Planta (2005) 221: 184–196
DOI 10.1007/s00425-004-1436-x

ORIGINAL ARTICLE

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Molecular and cell biology of arbuscular mycorrhizal symbiosis

Received: 31 August 2004 / Accepted: 25 October 2004 / Published online: 26 November 2004
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Abstract The roots of most extant plants are able to become engaged in an interaction with a small group of fungi of the fungal order Glomales (Glomeromycota). This interaction—arbuscular mycorrhizal (AM) symbiosis—is the evolutionary precursor of most other mutualistic root-microbe associations. The molecular analysis of this interaction can elucidate basic principles regarding such associations. This review summarizes our present knowledge about cellular and molecular aspects of AM. Emphasis is placed on morphological changes in colonized cells, transfer of nutrients between both interacting partners, and plant defence responses. Similarities to and differences from other associations of plant and microorganisms are highlighted regarding defence reactions and signal perception.

Keywords Arbuscular mycorrhizal fungi · Defence response · Induced systemic resistance · Morphology of arbuscule-containing cells · Nutrient transfer · Signal transduction

Abbreviations AM: Arbuscular mycorrhiza(l) · CCaMK: Calcium and calmodulin-dependent protein kinase · *DMI*: *DOES NOT MAKE INFECTIONS* · ER: Endoplasmatic reticulum · EST: Expressed sequence tag · GFP: Green fluorescent protein · NORK: Nodulation receptor kinase · NFR: Nod factor receptor · SYM: Mutants affected in symbioses · SYMRK: Symbiosis receptor-like kinase

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Introduction

Mycorrhizas are intimate and, in most cases, mutualistic associations of plant roots and fungi. They are crucial in the ecology and physiology of terrestrial plants, supporting plants under biotic (e.g. pathogen infection) or abiotic stress (e.g. nutrient or water deficiency). Exchange of nutrients—mineral nutrients supplied by the fungal microsymbiont versus carbohydrates provided by the plant—is considered to be the main benefit for the symbiotic partners (Smith and Read 1997). According to the phylogenetic position of these partners and according to the symbiotic structures, several types of mycorrhiza have been defined such as arbuscular mycorrhiza (AM), ectomycorrhiza, ericoid mycorrhiza, and orchid mycorrhiza.

The interaction reviewed in this article, arbuscular mycorrhiza (AM), is characterized by highly branched haustorium-like fungal structures within root cortical cells. It is formed by fungi from the order Glomales (Glomeromycota) and refers to more than 80% of all terrestrial plant species, including angiosperms, gymnosperms, pteridophytes, lycopods, and mosses. Only a few plant species, e.g. members of the Brassicaceae, Caryophyllaceae, Chenopodiaceae, or Urticaceae, do not engage in AM interactions (Smith and Read 1997; Vierheilig et al. 1996).

Because of the ancient origin of the symbiosis, which can be traced back to the Ordovician (Redecker et al. 2000), conservation of key mechanisms in molecular regulation can be anticipated. As a result of increasing availability of molecular tools, first steps have been taken towards understanding the molecular complexity of such mechanisms. Specific changes in root morphology and unique physiology during AM development suggest the existence of AM-specific regulatory pathways leading to the induction of AM-specific genes. The occurrence of defence responses in AM plants and the analysis of legume mutants defective in AM and symbiotic nitrogen fixation, however, indicate the existence of

common signal transduction pathways regulating AM, rhizobial, and pathogenic interactions (Dumas-Gaudot et al. 2000; Duc et al. 1989; Wegel et al. 1998).

The fungal partner

The fungi involved in AM symbiosis are obligate biotrophs. They reproduce asexually, forming multinucleate spores. Unusual polymorphism of ribosomal RNA in individual spores has led to the concept of internuclear variation in single spores, defining AM fungi as heterokaryotic organisms (Trouvelot et al. 1999; Kuhn et al. 2001). Heterokaryosis has been assumed to be of decisive importance to ecology and application of AM fungi. This concept, however, has recently been challenged by experiments suggesting that single spores contain a uniform population of nuclei characterized by intranuclear polymorphism (Pawlowska and Taylor 2004).

The systematic position of the order Glomales has always been problematic as no sexual form of the fungi is known. Phylogenetic analysis of 18S rRNA sequences (Simon et al. 1993) and of various protein sequences (Heckman et al. 2001) suggests the ancient origin of the Glomales and no close relation to the Zygomycetes, where the Glomales had traditionally been grouped. Accordingly, it has been proposed that the fungi be placed into a new phylum of their own, the Glomeromycota (Schüßler 2001; Schüßler et al. 2001). The ancient phylogenetic origin of the Glomales is confirmed by fossil findings, with symbiotic structures within fossil roots from the Devonian (about 400 Mio years ago; Remy et al. 1994; Taylor et al. 1995) and fossilized glomalean spores from the Ordovician (about 460 million years ago; Redecker et al. 2000).

In summary, these findings suggest a coevolution of AM fungi with the first land plants, pre-dating this association to all other plant–fungal interactions except for the lichens. This coevolution easily explains the nearly ubiquitous distribution of the AM symbiosis, in the plant kingdom as well as global ecosystems. While this review focuses on the plant side of the AM interaction, molecular work regarding the fungal partner has been summarized, e.g. by Franken and Requena (2001).

Model plants in AM research

The use of the model legumes *Medicago truncatula* (barrel medic) and *Lotus japonicus* has led to significant advances in our knowledge about the plant partner of the AM interaction. The genomes of *M. truncatula* and *L. japonicus* have been mapped physically and genetically (Hayashi et al. 2001; Kulikova et al. 2001; Kato et al. 2003) and are currently sequenced (http://www.genome.ou.edu/medicago.html; http://www.kazusa.or.jp/lotus/). A number of research groups have contributed EST sequences (see e.g. Asamizu et al. 2000; Bell et al. 2001; Lamblin et al. 2003), which are deposited in freely accessible internet databases (TIGR *Medicago truncatula* Gene Index (MtGI): http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=medicago; *Medicago truncatula* Data Base (MtDB): http://www.medicago.org/; TIGR *Lotus japonicus* Gene Index (LjGI): http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=l_japonicus; Kazusa *Lotus japonicus* EST-database: http://www.kazusa.or.jp/en/plant/lotus/EST/). These sequences have been used for performing Electronic Northern analysis (see, for example, Journet et al. 2002) and for constructing DNA microarrays for the analysis of differentially expressed genes (Liu et al. 2003; Wulf et al. 2003). Independent of these EST databases, differentially expressed transcripts have been searched using various plants and screening methods (Martin-Laurent et al. 1997; Burleigh and Harrison 1997; Murphy et al. 1997; Krajinski et al. 1998; van Buuren et al. 1999; Liu et al. 2003; Wulf et al. 2003). These approaches in combination with targeted approaches resulted in the elucidation of mycorrhiza-induced transcripts involved in the composition of the cytoskeleton (Rhody et al. 2003), in establishing the rhizobial interaction (Chabaud et al. 2002), the transportation of water (Krajinski et al. 2000), isoprenoid biosynthesis (Walter et al. 2002; Hans et al. 2004), and in the regulation of oxygen concentrations (Uchiumi et al. 2002).

Regarding the proteomic level, there are a number of reports documenting differential protein patterns in mycorrhizal and non-mycorrhizal roots (Pacovsky 1989; Schellenbaum et al. 1992; Arines et al. 1993; Garcia-Garrido et al. 1993; Dumas-Gaudot et al. 1994; Simoneau et al. 1994; Samra et al. 1997; Benabdellah et al. 1998; Dassi et al. 1999; Fester et al. 2002; Repetto et al. 2003). Using specific antibodies, Wyss et al. (1990) identified some of the induced proteins as nodulins. Using *N*-terminal sequencing techniques, Benabdellah et al. (2000) found an induced H^+-ATPase and Slezack et al. (2001) found an induced chitinase. The large amount of EST data available for *M. truncatula* and *L. japonicus* now allows the identification of low-abundant protein spots using MALDI-TOF analysis (Watson et al. 2003; Bestel-Corre et al. 2004), thus greatly facilitating the identification of proteins of interest in these plants.

Further tools for molecular work encompass protocols for transformation (Chabaud et al. 1996; Boisson-Dernier et al. 2001) and a TILLING approach for *L. japonicus* (Perry et al. 2003). Apart from mutants generated by TILLING, mutants related to mycorrhizal colonization have been described for pea (*Pisum sativum*, Duc et al. 1989; Gianinazzi-Pearson et al. 1991; Jacobi et al. 2003a, b), alfalfa (*M. sativa*; Bradbury et al. 1991), *M. truncatula* (Calantzis et al. 1998; Morandi et al. 2000; Ané et al. 2004), *L. japonicus* (Wegel et al. 1998) and tomato (*Lycopersicon esculentum*; Barker et al. 1998; David-Schwartz et al. 2001, 2003). The analysis of such mutants has provided insight into

signalling pathways between macro- and microsymbiont (see below).

Cytological features of AM plant roots

Fungal development starts with the germination of hyphae from resting spores. In the absence of a host plant, AM fungi show only limited hyphal growth whereas in the presence of root exudates growth and branching of hyphae is strongly increased (Tamasloukht et al. 2003). This presymbiotic fungal reaction is characterized by the activation of specific genes followed by subsequent physiological and morphological changes. In return, germinating spores produce diffusible factors which are perceived by plant roots leading to the expression of specific genes even in the absence of direct physical contact (Kosuta et al. 1998). The chemical nature of both plant and fungal diffusible factors is not yet known.

After the first physical contact between hyphae and plant roots, the fungus forms appressoria and subsequently penetrates the root surface colonizing the intercellular space of the root cortex. The plant actively mediates at least two steps allowing the fungus to penetrate the rhizodermis (Demchenko et al. 2004): (1) anticlinal cell walls of two adjacent epidermal cells separate from each other in the vicinity of fungal hyphae allowing the intercellular passage of the hyphae; and (2) fungal hyphae are allowed to pass intracellularly through an exodermal cell and an adjacent cell from the outermost cortical layer. After the subsequent penetration of the innermost cortical layers, tree-like fungal structures (arbuscules) are formed within individual root cortical cells by repeated dichotomous branching of fungal hyphae. Except for species from the genera *Scutellospora* and *Gigaspora*, all AM fungi form intra- or intercellular storage organs, lipid-rich vesicles, to varying degrees in late phases of the symbiosis (Smith and Read 1997).

The arbuscules are the key features of AM and are responsible for nutrient exchange (see below). They represent a dead end in the growth of AM fungi (Bonfante and Perotto 1995), because they finally senesce and collapse after 4–10 days of symbiosis (Sanders et al. 1977). The fungal structures are then degraded completely by the plant cell and the plant cell recovers its original morphology (Jacquelinet-Jeanmougin et al. 1987). This way, cortical cells are able to allow a second fungal penetration and arbuscule formation. The life cycle of AM fungi is completed by the formation of extraradical spores, which may enter another colonization process.

During colonization, the fungal arbuscule occupies a major portion of the plant cortex cell, but is separated from the cell protoplast by a part of the host plasma membrane, the periarbuscular membrane. This membrane completely surrounds the arbuscule, leading to up to a fourfold increase of the surface of the plasma membrane. Although it originates from the plant plasma membrane, the periarbuscular membrane exhibits different properties. In particular, phosphate transporters were shown to be located specifically in the periarbuscular membrane (Rausch et al. 2001; Harrison et al. 2002). Moreover, a high amount of H^+-ATPase activity (Gianinazzi-Pearson et al. 1991) accompanied by the highly acidic nature of the space separating plant and fungal plasma membranes has been found (Guttenberger 2000). These findings are consistent with the involvement of the periarbuscular membrane in the active transport of nutrients between the symbiotic partners (see below). The space separating plant and fungal plasma membranes corresponds to a new apoplastic compartment and represents the symbiotic interface. It is continuous with the peripheral plant cell wall, but its structure differs from it (Peterson and Bonfante 1994). Its components reflect the composition of the wall of the host cell that is being invaded. Pectins, xyloglucans, nonesterified polygalacturonans, arabinogalactans, and hydroxyproline-rich glycoproteins have been localized within this interface (Balestrini et al. 1994; Perotto et al. 1994; Bonfante and Perotto 1995). The mixture of primary plant cell wall components indicates that the arbusculated cells maintain their ability to synthesize and secrete cell wall material. This material, however, does not assemble further to build up a secondary wall (Peretto et al. 1995).

Colonization by an AM fungus induces dramatic changes in the shape and number of organelles of root cortical cells. As shown schematically in Fig. 1a, differentiated cells of the root cortex are extensively reorganized after penetration by an AM fungus (Bonfante and Perotto 1995). The central vacuole is fragmented, the volume of cytoplasm and the number of cell organelles increase significantly, and the nucleus moves into a central position. The nucleus of arbusculated cells undergoes hypertrophy (Balestrini et al. 1994) and is characterized by enhanced fluorochrome accessibility, increased nuclease sensitivity, and chromatin dispersion (Gianinazzi-Pearson 1996). These features reflect a higher transcriptional activity of the plant genome in colonized cells in comparison to non-colonized cells. The increase in the amount of host cytoplasm and the number of organelles surrounding the branching hyphae was shown first by electron microscopy (Carling and Brown 1982; Bonfante and Perotto 1995). The analysis of organelles labelled by the green fluorescent protein using confocal laser scanning microscopy provided new details and led to the discovery of network-like organelle structures in colonized cells. Such structures covering the developing arbuscule can be observed for plastids (Fig. 1b, Fester et al. 2001), mitochondria (unpublished data), and the ER (Fig. 1c), indicating a strong activation of the metabolism in the colonized root cortical cell. In the case of plastids, the networks are formed by tubular extensions, which have been referred to as stromules (*stroma*-filled tub*ules*, Köhler and Hanson 2000). In *Nicotiana tabacum*, the first step in the formation of these networks is the appearance of

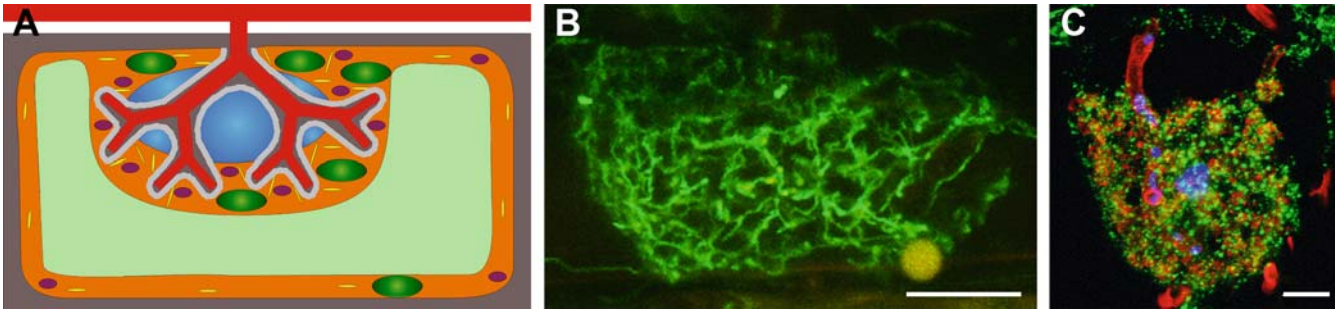

Fig. 1 Survey of the morphology of an arbuscule-containing root cortex cell. **a** Scheme of a young arbuscule (*red*) within a root cortex cell. The fungal hypha penetrates the cell wall (*grey*) and undergoes branching leading to the formation of an arbuscule. The arbuscule is surrounded by the plant cytoplasm (*orange*) which contains high numbers of organelles (plastids – *dark green*, mitochondria – *purple*, ER – *yellow*). Fungal hyphae and plant cytoplasm are separated by the periarbuscular membrane (*light grey*). The plant cell nucleus (*blue*) moves into the centre of the arbuscule; the vacuole (*light green*) fragments after the arbuscule is fully developed. **b** GFP-labelled plastids forming a network-like structure, which covers the arbuscule. Mycorrhizal roots of stably transformed tobacco plants, expressing a plastid-directed GFP (kindly provided by M. Hanson, New York, USA), were analysed by confocal laser scanning microscopy (CLSM). The superposition of 20 optical sections is shown. *Bar* represents 10 μm. **c** Visualization of ER in arbusculated cells. Mycorrhizal roots of transgenic *N. benthamiana* plants expressing ER-targeted GFP (kindly provided by D. Baulcombe, Norwich) were processed for immunolocalization according to Hans et al. (2004). Cross-sections were probed with anti-GFP antibody followed by a fluorescence-labelled secondary antibody. Micrographs were taken by CLSM showing GFP (*green*), DAPI-stained nuclei (*blue*) and fungal structures stained with WGA-TRITC (*red*). The superposition of 28 optical sections is shown. *Bar* represents 10 μm

"octopus"- or "millipede"-like plastid structures surrounding the plant cell nucleus (Fester et al. 2001).

Plant cells respond to colonization by an AM fungus by reorganization of microtubules and microfilaments (for review see Timonen and Peterson 2002). Microtubuli have been shown to undergo extensive remodelling from the early stages of arbuscule development until arbuscular collapse and senescence leading to different types of microtubular patterns (Genre and Bonfante 1997, 1998; Matsubara et al. 1999). Beside α- and β-tubulin, γ-tubulin also seems to be involved in cellular rearrangements as shown by the detection of microtubule organizing centres along the nuclear envelope and along the periarbuscular membrane (Genre and Bonfante 1999). Cytoskeletal rearrangements were observed not only in colonized cells but in adjacent cortical cells as well (Blancaflor et al. 2001), indicating exchange of signals prior to intracellular colonization. Accordingly, an active role of the plant cytoskeleton in mycorrhization appears more probable than a passive reaction to the physical pressure created by the fungus at the plant plasma membrane. The alterations of the microtubular network are also reflected in the mycorrhiza-specific up-regulation of an α-tubulin gene in mycorrhizal maize roots (Bonfante et al. 1996). Expression studies of the corresponding promoter:*uidA* fusions in tobacco revealed that this gene is induced specifically in cells containing developing arbuscules.

Transfer of nutrients between plants and fungi

Although the transfer of nutrients is not the only benefit for the symbiotic partners, it certainly represents an important factor of mycorrhizal interactions. Due to the inherent properties of the symbiotic partners, all mutualistic plant–microbe interactions essentially show the same trading patterns and similar structural features. Carbohydrates are provided by the plant in exchange for mineral nutrients provided by the microsymbiont. The nutrients have to be transported across the plant and the microsymbiont plasma membrane. These processes, in combination with the proteins identified to be involved in the AM symbiosis, have been summarized in Fig. 2. In contrast to the general pattern, some plants are able to invert the actual flow of carbohydrates, turning the mutualistic interaction into a parasitic one. This applies to some AMs (Imhof 1999; Bidartondo et al. 2002), as well as to ecto- and orchid mycorrhizas (Leake 1994).

Active transport of various metabolites across biological membranes is often powered by coupling the transport to the concomitant transport of protons in the same or in the opposite direction. This mechanism relies on the existence of a proton gradient across the membrane in question. Staining of mycorrhizal roots (*Allium porrum* and *Glomus versiforme*) using neutral red and Lyso-Sensor Green DND-189 revealed the existence of an acidic compartment in the periphery of arbuscules presumably identical with the periarbuscular space separating the plant and fungal plasma membranes (Guttenberger 2000). This acidification of the periarbuscular space corresponds to proton gradients across the fungal arbuscular and the plant periarbuscular membrane powering transport processes across these membranes. Enzymes responsible for the generation of such an acidification—H^+-ATPases—have been studied for a long time (Marx et al. 1982; Gianinazzi-Pearson et al. 1991). In recent years, genes of plant plasma membrane H^+-ATPases specifically induced in arbuscule-containing root cortical cells have been reported for barley (*Hordeum vulgare*; Murphy et al. 1997), tobacco (*N. tabacum*; Gianinazzi-Pearson et al. 2000), and barrel medic (Krajinski et al. 2002). Fungal H^+-ATPases involved in symbiotic nutrient transfer have been char-

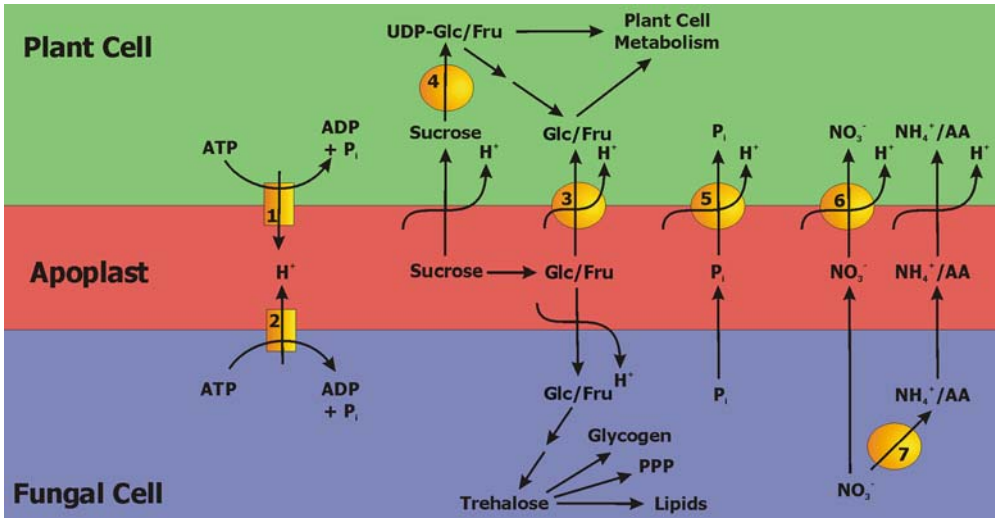

Fig. 2 Nutrient transfer in AM roots. Enzymes and transporters described to be specifically induced in AM roots are indicated. Membrane transport of most metabolites can be expected to be pH-dependent and to be powered by the activity of plant (*1*) and fungal (*2*) H^+-ATPases. Fungal H^+-ATPases have been described not to be restricted to arbuscules, suggesting active transport at intercellular hyphae as well. Sucrose from the phloem is either cleaved by apoplastic invertases and taken up by the plant (*3*) or fungal hexose transporters or imported into root cortical cells and cleaved there by a cytoplasmic sucrose synthase (*4*). The fungus transforms hexoses rapidly into trehalose, which is either metabolized by the pentose phosphate pathway, or used for the biosynthesis of glycogen and lipids. These compounds are then exported to fungal vesicles or to the external mycelium. The plant cell takes up phosphate from the periarbuscular space using specific, H^+-dependent plant phosphate transporters (*5*). Regarding nitrogen supply, AM-induced plant nitrate transporters (*6*) have been found, suggesting a similar transport mechanism as referring to phosphate. On the other hand, the observation of increased transcript levels of a fungal nitrate reductase (*7*) suggests the transfer of nitrogen in a reduced form (as ammonium or in an organic form). *AA* Amino acids

acterized by Ferrol et al. (2000) and Requena et al. (2003).

Regarding the transport of individual nutrients, the transfer of carbohydrates is thought to be the main benefit for the fungal symbiotic partner. One major factor explaining the obligate biotrophy of AM fungi was the finding that extraradical hyphae of these fungi are unable to take up carbohydrates. This has been shown by the application of various ^{13}C-labelled compounds to split petri dishes harbouring *Ri* T-DNA-transformed *Daucus carota* roots colonized by *G. intraradices* in one compartment and extraradical mycelium of the fungus alone in the other compartment (Pfeffer et al. 1999). The uptake of glucose and fructose by intraradical fungal structures has been documented by this study; however, the exact fungal structures responsible for this uptake are still a matter of debate (Douds et al. 2000). Fungal H^+-ATPases, possibly involved in the fungal uptake of carbohydrates from the apoplast, were found to be predominantly located on arbuscular trunks and intercellular hyphae, supporting an uptake of carbohydrate by these structures (Gianinazzi-Pearson et al. 1991). In pea mutants, however, where AM fungi were unable to form arbuscules, the formation of extraradical mycelium was strongly decreased as well, arguing in favour of an arbuscular role in the uptake of carbohydrates (Kling et al. 1996). The metabolic fate of hexoses taken up by intraradical fungal structures was determined by labelling experiments using mycorrhizal *D. carota* root culture in split petri dishes. In short-term experiments, glucose was mainly transformed to trehalose or glycogen (Douds et al. 2000). After longer incubation periods, glucose was either used directly for lipid biosynthesis or entered the pentose phosphate pathway, thus providing the reduction equivalents necessary for lipid biosynthesis (Pfeffer et al. 1999). Lipids and glycogen are then transferred to the extraradical mycelium (Bago et al. 2003), where the bidirectional movement of lipid bodies can be observed in vivo (Bago et al. 2002). Regarding the plant side, a cytosolic sucrose synthase (Hohnjec et al. 2003; Ravnskov et al. 2003) and a plasma membrane hexose transporter (Harrison 1996) have been described to be specifically induced in AM roots (Fig. 2). The corresponding induction of apoplastic and symplastic cleavage of sucrose probably reflects the increased need for carbohydrates in symbiotic root cortical cells.

Phosphate is a mineral nutrient limiting plant growth at many natural stands due to its poor solubility. AM fungi transport phosphate from distant reservoirs to the plant, extending the reach of plant root systems. Using ^{33}P provided in soil compartments only admissible to fungal hyphae and not to plant roots, it could be shown that even under non-limiting phosphate supply and without apparent growth effects of mycorrhizal colonization, colonized plant roots are reducing the activity of their own phosphate uptake system and rely mainly on their fungal symbionts for phosphate provision (Smith et al. 2003). Phosphate transporters involved in the uptake of phosphate from the external medium to fungal hyphae have been cloned from *G. versiforme* (Harrison and van Buuren 1995) and *G. intraradices* (Maldonado-

Mendoza et al. 2001). Both transporters are similar to members of the plant phosphate transporter family Pht1, which contains high-affinity H^+-dependent transporters (Rausch and Bucher 2002). Transporters from the same protein family are specifically expressed in AM roots and have been cloned from *Solanum tuberosum* (Rausch et al. 2001), *M. truncatula* (Harrison et al. 2002), and *Oryza sativa* (Paszkowski et al. 2002). Immunolocalization of the phosphate transporter MtPT4 from *M. truncatula* suggests its specific location in the periarbuscular membrane. This localization characterizes MtPT4 as an important transporter using the pH gradient established across the periarbuscular membrane in order to take up phosphate released from the fungal arbuscules to the periarbuscular space (see Fig. 2).

Besides phosphate, a number of mineral nutrients and most notably water are transported by AM fungi to their host plants. In the case of nitrate, AM fungi play an even more active role when compared to phosphate, because they are able to liberate nitrate from complex organic material within the soil (Hodge et al. 2001). Few proteins involved in nitrate transport in mycorrhizal roots have been found so far, amongst them a tomato nitrate transporter with increased transcript levels in AM colonized roots (Hildebrandt et al. 2002). In addition, a fungal (*G. intraradices*) nitrate reductase has been cloned, which is expressed in AM roots and therefore might be involved in the transfer of nitrogen as well (Kaldorf et al. 1998).

Defence reactions of plant roots during colonization by AM fungi

As described above, AM fungi extensively invade host root tissues. The fact that the spread of mycelium occurs only in the root cortex suggests that the host plant exerts some kind of control over fungal proliferation, confining it to specific root tissues. Defence processes, however, which are usually triggered as a plant response to microbial invasion are observed only in a modulated form in AM roots. The current knowledge about this modulated defence response has been summarized by a number of reviews (Dumas-Gaudot et al. 2000; García-Garrido and Ocampo 2002). Most host plants show remarkably little cytological reaction to appressorium formation or to the first steps of root colonization (Gianinazzi et al. 1996). Typical structural defence barriers such as papillae or wall appositions containing callose, phenolic compounds, or lignin are not elicited during plant responses to AM fungi. Other elements of the plant defence response like phenylpropanoid biosynthesis, enzymes involved in the response against oxidative stress, and pathogenesis-related (PR) genes including hydrolytic enzymes have been described for mycorrhizal roots. In most cases, however, such defence responses of the plant are weak, transient, uncoordinated, or strictly localized and therefore differ from that in plant-pathogen interactions (Gianinazzi-Pearson et al. 1996).

The phenylpropanoid metabolism is activated in typical AM interactions, but to a much lower extent than in plant-pathogen interactions (Harrison and Dixon 1994; Volpin et al. 1994; Peipp et al. 1997). Transcripts encoding enzymes of the flavonoid biosynthetic pathway, phenylalanine ammonia lyase (PAL), and chalcone synthase (CHS), but not the defence-specific enzyme isoflavone reductase (IFR), are induced specifically in cells containing arbuscules in *M. truncatula* (Harrison and Dixon 1994). It has been speculated that this induction rather reflects the biosynthesis of flavonoid compounds stimulating the growth of AM fungi than the biosynthesis of antimicrobial phytoalexins (Harrison 1999). AM-specific alterations in the pattern of anti-oxidative enzymes, such as catalase, peroxidase and superoxide dismutase, indicate that the corresponding genes might be expressed specifically during the colonization process (Arines et al. 1994a, b; Blilou et al. 2000; Lambais 2000). This activation corresponds to the occurrence of reactive oxygen species in arbusculated root cortical cells (Salzer et al. 1999). The expression of genes coding for PR proteins is strongly reduced when compared to plant-pathogen interactions, where PR proteins accumulate throughout infected root tissues (Tahiri-Alaoui et al. 1993). In AM roots, a structured wall material containing PR-1 protein and hydroxyproline-rich glycoprotein is transiently deposited around hyphae specifically in cortex cells containing developing arbuscules (Gianinazzi-Pearson et al. 1992). Regarding hydrolytic enzymes like chitinases and β-1,3-glucanases which play a well-documented role in plant development in general and in the inducible plant defence response in particular (Boller 1987; Collinge et al. 1993), only specific isoforms are expressed in AM roots of various plant-fungal combinations (Lambais and Medhy 1993, 1998; Blee and Anderson 1996; David et al. 1998). Most of the data argue in favour of a direct role in the formation/degradation of arbuscules. In *M. truncatula*, however, the class III chitinase gene family is expressed specifically in cells containing functionally active arbuscules (Salzer et al. 2000; Bonanomi et al. 2001). Accordingly, the induced chitinase has been proposed to play a role during formation and functioning rather than during degradation of arbuscules. One possible function for chitinases could be the cleavage of fungal elicitors, contributing to an attenuation of the defence response.

In summary, AM fungi fail to elicit the full cascade of non-specific defence responses in host roots. The differential activation of defence-related genes may reflect the partial elicitation of a general plant defence response to early stages of fungal invasion that is subsequently repressed as the symbiosis becomes established. This could be due to several mechanisms (Dumas-Gaudot et al. 2000): (1) low elicitation capacity of AM fungal elicitors, (2) regulation by additional fungal inhibitors, or (3) fungal compatibility factors which could counteract elicitor activity. Whether AM fungi synthesize such molecules has yet to be demonstrated. Evi-

dence for the involvement of plant genes in the modulation of defence processes in AM has been obtained from the study of mycorrhiza-resistant plant mutants. Upon contact with AM fungi, the roots of myc$^-$ mutants show higher levels of defence responses when compared to the wild type. These responses include the occurrence of characteristic defence molecules such as phenolics, callose, and PR proteins (Gollotte et al. 1993).

Induced systemic resistance in AM plants

AM can effectively reduce root disease caused by a number of soil-borne pathogens, such as *Fusarium* spp., *Aphanomyces euteiches* and *Phytophthora* spp. (Dumas-Gaudot et al. 2000). Such a kind of bioprotection by AM is similar to that of non-pathogenic microorganisms, which can protect plants systemically against different pathogens without first causing symptoms. This phenomenon has been called induced systemic resistance (ISR; Park et al. 1997). Reviews covering the mechanism of ISR by AM fungi (for overview, see Linderman 2000) have focused on nutritional effects, sink competition with infection sites, morphological changes in roots and root tissues, changes in chemical constituents in plant tissue, reduction of abiotic stress, and microbial changes in the mycorrhizosphere.

Data about ISR caused by AM are highly controversial. In general, mycorrhizal plants are more vigorous due to alterations of photosynthesis, leaf hydration, leaf osmotic potential, stomatal conductance, reproduction, and transpiration (Smith and Read 1997; Augé 2001). This could lead to an enhanced tolerance to pathogens, but might also provide a better substrate for some obligate pathogens (Meyer and Dehne 1986). Moreover, defence responses, normally elicited by pathogens, are suppressed in mycorrhizal plants (Guenoune et al. 2001). As shown for infection of leaves of mycorrhizal tobacco plants with *Botrytis cinera*, necrotic lesions appear earlier and grow larger (Shaul et al. 1999). This could be due to a systemic suppression of defence-related properties by AM leading to an increased susceptibility of leaves.

In contrast to leaves of mycorrhizal plants, there are reports of an induction of ISR by AM fungi in roots. This phenomenon was first described in mycorrhizal pea for the root pathogen *A. euteiches* (Kjoller and Rosendahl 1996). Although the spread of *A. euteiches* in mycorrhizal roots as analysed by staining was unaffected, no symptoms of the infection were visible. It could be clearly demonstrated that bioprotection by *G. mosseae* against *A. euteiches* was dependent on a fully established symbiosis with the presence of arbuscules (Slezack et al. 2000). In the interaction of mycorrhizal roots with *Phytophthora parasitica*, implications for plant bioprotection could be drawn from the accumulation of phenolic compounds in the plant cell wall reflecting increased lignification (Cordier et al. 1998). Lignification, considered as an important mechanism for disease resistance (Morandi et al. 1984), may contribute to reducing pathogen proliferation in mycorrhizal roots. Furthermore, the systemic induction of mycorrhiza-induced new isoforms of chitinase, chitosanase, glucanase, and superoxid dismutase was suggested to be responsible for reduced disease symptoms upon *P. parasitica* infections (Pozo et al. 1999, 2002).

In summary, various elements of ISR have been observed in roots colonized by AM fungi. Signals involved in the induction of ISR after formation of the AM symbiosis are completely unknown. It has been assumed that the low level of defence reactions upon mycorrhization could be sufficient for priming (Dumas-Gaudot et al. 2000). Whether plant hormones such as jasmonic acid, known to be involved in mycorrhization (Hause et al. 2002), could serve as putative endogenous signals in mycorrhiza-induced ISR remains to be elucidated.

Signalling pathways in AM

In contrast to the broad host specificity of AM fungi, successful infection with rhizobia, a phylogenetically diverse group of Gram-negative bacteria, is generally host-strain specific (Perret et al. 2000). 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 find the ideal environment to reduce atmospheric nitrogen to ammonia, a source of nitrogen which can be used by the plant (for review see Mylona et al. 1995; Long 1996). Rhizobia are recognized by legumes via specific Nod factors (lipochitooligosaccharides) and additional components, such as extracellular polysaccharides, lipopolysaccarides, and secreted proteins (Perret et al. 2000). Investigations of both AM and nodule formation in legume species have revealed a genetic overlap between both types of endosymbioses.

The first hints of a conservation of signal transduction pathways came from the characterization of genes which are expressed early in nodule development and in mycorrhizal roots. Several genes have been identified which are induced during both symbiotic interactions, e.g. early nodulin genes (van Rhijn et al. 1997), the leghaemoglobin gene *VFLb29* (Frühling et al. 1997), and an aquaporin encoding gene (Wyss et al. 1990). The most convincing evidence, however, resulted from the characterization of the so-called SYM mutants, which defined novel, genetically controlled steps common to AM colonization and nodule formation. A large collection of SYM mutants of *L. japonicus*, *M. truncatula*, and *P. sativus* has been isolated (for review, see Peterson and Guinel 2000). Phenotypically, these mutants do not form functional nodules after inoculation with *Rhizobia*. A subset of these mutants was also impaired in AM symbiosis.

The current knowledge about the signals necessary for nodule and AM formation in legumes is summarized in Fig. 3. Two nodulation-specific Nod factor receptor

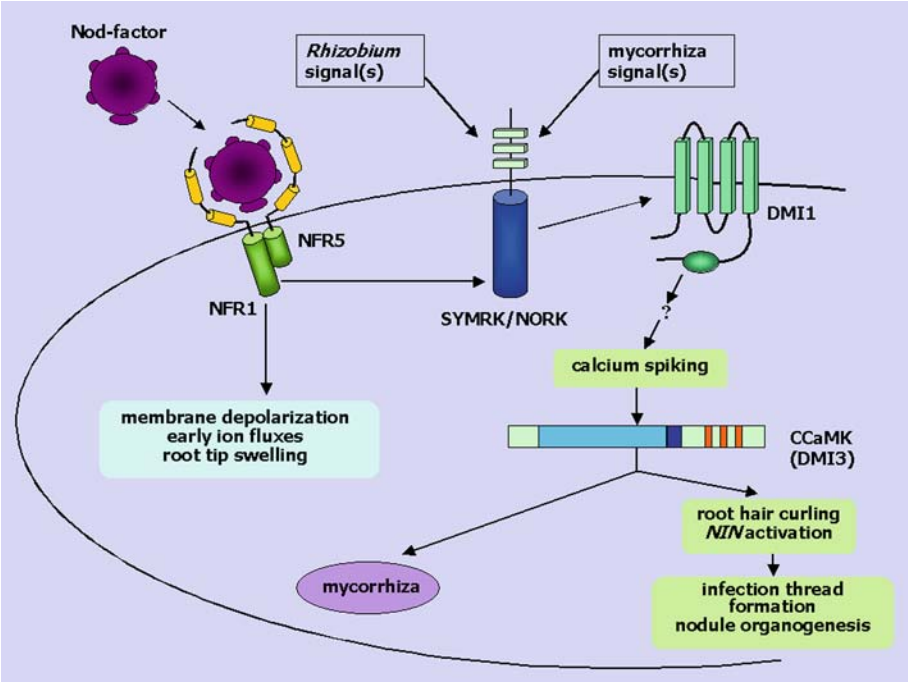

Fig. 3 Model of signalling pathways according to the genetic analysis of SYM genes (adapted from Radutoiu et al. 2003; Parniske 2004). NRF1 and NRF5 are receptor kinases specific for Nod-factor recognition and mediate specific responses to *Rhizobia*. SYMRK/NORK receptor kinases may be the earliest receptors acting in the AM signalling pathway. Both are also involved in the transduction of signals from *Rhizobia*. They activate, probably by phosphorylation, DMI1, a predicted ion channel. This ion channel is necessary for calcium spiking in root hairs interacting with *Rhizobia*. Whether calcium spiking is also necessary for AM formation is not yet clear. Nevertheless, DMI3, a calcium- and calmodulin-dependent protein kinase (CCaMK), is involved in both signalling pathways indicating that Ca^{2+} could also be a messenger in mycorrhizal signalling

(NFR) kinases, NFR1 and NFR5, have been identified in *L. japonicus* to act upstream of the common pathway (Radutoiu et al. 2003; Madsen et al. 2003). These might be involved in Nod-factor binding, because both kinases contain LysM motifs in their extracellular domain. Genes responsible for the first common step in the signal transduction pathways necessary for the rhizobial and AM symbiosis were identified by map-based cloning in *L. japonicus* and *M. truncatula*. The genes code for receptor-like kinases and are named SYMRK/NORK (*sym*biosis *r*eceptor-like *k*inase/*no*dulation *r*eceptor *k*inase; Endre et al. 2002; Stracke et al. 2002). Genetically, SYMRK/NORK acts downstream of the recognition of microbial signalling molecules and upstream of the activation of calcium spiking, which is one of the earliest detectable root-hair responses (Stracke et al. 2002). Both receptors contain extracellularly located leucine-rich repeats, which might be involved in the perception of microbial signal molecules. It is not yet clear, however, how SYMRK/NORK can integrate fungal and bacterial signals. Whether this occurs directly through the formation of SYMRK-NFR heterocomplexes or indirectly via secondary signals remains to be elucidated.

Analysis of a different set of *M. truncatula* mutants, also unable to establish nodulation as well as AM, and designated as *DMI* (*DOES NOT MAKE INFECTIONS*; Catoira et al. 2000; Ané et al. 2002), led to the discovery of two additional points in the Nod-factor-activated signal transduction pathway that leads to responses such as root-hair deformations, expressions of nodulin genes, and cortical cell divisions. DMI1 is required for Nod-factor-induced calcium spiking, whereas DMI3 acts downstream of calcium spiking. Cloning of *DMI1* revealed a membrane-spanning protein containing one domain showing homology to the ligand-gated cation channel domain of archeae (Ané et al. 2004). This protein might participate in the formation of a receptor complex for symbiotic signals and function in Nod-factor-induced calcium oscillation this way (Ané et al. 2004). In contrast, *DMI3* exhibits high similarity to genes encoding calcium- and calmodulin-dependent protein kinases (CCaMKs, Lévy et al. 2004). CCaMK might respond directly to oscillations in calcium concentrations, resulting in a phosphorylation event (Lévy et al. 2004; Mitra et al. 2004). This indicates that, most likely, calcium spiking is an essential component of the signalling cascade leading to successful mycorrhizal colonization, although it has not been shown to occur in the AM interaction.

Conclusions/perspectives

The use of the model legumes *Medicago truncatula* and *Lotus japonicus* has significantly advanced molecular research of mutualistic root-microbe interactions.

Common key elements of symbiotic signalling have been found in the AM and the rhizobial interaction, although both interactions have developed at a different time and with completely different partners. Because the AM symbiosis is the most ancient root-microbe interaction known, molecular research regarding this symbiosis can be expected to reveal further basic symbiotic mechanisms. The current sequencing programs regarding the genomes of both legumes in combination with the targeted generation of mutants will provide the basis for further progress in this direction.

Acknowledgements We thank D. Strack for critical reading of the manuscript and S. Schaarschmidt for help in preparing Fig. 3. We apologize to those colleagues whose work was not cited because of space limitations. Our work is supported by the Deutsche Forschungsgemeinschaft (DFG) within the Focus Program "Molecular Basics of Mycorrhizal Symbioses".

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