legumes. Proc. Natl. Acad. Sci. USA doi:10.1073/pnas.1904456116.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Timmers, A.C.J., Auriac, M., and Truchet, G. (1999). Refined analysis of early symbiotic steps of the *Rhizobium-Medicago* interaction in relationship with microtubular cytoskeleton rearrangements. Development 126: 3617–3628.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Trujillo, D.I., Silverstein, K.A., and Young, N.D. (2014). Genomic characterization of the LEED..PEEDs, a gene family unique to the *Medicago* lineage. G3 4: 2003-2012.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Trujillo, D.I., Silverstein, K.A.T., and Young, N.D. (2019). Nodule-specific PLAT domain proteins are expanded in the *Medicago* lineage and required for nodulation. New Phytol. 222: 1538–1550.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Tsikou, D., Yan, Z., Holt, D.B., Abel, N.B., Reid, D.E., Madsen, L.H., Bhasin H., Sexauer, M., Stougaard, J., and Markmann, K. (2018). Systemic control of legume susceptibility to rhizobial infection by a mobile microRNA Science 362: 233-236.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Udvardi, M. and Poole, P.S. (2013). Transport and metabolism in legume-rhizobia symbioses. Annu. Rev. Plant Biol. 64: 781–805.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

van Brussel, A.A.N., Bakhuizen, R., van Spronsen, P.C., Spaink, H.P., Tak, T., Lugtenberg, B.J.J., and Kijne, J.W. (1992). Induction of pre-infection thread structures in the leguminous host plant by mitogenic lipo-oligosaccharides of *Rhizobium*. Science 257: 70–72.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Van de Velde, W., Pérez Guerra, J.C., De Keyser, A., De Rycke, R., Rombauts, S., Maunoury, N., Mergaert, P., Kondorosi, E., Holsters, M., and Goormachtig, S. (2006). Aging in legume symbiosis. A molecular view on nodule senescence in *Medicago truncatula*. Plant Physiol. 141: 711–720.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

van Velzen, R. et al. (2018). Comparative genomics of the nonlegume *Parasponia* reveals insights into evolution of nitrogen-fixing rhizobium symbioses. Proc. Natl. Acad. Sci. USA 115: E4700-E4709.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

van Velzen, R., Doyle, J.J., and Geurts, R. (2019). A resurrected scenario: single gain and massive loss of nitrogen-fixing nodulation. Trends Plant Sci. 24: 49-57.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Van Zeijl, A., Op den Camp, R.H.M., Deinum, E.E., Charnikhova, T., Franssen, H., Op den Camp, H.J.M., Bouwmeester, H., Kohlen, W., Bisseling, T., and Geurts, R. (2015). *Rhizobium* lipo-chitooligosaccharide signaling triggers accumulation of cytokinins in *Medicago truncatula* roots. Mol. Plant 8: 1213–1226.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

van Zeijl, A., Wardhani, T.A.K., Seifi Kalhor, M., Rutten, L., Bu, F., Hartog, M., Linders, S., Fedorova, E.E., Bisseling, T., Kohlen, W., and Geurts, R. (2018). CRISPR/Cas9-mediated mutagenesis of four putative symbiosis genes of the tropical tree *Parasponia andersonii* reveals novel phenotypes. Front. Plant Sci. 9: 284.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Verdier, J. et al. (2013). A regulatory network-based approach dissects late maturation processes related to the acquisition of desiccation tolerance and longevity of *Medicago truncatula* seeds. Plant Physiol. 163: 757–774.

Pubmed: [Author and Title](#)
Google Scholar: [Author Only Title Only Author and Title](#)

Vernié, T., Moreau, S., de Billy, F., Plet, J., Combier, J.-P., Rogers, C. Oldroyd, G., Frugier, F., Niebel, A., and Gamas, P. (2008). EFD Is an ERF transcription factor involved in the control of nodule number and differentiation in *Medicago truncatula*. *Plant Cell* 20: 2696–2713.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Vernié, T., Kim, J., Frances, L., Ding, Y., Sun, J., Guan, D., Niebel, A., Gifford, M.L., de Carvalho-Niebel, F., and Oldroyd, G.E.D. (2015). The NIN transcription factor coordinates diverse nodulation programs in different tissues of the *Medicago truncatula* root. *Plant Cell* 27: 3410–3424.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Vinardell, J.M., Fedorova, E., Cebolla, A., Kevei, Z., Horváth, G., Kelemen, Z., Tarayre, S., Roudier, F., Mergaert, P., Kondorosi, A., and Kondorosi, E. (2003). Endoreduplication mediated by the anaphase-promoting complex activator CCS52A is required for symbiotic cell differentiation in *Medicago truncatula* nodules. *Plant Cell* 15: 2093–2105.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Wang, H., Moore, M.J., Soltis, P.S., Bell, C.D., Brockington, S.F., Alexandre, R., Davis, C.C., Latvis, M., Manchester, S.R., and Soltis, D.E. (2009). Rosid radiation and the rapid rise of angiosperm-dominated forests. *Proc. Natl. Acad. Sci. USA* 106: 3853–3858.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Wang, D., Griffiths, J., Starker, C., Fedorova, E., Limpens, E., Ivanov, S., Bisseling, T., and Long, S. (2010). A nodule-specific protein secretory pathway required for nitrogen-fixing symbiosis. *Science* 327: 1126–1129.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Wang, Y., Wang, L., Zou, Y., Chen, L., Cai, Z., Zhang, S., Zhao, F., Tian, Y., Jiang, Q., Ferguson, B.J., Gresshoff, P.M., and Li, X. (2014). Soybean miR172c targets the repressive AP2 transcription factor NNC1 to activate ENOD40 expression and regulate nodule initiation. *Plant Cell* 26: 4782–4801.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Wang, Y., Li, K., Chen, L., Zou, Y., Liu, H., Tian, Y., Li, D., Wang, R., Zhao, F., Ferguson, B.J., Gresshoff, P.M., and Li, X. (2015). MicroRNA167-directed regulation of the auxin response factors GmARF8a and GmARF8b is required for soybean nodulation and lateral root development. *Plant Physiol.* 168: 984-999.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Wang, C. et al. (2016). NODULES WITH ACTIVATED DEFENSE 1 is required for maintenance of rhizobial endosymbiosis in *Medicago truncatula*. *New Phytol.* 212: 176–191.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Wang L., Sun Z., Su C., Wang Y., Yan Q., Chen J., Ott T., and Li X. (2019). A GmNINa-miR172c-NNC1 regulatory network coordinates the nodulation and autoregulation of nodulation pathways in soybean. *Mol. Plant.* doi:10.1016/j.molp.2019.06.002.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Werner, G.D.A., Cornwell, W.K., Sprent, J.I., Kattge, J., and Kiers, E.T. (2014). A single evolutionary innovation drives the deep evolution of symbiotic N₂-fixation in angiosperms. *Nat. Commun.* 5: 4087.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Wildermuth, M.C., Steinwand, M.A., McRae, A.G., Jaenisch, J., and Chandran, D. (2017). Adapted biotroph manipulation of plant cell ploidy. *Annu. Rev. Phytopathol.* 55: 537-564.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Wu Z., Wang M., Yang S., Chen S., Chen X., Liu C., Wang S., Wang H., Zhang B., Liu H., Qin R., and Wang X. (2019). A global coexpression network of soybean genes gives insight into the evolution of nodulation in non-legumes and legumes. *New Phytol.* doi:10.1111/nph.15845.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Xiao, T.T., Schilderink, S., Moling, S., Deinum, E.E., Kondorosi, E., Franssen, H., Kulikova, O., Niebel A., and Bisseling, T. (2014). Fate map of *Medicago truncatula* root nodules. *Development* 141: 3517–3528.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Yan, Z., Shakhawat Hossain, M., Arikat, S., Valdés-López, O., Zhai, J., Wang, J., Libault, M., Ji, T., Qiu, L., Meyers, B.C., and Stacey, G. (2015). Identification of microRNAs and their mRNA targets during soybean nodule development: functional analysis of the role of miR393j-3p in soybean nodulation. *New Phytol.* 207: 748–759.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yan, Z., et al. (2016). Identification and functional characterization of soybean root hair microRNAs expressed in response to Bradyrhizobium japonicum infection. Plant Biotechnol. J. 14: 332-341.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

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

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yang, Z. (2008). Cell polarity signaling in Arabidopsis. Annu. Rev. Cell Dev. Biol. 24: 551–575.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yano, K. et al. (2008). CYCLOPS, a mediator of symbiotic intracellular accommodation. Proc. Natl. Acad. Sci. USA 105: 20540-20545.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Young, N.D. et al. (2011). The Medicago genome provides insight into the evolution of rhizobial symbioses. Nature 480: 520-524.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yu, H., Xiao, A., Dong, R., Fan, Y., Zhang, X., Liu, C., Wang, C., Zhu, H., Duanmu, D., Cao, Y., and Zhang, Z. (2018). Suppression of innate immunity mediated by the CDPK-Rboh complex is required for rhizobial colonization in Medicago truncatula nodules. New Phytol. 220: 425-434.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yuan, S.L., Li, R., Chen, H.F., Zhang, C.J., Chen, L.M., Hao, Q.N., Chen, S.L., Shan, Z.H., Yang, Z.L., Zhang, X.J., Qiu, Z., and Zhou, X.A. (2017). RNA-Seq analysis of nodule development at five different developmental stages of soybean (Glycine max) inoculated with Bradyrhizobium japonicum strain 113-2. Sci Rep. 7: 42248.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Zhou, P. et al. (2017). Exploring structural variation and gene family architecture with De Novo assemblies of 15 Medicago genomes. BMC Genomics 18: 261.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)