Chapter 10

COMPARISON BETWEEN ACTINORHIZAL AND LEGUME SYMBIOSIS

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1. INTRODUCTION

Two types of root-nodule symbioses exist between higher plants and N_2-fixing soil bacteria; these are the legume-rhizobia and actinorhizal symbioses. In both interactions, bacteria induce the formation of special plant organs, nodules, on the plant roots. Bacteria fix N_2 while being hosted within plant cells and are provided with carbon sources by the plant hosts. In the case of rhizobial symbioses, a diverse group of eleven genera of Gram-negative unicellular soil bacteria (*Rhizobium, Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, Sinorhizobium, Methylobacterium, Blastobacter, Devosia, Burkholderia and Ralstonia;* Chen *et al.,* 2001; Moulin *et al.,* 2001; Rivas *et al.,* 2002; van Berkum *et al.,* 2002*;* Vandamme *et al.,* 2002; Young *et al.,* 2001) induce nodules on the roots of legumes and one non-legume, *Parasponia* sp. (Ulmaceae). In the case of actinorhizal symbioses, Gram-positive actinomycetous soil bacteria of the genus *Frankia* induce nodules on the roots of dicotyledonous plants from eight different families, mostly trees or woody shrubs. Legume nodule primordia are formed in the root cortex and develop into stem-like organs with a peripheral vascular system and infected cells in the central tissue. In contrast, actinorhizal nodule primordia are formed in the root pericycle, like lateral root primordia. Mature actinorhizal nodules are coralloid organs composed of multiple lobes, each of which represents a modified lateral root without root cap, a superficial periderm, and infected cells in the expanded cortex.

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Interestingly, nodules of the only non-legume (*Parasponia*) that is able to enter a rhizobial symbiosis structurally and developmentally resemble actinorhizal nodules (Lancelle and Torrey, 1984; 1985).

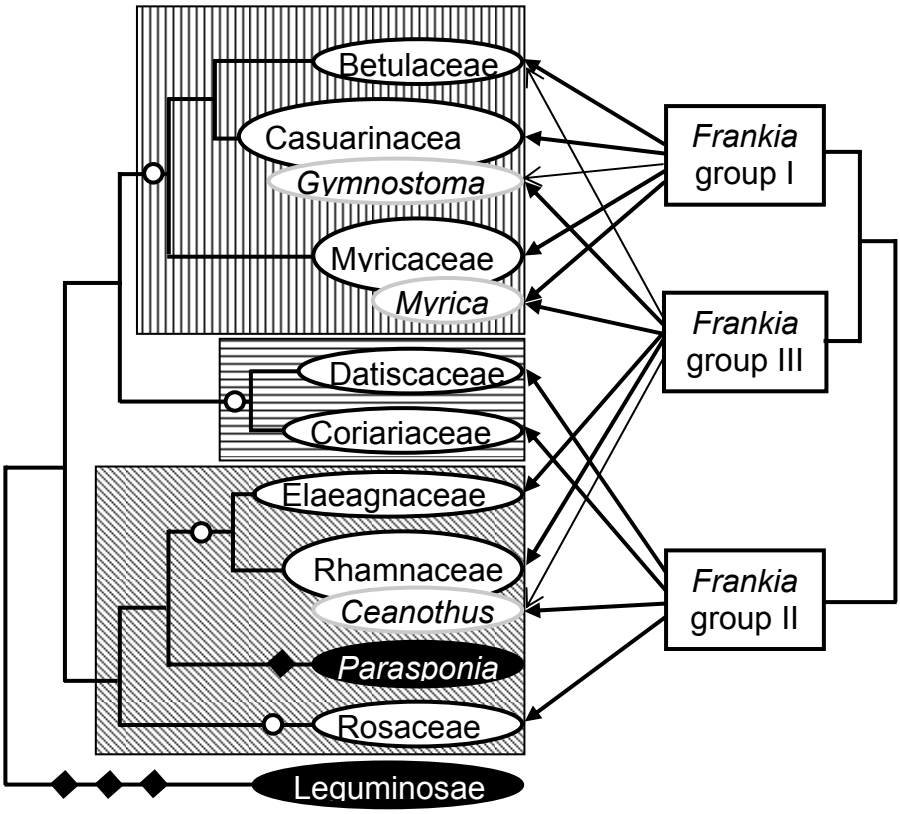

Figure 1: Simplified scheme of the phylogenetic relationship between actinorhizal plants as explained by Swensen and Benson (this volume) and Benson and Clawson (2000). Groups of plants infected by rhizobia are labeled by inverse print. Boxes indicate the three main groups of actinorhizal plants: higher Hamamelidae (vertical stripes); Cucurbitales (horizontal stripes); and Rosales (diagonal stripes). The phylogenetic relationship between the three clades of symbiotic Frankia *is included. Some actinorhizal genera (*Gymnostoma, Myrica, Ceanothus*) that differ in microsymbiont specificity from the rest of the family are indicated. Thick arrows connect* Frankia *clades with the members and plant groups that the clades are commonly associated with. Thin arrows indicate that members of that clade have been either isolated from or detected in an effective or ineffective nodule of a member of the plant group at least once. Host specificity exists within the* Frankia *clades,* i.e., *not all members of a* Frankia *clade can nodulate all plants associated with that clade. Circles indicate the four putative origins of the ability to enter into an actinorhizal symbiosis (Swensen, 1996); black rhombs indicate the four putative origins of the ability to enter a symbiosis with rhizobia (Doyle, 1998).*

Evolutionary analysis indicates that N_2-fixing root-nodule symbioses evolved 50-100 million years ago (Kistner and Parniske, 2002). As a comparison, plant symbioses with arbuscular mycorrhizal fungi are much older and fossil evidence dates them back for at least 400 million years (Remy *et al.,* 1994). Thus, mechanisms that were evolved for the fungal symbioses may have been exploited in the evolution of N_2-fixing root-nodule symbioses, which will be discussed later. Phylogenetic analysis has shown that all plants able to enter a root-nodule symbiosis belong to a single clade (Rosid I; see Figure 1), *i.e.*, they go back to a common ancestor (Soltis *et al.,* 1995). Within the Rosid I clade, rhizobial symbioses are proposed to have evolved four times independently, namely three times within the legume family and once for *Parasponia* (Doyle, 1998). Similarly, four independent origins have been hypothesized for actinorhizal symbioses (Swensen, 1996; Figure 1), although another proposal suggests that present-day actinorhizal symbioses go back to an ancestral symbiosis that emerged before the divergence of extant actinorhizal plants (Clawson *et al.,* 2004). At any rate, the phylogenetic data suggest that the common ancestor of the Rosid I clade had acquired a property upon which a root-nodule symbiosis could develop.

2. NODULE STRUCTURE

2.1. Legume Nodules

Two types of legume nodules are known, determinate and indeterminate (see Figure 2). The type of nodule depends on the host plant (Trinick and Galbraith, 1980). Indeterminate nodules have an apical meristem, the activity of which leads to the formation of a developmental gradient in the inner tissue. Close to the meristem, cells are infected by infection threads. More basal cells internalize rhizobia in an endocytosis-like process, surrounding them with a peribacteroid membrane (PBM) that is derived from the plant plasma membrane. Internalized rhizobia along with their PBMs are called symbiosomes. Within symbiosomes, rhizobia differentiate into bacteroids, express nitrogenase genes, and begin to fix N_2. In determinate nodules, the meristem stops its activity early in nodule development, and new infected cells mostly do not arise by infection, but by division of infected cells (Newcomb, 1981; Rolfe and Shine, 1984). Indeterminate nodules can be unbranched (caesalpinoid type) or lobed (mucunoid and crotalarioid type; Corby 1988). There are two types of determinate nodules, the desmodioid type, which occurs in the Phaseoleae and Loteae, and the aeschynomenoid type (Doyle, 1998; Sprent, 1995; 2001). The determinate nodules of *Sesbania rostrata*, ostensibly of the aeschynomenoid type, show some phenotypic plasticity in that they retain an inactive meristem that can be reactivated by ethylene leading to indeterminate growth (Fernandez-Lopez *et al.,* 1998). Phylogenetic analysis and the distribution of primitive features, like persistent infection threads, suggest that indeterminate caesalpinioid nodules represent the basal form and that determinate nodules are a phenomenon which evolved independently at different points during legume evolution (Sprent, 2001).

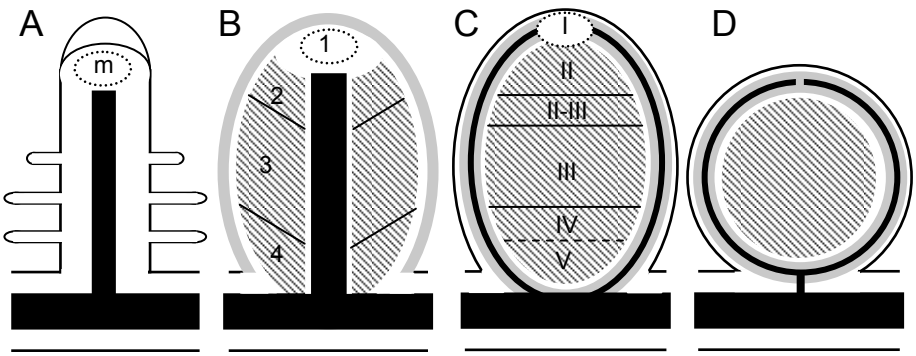

Figure 2: Comparison between lateral root (A), actinorhizal nodule lobe (B), indeterminate legume nodule (C), and determinate legume nodule (D). The vascular system is given in black. The actinorhizal nodule lobe is surrounded by a periderm (dark grey). The vascular system of legume nodules (C, D) is embedded in the nodule parenchyma that forms a turgor-controlled O_2-diffusion barrier. (A), (B), and (C) are indeterminate organs with an apical meristem (m, 1, I), the activity of which leads to the formation of a developmental gradient of infected cells in the tissue containing the infected cells (hatched). Zone of infection (2, II); interzone (II-III); zone of nitrogen fixation (3, III); and zone of senescence (4, IV) (Vasse et al., *1990; Ribeiro* et al., *1995). A zone of saprophytic growth (V) is found so far only in alfalfa nodules, where bacteria are released from remaining infection threads, re-invade senescent plant cells, and live saprophytically in a unique ecological niche (Timmers* et al., *2000).*

In some tropical legumes that form caesalpinioid nodules, *e.g.*, *Andira* and *Chamaecrista*, as well as in *Parasponia* sp., rhizobia are not internalized in infected cells in an endocytosis-like process, but they develop into their N_2-fixing form within branching infection threads (reviewed by Sprent, 2001). On the other hand, there are some aeschynomenoid nodules where no infection threads are formed at any point, but where rhizobia colonize the root cortex intercellularly and are taken up into nodule primordium cells from the apoplast (for *Arachis*, see Chandler 1978; and for *Stylosanthes,* see Chandler *et al.,* 1982).

2.1.1. Oxygen-diffusion Pathways

Nitrogenase is very sensitive to O_2, but the nitrogen-fixation process requires large amounts of ATP that have to be obtained by respiration. This situation leads to the so-called oxygen dilemma of nitrogen fixation - nitrogenase has to be protected from O_2, but O_2 has to be brought to the sites of respiration. In principle, because O_2 diffusion is far more rapid in air than in water, several different mechanisms are available to help solve this dilemma. These include: (a) restriction of O_2 diffusion by cell layers devoid of intercellular spaces, which could be achieved either by occlusion of the intercellular spaces or by turgor control; (b) metabolic protection, *i.e.*, the rapid removal of O_2 by highly active respiratory processes; and (c) the presence of a high-affinity O_2-binding protein that transports O_2 to the sites of respiration and not those of nitrogen fixation. In legumes as well as *Parasponia*,

infected cells contain large amounts of nodule-specific O_2-transport proteins, the leghemoglobins (Appleby, 1984; Appleby *et al.,* 1983). Furthermore, an O_2-diffusion barrier, the so-called nodule endodermis, surrounds the inner tissue (reviewed by Minchin, 1997). In legumes, this is possible because the vascular system is located in the periphery of the nodule, so the nodule endodermis can protect the infected cells without restricting the access of O_2 to the vascular system where it is needed to generate energy (ATP) for transport processes. In *Parasponia* nodules, however, with their actinorhizal-like morphology, this problem seems to be solved by having infected cells form two separate regions in the cortex, each of which is surrounded by its own O_2-diffusion barrier. Between these two areas, O_2 can pass to the central vascular system (see chapter 5 in this volume; Tjepkema and Cartica, 1982).

2.2. Actinorhizal Nodules

As described above, actinorhizal nodules are composed of multiple lobes each of which represents a modified lateral root. Due to the activity of the apical meristem, the infected cells in the expanded cortex are arranged in a developmental gradient (see Figure 2). In the infection zone, they become gradually filled with branching *Frankia* hyphae. In the nitrogen-fixation zone, *Frankia* vesicles have developed and bacterial nitrogen fixation takes place. In the zone of senescence, bacterial material is degraded by the plant. In most actinorhizal nodules, infected and uninfected cells are interspersed in the cortex of the nodule lobe. In the actinorhizal Cucurbitales, however, the infected cells form a continuous patch, kidney-shaped in cross-section, at one side of the acentric stele (Hafeez *et al.,* 1984; Newcomb and Pankhurst, 1982; see chapter 5 in this volume).

2.2.1. Oxygen-diffusion Pathways and Oxygen Protection in Actinorhizal Nodules

In contrast to most legume nodules, actinorhizal nodule lobes are surrounded by a superficial periderm that can be more or less impermeable to gas. To provide the nodules with O_2 as required to produce energy for nitrogen fixation, the nodule periderm may be disrupted by lenticels (as in *Alnus, Datisca,* and *Coriaria*) or nodule roots can be formed (as in Casuarinaceae, Myricaceae, and *Datisca*). Lenticels are also found on the surface of desmodioid legume nodules (Sprent, 2001). Nodule roots are agraviotropically growing roots with reduced root caps, without root hairs, and with large air spaces in the cortex that are formed at the tip of nodule lobes, *i.e.*, by a change in the activity of the nodule lobe meristem (Torrey, 1976). Subsequently, new nodule lobe meristems can be induced next to the origin of the nodule root. Nodule roots provide access to O_2 for nodules formed on roots of plants grown in wetlands, and their length depends on the pO_2 level with the length increasing with decreased pO_2 (Silvester *et al.,* 1988; Sprent and Scott, 1979). *Datisca glomerata* can form either nodule roots (in liquid culture) or lenticels (in soil).

Diverse protection systems for the O_2-sensitive process of bacterial nitrogen fixation have developed in different actinorhizal plant genera (see chapter 5 in this

volume). The morphology of actinorhizal nodule lobes does not allow for a legume-like O_2-diffusion control system, except for *Coriaria* nodules. These nodules have developed a comparable system, which uses a long lenticel at the non-infected side of the nodule lobe, with presumably turgor-based control in the cell layers between stele and periderm to regulate the access of O_2 to the infected cells (Silvester and Harris, 1989). It is likely to be due to this system that *Coriaria* is the actinorhizal plant, which shows the fastest adaptation to changes in external pO_2 as determined by nitrogen-fixation rates (Silvester and Harris, 1989).

Frankia can contribute to O_2 protection by forming vesicles. Furthermore, *Frankia* vesicles are sites of major respiratory activity, thereby achieving the metabolic removal of O_2 (Vikman, 1992). However, the differences in vesicle morphology among actinorhizal plants make it unlikely that *Frankia* vesicles contribute to O_2 protection in all cases. For instance, in *Casuarina* nodules, *Frankia* does not form vesicles (Berg and McDowell, 1987) and the plant seems to be solely responsible for O_2 protection of nitrogenase, which involves the production of hemoglobin in infected cells as is the case with legume nodules. Recently, bacteriohemoglobins have been discovered in *Frankia* and these may be involved in the protection of nitrogenase as well (Beckwith *et al.,* 2002).

In summary, O_2-protection mechanisms in actinorhizal nodules are diverse and may involve both the host and microsymbiont in exerting metabolic control to decrease O_2 levels, in physically restricting O_2 diffusion, and/or in regulating O_2 levels by the presence of hemoglobin.

3. NODULE-INDUCTION MECHANISMS

In both legume and actinorhizal symbioses, infection can take place either intra-cellularly *via* root hairs or inter-cellularly *via* penetration of the root epidermis and bacterial colonization of the root cortex. The pathway by which the bacteria enter the plant depends on the host-plant species. Mechanisms employed in both symbioses are summarized in Figure 3.

3.1. Legume Nodules

Components of the plant-root exudate induce the expression of the rhizobial *nod* genes, the products of which catalyse the production and export of rhizobial signal molecules, lipochitooligosaccharides, the so-called Nod factors (reviewed by Pueppke, 1996). Nod factors are essential for both inter- and intra-cellular infection. In most legumes examined, rhizobia enter the plant intra-cellularly *via* root hairs. However, different inter-cellular infection mechanisms have been characterized for some tropical legumes, most of them woody. In some cases, intermediates exist, *i.e.*, plants where both intra-cellular and inter-cellular infection is possible depending on the growth conditions (Subba-Rao *et al.,* 1995). During inter-cellular infection, rhizobia exploit gaps in the epidermis to enter the root; they cannot penetrate the epidermis directly.

During intracellular infection, Nod factors induce the deformation of root hairs in the root-hair extension zone by inducing the blockage and re-initiation of root-hair growth (Heidstra *et al.,* 1994). Nod factors also induce both the formation of pre-infection thread structures (PITs) in cortical cells, and cell divisions in the inner cortex (in plants forming indeterminate nodules) or in the outer cortex (in plants forming determinate nodules). When a rhizobium is trapped in a root-hair curl, local hydrolysis of the cell wall takes place (van Spronsen *et al.,* 1994), the plasma membrane invaginates, and new cell-wall material is deposited, leading to the formation of a tubular structure, called the infection thread (for reviews, see Brewin, 1991; Kijne, 1992; Newcomb, 1981). The ultrastructure of the infection thread wall is similar to that of the cell wall. Within the infection thread, rhizobia enter the plant root while still embedded in the infection-thread matrix that seems to consist of plant and bacterial compounds.

Concomitant with infection-thread formation, cortical cells are mitotically reactivated, leading to the formation of the nodule primordium. Infection threads grow toward primordium cells and release rhizobia into their cytoplasm. In plants that form indeterminate nodules, primordia are formed in the inner cortex and infection threads have to traverse outer cortical cells to reach them. Infection threads follow a tip-growth mechanism that requires the polarization of the cytoplasm. Therefore, cortical cells undergo morphological changes to support infection-thread growth and rearrange their cytoplasm to form a radially oriented conical structure, the cytoplasmic bridge that resembles a preprophase band, the so-called pre-infection thread (PIT; Kijne, 1992; van Brussel *et al.,* 1992). PIT formation can be induced by Nod factors alone, but infection thread formation, *i.e*., invagination of the root-hair plasma membrane and synthesis of the infection-thread wall and matrix, occurs only in presence of rhizobia. PITs, but no infection threads, are formed in response to rhizobial lipopolysaccharide mutants (Spaink, 2000). PIT-forming cells re-enter the cell cycle and most likely become arrested in the G2 phase, *i.e*., they undergo DNA re-duplication but get re-arrested before mitosis (Yang *et al.,* 1994).

During inter-cellular infection, bacteria enter the roots *via* gaps in the root epidermis, *e.g*., through the cracks at the junctions of emerging lateral or adventitious roots (for *Arachis*, see Chandler, 1978; Boogerd and van Rossum, 1997; for *Stylosanthes,* see Chandler *et al.,* 1982; for *Aeschynomene*, see Alazard and Duhoux, 1990; for *Neptunia*, see James *et al.,* 1992; and for *Sesbania rostrata*, see Ndoye *et al.,* 1994). For *Lotus uliginosus*, infection occurs *via* enlarged epidermal cells (James and Sprent, 1999), whereas with *Mimosa,* rhizobia enter directly between epidermal cells (de Faria *et al.,* 1988). Concomitantly, cell divisions, which lead to the development of a nodule primordium, are induced in the root cortex. In many cases, once rhizobia begin colonizing the apoplast, infection-thread formation can be induced within the root cortex (James *et al.,* 1992; James and Sprent, 1999; Ndoye *et al.,* 1994; Schaede *et al.,* 1940; Subba-Rao *et al.,* 1995). The infection of nodule primordium cells can occur *via* infection threads; rhizobia are released into the cytoplasm from an unwalled portion of the infection thread and surrounded by PBMs. In those cases where no infection thread formation takes place at all, inter-cellular rhizobia move through the cortex by abortive infection

and the resulting collapse of cortical cells, leading to the formation of infection strands (in *Stylosanthes*, see Chandler *et al.,* 1982; in *Mimosa*, see de Faria *et al.,* 1988; in *Aeschynomene*, see Alazard and Duhoux, 1990). They enter primordium cells through a structurally altered, probably partly degraded, part of the cell wall and become surrounded by PBMs (for *Arachis*, see Chandler, 1978; for *Aeschynomene*, see Alazard and Duhoux, 1990; for *Stylosanthes*, see Chandler *et al.,* 1982; for *Chamaecytisus*, see Vega-Hernández *et al.,* 2001).

Parasponia is also infected inter-cellularly. Here, the colonization of the root surface by rhizobia leads to the induction of cortical-cell divisions and the formation of a so-called prenodule, the presence of which leads to ruptures in the root epidermis (Bender *et al.,* 1987). Rhizobia enter the root cortex through these ruptures and colonize it inter-cellularly. This process is similar to the infection of the leguminous tree *Chamaecytisus* (Vega-Hernández *et al.,* 2001), where an abortive intra-cellular infection *via* root hairs leads to the formation of a nodule primordium. This, in turn, causes ruptures in the root epidermis through which rhizobia enter the root cortex and colonize it inter-cellularly. In *Chamaecytisus*, infection threads are not formed, but rhizobia move through the cortex by the collapse of cortical cells and are taken up directly into nodule primordium cells (Vega-Hernández *et al.,* 2001). In contrast, in *Parasponia*, infection threads are formed within the root at a later stage. They start from inter-cellular rhizobial colonies, when the formation of the nodule primordium has been induced in the root pericycle (Bender *et al.,* 1987; Lancelle and Torrey, 1984; 1985).

3.2. Actinorhizal Nodules

Like rhizobia, *Frankia* strains can enter the roots of their host plants either intra-cellularly *via* root hairs or inter-cellularly, and the mode of infection depends on the host plant species. Intra-cellular infection takes place in the higher Hamamelidae, *i.e.*, in plants of the families Betulaceae, Casuarinaceae and Myricaceae (Figure 1). The infection mechanisms are described in detail in chapter 6 of this volume, so they are only briefly summarized here (Figure 3).

Intra-cellular infection is similar to the corresponding process described for rhizobia. *Frankia* culture supernatants contain a factor of unknown chemical nature that induces the deformation of root hairs. When a hypha is trapped in a root hair curl, an infection thread-like structure ("encapsulation") develops by which the hypha enters the plant root. Within this tubular ingrowth, which grows by cell-to-cell passage like infection threads in legumes, the hypha is embedded in a cell wall-like matrix, the equivalent of the infection thread wall. There is no equivalent of the legume infection thread matrix in actinorhizal symbioses.

Concomitantly, cell divisions are induced in the root cortex. The infection thread-like structure grows to the dividing cortical cells and infects some of them by intense branching within the cells, filling them with *Frankia* hyphae from the inside outward. Once the cell is filled with branched hyphae in infection thread-like structures, vesicles develop and nitrogen fixation starts. This cortex-based structure is called the prenodule. While the prenodule develops, the formation of the nodule

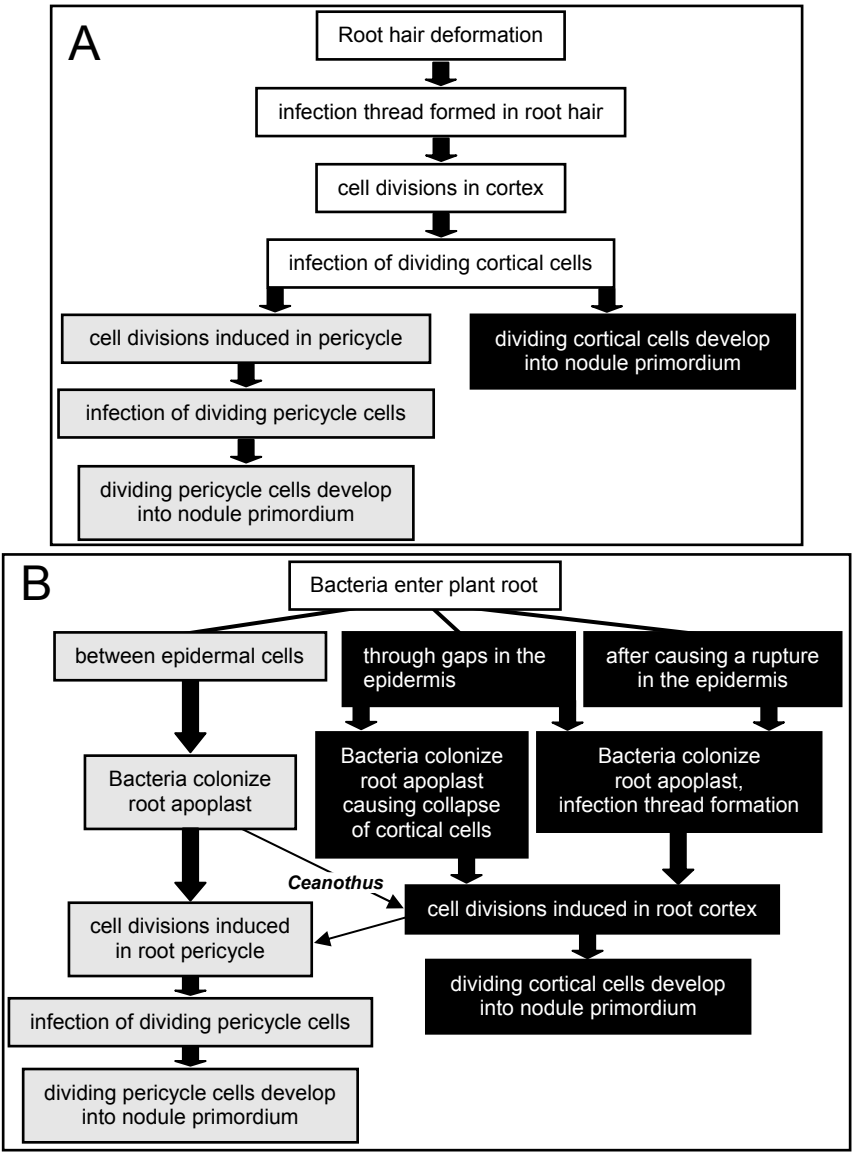

Figure 3. Comparison of nodule-induction mechanisms in actinorhizal plants and legumes. (A) Intracellular infection (higher Hamamelidae and legumes).(B) Intercellular infection (Rosales, Cucurbitales, legumes). Gray background indicates mechanisms specific for actinorhizal plants; black background indicates mechanisms specific for legumes; and white background indicates mechanisms present in both groups.

primordium is induced in the pericycle of the root vascular system, like in the case of lateral root primordia. Hyphae in infection thread-like structures ("encapsulated

hyphae") grow from the prenodule to the nodule primordium and infect primordium cells. The nodule primordium develops into a nodule lobe. Depending on the host-plant species, more than one nodule primordium can be formed per prenodule (Torrey and Callaham, 1979).

During inter-cellular infection, which has been described for actinorhizal Rosales, *i.e.,* Rhamnaceae, Elaeagnaceae and Rosaceae (Berry and Sunell, 1990; Miller and Baker, 1986; Racette and Torrey, 1989), *Frankia* hyphae enter the root by penetration between epidermal cells and colonize the root cortex inter-cellularly. In contrast to rhizobia, *Frankia* does not depend on gaps in the root epidermis. During the colonization of the cortex, the root cortical cells secrete an electron-dense protein-rich material into the intercellular spaces (Liu and Berry, 1991a; 1991b), and the formation of a nodule primordium is induced in the root pericycle. *Frankia* hyphae infect primordium cells from the apoplast by intense branching of invading hyphae, concomitant with continuous invagination of the plant plasma membrane and the synthesis of a cell wall-like matrix that embeds the invading hyphae. Once the host cell is filled with hyphae, vesicles develop and nitrogen fixation starts, as is the case during intra-cellular infection. No prenodules are formed during inter-cellular infection. However, in one inter-cellularly infected species, *Ceanothus griseus* (Rhamnaceae), cortical cell divisions are induced during bacterial colonization of the root cortex, but the dividing cells are not infected by *Frankia* (Berry and Sunell, 1990; Liu and Berry, 1991a; see Figure 3).

In host plants of the actinorhizal Cucurbitales (*Datisca* and *Coriaria*), the infection mechanism has not been examined in detail yet but, because no prenodules or infection thread-like structures are found in these plants, infection is assumed to follow the inter-cellular pathway. However, in contrast to all other host plants examined, infected cells of *Datisca* and *Coriaria* nodules are filled with branching *Frankia* hyphae from the periphery inward, instead of from the center outward, and they retain large central vacuoles (Mirza *et al.,* 1994; Newcomb and Pankhurst, 1982). Another unique feature of actinorhizal Cucurbitales is that their nodule cortical cells become multinucleate prior to infection.

4. HOST SPECIFICITY

In contrast to rhizobia, *Frankia* can fix N_2 in the free-living state, and hence could be expected to be less dependent in their distribution on their macrosymbionts than are rhizobia. *Frankia* has been detected in soils that had been devoid of actinorhizal plants for decades (Normand and Lalonde, 1982; Maunuksela *et al.,* 1999), but rarely in soils outside the normal area of distribution of actinorhizal plants (Huss-Danell *et al.,* 1999, but see chapter 8 in this volume). Although several rhizobial strains depend on their host plants for persistence (Woomer *et al.,* 1988), others are better adapted to saprophytic growth (Hirsch, 1996). Evidence suggests that non-symbiotic rhizobia persist in soils in the absence of legumes and can acquire symbiotic genes from inoculant strains upon introduction of host legumes (Sullivan *et al.,* 1995; 1996; Sullivan and Ronson, 1998). However, for several actinorhizal genera (*Datisca, Coriaria, Ceanothus* and those within the Rosaceae), it has not yet

been possible to isolate the microsymbiont (Swensen and Benson, this volume), which raises the suspicion that these *Frankia* strains might represent obligate symbionts.

4.1. Signal Exchange during Legume-nodule Induction

Flavonoids excreted by plant roots activate the rhizobial transcription factor NodD, which activates the transcription of the *nod/nol/noe* genes, the products of which produce specific lipochitooligosaccharides, the Nod factors (reviewed by Carlson *et al.,* 1994). Various substitutions at the terminal chitin monomers, as well as variations in the acyl group at the non-reducing end, lead to a high degree of structural variability among Nod factors (reviewed by Mergaert *et al.,* 1997). Host specificity is determined mostly by Nod factors (reviewed by Mergaert *et al.,* 1997), although it can be influenced by mechanisms increasing the concentration of rhizobia at the infection site (reviewed by Hirsch, 1999). Host range can vary between the extremes of one bacterial strain with one host plant, *i.e.*, *Azorhizobium caulinodans* ORS571/*Sesbania rostrata* (de Bruijn 1989), and one bacterial strain that can infect more than 112 genera of legumes as well as the non-legume *Parasponia andersonii, i.e., Rhizobium* NGR234 (Pueppke and Broughton, 1999).

Nod factors induce the following effects on host plants: (i) root-hair deformation; (ii) PIT formation; and (iii) cortical cell divisions, *i.e*., the formation of a nodule primordium. On some plants, Nod factors are sufficient to induce the formation of either nodule primordia or whole bacteria-free nodules (for *Medicago sativa*, see Truchet *et al.,* 1991; for *Glycine soja*, see Stokkermans and Peters, 1994).

It is interesting that among rhizobia, *nod* and *nif* (nitrogen fixation) gene phylogeny can differ from rRNA phylogeny (Haukka *et al.,* 1998), which indicates that lateral transfer of *nod* genes has taken place and has allowed the recipient strains to infect new hosts. An extreme case was shown by the finding of "symbiotic islands" in a *Mesorhizobium loti* strain (Sullivan *et al.,* 1995; Sullivan and Ronson, 1998). Analogous to the "pathogenicity islands" of bacterial animal pathogens (Lee, 1996), this strain contains a large DNA region with all genes required for an efficient root-nodule symbiosis, which was transferable into other soil bacteria.

4.2. Signal Exchange during Actinorhizal Nodule Induction

The *Frankia* Nod factor equivalent has not yet been characterized, partially due to the fact that, in contrast to legume symbioses, no convenient bioassay is available. Root-hair deformation on actinorhizal plants can also be induced by several non-symbiotic soil bacteria (Knowlton *et al.,* 1980). However, a partial purification of the root hair-deforming factor from the supernatant of *Frankia* cultures led to the conclusion that no lipochitooligosaccharides are involved in the infection of actinorhizal plants (Cérémonie *et al.,* 1999).

Host specificity of *Frankia* strains has been described in chapter 4 of this volume. Several factors hamper comparisons between the host ranges of rhizobia

and *Frankia* strains. First, the number of symbiotic genera among legumes by far exceeds the total number of actinorhizal genera, *e.g.*, there are 25 actinorhizal genera altogether, whereas the wide host-range *Rhizobium* strain NGR234 itself can infect more than 112 different leguminous genera. On the other hand, actinorhizal genera come from different plant families, making it difficult to compare the host specifity of NGR234 with that of a *Frankia* strain, which can infect *Myrica gale* (Myricaceae) and *Casaurina glauca* (Casuarinaceae)! To complicate matters further, some actinorhizal genera are more promiscuous than others. However, this property is dependent on the ecological context because strong differences were found between greenhouse and field trials (see chapter 4 of this volume).

Second, the infection of actinorhizal plants seems to be less strictly controlled than does that of legumes. Actinorhizal nodules can accommodate not only different *Frankia* strains, but also, in their outer cortex, non-N_2-fixing related actinomycetes that cannot re-infect the plant on their own (Mirza *et al.,* 1992; Ramirez-Saad *et al.,* 1998). In some cases, *Frankia* strains isolated from actinorhizal nodules could not re-infect the host plants from which they were isolated, but were instead able to infect other actinorhizal plants (*e.g.*, Torrey, 1990; see chapter 4 in this volume). At any rate, no *Frankia* strain specific to a single host-plant species has ever been characterized.

For *Alnus glutinosa*, it is known that the plant's degree of resistance against infection by ineffecient *Frankia* strains (strains that cannot fix N_2 in symbiosis) is genetically determined (Wolters *et al.,* 1999). However, it is not known at which stage infection by these strains is blocked.

5. ROOT NODULES AND OTHER ROOT SYMBIOSES

More than 80% of all terrestrial plants can enter arbuscular mycorrhizal (AM) symbioses with fungi (Remy *et al.,* 1994). These interactions go back at least 400 million years and involve the stable uptake of hyphae into root cortical cells, but the fungal structures ("arbuscules") are degraded by the infected plant cell after a few days (Harrison, 1999). In most cases, AM does not involve the formation of special lateral root-like structures. However, a few cases are known where AM fungi induce the formation of so-called myconodules on the roots of some tropical trees. Myconodules resemble single-lobed actinorhizal nodules but have only a short life-time, which might be due to the short life-time of arbuscule-containing cells in general. Myconodules have been found on some legume trees and on the actinorhizal *Gymnostoma* sp., but also on trees that do not enter N_2-fixing root nodule symbioses (Béreau and Garbaye, 1994; Duhoux *et al.,* 2001). Hence, also fungi can trigger the formation of lateral root-like structures on higher plants.

Legume genetics have shown that rhizobial and AM symbioses involve common components in plant-signal transduction. Several legume mutants have been identified that are affected in both symbioses (Duc *et al.,* 1989; Stougaard, 2001). In all cases, these mutants are affected in early steps of the interaction, *e.g.* in *symRK* mutants of *Lotus japonicus* or *nork* mutants of alfalfa, the response to rhizobial Nod factors is blocked before root-hair deformation, and the penetration of the root

epidermis by hyphae of AM fungi is blocked (Endre *et al.,* 2002; Stracke *et al.,* 2002). These results indicate that Nod-factor perception and signal transduction involves modules developed for the interaction of plants with AM fungi.

Another type of myconodule is known. It involves *Penicillium nodositatum*, which can use the actinorhizal intra-cellular infection pathway of *Alnus* to form parasitic myconodules (Pommer, 1956; Sequerra *et al.,* 1994; 1995). Again, these myconodules resemble single-lobed actinorhizal nodules, showing that *Frankia* Nod-factor equivalents can also be formed by fungi.

5.1. Which Phytohormones are Involved in Nodule Induction?

Rhizobial Nod factors seem to affect plant morphogenesis *via* auxin transport. Nod factor application results in a block in auxin transport in the root vascular system, which in turn causes an accumulation of auxin at the site of Nod factor application (Mathesius *et al.*, 1998a). Eventually, the auxin that accumulates in the stele at the Nod factor-application site leaks into the cortex and cell divisions occur. Once in the root tip, auxin from the stele is redirected and transported upward in the root cortex (Friml and Palme, 2002). Nod factors seem first to cause auxin depletion, *i.e*., a decrease of the auxin/cytokinin ratio, and then an auxin excess, *i.e*., an increase of the auxin/cytokinin ratio, in the root cortex. It is unclear which of these effects causes the induction of cell division. The effect of Nod factors on auxin concentrations might be mediated *via* plant flavonoids (Mathesius *et al.,* 1998b), which regulate enzymes involved in auxin degradation (Mathesius, 2001).

Support for the suggestion that nodule induction occurs *via* manipulation of plant-auxin transport arises from the fact that, in some legumes, exogenous application of auxin-transport inhibitors can lead to the induction of so-called pseudonodules, *i.e*., rhizobia-free nodules (Hirsch *et al.,* 1989). The induction of nodule-like structures on alfalfa roots by *E. coli*, which produce a cytokinin (Cooper and Long, 1994), might be seen in the same context, *i.e*., a localized change of the auxin-cytokinin balance seems to cause legume nodule induction. For actinorhizal plants, induction of pseudonodules by exogenously applied cytokinins has also been shown (Rodriguez-Barrueco and Bermudez de Castro, 1973). Pseudo-actinorhizal nodules also occur on plants in whose roots the auxin/cytokinin ratio has been changed by infection with *Agrobacterium rhizogenes* (Berg *et al.,* 1992). The differences in the localization of the pseudonodule primordium in legumes *versus* actinorhizal plants might be explained by differences in phytohormone balance in the two systems.

Another symbiotic interaction between plant roots and microorganisms that leads to morphological changes in the root system is the ectomycorrhizal symbiosis, which leads to the formation of short highly branched lateral roots (see, *e.g*., Tagu *et al.,* 2002). Like nodule formation, ectomycorrhization requires the manipulation of plant development *via* auxin produced by the fungi (reviewed by Barker and Tagu, 2000; Martin *et al.,* 2001). One ectomycorrhizal fungus has been also shown to produce an auxin antagonist, hypaphorine, a betaine of tryptophan (Ditengou and Lapeyrie 2000). Interestingly, through interfering with the auxin response,

hypaphorine blocks the extension of elongating root hairs and leads to the swelling of root hairs (Ditengou *et al.,* 2000), an effect similar to the initial response of elongating root hairs to rhizobial Nod factors (Heidstra *et al.,* 1994). It also affects membrane depolarization and ion currents similarly to Nod factors (Reboutier *et al.,* 2002), implying that the same signal-transduction pathway may be involved. In contrast to the effects of Nod factors, hypaphorine also has this effect on root hairs of non-host plants (Reboutier *et al.,* 2002). Obviously, symbiotic bacteria and fungi target the same plant signal-transduction pathway *via* different mechanisms.

Using plant mutants, ethylene has been implicated in the mechanism by which legumes control the number of nodules formed (called autoregulation; reviewed by Guinel and Geil, 2002). Due to the lack of actinorhizal plant mutants, the ethylene effect has not been examined in this system. However, in legumes, the repression of nodulation in response to soil-N is controlled by the same receptor kinases as autoregulation (Searle *et al.,* 2002), and nitrogen inhibition of nodulation in actinorhizal plants is well known and seems to involve similar combinations of root- and shoot-derived factors as in legumes (see chapter 6 in this volume). The receptor kinases involved in both combined-N inhibition and autoregulation of legume nodulation are closely related to CLAVATA1 from *Arabidopsis*. This receptor kinase is involved in cell fate determination in shoot apical meristems (De Young and Clark, 2001) and controls not only nodule, but also lateral-root, meristems. Therefore, it is likely that the same basic mechanisms that were recruited in legumes to control nodulation were also adapted by actinorhizal plants. Interestingly, the induction of pseudonodules on alfalfa roots by cytokinin is under autoregulatory control, whereas auxin transport inhibitor-dependent nodule formation is not (Cooper and Long, 1994; Hirsch *et al.,* 1989). This result might imply that autoregulation works *via* an ethylene effect of auxin transport or degradation.

6. EVOLUTION OF ROOT-NODULE SYMBIOSES

Generally speaking, root-nodule symbioses require: (i) the controlled penetration by a microsymbiont of the plant root, and the concomitant suppression of plant defense; (ii) the stable internalization of the microsymbiont into plant cells; and (iii) the induction of the formation of an organ with a vascular connection to the root stele. These processes have to be brought about *via* signal exchange between host and microsymbiont.

The first two requirements are already established for AM fungi. In exceptional cases, AM fungi even induce the formation of lateral root-like structures (Béreau and Garbaye, 1994; Duhoux *et al.,* 2001). The chitinaceous nature of the rhizobial Nod factors, in combination with the overlap in the signal-transduction pathways for legume-nodule formation and AM formation, has led to the hypothesis that perhaps N_2-fixing bacteria have copied fungal signal molecules. On the other hand, chitins occur in plant secondary cell walls (Benhamou and Asselin, 1989) and plant chitinases, which are involved in plant development, have been identified (Domon *et al.,* 2000; Zhong *et al.,* 2002). There is also evidence that chitooligosaccharide-like signal molecules exist in plants (de Jong *et al.,* 1995; Dyachok *et al.,* 2002;

Schmidt *et al.,* 1993) and that chitinases produce them by acting on apoplastic arabinogalactan proteins (Domon *et al.,* 2000; Passarinho *et al.,* 2001). Hence, rhizobial Nod factors may instead resemble endogenous plant-signal molecules.

6.1. Ways for Symbiotic Bacteria to enter Plant Cells

There are two types of internalization of bacteria within plant cells in root nodule symbioses. Infection-thread formation occurs in both actinorhizal and legume symbioses, whereas symbiosome formation occurs in legumes only. Bacterial nitrogen fixation in persistent infection threads occurs in actinorhizal symbioses, in *Parasponia*, and in some tropical legumes (*e.g.*, *Andira, Chamaecrista*; reviewed by Sprent, 2001), but endocytotic uptake into symbiosomes takes place in all other nodule-forming legumes. There are some legume symbioses in which infection threads are never formed and in which the bacteria enter the roots inter-cellularly and become internalized in symbiosomes (*e.g.*, for *Arachis*, see Chandler, 1978; for *Stylosanthes*, see Chandler *et al.,* 1982), but phylogenetic analysis implies that these are evolved cases (Sprent, 2001). It seems likely that the basal method of internalizating N_2-fixing bacteria was *via* infection threads.

As a mechanism for bacteria to enter plant roots, infection threads are formed only in root hairs. This is not surprising because infection threads are expanding by tip growth, which requires a polarization of the cytoplasm. Trichoblasts are already organized to support tip growth in that root hairs arising from them do so by a tip-growth mechanism. It seems possible that the corresponding reorganization of the cytoplasm cannot be achieved in atrichoblasts.

There is evidence implying that root-hair infection by rhizobia requires a specific structure of the epidermal cell wall (Matthysse and McMahan, 2001; Mort and Grover, 1988). This requirement, together with the fact that not all plants form numerous root hairs, might be taken to imply that root hair-independent infection-thread formation was the basal way of bacterial entry into the root. However, the uniformity in root-hair infection, when contrasted with the diversity of intercellular-infection mechanisms (reviewed, for legumes, by Sprent, 2001), points to the former mechanism as basal. Further, root-hair infection would be advantageous to the plant because it allows control of bacterial entry into the root.

For legumes, infection-thread growth through cortical cells requires the formation of pre-infection thread (PIT) structures formed by root cortical cells re-entering, but not completing, the cell cycle, which leads to endoreduplicaton and the formation of a cytoplasmic bridge through which the infection thread will grow (Yang *et al.,* 1994). PIT-like structures have also been observed in the infection zone of actinorhizal nodules, indicating that they form a general part of infection-thread growth (Berg, 1999). It is interesting that, in Cucurbitales, where *Frankia* is internalized from the apoplast in infection thread-like structures, nodule cells become multinucleate prior to and during the infection process (Hafeez *et al.,* 1984; Newcomb and Pankhurst, 1982). These data indicate that, also in plants with an intercellular infection mechanism, some cell-cycling is required before a cell can support the internalization of the microsymbiont. Because endoreduplication of

nuclear DNA has also been observed in cells infected by AM fungi (Berta *et al.,* 2000), it is possible that such a mechanism for the internalization of microsymbionts in plant cell was already in place (if only for fungi), when N_2-fixing symbioses evolved (Parniske, 2000). However, infection thread-like structures do not exist in AM symbioses, because the intracellular growth of AM fungal hyphae does not involve PIT formation. Clearly, the internalization mechanism for AM fungi had to be changed significantly to allow the internalization of bacteria and some new functions had to be recruited. For instance, the promoter of the nodule-specific subtilase, *cg12*, is active in infection thread-containing cells of *Allocasuarina verticillata* roots as well as in infected cells of nodules and prenodules, but not in cells that contain fungal arbuscules (Svistoonoff *et al.,* 2003).

The host range of *Frankia* is ostensibly wider than that of rhizobia and includes plants from eight different families, but there are far more legume genera that are able to enter rhizobial symbioses. Was an actinorhizal symbiosis an impediment to further evolution of the host-plant species? Interestingly, the only legumes, where bacteroids still fix N_2 in infection threads and where no complete endocytotic process takes place, are woody species; this situation is particularly obvious in the genus, *Chamaecrista* (Naisbitt *et al.,* 1992; Sprent, 2001). The structure of *Frankia* necessitates a symbiosis with persistent infection threads. The internalization of microsymbionts by continuous invagination of the plasma membrane without complete endocytosis has to counteract the turgor of the plant cell, putting high demands on the cytoskeleton, and perhaps requiring some stabilization of the cell walls of infected cells (as by lignified tissues). In contrast, the endocytotic internalization of rhizobia in symbiosomes allows turgor control by aquaporins in the peribacteroid membranes (Dean *et al.,* 1999). In the only intracellular symbiosis between a higher plant and the filamentous cyanobacterium *Nostoc,* the N_2-fixing *Gunnera* symbiosis, the microsymbiont is internalized by a complete endocytotic process (Bergman *et al.,* 1992). Although in arbuscular mycorrhizal symbioses, branching fungal hyphae are internalized in root cortical cells by continuing invagination of the plasma membrane as in the case of *Frankia* hyphae, arbuscules only have a life time of a few days (Harrison, 1999). It is tempting to speculate that this restriction, *i.e*., the necessity for persistent infection threads requiring a stabilization of the walls of infected cells, impaired the distribution of the actinorhizal symbiosis.

6.2. Signal-transduction Pathways for the Differentiation of Nodule Cells

Rhizobia and *Frankia* are only stably internalized (in contrast to infection-thread growth through cells) in cells formed after signal exchange with the microsymbiont, whereas AM fungi can form arbuscules in pre-existing root cortical cells. Thus, some novel differentiation appears necessary for stable internalization of a bacterial microsymbiont. This suggestion is supported by the fact that there are several fungal pathogens that enter plant cells (see, *e.g*. Ellis *et al.,* 2000), but no known bacterial pathogens (Kistner and Parniske, 2002). This new differentiation required for the internalization of symbiotic bacteria could share common features in legumes and

actinorhizal plants and, indeed, evidence exists that infected cell-specific transcription factors are conserved among legumes and actinorhizal Hamamelidae.

This evidence is based on the observations that a transcriptional fusion between the promoter of the gene encoding the symbiotic hemoglobin of *Casuarina glauca* and the ß-glucuronidase (*GUS*) reporter gene was found to be expressed specifically in the infected cells of nodules of the legume *Lotus corniculatus* (Jacobsen-Lyon *et al.,* 1995). Furthermore, a soybean leghemoglobin promoter-*GUS* fusion was expressed specifically in the infected cells of actinorhizal *Allocasuarina verticillata* nodules (Franche *et al.,* 1998), even though the infected cells are not at morphologically equivalent positions in both types of nodules. However, although the expression pattern of a *GUS* fusion with the hemoglobin promoter from *Parasponia andersonii* was conserved in the actinorhizal *A. verticillata* (Franche *et al.,* 1998), it was not conserved in the legume *L. corniculatus* (Andersson *et al.,* 1997). These results suggest that legumes and higher Hamamelidae are more closely related to each other than to the other N_2-fixing plant groups. On the other hand, the hemoglobin gene expressed in infected cells of *Parasponia* nodules, in contrast to the symbiotic (leg-)hemoglobin genes of *Casuarina* and legumes, is not nodule-specific (Landsmann *et al.,* 1988; Trinick *et al.,* 1989), so the discrepancy may be due to the promoter sequences responsible for expression during non-symbiotic development.

Another type of infection-related transcription factor may also be conserved among legumes and actinorhizal plants. The promoter of the nodule-specific subtilase gene from *Casuarina glauca, cg12* (Laplaze *et al.,* 2000b; Svistoonoff *et al.,* 2003) is active in infection thread-containing cells (beginning with infected root hairs) in both legumes and actinorhizal plants, as shown by *cg12* promoter-marker gene fusions in *Allocasuarina verticillata* and *Medicago truncatula* (S. Svistoonoff, L. Laplaze, C. Franche and D. Bogusz, unpublished data). In actinorhizal plants, *cg12* expression occurs throughout the nitrogen-fixation zone, whereas, in legume nodules, it is restricted to the distal part of the prefixation zone and is absent in bacteroid-containing cells. Hence, in both symbioses, *cg12* expression is linked to the presence of infection threads in cells, not infection *per se*. As mentioned in section 6.1., *cg12* is not expressed in arbuscule-containing cells, which underlines the difference in the internalization processes for bacteria and fungi in plant cells.

At any rate, in both legumes and Casuarinaceae, at the level of transcription factors, there is a distinction between cells through which infection threads pass, including root-hair cells and root cortical cells that exist before signal exchange with the microsymbiont, and cells that either have been filled or are in the process of filling with branching infection threads or bacteroids (all these cells having been formed after signal exchange with the microsymbiont). The former cells show *cg12* expression but no expression of (leg-)hemoglobin, whereas the latter cells, in both legumes and Casuarinaceae, show (leg-)hemoglobin expression and, in Casuarinaceae, also express *cg12*.

Consistent with the link between infection-thread growth and cell cycling, in *M. truncatula* (*i.e.*, in a heterologous system) weak *cg12* promoter-*GFP* expression is

also found in the root pericycle at the nodule-induction site, and in the nodule primordium cells that have not been entered by an infection thread, *i.e.*, in dividing cells and cells that are preparing to divide (S. Svistoonoff, L. Laplaze, C. Franche and D. Bogusz, unpublished data). Infection thread-containing cell-specific signal transduction seems to be derived from cell cycle-dependent signalling. In *Allocasuarina verticillata*, which is closely related to the source of the *cg12* promoter, *Casuarina glauca, cg12* promoter-*GFP* or -*GUS* are expressed exclusively in infection thread-containing cells (Svistoonoff *et al.,* 2003).

An unusual leghemoglobin gene (*Vflb29*) in *Vicia faba* has a promoter that was active specifically and exclusively in rhizobium bacteroid-containing and in arbuscule-containing cells (Vieweg *et al.,* 2004), so indicating some conservation of transcription factors between bacterium- and fungus-infected cells that cannot be related exclusively to the mechanism of infection-thread growth. Unfortunately, the activity of the *Vflb29* promoter has not been examined in actinorhizal plants.

Another data set indicates differences in the signal-transduction pathways involved in cell differentiation in nodules from Rosid I subgroups. Rubisco activase transcription is induced in *Datisca* nodules, but the transcript is not spliced correctly and does not leave the nucleus (Okubara *et al.,* 1999). Thus, the signal-transduction pathway that leads to the induction of rubisco activase transcription is active in *Datisca* nodules, implying that components of leaf light- or sugar-dependent signal-transduction pathways may have been recruited for the control of nodule development. However, no rubisco activase message could be detected in nodules of *Alnus glutinosa* (K. Pawlowski, unpublished data). Because differences in the regulation of a basic photosynthetic gene as conserved as rubisco activase are not likely between *Alnus* and *Datisca,* the induction of rubisco activase transcription in *Datisca,* but not *Alnus*, nodules seems to indicate that different signal-transduction pathways have been recruited for the control of nodule development in actinorhizal higher Hamamelidae and Cucurbitales, respectively. These data would support the idea that, based on the common precondition acquired by the Rosid I ancestor, the nodulation syndrome evolved independently in the different symbiotic subgroups of Rosid I (Soltis *et al.,* 1995; Swensen, 1996). On the other hand, an overlap between light signal-transduction pathways and the regulation of nodulation has been shown in legumes (Nishimura *et al.,* 2002), which are likely to be more closely related to actinorhizal Hamamelidae than to Cucurbitales (Pawlowski *et al.,* 2003). A homolog of the transcription factor HY5, which regulates photomorphogenesis and lateral root development in *Arabidopsis*, regulates photomorphogenesis and nodulation in *Lotus japonicus* (Nishimura *et al.,* 2002).

6.3. Structural Diversity of Nodules

Regarding the indeterminate and determinate growth pattern of legume nodules (Figure 2), it is obvious that the latter represents a derivative development (Doyle, 1998). Because perennial nodules require an indeterminate growth pattern, it is not surprising that, in spite of the structural diversity of actinorhizal nodules (Figure 3), a determinate nodule type never developed on woody host plants.

The main difference between legume- and actinorhizal-nodule development is the induction of cell divisions leading to the formation of the nodule primordium in the cortex (legumes) *versus* the pericycle (actinorhizal plants and *Parasponia*). It has been proposed that the stem-like organisation of legume nodules is a result of their induction in the root cortex instead of the pericycle, and is due to a predisposition of legumes to form lateral-root storage organs, a tendency that is not present in other symbiotic plant families (Joshi *et al.,* 1991). In some cases, legumes have been found to form intermediates between lateral roots and nodules (Hirsch, 1992). Furthermore, the necessity to obtain a vascular connection between nodule and root means that, in legumes also, divisions in the root pericycle are induced at an early time point in nodule development (Gualtieri and Bisseling, 2000), giving credibility to the argument that both legume and actinorhizal nodules are modified lateral roots.

As mentioned above, cortical cell divisions are also induced during intercellular infection of *Parasponia* and during intercellular infection of actinorhizal higher Hamamelidae and lead to the formation of the so-called prenodules, not to nodule primordia. Also, one inter-cellularly infected actinorhizal plant shows a putative relic of prenodule formation, in that cortical cells start dividing upon inter-cellular colonization of the root cortex, but do not become infected (*Ceanothus griseus*; see Liu and Berry, 1991a). Because prenodule cells show the same differentiation as their counterparts in mature nodule lobes, it has been proposed that prenodule-like structures were the origin of all root-nodule symbioses (Laplaze *et al.,* 2000a). In order to achieve both efficient removal of the fixed-N and an efficient supply of carbon sources by the host, a vascular connection of the symbiotic organ would have evolved later with different localization of the vascular system relative to the infected cells in legumes *versus* other plant families (Figure 3). In a similar vein, Kistner and Parniske (2002) have suggested that the evolution of N_2-fixing plants started with the ancestor of the symbiotic subclades of Rosid I (called "FaFaCuRo" for Fabales, Fagales, Cucurbitales and Rosales) acquiring the ability to internalize bacterial microsymbionts in infection threads, *i.e.*, that an infection-thread symbiosis was the origin of root-nodule symbioses (Figure 4). This hypothesis is supported by an electron-microscopy finding of bacterial cells in infection threads in roots of the non-nodulating legume tree *Gleditsia* sp. (Faria *et al.*, 1999). In all extant root-nodule symbioses examined, bacterial nitrogen fixation is only found in infected cells that arose by division after signal exchange with the microsymbiont, this infection of *Gleditisa* is probably consistent with this fact because the infected areas of the roots were macroscopically visible as small bumps that are probably indicative of cell division.

6.4. Microbial Signal Compounds Trigger Cell Cycling

Based on the assumption that the induction of incomplete cell cycling, namely endoreduplication, is required for infection-thread growth, the induction of nodule primordium formation - complete cell cycling - does not represent a complete new achievement apart from the internalization of the bacterial microsymbionts in

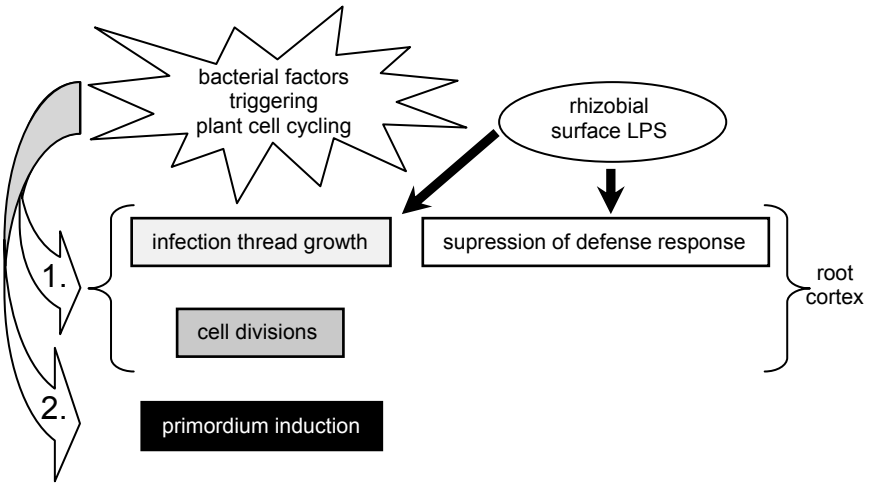

Figure 4. Manipulation of plant development by symbiotic bacteria (hypothetical). Triggering plant cell cycling, Nod factors could initially (1.) have caused infection thread growth and cell division, depending on the amount of receptors in the corresponding cells (indicated by different shades of grey). These events would have led to the formation of prenodule-like structures. Later, (2.) the induction of an organ primordium (i.e., sustained cell divisions) in root cortex and pericycle (legumes) or in the root pericycle (actinorhizal plants) could have been added, leading to nodule formation. In legumes, rhizobial surface lipopolysaccharides (LPS) are responsible for the suppression of the plant defence response and are required for infection-thread formation. It is not clear whether, and if yes, which, Frankia *surface molecules have similar functions.*

infection threads. On the other hand, the example of *Parasponia* shows that induction of cell division can cause ruptures in the plant root epidermis by which bacteria can enter the root cortex (Bender *et al.,* 1987). Thus, this might be interpreted to mean that the primary event was for bacteria to acquire the ability to manipulate plant cell division, with infection-thread formation being a later adjustment. However, the example of *Chamaecytisus* indicates that the induction of cortical cell division, to cause ruptures in the root epidermis by which bacteria enter the plant, is likely to represent an evolved mechanism derived from an earlier intracellular infection pathway (Vega-Hernández *et al.,* 2001).

It has been suggested that rhizobia with wide host ranges, such as NGR234, were basal in legume nodulation (Broughton *et al.,* 2000; Perret *et al.,* 2000) and that more specific associations only evolved later. Nevertheless, the difference between host specificity ranges of extant *Frankia* strains and rhizobia, as well as the obvious lack of chemical similarity between Nod factors of both groups of bacteria (Cérémonie *et al.,* 1999), implies that *Frankia* Nod factors do not use the same type of receptor as rhizobial Nod factors. A receptor-like kinase (RLK) containing three leucine-rich repeats (LRRs) and an unusual apoplastic domain is involved in the early stages of both AM and rhizobial symbioses and might represent the plant receptor of rhizobial Nod factors (called NORK or SYMRK; Endre *et al.,* 2002; Kistner and Parniske, 2002; Stracke *et al.,* 2002). RLKs differ in their apoplastic

domains (Becraft, 2002), and no RLK with an apoplastic domain like that of NORK/SYMRK has been found outside of the legume family (Kistner and Parniske, 2002). RLKs like NORK/SYMRK, though without the unusual apoplastic domain and instead with more LRRs, *e.g.*, CLAVATA1 and the receptors of systemin and sulfokinins, bind proteinaceous effectors (Matsubayashi *et al.,* 2002; Scheer and Ryan, 2002; Trotochaud *et al.,* 2000). On the other hand, SERK-like RLKs have five LRRs and an apoplastic proline-rich domain, and their LRRs seem to serve only for multimerization, not for receptor binding (Shah *et al.,* 2001). Thus, it is not clear whether the LRRs of NORK/SYMRK have anything to do with effector binding, and what type of signal molecule is bound by NORK/SYMRK. It has been suggested that the acquisition of a NORK/SYMRK-like RLK by exon shuffling occurred at the beginning of the evolution of plants able to enter N_2-fixing root nodule symbioses (Kistner and Parniske, 2002).

Although rhizobial Nod factors and AM fungi signal substances share part of a signal transduction chain (Stougaard, 2001), it is not assumed that AM signal substances are structurally similar to Nod factors. The host range of AM fungi would argue against that. It is quite possible that AM signal factors as well as Nod factors bind to different receptors, thereby causing the production of a signal molecule that is recognized by NORK/SYMRK.

As mentioned above, some legumes and at least one actinorhizal plant, *Gymnostoma*, can form myconodules to host AM fungi (Béreau and Garbaye, 1994; Duhoux *et al.,* 2001). Also, the myconodules of legume trees do not have a legume nodule-like structure and the myconodules of *Gymnostoma* do not show prenodules. Thus, even if the formation of a lateral root-like structure is induced by AM fungal signal factors, its ontogenesis will not follow the model of bacterially induced nodules. However, *Penicillium nodositatum,* which is not an AM fungus, can use the infection pathway of *Frankia* to induced parasitic myconodules (Sequerra *et al.,* 1994; 1995). This can only be explained by *P. nodositatum* being able to produce signal molecules similar to those of *Frankia.* It also seems to imply that the formation of infection thread-like structures through which *Frankia* enters the plant roots does not require *Frankia* surface lipopolysaccharides, whereas legume infection-thread formation does require rhizobial LPS, a conclusion that seems convincing in the context of the diversity of *Frankia*- and *Frankia*-like strains that can be found in actinorhizal nodules (see chapter 2 in this volume). Interestingly, rhizobial LPS plays a role in the suppression of an anti-pathogen response of the plant against rhizobia (Albus *et al.,* 2001). Thus, although there seem to be similarities in the way rhizobia, *Frankia* and AM fungi infect plants, there seem to be striking differences in the way they suppress the anti-pathogen response.

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