Signaling and Communication in Plants

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Signaling and Communication in Plant Symbiosis

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Preface

Because of their photosynthesis, plants are the major primary producers in terrestrial ecosystems and a precious source of organic carbon for all their microbial symbionts. Biotrophic microbial symbionst derive nutrients from the tissues of the living host, and they often colonise the plant cells where they form intracellular structures that are the site of nutrient uptake and exchange, as well as of interorganismal signaling and communication. To colonise the plant tissues via balanced endosymbiosis, all biotrophic microbes, irrespective of their trophic strategy, need to overcome the plant defense responses through an exchange of molecular signals.

Plants are unique as they are able to associate with both prokaryotic and eukaryotic microbes and establish with them well-balanced symbiotic interactions that range from mutualism to antagonism. No other multicellular organisms give rise to this variety of symbiotic interactions. Plants must be able to discriminate between mutualistic micro-organisms that may exchange organic carbon for essential nutrients such as nitrogen and phosphorus, thus promoting plant growth, and antagonistic pathogens that are only detrimental and cause disease. As this volume is making clear, a fine tuning of the signals in plant symbioses is very complex and still only partially understood.

This volume provides overviews of the current knowledge on a variety of symbiotic systems. The first section is dedicated to signalling during the formation of mutualistic symbioses in legume plants. Legume plants have been pivotal to understand the genetic bases of symbioses and the comparison between nodule and arbuscular mycorrhizal (AM) symbioses has revealed a common symbiosis (SYM) signalling pathway leading to intracellular accommodation of fungal and nitrogen fixing bacterial endosymbionts. The recent discovery that AM fungi secrete symbiotic signals that resemble rhizobial lipochito-oligosaccharides (Maillet et al. 2011) brings the similarities between these two symbioses even further. Despite the importance of legumes as model systems to study mutualistic symbioses, there is a great diversity of plant-microbe interactions that involve nitrogen fixing bacteria others than rhizobia, and a variety of other mycorrhizal and endophytic fungi. Some of the chapters in this book provide current knowledge on these diverse

interactions, and witness the progress in the unravelling of genetic determinants in plant-microbe signalling, and the impressive amount of data emerged from the use of both genomic and post-genomic approaches. The last section of the book is focused on the interaction of plants with antagonistic biotrophs, ranging from filamentous fungi to oomycetes, and to nematodes.

Torino — Silvia Perotto
Bonn — Frantisek Baluska

Reference

Maillet et al. 2011. Fungal lipochitooligosaccharides symbiotic signals in arbuscular mycorrhiza. Nature, 469:58–63

Contents

The Role of Diffusible Signals in the Establishment of Rhizobial and Mycorrhizal Symbioses

J. Benjamin Miller and Giles E.D. Oldroyd

Abstract The roots of at least 80% of all angiosperms are able to engage in symbiotic relationships with arbuscular mycorrhizal (AM) fungi of the group Glomeromycota in order to derive macro- and micro-nutrients from the environment (Brachmann and Parniske, PLoS Biol 4:e239, 2006). Legume roots also form a unique symbiosis with rhizobia in order to derive fixed nitrogen. The establishment of both of these symbioses depends upon signalling between the plant host and the microorganism, of which a number of diffusible signals are essential. Here we discuss the synthesis and role of these diffusible signals for the establishment of both rhizobial and mycorrhizal symbioses.

1 Introduction: A Molecular Dialogue Between Host Plant and Symbiont

In order for symbioses to be established between host and symbiont it is necessary that tightly regulated communication occurs. In the case of symbiotic interactions between plant roots and microorganisms in the rhizosphere, the plant must attract and promote the symbiotic partner to interact with its root, whilst in turn the microorganism must respond to distinguish itself as symbiotic rather than pathogenic and subsequently gain regulated entry into the root. This results in a situation where each organism is required to participate in an elaborate communication in order to allow the establishment and progression of symbiosis. The term "molecular dialogue" was originally coined to describe this communication which occurs between the roots of legumes and rhizobia (Denarie et al. 1993), and is also suitable when considering the interaction between mycorrhiza and host plants.

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2 Diffusible Signals During Nodulation

During the establishment of the legume-*Rhizobium* symbiosis, plant roots release flavonoids which are perceived by rhizobial bacteria. This perception leads to the induction of rhizobial *nod* genes which encode proteins required for the synthesis of Nodulation (Nod) factors. Nod factors are secreted by rhizobia and upon their detection act as signalling molecules to the host plant (Oldroyd and Downie 2006, 2008). Nod factors are essential for nodulation and are important mediators of host-range specificity.

2.1 Flavonoids

The earliest component of the molecular dialogue between legumes and rhizobia is the synthesis of flavonoids by the plant root. These are constitutively produced polyaromatic compounds that are perceived by rhizobia via the NodD protein (Peck et al. 2006). This LysR-type transcriptional regulator then promotes Nod factor biosynthesis by activating the transcription of *nod* genes (Fisher and Long 1993).

2.1.1 Structure and Synthesis

Flavonoids are diverse polyaromatic secondary metabolites consisting of a 15-carbon skeleton and are formed from a branch of the phenylpropanoid pathway. The first committed step of flavonoid biosynthesis is catalysed by chalcone synthase; this reaction involves the condensation of *4*-coumaroyl-CoA with three molecules of malonyl-CoA to form a chalcone flavonoid precursor (Fig. 1). This precursor feeds into further biosynthetic reactions which yield either *5*-deoxyflavonoids or *5*-hydroxyflavonoids. Flavonoids can be further sub-classed into flavonoids and isoflavonoids according to whether the phenyl group is attached to C2 or C3 (as with the flavonol kaempferol or the isoflavone daidzein, respectively; Fig. 1).

Flavonoid production is ubiquitous in plants and these compounds are typically associated with plant defence responses, in addition to lignin and anthocyanin production (Winkel-Shirley 2001). The first proof for the role of root-exuded flavonoids during symbiosis was the induced expression of *Sinorhizobium meliloti* nodulation genes (*nodABC*) by luteolin, a flavone (Peters et al. 1986), and the induction of *Rhizobium trifolii nod* genes by flavones from clover (Redmond et al. 1986). Subsequent research has identified several other flavonoids involved in the nodulation signalling of many plant species (reviewed by Broughton et al. 2000). It has been noted that some flavonoid structures are only produced by particular plants; for example, isoflavonoid production is limited to the Papilionoideae (or Faboideae) subfamily of the Leguminosae (Dixon et al. 2002). This diversity of flavonoid production has been associated with determining, at least in part, the

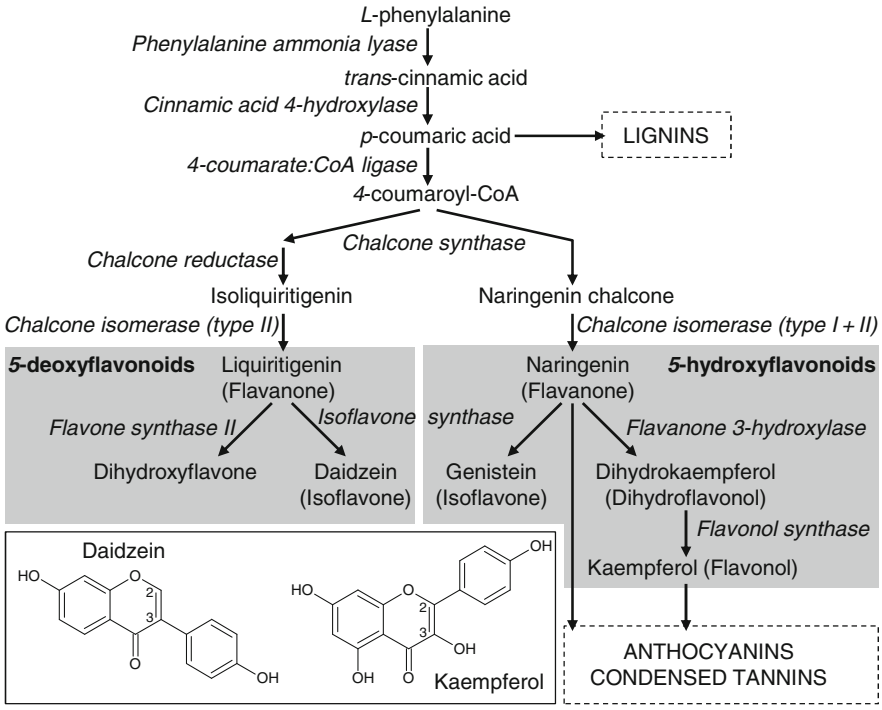


Fig. 1 Biosynthesis of flavonoids. Partial diagram of the phenylpropanoid pathway. Enzymes are depicted in italics; *5*-deoxyflavonoids and *5*-hydroxyflavonoids are denoted by shaded boxes; compounds in dashed boxes represent major side branches of the pathway. Inset shows chemical structures of two example flavonoids isolated from root exudates. Figure adapted from Shaw et al. (2006), Subramanian et al. (2007), Winkel-Shirley (2001) and Zhang et al. (2009)

specificity of *Rhizobium* responses (Cooper 2007; Gibson et al. 2008); for instance, some flavonoids will act as *nod* gene inducers in one context, but as repressors in another (Firmin et al. 1986).

2.1.2 Biological Activity

Two distinct roles for flavonoids and their related molecules have been suggested in the context of early communication during the legume-*Rhizobium* symbiosis: (1) they are involved in chemotaxis responses of rhizobia; (2) they modulate *nod* gene expression of rhizobia (Shaw et al. 2006).

It is essential that rhizobia present in the rhizosphere are attracted to host roots in order for the symbiosis to be formed. Plant roots secrete many compounds into the rhizosphere and in doing so modulate microorganism populations (Dennis et al. 2010). Indeed, it has been estimated that 27% of carbon allocated to roots is

deposited in the rhizosphere (Jones et al. 2009). Legumes must therefore selectively encourage the chemotaxis of rhizobia and flavonoid secretion is important in this process. Positive chemotaxis of *S. meliloti* was specifically demonstrated with the flavone luteolin which is produced by the *S. meliloti* host *Medicago sativa*; this response did not occur with naringenin or apigenin, two closely related flavonoids not produced by *M. sativa* (Caetano-Anolles et al. 1988). Additionally, flavonoid production in host roots is increased upon the addition of compatible *Rhizobium* species (Schmidt et al. 1994). This increased flavonoid synthesis was dependent upon Nod factor structure, implying a positive feedback loop in the regulation of flavonoid production specifically in legume–rhizobia interactions which are symbiotically favourable.

Arguably the most important and most well-characterised rhizobial response to flavonoids is the induction of *nod* gene expression. At least 30 *nod* gene inducing flavonoids have been identified, and their activity is typically in the low micromolar to nanomolar range (Cooper 2004). The exact mechanism by which flavonoids are perceived in rhizobia is unclear; however, the importance of NodD proteins is apparent. NodDs are transcriptional regulators of the LysR-type family and primarily control the expression of *nod* genes, which are responsible for Nod factor biosynthesis (Sect. 2.2). Rhizobia usually contain one to five NodD homologues, depending on species; for example, *S. meliloti* contains three NodDs which share greater than 77% amino acid identity (Honma and Ausubel 1987; Peck et al. 2006). NodDs bind to conserved 55 bp DNA sequences of the promoter region of inducible *nod* genes, the so-called *nod* box, and in doing so induce a bend in the DNA (Fisher and Long 1993). This DNA bending appears to sharpen upon appropriate flavonoid treatment, resulting in subsequent RNA polymerase binding and thereby activating transcription (Chen et al. 2005).

Much genetic evidence suggests that NodD is involved in flavonoid perception. NodD is necessary and sufficient for *nodC* expression in the presence of flavonoids (Mulligan and Long 1985). NodD from *Rhizobium leguminosarum* bv. *viciae* localises to the cytoplasmic membrane (Schlaman et al. 1989), which is also where the flavonoid inducer naringenin accumulates (Recourt et al. 1989). Point mutation of NodD proteins extends *nod* gene expression to include flavonoids which are usually non-inducing (Burn et al. 1987; McIver et al. 1989). Together with additional research this has led to the suggestion that NodD controls rhizobial responses to flavonoids in a species-specific fashion (Horvath et al. 1987; Spaink et al. 1987; Zaat et al. 1989). However, the direct biochemical interaction of NodD and flavonoids has been difficult to prove, although more recent work has begun to demonstrate this interaction (Li et al. 2008; Peck et al. 2006). The work of Peck et al. (2006) has importantly shown that inducing and non-inducing flavonoids promote binding of *S. meliloti* NodD1 to the *nod* box, suggesting that competitive inhibition between inducing and non-inducing flavonoids may be important in regulating *nod* gene expression and thus nodulation efficiency. In vivo binding of *S. meliloti* NodD1 to the *nod* box upon luteolin treatment also requires the activity of the chaperonin GroEL (Ogawa and Long 1995; Yeh et al. 2002).

Flavonoid induction of NodD via the *nod* box has been well characterised, and 14 of the 16 genes required for Nod factor biosynthesis are regulated in this manner in *Sinorhizobium* sp. strain NGR234 (Freiberg et al. 1997; Kobayashi et al. 2004). The promoters of many other *Rhizobium* genes also contain *nod* boxes, as demonstrated in NGR234 which responds by increasing transcription of 147 open reading frames upon daidzein treatment (Perret et al. 1999). Indeed, flavonoid treatment can also act to repress gene expression (Firmin et al. 1986), for example coumestrol and medicarpin, flavonoids secreted by *M. sativa* roots, repress *nodC* expression in *S. meliloti* (Zuanazzi et al. 1998). Different flavonoids therefore play different roles to positively and negatively regulate *nod* gene expression in *Rhizobium* species.

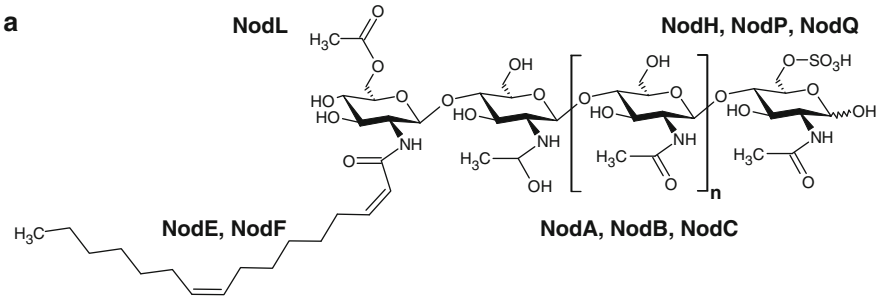
Transient increases in intracellular calcium in *R. leguminosarum* bv. *viciae* have recently been detected upon treatment with flavonoid inducers (Moscatiello et al. 2010). These calcium transients were NodD independent, suggesting that an additional flavonoid-perception mechanism remains to be characterised in *R. leguminosarum* bv. *viciae*. Flavonoid non-inducers did not activate the calcium response in *R. leguminosarum* bv. *viciae* (Moscatiello et al. 2010), therefore this alternative flavonoid-perception mechanism must be specifically activated by only flavonoid inducers. It will therefore be interesting to know the exact interplay between different flavonoids, NodD and calcium in the role of *nod* gene induction.

Silencing of enzymes involved in the biosynthesis of flavonoids has confirmed the importance of these secondary metabolites in establishing symbioses with rhizobia. *Medicago truncatula* plants silenced for chalcone synthase, chalcone reductase and flavone synthase II expression show decreased or no nodulation with *S. meliloti* (Wasson et al. 2006; Zhang et al. 2009). Similar silencing experiments in *Glycine max* also show decreased nodulation (Subramanian et al. 2006). These results are consistent with the importance of flavonoids in activating Nod factor biosynthesis, although it has also been suggested that auxin transport in host roots may be regulated by flavonoids and this has been proposed to be involved in nodule organogenesis (Subramanian et al. 2007). However, the relative importance of flavonoids activating Nod factor biosynthesis versus regulating auxin for nodule organogenesis remains to be resolved.

Non-flavonoid diffusible signals are also produced by legumes and perceived by rhizobia, although their role appears to be relatively minor due to the higher concentrations required for biological activity (Brencic and Winans 2005; Cooper 2007). The first non-flavonoid compounds to be identified were the betaines trigonelline and stachydrine from *M. sativa* which activated *nod* gene expression in *S. meliloti* (Phillips et al. 1992). Jasmonates also stimulate *nod* gene expression in *R. leguminosarum* (Rosas et al. 1998) and *Bradyrhizobium japonicum* (Mabood et al. 2006), whilst *B. japonicum nod* gene induction has also been described with xanthones (Yuen et al. 1995). Interestingly, simple phenolics from wheat, such as vanillin and isovanillin, are also able to act as *nod* gene inducers in *Sinorhizobium* sp. strain NGR234 (Le Strange et al. 1990).

2.2 *Nod Factors*

Upon perception of flavonoids, rhizobial NodD proteins induce *nod* gene transcription. Some of these *nod* gene products are enzymes involved in the production of a suite of lipochitooligosaccharides (LCOs) called Nod factors. The first Nod factor structure to be presented was that of *S. meliloti* (Fig. 2a; Lerouge et al. 1990). Nod factors have a generalised structure consisting of a chitin backbone of usually three to five *β-1,4*-linked *N*-acetylglucosamine residues to which additional decorations and substituents are added, including an acyl (fatty acid) chain at the non-reducing terminus. These decorations vary between *Rhizobium* strains and may include the addition of acetyl, methyl, sulfate and sugar moieties (Fig. 2b). A single species of rhizobia may produce several different Nod factors; for example, *Rhizobium tropici* CIAT899 produces 52 different LCOs at acidic pH and 29 LCOs at neutral pH, yet only 15 structures are common to both growth conditions (Morón et al. 2005).



Acyl	R_1	R_2	R_3	R_4	R_5	R_6	n
C16, C18, C20 (varying degrees of saturation; between 0 and 4 double bonds)	H, Me	H, OH, Cb	H, Ac, Cb	H, S, Ac, Fuc, MeFuc, AcFuc, AcMeFuc, SMeFuc	H, Ara	H, OH, Ac, Fuc	-1, 0, 1, 2, 3

Fig. 2 (**a**) Structure of *Sinorhizobium meliloti* Nod factor. The *N*-acetylglucosamine backbone is synthesised by NodA, NodB and NodC; the acyl chain group is determined by Nod E and NodF; *O*-sulfation is performed by NodH, NodP and NodQ; *O*-acetylation is performed by NodL. (**b**) Generalised structure of naturally occurring Nod factors. Table shows major decorations and variations in Nod factor structure based on species studied to date. *Ac* acetyl, *Ara* arabinosyl, *Cb* carbamoyl, *Fuc* fucosyl, *H* hydrogen, *Me* methyl, *OH* hydroxyl, *S* sulfate, *AcFuc* acetylated fucose, *MeFuc* methylfucose, *AcMeFuc* acetylated methylfucose, *SMeFuc* sulfated methylfucose. Figure adapted from Perret et al. (2000) and Wais et al. (2002)

Rhizobia also produce many other compounds (including type I and type III secreted proteins and surface polysaccharides) which have varying degrees of importance during nodulation. These will not be discussed here as Nod factors are the most important diffusible signalling molecules when considering the establishment of the legume-*Rhizobium* symbiosis. A recent detailed review of these additional signals is provided by Downie (2010).

2.2.1 Nod Factor Core (NodA, NodB, NodC)

The core structure of Nod factors is produced by the enzymes encoded for by the *Rhizobium* genes *nodABC*, which form an operon in many species. The first step of Nod factor biosynthesis involves the assembly of the chitin backbone through the activity of NodC, an *N*-acetylglucosaminyltransferase, which causes chain elongation at the non-reducing terminus (Kamst et al. 1997, 1999; Mergaert et al. 1995; Spaink et al. 1994). NodB, an *N*-deacetylase, then removes the *N*-acetyl moiety from the non-reducing terminus (John et al. 1993; Spaink et al. 1994), allowing for the subsequent addition of an acyl chain to the chitin oligomer via NodA, an *N*-acyltransferase (Fig. 2a; Atkinson et al. 1994; Debelle et al. 1996; Rohrig et al. 1994). NodA represents a unique biosynthetic enzyme since it allows the addition of an acyl chain to a polysaccharide without going through a nucleotide-activated intermediate.

The *nodABC* genes are absolutely essential for Nod factor synthesis and bacterial mutants in these genes are unable to initiate plant signalling (Wais et al. 2002) or nodulate their host (Fisher et al. 1985; Marvel et al. 1985). Cross-species complementation experiments have demonstrated that *nodABC* loci of a number of *Rhizobium* strains are able to complement mutants of a different strain and allow successful nodulation (Krishnan and Pueppke 1991; Marvel et al. 1985). Therefore, *nodABC* were previously referred to as the common nodulation genes. However, recent sequencing of two *Bradyrhizobium* species (BTAi1 and ORS278) revealed the absence of the *nodABC* genes from these genomes; despite this lack of *nodABC*, *Bradyrhizobium* species ORS285 efficiently formed fully functional nodules (Giraud et al. 2007). This exception to the rule therefore provides a unique example of Nod factor-independent establishment of a root nodule symbiosis.

NodA and NodC have also been implicated in determining host-range specificity: *S. meliloti* NodA is able to transfer unsaturated C16 fatty acids to the *N*-acetylglucosamine backbone whilst *R. tropici* NodA is unable to perform this reaction (Roche et al. 1996). Characterisation of NodC from different *Rhizobium* species has demonstrated that this protein controls the number of *N*-acetylglucosamine residues which condense together: *S. meliloti* NodC forms chitotetraoses whilst *Mesorhizobium loti* NodC forms chitopentaoses (Kamst et al. 1995, 1997). However, it is the decorations added to the core Nod factor structure which play a more important role in determining host-range specificity.

2.2.2 Nod Factor Decorations

Research into Nod factor structural requirements began through a series of cross-inoculation experiments whereby *Rhizobium* isolates from one legume were inoculated onto other legume species to determine whether nodulation was possible. Although the precise structures of Nod factors had not been determined, these experiments provided a wealth of data on host-range specificity. Now that many of these Nod factor structures have been determined it is clear that the decorations added to the core Nod factor structure (Fig. 2b) are important for determining host-range specificity. For example, expression of *nodABC* in *Escherichia coli* is alone sufficient to trigger root hair deformation in clover but not *M. sativa*; however, root hair deformation is observed in both species upon the additional expression of *nodH* (Banfalvi and Kondorosi 1989). Similar experiments have established roles for other host-specific *nod* genes in controlling host-range specificity through the decorations added to the core Nod factor structure (Lopez-Lara et al. 1996; Lorquin et al. 1997a, b; Mergaert et al. 1996; Spaink et al. 1991). Additionally, this role for different Nod factor structures in determining host-range specificity is particularly important when considering the broad host-range *Sinorhizobium* sp. strain NGR234 which is able to nodulate many host plants because it produces a wide variety of Nod factor structures (Price et al. 1992).

Acylation (NodE, NodF)

NodE and NodF determine which acyl chain(s) are added to the core Nod factor structure by NodA. NodF is an acyl carrier protein, whilst NodE is a β-ketoacyl synthase implicated in determining the degree of acyl chain saturation (Bloemberg et al. 1995a; Debelle and Sharma 1986; Geiger et al. 1991; Ritsema et al. 1997; van der Drift et al. 1996). In *R. leguminosarum* bv. *viciae,* NodE activity leads to the production of a Nod factor with a polyunsaturated C18:4 acyl chain. Inactivation of this gene instead results in the incorporation of vaccenic acid, an unsaturated C18:1 acyl chain (Spaink et al. 1991), and subsequently renders the strain unable to nodulate *Vicia sativa* (Canter Cremers et al. 1989). Deletion of *S. meliloti nodF* yields Nod factors with similar acyl chain compositions to those obtained from *nodE* deletion mutants, suggesting that the combined action of NodE and NodF is required for appropriate acyl chain addition (Demont et al. 1993). Indeed, exchanging the *nodEF* genes of *S. meliloti* with those of *R. leguminosarum* bv. *viciae* extends the production of Nod factors to include structures with polyunsaturated C18:2, C18:3 and C18:4 acyl chains (Demont et al. 1993). Methyl-branched acyl chains are added to the Nod factor of the arctic *Mesorhizobium* sp. strain N33 (*Oxytropis arctobia*) and this requires a fully functional *nodE* gene (Poinsot et al. 2001). Poinsot et al. (2001) speculate that incorporation of these unusual acyl chains is important for this species to tolerate extreme cold.

Some species of legumes utilise specific recognition of the acyl chain as a stringent measure of Nod factor during rhizobial infection (Ardourel et al. 1994; Walker and Downie 2000). However, this recognition occurs in combination with either the *O*-acetylation of Nod factor by NodL (Sect. 2.2.2.3; Ardourel et al. 1994), or the action of other Nod proteins (Walker and Downie 2000).

Glycosylation (NoeC, NodZ, NolK)

Two forms of glycosylation have been described as Nod factor decorations: arabinosylation and fucosylation. The importance of both modifications has been described for Nod factors from *Azorhizobium caulinodans*, the symbiont of *Sesbania rostrata* (Mergaert et al. 1997). Arabinosylation and fucosylation also appear to be required by other symbionts of *S. rostrata*, suggesting a possible responsibility of these glycosyl decorations for determining host-range specificity (Lorquin et al. 1997a).

D-arabinosylation on C3 of the reducing terminus of Nod factor is dependent on *noeC* and/or downstream genes in *A. caulinodans* (Mergaert et al. 1996). Presence of this *D*-arabinosyl group on Nod factors from *A. caulinodans* results in higher numbers of nodules on *S. rostrata* roots than Nod factors without this decoration (Fernandez-Lopez et al. 1998). However, other host species of *A. caulinodans* show a preference for fucosylated Nod factors, implying that arabinosylation is particularly important for nodulation of *S. rostrata* (Fernandez-Lopez et al. 1998).

L-fucosylation of *A. caulinodans* Nod factors on C6 of the reducing terminus depends on both *nodZ* and *nolK*. NolK is involved in the biosynthesis of GDP-fucose, which is a substrate for the fucosyltransferase NodZ (Mergaert et al. 1996). These authors also detected a NodZ-independent Nod factor fucosyltransferase activity, although this activity was not encoded for by any of the known *nod* genes.

NodZ also decorates the Nod factors of other *Rhizobium* species with fucosyl groups (Lopez-Lara et al. 1996; Quesada-Vincens et al. 1997; Quinto et al. 1997; Stacey et al. 1994). NodZ from *Sinorhizobium* sp. strain NGR234 preferentially fucosylates chitopentaoses over single *N*-acetylglucosamine residues or non-fucosylated Nod factors, implying that fucosylation occurs before acylation (Quesada-Vincens et al. 1997). Nodulation of *Macroptilium atropurpureum* by *B. japonicum* (Stacey et al. 1994) and *Pachyrhizus tuberosus* by *Sinorhizobium* sp. strain NGR234 (Quesada-Vincens et al. 1997) is blocked by mutation of *nodZ*. However, expression of *B. japonicum nodZ* in *R. leguminosarum* bv. *viciae* results in fucosylated Nod factor production and extends the host range of the *R. leguminosarum* strain (Lopez-Lara et al. 1996). It is also interesting to note that despite the absolute necessity of *B. japonicum* for NodZ in order to nodulate *M. atropurpureum*, *nodZ* expression is not controlled by NodD, a trait which is unique amongst the *nod* genes (Stacey et al. 1994).

An additional rare fucosylation site has been identified in *Mesorhizobium loti* strain NZP2213 where the fucosyl residue is found on a non-terminal *N*-acetylglucosamine residue of the Nod factor structure (Olsthoorn et al. 1998).

Acetylation (NodL, NodX, NolL)

An *O*-acetyl group can be added on C6 of either the reducing or non-reducing terminal *N*-acetylglucosamine residue of Nod factor through the action of NodX or NodL, respectively (Bloemberg et al. 1994). NodX from *R. leguminosarum* bv. *viciae* is able to only use chitopentaoses as a substrate for *O*-acetylation (Firmin et al. 1993) and the quantities of *O*-acetylated Nod factor produced by NodX is temperature-dependent (Olsthoorn et al. 2000). Mutation of *nodX* in *R. leguminosarum* bv. *viciae* strain TOM abolishes the ability of this strain to nodulate *Pisum sativum* cv. Afghanistan (Davis et al. 1988). This *nodX* mutant produces other LCOs identical to wild-type bacteria (Ovtsyna et al. 1999), suggesting that the specificity of the interaction between strain TOM and *P. sativum* cv. Afghanistan is due to *O*-acetylation. However, nodulation with this *nodX* mutant can be restored by expression of *nodZ* from *B. japonicum* (Sect. 2.2.2.2; Ovtsyna et al. 1998), implying that *O*-acetylation alone cannot be the only mechanism for determining specificity in this interaction.

The NodL *O*-acetylation reaction is dependent upon a non-reducing terminally de-*N*-acetylated chitin oligosaccharide substrate (i.e. the product of NodB and NodC activity; Bloemberg et al. 1995b). The stringency for NodL-mediated *O*-acetylation appears to be low since fully functional nodulation of *M. sativa* by the *S. meliloti nodL* mutant is possible, although significantly decreased infection thread formation and a delay in nodulation was noted (Ardourel et al. 1994).

In addition to acetylation by NodX or NodL, acetyl groups can be added onto fucose decorations via NolL. NolL from *Sinorhizobium* sp. strain NGR234 leads to Nod factor structures with 3-*O*- or 4-*O*-acetylation on fucose (Berck et al. 1999), whilst NolL from *Rhizobium etli* yields Nod factors with only 4-*O*-acetylation on fucose (Corvera et al. 1999). NolL is not essential for nodulation of *Phaseolus vulgaris* by *R. etli*, although nodulation of the *nolL* mutant was less efficient than the wild-type strain on some *P. vulgaris* cultivars (Corvera et al. 1999). Heterologous expression of *nodZ* or *nodZ* and *nolL* has demonstrated that NolL is necessary for efficient nodulation of *Lotus japonicus* by *R. leguminosarum* bv. *viciae* (Pacios Bras et al. 2000). Different *Lotus* species also have different requirements for NolL-mediated acetylation on fucose: the *M. loti nolL* mutant is unable to form infected nodule primordia on *L. filicaulis* and *L. corniculatus* yet can successfully nodulate *L. japonicus* (Rodpothong et al. 2009). This apparent discrepancy for the requirement of NolL for successful nodulation of *L. japonicus* was explained by Rodpothong et al. (2009) as being due to other differences, notably the acyl chain structure, between the Nod factors of *M. loti* (the true symbiont of *L. japonicus*) and *R. leguminosarum* bv. *viciae* expressing *nodZ* and *nolL*.

A rare acetylation site has been determined in the *M. loti* strain N33 where *6-O*-acetylation occurs on the residue proximal to the non-reducing *N*-acetylglucosamine, although the gene encoding the enzyme responsible for this modification has not been identified (Poinsot et al. 2001).

Methylation (NodS, NoeI)

Two forms of methylation can occur on Nod factors: *N*-methylation on the non-reducing terminus controlled by NodS (Geelen et al. 1993; Jabbouri et al. 1995) or *2-O*-methylation on fucose mediated by NoeI (Jabbouri et al. 1998). NodS is an *N*-methyltransferase and in *A. caulinodans* or *Sinorhizobium* sp. strain NGR234 methylates end-deacetylated chitooligosaccharides using an *S*-adenosyl-*L*-methionine-binding protein as a methyl donor (Geelen et al. 1995). Indeed, the structure of NodS from *B. japonicum* has recently been solved, representing the first crystal structure of an *S*-adenosyl-*L*-methionine-dependent methyltransferase (Cakici et al. 2010). The *R. etli nodS* mutant is less able to induce root hair curling and actin cytoskeleton rearrangements in *P. vulgaris* than wild-type *R. etli*, suggesting that *N*-methylation is key in regulating these Nod factor-dependent responses (Cardenas et al. 2003). *N*-methylation by NodS biosynthetically precedes any *O*-acetylation reactions by NodL (Lopez-Lara et al. 2001).

2-O-methylation of fucose by NoeI is common in *Sinorhizobium* sp. strain NGR234 and *S. fredii* strain USDA257 (Jabbouri et al. 1998). Mutation of this gene leads to production of LCOs which are non-methylation on fucose, although as this appears to have no effect on nodulation (Jabbouri et al. 1998) this Nod factor decoration is of lesser significance.

Carbamoylation (NodU, NolO)

Carbamoylation on the non-reducing terminal *N*-acetylglucosamine residue is controlled by the carbamoyltransferases NodU and NolO. Expression of *Sinorhizobium* sp. strain NGR234 *nodU* in *S. fredii* strain USDA257 (which does not produce carbamoylated Nod factors) allows *6-O*-carbamoylated Nod factors production (Jabbouri et al. 1995). Likewise, expression of *Sinorhizobium* sp. strain NGR234 *nolO* in *S. fredii* strain USDA257 has confirmed the role of NolO in controlling *3-O*- and *4-O*-carbamoylation at the non-reducing terminus (Jabbouri et al. 1998). The host range of *S. fredii* expressing *nolO* is increased to include non-host species (Jabbouri et al. 1998). However, the nodulation phenotype of the *S. fredii nolO* mutant was not different from the wild-type strain, although the mutant showed decreased competitiveness to nodulate *G. max* (Madinabeitia et al. 2002). Interestingly, Jabbouri et al. (1998) suggest the existence of a third (as yet uncharacterised) carbamoyltransferase in *Sinorhizobium* sp. strain NGR234 since mutation of *nodU* and *nolO* failed to result in Nod factors entirely devoid of carbamoylation.

Sulfation (NodH, NodP, NodQ, NoeE)

Addition of an *O*-sulfate group is common to many Nod factors and this reaction is performed by the sulfotransferases NodH (Del Papa et al. 2007; Ehrhardt et al. 1995; Laeremans et al. 1996; Lerouge et al. 1990; Roche et al. 1991; Schultze et al.

1995) and NoeE (Hanin et al. 1997). NodH activity results in sulfation on C6 of the reducing terminus, while NoeE only gives sulfation on fucose residues attached to C6 of the reducing terminus (Quesada-Vincens et al. 1998). NodP and NodQ are also essential for Nod factor sulfation and act as sulfur activators by synthesising the sulfur donor 3′-phosphoadenosine 5′-phosphosulfate (Schwedock and Long 1990; Schwedock et al. 1994).

The *R. tropici* strain CFN299 *nodP* mutant shows decreased nodulation on *P. vulgaris* cv. Negro Xamapa, while *nodH* and *nodP* mutants acquire an increased capacity to nodulate the two other cultivars (Laeremans et al. 1996). Likewise, the *nodH* mutant of *R. tropici* shows decreased nodulation in comparison to wild-type when nodulating *Leucaena leucocephala* (Folch-Mallol et al. 1996). *R. fredii* expressing *noeE* produced sulfated LCOs and therefore acquired the ability to nodulate *Calopogonium caeruleum*, whilst mutation of *noeE* from *Sinorhizobium* sp. strain NGR234 abolished the production of sulfated LCOs and prevented nodulation of *P. tuberosus* (Hanin et al. 1997). Importantly, *S. meliloti* Nod factor sulfation is essential for root hair deformation and nodulation of *M. sativa* (Roche et al. 1991). These findings all support a role for sulfation as a major determinant of symbiont specificity for their host plant species, yet in other interactions the stringency for sulfation appears to be low. Mutation of the *nodHPQ* genes of *Rhizobium* sp. strain N33 appears to have no effect on nodulation of two host species tested (Cloutler et al. 1996). Remarkably, a recent report suggests that the *nodH* mutant of *Sinorhizobium* sp. strain BR816 shows increased nitrogen fixation relative to the wild-type strain despite there being no other nodulation phenotype (Remans et al. 2007). The authors attribute this interesting result to the availability of activated sulfate inside nodules which they argue is likely to be greater with the *nodH* mutant.

2.2.3 Nod Factor Secretion

NodI and NodJ act as an ATP-binding cassette (ABC) transporter (Higgins et al. 1986) and are involved in Nod factor secretion. Secretion of Nod factor is impaired in *nodI* and/or *nodJ* rhizobia mutants (Cardenas et al. 1996; Fernandez-Lopez et al. 1996; Spaink et al. 1995), whilst *E. coli* engineered for the biosynthesis of Nod factors only secreted these LCOs in the presence of NodI and NodJ (Fernandez-Lopez et al. 1996). Interestingly, the *nodIJ* mutant of *R. etli* is able to nodulate *P. vulgaris*, although the mutant shows a delayed and decreased nodulation phenotype in comparison to the wild-type. This non-essential role of NodI and NodJ therefore suggests a possible additional component involved in rhizobial secretion of Nod factors which has yet to be characterised.

2.3 Plant Responses to Nod Factor

Nod factors are perceived in epidermal and root hair cells since fluorescent Nod factors added to plant roots accumulate in the walls of these cells (Goedhart et al. 2000). Host plant cells are able to perceive Nod factor concentrations as low as 10^{-12} M (Oldroyd and Downie 2004), suggesting that the receptor able to perceive Nod factors is highly sensitive. In *L. japonicus,* two Nod factor receptors have been identified: NFR1 and NFR5 (Madsen et al. 2003; Radutoiu et al. 2003). The *M. truncatula* gene equivalent to *NFR5* is *NFP* (Nod factor perception), whilst *LYK3* is orthologous to *NFR1* (Amor et al. 2003; Smit et al. 2007). Interestingly, LYK3 has been described as an entry receptor which controls rhizobial infection in a manner dependent upon Nod factor structure (Smit et al. 2007), therefore suggesting an additional element for Nod factor structural specificity in establishing the legume-*Rhizobium* symbiosis. These Nod factor receptors are receptor-like kinases and contain LysM domains which are involved in binding *N*-acetylglucosamine, making them likely Nod factor receptors. Importantly, *M. truncatula* transformed with *NFR1* and/or *NFR5* was able to form nodules with a rhizobial species usually specific to *L. japonicus* (Radutoiu et al. 2007). This directly implicates NFR1 and NFR5 as Nod factor receptors whilst also demonstrating the importance of these receptors and the structure of Nod factors themselves for determining symbiont specificity. However, binding assays between Nod factors and their potential targets have yet to provide formal evidence for a direct physical interaction between ligand and receptor.

Nod factors trigger a range of molecular responses in legumes (reviewed by D'Haeze and Holsters 2002; Oldroyd et al. 2001a). These responses include rapid pH changes (Felle et al. 1996, 2000), root hair deformation (Roche et al. 1991; Spaink et al. 1991), lateral root formation (Olah et al. 2005), reactive oxygen species production (Cardenas et al. 2008; Cardenas and Quinto 2008), induction of calcium flux (Ehrhardt et al. 1992), induction of calcium spiking (Ehrhardt et al. 1996), and gene expression changes (Mitra et al. 2004). Nod factors are required for infection thread development but are insufficient to activate this response alone (Dazzo et al. 1991), although the formation of pre-infection thread structures has been described (van Brussel et al. 1992). At sufficiently high concentrations Nod factors can also induce cortical cell division and the formation of nodule primordia (Truchet et al. 1991). The diversity of these responses only goes to demonstrate the critical importance of Nod factors as signalling molecules in the early stages of nodulation.

The sensitivity of the responses triggered by Nod factor can vary by several orders of magnitude; for example, the two Ca^{2+} signatures (flux and spiking) can be separated, such that high concentrations ($>10^{-9}$ M) of Nod factor induce flux followed by spiking, while low Nod factor concentrations ($<10^{-10}$ M) induce only calcium spiking (Shaw and Long 2003). *Pisum sativum* plants treated with chitin oligomers of four or five residues also show calcium spiking, but not calcium flux (Walker et al. 2000). These observations suggest that the activation of calcium

spiking has a lower stringency for Nod factor structure and concentration than the induction of calcium flux. The structural requirements of *S. meliloti* Nod factors to trigger calcium spiking have been analysed and effects due to missing decorations, such as *O*-acetylation, *N*-acylation or *O*-sulfation, can be overcome by treating with high enough concentrations of Nod factor (Oldroyd et al. 2001b; Wais et al. 2002). This work has formally shown that Nod factor decorations, in addition to determining host-range specificity, play a role in determining the potency of the Nod factor signal to the plant and that concentration of LCOs must therefore be considered when determining biological activity.

Calcium spiking, the rapid oscillation of calcium concentration in the nucleus and peri-nuclear region of root hair cells (Ehrhardt et al. 1996), is central to the signalling pathway activated by Nod factor (Oldroyd and Downie 2004). Components of this common symbiosis signalling pathway are required for both nodulation and mycorrhization in legumes; mutation in any of these genes blocks the formation of either symbiosis.

3 Diffusible Signals During Mycorrhization

The molecular dialogue between AM fungi and host plant roots has been less well characterised than that of legumes and rhizobia, partly because the fungus is an obligate biotroph which makes it less amenable to study. An additional problem for the study of AM fungi is the asynchronous nature of the infection process. Despite these limitations, research in this area has begun to provide some interesting parallels between signalling during nodulation and mycorrhization (reviewed by Bonfante and Genre 2010; Harrison 2005; Parniske 2008). Diffusible signals again play a key role in the establishment of mycorrhizal interactions. Strigolactones are released from plant roots and these promote AM fungal spores to germinate. Germinated spores produce a diffusible signal, a so-called "Myc" factor, which triggers signalling in the plant (mediated by the symbiosis signalling pathway in both legumes and non-legumes; Gutjahr et al. 2008). The chemical nature of "Myc" factor has proved elusive, although recent work has characterised LCOs derived from AM fungi which act in an analogous fashion to Nod factor.

3.1 Strigolactones

Strigolactones play an important role in establishing mycorrhizal symbioses, serving as germination and hyphal branching cues for dormant AM fungal spores. Nothing is known about strigolactone perception by the fungus or the requirement for different chemical structures of strigolactones. Other diffusible signals, including flavonoids, have also been implicated in triggering spore germination.

3.1.1 Structure and Synthesis

The first strigolactone isolated from root exudates was strigol (Cook et al. 1966). Numerous subsequent experiments have demonstrated the presence of strigolactones in root exudates from different species, including both monocotyledonous (Awad et al. 2006) and dicotyledonous plants (Yoneyama et al. 2008). It was not until 2003 though that strigolactones were formally proved to be derived from roots, as demonstrated through aseptic plant culture experiments (Yasuda et al. 2003). Many chemical structures of naturally occurring strigolactones have now been proposed (Yoneyama et al. 2009); these vary primarily in the position and number of hydroxyl, methyl and acetyl groups present on the core ring structure. Interestingly, *Arabidopsis thaliana*, a non-mycorrhizal species, produces low concentrations of strigolactones relative to other plant species which can form symbioses with AM fungi (Westwood 2000).

Relatively little is known about the biosynthesis of strigolactones, which were originally considered to be a group of sesquiterpene lactones. However, the tricyclic ring structure of strigolactones is now known to be derived from carotenoid biosynthesis (Fig. 3; Jamil et al. 2010; Lopez-Raez et al. 2008; Matusova et al. 2005). Two proteins characterised in *A. thaliana* as carotenoid cleavage dioxygenase enzymes have been implicated specifically in the biosynthesis of strigolactones: CCD7 (Booker et al. 2004) and CCD8 (Sorefan et al. 2003). *CCD7* and *CCD8* were originally studied for their mutant phenotypes in shoot branching and a role for branching inhibition by strigolactones is now established (Gomez-Roldan et al. 2008; Umehara et al. 2008). Mutation or silencing which causes decreased *CCD7* expression in tomato plants gives rise to strigolactone-impaired lines which show decreased colonisation with AM fungi, thus demonstrating the importance of this enzyme for the synthesis of strigolactones and the importance of strigolactones for mycorrhization (Koltai et al. 2010; Vogel et al. 2010).

3.1.2 Biological Activity

Strigolactones have been well characterised for their role in the interaction between plants and weeds of the genus *Striga*, from where these molecules derive their name. During this parasitic interaction, strigolactones released by the host plant promote germination of *Striga* species (reviewed by Bouwmeester et al. 2003). However, the role of strigolactones during symbiotic interactions with AM fungi was not determined until 2005 (Akiyama et al. 2005).

The amount of strigolactone secretion by roots is thought to be very low, but these molecules are highly potent and are able to induce fungal hyphal branching at picogram to nanogram quantities (Akiyama and Hayashi 2006; Bucher et al. 2009). For this reason, synthetic strigolactones have also been used in the study of strigolactones and AM fungi. Fungal responses after the application of synthetic

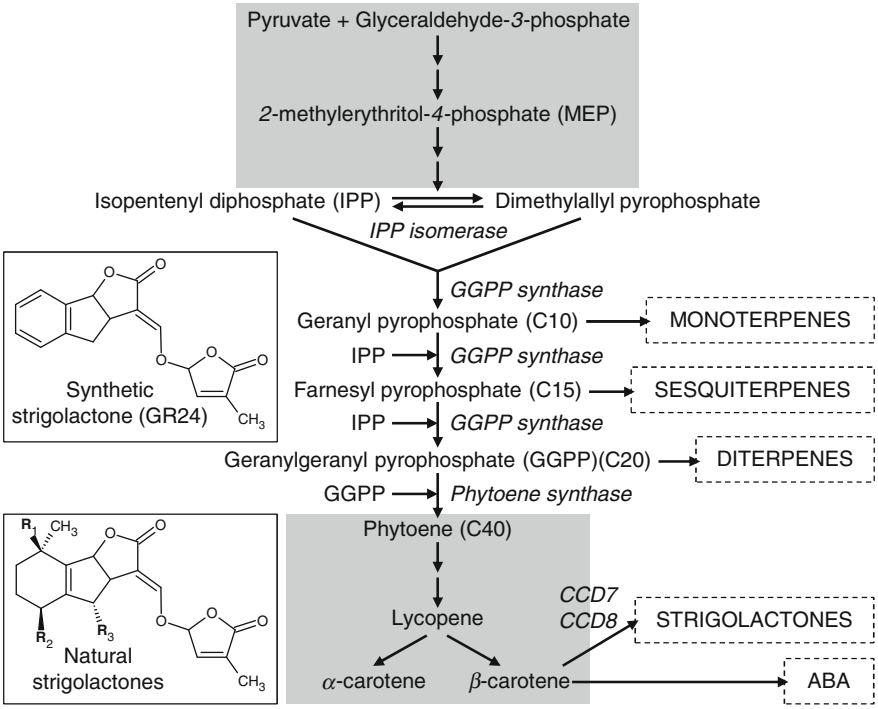


Fig. 3 Biosynthesis of strigolactones. Partial diagram of the carotenoid biosynthesis pathway. Enzymes are depicted in italics; the *2*-methylerythritol-*4*-phosphate (MEP) pathway and the first committed steps of carotenoid biosynthesis are denoted by upper and lower shaded boxes, respectively; compounds in dashed boxes represent major side branches of the pathway. *ABA* abscisic acid, *CCD7*/*8* carotenoid cleavage dioxygenase, *GGPP* geranylgeranyl pyrophosphate, *IPP* isopentenyl diphosphate. Insets show chemical structures of example synthetic and naturally occurring strigolactones: strigol: $R_1 = CH_3, R_2 = OH, R_3 = H$; strigyl acetate: $R_1 = CH_3, R_2 = OAc, R_3 = H$; sorgolactone: $R_1 = H, R_2 = H, R_3 = H$. Figure adapted from Akiyama and Hayashi (2006) and Matusova et al. (2005)

strigolactones, such as GR24, are comparable to treatment with naturally occurring strigolactones (Akiyama et al. 2005). Strigolactones (or "branching factors" as they were originally named before their identification) promote spore germination and branching in *Gigaspora* spp. (Akiyama et al. 2005; Buee et al. 2000; Nagahashi and Douds 1999). In addition, strigolactone treatment promotes a number of other fungal pre-symbiotic responses, including the induction of mitosis (Buee et al. 2000), increased expression of mitochondrial-related genes (Tamasloukht et al. 2003), increased density of mitochondria (Besserer et al. 2009), and thus increased respiratory activity (Tamasloukht et al. 2003).

Mycorrhization is impaired in the strigolactone-deficient *ccd8* mutant of *P. sativum*, although this phenotype can be partially recovered by exogenous application of GR24 (Gomez-Roldan et al. 2008). As full complementation is not achieved with exogenous treatment of strigolactone it is possible that directionality

of the diffusible signal via a concentration gradient is important in order to encourage germinating AM spores to grow towards host roots. This existence and importance of a natural concentration gradient is especially likely when considering that strigolactones are readily hydrolysed in the soil (Akiyama and Hayashi 2006). Evidence for chemotaxis responses of AM fungi to root diffusible signals supports this hypothesis (Sbrana and Giovannetti 2005).

The involvement of flavonoids as diffusible signals during the establishment of interactions with AM fungi remains unclear (as discussed by Larose et al. 2002; Vierheilig et al. 1998). For example, the flavonoid medicarpin accumulates in *M. sativa* roots and strongly inhibits *Glomus intraradices* hyphal growth (Guenoune et al. 2001), whilst a flavonoid from melon roots enhances mycorrhization in this species (Akiyama et al. 2002). Likewise, the flavonoid quercetin stimulates AM fungal spore growth and branching (Becard et al. 1992; Tsai and Phillips 1991). Other studies have suggested that flavonoids are not absolutely essential for hyphal growth (Becard et al. 1995). The most likely conclusion is that AM fungal responses to flavonoids are compound and genus specific (Scervino et al. 2005a, b), therefore making it difficult to assign a definitive role for these diffusible signals during the establishment of symbioses with AM fungi. Contrasting this with nodulation, recent evidence suggests that nodulation of *M. sativa* by *S. meliloti* is increased by strigolactone treatment (Soto et al. 2010); it will therefore be interesting to know whether the interplay between these diffusible signals is important for the establishment of both symbioses.

3.2 "Myc" Factors

A diffusible signal originating from the fungus, the so-called "Myc" factor, has long been hypothesised, but until recently had not been characterised. It has been shown that a diffusible factor released from germinating AM fungi is able to induce expression of *ENOD11*, a symbiosis-specific gene in *M. truncatula* (Kosuta et al. 2003). Olah et al. (2005) used a membrane separating AM fungi from plants to demonstrate that this diffusible fungal factor identified by Kosuta et al. (2003) activates root branching in *M. truncatula* (a response also observed with Nod factor). AM fungi are able to induce calcium spiking in *M. truncatula* root hair cells associated with highly branched fungal hyphae and this occurs prior to physical contact between the fungus and the root (Kosuta et al. 2008). Calcium spiking has also been detected upon contact of fungal hyphopodia with non-trichoblastic root cells of *M. truncatula* and *Daucus carota*, and also upon treatment of *M. truncatula* roots with a concentrated extract from germinating fungal spores (Chabaud et al. 2011). This Ca^{2+} spiking in *M. truncatula* is dependent on components of the symbiosis signalling pathway but is *NFP*-(in)dependent, therefore implying different plant machineries for the recognition of "Myc" and Nod factors (Chabaud et al. 2011; Kosuta et al. 2008). Calcium transients have also been detected in *G. max* cell cultures exposed to germinating spores from *Glomus* species

(Navazio et al. 2007), although this signal was also released by non-germinating spores so perhaps represents a triggering of defence responses.

3.2.1 LCOs as a "Myc" Factor

An exciting recent development has proposed the structures of two LCOs produced by the AM fungus *G. intraradices* (Fig. 4; Maillet et al. 2011). One of these LCOs contains an unsaturated C18:1 acyl chain and is non-sulfated on the reducing terminus (Fig. 4a), whilst the other LCO has a saturated C16 acyl chain and is *O*-sulfated at the reducing terminus (Fig. 4b). These LCOs were characterised for their ability to induce *pENOD11::GUS* expression and root hair deformation in *M. truncatula*. Application of these Myc LCOs to *M. truncatula*, *Tagetes patula* or *D. carota* resulted in increased mycorrhizal colonisation. Increased lateral root formation in *M. truncatula* was also observed upon Myc LCO treatment and importantly this was *NFP*-dependent (Maillet et al. 2011). It has been previously

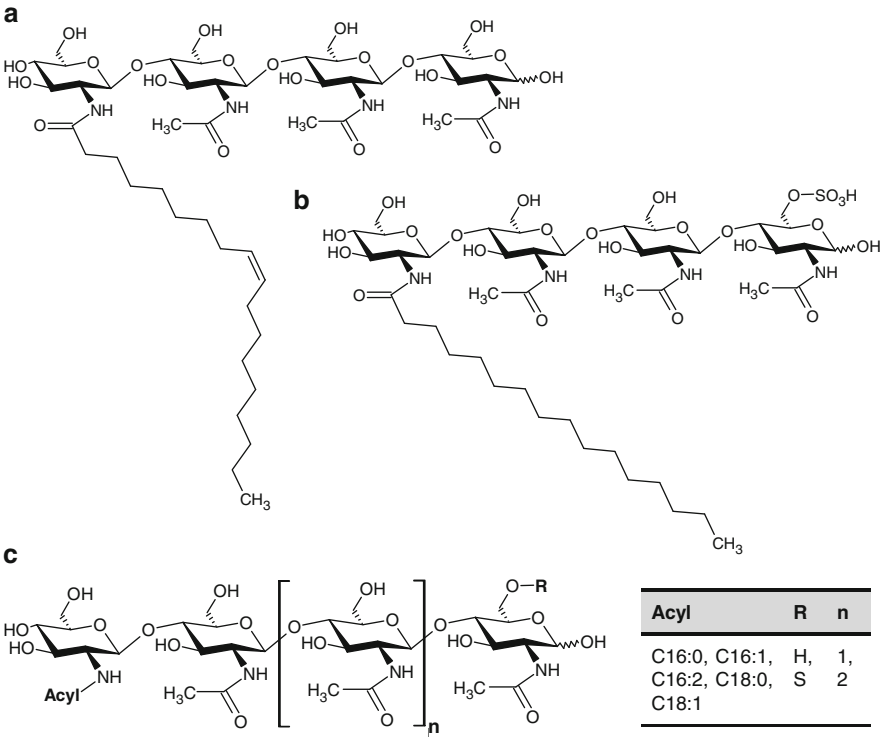


Fig. 4 (**a**) Structure of *Glomus intraradices* non-sulfated lipochitooligosaccharide (LCO). (**b**) Structure of *G. intraradices* sulfated LCO. (**c**) Generalised structure of naturally occurring *G. intraradices* LCOs. Table shows decorations and variations in Myc LCOs. Abbreviations as in Fig. 2. Figure adapted from Maillet et al. (2011)

shown that *NFP* is not required for mycorrhizal associations (Amor et al. 2003); therefore the apparent discrepancy for the requirement of *NFP* for inducing different mycorrhizal responses in *M. truncatula* could be due to the existence of other as yet uncharacterised diffusible signals derived from AM fungi. The *NFP*-independent diffusible fungal signals described by Olah et al. (2005) and Chabaud et al. (2011) may therefore represent different novel classes of diffusible signalling molecules (i.e. non-LCOs) involved in establishing the mycorrhizal symbioses. The apparent dual role of NFP in signalling during mycorrhization and nodulation could also be explained by the existence of a receptor complex consisting of NFP and other currently uncharacterised receptors. This is supported by the dual role of *Parasponia* NFP, which is involved in both nodulation and mycorrhization (Op den Camp et al. 2011). Alternatively, if early mycorrhizal signalling occurs in a directly analogous fashion to that of Nod factor, the "Myc" factor which causes *NFP*-independent root branching (Olah et al. 2005) and calcium spiking (Chabaud et al. 2011; Kosuta et al. 2008) would require an additional separate receptor. However, such a "Myc" factor receptor has yet to be identified.

Given the diversity of Nod factor structures and the fact that at least 80% of land plants are able to engage in symbiotic interactions with mycorrhiza, it is almost certain that other Myc LCOs exist in nature and have yet to be isolated and characterised. It is also tempting to speculate that *G. intraradices* and other AM fungi produce a broad spectrum of LCOs in order to colonise a wide range of host species, as with the broad host-range *Sinorhizobium* sp. strain NGR234.

4 Conclusions and Perspectives

The importance of diverse diffusible signals during the establishment of nodulation and mycorrhization is clear. Nod factor structural diversity has been implicated as a key determinate of host-range specificity, as discussed here and reviewed extensively by D'Haeze and Holsters (2002) and Perret et al. (2000). However, this structural diversity alone does not determine host-range specificity in all legume-*Rhizobium* symbioses; for example, *R. etli* and *M. loti* produce identical Nod factors yet nodulate different plant species (Cardenas et al. 1995). The recent identification of LCOs produced by the AM fungus *G. intraradices* will no doubt result in detailed research into the structural importance of these molecules during mycorrhizal interactions. Developments in signalling by nodulation- and mycorrhization-specific LCOs will also prove exciting, particularly in addressing the question of specificity in symbiosis signalling.

When considering the signals released from symbionts it is important to bear in mind that mycorrhization evolved ~400 million years before nodulation (Kistner and Parniske 2002). Chitin, the major component of fungal cell walls, therefore becomes a key molecule: chitin fragments released from cell walls of symbiotic or pathogenic fungi would act as a trigger of plant defence responses. In order to differentiate themselves as symbiotic (and also to protect against hydrolysis by