Plant Mol Biol (2016) 90:613–622
DOI 10.1007/s11103-016-0450-2

Recent advances in actinorhizal symbiosis signaling

Emilie Froussart[1] · Jocelyne Bonneau[1] · Claudine Franche[1] · Didier Bogusz[1]

Received: 13 November 2015 / Accepted: 5 February 2016 / Published online: 12 February 2016

Abstract Nitrogen and phosphorus availability are frequent limiting factors in plant growth and development. Certain bacteria and fungi form root endosymbiotic relationships with plants enabling them to exploit atmospheric nitrogen and soil phosphorus. The relationships between bacteria and plants include nitrogen-fixing Gram-negative proteobacteria called rhizobia that are able to interact with most leguminous plants (Fabaceae) but also with the non-legume Parasponia (Cannabaceae), and actinobacteria *Frankia,* which are able to interact with about 260 species collectively called actinorhizal plants. Fungi involved in the relationship with plants include Glomeromycota that form an arbuscular mycorrhizal (AM) association intracellularly within the roots of more than 80 % of land plants. Increasing numbers of reports suggest that the rhizobial association with legumes has recycled part of the ancestral program used by most plants to interact with AM fungi. This review focuses on the most recent progress made in plant genetic control of root nodulation that occurs in non-legume actinorhizal plant species.

Keywords Actinorhizal plants · Signaling · Nitrogen-fixing root nodule symbiosis · Nodulation · *Frankia* · Rhizobia

Introduction

In the context of global change, priority needs to be given to biological nitrogen fixation to sustain both population growth and ensure a clean and healthy environment (Steffen et al. 2015). The Haber–Bosch process, which turns the nitrogen in the air into ammonia to make chemical fertilizers, involves the production of large amounts of CO_2 which largely contribute to global warming. Furthermore, nitrogen pollution caused by chemical fertilizers in the runoff from agricultural fields is a major threat to human and animal health.

The ability to reduce atmospheric nitrogen to ammonia (nitrogen fixation) is restricted to prokaryotes, some of which form mutualistic symbioses with plants (Franche et al. 2009). Two kinds of associations lead to endosymbiotic plant-bacterial associations in which different nitrogen-fixing soil eubacteria are hosted in root nodules: filamentous Gram-positive *Frankia* bacteria associate with about 260 plant species belonging to eight different families called actinorhizal plants (Dawson 2007), whereas Gram-negative rhizobia associate only with legumes (Fabaceae) and Parasponia (Cannabaceae) (Trinick 1973, 1979).

Legumes, Parasponia and actinorhizal plants all belong to the same clade (Fabid), suggesting that a predisposition to evolve nitrogen-fixing root nodule symbioses appeared only once during evolution (Soltis et al. 1995; Doyle 1998, 2011). Recent work using evolutionary models supports the hypothesis of predisposition suggesting that a single evolutionary innovation occurred over 100 million years ago (MYA), and was followed by several evolutionary events leading to the emergence of the different kinds of symbiotic associations (Werner et al. 2014). In contrast to nitrogen fixing symbioses, the association between

---

✉ Claudine Franche
claudine.franche@ird.fr

[1] Equipe Rhizogenèse, UMR DIADE (IRD-UM), Institut de Recherche pour le Développement (IRD), 911 avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France

arbuscular mycorrhizal (AM) fungi involves about 80 % of land plants and originated more than 400 MYA (Remy et al. 1994). Several observations including the fact that some legume mutants defective in nodulation also lack the AM interaction, and the sharing of common signaling components, suggest that the evolutionary younger nitrogen-fixing symbiosis recruited functions from the older AM symbiosis (Parniske 2008).

Symbiotic associations with rhizobia and AM involve the exchange of signals enabling mutual recognition and activation of a signaling cascade. In the case of rhizobial associations, lipo-chitooligosaccharides (LCOs), called Nod factors, are synthesized in response to the flavonoids released in plant root exudates (Oldroyd et al. 2011). AM interactions begin when the plant hormones strigolactones stimulate the branching and growth of AM fungi (Buee et al. 2000), which respond by releasing chitin oligomers (COs) and LCOS, the latter being structurally similar to Nod factors (Maillet et al. 2011; Genre et al. 2013). Genetic analyses demonstrated that rhizobia-legumes and AM associations share a single signaling pathway, or «common symbiosis pathway» (CSP) (Kistner and Parniske 2002; Oldroyd et al. 2011). This pathway contains a receptor-like kinase SYMRK/DMI2, nuclear pores, and the potassium channel proteins required for the induction of calcium oscillations (Capoen et al. 2011; Gutjahr and Parniske 2013). A nuclear calcium- and calmodulin-dependent kinase (CCaMK/DMI3) interacting with transcription factors (Cyclops/IPD3) is also part of this common pathway. The nuclear Ca^{2+}-spiking induced by AM fungi and rhizobia is likely decoded by CCaMK triggering infection and organogenesis programs (Levy et al. 2004; Mitra et al. 2004; Miwa et al. 2006). Among the infection related genes, vapyrin (VPY), a protein consisting of a major sperm protein (MSP) domain and an ankyrin domain, was found to be involved in AM and rhizobia infection (Murray et al. 2011; Gutjahr and Parniske 2013). The *vpy-1* mutant displays normal Ca^{2+}-spiking in root hairs in response to supplied Nod factors, and the elevation of VPY transcripts upon application of Nod factors depends on NFP, DMI1, and DMI3. These findings thus show that VPY acts downstream of the common signalling pathway (Murray et al. 2011). Likewise, a set of symbiotic genes are controlled by specific early transcription factors (TFs) such as CYCLOPS/IPD3 (Singh et al. 2014), NSP1 (Smit et al. 2005), NSP2 (Kalo et al. 2005), ERN1/ERN2 (Andriankaja et al. 2007) and NIN (Schauser et al. 1999).

The lack of a genetic system for *Frankia* together with the difficulty involved in obtaining actinorhizal plant mutants are two major obstacles to deciphering the molecular dialogue between the symbiotic partners. However, the use of RNA interference (RNAi) to downregulate candidate genes in plants and the growing number of sequenced *Frankia* genomes has enabled significant advances in our knowledge of actinorhizal symbioses in the last few years (Franche and Bogusz 2012; Bogusz and Franche 2015).

The aim of this review is to highlight the most recent findings in symbiotic signaling in actinorhizal symbiosis.

The infection process

Frankia can infect actinorhizal plants in two different ways, intracellular infection via root hair penetration (Fagales) or intercellular penetration (Rosales and Cucurbitales) (Berry and Sunell 1990). Intracellular infection occurs in several actinorhizal genera (*Myrica*, *Comptonia*, *Alnus* and *Casuarina*), where the infection process proceeds through root hair deformation and penetration. In addition to *Frankia*, other soil bacteria, including *Pseudomonas* spp., deform the root hairs of *Alnus* sp. (Knowlton et al. 1980). It has been hypothesized that these «helper» bacteria in the rhizosphere prepare the root hair surface for penetration of the root hair cell wall by *Frankia* (Knowlton et al. 1980). After invagination of growing filaments of *Frankia* into the curled root hairs, infection proceeds intracellularly in the root cortex (Berry et al. 1986). In the host cell, *Frankia* is embedded within a host derived growing tubular matrix enriched in polygalacturonans known as the infection thread (Berg 1999). Upon infection, cell divisions occur in the root cortex leading to a small protuberance called the prenodule, which consists of *Frankia*-infected and uninfected cells (Laplaze et al. 2000) (Fig. 1). While the prenodule develops, the nodule primordium results from mitotic activity in pericycle cells located opposite a protoxylem pole (Callaham and Torrey 1977). As the nodule primordium grows, the nodule cortex becomes infected intracellularly by *Frankia* hyphae coming from the prenodule (Fig. 1). No root hair deformation is observed during the intercellular root invasion process (*Discaria*, *Eleagnus*, *Ceanothus* and *Cercocarpus)* (Valverde and Wall 1999). *Frankia* hyphae penetrate the middle lamella between adjacent root epidermal cells and progress apoplastically between cortical cells, within an electron-dense matrix. Cell divisions are induced in the root pericycle opposite a protoxylem pole, leading to the formation of the nodule primordium. *Frankia* hyphae infect primordium cells in the nodule lobe where intracellular colonization occurs (Wall and Berry 2008). The prenodule step has not been reported in intercellular infection. In general, mature actinorhizal nodules are multilobed structures, each nodule lobe is a modified lateral root without a root cap, including a central vascular bundle, infected cells restricted to the cortex, and a meristem at the apex.

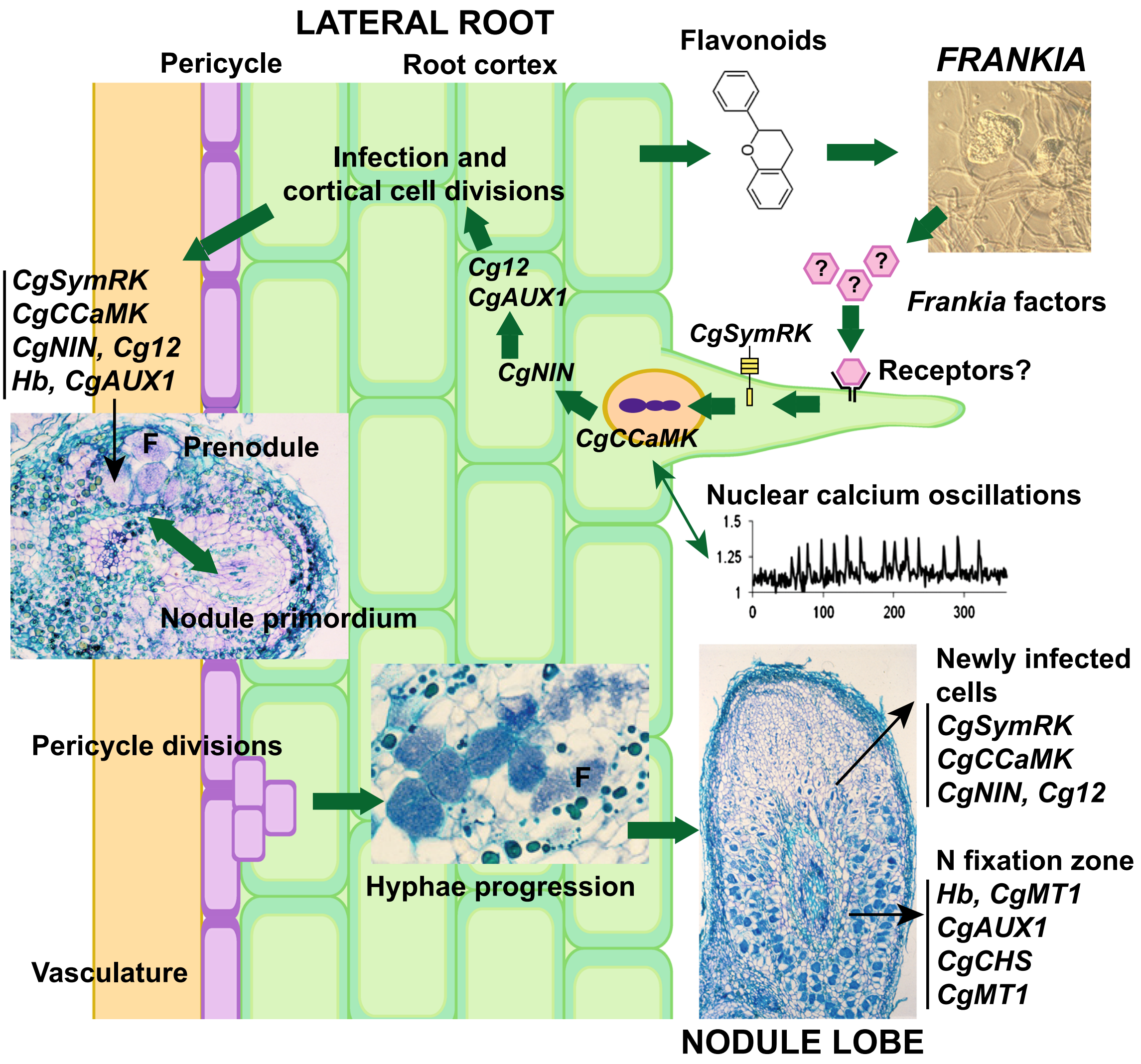

Fig. 1 Schematic diagram of actinorhizal signaling in the intracellular infected *C. glauca*. Actinorhizal plant roots release flavonoids that trigger the production of *Frankia* signals. The nodulation signaling pathway is triggered when as yet unknown receptors sense *Frankia* signals. Activation of the receptors produces oscillations of calcium concentration (calcium spiking). Two members of the CSP, a leucine-rich repeat receptor kinase (SYMRK) and a calcium calmodulin-dependent protein kinase (CCaMK), are expressed following *Frankia* infection. Like in legumes, the *NIN* gene is expressed during preinfection stages in developing root hairs and during *Frankia* infection. Expression of *Cg12* encoding a subtilisin-like serine protease and of *CgAUX1* encoding an auxin influx carrier have been shown to be specifically linked to plant cell infection by *Frankia* (Svistoonoff et al. 2003; Péret et al. 2007). Expression in prenodules and in mature nodule lobes has also been demonstrated, suggesting that some of the genes involved in symbiotic signaling may have other roles in nodule function. Hemoglobin (*CgHb*) and metallothionein (*CgMT1*) genes have been shown to be highly expressed in cells filled with *Frankia* both in prenodules and in mature nodule lobes (Gherbi et al. 1997; Laplaze et al. 2000; Obertello et al. 2007)

Plant symbiotic signaling molecules

By acting as auxin transport inhibitors, flavonoids play many different roles in signaling and in nodule development at different stages of rhizobium symbiosis (Wasson et al. 2006). It has long been postulated that flavonoids also play a significant role in actinorhizal symbioses including chemo-attraction, proliferation of *Frankia,* and plant host specificity (Smolander and Sarsa 1990; Benoit and Berry 1997; Van Ghelue et al. 1997; Hughes et al. 1999; Popovici et al. 2010). However, the mechanisms underlying the movement of the non-motile *Frankia* are not yet known. Recently, *Casuarina cunninghamiana* root extracts containing flavonoids were shown to be causing alterations to the surface components of the compatible *Frankia* strain CcI3 in relation to infectivity (Beauchemin et al. 2012). In *C. glauca,* flavonoids were shown to accumulate in nodules, and a possible role in nodule compartmentation between *Frankia*-infected and uninfected cortical cells was suggested (Laplaze et al. 1999). When an RNAi approach was used to silence the expression of the chalcone synthase gene from *C. glauca*, a major reduction in nodulation was observed (Abdel-Lateif et al. 2013). Since in *CgCHS1*-RNAi plants, the nodulation rate was affected whereas the nodule structure was not, it was suggested that flavonoids act during the early stages of symbiotic interactions. Future work should address the chemical nature of the specific flavonoids that act as early signals, and identify their specific targets, even if no homologs of canonical *nod* genes have been found in the *Casuarina* infective *Frankia* strain CcI3 (Normand et al. 2007). Previous studies in legumes that form indeterminate nodules have shown that certain flavonoids are required to control auxin transport during nodule initiation (Wasson et al. 2006; Zhang et al. 2009). To study this hypothesis in actinorhizal symbiosis, auxin transport was investigated in *C. glauca* as described in the following paragraph.

The role of auxin in actinorhizal symbiosis

Auxins play a crucial role in different aspects of plant growth and development (Ludwig-Muller 2011). They contribute to the development of lateral roots, shoots, and flowers, and are involved in defense mechanisms against pathogens. Auxins also play an important role in different growth processes associated with symbionts. The role of auxin has been described in ectomycorrhizal infection (Sukumar et al. 2013) and in the formation of arbuscules in AM symbiosis (Etemadi et al. 2014). In legumes, auxin is involved in indeterminate nodule formation (Mathesius 2008). More recently, auxin signaling was shown to contribute to rhizobial infection of *M. truncatula* root hairs (Breakspear et al. 2014).

Recent data suggest that auxin is a signal that regulates the *Frankia* infection process and nodule organogenesis. As treatment of *C. glauca* roots with the auxin influx inhibitor 1-naphtoxyacetic acid (1-NOA) perturbs the nodulation process, two genes encoding putative *C. glauca* auxin influx carriers were cloned and characterized (Péret et al. 2007). One of them, *CgAUX1*, was shown to be expressed in *Frankia*-infected cells during nodule development, suggesting that an influx of auxin could be involved in the infection process. It was also shown that auxins accumulate in *Frankia*-infected cells and that accumulation is driven by coordinated cell-specific expression of influx and efflux auxin transporters (Perrine-Walker et al. 2010). *Frankia* strains have the ability to produce auxins in pure culture (Wheeler et al. 1984; Hammad et al. 2003). However, since *Frankia* mutants defective in auxin biosynthesis are not available, the exact source of the auxin, i.e. plant or bacteria, is not known (Perrine-Walker et al. 2010). Transcriptional analyses in *C. glauca* expanded the range of transcription factors putatively involved in nodulation (Hocher et al. 2011; Diédhiou et al. 2014). *CgIAA7,* one of the auxin-response factor (AEF) genes in the AUX/IAA family, was identified in *C. glauca* (Champion et al. 2015). *CgIAA7* is expressed both in plant cells infected by *Frankia* and in nodule primordia in *C. glauca*. This transcription factor, together with the auxin response factors (ARF) family of transcription factors, controls the expression of downstream auxin-responsive genes (Peer 2013). To interfere in the signaling pathway, dominant negative and positive versions of *CgIAA7* were expressed during nodulation in *C. glauca*. Based on the impacts of the expression of different versions of *IAA7* on the establishment of the symbiosis, these authors concluded that auxin could be a negative regulator of symbiosis. In the proposed model, which resembles that of the long-distance regulation mechanism described for AM symbiosis (Meixner et al. 2005), it is hypothesized that auxin accumulation in infected plant cells triggers the synthesis of an inhibitory diffusible factor, resulting in the inhibition of nodulation (Champion et al. 2015). This suggests that high auxin concentrations control nodulation to balance the nitrogen gains with energy demands. It is also possible that at low concentrations, auxin is involved in other processes such as the cell-wall remodelling that occurs during infection, limitation of plant defense mechanisms, or control of the division of pericycle cells leading to the initiation of nodule primordia.

In contrast to *C. glauca*, in the actinorhizal plant *Discaria trinervis*, nodulation is initiated by intercellular infection. Since treatment with 1-NOA also impaired nodulation efficiency in *D. trinervis,* the role of auxin during the nodulation process was investigated (Imanishi et al. 2014). Immunolocalization experiments indicated that, as previously reported in *C. glauca,* auxin accumulated in *Frankia* infected cells. However, in contrast to the findings concerning *CgAUX1* in *C. glauca,* the activity of the promoter *DtAUX1* from *D. trinervis* was restricted to the meristem region of the nodules and was not detected during infection by *Frankia*. This may be explained by the fact that (i) the role of auxin differs between *Frankia* intercellular penetration and intracellular hairy root infection mechanisms (ii) different auxin transporters may be involved in *C. glauca* and *D. trinervis.*

New insights into the actinorhizal signal transduction pathway

As mentioned above, phylogenetic studies have shown that all plants able to enter a root nodule symbiosis belong to the same clade, suggesting that they share a predisposition for symbiosis (Soltis et al. 1995; Doyle 1998). This finding prompted investigation of the possibility that the CSP is also shared by actinorhizal symbioses. The legume homolog of the receptor-like kinase gene *SYMRK/DMI2* positioned upstream from the genes involved in calcium spiking, was isolated and its function in signaling was assessed in *C. glauca* (Gherbi et al. 2008) and *Datisca glomera* (Markmann et al. 2008). Following downregulation of *SYMRK/DMI2* in both *C. glauca* and *D. glomerata,* a severe reduction in nodulation by *Frankia* and in colonization by AM fungi was shown in the transgenic plants. Furthermore *C. glauca* and *D. glomerata SYMRK/DMI2* genes had the ability to complement knockout *Lotus japonicus symrk* mutants. These results point to the existence of the CSP in actinorhizal plants.

Transcriptional analysis of *C. glauca* and *A. glutinosa* expanded the range of CSP genes reported to be involved in AM and legume-rhizobium symbioses (Hocher et al. 2011; Demina et al. 2013; Diédhiou et al. 2014). Most of the CSP genes were identified in these two actinorhizal plants, including *CCaMK*, the calmodulin-dependent protein kinase (CCaMK), which may decode calcium spiking in response to a *Frankia* signal. In *C. glauca*, silencing of *CgCCaMK* resulted in lower nodulation and AM fungal colonization (Svistoonoff et al. 2013). The function of CgCCaMK in endosymbioses was supported by full complementation of the *M. truncatula dmi3* mutant for nodulation and colonization by AM fungi (Svistoonoff et al. 2013). In legumes and *Parasponia andersonii*, a gain-of-function mutation of *CCaMK* has been shown to cause spontaneous nodulation without rhizobia (Gleason et al. 2006; Tirichine et al. 2006). Interestingly, when an autoactive form of *CgCCaMK* lacking the auto-inhibitory/CaM domain was expressed in *C. glauca* and *D. trinervis*, nodule organogenesis was also observed in absence of *Frankia* (Svistoonoff et al. 2013). Since *D. trinervis* is an actinorhizal member of the Rosales infected by *Frankia* via the primitive intercellular infection pathway, one can conclude that *CCaMKs* are central components of actinorhiza organogenesis, regardless of the mode of infection (Svistoonoff et al. 2013). Furthermore, since legume and actinorhial nodules differ anatomically, these results raise the question of the specific mechanisms that determine the organogenesis of the two different types of nodules. Additional evidence for the conservation of CSP in actinorhizal plants was provided by the observation of Ca^{2+} oscillations in *A. glutinosa* and *C. glauca* root hairs in response to exsudate to *Frankia* (Granqvist et al. 2015; Chabaud et al. 2016).

In legumes, the transcription factor NODULE INCEPTION, NIN, is linked to a «NOD»-specific pathway downstream from the CSP. The *NIN* gene is expressed early during the interaction with rhizobia, in both root epidermis and nodule promordia. NIN fulfills many functions by targeting genes involved in bacterial infection, nodule organogenesis, and autoregulation of nodulation (Marsh et al. 2007; Soyano et al. 2013; Yoro et al. 2014). *CgNIN*, a *C. glauca* sequence homolog to the *Lotus japonicus LjNIN* gene, was recently identified (Clavijo et al. 2015). This relationship and the finding that *CgNIN* belongs to the same cluster that includes legume *NIN* genes involved in symbiosis, led to its further characterization in the control of nodulation by *Frankia*. *CgNIN-RNAi* plants showed reduced nodulation, but AM colonization was not affected. Complementation of a *M. truncatula nin* mutant with a *CgNIN* under the control of *MtNIN* promoter revealed the absence of infection events, but small nodule-like structures were observed. These authors concluded that *CgNIN* can only partly complement the *MtNIN* mutant by activating early cell divisions, leading to nodule organogenesis. During the symbiotic process, the *CgNIN* promoter was shown to drive some expression in early infected cells including root hairs (Clavijo et al. 2015). Altogether, these data show that, like in legumes, NIN plays a central role in actinorhizal symbiosis suggesting, in addition to the CSP, conservation of a « NOD » specific pathway in the host plants nodulated by rhizobia and *Frankia*. In addition to NIN, several transcription factors of the GRAS and NF-Y families have been shown to play a critical role in rhizobia and AM symbioses (for a review, see Rípodas et al. 2014). Their role is to trigger the developmental program of nodule organogenesis and the infection process. Recently, transcriptional and phylogenetic analyses in *C. glauca* and *A. glutinosa* revealed several other potential transcription factors homologous to those involved in legume-rhizobia and AM symbioses (Diédhiou et al. 2014). Once again, these results suggest conservation of the mechanisms regulating gene expression in these three endosymbioses and extending far beyond the CSP.

The *Frankia* nodulation signal

In all rhizobia -except photosynthetic *Bradyrhizobia*- that elicit root-and stem nodules of legumes belonging to the *Aeschynomene* genus (Giraud et al. 2007), the synthesis of the N-acylated chitin oligomers of Nod factors is controlled by three specific enzymes, encoded by canonical *nod* genes *nodABC* (NodA—acyl transferase, NodB—chitin deacetylase, NodC—chitin synthase) (Oldroyd et al. 2011). The differences in the structure of Nod factors made by different rhizobia species are major determinants of host specificity) (Oldroyd et al. 2011).

Compared to rhizobia, one distinguishing feature of *Frankia* is that individual strains may have a gradient of specificity for different plant species, host origin is not always a determining characteristic for the classification of *Frankia* (Swensen and Benson 2008). Several phylogenetic chronometers including RAPD PCR, 16S and 23 s-rRNA, gene sequences (ITS region) between 16S and 23S-rRNA, and *nif* gene sequences have been used to identify the genetic variability and relationships among the *Frankia* strains isolated from different host plants (reviewed in Hahn 2008). Based on the 16S database, Normand et al. (1996) grouped the *Frankia* genus in four clusters: cluster 1, strains effective in *Alnus* (Betulacea), *Casuarina*, *Allocasuarina* (Casuarinaceae) and *Comptonia*, *Myrica* and *Morella* (Myricaceae); cluster 2, uncultured strains present in nodules of *Dryas* (Rosaceae), *Coriaria* (Coriariaceae) and *Datisca* (Dastscaceae); cluster 3, strains effective in Elaeagnaceae and *Gymnostoma* (Casuarinaceae); and cluster 4, non-infective or non-effective strains isolated from a range of host plants. The recent determination of several *Frankia* genomes made it possible to look for canonical *nod* homologs from rhizobia. In the *Frankia* genomes of symbionts belonging to cluster 1 (*Frankia* CcI3 and *Frankia* ACN14a) and cluster 3 (EAN1pec), several *nodC* and *nodB* distant homologs were observed spread throughout the actinobacterial genomes. Furthermore, they did not form a SYM-island and no *nodA* gene was detected (Normand et al. 2007). These findings are in accordance both with those of previous studies in which no molecules ressembling Nod factors were detected in *Frankia* supernatants (Cérémonie et al. 1999) and with the absence of rhizobial complementation of *nod* mutants with *Frankia* DNA (Cérémonie et al. 1998). Transcriptomics and proteomics approaches were used to identify *Frankia* genes that are essential for the production of symbiotic factor (Alloisio et al. 2007, 2010; Bickhart and Benson 2011; Udwary et al. 2011). However, only genes that fulfill metabolic functions in symbiosis have been identified so far. The difficulty involved in isolating *Frankia* symbiotic genes is amplified by the fact that, in the field of plant–microbe interaction, Gram-positive bacteria have received little attention compared with the intensive study of Gram-negative bacteria (Francis et al. 2010). Whether « omics » technologies will allow the identification of candidate genes involved in the synthesis of the symbiotic signals emitted by culturable *Frankia* strains remains to be seen. Recently, the genome sequence of the unculturable cluster 2 *Frankia datiscae* Dg1 revealed the presence of canonical *nod* genes *nodABC* (Persson et al. 2011). Although Nod factors were not purified, transcripts of these genes were detected, suggesting they play a role in the *Frankia/Datisca* symbiosis (Persson et al. 2015). Since actinorhizal symbioses involving cluster 2 *Frankia* strains are thought to be the oldest actinorhizal symbioses, it was hypothesized that canonical *nod* genes were probably lost during the evolution of *Frankia* symbionts (Persson et al. 2015). The BMG5.1 strain, which also belongs to the recalcitrant cluster 2, was isolated from *Coriaria myrtifolia* and reported to fulfill Koch's postulates by reinfecting its host plants (Gtari et al. 2015). The genome of this isolate does not contain the canonical *nodABC* genes (M. Gtari, personal communication). This result contradicts the hypothesis that most *Frankia* symbionts lost their *nod* genes, and implies that *Frankia dastiscae* Dg1 may have recently acquired them.

The root hair deformations observed after contact with *Frankia* hyphae are similar to those observed following root incubation with a filtrate from a *Frankia* culture. Furthermore, a correlation was found between root hair deformation and the host range of the *Frankia* strains tested, suggesting that a *Frankia* diffusible factor involved in actinorhizal symbiosis is present in *Frankia* culture supernatant (Van Ghelue et al. 1997; Cérémonie et al. 1999). Initial attemps to isolate specific signal molecules with bioassays based on root hair deformation with *Frankia* culture supernatants were not sucessful (Van Ghelue et al. 1997; Bhuvaneswari and Solheim 2000). Of great interest is the demonstration that the *CgNIN* promoter was activated by diffusible factors synthetized by *Frankia* at a very early stage (14 h) after the incubation of the root system with a supernatant of *Frankia* CcI3. Consistent with the expression of *CgNIN* at the pre-infection stage, the inhibition of root hair deformation was observed in *CgNIN-RNAi* plants (Clavijo et al. 2015). This result paves the way for using transgenic plants Pro*CgNIN* driving the expression of a reporter gene as a biological test to purify the diffusible *Frankia* symbiotic factors (Clavijo et al. 2015). Thus, transgenic *C. glauca* expressing the Pro*CgNIN:GFP* fusion were used to identify the biologically active molecules in the *Frankia* supernatant (Chabaud et al. 2016). The candidate molecules for *Frankia* signals were found to be hydrophilic and chitinase-resistant (Chabaud et al. 2016), thereby confirming the results of previous studies (e.g. Cérémonie et al. 1999). Interestingly, these diffusible biologically active molecules were found to induce calcium spiking in *C. glauca*, strengthening the hypothesis of their role as signaling molecules (Chabaud et al. 2016). The purification and complete characterization of *Frankia* signaling molecules involved in symbiotic process will indeed be of great interest.

Conclusions

Recent research in actinorhizal signaling has highlighted the function of already known legume signaling components of the CSP, such as SYMRK/DMI2 and CCaMK/DMI3, or required for nodulation, such as NIN (see Fig. 1 for summary). These findings reinforce the hypothesis that these genes are part of the predisposition to evolve nodulation in Fabids. More research is needed to understand the mechanisms that determine actinorhizal specificity. First, the isolation and characterization of the *Frankia* symbiotic factor and its plant receptor complexes must be pursued.

The identification of *CgNIN* as a potential marker for pre-infection events should help identify the active fractions obtained from *Frankia* culture supernatant. In addition to NIN, functional analyses of the transcription factors recently identified in *C. glauca* and *A. glutinosa* are essential to understand their specific role in actinorhizal symbiosis (Diédhiou et al. 2014). Since actinorhizal nodules are developmentally related to lateral roots, it would be interesting to focus on transcription factors, as these are also known to play a role in lateral root growth. In a recent work, a new GRAS-domain containing protein, named SIN1, involved in both lateral root and nodule formation, was characterized in common bean (Battaglia et al. 2014). How actinorhizal and legume plants recruited a pre-existing lateral root genetic program in response to *Frankia* and rhizobia, is a key question for the future transfer of nitrogen-fixing symbiosis to major non-legume crops.

Acknowledgments We apologize to colleagues for not being able to cite all relevant and earlier papers because of space limitations and the focus of the review on recent research. Research conducted in the Rhizogenesis laboratory on actinorhizal plants was supported by the Institut de Recherche pour le Développement (IRD) and the Agence Nationale de la Recherche (ANR) Blanc project NewNod (ANR-06-BLAN-0095) and SESAM (BLAN-1708-01).

References

Abdel-Lateif K, Vaissayre V, Gherbi H, Verries C, Meudec E, Perrine-Walker F, Cheynier V, Svistoonoff S, Franche C, Bogusz D, Hocher V (2013) Silencing of the chalcone synthase gene in *Casuarina glauca* highlights the important role of flavonoids during nodulation. New Phytol 199:1012–1021. doi:10.1111/nph.12326

Alloisio N, Felix S, Marechal J, Pujic P, Rouy Z, Vallenet D, Medigue C, Normand P (2007) *Frankia alni* proteome under nitrogen-fixing and nitrogen-replete conditions. Physiol Plant 130:440–453. doi:10.1111/j.1399-3054.2007.00859

Alloisio N, Queiroux C, Fournier P, Pujic P, Normand P, Vallenet D, Médigue C, Yamaura M, Kakoi K, Kucho K (2010) The *Frankia alni* symbiotic transcriptome. Mol Plant Microbe Interact 23:593–607. doi:10.1094/MPMI-23-5-0593

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

Battaglia M, Rípodas C, Clúa J, Baudin M, Aguilar OM, Niebel A, Zanetti ME, Blanco FA (2014) A nuclear factor Y interacting protein of the GRAS family is required for nodule organogenesis, infection thread progression, and lateral root growth. Plant Physiol 164:1430–1442. doi:10.1104/pp.113.230896

Beauchemin NJ, Furnholm T, Lavenus J, Svistoonoff S, Doumas P, Bogusz D, Laplaze L, Tisa LS (2012) *Casuarina* root exudates alter the physiology, surface properties, and plant infectivity of *Frankia* sp. strain CcI3. Appl Environ Microbiol 78:575–580. doi:10.1128/AEM.06183-11

Benoit LF, Berry AM (1997) Flavonoid-like compounds from seeds of red alder (*Alnus rubra*) influence host nodulation by *Frankia* (Actinomycetales). Physiol Plant 99:588–593. doi:10.1111/j.1399-3054.1997.tb05361

Berg RH (1999) *Frankia* forms infection threads. Can J Bot 77:1327–1333. doi:10.1139/b99-073

Berry AM, Sunell LA (1990) The infection process and nodule development. In: Schwintzer CR, Tjepkema JD (eds) The biology of *Frankia* and actinorhizal plants. Academic Press, New York, pp 61–81

Berry AM, McIntyre L, McCully ME (1986) Fine structure of root hair infection leading to nodulation in the *Frankia-Alnus* symbiosis. Can J Bot 64:292–305. doi:10.1139/b86-043

Bhuvaneswari TV, Solheim B (2000) Root-hair interactions in actinorhizal symbioses. In: Ridge RW, Emons AMC (eds) Root hairs: cell and molecular biology. Springer, Paris, pp 311–327

Bickhart DM, Benson DR (2011) Transcriptomes of *Frankia* sp. strain CcI3 in growth transitions. BMC Microbiol 11:192. doi:10.1186/1471-2180-11-192

Bogusz D, Franche C (2015) Signaling and communication between actinorhizal plants and *Frankia* during the intracellular symbiotic process. In: de Bruijn FJ (ed) Biological nitrogen fixation. Wiley, Hoboken, pp 547–554

Breakspear A, Liu C, Roy S, Stacey N, Rogers C, Trick M, Morieri G, Mysore KS, Wen J, Oldroyd GE, Downie JA, Murray JD (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. doi:10.1105/tpc.114.133496

Buee M, Rossignol M, Jauneau A, Ranjeva R, Becard G (2000) The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. Mol Plant Microbe Interact 13:693–698. doi:10.1094/MPMI.2000.13.6.693

Callaham D, Torrey JG (1977) Prenodule formation and primary nodule development in roots of *Comptonia* (Myricaceae). Can J Bot 17:2306–2318. doi:10.1139/b77-262

Capoen W, Sun J, Wysham D, Otegui MS, Venkateshwaran M, Hirsch S, Miwa H, Downie JA, Morris RJ, Ané JM, Oldroyd GE (2011) Nuclear membranes control symbiotic calcium signaling of legumes. Proc Natl Acad Sci USA 108:14348–14353. doi:10.1073/pnas.1107912108

Cérémonie H, Cournoyer B, Maillet F, Normand P, Fernandez MP (1998) Genetic complementation of rhizobial *nod* mutants with *Frankia* DNA: Artifact or reality? Mol Gen Genet 260:115–119. doi:10.1007/s004380050877

Cérémonie H, Debellé F, Fernandez MP (1999) Structural and functional comparison of *Frankia* root hair deforming factor and rhizobia nod factor. Can J Bot 77:1293–1301. doi:10.1139/b99-060

Chabaud M, Hassen Gherbi H, Pirolles E, Vaissayre V, Fournier J, Moukouanga D, Franche C, Bogusz D, Tisa LS, Barker DG, Svistoonoff S (2016) Chitinase-resistant hydrophilic symbiotic factors secreted by *Frankia* activate both Ca^{2+} spiking and *NIN* gene expression in the actinorhizal plant *Casuarina glauca*. New Phytol. doi:10.1111/nph.13732

Champion A, Lucas M, Tromas A, Vaissayre V, Crabos A, Diédhiou I, Prodjinoto H, Moukouanga D, Pirolles E, Cissoko M, Bonneau J, Gherbi H, Franche C, Hocher V, Svistoonoff S, Laplaze L (2015) Inhibition of auxin signaling in *Frankia* species-infected cells in *Casuarina glauca* nodules leads to increased nodulation. Plant Physiol 167:1149–1157. doi:10.1104/pp.114.255307

Clavijo F, Diedhou I, Vaissayre V, Brottier L, Ascolate J, Moukouanga D, Crabos A, Auguy F, Franche C, Gherbi H, Champion A, Hocher V, Barker D, Bogusz D, Tisa L, Sivistoonoff S (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. doi:10.1111/nph.13506

Dawson JO (2007) The ecology of actinorhizal plants. In: K Pawlowski, WE Newton (eds) Nitrogen-fixing actinorhizal symbioses, vol. 6, Nitrogen fixation: applications and research progress. Springer, Dordrecht, pp 199–234. doi: 10.1007/978-1-4020-3547-0_8

Demina IV, Persson T, Santos P, Plaszczyca M, Pawlowski K (2013) Comparison of the nodule versus root transcriptome of the actinorhizal plant *Datisca glomerata*: actinorhizal nodules contain a specific class of defensins. PLoS One 8:e72442. doi:10.1371/journal.pone.0072442

Diédhiou I, Tromas A, Cissoko M, Gray K, Parizot B, Crabos A, Alloisio N, Fournier P, Carro L, Svistoonoff S, Gherbi H, Hocher V, Diouf D, Laplaze L, Champion A (2014) Identification of potential transcriptional regulators of actinorhizal symbioses in *Casuarina glauca* and *Alnus glutinosa*. BMC Plant Biol 14:342. doi:10.1186/s12870-014-0342-z

Doyle JJ (1998) Phylogenetic perspectives on nodulation: an evolving views of plants and symbiotic bacteria. Trends Plant Sci 3:473–478. doi:10.1016/S1360-1385(98)01340-5

Doyle JJ (2011) Phylogenetic perspectives on the origins of nodulation. Mol Plant Microbe Interact 24:1289–1295. doi:10.1094/MPMI-05-11-0114

Etemadi M, Gutjahr C, Couzigou JM, Zouine M, Lauressergues D, Timmers A, Audran C, Bouzayen M, Bécard G, Combier JP (2014) Auxin perception is required for arbuscule development in arbuscular mycorrhizal symbiosis. Plant Physiol 166:281–292. doi:10.1104/pp.114.246595

Franche C, Bogusz D (2012) Signalling and communication in actinorhizal symbiosis. In: Perotto S, Baluska F (eds) Signalling and communication in plant symbiosis. Springer, Berlin, pp 73–92. doi:10.1007/978-3-642-20966-6

Franche C, Lindstrom K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59. doi:10.1007/s11104-008-9833-8

Francis I, Holsters M, Vereecke D (2010) The Gram-positive side of plant-microbe interactions. Environ Microbiol 12:1–12. doi:10.1111/j.1462-2920.2009.01989.x

Genre A, Chabaud M, Balzergue C, Puech-Pagès V, Novero M, Rey T, Fournier J, Rochange S, Bécard G, Bonfante P, Barker DG (2013) Short-chain chitin oligomers from arbuscular mycorrhizal fungi trigger nuclear Ca^{2+} spiking in *Medicago truncatula* roots and their production is enhanced by strigolactone. New Phytol 198:190–202. doi:10.1111/nph.12146

Gherbi H, Duhoux E, Franche C, Pawlowski K, Nassar A, Berry AM, Bogusz D (1997) Cloning of a full-length symbiotic hemoglobin cDNA and *in situ* localization of the corresponding mRNA in *Casuarina* root nodule. Physiol Plant 99:608–616. doi:10.1111/j.1399-3054.1997.tb05364.x

Gherbi H, Markmann K, Svistoonoff S, Estevan J, Autran D, Giczey G, Auguy F, Péret B, Laplaze L, Franche C, Parniske M, 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. doi:10.1073/pnas.0710618105

Giraud E, Moulin L, Vallenet D, Barbe V, Cytryn E, Avarre JC, Jaubert M, Simon D, Cartieaux F, Prin Y, Bena G, Hannibal L, Fardoux J, Kojadinovic M, Vuillet L, Lajus A, Cruveiller S, Rouy Z, Mangenot S, Segurens B, Dossat C, Franck WL, Chang WS, Saunders E, Bruce D, Richardson P, Normand P, Dreyfus B, Pignol D, Stacey G, Emerich D, Verméglio A, Médigue C, Sadowsky M (2007) Legumes symbioses: absence of *nod* genes in photosynthetic bradyrhizobia. Science 316:1307–1312. doi:10.1126/science.1139548

Gleason C, Chaudhuri S, Yang T, Munoz A, Poovajah BW, Oldroyd GE (2006) Nodulation independent of rhizobia induced by a calcium-activated kinase lacking autoinhibition. Nature 441:1149–1152. doi:10.1038/nature04812

Granqvist E, Sun J, Op den Camp R, Pujic P, Hill L, Normand P, Morris RJ, Downie JA, Geurts R, Oldroyd GE (2015) Bacterial-induced calcium oscillations are common to nitrogen-fixing associations of nodulating legumes and non-legumes. New Phytol 207:551–558. doi:10.1111/nph.13464

Gtari M, Ghodhbane-Gtari F, Nouioui I, Ktari A, Hezbri K, Mimouni W, Sbissi I, Ayari A, Yamanaka T, Normand P, Tisa LS, Boudabous A (2015) Cultivating the uncultured: growing the recalcitrant cluster-2 *Frankia* strains. Sci Rep 5, 13112. doi:10.1038/srep13112

Gutjahr C, Parniske M (2013) Cell and developmental biology of arbuscular mycorrhiza symbiosis. Annu Rev Cell Dev Biol 29:593–617. doi:10.1146/annurev-cellbio-101512-122413

Hahn D (2008) Polyphasic taxonomy of the genus *Frankia*. In: Pawlowski K, Newton WE (eds) Nitrogen–fixing actinorhizal symbioses. Springer, Dordrecht, pp 25–45. doi:10.1007/978-1-4020-3547-0_2

Hammad Y, Nalin R, Marechal J, Fiasson K, Pepin R, Berry AM, Normand P, Domenach AM (2003) A possible role for phenyl acetic acid (PAA) on *Alnus glutinosa* nodulation by *Frankia*. Plant Soil 254:193–205. doi:10.1007/978-94-017-1601-7_21

Hocher V, Alloisio N, Auguy F, Fournier P, Doumas P, Pujic P, Gherbi H, Queiroux C, Da Silva C, Wincker P, Normand P, Bogusz D (2011) Transcriptomics of actinorhizal symbioses reveals homologs of the whole common symbiotic signaling cascade. Plant Physiol 156:700–711. doi:10.1104/pp.111.174151

Hughes M, Donnelly C, Crozier A, Wheeler CT (1999) Effects of the exposure of roots of *Alnus glutinosa* to light on flavonoids and nodulation. Can J Bot 77:1311–1315. doi:10.1139/b99-077

Imanishi L, Perrine-Walker FM, Ndour A, Vayssières A, Conejero G, Lucas M, Champion A, Laplaze L, Wall L, Svistoonoff S (2014) Role of auxin during intercellular infection of *Discaria trinervis* by *Frankia*. Front Plant Sci 5:399. doi:10.3389/fpls.2014.00399

Kalo P, Gleason C, Edwards A, Marsh J, Mitra R, Hirsch S, Jakab J, Sims S, Long S, Rogers J, Kiss G, Downie J, Oldroyd G (2005) Nodulation signaling in legumes requires NSP2, a member of the GRAS family of transcriptional regulators. Science 308:1786–1789. doi:10.1126/science.1110951

Kistner C, Parniske M (2002) Evolution of signal transduction in intracellular symbiosis. Trends Plant Sci 7:511–518. doi:10.1016/S1360-1385(02)02356

Knowlton S, Berry A, Torrey JG (1980) Evidence that associated soil bacteria may influence root hair infection of actinorhizal plants by *Frankia*. Can J Microbiol 26:971–977

Laplaze L, Gherbi H, Frutz T, Pawlowski K, Franche C, Macheix JJ, Auguy F, Bogusz D, Duhoux E (1999) Flavan-containing cells delimit *Frankia*-infected compartments in *Casuarina glauca* nodules. Plant Physiol 121:113–122. doi:10.1104/pp.121.1.113

Laplaze L, Duhoux E, Franche C, Frutz T, Svistoonoff S, Bisseling T, Bogusz D, Pawlowski K (2000) *Casuarina glauca* prenodule cells display the same differentiation as the corresponding nodule cells. Mol Plant Microbe Interact 13:107–112. doi:10.1094/MPMI.2000.13.1.107

Levy J, Bres C, Geurts R, Chalhoub B, Kulikova O, Duc G, Journet EP, Ané JM, Lauber E, Bisseling T, Dénarié J, Rosenberg C, Debellé F (2004) A putative Ca^{2+} and calmodulin-dependant protein kinase required for bacterial and fungal symbioses. Science 303:1361–1364. doi:10.1126/science.1093038

Ludwig-Muller J (2011) Auxin conjugates: their role for plant development and in the evolution of land plants. J Exp Bot 62:1757–1773. doi:10.1093/jxb/erq412

Maillet F, Poinsot V, André O, Puech-Pagès V, Haout A, Gueunier M, Cromer L, Giraudet D, Formey D, Niebel A, Martinez EA, Driguez H, Bécard G, Dénarié J (2011) Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature 469:58–63. doi:10.1038/nature09622

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

Marsh JF, Rakocevic A, Mitra RM, Brocard L, Eschstruth A, Long SR, Schultze M, Ratet P, Oldroyd GE (2007) *Medicago truncatula NIN* is essential for rhizobial-independent nodule organogenesis induced by autoactive calcium/calmodulin-dependent protein kinase. Plant Physiol 144:324–335. doi:10.1104/pp.106.093021

Mathesius U (2008) Goldacre paper: auxin: At the root of nodule development? Funct Plant Biol 35:651–668. doi:10.1071/FP08177

Meixner C, Ludwig-Müller J, Miersch O, Gresshoff P, Staehelin C, Vierheilig H (2005) Lack of mycorrhizal autoregulation and phytohormonal changes in the supernodulating soybean mutant nts1007. Planta 222:709–715. doi:10.1007/s00425-005-0003-4

Mitra RM, Gleason CA, Edwards A, Hadfield J, Downie JA, Oldroyd GED, Long SR (2004) A Ca2 +/calmodulin-dependent protein kinase required for symbiotic nodule development: gene identification by transcript-based cloning. Proc Natl Acad Sci USA 101:4701–4705. doi:10.1073/pnas.0400595101

Miwa H, Sun J, Oldroyd GED, Downie JA (2006) Analysis of nod-factor-induced calcium signaling in root hairs of symbiotically defective mutants of *Lotus japonicus*. Mol Plant Microbe Interact 19:914–923. doi:10.1094/MPMI-19-0914

Murray JD, Muni RRD, Torres-Jerez I, Tang Y, Allen S, Andriankaja M, Li G, Laxmi A, Cheng X, Wen J, Vaughan D, Schultze M, Sun J, Charpentier M, Oldroyd G, Tadege M, Ratet Mysore KS, Chen R, Udvardi MK (2011) Vapyrin, a gene essential for intracellular progression of arbuscular mycorrhizal symbiosis, is also essential for infection by rhizobia in the nodule symbiosis of *Medicago truncatula*. Plant J 65:244–252. doi:10.1111/j.1365-313X.2010.04415.x

Normand P, Orso S, Cournoyer B, Jeannin P, Chapelon C, Dawson J, Evtushenko L, Misra AK (1996) Molecular phylogeny of the genus *Frankia* and related genera and emendation of the family *Frankiaceae*. Int J Syst Bacteriol 46:1–9. doi:10.1099/00207713-46-1-1

Normand P, Lapierre P, Tisa LS, Gogarten JP, Alloisio N, Bagnarol E, Bassi CA, Berry AM, Bickhart DM, Choisne N, Couloux A, Cournoyer B, Cruveiller S, Daubin V, Demange N, Francino MP, Goltsman E, Huang Y, Kopp OR, Labarre L, Lapidus A, Lavire C, Marechal J, Martinez M, Mastronunzio JE, Mullin BC, Niemann J, Pujic P, Rawnsley T, Rouy Z, Schenowitz C, Sellstedt A, Tavares F, Tomkins JP, Vallenet D, Valverde C, Wall LG, Wang Y, Medigue C, Benson DR (2007) Genome characteristics of facultatively symbiotic *Frankia* sp. strains reflect host range and host plant biogeography. Genome Res 17:7–15. doi:10.1101/gr.5798407

Obertello M, Wall L, Laplaze L, Nicole M, Auguy F, Gherbi H, Bogusz D, Franche C (2007) Functional analysis of the metallothionein gene *cgMT1* isolated from the actinorhizal tree *Casuarina glauca*. Mol Plant Microbe Interact 20:1231–1240. doi:10.1094/MPMI-20-10-1231

Oldroyd GE, Murray JD, Poole PS, Downie JA (2011) The rules of engagement in the legume-rhizobial symbiosis. Annu Rev Genet 45:119–144. doi:10.1146/annurev-genet-110410-132549

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6:763–775. doi:10.1038/nrmicro1987

Peer WA (2013) From perception to attenuation: auxin signalling and responses. Curr Opin Plant Biol 16:561–568. doi:10.1016/j.pbi.2013.08.003

Péret B, Swarup R, Jansen L, Devos G, Auguy F, Collin M, Santi C, Hocher V, Franche C, Bogusz D, Bennett M, Laplaze L (2007) Auxin influx activity is associated with *Frankia* infection during actinorhizal nodule formation in *Casuarina glauca*. Plant Physiol 144:1852–1862. doi:10.1104/pp.107.101337

Perrine-Walker F, Doumas P, Lucas M, Vaissayre V, Beauchemin NJ, Band LR, Chopard J, Crabos A, Conejero G, Péret B, King JR, Verdeil JL, Hocher V, Franche C, Bennett MJ, Tisa LS, Laplaze L (2010) Auxin carriers localization drives auxin accumulation in plant cells infected by *Frankia* in *Casuarina glauca* actinorhizal nodules. Plant Physiol 154:1372–1380. doi:10.1104/pp.110.163394

Persson T, Benson DR, Normand P, Vanden Heuvel B, Pujic P, Chertkov O, Teshima H, Bruce DC, Detter C, Tapia R, Han S, Han J, Woyke T, Pitluck S, Pennacchio L, Nolan M, Ivanova N, Pati A, Land ML, Pawlowski K, Berry AM (2011) Genome sequence of "*Candidatus* Frankia datiscae" Dg1, the uncultured microsymbiont from nitrogen-fixing root nodules of the dicot *Datisca glomerata*. J Bacteriol 193:7017–7018. doi:10.1128/JB.06208-11

Persson T, Battenberg K, Demina IV, Persson T, Battenberg K, Demina IV, Vigil-Stenman T, Vanden Heuvel B, Pujic P, Facciotti MT, Wilbanks EG, O'Brien A, Fournier P, Cruz Hernandez MA, Mendoza Herrera A, Médigue C, Normand P, Pawlowski K, Berry AM (2015) *Candidatus* Frankia datiscae Dg1, the actinobacterial microsymbiont of *Datisca glomerata*, expresses the canonical *nod* genes *nodABC* in symbiosis with its host plant. PLoS One 10:e0127630. doi:10.1371/journal.pone.0127630

Popovici J, Comte G, Bagnarol E, Alloisio N, Fournier P, Bellvert F, Bertrand C, Fernandez MP (2010) Differential effects of rare specific flavonoids on compatible and incompatible strains in the *Myrica gale-Frankia* actinorhizal symbiosis. Appl Environ Microbiol 76:2451–2460. doi:10.1128/AEM.02667-09

Remy W, Taylor TN, Hass H, Kerp H (1994) Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc Natl Acad Sci USA 91:11841–11843

Rípodas C, Clúa J, Battaglia M, Baudin M, Niebel A, Zanetti ME, Blanco F (2014) Transcriptional regulators of legume-rhizobia symbiosis: nuclear factors Ys and GRAS are two for tango. Plant Signal Behav 9:e28847. doi:10.4161/psb.28847

Schauser L, Roussis A, Stiller J, Stougaard J (1999) A plant regulator controlling development of symbiotic root nodules. Nature 402:191–195. doi:10.1038/46058

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

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

Smolander A, Sarsa ML (1990) *Frankia* strains of soil under *Betula pendula*: behaviour in soil and in pure culture. Plant Soil 122:129–136. doi:10.1007/BF02851920

Soltis DE, Soltis PS, Morgan DR, Swensen SM, Mullin BC, Dowd JM, Martin PG (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. doi:10.1073/pnas.92.7.2647

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

Steffen W, Richardson K, Rockström J, Cornell SE, Fetzer I, Bennett EM, Biggs R, Carpenter SR, de Vries W, de Wit CA, Folke C, Gerten D, Heinke J, Mace GM, Persson LM, Ramanathan V, Reyers B, Sörlin S (2015) Planetary boundaries: guiding human development on a changing planet. Science. doi:10.1126/science.1259855

Sukumar P, Legué V, Vayssières A, Martin F, Tuskan GA, Kalluri UC (2013) Involvement of auxin pathways in modulating root architecture during beneficial plant-microorganism interactions. Plant Cell Environ 36:909–919. doi:10.1111/pce.12036

Svistoonoff S, Laplaze L, Auguy F, Runions J, Duponnois R, Haseloff J, Franche C, Bogusz D (2003) *cg12* expression is specifically linked to infection of root hairs and cortical cells during *Casuarina glauca* and *Allocasuarina verticillata* actinorhizal nodule development. Mol Plant Microbe Interact 16:600–607. doi:10.1094/MPMI.2003.16.7.600

Svistoonoff S, Benabdoun FM, Nambiar-Veetil M, Imanishi L, Vaissayre V, Cesari S, Diagne N, Hocher V, de Billy F, Bonneau J, Wall L, Ykhlef N, Rosenberg C, Bogusz D, Franche C, Gherbi H (2013) The independent acquisition of plant root nitrogen-fixing symbiosis in Fabids recruited the same genetic pathway for nodule organogenesis. PLoS One 8:e64515. doi:10.1371/journal.pone.0064515

Swensen SM, Benson DR (2008) Evolution of actinorhizal host plants and Frankia endosymbionts. In: Pawlowski K, Newton WE (eds) Nitrogen fixing actinorhizal symbioses. Springer, Netherlands, pp 73–104

Tirichine L, Imaizumi-Anraku H, Yoshida S, Murakami Y, Madsen L, Miwa H, Nakagawa T, Sandal N, Albrektsen A, Kawaguchi M, Downie A, Sato S, Tabata S, Kouchi H, Parniske M, Kawasaki S, Stougaard J (2006) Deregulation of a Ca^{2+}/calmodulin-dependent kinase leads to spontaneous nodule development. Nature 441:1153–1156. doi:10.1038/nature04862

Trinick MJ (1973) Symbiosis between *Rhizobium* and the non-legume, *Trema aspera*. Nature 244:459–460. doi:10.1038/244459a0

Trinick MJ (1979) Structure of nitrogen-fixing nodules formed by *Rhizobium* on roots of *Parasponia andersonii* planch. Can J Microbiol 25:565–578. doi:10.1139/m79-082

Udwary DW, Gontang EA, Jones AC, Jones CS, Schultzb AW, Winterb JM, Yang JY, Beauchemin N, Capsonb TL, Clark BR, Esquenazi E, Eustáquiob AS, Freel K, Gerwick L, Gerwick WH, Gonzaleze D, Liu WT, Malloy KL, Maloney KN, Nett M, Nunnery JK, Penn K, Prieto-Davo A, Simmons TL, Weitz S, Wilson MC, Tisa LS, Dorrestein PC, Moore BS (2011) Significant natural product biosynthetic potential of actinorhizal symbionts of the genus *Frankia*, as revealed by comparative genomic and proteomic analyses. Appl Environ Microbiol 77:3617–3625. doi:10.1128/AEM.00038-11

Valverde C, Wall LG (1999) The regulation of nodulation in *Discaria trinervis* (Rhamnaceae)—*Frankia* symbiosis. Can J Bot 77:1302–1310. doi:10.1139/b99-072

Van Ghelue M, Løvaas E, Ring E, Solheim B (1997) Early interactions between *Alnus glutinosa* and *Frankia* strain ArI3. Production and specificity of root hair deformation factor(s). Physiol Plant 99:579–587. doi:10.1111/j.1399-3054.1997.tb05360

Wall LG, Berry AM (2008) Early interactions infection and nodulation in actinorhizal symbiosis. In: Pawlowski K, Newton WE (eds) Nitrogen fixing actinorhizal symbiosis, vol 6. Springer, New York, pp 147–166

Wasson AP, Pellerone FI, Mathesius U (2006) Silencing the flavonoid pathway in *Medicago truncatula* inhibits root nodule formation and prevents auxin transport regulation by rhizobia. Plant Cell 18:1617–1629. doi:10.1105/tpc.105.038232

Werner GDA, Cornwell WK, Sprent JI, Kattge J, Kiers ET (2014) A single evolutionary innovation drives the deep evolution of symbiotic N_2-fixation in angiosperms. Nat Commun 5:4087. doi:10.1038/ncomms5087

Wheeler CT, Crozier A, Sandberg G (1984) The biosynthesis of indole-3-acetic acid by *Frankia*. Plant Soil 78:99–104. doi:10.1007/BF02277843

Yoro E, Suzaki T, Toyokura K, Miyazawa H, Fukaki H, Kawaguchi M (2014) A positive regulator of nodule organogenesis, NODULE INCEPTION, acts as a negative regulator of rhizobial infection in *Lotus japonicus*. Plant Physiol 165:747–758. doi:10.1104/pp.113.233379

Zhang J, Subramanian S, Stacey G, Yu O (2009) Flavones and flavonols play distinct critical roles during nodulation of *Medicago truncatula* by *Sinorhizobium meliloti*. Plant J 57:171–183. doi:10.1111/j.1365-313X.2008.03676.x