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Legume nodulation and mycorrhizae formation; two extremes in host specificity meet

Cathy Albrecht, René Geurts and
Ton Bisseling¹

Department of Molecular Biological Sciences, Laboratory of
Molecular Biology, Agricultural University, Dreijenlaan 3,
6703 HA Wageningen, The Netherlands

¹Corresponding author
e-mail: Ton.Bisseling@mac.mb.wau.nl

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Introduction

Most higher plants have the ability to form arbuscular endomycorrhiza (AM); a symbiotic association of the plant root with fungi belonging to the order of *Glomales*. These fungi grow towards the inner cortical cells of the root where they differentiate into highly branched structures, the arbuscules (Figure 1). In AM symbiosis, the fungus also forms hyphae outside the plant and these provide a connection between the soil and the inner part of the plant and they facilitate the uptake of nutrients such as phosphate (for reviews see: Gianinazzi-Pearson, 1996; Harrison, 1997).

In contrast to AM formation, only a few plant species have the ability to interact symbiotically with bacteria of the genera *Azorhizobium*, *Bradyrhizobium*, *Rhizobium* and *Sinorhizobium* (here collectively called *Rhizobium*). This interaction is almost completely restricted to leguminous plants and results in the formation of a completely new organ, the root nodule. In these nodules the bacteria are hosted intracellularly and there they find the ideal environment to reduce atmospheric nitrogen into ammonia, a source of nitrogen which can be used by the plant (for reviews see: Mylona *et al.*, 1995; Long, 1996).

At first glance the interactions of plants with rhizobia and AM fungi seem to have little in common. The induced morphological responses of the host plants are different. Furthermore, both interactions are extremes in terms of host specificity. Whereas in AM formation there is very little host specificity, the *Rhizobium*–legume interaction is highly specific. However, genetic studies have shown that several common steps are involved in establishing these symbioses (Duc *et al.*, 1989; Bradbury *et al.*, 1991; Gianinazzi-Pearson, 1996). Furthermore, some host genes are induced during the initial steps of both interactions. In the first part of this overview, the *Rhizobium*–plant interaction is described with an emphasis on factors that determine the specificity of the interaction. In the second section, AM formation is described as well as the common aspects of both symbioses.

Rhizobium-induced nodule formation

Nod factors

Root-nodule formation involves growth responses in the epidermis as well as cortex of the root. This implies that the bacteria redirect the development of fully differentiated plant cells. The bacterial signals that set this in motion are the so-called nodulation (Nod) factors. Nod factors of the different *Rhizobium* species have a common basic structure; a β -1,4-linked *N*-acyl-D-glucosamine backbone of mostly four or five units, containing a fatty acid at the non-reducing terminal sugar (Figure 2; for a review, see Carlson *et al.*, 1994; Long, 1996).

The biosynthesis of the basic Nod-factor structure is catalysed by the bacterial NodA, NodB and NodC proteins. NodC is an *N*-acetylglucosaminyl-transferase and catalyses the synthesis of the chitin oligomer and controls the length of this backbone. The terminal non-reducing glucosamine unit of this oligomer is deacetylated by NodB, and subsequently substituted with an acyl chain by NodA. Several other Nod proteins, which can be specific for a certain *Rhizobium* species, modify a terminal sugar residue or determine the nature of the acyl chain (Carlson *et al.*, 1994). These modifications define the biological activity and host specificity (see below) of Nod factors. As an example, Nod factors produced by *Sinorhizobium meliloti* (previously named *Rhizobium meliloti*) and *Rhizobium leguminosarum* biovar *viciae*, are shown in Figure 2. The major difference between both Nod factors concerns the presence of a sulfate group at the reducing terminal sugar of the *S. meliloti* factor and the structure of the acyl chain.

Since bioactivity of Nod factors is controlled by their structure it is very likely that they are recognized by receptors of the host. However, such receptors have not been cloned. Biochemical studies have shown that a few Nod-factor binding proteins occur, but it is not yet clear whether these are Nod-factor receptors (Bono *et al.*, 1995; Niebel *et al.*, 1997).

Nodulation process

Nod-factor-secreting rhizobia induce 'shepherd's crook'-like curling of root hairs within 1–2 days of inoculation (Figure 1). *Rhizobium* uses the microenvironment within such curls to establish an infection site. They locally degrade the plant cell wall and enter the root hair via invagination of the plasma membrane (Turgeon and Bauer, 1985). Vesicles are directed to the invaginated membrane, leading to the formation of an 'inward tip growing' tubular structure, the infection thread (Figure 1). In general, Nod factors are not sufficient to trigger root-hair curling and infection-thread formation, but they play a crucial role in the infection process, since infections can only be initiated when the bacteria secrete specific Nod factors (Ardourel *et al.*, 1994; Geurts *et al.*, 1997).

Results obtained with bioassays have provided some

Fig. 1. Pea root cortex infected by the mycorrhizal fungus *Glomus intraradices* (A) and a *Rhizobium leguminosarum* bv *viciae* induced infection thread in a vetch-root hair (B). The AM fungus has entered the root intercellularly and it has formed an arbuscle [(A), Trypan blue staining]. In contrast, *Rhizobium* enters its host plant intracellularly via an infection thread [(B), Vetch-root hair with an infection thread containing *R.leguminosarum* bv *viciae* bacteria expressing green fluorescent protein (GFP) (Spaink *et al.*, 1998)].

insight into the mechanism by which Nod factors alter the growth pattern of root hairs. Such studies have most extensively been carried out using vetch (*Vicia sativa*). Vetch-root hairs which respond morphologically to the application of Nod factor have almost terminated growth. The morphological changes start with swelling of the root-hair tip, which occurs within 1 h of Nod-factor application (Heidstra *et al.*, 1994). This swelling is the result of isotropic growth, is accompanied by the formation of a calcium gradient at the plasma membrane and requires protein synthesis (Vijn *et al.*, 1995; De Ruijter *et al.*, 1998). At these swollen tips, new tip growth is initiated and the cyto-architecture of the resulting outgrowth shows a strong resemblance to that of normal growing root hairs. Such studies show that Nod factors can re-induce (tip) growth in root hairs. However, it remains unclear how Nod-factor secreting bacteria can redirect tip growth in such way that shepherd's crook-like curls are formed.

Furthermore, whether/how the bacteria exploit and modify this growth process for infection-thread formation remains to be resolved.

Nod-factor-induced growth responses in root hairs are preceded by rapid physiological changes. These involve a rapid influx of calcium into the hairs (Gehring *et al.*, 1997; Felle *et al.*, 1998). Shortly after this calcium flux, an opposite-directed flux of chloride ions occurs, which is accompanied by a depolarization of the root-hair membrane (Ehrhardt *et al.*, 1992; Felle *et al.*, 1998). These processes are followed by an alkalinization of 0.2–0.3 pH units of the root-hair cytoplasm (Felle *et al.*, 1996). Several minutes after the application of Nod factors, a regular oscillation of cytoplasmic calcium occurs around the nucleus (Ehrhardt *et al.*, 1996). Whether and how these physiological changes are involved in the alteration of growth of hairs is unknown.

Nod factors can mitotically activate clusters of cortical

Fig. 2. The major Nod factor produced by *Sinorhizobium meliloti* (top) and one of the *Rhizobium leguminosarum* bv *viciae* secreted factors (bottom). The major difference between both Nod factors concerns the specific decoration at the reducing terminal sugar unit and the structure of the acyl chain. The *S. meliloti* Nod factor contains four glucosamine units, an acyl chain of 16 C-atoms in length with two unsaturated bonds, an acetyl group at the non-reducing end and a sulfate group at the reducing terminal sugar residue (Lerouge *et al.*, 1990). In contrast, *R. leguminosarum* bv *viciae* produces a mixture of factors which contains several major compounds. The length of the glucosamine backbone is four or five units carrying an acyl chain of 18 C-atoms with either one or four unsaturated bonds. These Nod factors can be *O*-acetylated at the non-reducing terminal sugar residue (Spaink *et al.*, 1991). Pentameric Nod factors can be partially acetylated at their reducing terminal sugar residue (gray box) when the bacterium contains the *nodX* gene, whereas in the absence of this *nod* gene no substitution is present (Firmin *et al.*, 1993).

cells by which nodule primordia are formed. Which cortical cells will form a nodule primordium is determined by the host plant. Primordia are mainly formed opposite the protoxylem poles and furthermore, the host species determines whether inner or outer cortical cells are involved in primordium formation. When the nodule primordia are formed in the outer cortical cell layers, as in soybean (*Glycine max*), the infection thread grows through the root hair and can immediately invade the primordium. In contrast, in legumes, e.g. pea (*Pisum sativum*) and *Medicago* species, in which nodules are formed in the inner cortex, the infection thread has to cross several cortical cell layers before reaching the primordium. The cortical cells which will be traversed by an infection thread re-allocate their nuclei to their centre from where microtubules and cytoplasmic strands are positioned anticlinically to the advancing infection thread (Van Brussel *et al.*, 1992). By using cell-cycle phase-specific markers it has been shown that these cells have entered the cell cycle but become arrested in G₂, which indicates that the cytological structure found resembles a phragmoplast (Yang *et al.*, 1994). A radial array of cortical cells containing such phragmoplast-like structures provides a track for the infection thread to support its growth and

to guide it to the primordia. The formation of phragmoplast-like structures during infection shows that rhizobia recruit and modify a common process, namely cell division, and use this for a completely different purpose, the infection process.

When the infection thread reaches the primordium, the bacteria are released, and enter the cytoplasm via an invagination of the host-cell membrane. Within the host cytoplasm the bacteria remain surrounded by a host membrane, and together they form a so-called symbiosome that divides. In this way the bacterial surface is never in direct contact with the plant cytoplasm. Upon infection the nodule primordia simultaneously form a meristem as well as the different tissues that form a nodule. In most species nodules are macroscopically visible 4–7 days after inoculation. The meristems maintain their mitotic activity, at least during a substantial part of the lifetime of a nodule, and they add cells to the different nodule tissues by which the organ grows.

Host specificity

Nod-factor-controlled host specificity. An intriguing property of the *Rhizobium*–legume interaction is its host

specificity. Most rhizobia have a very narrow host range. For example, *S. meliloti* is able to interact with *Medicago*, e.g. alfalfa (*Medicago sativa*), *Trigonella* and *Melilotus* species, whereas *R. leguminosarum* bv *viciae* forms nodules on species of the genera *Pisum* (e.g. pea), *Vicia* (e.g. vetch), *Lathyrus* and *Lens*.

In most cases host specificity is controlled at several levels, but synthesis and the structure of Nod factors often play a prominent role. The rhizobial genes encoding the enzymes involved in the biosynthesis of Nod factors are activated when the bacteria grow in the vicinity of the root system of their host plants. There, rhizobia sense signal molecules, which are usually flavonoids, secreted by the host. These molecules activate the constitutively formed transcriptional regulator(s), NodD, that induces the expression of the other *nod* genes of which most are involved in the biosynthesis and secretion of Nod factors. The nature of the flavonoids secreted by the host can play a key role in controlling host specificity. For example, *Rhizobium etli* and *Rhizobium loti* secrete Nod factors with an identical structure, but their NodDs are only activated by elicitors secreted by their specific host plant. By introducing a gene encoding a constitutively active NodD protein these bacteria obtained a broader host range and could infect each other host plant (Cardenas *et al.*, 1995; Lopez-Lara *et al.*, 1995).

The length of the glucosamine backbone, the structure of the acyl chain and specific substitutions at the terminal sugar residues of the produced Nod factors can all contribute to the ability of the bacterium to nodulate their host plants. Changes in Nod-factor structure, either by *nod* gene mutations or introduction of *nod* genes of other *Rhizobium* species, mostly results in a decreased nodulation potential of the strain, but in some cases it obtains the ability to nodulate non-host plants. For example, mutating the sulfotransferase-encoding gene *nodH* of *S. meliloti*, which is involved in sulfation of the reducing sugar terminus of the Nod factor (Roche *et al.*, 1991), results in the inability of such strains to interact with alfalfa, but it has gained the ability to interact with vetch (Debellé *et al.*, 1988; Faucher *et al.*, 1989). Such experiments demonstrate that the plant discriminates the different *Rhizobium* species by recognizing their specific Nod factors. Some rhizobia have overcome this restriction by producing a great variety of Nod factors, e.g. *Rhizobium* sp. NGR234 (Price *et al.*, 1992), which can nodulate a broad host range encompassing legume species of >110 genera (Freiberg *et al.*, 1997) as well as the only non-legume known to establish a symbiosis with rhizobia, *Parasponia andersonii*.

Strict regulation of bacterial entry. Of all Nod-factor-controlled responses, bacterial entry appears to be the most stringently controlled. Infection-thread initiation/growth in the root epidermis will only occur efficiently when the rhizobia produce Nod factors with a specific structure, whereas other responses depend less on Nod-factor structure. For example, *S. meliloti* strains mutated in either *nodL*, leading to an absence of the *O*-acetylation of the non-reducing terminal sugar residue, or mutated in *nodFE*, leading to the absence of specific unsaturated bonds in the acyl chain, are both seriously hampered in the infection process, whereas other Nod-factor-induced plant responses are not affected (Ardourel *et al.*, 1994).

A host gene that is specifically involved in controlling infection is *SYM2* (Geurts *et al.*, 1997). *SYM2* was first identified in the wild pea ecotype Afghanistan (*SYM2^A*), where it inhibits nodulation of *R. leguminosarum* bv *viciae* strains lacking *nodX*. NodX *O*-acetylates pentameric *R. leguminosarum* bv *viciae* Nod factors at the reducing terminal sugar residue (Firmin *et al.*, 1993; Figure 2), which does not harbour a specific substitution in the absence of *nodX* (Spaink *et al.*, 1991). Thus, the activity of *SYM2* depends on the structure of the Nod factors secreted by the infecting rhizobia. However, the correlation between infection-thread formation on *SYM2^A* harbouring peas, and Nod-factor structure is not very strict since *nodZ* of *Bradyrhizobium japonicum*, which *O*-fucosylates the reducing sugar unit of pentameric Nod factors, can in part replace *nodX* (Ovtsyna *et al.*, 1998).

In the incompatible interaction of *SYM2^A*-harbouring peas with *R. leguminosarum* bv *viciae* strains secreting Nod factors which lack the proper substitution, infection threads are formed, but they become arrested in the root epidermis. However, occasionally the incompatible interaction results in a successful infection leading to a nodule (Geurts *et al.*, 1997). This suggests that incompatibility is not due to the inability to induce infection-thread formation, but rather it is due to a defect in bypassing a negative-acting mechanism controlling infection-thread growth. Compatible strains appear to have this ability by producing a specifically decorated Nod factor.

The regulation of infection-thread initiation/growth at different stages shows analogies with the regulation of pollen-tube growth in self-incompatible plants. After initiation of pollen-tube formation, the continuation of their growth is controlled by a self-incompatibility system. This mechanism is based on pollen–pistil recognition and aims to avoid self-fertilization of plants (for a review see Hiscock *et al.*, 1996). It seems probable that the incompatibility mechanism controlling infection-thread growth will also involve the recognition of epitopes at the infection-thread membrane. Since this incompatibility mechanism depends on Nod-factor structure it is possible that the host senses the structure of Nod factors secreted by the bacteria inside the infection thread when the infection thread grows in the epidermal cell. This model implies that infection-thread formation involves Nod-factor activity at two stages; the initiation of an infection thread as well as the suppression of the incompatibility response. Whereas the formation of infection threads is initiated by a relatively wide range of Nod factors, suppression of the incompatibility response requires a Nod factor with a more specific structure.

Alternatively, it is possible that Nod factors can induce the infection response at variable levels depending on the structure of the Nod factor. This would imply that the ability of the plant to block the growth of infection threads is lower when the infection response is higher.

Besides Nod factors, other components can also facilitate infection-thread growth. For several plants it has been shown that deficiencies in Nod-factor structure, by which infections are hampered, can be complemented, in part, by the rhizobial NodO protein (Economou *et al.*, 1994; Geurts *et al.*, 1997; Vlassak *et al.*, 1998). NodO is a secreted protein, which is not involved in Nod-factor production or secretion (Economou *et al.*, 1990). It has

been shown that NodO is able to bind calcium and it can integrate into artificial membranes where it forms ion channels (Economou *et al.*, 1990; Sutton *et al.*, 1994). Therefore, it has been postulated that it will form ion channels in the host plasma membrane as well, where it could contribute to the suppression of the incompatibility mechanism or the induction of the infection responses.

Other host proteins involved in the regulation of infection-thread growth are lectins. In the root, lectins are present in relatively low amounts and are localized on the external surface of elongated epidermal cells and on the tips of developing root hairs (Diaz *et al.*, 1995a; Van Rhijn *et al.*, 1998). Introduction of the pea lectin into white clover (*Trifolium repens*) showed to increase nodulation by its host strain *R. leguminosarum* bv *trifolii* (Diaz *et al.*, 1995b). Strikingly, expression of heterologous lectins also facilitates infection by non-host rhizobia, showing that lectins—in analogy to NodO—decrease the threshold level for the infection response (Diaz *et al.*, 1989, 1995b; Van Rhijn *et al.*, 1998). How this is achieved is not exactly known; however, data obtained with a lectin mutated in the carbohydrate-binding site shows that this protein is unable to extend the host range and can also no longer facilitate attachment of the bacterium to the root-hair surface (Van Rhijn *et al.*, 1998).

Endomycorrhizal symbiosis and common aspects of both interactions

AM formation

In nature, most plants do not only have roots; instead they have mycorrhizae, the symbiotic association of a fungus and plant roots. AM are by far the most common root endosymbiotic association, and are formed between the roots of most higher plants and fungi belonging to the order *Glomales*. AM fungi are obligate biotrophs and strictly dependent on their host plant for survival. As with the *Rhizobium*–legume interaction, this symbiosis is set in motion by the exchange of signals between the two symbionts, although the nature and the mechanism of action of these molecules are unknown. Exudates from a host root, especially (iso) flavonoids, enhance spore germination, and elongation and branching of hyphae (Nair *et al.*, 1991; Giovannetti *et al.*, 1993). At the root surface the hyphae form swollen structures, named appressoria. The formation of appressoria is initiated upon contacting the cell wall of a root epidermal cell. In contrast, appressoria are not formed when contacting cortical or vascular cell walls, indicating that the fungus recognizes specific epitopes present in the cell wall of root epidermal cells (Nagahashi and Douds, 1997).

The appressoria become firmly attached to the root epidermis and subsequently new hyphae develop which will enter the root. Depending on the host plant this can occur either intercellularly or intracellularly. Since AM involving intercellular infection, the Arum type, is found predominantly in cultivated herbs, it has become more frequently studied than the AM involving intracellular infection, the Parish type (for a review see Smith and Smith, 1997). Therefore, in this review we will focus on the Arum-type interaction in which the fungus enters the root between two epidermal cells. The plant accommodates the invasion of the fungus by secreting new cell wall

material which surrounds the infecting hyphae. In the inner cortex, the fungus invades cells and there they differentiate into highly ramified structures, the arbuscules (Figure 1). These structures are thought to facilitate the exchange of nutrients between both organisms.

Although arbuscules occur intracellularly, they are never in direct contact with the cell cytoplasm. A perifungal membrane, originating from the plant plasma membrane invaginates and surrounds the arbuscules. During the formation of arbuscules, the plant cell becomes cytoplasmically dense, its vacuole fragments, and the number of Golgi bodies increases. Furthermore, the nucleus moves to a more central position in the cell (Balestrini *et al.*, 1992).

When the mycorrhizal fungi colonize the roots and appressoria have been formed, the fungus rapidly enters the cortex. Upon entry the root arbuscules are formed within a few days (Albrecht *et al.*, 1998). Arbuscules have a similar morphology as haustoria; the feeding structures which are formed by several pathogenic fungi during a compatible interaction. During both haustorium and arbuscle formation, plant defense responses are induced, but only at a low level. For these reasons it seems probable that haustorium and arbuscle formation involve similar mechanisms.

In contrast to the *Rhizobium*–legume interaction, there is very little host specificity in AM symbioses. A fungus can interact with a diverse range of host plant species, whereas a certain host plant can interact with several fungal species. However, several plant families can be considered as ‘non-mycorrhizal’ or ‘rarely mycorrhizal’, e.g. the *Brassicaceae*.

Common genes are involved in mycorrhizae and nodule formation

The morphological responses that take place in the epidermal and cortical cells when roots become infected by rhizobia or AM fungi, respectively, seem at first sight to involve unrelated processes. However, molecular and genetic studies show that the infection processes are strikingly similar. Several genes have been identified which are induced during both symbiotic interactions, e.g. the early nodulin genes *ENOD2*, *ENOD40* (Van Rhijn *et al.*, 1997), *ENOD5*, *ENOD12* (Albrecht *et al.*, 1998), the leghemoglobin gene *VFLb29* (Frühling *et al.*, 1997) and the aquaporin encoding gene *NOD26* (Wyss *et al.*, 1990). However, the most convincing evidence that the infection processes used by both microsymbionts involve common steps came from studies with legume mutants which have lost the ability to form nodules. A large proportion of the nodulation-resistant mutants were also completely resistant to AM fungi, while their interaction with soil pathogens was not affected (for reviews: Gianinazzi-Pearson, 1996; Harrison, 1997). In pea, four genes have been identified that are essential for early steps of both the rhizobial and mycorrhizal interactions. Mutants of three of these genes, *sym8*, *sym9* and *sym19*, have been studied in more detail at a cytological level. These mutants are unable to form an infection thread (LaRue and Weeden, 1994) and although AM fungi still can form appressoria on these Nod[−]/Myc[−] mutants, they fail to develop intercellular hyphae (Gianinazzi-Pearson, 1996). This shows that

rhizobial and mycorrhizal infection involves common mechanisms.

Do signals from AM fungi and rhizobial Nod factors activate common signal-transduction pathways?

The availability of marker genes which are activated during both symbiotic interactions, as well as host mutants blocked in the infection by either microsymbiote, have provided the means to study whether the induction of the infection-related genes involve common mechanisms. Here we will focus on two early nodulin genes, *ENOD12* and *ENOD40*.

***ENOD12*.** *ENOD12* is the best studied marker gene for early rhizobial Nod-factor-induced responses. The gene is induced in cells involved in, or getting prepared for infection by rhizobia (Scheres *et al.*, 1990; Journet *et al.*, 1994). Using a spot-inoculation assay it was shown that in the AM interaction *ENOD12* also is activated when the fungus infects the roots (Albrecht *et al.*, 1998). Since the encoded protein might be a cell wall component, it could be part of the matrix secreted by the host that surrounds the microsymbionts.

Studies with transgenic *Medicago* plants, carrying the promoter of the *ENOD12* gene in front of the β -glucuronidase reporter gene (*GUS*), it was shown that the *ENOD12*-inducing activity of Nod factors can be mimicked by mastoparan, a compound thought to activate G proteins (Pingret *et al.*, 1998). Furthermore, studies using various putative phospholipase C (PLC) antagonists suggest that inositol phosphate signalling plays a role. Whether inositol phosphate signalling plays a role in *ENOD12* induction by AM fungi is unknown. However, *ENOD12* is neither induced by Nod factors nor by mycorrhiza in the root epidermis of a pea *sym8* mutant (Albrecht *et al.*, 1998). Therefore, it is probable that the signal-transduction cascades leading to *ENOD12* expression, which are activated by rhizobial Nod factors and mycorrhiza, at least have *SYM8* in common.

***ENOD40*.** Like *ENOD12*, the early nodulin gene *ENOD40* also is activated by both *Rhizobium* and AM fungi (Van Rhijn *et al.*, 1997). In the *Rhizobium* interaction this gene is first expressed in pericycle cells opposite the protoxylem poles within a few hours of Nod-factor application and markedly before the first cell divisions occur in the root cortex (W.C.Yang and T.Bisseling, unpublished results). Later, when cell divisions are induced, *ENOD40* is also expressed in the dividing cells (Kouchi and Hata, 1993; Yang *et al.*, 1993). Strikingly, the expression in the pericycle opposite xylem poles, is complementary to the expression pattern of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, the gene encoding the enzyme catalysing the last step of the biosynthesis of ethylene (Figure 3). Since ethylene is an inhibitor of cortical cell division, the localized expression of ACC synthase contributes to the positioning of nodule primordia (Heidstra *et al.*, 1997). Overexpression of *ENOD40* as well as ballistic introduction of an *ENOD40*-expression construct induces cell divisions in the root inner cortex (Charon *et al.*, 1997). Hence the local induction of *ENOD40* expression in the region of the

Fig. 3. (A) Accumulation of ACC oxidase mRNA in the regions of the pericycle opposite the phloem poles of an uninoculated pea root, visualized by *in situ* hybridization with a digoxigenin (DIG)-labelled ACC oxidase antisense RNA (Heidstra *et al.*, 1997). (B) Induction of *ENOD40* in the pericycle of the root opposite protoxylem poles by *Rhizobium leguminosarum* bv *viciae* Nod factor 2 days after application (Vijn *et al.*, 1993). *ENOD40* mRNA is visualized by *in situ* hybridization with ^{35}S -labelled *ENOD40* antisense RNA (dark field). The protoxylem poles are marked by arrow heads.

pericycle opposite the phloem poles could provide additional positional information for nodule primordium formation.

ENOD40 has been isolated from several legumes as well as from some non-legume species. All these *ENOD40* genes contain two regions which are highly conserved. However, only the 5' located box 1 contains a short conserved open reading frame encoding a small peptide of 10–13 amino acids. Ballistic introduction in *Medicago varia* roots of a DNA construct encoding this small peptide is sufficient to induce cortical cell divisions (Charon *et al.*, 1997), but experiments which showed that this small peptide conferred tolerance to high auxin to tobacco protoplasts are under debate (Van de Sande *et al.*, 1996; Schell *et al.*, 1998).

The question remains how *ENOD40* is induced by the AM fungus. Since the gene can also be activated by cytokinin (Minami *et al.*, 1996) and it has been shown that AM fungi produce cytokinin, it can be postulated that

this hormone induces *ENOD40* in the AM interaction (Van Rhijn *et al.*, 1997). Also, it has been proposed that Nod factors cause an increase in the level of cytokinin in root tissues (Cooper and Long, 1994), and this could explain why *ENOD40* is induced by both microsymbionts (van Rhijn *et al.*, 1997). However, it seems probable that in addition to cytokinin, at least one other molecule in the *ENOD40*-activating cascade is in common. Nod factors can not induce *ENOD40* in the alfalfa Nod⁻/Myc⁻ mutant MN NN1008, whereas cytokinin is able to do so (Bradbury *et al.*, 1991; W.C. Yang and T. Bisseling unpublished). This suggests that MN NN1008 is mutated in a gene that is active upstream of cytokinin. Furthermore, it is blocked in Nod-factor-activated calcium spiking which is induced within minutes after Nod-factor application (Ehrhardt *et al.*, 1996). This suggests that the mutated gene is probably involved in an early step of the activated signalling cascades.

Conclusion

The above reviewed studies have revealed that common host genes are involved in both the rhizobial and mycorrhizal interactions. This finding has an important implication, since in contrast to *Rhizobium*, AM fungi have the ability to interact with a wide range of higher plants. Assuming that the mechanisms by which AM fungi infect their various hosts are similar, it implies that *SYM* and *ENOD* genes, required for the interaction of legumes with both micro-symbionts, are most probably widespread in the plant kingdom. Present studies with transgenic rice (*Oryza sativa*) are consistent with this idea (Reddy *et al.*, 1998). These studies show that a *Medicago ENOD12* promoter in transgenic rice can be activated by rhizobial Nod factors, demonstrating that a signal-transduction cascade involved in the activation of this leguminous promoter is present in rice. In legumes *SYM8* is essential for the induction of *ENOD12* either by rhizobial Nod factors or AM fungi. Therefore, it is likely that this gene will be present in non-legumes (e.g. rice) as well. Although, with the exception of *Parasponia andersonii*, non-leguminous plants are unable to establish a symbiosis with *Rhizobium*, they seem to harbour a perception mechanism by which Nod factors can be recognized. Obviously this perception mechanism is not maintained by non-legumes to recognize rhizobial Nod factors. The natural ligands for this perception mechanism are unknown. However, since its activation leads to *ENOD12* transcription it is worthwhile to study whether molecules of AM fungi are natural ligands. Although the function of the non-leguminous perception mechanism is not clear, it seems probable that it has a widespread occurrence, and that the Nod factor perception mechanism of legumes has evolved from it.

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